Rapid on-site monitoring of pesticide residues with MIP sensors

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BSc. (Chemistry), MSc. (Physical chemistry)

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School of Applied Sciences
RMIT University
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Statement of Authenticity

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; and, any editorial work, paid or unpaid, carried out by a third party is acknowledged.

The work was carried out in the Applied Chemistry, RMIT University, Melbourne, during the official time frame allocated.

Mohammad Al Kobaisi

October 2007
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and sister and brothers, Fatima, Ali and Hassan
Publications
Mohammad Al Kobaisi, Margaret Glewis, Colin Rix, Timur S. Jakubov and David E. Mainwaring,

News items

Peter Dry, Lauren Jones, Researchers a step closer to on site testing of pesticide residue levels in wine, Australian and New Zealand Wine Industry Journal 2006, 21(5), 103.

Lyall Corless, Faster residue testing in sight for wine and grape juice, Mildura Midweek, Tuesday June 26 2007, page 10.

Conferences
Microbial Forensics Workshop (New Diagnostic Technologies), Australasian Plant Pathology Society 2005 conference at Deakin University, Waterfront Campus, Geelong, 26th September 2005, presentation and poster display on MIPs for pesticide residue detection.

Presentations
Forum at Department of Primary Industries (Knoxfield) entitled “Aspects of Microtechnology and potential applications to Agriculture” 9th May 2005 including results up to that date for this project.

Presentation to the Agriculture and Environment Sector Initiatives Group Meeting, Department of Primary Industry, Spring St Office, Melbourne entitled “Rapid On-Line Monitoring of Pesticide Residues with Low Cost Molecularly Imprinted Polymer (MIP) Sensors”, 10th April 2006.

Mohammad Al Kobaisi, Margaret Glewis, Colin Rix, David E. Mainwaring, Michelle Warren, David Riches, Jaqueline Edwards, An Iprodione sensing prototype capable of on-site measurement, Oral presentation at the 5th Symposium of In Vino Analytia Scientia, 24th July 2007, Analytical Chemistry for Wine, Brandy and Spirits; Session five: Chemical and Biochemical Analysis.

Demonstration of the prototype on-site iprodione sensor at Bulong Estate, Yarra Junction, Friday 15th June 2007.
Abstract

Molecularly imprinted polymer coated quartz crystal (QCM-MIP) sensors were fabricated for the detection of “iprodione” in wine and grape juice liquid phases. A set of polymers was templated with iprodione and pyrimethanil to study the imprinting process and the properties of the imprinted and non-imprinted polymers (NIP). The effect of solvent type and ratio, functional monomers (FMs), and templates on the polymer properties was studied. Porogenic solvents produced mesoporous polymers with high surface areas of up to 510 m²/g as measured using the BET method. Non-porogen solvents produced macroporous polymers with much lower surface areas between 50 – 150 m²/g. Low solvent ratios (~5%) produced glassy polymers with very low surface areas, while high solvent ratios (~95%) produced polymer microspheres. Between the above limits porous polymeric systems are produced. Nitrogen adsorption, scanning electron microscopy (SEM) and thermogravimetric analysis (TGA) were used to characterize the morphology and microstructure of the polymers.

The comparison of TGA decomposition profiles of the templated and non-templated polymers showed higher stability for the MIPs when π-π interaction between the template – FM was utilized in the imprinting process. This is due to the higher geometrical order resulting from the π-π stacking of the template and FMs.

Templating processes had a significant effect on the polymers morphology and functionality when the imprinting was based on strong interactions between the template and functional monomers. Iprodione, as a template, had a minimal effect on the EGDMA – MAA polymer system when compared to the effect of pyrimethanil. The stronger pyrimethanil – MAA interactions caused the monomers to associate closely around the template in the pre-polymerization solution, and reduces the pore sizes of the EGDMA – MAA polymer to the minimum possible in the mesopore range causing the shoulder above 4.5 nm in BJH pore size distribution to disappear in the templated polymer. Relying on weak interactions left smaller effects on the polymer properties. It was observed that iprodione leaves stronger imprinting effect in polymer structure when aromatic FMs are used. This effect was maximised using FMs with larger aromatic systems.

The stability of the porous system in EGDMA – DVB – 4VPy polymers was examined at high temperatures, no significant changes were observed in BJH pore size distribution up to 200°C. The FTIR spectra also showed no significant change in the polymers functionality.

The adsorption isotherms of iprodione were studied in model and real solutions, and the selectivity of adsorption was examined in presence of the major components present in the real media. It was observed that iprodione adsorption is strongly influenced by the nature of the medium and although imprinting improved the polymers selectivity and capacity, it had smaller role in the adsorption process.

The MIP and NIP adsorption thermodynamics were studied and the results showed there is a major difference in their re-binding mechanisms. This study also resulted in in-sight into the polymer
structure heterogeneity. Imprinting resulted in larger hydrophobic segments in the polymer network allowing for the interaction between the adsorbed iprodione molecules.

The EGDMA – DVB – 4VPy iprodione templated and non-templated polymers were applied to QCM device using a sandwich method. Pre-polymerization solution volumes of 0.5 – 1 µL were used to synthesize the polymer coatings on the QCM, which caused an approximately 2 kHz shift in the QCM resonator frequency, while maintaining a high quality factor $Q$ for the crystal enabling the QCM-MIP to lock to the phase lock loop (PLL) system. The polymer films response to several aqueous media, and to iprodione solution in these media was then studied. The results showed a novel means to investigate water clustering in contact with solids and adsorbed species exchange on the surface in the adsorption process. Similar to iprodione adsorption in the bulk studies, iprodione sensing using the QCM-MIP system showed great sensitivity to the medium composition. In a controlled medium environment the method shows a near-linear range between 5 and 100 ppm iprodione in white grape juice, with relative standard deviations between 10 and 4%.

The results obtained from this research are important in the areas of the agricultural industry and food quality monitoring. The technology developed has the capacity to provide efficient, low cost, easy to use devices for the on-site evaluation of specific targeted analytes. For example in this research, iprodione detection and quantification in wine and grape juice was demonstrated. These devices may potentially have an improvement in performance in simpler media, such as in aqueous based samples. This may lead to improved food and water safety, and further utilization in the agrochemicals sector, increasing international market competitiveness for exported goods.

Future development of QCM-MIP sensors for the detection of pesticides in viticulture may include QCM arrays in combination with partially selective adsorbent polymer coatings for targeted species and other components in the medium. An array of sensors can efficiently compensate for the influence of other species present in the sample on the response to the targeted analyte. A QCM array can produce a potentially sample “finger-print” that may give a more accurate analysis. The response of the QCM array would then be analysed using a neural network, chemometrics or pattern recognition techniques to identify the chemical being detected and determine its concentration. The quality and accuracy of the QCM-MIP response can be improved by further development of the selectivity and capacity of the sensing materials i.e. MIPs in the QCM – MIP array.
## Abbreviations

<table>
<thead>
<tr>
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<th>Definition</th>
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<tbody>
<tr>
<td>4VPy</td>
<td>4-Vinyl Pyridine</td>
</tr>
<tr>
<td>$a_i$</td>
<td>The activity of component $i$</td>
</tr>
<tr>
<td>ACM</td>
<td>Acrylamide</td>
</tr>
<tr>
<td>AGC</td>
<td>Automatic Gain Control</td>
</tr>
<tr>
<td>AIBN</td>
<td>$\alpha,\alpha'$-Azoisobutyronitrile</td>
</tr>
<tr>
<td>APT</td>
<td>Atomic Polar Tensors</td>
</tr>
<tr>
<td>AT-cut</td>
<td>Quartz plate cut at 35°15’ angle to the z axis</td>
</tr>
<tr>
<td>$B$</td>
<td>Adsorption</td>
</tr>
<tr>
<td>BET</td>
<td>S. Brunauer, P. H. Emmett, and E. Teller</td>
</tr>
<tr>
<td>BJH</td>
<td>E. P. Barrett, L. G. Joyner, and P. P. Halenda</td>
</tr>
<tr>
<td>BT-cut</td>
<td>Quartz plate cut at -49° angle to the z axis</td>
</tr>
<tr>
<td>BVD</td>
<td>Butterworth-van Dyke model</td>
</tr>
<tr>
<td>$C$</td>
<td>Capacitor</td>
</tr>
<tr>
<td>$c$</td>
<td>Number of Components</td>
</tr>
<tr>
<td>$C_{eq}$</td>
<td>The adsorbate concentration at equilibrium</td>
</tr>
<tr>
<td>$c_{ijkl}$</td>
<td>elastic stiffness constant</td>
</tr>
<tr>
<td>$C_P$</td>
<td>Frequency shift pressure coefficient</td>
</tr>
<tr>
<td>$C_{solubility}$</td>
<td>The solubility of the adsorbate in the adsorption medium</td>
</tr>
<tr>
<td>$D_j$</td>
<td>electrical displacement</td>
</tr>
<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinylbenzene</td>
</tr>
<tr>
<td>$E_j$</td>
<td>electric field</td>
</tr>
<tr>
<td>$E_{i,\text{Solvated}}$</td>
<td>The energy of formation of $i$ in its solvated state</td>
</tr>
<tr>
<td>$E_i^\Theta$</td>
<td>The energy of formation of $i$</td>
</tr>
<tr>
<td>$e_{ij}$</td>
<td>piezoelectric stress constant</td>
</tr>
<tr>
<td>EGDMA</td>
<td>Ethylene Glycol Dimethacrylate</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>$f$</td>
<td>oscillation frequency</td>
</tr>
<tr>
<td>$f_o$</td>
<td>QCM fundamental resonance frequency</td>
</tr>
<tr>
<td>$f_a$</td>
<td>Anti-resonance Frequency</td>
</tr>
<tr>
<td>$f_N$</td>
<td>The resonance frequency of the $N^{th}$ harmonic</td>
</tr>
<tr>
<td>$f_r$</td>
<td>Resonance Frequency</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform – Infra Red</td>
</tr>
<tr>
<td>FM</td>
<td>Functional Monomer</td>
</tr>
<tr>
<td>$G$</td>
<td>The complex shear modulus</td>
</tr>
<tr>
<td>$G'$</td>
<td>The storage modulus</td>
</tr>
<tr>
<td>$G^*$</td>
<td>The loss modulus</td>
</tr>
<tr>
<td>$h$</td>
<td>hour</td>
</tr>
<tr>
<td>$h_i$</td>
<td>Thickness of the Crystal Plate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance Liquid Chromatography</td>
</tr>
<tr>
<td>$k$</td>
<td>wavenumber</td>
</tr>
<tr>
<td>$L$</td>
<td>The thickness of the adsorbed layer or film on QCM surface</td>
</tr>
<tr>
<td>$L$</td>
<td>Inductor</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic Acid</td>
</tr>
<tr>
<td>MCMM</td>
<td>Monte Carlo Multiple Minimum algorithm</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MIP</td>
<td>Molecularly Imprinted Polymer</td>
</tr>
<tr>
<td>MMFF</td>
<td>Merck Molecular Force-Field</td>
</tr>
<tr>
<td>$N$</td>
<td>Resonator Harmonic Number</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NIP</td>
<td>Non-Imprinted polymer</td>
</tr>
<tr>
<td>$p$</td>
<td>Number of Phases</td>
</tr>
<tr>
<td>PES</td>
<td>potential energy surface</td>
</tr>
<tr>
<td>PLL</td>
<td>Phase Lock Loop</td>
</tr>
<tr>
<td>$Q$</td>
<td>Resonating Crystal Quality Factor</td>
</tr>
<tr>
<td>$q$</td>
<td>Heat of Process</td>
</tr>
<tr>
<td>QCM</td>
<td>Quartz Crystal Microbalance</td>
</tr>
<tr>
<td>$R$</td>
<td>Ideal Gas Constant</td>
</tr>
<tr>
<td>$R$</td>
<td>Resistor</td>
</tr>
<tr>
<td>$S_{ij}$</td>
<td>strain matrix</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>$T_{ij}$</td>
<td>Stress matrix</td>
</tr>
<tr>
<td>$T_{xy}$</td>
<td>Surface Stress</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>TAC</td>
<td>Triallyl Cyanurate</td>
</tr>
<tr>
<td>TAIC</td>
<td>Triallyl Isocyanurate</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyme</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermal Gravimetry Analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TSM</td>
<td>Thickness Shear Mode</td>
</tr>
<tr>
<td>VC</td>
<td>1-Vinyl Carbazole</td>
</tr>
<tr>
<td>VI</td>
<td>1-Vinyl Imidazole</td>
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<table>
<thead>
<tr>
<th>Symbol</th>
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</thead>
<tbody>
<tr>
<td>$w$</td>
<td>Work done during a process</td>
</tr>
<tr>
<td>WGJ</td>
<td>White grape Juice</td>
</tr>
<tr>
<td>WW</td>
<td>White Wine</td>
</tr>
<tr>
<td>$x_i$</td>
<td>Molar Fraction of $i$</td>
</tr>
<tr>
<td>$X$</td>
<td>Mechanical Reactance</td>
</tr>
<tr>
<td>$Z_s$</td>
<td>Mechanical Impedance</td>
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<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>$\Gamma_i$</td>
<td>The surface excess</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>The interfacial tension</td>
</tr>
<tr>
<td>$\gamma_i$</td>
<td>Activity Coefficient of $i$</td>
</tr>
<tr>
<td>$\Delta f_{\eta}$</td>
<td>Frequency shift caused by viscosity change</td>
</tr>
<tr>
<td>$\Delta f_r$</td>
<td>Frequency shift caused by</td>
</tr>
<tr>
<td>$\Delta f_{m}$</td>
<td>Frequency shift caused by mass</td>
</tr>
<tr>
<td>$\Delta f_T$</td>
<td>Frequency shift caused by temperature change</td>
</tr>
<tr>
<td>$\Delta f_p$</td>
<td>Frequency shift caused pressure change</td>
</tr>
<tr>
<td>$\Delta f_{total}$</td>
<td>Total frequency shift</td>
</tr>
<tr>
<td>$\Delta G^{Ads}$</td>
<td>Gibbs free energy of adsorption</td>
</tr>
<tr>
<td>$\Delta G^{Sol\rightarrow Ads}$</td>
<td>The free energy of changing the solvated species to an adsorbed species</td>
</tr>
<tr>
<td>$\Delta G^{Sol}$</td>
<td>Gibbs free energy of the solution</td>
</tr>
<tr>
<td>$\Delta H^{Ads}$</td>
<td>Entalpy of adsorption</td>
</tr>
<tr>
<td>$\Delta m$</td>
<td>Mass load on the QCM surface</td>
</tr>
<tr>
<td>$\Delta S^{Ads}$</td>
<td>Entropy of adsorption</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Chemical shifts (ppm)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Acoustic Wave Decay Length</td>
</tr>
<tr>
<td>$\varepsilon_{ij}$</td>
<td>permittivity constants</td>
</tr>
<tr>
<td>$\varepsilon_{\text{liquid}}$</td>
<td>Liquid Permittivity</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Liquid viscosity</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>wavelength</td>
</tr>
<tr>
<td>$\mu$</td>
<td>The chemical potential</td>
</tr>
<tr>
<td>$\mu_q$</td>
<td>Shear stiffness of the quartz crystal</td>
</tr>
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<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>$\nu_s$</td>
<td>Shear Wave Velocity</td>
</tr>
<tr>
<td>$\rho_q$</td>
<td>The mass density of the quartz crystal</td>
</tr>
<tr>
<td>$\rho_{\text{liquid}}$</td>
<td>Density of the liquid</td>
</tr>
<tr>
<td>$\sigma_{\text{liquid}}$</td>
<td>Liquid Conductance</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Relaxation Time</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Degrees of freedom</td>
</tr>
<tr>
<td>$\omega$</td>
<td>The angular excitation frequency of an oscillator</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>$\delta$</td>
<td>Chemical shifts (ppm)</td>
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<td>$\lambda$</td>
<td>wavelength</td>
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<tr>
<td>$\mu$</td>
<td>The chemical potential</td>
</tr>
<tr>
<td>$\mu_q$</td>
<td>Shear stiffness of the quartz crystal</td>
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Appendix A
1 Introduction

1.1 Preface

The work described in this thesis is concerned with a detailed examination of the preparation and properties of the molecularly imprinted polymers (MIPs) designed for use in a QCM device for the detection of iprodione, a fungicide widely used in viticulture. Most of the analytical methods reported for quantitation of iprodione residues in various matrices, including grape juice and wine, are based on gas chromatography (GC) or high performance liquid chromatography (HPLC).

Recently, enzyme-linked immunosorbent assay (ELISA) was investigated for the detection of iprodione in agricultural samples, although it has not yet been adopted by the industry. In this method, iprodione was extracted from the homogenized sample using methanol, and then an ELISA kit based on optical density measurements at 450 nm was used for detection.

Chromatography based techniques have several disadvantages for the industry which include the need for trained personnel, and the fact that sample collection and delivery to an analytical laboratory is costly and time consuming. Additionally the extraction and clean up steps in the sample preparation require significant time as well as large amounts of solvents. Clearly, there is a need for a low cost, rapid and on-the-spot assay technique for species such as iprodione.

This thesis addresses this matter, and is based on utilizing molecularly imprinted polymers (MIPs) as a recognition system mounted on a quartz crystal microbalance (QCM) as a mass sensitive transducer producing a detectable electrical signal from iprodione adsorption to the attached MIP film.

1.2 Molecularly imprinted polymers (MIPs)

Developing a material with selective recognition is a core requirement for such chemical sensing, and molecular imprinting is one method used to produce materials with this property. The Soviet chemist, M. V. Polyakov, was the first to investigate the field of molecular imprinting in his quest to develop silica for use in chromatography (1931). But it wasn’t until 1972 that this technique was rediscovered by Klotz and Wulff to produce synthetic polymers with selective recognition.
properties. Since then, the field has shown rapid growth and MIPs have found their way into a vast range of applications in the technical and scientific disciplines.

Molecular recognition plays a central role in biological systems and is based on the fundamental chemical interactions between molecules. When a molecular recognition event undergone by an enzyme, protein or a nucleic acid is examined in detail, it is found that the process involves a combination of various intermolecular chemical interactions, based mainly on hydrogen-bonding, dipole – dipole and ionic interactions. Such a process occurs within an “evolutionary designed” functionality and geometrical space, which enables the complex recognition process to be selective, sensitive and dynamic.

Polymer imprinting is a synthetic strategy originally adopted to mimic biological antibodies, but in a vastly simplified manner, so as to tailor specific and selective binding sites for a particular target molecule. This approach is based on freezing in multiple interactions between a template molecule and functional groups on functional monomers which form an association prior to polymerization. Removing the template molecule from the “frozen” association after polymerization is expected to provide a “tuned” site capable of rebinding molecules with the same geometry and physicochemical characteristics. Simultaneous weak interactions at the correct distances and in the correct orientation, i.e. the desired topography, can provide sufficient energy and specificity for selective rebinding.

Some of the important advantages listed by Dickert et al. for the use of MIPs in mass sensing devices are:

1. Fast and relatively uncomplicated polymerisation reactions,
2. The MIP sensing material can be either a bulk material or a thin sensor layer, which allows tuning of material properties towards the desired behaviour,
3. Polymeric materials can be deposited on most transducers using standard industrial processes, and
4. Polymeric matrices usually exhibit very favourable chemical, mechanical and thermal stability.

The aim of the present project was to examine the physicochemical properties of the target analyte, iprodione, and the types of interactions that could be used in the polymer imprinting process. Thus, it was necessary to prepare a range of polymers, and to study the morphology, adhesion, solvent effects and film forming properties of both the control and imprinted polymers. The adsorption of iprodione by the polymers was investigated, especially with regard to the influence of the solvent media on adsorption. The selectivity of iprodione adsorption was then studied to determine the crucial factors that control optimal sensitivity for the preparation of a QCM based sensor utilizing an imprinted polymer coating.
1.3 Chemical sensing

In general terms “sensing” is the production of a detectable, or measurable, digital or analogue signal in response to a physical, chemical or biological stimulus. A sensor device includes the sensing surface or bulk material and electronic circuits, which act as transducers to convert the stimulus to an electrical signal, amplify the signal and filter the noise. This signal may appear as a read-out, a warning alarm, or be used as input to activate another system 13, 14.

Chemical sensing is based on the interaction of a particular chemical species with the sensing part of the sensor. Ions, small and large molecules, in either gas or liquid media, will cause the stimulus event, and produce a signal to assay their concentration or detect their presence. The factors determining the overall performance of a sensor are the response mechanism, selectivity, detection limit, sensing range, response time, reusability and lifetime.

A level of recognition is necessary to produce a meaningful signal. Introducing selectivity to a chemical sensor depends on the specific interaction used to produce the signal, and the number and concentration of interfering species present in the matrix to be analysed. The selectivity toward species such as drugs in biological fluids, hazardous chemicals in drinking or wastewater, toxic substances in industrial effluents, or particular gas species in a closed or open atmosphere, must consider the matrix and competing species likely to be present.

Traditional chemical sensing is based on potentiometric, amperometric, optical and acoustic properties 15. Many of the assay methods are generally time consuming and expensive. Thus, there is great interest in developing new micro-sensors and miniaturizing traditional sensing systems that can be used to produce portable, affordable and rapid response devices.

1.4 Acoustic resonators

Acoustic based sensing devices use elastic waves at frequencies well above the audible range (about 1 MHz to 1000 MHz) propagating in a specially designed solid sensing structure as the sensing mechanism. These sensors exhibit changes in their resonant frequency as mass changes occur on their surface, and this offers detection with high sensitivity. Such devices can also monitor other solid and fluid properties such as polymer modulus, electrical conductivity, liquid density and viscosity 16. The wide variety of sensors in this class enables them to extend their application to different areas of health, science and engineering.

Most acoustic wave devices are fabricated by applying metal electrodes to a piezoelectric substrate. The electrodes act as transducers to launch acoustic waves into the material at these ultrasonic frequencies. A distinction can be made between ‘one port’ acoustic devices such as the QCM resonator, and ‘two port’ devices including Surface Acoustic Wave (SAW) device, Acoustic Plate Mode (APM) sensor and Flexural Plate Mode (FPM) device 15.
Table 1.1 The summary of the characteristics of the most commonly utilized acoustic piezoelectric microsensors, from Ref. 17,18.

<table>
<thead>
<tr>
<th>Resonator</th>
<th>Plate thickness</th>
<th>Medium</th>
<th>Wave type</th>
<th>Operating frequency (MHz)</th>
<th>Temperature stability</th>
<th>Mass sensitivity</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCM</td>
<td>$\lambda/2$</td>
<td>Gas-liquid</td>
<td>Bulk</td>
<td>4-30</td>
<td>High</td>
<td>$-\frac{1}{\rho_\nu d}$</td>
<td>Quartz, 6 MHz, $d = 277 \mu m$, $\nu_\lambda = 3330 \text{ ms}^{-1}$, $\lambda = 554 \mu m$</td>
</tr>
<tr>
<td>SAW</td>
<td>$\gg \lambda$</td>
<td>Gas</td>
<td>Surface</td>
<td>30-500</td>
<td>High/medium</td>
<td>$-\frac{f_0}{\rho_\nu V}$</td>
<td>Quartz, 158 MHz, $d = 760 \mu m$, $\nu_\lambda = 3360 \text{ ms}^{-1}$, $\lambda = 20 \mu m$</td>
</tr>
<tr>
<td>FPW</td>
<td>$\ll \lambda/2$</td>
<td>Gas-liquid</td>
<td>Plate</td>
<td>2-7</td>
<td>Medium</td>
<td>$-\frac{1}{2\rho_\nu d}$</td>
<td>ZnO, 5.5 MHz, $d = 3.5 \mu m$, $\nu_\lambda = 3330 \text{ ms}^{-1}$, $\lambda = 554 \mu m$</td>
</tr>
<tr>
<td>SH-APM</td>
<td>$3-10\lambda$</td>
<td>Gas-liquid</td>
<td>Plate</td>
<td>25-200</td>
<td>High</td>
<td>$-\frac{f}{\rho_\nu d}$</td>
<td>Quartz, 101 MHz, $d = 203 \mu m$, $\nu_\lambda = 550 \text{ ms}^{-1}$, $\lambda = 100 \mu m$</td>
</tr>
</tbody>
</table>

The efficiency of the sensing mechanism in acoustic wave devices depends on the selectivity of the binding of the targeted species to the sensing device. Various mechanisms have been used to adsorb particular species, in biological and chemical media, in gas and liquid phases. Antibodies, selective adsorbing membranes, functional surfaces etc. have been used to exclude or minimize, the effect of interfering components present in the sample.

1.5 Quartz crystal microbalance (QCM)

The QCM is a transducer that functions on the basis of acoustic wave propagation in a piezoelectric material 15. Extensive research has been undertaken examining the prospect of the QCM as a chemical and gravimetric sensor in different areas, and several commercial chemical sensing applications of QCMs have been developed, especially for gaseous species.

The basic principles on which acoustic wave devices function are summarized in Appendix A, including the effect of density and viscosity of the working media, the mass load, elasticity, and surface roughness. Understanding the effects of these parameters is essential for developing a sensor based on the combination of a QCM with a MIP.

The QCM is an ideal choice as a transducer in this application since:

1. It possesses a high mass sensitivity 19.
2. It has a linear response over a wide range of mass load.
3. The response stability around room temperature for an AT-cut QCM device when operated in the thickness shear mode, provides a zero temperature coefficient of frequency at 25 °C, and a remarkable temperature stability around room temperature 20.
4. Unlike surface acoustic wave (SAW) devices, the QCM stability is less sensitive to a high mass load and the density and viscosity of the medium. These factors allow it to be used in liquids and with the attachment of a heavy MIP sensor film much more effectively.

1.6 QCM – MIP

To produce a meaningful signal using a QCM device it is necessary to have a detectable selective mass change on the device surface. The selectivity of the mass change has to be arranged to target a particular species in a specific medium in the presence of other competing or inert species. Modified metal electrode surfaces, different types of coatings, adsorbent films, membranes, tethered antibodies, reactive surfaces, and many other techniques and mechanisms have been used to detect the presence of targeted species or study fundamental processes using a QCM.

Recently, Molecularly Imprinted Polymers (MIPs) have been considered as selective adsorbents and used as coatings for QCM devices. The relationship between the detection limit of the sensing device and the capacity, surface area and porosity of MIPs is crucial to the performance of a QCM-MIP combination, and these factors will be reviewed in the following chapter.

In this thesis, a MIP has been applied to a QCM for use as a sensor in the detection of the viticulture antifungal species, iprodione, in the liquid phase. The details of the applications of a QCM – MIP device combination are reviewed in the following chapter, which suggests that a combination of molecular imprinting and acoustic wave based sensing is capable of producing a portable and low cost sensing device able to assay iprodione in grape juice and wine.

The performance of a QCM – MIP combination depends on the properties of the individual components involved in the sensing event, this includes the adsorption kinetics of the analyte on the MIP and the capacity and affinity of the MIP towards the analyte itself.

Fundamental considerations in using such a QCM – MIP device are:

1. The kinetics of the sensing process; a slow adsorption process causes a long response time, and this may detract from the long term stability of the QCM response.

2. The stability of the system response especially in aqueous media. This is a critical factor arising from cluster formation of water molecules in solution, and the sensitivity of the cluster size to other components present in the solution. This has been studied and discussed in this work.

3. Low sensitivity at lower concentrations, which depends on the adsorbent capacity.

The material behaviour of the MIP film on a QCM device is also important and must be considered:

1. The quality of the sensing material attached to the QCM, this includes; mass load, mass distribution, film thickness, physical strength, the MIP adhesion to the transducer surface and the surface roughness of the material in contact with the analysis medium.

2. The MIP film functionality, this includes; its swelling in the working medium, and its stability in the medium under real operating conditions.
1.7 Iprodione - the target analyte

Iprodione (1) is a dicarboximide fungicide widely used in agriculture. Extensive information about the material, its toxicity, residues in food, and its usage rules and regulations worldwide are contained in the International Programme on Chemical Safety (IPCS) website, and although the material is an appropriate fungicide, its presence in food products must be minimized.

Based on an amendment to the Australia and New Zealand Food Standards Code, the legally permissible maximum residue level (MRL) for Iprodione in Australian wine grape juice is 20 mg/kg (20 ppm). The same MRL was maintained for iprodione in grapes according to the Australian Wine Research Institute MRL Database, without specifying an MRL for iprodione in wine, although there was no mention of an iprodione MRL in wine in the Explanatory Statement of Application A582 on Maximum Residue Limits (April, May, June 2006).

Iprodione is used in pre-harvest viticulture treatment to prevent grey mould (caused by Botrytis cinerea) and other fungal diseases. Botrytis cinerea is one of the most destructive fungal pathogens in viticulture; its growth causes serious production losses and adversely affects wine quality. Since the iprodione is applied late in the growing season, there is a high probability of transferring the residues to grape juice and wine products. Mlikota et al. examined the effect of iprodione on the must fermentation of grape juice as part of a comparative study of five fungicides. In this case they found that four of the compounds, iprodione, vinclozoline, procymidone and dichlofluanid did not affect the fermentation process, whereas the fifth, folpet, strongly inhibited the beginning and continued progress of fermentation.

1.8 References


2 Detection using a MIP applied to a QCM

2.1 Selectivity

The sensing ability of acoustic wave devices is based on gravimetric changes occurring on the sensing surface, which has one side in contact with the oscillating piezoelectric crystal and the other side in contact with the analyte matrix. Depending on the operating conditions and environment, it is necessary to introduce sufficient selectivity to the sensing material to minimize the effect of interfering species present in the analyte matrix. Size exclusion, shape, chemical functionality, antibodies, pre-constructed adsorption sites, and recently, molecularly imprinted polymers (MIPs), have all been used to improve the selectivity, and increase the sensitivity of acoustic wave sensors.

Examples of such approaches in mass sensors \textsuperscript{1-12} include cavitand molecules such as calix[n]arenes, calix[n]resorcinarenes and cyclodextrins which are all being used as recognition sites that exploit their size exclusion and chemical interaction characteristics. In addition, functionalizing the sensor surface, or depositing Langmuir-Blodgett monolayer films, have been utilized to target particular species in the analyte matrix or to study a specific interaction \textsuperscript{13-20}. The ultimate binding selectivity is demonstrated by antibodies due to their lock and key relationship with the target molecule. However, these have their own drawbacks and limitations particularly temperature range, medium and pH sensitivity, and storage stability \textsuperscript{13, 21-28}. Both synthetic and natural antibodies have been used as selective sites in mass sensing devices.

2.2 MIPs

Polymer imprinting is a synthetic strategy adopted to mimic biological antibodies to tailor specific and selective binding sites for a particular target molecule. This approach is based on freezing multiple interactions between a template molecule and functional groups on functional monomers formed by association prior to polymerization. Removing the template molecule from this association after polymerization is expected to provide a specific site capable of rebinding molecules with the same geometry and physicochemical characteristics. Simultaneous weak interactions at the right distance and direction, i.e., the desired topography, can provide sufficient energy and specificity for selective rebinding.
When the interaction energy between the template and the functional monomer is sufficient to form a stable association prior to polymerization, the imprinting method is called non-covalent imprinting. But if the template – functional monomer interaction does not form a strong and stable association, covalent imprinting may be used to form an easily cleavable covalent bond between the template and a polymerizable unit prior to polymerization. After polymerization, the binding site can be created by cleaving the bond between the template and functional monomer. Table 2.1 lists the types of intermolecular interaction and energies involved in molecular recognition processes.

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Energy (kJ/mol)</th>
<th>Stability</th>
<th>Lability</th>
<th>Distance (r) dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalent Carbon</td>
<td>150 - 500</td>
<td>High</td>
<td>Low</td>
<td>1st Row M-L</td>
</tr>
<tr>
<td>Covalent coordinate bond</td>
<td>80 - 340</td>
<td>High</td>
<td>High</td>
<td>2nd Row M-L</td>
</tr>
<tr>
<td>Hydrogen bond</td>
<td>5 - 60</td>
<td>Medium</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Ion-Ion</td>
<td>40 - 380</td>
<td>High</td>
<td>High</td>
<td>1/r</td>
</tr>
<tr>
<td>Ion-Dipole</td>
<td>40 - 210</td>
<td>High</td>
<td>High</td>
<td>1/r², 1/r⁴</td>
</tr>
<tr>
<td>Dipole-Dipole</td>
<td>5 - 40</td>
<td>Low</td>
<td>High</td>
<td>1/r³, 1/r⁶</td>
</tr>
<tr>
<td>Cation-π stacking</td>
<td>5 - 80</td>
<td>Medium</td>
<td>High</td>
<td>1/r², 1/r⁴</td>
</tr>
<tr>
<td>Dispersive stacking</td>
<td>5 - 20</td>
<td>Low</td>
<td>High</td>
<td>1/r³, 1/r⁶</td>
</tr>
<tr>
<td>Solvent effect</td>
<td>5 - 40</td>
<td>High</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>

The stability of MIPs and their ability for molecular recognition provide a vast range of applications for imprinted polymers in chromatography, chemical sensing, and specific assays. Some imprinted polymers have demonstrated catalytic and enzymatic activity and, some drug delivery systems have also been developed. The imprinting technique is described and reviewed in many monographs and articles, and is a growing area of scientific research.

### 2.3 QCM-MIP

The ability to tailor specific sensing interfaces using polymer imprinting has produced materials for sensing applications. The antibody mimicking sites residing in these synthetic imprinted polymers makes them amenable for application to different areas of science and technology. The main advantage of MIPs versus natural anti-bodies is their chemical and thermal stability. The ability to use MIPs in organic media and under harsh acidic or basic conditions gives these polymers a robust and competitive edge. Additionally, the MIP thermostability and their resistance to harsh environmental conditions, provide a long shelf life. The nature of the reversible rebinding process enables MIPs to be reused without losing their performance efficiency. Low-cost large-scale production, ease of synthesis, and the predictability of specificity by logical design, provide economic advantages which have encouraged researchers to further develop the methodology and applications of MIPs.
On the other hand, using the imprinting approach to produce sensing materials does have some limitations. Generally, MIPs contain a low density of recognition sites, which will not provide a high level of sensitivity in device applications. Since most of the imprinted sites are buried within the polymer structure, the response time is very slow due to the diffusion barrier for the analyte molecule to migrate to the imprinted site destination. The macroscopic dimensions of the polymers, and the poor electrical conductivity of MIPs as sensing materials, make communication of the rebinding event with electronic transducers difficult.

Dickert et al. have reviewed the application of MIPs in combination with a QCM sensing device. Ye and Haupt focused on the application of MIPs as antibody and receptor mimics for assays, sensors and drug discovery. And Alexander et al. included a section dedicated to QCM-MIP in their survey of the literature up to the year 2003. But to date, there is no collective dedicated review which analyses and categorizes the different approaches taken to incorporate sensing in a QCM detection system.

2.3.1 Detection

Response time, detection limit, saturation limit, signal-to-noise ratio and working conditions (e.g. pH, humidity, temperature, etc.) are the most important factors in the sensing process. To control these factors in designing a QCM-MIP based sensor, film thickness, surface roughness, rebinding site density, rebinding kinetics, working media, and the mass uptake or molecular weight of the targeted species must be optimized.

Although MIPs are stable, easy to prepare, and inexpensive, there are still challenges to overcome in their fabrication and use. Different approaches have been used to apply MIP materials to a QCM in order to optimize selective mass uptake and detection of targeted analytes. Difficulties with material properties and methods have forced researchers to develop efficient techniques to apply MIPs to QCM substrates in order to obtain the necessary response from the sensing device. These techniques are reviewed here.

Having a stable base-line and high signal-to-noise ratio of the QCM response in the absence of targeted analyte is essential for quantifying the analyte signal. The swelling and reactivity of the sensing membrane in the presence of the matrix are often limiting properties. Usually, a MIP membrane composed of highly cross-linked material can minimise the swelling of the film. Using a secondary linear polymer as the matrix for the MIP particles, or imprinting linear polymers by electrochemical means worsens this problem if the working matrix is inappropriate.

Sensing membranes with rigid structures have a very short response time, and several reports of imprinted TiO$_2$ and silica-alumina have exhibited response times from a few seconds to 1 min. By reducing the film thickness of methacrylic acid (MAA) – trimethylolpropane trimethacrylate (TRIM) imprinted polymer to less than 50 nm using surface initiated imprinting, the response time for propranolol detection was reduced to less than 1 min.

Analyte adsorption by the polymer does not follow the surface theory model of hard sphere adsorption. This arises because the polymeric structure of the sensing membrane is flexible and
elastic, despite a high degree of cross-linking, and the conformation of organic analytes is too dynamic to be considered as a hard sphere. Thus, the response time of the acoustic wave device depends on both the analyte adsorption mechanism and the relaxation mechanism of the polymer structure, after the analyte molecules have diffused into the structure and have become accommodated at the binding sites. This relaxation process depends on the polymer swelling properties, solvent medium and analyte.

The kinetics of analyte rebinding to the sensing film is another factor that affects the response time of the sensor. A thick or poorly porous sensing membrane, will introduce a diffusion barrier to the rebinding kinetics, which includes the time necessary for the analyte molecule to reach a rebinding site buried away from the matrix-MIP film contact surface, as seen in Figure 2.1. The use of a thinner film for the MIP can thus minimize the response time of the sensor.

![Figure 2.1 The analyte sensing film membrane/liquid interface](image)

The signal magnitude and detection limit of the sensor depend on the density of rebinding sites in the sensing film. A smaller amount of the selective adsorbent or a thinner MIP film will reduce the sensitivity of the sensor. This is the reason behind efforts to produce high capacity, highly selective sensing materials. Dickert et al. 56 have studied the effect of film thickness on the magnitude of the sensor response. Using a polyurethane (PU) film, their results demonstrated that the rebinding of the analyte degraded and fresh engine oil was not surface dependent, but a bulk phenomenon. The size of the molecule was another factor determining the nature of the adsorption process. The short response time (~10 min) of an albumin imprinted coating on a QCM device, suggested that the albumin adsorption on the polymer film was primarily surface adsorption 57. A similar response time was obtained for surface adsorption, as the results of Lieberzeit et al. showed for stamp-printed insulin on the surface of a PU film 53.

The importance of the coating film properties can be seen by comparing the results of Fu et al. 58 and Matsuguchi et al. 59 who designed QCM-MIP sensors for volatile organic compounds (VOC). A porous MIP system held in a poly(isobutylene) matrix as a sensing coating exhibited response time of less than 2 min 58, while less porous MIP particles held in a poly (methylmethacrylate) matrix demonstrated a much slower response time of about 60 min 59.

It is clear that the sensing membrane affinity for the targeted analyte will determine the partition equilibrium between the working medium and the sensing surface. Increased mass uptake occurs at lower concentrations of analyte in high affinity MIP films, and this lowers the detection limit of the
sensing device. Percival et al. 60 and Stanly et al. 61 developed a film with a saturation level around 1 ppm for terpenes and L-serine, but with a very low detection limit of 200 ppb for terpenes and 2 ppb for L-serine, demonstrating the compromise between the high affinity and low capacity of the sensing film.

2.3.2 Sensing film development

The essential parameters monitored to detect changes on QCM based sensors are the frequency shift of the maximum real-part of the crystal admittance, and the series resistance in the Butterworth–van-Dyke (BVD) equivalent circuit 62. In liquid media, the frequency shift is dependent on the mass loading effect, the viscous coupling with the liquid, surface roughness, and the occurrence of other surface stresses. The commonly used Sauerbrey equation 63 will not relate the mass loading to the frequency shift quantitatively when one or more of these factors become dominant.

Using a MIP as the sensing material in a QCM based device must be well considered to ensure that the sensing signal is optimized, therefore minimizing deviation from the ideal conditions necessary for the application of device. The MIP film applied to the QCM surface must meet certain criteria, otherwise the oscillation may fail due to high damping, due to a high mass load (film thickness), a high degree of roughness, or specific visco-elastic properties (due to a low degree of cross-linking or the MIP particles holding matrix). The uniformity of the distribution of the sensing film on the QCM surface is another parameter which can affect the performance of the sensing device.

The importance of surface recognition in MIP technology was recognized in the early stages of development. Kempe et al. 64 improved the selectivity of silica particles toward the enzyme ribonuclease A by surface imprinting. In this case, the imprinted material was used as a stationary phase and its behaviour investigated by HPLC.

In early work, Dickert et al. 42, 65 synthesized a MIP film, in an open, but controlled, atmosphere and using thermo-polymerization. The film was designed to detect polycyclic aromatic hydrocarbons in water on a QCM plate. Since then, different approaches have been developed to make a MIP film function in acoustic wave devices such as QCM. Spin coating, electro-polymerization, surface polymerisation, and sandwich techniques have all been utilized to cast MIPs on QCMs.

2.3.2.1 Spin coating

Luo et al. 66 and Liang et al. 67 cast films of MIP particles by spreading a uniform suspension of MIP particles in a PVC solution prepared in tetrahydrofuran (THF) on the QCM electrode surface and leaving the solvent to dry at room temperature.

In order to produce a thinner and more evenly distributed coating, Percival et al. 68 spin coated a suspension of nandrolone imprinted particles in a THF solution of PVC on the QCM device. The same method was used by Yan et al. 69 to coat a QCM device with daminozide imprinted MAA – EGDMA microspheres.
Fu et al. have used poly (isobutylene) (PIB) as an adhesive for binding the imprinted polymer particles to the QCM. A solution of PIB in trichloroethylene (TCE) was spin coated on one side of the QCM at high speed to produce a thin uniform coating, then a suspension of very fine MIP particles in ethanol was spread on the PIB coating, and the solvent left to evaporate at room temperature: this left a MIP coating that was more exposed compared to the technique used by Percival et al.

Matsuguchi et al. have used poly(methyl methacrylate) (PMMA) as the matrix polymer to attach MIP particles to the QCM surface. The MIP particles were homogenized by ultrasonic stirring in an acetone solution of PMMA and then spin coated on the QCM. The film was then heated at 80°C under reduced pressure for 2 hours, resulting in a film having a thickness of several microns.

Das et al. irradiated a MIP polymerization solution while spin coating. Using this technique a thin, uniform coating of the sensing material was formed directly on the crystal without the need for binder.

Hyden et al. spin coated a polymerization solution to produce a thin, uniform film of the polymer, prior to being imprinted by lyzozyme using a stamping technique in which stamps were pressed on the QCMs during the UV polymerization. A different approach was taken to coat the QCM surface with a polymer film to imprint Tobacco Mosaic Virus (TMV) using the stamping technique. In that case, the polymerization solution was partially pre-polymerized by heating at 70 °C for 5 min prior to spin coating. Stamps were again clipped against the crystal surface during UV polymerization. To imprint a larger template, such as a red blood cell, the device surface was coated using the drop – coating technique, and then the film was stamped with a layer of the template on a glass slide. In a study to imprint albumin, Lin et al. spin coated the polymerization solution in two steps before UV irradiating the film under nitrogen for 36 hours.

Lieberzeit et al. studied the effect of the coating roughness on the electronic response of a screen printed QCM based sensor. They imprinted templates ranging from small molecules to erythrocytes to study the sensors' response in each case. Schmidt et al. investigated the factors affecting the spin coating of MIPs under UV irradiation. The effect of different porogens, initiators and spinning rates on the morphology and imprinting efficiency of the spin coated MIP films were studied.

Ersoz et al. used methacryloylamidocysteine to activate the QCM gold electrode surface prior to forming a polymer film using UV irradiation of a dropped and spread polymerization solution under nitrogen atmosphere.

### 2.3.2.2 Electro-synthesis

In an attempt to reduce the MIP film thickness and overcome the diffusion barrier, several other approaches have been taken to produce MIP thin films or monolayers. Reducing the film thickness also improves another aspect of MIP based sensors; it increases the electrical contact between the recognition site and the electronic transduction.

Sallacan et al. used electro-polymerization to produce polymer modified electrodes. Electro-synthesis of the polymer enabled the construction of the MIP film in a controlled way using a multi-
step procedure. Fing et al. 77 and Liao et al. 78 also used cyclic voltammetry for the electro-synthesis of a polymer film on the QCM gold electrode. This method demonstrated an efficient way to imprint sorbitol and 5-fluoro-1-(tetrahydro-2-furyl)uracil (tegafur) in poly(o-phenylenediamine) and poly(m-aminophenol) respectively.

The limiting weakness of the electro-synthesis of MIP films is the solubility of the monomers and the template in the electrolyte solution. In addition, the formation of the template – functional monomer association in electrolyte media will be dominated by the ionic and hydrogen-bond interactions present in the solution, which may reduce the efficiency of the imprinting process.

2.3.2.3 Sandwich techniques

The sandwich technique was one of the first methods to be reported for coating QCM devices with a MIP film. The major shortcoming of this technique is the failure to consistently reproduce MIP film quality and thickness.

Haupt et al. 79 imprinted S-propranolol in a MAA – TRIM copolymer by UV irradiation of a pressed sandwich of the polymerization solution. Using TRIM as the cross-linker improved the film adhesion to the device enabled researchers to reduce its ratio to such a level that the MIP retained its high specificity when used in binding assays.

Kugimiya et al. 80 used the sandwich method to coat a 9MHz platinum electrode AT-cut QCM oscillating plate. To improve film adhesion to the QCM, vinyl groups were introduced to the surface by thiolizing the platinum electrode with a solution of 1-butanethiol and allyl mercaptan in ethanol/water. Then, a sandwich of the polymerization mixture between the platinum electrode of the QCM and a glass microcover was prepared. Polymer synthesis was initiated by UV irradiation at room temperature under a nitrogen atmosphere. Later, the same group reported the successful casting of a sialic acid imprinted polymer on a QCM crystal using the same technique 81. Subsequently Percival et al. 60 used the Kugimiya and Takeuchi method to cast a MIP film on a QCM plate, and showed that a QCM loaded with a thick MIP film performed well in the liquid phase to detect L-menthol selectively.

Stanley et al. 61 used the same method to imprint L-serine. They investigated the effect of the cross-linker ratio on the film adhesion to the QCM, and found that a high cross-linker ratio can lead to a rigid polymer film with significantly reduced adhesion to the electrode surface. Liu et al. 82 have used the same method to cast a polymer imprinted with L-tryptophan on a QCM electrode.

Feng et al. 83 used the sandwich method to produce a formaldehyde imprinted coating on a QCM for use as a gas phase sensor. They compared the effect of different diluents on the imprinting process and found that in the case of imprinting formaldehyde in MAA – EGDMA polymer toluene showed better capacity, selectivity and polymerization quality compared to MeCN, chloroform, and hexane. This was attributed to due to poor hydrogen-bonding between the solvent molecules and the template and functional monomer, which allows the association interaction between formaldehyde and MAA in the pre-polymerization solution to be unhindered by the solvent.
2.3.2.4 Surface polymerization

This technique is based on attaching a convenient organic functionality to the surface so that polymerization can start from the surface, or involve the functionalized surface in the polymerization process. Activating this functionality in the presence of monomers in the solution allows polymer growth from the surface, thus forming a layer of the polymer which is chemically bonded to the surface. Bossi et al. and Piletsky et al. have used such surface grafting to prepare a MIP for the recognition of protein and β-agonists using optical detection. Many other investigations have used surface grafting of a MIP for purposes other than acoustic wave sensing. Turner et al. reviewed the methods used to imprint proteins, from conventional bulk techniques to novel thin film and monolayer surface imprinting approaches.

The surface polymerization technique has been used to modify a QCM electrode with a MIP sensing layer. Lin et al. have developed a biosensor for peptides that employed a new cross-linker, (N-Acr-L-Cys-NHBN)₂ (1), to deposit the imprinted film. A monolayer of this cross-linker was immobilized on the electrode surface, then, after depositing 4 μL of the polymerization solution on top, the chip was placed in a vial containing 3 mL of acetonitrile and irradiated with UV light. The polymer formed a thin film on the gold surface.

Piacham et al. functionalized a gold electrode surface using 2-ethyl-5-phenylisoxazolium-3'-sulfonate to produce carboxyl groups on the surface, by forming a self assembled monolayer (SAM) as shown in the following scheme, Figure 2.2. The initiator, 2,2'-azobis(2-amidinopropane) hydrochloride (ABAH), was attached to the carboxyl groups. The initiator-coated resonator was immersed in the polymerization solution and the polymer film synthesis initiated by UV irradiation.

Bunte et al. used spray coating to apply a MIP layer on a QCM device. Parameters such as number of sprays and the spraying distance were optimized. A rigid film and strong adhesion to the crystal were not necessary due to the gas phase application of the QCM-MIP sensor to monitor TNT vapours.

2.3.3 Film adhesion

The physical properties of the film applied to the QCM crystal are one of the more important parameters to consider for the regeneration and reusability of the sensing device. Strong adhesion of the sensing material to the surface of the device improves its robustness. Some of the techniques used to improve MIP – surface adhesion include using binding matrices, the introduction of polymerizable functional groups to the QCM device surface using thiolizing agents, and using monomers having a strong interaction with the QCM electrode surface.
To increase the polymer film adhesion to the electrode of the QCM device the electrode surface must first be cleaned. The commonly used reagent for this is warm piranha solution (30% wt. \( \text{H}_2\text{O}_2 \) – concentrated \( \text{H}_2\text{SO}_4 \), 3:7 v/v, 60-70°C). Treating the gold electrode surface on the crystal with this solution for 10 minutes removes organic matter from the surface and produces an active surface for film application. Alternatively, Ersoz et al. \(^{75}\) have used a boiling solution of \( \text{H}_2\text{O}_2 \) (33%), \( \text{NH}_3 \) (33%) and deionized water in a 1:1:5 ratio to clean the gold electrodes.

Piacham et al. \(^{55}\) used surface initiation to chemically bind the polymer film to the substrate as shown in Figure 2.2. In this process, initiator, ABAH, was bond to the gold electrode using a thiolizing agent. In order to improve polymer film adhesion, Kugimiya et al. \(^{81}\) introduced a vinyl functional group to the QCM platinum electrode surface by immersion in an allyl mercaptan (3.0 mM) and 1-butanol (1.5 mM) solution in 4:1 ethanol-water for 12 hours. The saturated 1-butanol was used as a spacer between vinyl groups adsorbed on the surface.

For the same purpose, Liu et al. \(^{82}\) immersed a cleaned crystal in an ethanol solution containing 3 mM thiocic acid (2), modified glycyl methacrylate (GMA) (3) and 3 mM thiocic acid dodecane ester for 12 h in order to introduce vinyl groups onto the gold electrode of the QCM. After the self-assembly process, the crystal was rinsed thoroughly with ethanol and then dried under \( \text{N}_2 \).

In order to prepare a monolayer of methacryloyl groups on the surface, Ersoz et al. \(^{75}\) treated the cleaned gold surface of the QCM electrode with a 10 mM solution of methacryloylamidocysteine (MAC) in ethanol/water (4:1, v/v) for 24 h.

Lin et al. \(^{87}\) and Tai et al. \(^{89, 90}\) designed a new cross-linking monomer containing a chiral center and a disulfide functional group. The gold electrode surface was functionalised using the polymerizable acrylamide groups after treatment with (N-Acr-L-Cys-NHBn)\(_2\) (1).
Das et al. 70 found that using the 1,5-bis(2-acetamidoacryloyloxy)pentane cross-linker dramatically improved adhesion of the thin film to the gold surface. While Haupt et al. 79, Stanley et al. 61 and Percival et al. 60 used an unpolished QCM device to improve adhesion of the polymer film to the device.

Lin et al. 57 used functionalised gold electrode QCM devices to compare albumin adsorption between three functionalized surfaces (Au–NH₂, Au–OH, Au–COOH) and the bare gold electrode coated with MIP. Albumin was dissolved in the monomer, DMAPMA (3-dimethylaminopropyl methacrylamide), and then the cross-linker and initiator were added to the solution. This solution was spin coated onto the bare and functionalized QCM devices and polymerized using UV irradiation. No adhesion problems were reported. When a suspension of MIP particles in PVC, PIB, and PMMA solution are spin coated on the QCM device, the polymers play the dual role of binding matrix and adhesive interface to the crystal surface 66, 68, 91-93.

In the process of developing a new technique of molecular imprinting, Kanekiyo et al. 94 used 3-mercaptopropionic acid to bond the deposited polyionic polymer to the QCM gold electrode surface. Electrostatic interactions between the carboxyl groups of the thiolizing agent and polyionic polymer were employed in this technique. The subsequent layer-by-layer deposition of the polycationic and polyanionic polymers on the negatively charged thiolized gold surface in the presence of anionic template formed a thin multilayer imprinted coating on the gold surface. The multilayer coating thickness was 320 Å after the deposition of ten layers. The same group 95 reported using 2-aminoethanethiol to adhere poly(MAA co-EGDMA), and 11-mercaptoundecanoic acid to produce poly(4-VP co-EGDMA) films. Using a similar acid – base interaction, Kikochi et al. 96 deposited a 2-aminoethanethiol self-assembled monolayer (SAM) to adhere terpene imprinted MAA-EGDMA to the QCM gold electrode surface.

2.3.4 Film properties

Thickness, uniformity of distribution, surface roughness, porosity and elasticity are the main characteristics that need to be controlled when applying a MIP, or any sensing film, to a QCM surface. The effect of these properties is crucial when working in liquid media. Extensive studies have been conducted to investigate the effect of these parameters on the response of QCM based devices. An overview and analysis of the literature related to MIP films on QCM gold electrodes is the focus of the following discussion.

It was found that a higher cross-linker ratio in the MIP preparation leads to a rigid polymer film 97 and significant reduction of adhesion to the electrode surface 60, 61. However, the lack of frequency shift at lower ratios of cross-linker indicates that the increased flexibility of the polymer results in the relaxation or collapse of the imprinted sites with no response observed. Using the sandwich method to cast the polymer film on the QCM electrode produces relatively thick polymer films so that the cross-linking surface tension force within the film exceeds the adhesion force between the film and the device.

Spin coating has repeatedly been used to cast MIP polymers on QCM devices. Reducing the film thickness, and consequently the QCM mass load, increases the device performance and reduces the
baseline noise, especially in liquid media. The effect of the cross-linker on film quality has been reported by Lin et al. In the process of imprinting albumin, three different cross-linkers were used, pentaerythritol tetraacrylate (PETTA) (4), trimethylolpropane trimethacrylate (TMPTMA) (5), and tetraethyleneglycol dimethacrylate (TEGDMA) (6). The largest film thickness was produced by PETTA (4) due to the high viscosity of the polymerization solution in comparison to TMPTMA (5). The increased thickness of the thin film caused diffusion resistance, and reduced the analyte adsorption capacity compared with films prepared using (4) and (6).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figs}
\caption{Structures of cross-linkers used in the study. (4) Pentaerythritol tetraacrylate (PETTA), (5) Trimethylolpropane trimethacrylate (TMPTMA), (6) Tetraethyleneglycol dimethacrylate (TEGDMA).}
\end{figure}

### 2.3.4.1 Thickness
Optimizing the amount, distribution, morphology, and viscoelastic properties of the sensing material loaded on the QCM crystal is crucial to obtain a well defined signal from analyte binding. The thinner the sensing film, the lower the diffusion barrier and the faster the response time of the sensor. The other advantage of thin films is low mass load on the QCM, which reduces the noise and minimises the system fluctuation. Thicker films require a longer time to establish a stable baseline due to the swelling of the polymeric system, or equilibration of the bulk of the polymer with solvent molecules in the liquid phase. On the other hand, the thinner the film, the less adsorption sites, the lower the selective mass uptake, and the smaller the signal. Humidity and diffusion limitations are also problematic in gas phase measurements.

Piacham et al. reduced the response time to below 1 min using a surface initiated molecular imprinting polymerization technique, which displayed some chiral selectivity towards the template, (S)-propranolol. The thickness of the MIP films obtained using this method was below 50 nm.

Using a spin coated viscose polymerization solution followed by UV curing, gave a 400 nm thick film with a response time of about 10 sec. These response times with thin films are far superior to the values obtained for thick films prepared by the sandwich coating technique. For example, Lin et al. reported a 30 min response time for an L-tryptophan imprinted polymer obtained using the sandwich method.

In the case of gas phase sensing, Fu et al. found that the relation between film thickness and sensor response was not linear. Attempts to obtain MIP coating thicknesses producing frequency shifts greater than 10 kHz for the coating led to a long-term baseline frequency shift, unstable oscillation, and even cessation of oscillation of QCM devices. The researchers found that thinner MIP films exhibited a more rapid response to vapour pulses, while thicker films gave somewhat slower rise and decay times. For these reasons, typical MIP polymer coatings with frequency shift from 2 to 5 kHz were used in the study of Fu et al. Luo et al. reported similar observations and used about a 10 kHz shift for the coating in their study.
2.3.4.2 Viscoelasticity and surface roughness

The viscoelasticity of a polymer can be described by a complex modulus. The modulus is defined as the stress associated with a unit strain, and has units of force/unit area (N/m²). It can be considered as the stiffness, or rigidity, of the polymer, and is related to the inter- and intra-molecular forces within the polymer. In general, polymer film-acoustic wave interactions are dominated by the shear component of displacement. Thus, it is the shear modulus which can be effectively probed with acoustic wave devices. This shear modulus can be represented by $G = G' + jG''$ where $G'$, the storage modulus, is associated with energy storage and released during periodic deformation associated with the oscillating stress, and $G''$, the loss modulus, is associated with the dissipation of energy, usually as heat, and $j = \sqrt{-1}$. The overall modulus depends on the molecular structure of the polymer, the average molecular weight, the temperature, and in general, the rate (frequency) of applied shear stress.

Tan et al. have studied the coating viscoelasticity effect on the QCM response. They spin coated MIP particles suspended in PVC solution in THF, forming a coating of MIP particles embedded in a PVC matrix. The ratio of MIP to PVC matrix was changed, and the difference in half band width of the conductance spectrum between the bare and the coated electrodes was monitored for any difference either in gas or liquid environment, or with time. In air, the half band width of the conductance peak for the bare electrode was 400 Hz while for the coated electrode it was 2200 Hz. When the electrodes were immersed, the value of $G_{\text{max}}$ decreased rapidly to one tenth the value for the bare electrode, and to one quarter for the coated electrode, and the half band width increased due to the effect of the liquid phase. Results obtained by Chance et al. suggested that QCM sensor sensitivities can be significantly enhanced for selected analytes by the careful selection of an appropriate swellable polymer coating.

The atomic force microscope (AFM) study of Lieberzeit et al. demonstrated that the electronic quality of the QCM response is dependent on the surface roughness. The reduction in surface unevenness leads to a substantial improvement in the electronic properties of the resulting devices. A gold layer was prepared by (i) screen printing a gold paste and subjecting it to thermal treatment at 400 °C (average roughness of 100 nm); (ii) spinning the substrate after screen printing for 1 min at 6000 rpm, which reduced the roughness to 40 nm; further, and (iii) placing the printed QCM into a dichloromethane saturated chamber (a dilutor for the gold paste) for 1 min, which reduced the roughness to 5 nm. Frequency analysis studies indicated the first film had a damping of -3.2 dB, after spinning it was reduced to -1.9 dB, while incubation in CH₂Cl₂ led to -1.1 dB damping. Considering the logarithmic scale of impedance, these represent substantial improvements.

The use of a blended polymer solution as matrix to hold and adhere the MIP particles to the QCM device introduces new parameters to the sensor performance. As mentioned above, poly(isobutylene) (PIB) in trichloroethylene (TCE), PVC in tetrahydrofuran (THF), and an acetone solution of poly(methyl methacrylate) (PMMA) have all been reported as matrices for MIP coatings. There are several major disadvantages in using this technique. Having the MIP particles as a suspension in the polymer solution reduces the exposure of the sensing material to the analyte after
spin coating, so that the supporting polymer matrix introduces a diffusion barrier which increases the response time to 60 min \(^{59}\), even in the gas phase. Another disadvantage relates to the properties of the matrix polymer in the sensing process, since elasticity, swelling, and the functionality of the polymer have to be considered with regard to its interaction with the medium and the targeted analyte. Due to the non-rigid structure of these linear-chain polymer matrices, they have elastic properties that may damp the crystal response impedance, and increase noise in the signal.

2.3.4.3 Porosity

The aim of the majority of sensing applications is to extend the operating range to lower concentrations and reduce the detection limit. The response of mass sensing devices can be maximised by optimizing the mass uptake by the sensing film applied on the QCM device or any other acoustic based sensor. This optimization includes controlling the film thickness while maintaining a high binding capacity. In this process, there is a compromise between the film thickness, surface roughness, porosity, and the sensor response. The greater the roughness of the surface and the higher the porosity of the sensing film, the larger the contact area between the matrix containing the analyte and the sensing device, and therefore the higher the mass uptake capacity of the sensing film.

Schmidt et al. \(^{73, 74, 100}\) have reported the introduction of highly porous, thin MIP films, without using low boiling point, high vapour pressure, porogen solvents. The porosity of Schmidt’s system is introduced by the reaction – induced phase separation of a sacrificial polymeric porogen. Using this approach, R- and S-propranolol in a TRIM – MAA system, have been imprinted in diglyme or triglyme as solvent, in the presence of poly vinyl acetate (PVAc) as the polymeric porogen, using the spin coating method. The problem of rapid solvent evaporation was solved by replacing toluene with the less volatile diglyme, and much faster polymerization rates were achieved by replacing the \(\alpha,\alpha'\)-Azoisobutyronitrile (AIBN) with 2,2-dimethoxy-2-phenylacetophenone (DMPA). The thickness and morphology of the polymer film varied with the PVAc ratio: increasing the PVAc ratio to 2\% resulted in a higher rebinding capacity, no further increase in capacity was observed with higher PVAc ratios. The film thickness was increased by increasing the PVAc ratio. This is due to the higher viscosity of the polymerization solution containing more PVAc. Below 2\% PVAc, the rate of increase of rebinding capacity was much higher than the increase in the film thickness. AFM studies of the morphology of the polymers prepared in the presence of a sacrificial polymeric porogen showed that the porous system develops by adding PVAc, and increasing the PVAc ratio to 2\% increased the pore size and depth. Further increasing the PVAc ratio changes the morphology to agglomerate formation, after passing through a transition state between the two morphologies. Increasing the molecular weight of the linear sacrificial polymer intensified the effect of its presence \(^{73}\). These three reports of Schmidt et al. \(^{73, 74, 100}\) give details of the parameters and effects involved when a linear sacrificial porogen polymer is included in the MIP formulation.
2.3.5 Polymeric systems

2.3.5.1 Free radical polymerization systems

This approach is based on the radical polymerization of vinyl- or acrylic- group based functional monomers and cross-linker monomers. Due to the simplicity and flexibility of this approach it is the most popular system reported in the literature.

EGDMA (ethylene glycol dimethacrylate) (7) 58, 60, 61, 66, 68, 69, 75, 80, 81, 83, 88, 93, 95, 96, 101-104, TRIM 55, 79, 82, 105, DVB (divinylbenzene) 59, 70, 106-108, TEGDMA (6) 57, TMPTMA (5) 57, PETTA (4) 57, 1,5-bis(2-acetamidoacryloyloxy)-pentane (8) and 1,4-diacyrlyloxybenzene (9) 70 have all been used as cross-linkers in preparing MIP films on QCM devices.

When EGDMA and DVB, the traditional cross-linkers used in bulk polymers are used, some difficulties are encountered due to their low viscosities and the requirement to use heat to initiate the polymerization of DVB, which restricts the casting of a MIP film with a required thickness and porosity. Most systems that use these cross-linkers employ a secondary polymeric medium to hold and adhere the MIP particles to the sensing device.

Physical properties, such as the higher viscosity of the TRIM cross-linker, have enabled researchers to spin coat the pre-polymerization solution under UV irradiation and still maintain the required film thickness to produce a detectable signal.

To imprint the protein albumin 57 the geometrical scale of the template required Lin et al. to use a large cross-linker such as TEGDMA (6), in combination with multi-polymerizing functional group cross-linkers, TMPTMA (5) and PETTA (4), in order to appropriately tailor a rigid binding site structure.

In order to use hexachlorobenzene as a template, Das et al. 70 used 1,4-diacyrlyloxybenzene as both the functional monomer and cross-linker, and utilised the π-π stacking interaction of this monomer with the template, to construct the binding site in the MIP. 1,5-bis(2-acetamidoacryloyloxy)-pentane was used to improve the polymer film adhesion to the gold electrode surface.

To provide a detection technique for dengue virus 89, 90 the antigen of NS1, a protein associated with the infected cells (pentadecapeptide epitope) was imprinted in a copolymer of AA, AAm, and N-benzylacrylamide after functionalizing the surface of the gold electrode of the QCM device to improve the polymer film adhesion.

2.3.5.2 Electropolymerization

Cyclic voltammetry has been used to synthesize a MIP film on the electrode of a QCM. To be successful, the template must show no electroactivity in the potential range used for the polymerization, in order to avoid reactions which would alter the template molecular structure and
functionality. Control of the film thickness depends on the polymer film conductivity, and a poor conductivity of the polymer film causes current decrease and cessation of further polymerization. Another important factor is the solubility of the template and polymer components in an electrolyte (usually an aqueous system). In electrochemical polymer imprinting, hydrophilic interactions between the template and the monomers are utilised in the associative step prior to polymerization.

In this manner, o-phenylenediamine (o-PD) (10) has been used to imprint glucose \( ^{109} \), D/L-phenylalanine \( ^{110} \) and sorbitol \( ^{7} \). Slightly acidic conditions were used, and scanning 20-30 times between 0 and 0.8V at 50mV/s rate produced the imprinted membrane sensor.

\[
\text{NH}_2\text{NH}_2\quad (10)
\]

Different functional groups were identified and quantified using spectroscopic investigation on poly(o-PD) films obtained by electrosynthesis. The presence of carbonyl, primary/secondary aminic and oximic (C=N–OH) groups was observed, together with iminic (phenazinic) (11) nitrogen. In particular, the \( \text{NH}_2 \) groups were present even at pH 1 and their amount was found to increase with polymerization pH, thus indicating that the polymer structure is not completely conjugated. A 1,4-substituted benzenoid–quinoid structure (12) seems to be more appropriate to describe the polymer structural, although a higher degree of conjugation was assumed for poly(o-PD) electrosynthesised at low pH \( ^{111} \).

\[
\begin{align*}
\text{NH}_2\text{NH}_2 \quad \text{NH}_2\text{NH}_2 \\
\text{NH}_2\text{NH}_2 \\
\end{align*}
\]

\[
\begin{align*}
\text{NH}_2\text{NH}_2 \\
\text{NH}_2\text{NH}_2 \\
\end{align*}
\]

These results demonstrated that this polymeric system contains a large number of polar hydrophilic functionalities, and aromatic conjugated systems, which may broaden future applications for both types of targeted analytes.

The possibility of combining the gravimetric and electrochemical functions in this type of sensing film is another advantage of this imprinting approach. Liao et al. \( ^{78} \) have imprinted poly(m-aminophenol) (13) with 5-fluoro-1-(tetrahydro-2-furyl) uracil (tegafur) using electrochemical polymer film synthesis.

\[
\text{NH}_2\text{NH}_2 \\
\text{NH}_2\text{NH}_2 \\
\text{NH}_2\text{NH}_2 \\
\]

Electropolymerization was used to imprint nitrobenzene in a polymer of o-aminothiophenol (o-AT) (14) in an organic solvent (\( \text{CH}_2\text{Cl}_2 \)) containing 10 mM o-AT, 10 mM nitrobenzene and tetrabutylammonium perchlorate \( ^{112} \). Huan et al. demonstrated that organic molecules can be
imprinted using electropolymerization on a gold electrode surface, where they also utilized electrochemical methods to produce the signal for the targeted analyte.

\[
\text{Imprint molecule} \quad (14)
\]

Deore et al. \cite{113} imprinted L-glutamate in an overoxidized polypyrrole (15) film prepared by electropolymerization. Polypyrrole is a conductive polymer, which loses conductivity by over oxidation which develops into a more functional porous coating, showing enantioselectivity toward the templated molecule.

\[
\text{Polypyrrole film} \quad (15)
\]

N,N-methylenebisacrylamide (16) in combination with acrylamide and m-acrylamidephenylboronic acid (17) were used to imprint nucleotides and monosaccharides. Unlike the previous two polymeric systems, Sallacan et al. \cite{76} stated that the selectivity was enhanced by increasing the degree of cross-linking of the polymer upon imprinting. This was explained by an increase in the rigidity of the imprinted sites at higher degrees of cross-linking.

\[
\text{Acrylamide and m-acrylamidephenylboronic acid} \quad (16, 17)
\]

### 2.3.5.3 Polyurethane (PU) systems

Dickert et al. \cite{65, 114} used bisphenol A (2,2-bis(4-hydroxyphenyl)propane) (18), phloroglucinol (19), and p,p’-diisocyanatodiphenylmethane (20) containing 30% of the respective triisocyanate (21) (mixture of isomers) dissolved in THF to form a stoichiometric solution to be polymerized in the presence of polycyclic aromatic hydrocarbons (PAHs) to produce the MIP film. This system provided an optimized π-π interaction between the PAHs and the monomeric aromatic components.

\[
\text{Polyurethane components} \quad (18, 19, 20, 21, 22)
\]

Lieberzeit et al. \cite{53} used this polymeric system to stamp print insulin onto a spin coated PU film. In other work, Dickert et al. \cite{56} used a similar system to produce a MIP film sensitive to oil degradation
compounds. Here, they used triethanolamine (22) as both a powerful cross-linker and also to utilise the basic properties of the amine to interact with acidic groups on the oil degradation products.

Bromobenzene was employed as a template to produce a PU film MIP for a gas phase sensor to determine the end point of an alkylation reaction. The polymerization mixture was drop coated on the QCM electrode and cured at room temperature. Toluene was used to remove the template and other unreacted components 53.

To cast a polymer film on the QCM crystal, a freshly prepared mixture of monomers and template was applied to the quartz plate. Polymerization was then performed under ambient conditions (25 °C in air), and at temperatures up to 70 °C, in a saturated solvent atmosphere. The polycondensation was complete within several hours, forming clear transparent coatings on the quartz substrate.

2.3.5.4 Inorganic imprinted systems

Another approach used to produce imprinted films on a QCM electrode employs inorganic supports. Silica, titania, alumina, or a combination of these components, was utilized to produce a sensing membrane 51, 52, 54, 115-117.

Lee et al. imprinted carboxylic acid based templates 51, 52, amino acid derivatives 117, and D-glucose 116 template molecules in TiO₂ ultrathin films or nanoparticles, and used them as sensing materials. The QCM gold electrode surface was first functionalised using mercaptoethanol to provide the necessary adhesion of the TiO₂ film. Then, using a surface sol-gel process, titanium butoxide was allowed to react with the template to form a covalently bound template – Ti alkoxide complex, which was subsequently hydrolysed with a small amount of water used as a dipping solution for the QCM coating. The film thickness was controlled by the number of coatings applied to the device.

Ichinose et al. 115 found that low temperature oxygen plasma treatment of surface sol-gel films of titania, titania-silica and TiO₂ – polyacrylic acid nanocomposites was advantageous to form porous metal oxide films.

Ling et al. 54 produced a silica – alumina gel with size selective recognition by imprinting catecholamine as a template. The Al/Si ratio was found to be very important, and the Al(III) sites acted as Lewis acid sites in the rebinding process.

2.3.5.5 Surface imprinting

Surface imprinting is based on the immobilization of the template on a solid support followed by creating a binding site by modifying the surface around the template. Surface imprinting strategies provide substantial advantages for the recognition of large templates such as proteins, viruses and cells. The binding sites are more readily accessible at the surface, and this eliminates the diffusion limitation of large species passing into the imprinted material to reach the recognition site. Thus, the binding kinetics are less limiting, given that template rebinding only requires the presence of the molecule at, or close to, the recognition surface.
Shi et al. \textsuperscript{118} reported a method for imprinting surfaces with protein-recognition sites. The template proteins were arrayed on an atomically flat mica substrate and then disaccharides were applied to form the functional matching moieties for the arrayed protein molecules, which also provided a protective shell against denaturation and degradation. This shell was then coated with a fluoropolymer film such as hexafluoropropylene (C\textsubscript{3}F\textsubscript{6}) applied by radio-frequency glow-discharge plasma deposition. Reactive species link with each other, as well as to the disaccharides on the surface, making them covalently attached to the polymer film. This yields a smooth, conformationally stable film with good mechanical and chemical stability. Then, the outer surface of the film was supported on a glass substrate and the mica substrate peeled off and the template protein extracted from the hemispherically shaped binding pockets with a basic solution. This procedure created cavities that exhibited highly selective recognition via the disaccharide functionalities for a variety of template proteins, including albumin, immunoglobulin G, lysozyme, ribonuclease and streptavidin.

Friggeri et al. \textsuperscript{119} described a solution-to-surface imprinting method based on the different higher-order conformations adopted by boronic acid-appended poly(L-lysine), in the presence of sugars. This polymeric adsorbate contained boronic acid moieties, which bind to saccharides and also 10-sulfanyldecyl moieties, which anchor the polypeptide to a gold surface without hindering its conformational flexibility stabilized by pH control. The polymer – glucose complex initially formed in solution was subsequently anchored to the metal surface, which was then rinsed to remove the template molecules, resulting in a glucose selective molecularly imprinted interface.

Piacham et al. \textsuperscript{55} used surface polymerization as another approach for surface imprinting to imprint propranolol in a copolymer of TRIM and MAA. But the main surface imprinting approaches that have been used for large protein molecules, viruses and cells, are the so called “stamp printing” and screen printing techniques \textsuperscript{71, 72, 120-123}.

Gold paste \textsuperscript{72, 122}, PU \textsuperscript{120, 122}, and poly(MAA – DVB – styrene) \textsuperscript{71, 121} have been used in screen printing of the sensing film on QCM devices.

In a comparative study, Dickert et al. \textsuperscript{122} imprinted tobacco mosaic virus (TMV) using stamp printing, screen printing and a radical initiated acrylic acid (AA) – EGDMA copolymerization in an aqueous buffer. They screen printed TMV in gold paste and burned-off the organic matter at 420 °C overnight to fix the impressions. To stamp the same template on a PU film, the monomers were pre-polymerized up to the gel point, and then diluted and spin coated on a QCM device, and hardened under nitrogen at 95°C. Self-assembled virus layers at different concentrations on a very flat glass slide served as the stamp, which was then pressed into the thin film (200 nm), to generate the surface patterning desired. The sensor response indicated that the highly ordered stamp produced a higher sensitivity to TMV when compared to the monolayer stamps and the acrylic film.

Escherichia coli bacteria \textsuperscript{120}, erythrocytes \textsuperscript{71, 72}, yeast cells \textsuperscript{121, 124}, TMV \textsuperscript{71, 122}, and human rhinovirus (HRV) \textsuperscript{72} were able to be stamped on PU film.

Poly(MAA – DVB – styrene) \textsuperscript{121, 125} was used to stamp TMV, and trypsin and lysozyme \textsuperscript{71} proteins. The monomer mixture was thermally pre-polymerized and cast on the sensing device surface, then
stamped with a self-assembled layer of template. The polymer film was cured by overnight UV irradiation. The trypsin imprinted film showed eight times the frequency shift that was observed with the non-imprinted film.

In another approach, parapox ovis virus solution was spread onto a spin coated polystyrene film and left to dry at 4 °C, then a solution of vinyl pyrrolidone was deposited and cured overnight using UV irradiation.

The traditional method of imprinting, which relies on creating a binding site based on organizing the matching functional groups of the functional monomers around the template, has been rarely used to imprint large molecules such as proteins, enzymes, and viruses. In addition to forming the matching functionality association, the template leaves the impression of its size and geometry in the forming polymer structure. Considering the size of protein molecules, viruses and cells, finding the position of the originally imprinted molecule during the rebinding of another molecule is less probable, therefore entropic parameters such as matching size and geometry influence the rebinding process.

The comparative study of Dickert et al. showed that the response of a membrane produced by stamping an unorganized layer of viruses, and a membrane prepared using the traditional method of free radical initiation polymerization, did not demonstrate any significant difference.

Lieberzeit et al. used the stamping technique to imprint insulin on a spin coated, pre-polymerized PU film surface. Rick et al. studied the rebinding of independent lysozyme and cytochrome c and their protein – protein association complex imprinted in polymer films on a QCM device. 3-Aminophenylboronic acid was polymerized using ammonium persulfate to synthesise the MIP film. The strong hydrogen bonds formed between the boronic acid functional groups and the amine and hydroxyl groups on the protein provide a rigid pre-polymerization association. It was shown that the lysozyme – cytochrome c association imprinted polymer did not show specific binding to the individual proteins of the association, or an association of lysozyme or cytochrome c with another protein, such as albumin or myoglobin.

The photo-initiated polymerization of DMAPMA (3-dimethylaminopropyl methacrylamide) as functional monomer, and TEGDMA (6), PETTA (4) or TMPTMA (5) as cross-linkers to imprint albumin produced another selective film. Comparing the response of the MIP films made using the different cross-linkers highlighted the factors affecting the performance of the MIP. It was found that a higher film thickness was produced when a more viscous PETTA was used, and this caused a higher diffusion barrier for the analyte to rebind to the MIP. PETTA has four polymerizing groups while TMPTMA has three and TEGDMA has two. A TMPTMA cross-linked polymer showed the highest response to albumin rebinding.

The presence of the tetraethyleneglycol chain in the TEGDMA cross-linker allows its structure to be very flexible, and this may cause the collapse or deformation of the constructed binding site after template removal, thereby reducing the rebinding probability of the template.

The four polymerizing groups in the PETTA cross-linker form a highly rigid structure which is less accommodating for the geometry of the template molecule, making the constructed site more
hindered by the hydrogel internal structural forces. This also increases the chance of entrapment of the template during polymer synthesis and decreases the efficiency of the template removal process.

Lin et al. imprinted vasopressin polypeptide using the traditional method of polymerizing small functional monomers to fix an association with the template, but have not reported a comparative rebinding study between the imprinted and non-imprinted polymers, thus, it does not appear possible to judge the imprinting efficiency of their technique.

2.3.5.6 Other approaches

Kanekiyo et al. exploited polyion complex formation to produce recognition sites in polymeric structures. This approach involved two steps, (i) polyion complex formation between a boronic acid-containing polyanion and polycation in the presence of an anionic template which is bound to the boronic acid group, and, (ii) removal of the anionic template by extensive extraction of the precipitate. The 'cleft' created in the polyion complex has been shown to possess a 'memory' for the original anionic template molecule. This method has been applied to the imprinting of AMP in a bulk precipitation using the interaction between the adenosine monophosphate (AMP) and a phenyl boronic acid functional monomer.

Kanekiyo et al. demonstrated that the same approach can be utilized to form an imprinted film. They found that the multi-layer deposited by the alternating adsorption (layer by layer deposition of polyanion and polycation) method on the QCM surface only shows selective response to the template, AMP, when it is imprinted during the multi-layer deposition process. They showed that only the imprinted template can cause significant shrinkage in the swollen film during the rebinding. This causes an increase in frequency due to the loss of solvent molecules in the polyion structure. The non-imprinted polymer shows a frequency decrease in the presence of template. This sensing system demonstrated high sensitivity and selectivity toward the template.

Kobayashi et al. developed a method to produce porous membranes to investigate caffeine imprinting, using the phase inversion of copolymers of acrylonitrile (AN) and acrylic acid (AA), vinyl pyridine (VP), vinyl pyridine (2VP), or styrene, with elimination of the cross-linker from the traditional imprinting method. The polymers were prepared in DMSO at 50 °C in the presence of caffeine as template. The solution was spread on the substrate to form a 0.1 mm thick film and coagulated in water at 10 °C, and remained in contact with water overnight to remove the DMSO. This imprinted system showed an improvement in the selectivity and capacity compared to a non-imprinted polymer.

Richter et al. studied the morphology of a MIP of nylon-6 film imprinted with alanine and L-glutamine amino acids using formic acid as the solvent. Here they employed hydrogen bonding interactions as the primary cross-linking force between the polymer chains.
2.3.6 Media

2.3.6.1 Gaseous media

The use of a QCM device for gas phase quantitation has many drawbacks such as low selectivity, slow response, lack of reproducibility, drift, humidity effects, temperature control, etc. Nevertheless, these problems have motivated researchers to seek new materials to enhance the sensor parameters in gas phase detection systems.

Kikuchi et al. developed a MIP-QCM sensor for the recognition of terpenes in air. They examined the influence of humidity on the system response, and found the sensitivity was decreased at lower humidity. It was believed that the hydrophilicity of the functional monomer used (MAA) influenced the system sensitivity. So, to determine the concentration of the analyte gas (limonene), they suggested the humidity should be measured at the same time. Kikuchi et al. stated that a better selectivity is needed for determining the targeted molecular species.

Matsuguchi et al. investigated a molecular imprinting strategy for solvent molecules, and its application to QCM-based volatile organic compound (VOC) sensing. The QCM response time to p-xylene was about 60 min, but it was not clear whether this slow response time was due entrapment of the MIP particles in the PMMA coating matrix, or because of the diffusion barrier restricting the rate at which analyte molecules reach the sites buried deep inside the MIP particles in the film.

Fu et al. used hydroquinone and phenol as noncovalently bound templates to generate shape-selective cavities in a poly(acrylic) or poly(methacrylic) polymer matrix. Organic vapours with different molecular shapes and sizes such as toluene, benzene, trichloroethylene, carbon tetrachloride, and heptanes, were selected as the target vapours for the sorption/desorption tests. Imprinted polymers exhibit greater sensitivity and higher selectivity than the nonimprinted polymers toward organic vapours that were structurally related to the templates. The response time was quick (<1 min) and the desorption time was longer for the imprinted polymers, which indicated a stronger interaction with the analyte. A formaldehyde sensor produced by Feng et al. showed a 5 min response time for measurements performed at room temperature in the gas phase.

2.3.6.2 Liquid media

Most of the reports utilizing MIP films on QCM electrodes refer to liquid phase media. The effect of the surface morphology and the medium on the QCM response has been studied in detail by Martin et al., Urbakh et al., and Ha et al.

Urbakh et al. found that the frequency changes were due to both the inertial motion of a liquid rigidly coupled to the surface, and to the additional viscous energy dissipation induced by surface roughness. The resonance frequency of the QCM was dependent on both the properties of the fluid and the morphology of the interface surface. The surface roughness length scale can be defined by the Navier-Stokes equation for liquid velocity and by the wave equation for the elastic displacement in the crystal. The decay length of liquid velocities is \( \delta = (2\eta/\omega\rho)^{1/2} \), where \( \eta \) is the liquid viscosity and \( \rho \) is the liquid density.
Using a high ratio of cross-linker to monomer in the MIP synthesis limits, to a certain extent, the swelling of the polymer film and rigidifies the polymer structure. It is also necessary to maximize the active surface area of the polymer by creating a film with an open, porous morphology, in order to increase the mass uptake in the rebinding process.

Depending on the varying approaches used to apply the MIP to the QCM device (sandwich method, electrochemical synthesis, surface initiated polymerization, spin coating a suspension of MIP particles, spin coating the polymerization solution) a unique MIP film surface morphology may be produced.

Schmidt et al. 73, 74, 100 found that spin coating and open polymerisation of the MIP film without using a sacrificial linear chain porogen produced a nonporous polymer. The porous morphology of the film starts developing by including PVAc in the polymerization solution; a 1% PVAc produces films with a degree of roughness of 75 nm, 2% PVAc increases the roughness to 250 nm, 3% PVAc to 400 nm, while the presence of 10% PVAc in the polymerization solution caused agglomeration, in which the degree of roughness of the film was 1000 nm. Surface roughness was calculated as the root mean square (RMS) of the AFM measurements in the contact mode.

Lin et al. 57 reported the surface roughness observed using an AFM was about 100 nm for an albumin imprinted film, cast using the spin coating method. Wu et al. 106 reported a DVB – 4VP bilirubin imprinted polymer film prepared under nitrogen to have a film thickness of approximately 150 nm. The SEM images of the film showed a very rough surface with agglomeration morphology.

Das et al. 70 reported that reducing the film thickness to about 400 nm showed an improvement in the response time, independent of the morphological nature of the film, although they did not discuss the morphology or roughness of the film.

Spin coating of MIP particles suspended in a secondary linear polymer solution on the quartz plate surface will produce varying degrees of roughness on the coating surface, depending on the concentration of the MIP particles in the matrix polymer. Matsuguchi et al. 59 obtained a coating, several microns thick, by spin coating a suspension of MIP particles in PMMA solution in acetone. The SEM image of the coating showed it to be quite a rough surface. This coating was intended to detect VOC’s in the gas phase, illustrating here the morphology of similar coatings used in the liquid phase. There was no discussion on the effect of the matrix polymer and surface roughness on the QCM performance. The coating frequency shift was maintained at or below 10 kHz to avoid long-term base line shift, unstable oscillations or cessation of oscillation 58, 66, 93.

Friggeri et al. 119 and Piacham et al. 55 used surface imprinting to introduce selectivity for analyte rebinding to the QCM device. This method did not change the QCM electrode surface morphology dramatically due to the molecular scale surface modification, and so this minimized the roughness of the sensor surface – media interface, thus minimizing impedance damping. On the other hand, the low surface area limits the detectable response of the sensor to high analyte concentrations.

The sandwich method is a crude way to apply a MIP to a QCM device. Thick films deposited by this procedure showed varying morphology across the film profile. When the cover slip was silanized to
minimize polymer adhesion, the smooth film surface (skin) facing the cover slip is a plane with channels leading to a highly porous system in the bulk of the polymer film. Particles of the polymer that are exposed may be removed by sonication.

Figure 2.3 SEM images of EGDMA-DVB-4VP polymer film prepared in this work using the sandwich method in 80% (v/v) TG as solvent and AIBN as initiator.

Removing the film skin exposes the highly porous system for the analyte and increases the rebinding process, but this increases the surface roughness and QCM response quality. This point has not been discussed thoroughly in the reports which have used this method 60, 61, 79-82.

Haupt et al. 79 estimated the film thickness produced by this method to be about 2 µm. AFM studies by Liu et al. 82 of the bare gold electrode surface, the thiolized interface and the MIP film showed the roughness changes in the process of applying the MIP film. They reported a 10 nm fluctuation range on the film surface over an area of 1 µm × 1 µm, but to consider the porosity of the MIP film a larger area would have been needed to be considered.
2.3.7 Classes of targeted molecules and their interaction with functional monomers

2.3.7.1 Carbohydrates

Glucose has been targeted for imprinting using various strategies. Ersoz et al. used the ability of the cis-diol functionality of glucose (23) and a histidine-based functional monomer to chelate Cu(II) and form a strong association, prior to fixing the association using UV initiated polymerization in DMSO. The rebinding was performed in a carbonate buffer.

\[
\text{HO} - \text{CH}_2 - \text{OH} \quad \text{HO} - \text{CH}_2 - \text{OH} \quad \text{OH} - \text{CH}_2 - \text{OH}
\]

(23)

Malitest et al. reported the first example of an electro-synthesized polymer imprinted with glucose using o-phenylenediamine as the monomer to create the selective site. They defined the specific interaction between the template and the deposited coating using a Scatchard plot. The same methodology was used by Feng et al. to imprint sorbitol (25) electrochemically.

\[
\text{HO} - \text{CH}_2 - \text{OH} \quad \text{HO} - \text{CH}_2 - \text{OH} \quad \text{OH} - \text{CH}_2 - \text{OH}
\]

(25)

Sallacan et al. utilized the same cis-diol functionality to form a covalent bond with a phenylboronic acid functional monomer (26), and then fixed the complex (27) using electropolymerization as shown schematically below. In this instance, acrylamide and m-acrylamidephenylboronic acid (26) were used as functional monomers, and N,N'-methylenbis acrylamide was the cross-linker.

\[
\text{HO} - \text{CH}_2 - \text{OH} \quad \text{HO} - \text{CH}_2 - \text{OH} \quad \text{OH} - \text{CH}_2 - \text{OH}
\]

(26)

(27)

Using the same strategy and mechanism, Sallacan and co-workers imprinted the AMP (28), GMP (29), CMP (30), UMP (31) nucleotides and the sugars β-D(+)-glucose (32), D(+)-galactose (33), and β-D(-)-fructose (34). Friggeri et al. surface imprinted D-fructose (34) and D(+)-glucose (32) using a boronic acid based functional monomer appended to a linear poly(L-lysine) polymer.
adhered to the gold electrode surface of a QCM device. Lee et al.  took a completely different approach to produce specific sites for glucose rebinding by imprinting D(+)−glucose (32) molecules in TiO₂ nanoparticles and immobilizing them on the QCM gold electrode surface.

Kugimiya et al.  imprinted sialic acid (35) using p-vinylbenzeneboronic acid (36) as the functional monomer, by first esterifying sialic acid with, p-vinylbenzeneboronic in dry pyridine via azeotrophic distillation. The covalently bonded template−functional monomer (37) was polymerized with EGDMA, 2-hydroxyethyl methacrylate (HEMA), 4VP and 2,2′-azobis (dimethylvaleronitrile) in DMF using the sandwich method. The rebinding was performed in methanol/pyridine (99:1).

2.3.7.2 Acids, alcohols, amines, phenols, and nitro-groups

The imprinting efficiency is determined by the strength of the interaction between the functional monomer and the templated molecule, and the number of potential interactions on the template molecule. Acid − base, hydrogen − bonding and dipole − dipole interactions are the most widely used in tailoring imprinted polymers.

2,4-Dichlorophenoxyacetic acid (2,4-D) (38), L-tryptophane (39), and indoleacetic acid (40) are templates with acidic functionality, and 2,4-D, was imprinted using MAA as functional monomer via a carboxylic acid dimer association. Acrylamide (ACM) with a balanced acid − base functionality, was used to imprint (39), and N,N′-dimethylaminoethyl methacrylate with basic properties was used to imprint (40). Hydrogen-bonding was used as the associative binding to imprint bisphenol A (41) with both 4VP and MAA used as functional monomers. Protonated and deprotonated forms of these functional monomers are capable of producing stronger interactions with the template.
Vanillin (42) and trinitrotoluene (43) and dinitrotoluene were imprinted using MAA functional monomers, utilizing hydrogen bonding to construct adsorption sites. In the imprinting studies of (43), the template–functional monomer interaction was optimized by changing the diluent and utilizing functional monomers with different functionalities and structures. MeCN, dimethylformamide (DMF) and chloroform were used as diluent and MAA, acrylamide (AA), methylacrylamide (MAAM), 2-hydroxypropylmethacrylate (HPMA), and butandiolmonoacrylate (BDMA) were compared as functional monomers. MIP coating of an AA–EGDMA synthesized in chloroform diluent showed the highest sensitivity to the template.

Basic aromatic species such as atrazine (44) and nicotine (45) were imprinted using MAA as functional monomer. The larger heterocyclic molecule, bilirubin (46) was imprinted using 4VP as functional monomer and DVB as cross-linker. The adsorption site was created utilizing multiple π-π stacking and dipole-dipole interactions. Propranolol (47) has been imprinted using MAA as functional monomer with an acid–base interaction and a hydrogen–bond assisting association prior to polymerization. Schmidt et al. have compared DVB and TRIM as cross-linkers in the study of propranolol imprinting.

Organic compounds have been imprinted in inorganic systems. For example, dopamine (48) has been imprinted in a silica-alumina gel, while carbobenzyloxy-L (and D) amino acids (49) and azobenzene carboxylic acid (50), anthracencarboxylic acid isomers (51, 52) and 4-(4-propoxyphenylazo)benzoic acid (53) were imprinted in a TiO₂ gel. The main factors creating the memory in these cases are the size and geometry of the voids left by the template molecule. Aluminium (III) centres in the alumina-silica gel provide Lewis acid sites, for interaction with the lone pairs on the amine and hydroxyl functional groups. In a TiO₂ gel, titanium (IV) can act as an electron-pair acceptor and oxygen as an electron donor or hydrogen – bond site, but, in all cases, the size and geometry of the rigid structure is the dominant factor in the specific recognition and rebinding process.
2.3.7.3 Small molecules

The use of MIP films for the detection of very small molecules has been the focus of some reports, although high capacity MIP films are necessary for a gravimetric QCM sensors. Formaldehyde (54), acetaldehyde (55), L-serine (56), and trichloroacetic acid (57) were imprinted and applied as coatings to the QCM; (54, 55 and 56) were imprinted in MAA – EGDMA, while (57) was imprinted in 4VP – EGDMA.

Daminozide (58) contains both acidic and basic functionalities and was imprinted in a MAA – EGDMA polymer system.

Less interactive molecules possessing fewer polar functionalities have been successfully imprinted by employing a matching polymeric system to provide enhanced selectivity and sensitivity towards the targeted analyte. In this manner, L-menthol (59) was imprinted in an MAA – EGDMA system. The rebinding measurements were performed in ethanol, and small, but stable, frequency shifts obtained. The sensitivity range of the sensor was between 0.2 to 1.1 ppm. Nandrolone has also been imprinted (60) in the same polymeric system. Again a very low detection limit (0.01 ppm) and saturation level (0.2 ppm) were obtained. In the same polymeric system, Kikuchi et al. imprinted limonene (61), limonene oxide (62) and α-pinene (63) for detection on a QCM coating to be used in air. Tegafur (64) was imprinted electrochemically in poly(m-aminophenol). The QCM gravimetric response was compared with the same polymeric system in capacitive response mode.
2.3.7.4 Aromatics

Aromatic solvents are widely used in research and industry, and detecting the presence of these solvents in liquid and gaseous phases is important in the environment and work place OH&S. Dickert et al. have imprinted benzene, toluene, and xylene in a DVB – styrene system using biphenyl as porogen. The same group have imprinted polycyclic aromatic hydrocarbons (PAHs) in a PU system. The polyurethanes were chosen because they provide a hydrophilic polymer to ensure sufficient wetting for applications in water. To provide an optimized interaction via π–π-bonds for PAHs, aromatic monomeric components were selected. These sensing films were imprinted utilizing van der Waals and π–π interactions, allowing detection of these analytes despite the presence of any pronounced functionality. These weakly polar forces will provide the predominant interaction considering the low solubility of these analytes in water (the working medium) and the hydrophobic sensing surface.

Another approach has been taken by Fu et al. to produce selective sites for the detection of benzene, toluene and trichloroethylene vapours. Hydroquinone and phenol were used as templates to create shape selective cavities in a polymer coating based on MAA – EGDMA in DMSO as solvent. An initial functional monomer – template interaction is necessary to create the adsorption sites with the proper size and geometry. The affinity of the adsorption sites was confirmed by slow desorption kinetics. Hexachlorobenzene was imprinted in a non-polar aromatic system which utilized π–π stacking and the lipophilic interaction between the template and the adsorption site in the polymer matrix to produce a highly sensitive and selective sensor. The quick response time of the sensor (10 sec) was due to the polar nature of the working solvent (water) and the thin adsorbent film (400 nm).

Matsuguchi and Uno have imprinted toluene and p-xylene in a MMA-DVB system. Using a phenomenon called the “porogen imprinting effect” reported by Yoshizako et al. and Hosoya et al. to create a memory for the targeted porogen solvent molecules. Furthermore, DVB is likely to be more compatible with the aromatic molecules, potentially favoring toluene or p-xylene adsorption via additional π–π stacking interactions in the imprint sites.

2.3.7.5 Engine oil degradation

Dickert et al. used two different approaches to imprint engine oil degradation species. The degradation mostly affects the oil additives and surfactants, as oxidation inhibitors become exhausted, the polymeric compounds are cracked, and the base oil components are oxidized, yielding acids. The recognition sites were created using an acid – base interaction between the template and a basic amine centre in the polymeric matrix. The other approach was based on TiOz gel imprinting.

2.3.7.6 Proteins, viruses and cells

High molar mass molecules such as oxytocin, vasopressin, albumin, lysozyme, and cytochrome c, were imprinted using the traditional approach in a monomer solution.
Considering the size of proteins, viruses and cells, and the rebinding diffusion barrier, most workers have used the screen printing approach, which was discussed earlier in section 1.3.5.5.

2.4 References


69. Yan, S.; Fang, Y.; Gao, Z., Quartz crystal microbalance for the determination of daminozide using molecularly printed polymers as recognition element. *Biosensors and Bioelectronics* 2007, 22, (6), 1087-1091.


3 Materials and Methods

3.1 Materials

Ethylene glycol dimethacrylate (EGDMA) and technical grade divinylbenzene (DVB) (ca. ~50% GC) were obtained from Fluka. Both cross-linker monomers were purified by extraction with aqueous 10% NaOH, washed with water, dried over anhydrous magnesium sulfate (MgSO₄), filtered and distilled under reduced pressure (~45 °C/1 mmHg for DVB and ~100 °C/5 mmHg for EGDMA).¹⁻³

Methacrylic acid (MAA), 4-vinyl pyridine (4VPy) and 1-vinylimidazole (VI) (99%) were obtained from Aldrich Chemicals. 4-VP, VI and MAA were purified by distillation under reduced pressure (80 °C/10 mmHg, 78-79 °C/13 mmHg and 60 °C/15 mmHg respectively).²⁻⁴

Acrylamide (ACM) (electrophoresis grade 99+) and was from Aldrich Chemicals and used without further purification.

Triallyl cyanurate † (TAC) (purum ≥98% GC), triallyl isocyanurate † (TAIC) (purum ≥98% GC) and 1-vinyl carbazole (VC) (purum ≥98% GC) were from Fluka and used as received.

Cobalt(II) acetate tetrahydrate, (CH₃COO)₂Co·4H₂O, was from Aldrich Chemicals.

Tetrahydrofuran (THF), acetonitrile (MeCN) and methanol were HPLC grade, which were filtered and degassed prior to use. Triglyme (TG) was distilled and stored over molecular sieves. Milli-Q water was used for all experiments.

α,α’-Azoisobutyronitrile (AIBN) was purified by recrystallization from MeCN prior to use. 2, 2-Dimethoxy-2-phenyl acetonaphone (purum ≥98% GC) was from Fluka and used as received.

D-Glucose, D-fructose, ethanol, tartaric acid, malic acid, catechin, catechol and potassium chloride were analytical grade and used without further purification.

2-Propene-1-thiol (technical grade 60%) and 1-propane thiol (purum 97%) were obtained from Fluka.

¹ 2,4,6-tris(2-propenloxy)-1,3,5-triazine
† 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)trione
Iprodione \(^\dagger\) (technical grade 98.3\%) and pyrimethanil \(^\ddagger\) (technical grade 98.1\%) were donated by Bayer Cropscience Pty. Ltd. Iprodione was recrystallised twice from MeCN, and pyrimethanil was recrystallized from 10\% acetone in hexane before use.

The deuterated chloroform used in the NMR studies was obtained from Cambridge Isotopes (99.9\% D) and stored over molecular sieves. A drop of tetramethylsilane (TMS) was added to the deuterated solvent for referencing.

### 3.2 Instrumentation

**Photochemical Reactor**

A 125 W mercury lamp, cooled using a combined water and air jacket, was used as UV source. To irradiate the monomer solutions, a frame was designed to hold the UV lamp in a central position with eight radially located sample holders at a distance of 2 cm, as shown in Figure 3.1.

![Photochemical reactor](image)

**Figure 3.1** The photochemical reactor used in the photo-polymerizations.

**Active surface area analysis (BET)**

Surface area and porosimetry was determined by nitrogen gas adsorption/desorption. Nitrogen sorption measurements were performed using a Micromeritics ASAP 2000 (USA). A 100-150 mg of the sample was degassed at 70 °C under high vacuum for at least 12 h, prior to measuring the

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\(^\dagger\) 1-(1-methylethylaminocarbonyl)-3-(3,5-diochlorophenylimidazolidine)-2,4-dione

\(^\ddagger\) 4,6-dimethyl-N-phenyl-2-pyrimidinamine
isotherms at liquid nitrogen temperature (77 °K). The adsorption and desorption isotherms were recorded using an 89-point pressure table with 15sec equilibration intervals. The surface areas were evaluated using the BET method, and the average pore diameter and pore size distribution were evaluated using the BJH theory.

**Thermo Gravimetric Analysis (TGA)**
The polymer decomposition temperature was determined using a Perkin Elmer TGA7 analyzer attached to a TAC controller, gas changer and computer (USA). The samples were heated to 800 °C with a heating rate of 10 °C/min under nitrogen (20 mL/min). A platinum crucible was used as the reference.

**Scanning Electron Microscope (SEM)**
Polymer particles were affixed to aluminium pegs with carbon tape and sputter coated with gold for 60 sec at 0.016 mA (Ar plasma) using a SPI-Module Sputter Coater, (SPI Supplies Division of Structure Probe, Inc). SEM images were then obtained using a Philips XL30 or FEI Quanta 200 ESEM fitted with an Si(Li) X-ray detector and Gatan Al Cyro stage, operating at high vacuum and 25 kV, in order to study the morphology of the polymers.

**High Performance Liquid Chromatography (HPLC)**
The HPLC measurements were carried out using a Shimadzu LC-10AD Liquid chromatograph, fitted with an SPD-M10A Diode array detector, SIL-10A Auto injector, CBM-10A Communication bus module and CTO-10A Column oven (Japan).

**Fourier Transform Infrared spectroscopy (FTIR)**
FT-IR spectra were obtained in KBr disks using a Perkin Elmer 2000 (USA) spectrometer. IR was used to confirm the removal of the template molecule and study the polymers’ molecular structure.

**Nuclear Magnetic Resonance (NMR)**
Proton and $^{13}$C NMR spectra were measured in CDCl$_3$ solution on a Bruker Avance 300 (300MHz, USA) spectrometer. Chemical shifts ($\delta$) are given in ppm, internally referenced to TMS (0 ppm) or residual chloroform peaks (7.24 ppm, $^1$H) unless otherwise indicated.

**Phase lock oscillator and QCM resonators**
The quartz crystal microbalance (QCM) consisted of a Maxtek PLO10 phase locked oscillator (USA) connected to a computer. The quartz crystals used were polished AT-cut, 25 mm diameter, with Cr/Au contacts, operating at a fundamental resonant frequency of 5 MHz. The electrode area was approximately 133 mm$^2$ (Maxtek model No. 149211-2) and the crystals were mounted in a Maxtek CHT-100 crystal holder.

**Network analyser**
An Agilent E5100A universal counter was used to study the impedance profile of the crystal. A Pi-network test fixture (Figure 3.2) was used to study the QCM resonator coating properties in the sensor development process. All the circuit connections were BNC and all the leads RG 58 coaxial cables.
3.3 Methods

3.3.1 Bulk polymer preparation

3.3.1.1 Polymer microsphere synthesis
Polymer beads of MAA and 4VPy functional monomers with EGDMA cross-linker were prepared in MeCN solvent, with a solvent to monomers ratio of 95% v/v. The polymer synthesis was conducted using AIBN initiator (0.25 % w/w) and UV irradiation for 16 h. MAA : 4VPy : EGDMA ratios of 1:3:20, and 3:1:20, and MAA : EGDMA ratios of 1:2 and 1:5 were prepared.

In a typical preparation 52 µL of MAA, 195 µL of 4VPy, and 2.29 mL of EGDMA were mixed with 5 mg of AIBN in 50 mL MeCN. The oxygen was removed from the solution by sparging nitrogen for 10 min, and then, irradiated with UV for 16 h at room temperature.

3.3.1.2 Bulk polymer synthesis
In a typical MIP synthesis, the polymerisation mixture consisted of solvent, monomers, initiator, and the template molecule. The polymerisation mixture was sparged with nitrogen for 5-10 min to remove oxygen prior to starting the reaction. Polymerisation was initiated either by UV irradiation using a 125 W medium pressure mercury lamp photochemical reactor for at least 16 h, or by heating at 65-75 °C in a controlled temperature water bath for at least 16 h. Control samples of non-imprinted polymers (NIPs) were synthesised in a similar manner, without the addition of the template molecule to the polymerisation solution.

EGDMA
The cross-linker EGDMA was polymerised in MeCN using AIBN as initiator and UV irradiation for 16 h. The solvent ratio was 55% v/v and the initiator to monomer ratio was 2% w/w.

MAA – EGDMA
The MAA – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The MAA : EGDMA molar ratio was 1:5. The solvent ratio was 50% v/v and the initiator to monomer ratio was 2% w/w. The MIP template : functional-monomer molar ratio was 1:5.
4VPy – EGDMA
The 4VPy – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The 4VPy : EGDMA molar ratio was 1:5. The solvent ratio was 50% v/v and the initiator to monomer ratio 2% w/w. The MIP template/functional-monomer molar ratio was 1:5.

4VPy – MAA – EGDMA
The 4VPy – MAA – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The 4VPy : MAA : EGDMA molar ratio was 1:1:10. The solvent ratio was 50% v/v and the initiator to monomer ratio 2% w/w. The MIP template/4VPy/MAA molar ratio was 2:5:5.

4VPy – DVB – EGDMA
The 4VPy – DVB – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The 4VPy : DVB : EGDMA molar ratio was 2:3:5. The solvent ratio was 40% v/v and the initiator to monomer ratio 2% w/w. The MIP template/4VPy/DVB molar ratio was 1:2:3.

DVB – EGDMA
The DVB – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The DVB : EGDMA molar ratio was 1:2. The solvent ratio was 40% v/v and the initiator to monomer ratio 2% w/w. The MIP template/DVB molar ratio was 1:3.

TAIC – EGDMA
The TAIC – EGDMA polymers were synthesized in MeCN using AIBN as initiator and heat at 75 °C for 16 h. The TAIC : EGDMA molar ratios were 1:6 and 1:3. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. The MIP template/LACIC molar ratios were 1:1 and 1:2.

TAC – EGDMA
The TAC – EGDMA polymers were synthesized in MeCN using AIBN as initiator and heat at 75 °C for 16 h. The TAC : EGDMA molar ratio was 1:6. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. The MIP template/TAC molar ratio was 1:1.

VC – EGDMA
The VC – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The VC : EGDMA molar ratio was 1:6. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. The MIP template/VC molar ratio was 1:1.

VI – EGDMA
The VI – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The VI : EGDMA molar ratio was 1:3. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. The MIP template/VI molar ratio was 1:2.

ACM – EGDMA
The ACM – EGDMA polymers were synthesized in MeCN using AIBN as initiator and UV irradiation for 16 h. The ACM : EGDMA molar ratio was 1:4. The solvent ratio was 30% v/v and the
initiator to monomer ratio 2% w/w. This polymer was imprinted with two templates, iprodione and pyrimethanil. The MIP template/ACM molar ratio was 1:2.

**4VPy – DVB**
The 4VPy – DVB polymers were synthesized in methanol/CHCl₃ (1:3) using AIBN as initiator and heating at 65 °C in a controlled temperature water bath for 16 h. The DVB used was 55% pure. The 4VPy : DVB molar ratio was 1:10. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. This polymer was imprinted with two templates, iprodione and pyrimethanil. The MIP template/4VPy molar ratio was 1:2.

**4VPy – DVB – Co(II)**
The 4VPy – DVB – Co(II) polymers were synthesized in MeOH/CHCl₃ (1:3) using AIBN as initiator and heating at 65 °C in a controlled temperature water bath for 16 h. The DVB used was 55% pure. Cobalt acetate salt was dissolved in the solvent first and then the monomers were added to the solution. The 4VPy : DVB molar ratios were 1:20 and 1:10 and 4VPy : Co(II) molar ratios were 1:1 and 2:1. The solvent ratio was 60% v/v and the initiator to monomer ratio 2% w/w. These polymers were templated with iprodione, and the template : Co(II) molar ratio was 1:1.

In a typical preparation, 598 mg of CoAcO₂·4H₂O was dissolved in 16 mL MeOH/CHCl₃, and then 427 µL of 4VPy, and 10.4 mL of DVB with 100 mg of AIBN were added to the solution and stirred to dissolve. The oxygen was removed from the solution by sparging nitrogen for 10 min, and then, placed in 65 °C in a controlled temperature water bath for 16 h to polymerize.

### 3.3.1.3 Polymer processing and BET study
After crushing and wet grinding in acetone using a mortar and pestle, the polymers were extracted with acetone in a Soxhlet apparatus for 72 h, and then dried at 70 °C, in order to remove the template and the unreacted monomers. FT-IR spectra of the polymers were taken before and after the extraction process to confirm the template removal, and the structural stability of the polymers during the extraction process.

### 3.3.2 Polymer film preparation

#### Polymer adhesion to gold surface
Prior to the application of the polymer film to the electrode area, the crystals were cleaned by immersing in a Pirannha etch solution (1:3 30% (v/v) H₂O₂: conc. H₂SO₄) at 60 °C for 10 min, then rinsing with Milli-Q water and ethanol, before being finally dried in a stream of nitrogen. The electrode was then immersed in an ethanol/water (4:1, v/v) solution containing allyl mercaptan (53 µM) and 1-butanethiol (13 µM) for at least 2 h, to introduce vinyl groups onto the surface of the gold electrode. Then the crystal was again dried with nitrogen gas.

#### Polymer Film preparation
Polymer films were cast using the sandwich method by dispensing 1 µL of the polymerisation solution directly onto the thiolized surface of the Cr/Au electrode and then covering it immediately with a 12 or 15 mm diameter glass microscope cover-slip. Polymerisation was initiated either by UV
light radiation using a 125 W medium pressure mercury lamp for at least 10 h, or by heating at 75 °C in a controlled temperature oven for at least 6 h.

The cover slip was removed, and the coated QCM was rinsed in acetone for 4-6 h and washed several times with water and acetone to remove the template, solvent, and other soluble components present in the polymer coating. The coated crystal was dried in an oven at 70 °C in air.

### 3.3.3 Batch adsorption studies

A 10mg sample of the polymer powder was weighed into a screw cap vial and mixed with 10 mL of the adsorbate under study, and placed on an orbital shaker (Ratek Instruments, Australia) to allow equilibration while shaking at room temperature for 2 h. After equilibration, a sample of the solution was filtered using a Nylon syringe filter (0.45 µm) which was washed with ~4 mL of the same solution, to eliminate any adsorption by the filter, before analysing the final sample by HPLC.

For isothermal adsorption, a controlled temperature, water jacketed apparatus was constructed in which to immerse the vials containing the mixture of polymer powder and iprodione solution. The temperature of the water circulating in the jacket was maintained at the desired temperature (±0.2 °C) with a MGW Lauda model K4R Electronic thermostated bath. The water jacket was placed on the orbital shaker used to stir the adsorption mixtures.

The HPLC analysis used the following conditions: 20 µL injection volume, 70:30 MeCN and 0.01% formic acid in water mobile phase, flow rate of 1 mL/min, 210 nm detection wavelength, LiChrospher100 RP-18 C18 column (125 mm × 4mm i.d.), 5 µm particle size, bore size 100 Å and LiChroCART® (125 mm × 4 mm i.d.), HPLC guard cartridge, Merck.

### 3.3.4 Film adsorption studies

Syringe pumps (Model A-99, Razel Science instruments Inc., USA) were used to create the necessary flow in the QCM flow cell. The QCM crystal holder flow cell was made of Teflon with a 170 µL volume on top of the sensing surface, Figure 3.3.

![Figure 3.3](image.png) The flow system, consisting of syringe pumps, syringes, three way valve, and QCM holder flow cell, connected with 1/16 inch o.d. Teflon tubing.
The design of the Maxtek cell holder was modified to allow for the escape of bubbles from the chamber, and absorb the acoustic waves using the cone shaped surface as the top part of the flow cell chamber, as shown in Figure 3.4.

![Figure 3.4](image)

**Figure 3.4** The designed flow cell crystal holder for QCM adsorption/desorption measurements.

To switch between the various solutions or solvents, a VICI Instruments Co. Inc., Cheminert® model C22Z, three way valve was used; see Figure 3.5.

![Figure 3.5](image)

**Figure 3.5** Three way valve connections to the flow cell and the syringes

The QCM response was adjusted to base line with a solvent flow rate of 2.83 mL/h (±1 Hz/min) before conducting the analyte adsorption/desorption measurements.

### 3.4 References


4 The morphology of MIPs

The number of imprinted sites in a molecularly imprinted polymer (MIP) material, and their accessibility, depends on both the porosity and active surface area, which in turn are controlled by the polymerization mechanism and conditions. In general, the parameters affecting the kinetics of MIP formation are the initiation energy source, initiator activity, and polymerization temperature, while the thermodynamics of site formation is affected by phase separation, which is determined by the diluent type (which may also be a pore forming agent or porogen). The resultant morphology of a MIP will thus depend on the kinetic and thermodynamic parameters as well as the mole fractions of the system components. The presence of template molecules or sacrificial polymer chains are other parameters that direct the formation of pores with specific sizes.

The porosity of a MIP and a non-imprinted polymer (NIP) can be studied at three levels; macroporosity (with cavity diameters larger than 500 Å), mesoporosity (20 – 500 Å) and microporosity (less than 20 Å), all of which are controlled by factors involved in the polymer synthesis.

Active surface area and SEM studies of MIPs and NIPs can yield valuable information about the polymer morphology and reveal physical characteristics of the polymer during uptake of a particular adsorbate. For example, while investigating the poly(glycidyl methacrylate – EGDMA) polymer, Viklund et al. found that reducing the temperature of thermal polymerization in the presence of a dodecanol/cyclohexanol porogen increased the pore diameter and shifted the pore size distribution to higher ranges. They also found that increasing the dodecanol ratio in mixtures of dodecanol/cyclohexanol and dodecanol/toluene porogens shifted the pore size distribution to higher ranges.

Lammerhofer et al. studied the effect of initiation energy source and porogen solvent composition on the porosity of 2-hydroxyethyl methacrylate (HEMA) and EGDMA-based polymers using dodecanol/cyclohexanol solvent as porogen. By increasing the dodecanol ratio in the porogen, the pore diameter in EGDMA-based polymers increased, regardless of the initiation method.

\[1 \text{ Å} = 10^{10} \text{ m} = 0.1 \text{ nm}.\]
decrease in pore diameter was observed when using UV initiation, which was the opposite to when thermal initiation was used in the preparation of HEMA-based polymers.

4.1 The effect of solvent

The term solvent refers to the solvation interactions between solute and solvent species in solution. During the process of polymer synthesis in solution, the solvent properties play a dynamic role, and is dependent on the polymer growth stage. A good solvent is able to keep the polymer solvated while it is growing until high molecular weights are achieved, so the reaction continues on the interface of the polymer particles in solution, producing large a homogeneous network structure. Bad solvents or non-solvents initially solubilize the components involved in the polymer formulation, but as the polymer centres grow they cease to be soluble due to their high molecular weight, causing phase separation to occur. Then polymer beads crash out of the polymerization mixture forming porous morphology of aggregates. These solvents are called *porogens*, otherwise the more general term *diluent* is used with no regard to the solvent properties and behaviour in the polymerization process.

4.1.1 Solvent type

The solvent properties determine whether the polymer precipitates or remains suspended in solution during polymerization. When phase separation occurs during polymer growth, solid beads are produced from the liquid solution. The choice of porogen dictates the porosity of the polymer by determining the stage at which the polymer beads precipitate. Poor solvents produce smaller pores due to earlier phase separation in the polymerization process, larger pores are produced in solvents that solvate the growing polymer network for longer times during growth, allowing phase separation to occur later in the polymerization process.

In the present work, copolymers containing MAA and 4VPy functional monomers (FM) with EGDMA cross-linker (CL), (FM:CL molar ratio of 1:5) were prepared in MeCN and water/MeOH diluents (at a 50% v/v ratio) to study the effect of solvent on the polymer porosity; Table 4.1 outlines the polymers prepared.

<table>
<thead>
<tr>
<th>Polymer #</th>
<th>Monomers (FM)</th>
<th>Cross-linker</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MAA</td>
<td>EGDMA</td>
<td>MeCN</td>
</tr>
<tr>
<td>2</td>
<td>MAA:4VPy (1:1)</td>
<td>EGDMA</td>
<td>MeCN</td>
</tr>
<tr>
<td>3</td>
<td>MAA:4VPy (3:1)</td>
<td>EGDMA</td>
<td>MeCN</td>
</tr>
<tr>
<td>4</td>
<td>MAA:4VPy (1:3)</td>
<td>EGDMA</td>
<td>MeCN</td>
</tr>
<tr>
<td>5</td>
<td>MAA:4VPy (1:1)</td>
<td>EGDMA</td>
<td>Water:MeOH (1:3)</td>
</tr>
<tr>
<td>6</td>
<td>MAA:4VPy (1:3)</td>
<td>EGDMA</td>
<td>Water:MeOH (1:3)</td>
</tr>
<tr>
<td>7</td>
<td>4VPy</td>
<td>EGDMA</td>
<td>Water:MeOH (1:3)</td>
</tr>
<tr>
<td>8</td>
<td>4VPy</td>
<td>EGDMA</td>
<td>MeCN</td>
</tr>
</tbody>
</table>

Table 4.1 Polymers synthesized to study the effect of solvent (the functional monomers/cross-linker molar ratio in these polymers is 1:5).
The SEM analysis of these polymers showed a major difference in the morphology of the polymers prepared in MeCN compared to those prepared in water/MeOH, regardless of the monomer ratios used in the preparation, as shown by the typical images in Figure 4.1.

![SEM images of polymers #6 and #8](image)

**Figure 4.1** SEM images of polymers #6 (EGDMA – MAA – 4VPy) and #8 (EGDMA – 4VPy).

The pore volume distribution shows a smaller pore volume (in the mesopore range) for the polymers prepared in water/MeOH compared to the polymers prepared in MeCN, as indicated in Figure 4.2 (A and B).

![Pore volume distribution graphs](image)

**Figure 4.2** Pore volume distribution analysis based on BJH theory of polymers prepared in; (A) water/MeOH (polymers #5, #6 and #7), and (B) MeCN (polymers #1, #2, #3, #4 and #8), solvents.

The active surface area measurements shown in Figure 4.3 (A), using nitrogen (N$_2$) adsorption at liquid nitrogen temperature indicates that different polymer morphologies are produced depending on the solvents used in the synthesis.
Figure 4.3 (A) $N_2$ adsorption isotherms, and, (B) pore size distribution based on BJH analysis of desorption branch of #1 – 8 polymer systems; #5, #6 and #7 were prepared in water/MeOH, and #1, #2, #3, #4 and #8 were prepared in MeCN diluents, EGDMA was used as a cross-linker in all polymers.
Lower surface areas were obtained for the polymers synthesized in water/MeOH diluent with a trend dependent on the ratio of 4VPy:MAA monomers. The polymers synthesized in MeCN produce a similar group of N₂ adsorption isotherms, and Figure 4.3 (B) shows that when polymers are prepared in water/MeOH solvent (#5, #6 and #7) large pores (around 1000 Å) are produced, but when using MeCN as diluent (#1, #2, #3, #4 and #8), smaller pores ranging from 250 – 550 Å are observed.

These results are indicative of the better solvency of the water/MeOH diluent for the EGDMA-based polymer systems. Utilizing 4VPy as FM in the polymer reduces the polymer's solvency in water/MeOH diluent and shifts the pore diameters to lower values. This is due to the lower polarity of the 4VPy FM compared to MAA. A lower interaction energy with the solvent molecules reduces the solvency of the growing polymer, resulting in earlier phase separation and smaller pore diameters. The solvency effect of MeCN diluent on different 4VPy:MAA ratios is small, and is seen more clearly in polymers #9 – #16, listed in Table 4.2 and shown in Figure 4.5. The presence of 4VPy in this set of polymers exhibited an opposite effect to the polymers prepared in water/MeOH diluent, since the enhanced polymer solvency allowed the polymer beads to grow larger during polymerization.

### 4.1.2 Solvent ratio

The effect of solvent ratio on the morphology of an EGDMA – MAA – 4VPy (20:1:3) polymer was observed using 5%, 50% and 95% MeCN diluent. The SEM images of the polymers prepared, shown in Figure 4.4, allow a description of the mechanism leading to pore formation during polymerization.

![Figure 4.4](image)

**Figure 4.4** SEM images of the polymers prepared in (A) 5%, (B) 50% and (C) 95% MeCN diluent

At very low ratios of diluent a glassy system is formed with negligible pore volume and surface area as shown in Figure 4.4 (A). At high ratios of diluent, which create very low concentrations of the monomers in the polymerization mixture, the polymer beads continue to grow without aggregation or fusion due to the distance between the growing polymer nucleation centres, and individual beads are produced as shown in Figure 4.4 (C). At intermediate concentrations a porous polymer is formed due to polymer bead aggregation and fusion, as seen in Figure 4.4 (B).

Yoshimatsu et al. investigated the factors involved in controlling MIP bead formation. An understanding of these factors is necessary in order to control the polymerization mechanism so that MIP beads can be produced in different sizes and forms for potential applications. Identifying the parameters determining the bead size leads to an understanding of the mesoporosity of the MIP
material in porous systems. To further study the formation of polymer beads in 95% (v/v) MeCN as diluent, the eight combinations of monomers listed in Table 4.2 were examined. The presence and absence of iprodione as template without stirring during polymerization was also considered.

**Table 4.2** Polymers prepared to study the parameters affecting polymer bead formation (EGDMA was used as CL and MeCN 95% v/v as solvent in the preparation of this group of polymers, the Template : FMs ratio in the templated polymers was 1:5).

<table>
<thead>
<tr>
<th>Polymer #</th>
<th>Monomers (FM)</th>
<th>MAA:4VPy:EGDMA</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>MAA</td>
<td>(1:0:5)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>10</td>
<td>MAA</td>
<td>(1:0:2)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>11</td>
<td>MAA:4VPy (1:3)</td>
<td>(1:3:20)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>12</td>
<td>MAA:4VPy (3:1)</td>
<td>(3:1:20)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>13</td>
<td>MAA</td>
<td>(1:0:5)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>14</td>
<td>MAA</td>
<td>(1:0:2)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>15</td>
<td>MAA:4VPy (1:3)</td>
<td>(1:3:20)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>16</td>
<td>MAA:4VPy (3:1)</td>
<td>(3:1:20)</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

It was found that different bead sizes were obtained using different monomer ratios, and the presence of iprodione in the polymerization solution also affected bead formation. Both non-templated and templated copolymers of EGDMA – MAA form stable colloids, but copolymers of EGDMA – MAA – 4VPy produced a precipitate of polymer beads. In Figure 4.5 the SEM images of the particles show the effect of monomer ratios and the presence of iprodione in the polymerization solutions.

In copolymers of EGDMA – MAA – 4VPy, the higher ratios of the MAA to 4VPy FMs reduce the final bead size formed during polymerization from 0.5-3.0 µm to 0.1-0.3 µm. This confirms the solvent effect on the phase separation during bead growth. The presence of the 4VPy monomer enhanced the solubility of the polymer beads in MeCN diluent, allowing the polymer beads to grow larger without phase separation. In the MeCN polymer solutions containing only MAA and EGDMA monomers, the bead size was relatively small and phase separation occurred in the early stages of bead growth, terminating the polymerization chain reaction such that the beads were too small to precipitate, which remained suspended at the conclusion of the polymerization process. Figure 4.6 shows the TEM images of the EGDMA – MAA polymers prepared in MeCN. The bead size distribution of these particles is between 20 – 50 nm diameters.

The effect of the presence of the template iprodione in the polymerization solution on bead size distribution is shown in the SEM images in Figure 4.5. Based on SEM observations, the presence of iprodione reduced the bead size of #15 (EGDMA – MAA – 4VPy) by about a half (from ~2 to ~1 µm) compared to #11, where MAA:4VPy is 1:3, but, it did not significantly change the bead size between templated #16 and non-templated #12 polymers, where the MAA:4VPy ratio is 3:1.
Figure 4.5 SEM images of the #11 MAA – 4VPy – EGDMA (1:3:20) and #12 MAA – 4VPy – EGDMA (3:1:20) non-templated polymers, and #15 MAA – 4VPy – EGDMA (1:3:20) and #16 MAA – 4VPy – EGDMA (3:1:20) templated polymer beads produced using 95% MeCN diluent, the bar represent 1 µm.

Figure 4.6 TEM images of the EGDMA – MAA polymer (#9) prepared in MeCN diluent, TEM samples were prepared by dropping a highly diluted suspension of the original polymer bead suspension on the TEM grid (1 drop of the original suspension was diluted to 5 mL). Note the scale bars are in nm.
The formation of polymer beads at high diluent ratios clarified the formation of the porous system at lower ratios of diluent. Better solvency allowed the beads to grow larger, while poor solvency allowed phase separation to occur earlier and thus produce smaller beads. The SEM and TEM images seen in Figure 4.5 and Figure 4.6 show a distribution of sizes for the beads produced. The porosity of the non-templated polymer is due to the aggregation of these beads produced during the polymerization process.

When polymer beads with a certain range of sizes aggregate they will produce a range of pore sizes in the void space between the solid beads. This porosity follows the solvent strength of the diluent. If the diluent ratio is sufficiently low, and the polymer solvency is good, such that the beads reach each other during growth before phase separation occurs, they will fuse and continue to grow forming a channel type of porosity. In contrast, when the polymer solvency is poor, regardless of the diluent ratio, phase separation occurs before the beads aggregate, and this leads to a “cauliflower” type of porosity.

Figure 4.6 clearly shows that, for #9 polymer beads, the aggregation of the nano-spheres formed the porous system, in which the larger beads formed the major framework while the smaller beads fused the structure together as show schematically in Figure 4.7.

Figure 4.7 A schematic representation of the dried nano-spheres on the TEM grid.

4.2 Monomer ratio

A comparison of the polymers #5 and #6 (EGDMA – MAA – 4VPy) and #7 (EGDMA – 4VPy) shows the effect of monomer ratio on polymer morphology. In Figure 4.8 three SEM images of these polymers are shown. Increasing the 4VPy ratio reduces the particle size in the polymer aggregate. No variation in the morphology of polymers prepared in MeCN can be observed from the SEM images due to the very fine features of their shape.

Active surface area and pore size distribution analysis of this set of polymers confirms the SEM results. The macropores visible by SEM at the magnification used in Figure 4.8 are not detectable using Barrett, Joyner, and Halenda (BJH) theory to analyse the nitrogen adsorption experiments. However, the mesopores and micropores of these polymer systems can be determined using active surface area analysis. Figure 4.2 (A) shows the pore size distribution of polymers #5 EGDMA – MAA – 4VPy (20:2:2), #6 EGDMA – MAA – 4VPy (20:3:1), and #7 EGDMA – 4VPy (20:4).
Polymer #5 exhibits no distinct evidence of pores between 10 and 100 nm, while pores about 105 nm in diameter appear in #6. These pores are due to interparticle spaces between the polymer aggregates. The smaller particles of polymer #7 produced smaller interparticle spaces between polymer aggregates. This trend follows the increasing 4VPy content in these polymers.

![SEM images](image)

**Figure 4.8** SEM images of #5 EGDMA – MAA – 4VPy (20:2:2), #6 EGDMA – MAA – 4VPy (20:1:3), and #7 EGDMA – 4VPy (20:4) polymers prepared in water/MeOH diluent.

Figure 4.3 (B) illustrates the pore size distribution of polymers #1, #2, #3, #4, and #8 prepared in MeCN diluent, and exhibit a trend based on the MAA:4VPy monomer ratio in the polymer structure. All these polymers showed a broad pore size distribution, with the main difference being in the 200 – 800 Å range. Polymer #1 MAA showed a peak between 500 – 600 Å with a shoulder at 350 – 400 Å; #3 with a 3:1 MAA:4VPy molar ratio, has the 500 – 600 Å peak shifted to a lower value; around 500 Å; in #2 with a 1:1 MAA:4VPy molar ratio, the peak maximum shifted even lower to 390 Å, and a small peak began to appear at 270 Å pore diameter. This peak grows as the 4VPy fraction increases to 1:3 in polymer #4; while in polymer #8 4VPy the peak at 400 Å completely disappears and there is only a broad distribution with a maximum at 310 Å.

The increase in the 4VPy:MAA monomer ratio reduces the pore diameter in the polymeric systems when MeCN was used as diluent. This reduction is not a gradual change of porosity but arises because of an existing distinct pore structure for the polymeric domains containing 4VPy or MAA monomers. The acid – base interaction between these two FMs may be another reason contributing to the change in pore size, in addition to the solvent effect on the morphology. MAA, 4VPy and the ionic species of these FMs, and the distribution of such species in the polymer structure, affects the polymeric centers solubility in MeCN during polymer growth.

MAA is able to form a hydrogen bond with MeCN as the solvent, but 4VPy is less similar to MeCN and so has a lower solubility than MAA in MeCN. Ionic species formed by MAA – 4VPy combinations are likely to be less soluble than the uncharged species in an organic solvent such as MeCN. Based on this solubility, it is expected that the polymers with combinations of MAA – 4VPy would have smaller pores when compared to the respective 4VPy functionalized polymers.

### 4.3 Surface area analysis

The higher the surface area of a MIP the greater the number of potential adsorption sites available for adsorbate uptake, and hence the higher the capacity. Therefore the active surface area will determine the performance of MIP materials as selective adsorbents. The active surface area
analysis of the polymers showed the effect of the parameters determining the porosity of the material during the polymerization process.

Polymers synthesised in MeCN diluent gave high surface area materials due to their mesoporous morphology. Table 4.3 lists the active surface area analysis of polymers #1 – #8 prepared in either MeCN or water/MeOH.

**Table 4.3** Summary of active surface area analysis of polymers prepared in MeCN diluent (#1, #2, #3, #4, and #8) and polymers prepared in water/MeOH (1:3) diluent (#5, #6, and #7).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#1</th>
<th>#3</th>
<th>#2</th>
<th>#4</th>
<th>#8</th>
<th>#5</th>
<th>#6</th>
<th>#7</th>
</tr>
</thead>
<tbody>
<tr>
<td>4VPy : MAA</td>
<td>0:1</td>
<td>1:3</td>
<td>1:1</td>
<td>3:1</td>
<td>1:0</td>
<td>3:1</td>
<td>1:1</td>
<td>1:0</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>327</td>
<td>359</td>
<td>317</td>
<td>318</td>
<td>304</td>
<td>63</td>
<td>46</td>
<td>155</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>320</td>
<td>350</td>
<td>310</td>
<td>309</td>
<td>296</td>
<td>62</td>
<td>45</td>
<td>152</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>212</td>
<td>234</td>
<td>212</td>
<td>221</td>
<td>212</td>
<td>26</td>
<td>18</td>
<td>83</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>207</td>
<td>232</td>
<td>208</td>
<td>220</td>
<td>211</td>
<td>30</td>
<td>16</td>
<td>73</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>89</td>
<td>89</td>
<td>81</td>
<td>68</td>
<td>65</td>
<td>22</td>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.732</td>
<td>0.772</td>
<td>0.794</td>
<td>0.735</td>
<td>0.672</td>
<td>0.052</td>
<td>0.067</td>
<td>0.283</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.792</td>
<td>0.846</td>
<td>0.817</td>
<td>0.746</td>
<td>0.640</td>
<td>0.042</td>
<td>0.068</td>
<td>0.311</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.835</td>
<td>0.891</td>
<td>0.796</td>
<td>0.731</td>
<td>0.624</td>
<td>0.035</td>
<td>0.062</td>
<td>0.397</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.038</td>
<td>0.037</td>
<td>0.035</td>
<td>0.028</td>
<td>0.027</td>
<td>0.009</td>
<td>0.009</td>
<td>0.025</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>90</td>
<td>86</td>
<td>100</td>
<td>92</td>
<td>88</td>
<td>33</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>150</td>
<td>144</td>
<td>154</td>
<td>135</td>
<td>121</td>
<td>64</td>
<td>153</td>
<td>149</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>161</td>
<td>153</td>
<td>153</td>
<td>133</td>
<td>118</td>
<td>47</td>
<td>158</td>
<td>216</td>
</tr>
</tbody>
</table>

Polymers synthesized in water/MeOH (1:3) diluent exhibited much lower surface areas. This is due to the stronger solvency of protic solvents and hence the larger polymer particles produced during polymer growth in this diluent. The solubility of the 4VPy functionalized polymer is much lower than the MAA functionalized polymer, and thus it has a higher surface area. When the MAA:4VPy ratio is 1:1 in the polymer mixture, ionic species are produced in the polymerization solution, the protic solvents enhance solubility and the fused aggregates formed have a lower surface area. The pore size distribution of these polymers arises from the morphology of the external surface of the aggregated particles.
The solvency effect in MeCN is opposite to that in water/MeOH. The MAA functionalized polymers have a higher surface area compared to the 4VPy functional polymers due to the better solvency of the latter in MeCN, which gives rise to a channel network morphology. The solubility of functionalized polymers is much lower when MAA – 4VPy combinations are used in the polymer formulation and ionic species are produced, which lead to higher surface areas in comparison to polymers with formulations including only MAA or 4VPy.

4.4 Template effect

The effect of the template molecules, iprodione and pyrimethanil, on the porosity of bulk polymers is discussed in this section, where focus is given to the interaction between the FM and template.

The presence of iprodione in the polymerization solution was found to reduce the bead size in polymers #15 and #16 in comparison to #11 and #12. This effect can be due to two factors; (i) changing the solvent strength to solubilize the evolving polymer chains and networks, and, (ii) the interaction between the monomers and the template in the pre-polymerization solution. The presence of the hydrophobic iprodione reduces the solvency of FMs ionic species in MAA – 4VPy combinations. The poorer solvency for polar intermediates causes earlier phase separation of the polymer, and thus smaller beads. In the absence of the 4VPy FM in polymers #9, #10, #13 and #14, the solvency is much worse, and the beads are reduced to about 50 nm in diameter as shown in Figure 4.6.

The interaction between the cross-linker EGDMA and the template, iprodione, was studied by templating iprodione in an EGDMA polymer, and preparing the corresponding non-imprinted polymer (NIP). The composition of each polymer is given in Table 4.4. The active surface area deduced from the N₂ adsorption isotherms in Figure 4.9 shows no peak shift in the pore size distribution of the templated polymer compared to the non-templated polymer, thus it can be concluded that there is little or no interaction between the EGDMA monomer and the iprodione template molecule.

**Table 4.4** Non-templated and imprinted EGDMA polymers used to study the iprodione template effect (no FM was used).

<table>
<thead>
<tr>
<th>#</th>
<th>Cross-linker</th>
<th>Iprodione:EGDMA</th>
<th>Solvent</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>EGDMA</td>
<td>(0:30)</td>
<td>MeCN</td>
<td>Non-templated</td>
</tr>
<tr>
<td>18</td>
<td>EGDMA</td>
<td>(1:30)</td>
<td>MeCN</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

To further investigate the template interaction with FMs a set of polymers based on EGDMA, MAA and 4VPy monomers was prepared both with and without iprodione as the template, as outlined in Table 4.5. The molar ratio of FM to iprodione was 5:1. To ensure equilibrium of the associations between the template and the monomers in solution, the pre-polymerization solutions were sonicated for an hour after sparging with nitrogen. This study would also indicate the effect of the template on polymer morphology.
Table 4.5 Non-templated and imprinted polymers synthesized to study the template effect (EGDMA was used as CL and MeCN 50% v/v as solvent in the preparation of this group of polymers, the Template : FMs ratio in the templated polymers was 1:5).

<table>
<thead>
<tr>
<th>#</th>
<th>Monomers</th>
<th>MAA:4VPy:EGDMA</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>MAA</td>
<td>(1:0:5)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>20</td>
<td>4VPy</td>
<td>(0:1:5)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>21</td>
<td>MAA:4VPy</td>
<td>(1:1:10)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>22</td>
<td>MAA</td>
<td>(1:0:5)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>23</td>
<td>4VPy</td>
<td>(0:1:5)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>24</td>
<td>MAA:4VPy</td>
<td>(1:1:10)</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

Active surface area analysis was performed after extraction of the template and unreacted species from the polymers and subsequent drying, and the results are listed in Table 4.6. The sonication step included here changes the nitrogen adsorption results for this set of polymers, compared to polymers #1, #2 and #8 prepared with the same method but without the sonication step.

Table 4.6 Summary of active surface area analysis of non-templated and iprodione-imprinted polymers prepared in MeCN diluent.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#17</th>
<th>#18</th>
<th>#19</th>
<th>#20</th>
<th>#21</th>
<th>#22</th>
<th>#23</th>
<th>#24</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA:4VPy</td>
<td>-</td>
<td>-</td>
<td>1:0</td>
<td>0:1</td>
<td>1:1</td>
<td>1:0</td>
<td>0:1</td>
<td>1:1</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>346</td>
<td>363</td>
<td>287</td>
<td>208</td>
<td>138</td>
<td>284</td>
<td>185</td>
<td>101</td>
</tr>
<tr>
<td>Single point surface area at P/Po = 0.2027 (m²/g)</td>
<td>332</td>
<td>348</td>
<td>276</td>
<td>200</td>
<td>132</td>
<td>272</td>
<td>178</td>
<td>96</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>285</td>
<td>300</td>
<td>236</td>
<td>169</td>
<td>129</td>
<td>232</td>
<td>147</td>
<td>98</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>291</td>
<td>306</td>
<td>251</td>
<td>186</td>
<td>147</td>
<td>251</td>
<td>177</td>
<td>121</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>40</td>
<td>41</td>
<td>39</td>
<td>36</td>
<td>16</td>
<td>32</td>
<td>34</td>
<td>8</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.768</td>
<td>0.801</td>
<td>0.508</td>
<td>0.398</td>
<td>0.289</td>
<td>0.480</td>
<td>0.311</td>
<td>0.236</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.805</td>
<td>0.818</td>
<td>0.507</td>
<td>0.388</td>
<td>0.305</td>
<td>0.468</td>
<td>0.311</td>
<td>0.259</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.798</td>
<td>0.811</td>
<td>0.502</td>
<td>0.387</td>
<td>0.300</td>
<td>0.466</td>
<td>0.311</td>
<td>0.272</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.005</td>
<td>0.011</td>
<td>0.013</td>
<td>0.002</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>89</td>
<td>88</td>
<td>71</td>
<td>77</td>
<td>84</td>
<td>68</td>
<td>67</td>
<td>93</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>113</td>
<td>109</td>
<td>86</td>
<td>92</td>
<td>95</td>
<td>81</td>
<td>85</td>
<td>106</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>110</td>
<td>106</td>
<td>80</td>
<td>83</td>
<td>81</td>
<td>74</td>
<td>70</td>
<td>90</td>
</tr>
</tbody>
</table>
Figure 4.9 (A) N₂ adsorption isotherms, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) SEM images of non-templated (#17) and templated (#18) EGDMA polymers.
Figure 4.10 (A) N$_2$ adsorption isotherms, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) SEM images of non-templated (#19) and templated (#22) EGDMA – MAA.
Figure 4.11 (A) \( N_2 \) adsorption isotherms, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) SEM images of non-templated (#20) and templated (#23) EGDMA–4VPy polymers.
Figure 4.12 (A) $N_2$ adsorption isotherms, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) SEM images of non-templated (#21) and templated (#24) EGDMA – MAA – 4VPy polymers.
Comparison of the nitrogen adsorption isotherms, and the BJH desorption branch pore-size distribution analysis of the templated and non-templated polymers, shows that the BET surface area decreases by templating the polymers. For the pore size distribution in the range 20 – 120 Å, this change is due to two factors. The first is the change in the macroporosity of the polymers above 1000 Å because of the diluent solvency change, and the second is the effect of the template in the micropore range. The non-templated EGDMA – MAA (#19) and imprinted (#22) polymers show no significant difference in pore size distribution compared to the polymers containing 4VPy as functional monomer, as can be seen in Figure 4.10 (B). This is due to the very weak interaction between the hydrophobic iprodione and the hydrophilic MAA monomer.

The changes in the pore size distribution are significant for the EGDMA – 4VPy and EGDMA – MAA – 4VPy polymers, as shown by the data in Figure 4.11 and Figure 4.12 respectively. In the EGDMA – 4VPy polymers (#20 and #23) the pore size distribution of the templated polymer showed a decrease in the peak at about 102 Å whilst the peak at about 30 Å increased compared to the non-templated polymer. Both the BJH cumulative adsorption and desorption pore volume of pores between 17 and 3000 Å diameter decreased by templating the polymer, as detailed in Table 4.6. The reason for this decrease is believed to be due to the stronger hydrophobic interactions between the template and FM which are not present in the non-templated polymer.

In the case of the NIP and MIP of EGDMA – MAA – 4VPy (#21 and #24), similar hydrophobic interactions may be expected between 4VPy and the template. In addition, the acid – base equilibrium between the 4VPy and MAA FMs produces ionic species which may strongly interact with each other as well as the template molecule. The lower surface area and BJH cumulative adsorption and desorption pore volume of pores between 17 and 3000 Å diameter is evidence for these interactions, as detailed in Table 4.6, with data taken from Figure 4.12.

From these results, it was difficult to quantify the dimensions of the pores created by the template molecule due to the large pore volume arising from the porogen effect. Indeed, the diluent ratio had to be reduced to make the pores left by the template molecule more prominent in the pore size distribution profile. To conduct this study, where template-created pores could be identified in the polymer structure, one non-templated, and two EGDMA – ACM polymers templated with iprodione and pyrimethanil, were prepared in 28% MeCN diluent. Hydrophilic (hydrogen-bonding or dipole – dipole) interactions are possible between the ACM FM and the templates, as shown in Figure 4.13. The compositions of these polymers are listed in Table 4.7.

![Figure 4.13](image-url) Possible interaction between, (A) iprodione, and, (B) pyrimethanil and the ACM functional groups in the polymer.
Table 4.7 Non-templated and templated ACM – EGDMA polymers synthesized to study the template effect (EGDMA was used as CL and MeCN 28% v/v as solvent in the preparation of this group of polymers).

<table>
<thead>
<tr>
<th>#</th>
<th>Monomers</th>
<th>ACM:Template:EGDMA</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>ACM</td>
<td>(2:0:8)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>26</td>
<td>ACM</td>
<td>(2:1:8)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>27</td>
<td>ACM</td>
<td>(2:1:8)</td>
<td>Pyrimethanil</td>
</tr>
</tbody>
</table>

The active surface area analysis of these polymers revealed the different size of pores left by the different template molecules as shown in Figure 4.14 and Table 4.8.

Table 4.8 Summary of active surface area analysis of non-templated, and iprodione and pyrimethanil imprinted, EGDMA – ACM polymers prepared in low ratio MeCN diluent.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#25</th>
<th>#26</th>
<th>#27</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>217</td>
<td>317</td>
<td>301</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>212</td>
<td>309</td>
<td>293</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>161</td>
<td>228</td>
<td>208</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>184</td>
<td>253</td>
<td>239</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>45</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.353</td>
<td>0.450</td>
<td>0.407</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.345</td>
<td>0.437</td>
<td>0.379</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.340</td>
<td>0.436</td>
<td>0.378</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.018</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>65</td>
<td>57</td>
<td>54</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>86</td>
<td>77</td>
<td>73</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>74</td>
<td>69</td>
<td>63</td>
</tr>
</tbody>
</table>

The comparison between the templated EGDMA – ACM (#26 and #27) and the non-templated (#25) polymers shows little difference in the pore size distribution profile due to the dominant cross-linker ratio, see Figure 4.14. The BJH analysis (Figure 4.14 (B)) however clearly shows two new peaks for the iprodione and pyrimethanil templated polymers (#26 and #27) compared to the non-templated (#25) polymer.

Deconvoluting the numerical data of the pore size distribution to extract the pore groups of the polymers may be done using the following Gaussian distribution function;

\[
y = a_0 \exp \left[ -\frac{1}{2} \left( \frac{x-a_1}{a_2} \right)^2 \right]
\]
and using the amplitude \(a_0\) as a variable parameter, which after centering the data, peaks appeared at 63 Å for iprodione, and 49 Å for pyrimethanil, in addition to the two peaks appearing in the non-templated polymer at 38 Å and 103 Å. These sizes are of the same order of magnitude as pentameric aggregates of the imprinted molecules (iprodione 12.1 Å and pyrimethanil 10.5 Å). For example, considering the hydrogen-bond distance between the template molecule and the functional groups of the polymer, the pore sizes suggest the association of several template molecules in the pre-polymerization solution or during polymer growth, which exert a major effect on the subsequent morphology of the polymer.

The reduction of the amplitude of the peak at 38 Å in the iprodione imprinted polymer observed in Figure 4.14 (B) shows the repulsion effect of the template, increasing the pore diameter and the pore volumes in the pore size distribution. The area of this peak is still larger than the non-templated polymer which means some of the template molecules are accommodated in these pores within the polymer structure. The opposite is observed in the case of the pyrimethanil templated polymer, where the amplitude of the peak at 38 Å increased, and the pore volume at the larger diameter decreased because of the presence of the template, which indicates a strong hydrogen-bonding interaction between the FM and the template. This interaction pulls the monomers closer to each other before and during polymerization, forming a more closely packed structure.

The peaks calculated using this deconvolution method provide a good fit in agreement with the BJH incremental desorption pore volume analysis, as shown in Table 4.9.

**Table 4.9** Pore size distribution from deconvolution results of non-templated (#25), iprodione templated (#26), and pyrimethanil templated (#27) polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Center</th>
<th>Amplitude</th>
<th>Area</th>
<th>(a_0)</th>
<th>(a_1)</th>
<th>(a_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#25</td>
<td>38.45</td>
<td>0.43</td>
<td>1.97</td>
<td>0.4294</td>
<td>38.4523</td>
<td>1.8267</td>
</tr>
<tr>
<td></td>
<td>103.24</td>
<td>0.51</td>
<td>52.92</td>
<td>0.5090</td>
<td>103.2440</td>
<td>41.4733</td>
</tr>
<tr>
<td>#26</td>
<td>38.36</td>
<td>0.37</td>
<td>2.44</td>
<td>0.3668</td>
<td>38.3601</td>
<td>2.6497</td>
</tr>
<tr>
<td></td>
<td>63.81</td>
<td>0.36</td>
<td>13.56</td>
<td>0.3629</td>
<td>63.8142</td>
<td>14.9083</td>
</tr>
<tr>
<td></td>
<td>112.94</td>
<td>0.62</td>
<td>54.09</td>
<td>0.6198</td>
<td>112.9351</td>
<td>34.8115</td>
</tr>
<tr>
<td>#27</td>
<td>38.38</td>
<td>0.61</td>
<td>3.31</td>
<td>0.6142</td>
<td>38.3756</td>
<td>2.1494</td>
</tr>
<tr>
<td></td>
<td>49.15</td>
<td>0.24</td>
<td>6.76</td>
<td>0.2389</td>
<td>49.1482</td>
<td>11.2928</td>
</tr>
<tr>
<td></td>
<td>117.67</td>
<td>0.48</td>
<td>61.91</td>
<td>0.4786</td>
<td>117.6679</td>
<td>51.5997</td>
</tr>
</tbody>
</table>
Figure 4.14 (A) N₂ adsorption isotherms, and, (B) pore size distribution based on BJH analysis of desorption branch of non-templated (#25) and iprodione and pyrimethanil templated (#26 and #27) EGDMA – ACM polymers, and, (C) SEM images of polymers #25 and #26.
To study the effect of the interaction between the FM and the template on the porosity of the MIPs, pyrimethanil was imprinted in EGDMA – MAA in 36% v/v CHCl₃ diluent; the compositions of the polymers prepared are listed in Table 4.10. Baggiani et al.19 studied the templated polymers in bead and bulk forms using pyrimethanil as a template, and characterized the imprinting effect by chromatography. The strong acid–base interaction between the FM and the template was reported to embed the template molecule in the polymer chemical structure, as shown schematically in Figure 4.15.

![Figure 4.15 Pyrimethanil – MAA interaction in the MIP molecular structure.](image)

Table 4.10 Non-templated and pyrimethanil imprinted, EGDMA – MAA polymers used to study the template and the solvent ratio effect on the polymer’s morphology.

<table>
<thead>
<tr>
<th>#</th>
<th>MAA:Template:EGDMA</th>
<th>Solvent (v/v %)</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>(3:0:18)</td>
<td>CHCl₃ (36%)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>29</td>
<td>(3:1:18)</td>
<td>CHCl₃ (36%)</td>
<td>Pyrimethanil</td>
</tr>
<tr>
<td>30</td>
<td>(3:0:18)</td>
<td>CHCl₃ (60%)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>31</td>
<td>(3:1:18)</td>
<td>CHCl₃ (60%)</td>
<td>Pyrimethanil</td>
</tr>
</tbody>
</table>

In the present work, the MAA – pyrimethanil interaction was monitored by NMR titration, by following the change in the chemical shift of the N–H functionality in pyrimethanil as an indicator of interaction between MAA and the template, which reached a maximum at a 3:1 molar ratio, as shown in Figure 4.16, suggesting 3 moles of MAA are able to associate with one pyrimethanil molecule in the pre-polymerization solution, as used in polymers #29, #30 and #31.

![Figure 4.16 NMR titration of pyrimethanil using MAA in CDCl₃.](image)
Active surface area analysis of the polymers both before and after extraction of the template and other soluble materials shows that the presence of pyrimethanil in the polymerization solution changed the pore size distribution pattern compared to the NIP. The N₂ adsorption isotherms, shown in Figure 4.17 (A), indicate the extraction process for the NIP did not change the non-templated polymer (#28) morphology profile, but did have a significant effect on the templated polymer (#29), increasing the BET surface area from 163 to 249 m²/g, as shown in the values in Table 4.11. In addition, the cluster of pores appearing as a shoulder on the narrow peak at 39 Å in Figure 4.17 (B) disappeared, due to the strong interaction between the MAA and pyrimethanil reducing the pore size distribution to the minimum possible in the mesopore range for the MIP structure.

Table 4.11 Summary of surface area analysis of non-templated #28, #30 and pyrimethanil imprinted #29, #31 EGDMA – MAA polymers prepared in 36% and 60% v/v CHCl₃ diluent.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Before extraction</th>
<th>After extraction</th>
<th>After extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#28</td>
<td>#29</td>
<td>#28</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>283</td>
<td>163</td>
<td>291</td>
</tr>
<tr>
<td>Single point surface area at P/P₀ 0.2027 (m²/g)</td>
<td>276</td>
<td>157</td>
<td>285</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>179</td>
<td>125</td>
<td>176</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>225</td>
<td>164</td>
<td>219</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>80</td>
<td>24</td>
<td>87</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.308</td>
<td>0.183</td>
<td>0.303</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.280</td>
<td>0.182</td>
<td>0.263</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.283</td>
<td>0.188</td>
<td>0.264</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.034</td>
<td>0.009</td>
<td>0.038</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>44</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>62</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>50</td>
<td>46</td>
<td>48</td>
</tr>
</tbody>
</table>

To compare the solvent ratio effect with the FM – template interaction effect in the micropore range, the non-templated and templated EGDMA – MAA polymeric systems (#30 and #31) were prepared with a higher ratio of diluent (60% v/v of CHCl₃). Changing the solvent ratio from 36 to 60% did not change the morphology of the polymer, since the surface area analysis of the non-templated and templated polymers have very similar peak profiles and values, as can be seen in Figure 4.18.
Figure 4.17 (A) N$_2$ adsorption isotherms, (B) pore size distribution based on BJH analysis of desorption branch before and after extraction, and (C) SEM images of non-templated, and pyrimethanil templated, EGDMA – MAA polymers prepared in 36% v/v CHCl$_3$ (#28 and #29).
Figure 4.18 (A) $N_2$ adsorption isotherms, and, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) the SEM images of non-templated, and pyrimethanil templated, EGDMA – MAA polymers prepared in 60% v/v CHCl₃ (#30 and #31) after extraction.
The π-π interaction energy in these polymers is very weak compared to any acid – base interaction (see Table 1 of Chapter 2), although a polymer composed of monomers possessing functionalities capable of participating in these weak π-π interactions would potentially enhance the interaction strength between the template and the polymer. When such polymers were prepared, by including a monomer with an aromatic π-system such as DVB in the EGDMA-MAA polymer, see Table 4.12, an expansion of the micropore remaining after template molecule removal was observed when a 53% v/v diluent ratio was used in the synthesis.

**Table 4.12** Non-templated, and pyrimethanil imprinted, EGDMA-MAA-DVB polymers prepared to study the template effect on the pore size distribution.

<table>
<thead>
<tr>
<th></th>
<th>Monomers</th>
<th>Cross-linker</th>
<th>MAA:DVB:Template:EGDMA</th>
<th>Solvent (v/v%)</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>MAA, DVB</td>
<td>EGDMA</td>
<td>(3:3:0:21)</td>
<td>CHCl$_3$ (53%)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>33</td>
<td>MAA, DVB</td>
<td>EGDMA</td>
<td>(3:3:1:21)</td>
<td>CHCl$_3$ (53%)</td>
<td>Pyrimethanil</td>
</tr>
</tbody>
</table>

The $^1$H NMR spectra, Figure 4.19 and Figure 4.20, showed very little interaction between pyrimethanil and styrene. Styrene was used because of its high purity and the simplicity of the proton NMR spectrum. Indeed, a comparison of the small N-H chemical shift of the pyrimethanil in the mixture ($\delta = 7.091$ ppm) with pure pyrimethanil ($\delta = 7.154$ ppm) and other minor shifts in the aromatic protons, show only weak interactions occur in the mixture. In general the formation of a hydrogen bond shifts the proton signal to higher fields (lower $\delta$), in this case the N-H has shifted slightly to higher field in the presence of styrene, which indicates the formation of a hydrogen bond between pyrimethanil molecules, encouraging the formation of dimers of pyrimethanil.

There were no chemical shift changes in the styrene protons between the pure sample and the mixture with pyrimethanil in CDCl$_3$, this suggests that the styrene molecule merely changes the solvency and solvation arrangement of pyrimethanil.

**Figure 4.19** $^1$H NMR spectra of styrene, pyrimethanil and a mixture of styrene and pyrimethanil in CDCl$_3$. 

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Thus, aromatic π-π stacking between aromatic rings (styrene and pyrimethanil) is not expected to significantly contribute to the template association in the pre-polymerization solution.

Figure 4.20 An expansion of the aromatic proton region in the ¹H NMR spectra of styrene, pyrimethanil and a mixture of styrene and pyrimethanil in CDCl₃.

The surface area and BJH analysis of the desorption branch of the N₂ adsorption isotherms of the EGDMA – MAA – DVB polymers (#32 and #33) before and after the extraction process, (see Figure 4.21 and Table 4.13), show that the inclusion of DVB in the polymer (compared with the EGDMA – MAA (#28 – #31) polymers) merely introduced a level of flexibility to the polymer structure.

When the surface area analyses of the non-templated polymer (#32) before and after extraction are compared, a significant change in shape of the N₂ adsorption isotherm and BJH pore size distribution is observed. For example, after Soxhlet extraction with acetone for 72 h, a pore size peak develops at 56 Å as a shoulder on the 38 Å peak. Considering that the DVB used in these experiments was only 50% pure, and the other 50% consisted of vinylethylbenzene isomers, this flexibility was attributed to the lower ratio of cross-linking monomers compared to the EGDMA – MAA polymers (#28 – #31).

The pyrimethanil templated EGDMA – MAA – DVB polymer (#33) showed similar relaxation behaviour during extraction, as shown in Figure 4.21, but the relaxation of the templated polymer had a smaller effect on the polymer structure due to initial alignment of the aromatic monomers around the template molecule in the pre-polymerization solution. This suggests that pores with
Figure 4.21 (A) N$_2$ adsorption isotherms, and, (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – MAA – DVB NIP and pyrimethanil imprinted polymer (#32 and #33) prepared in 53% v/v CHCl$_3$ diluent before and after extraction process: the pore size distribution is scaled up by 0.5 unit steps to clarify the difference between the data sets.
similar size and geometry are formed in the process of imprinting pyrimethanil in EGDMA – MAA – DVB, but the flexibility of the polymer structure deforms some of these sites during the extraction process as the polymer relaxes to reduce structural stresses and strains.

Figure 4.21 indicates that when pyrimethanil is removed from the templated polymer during the extraction process, a shoulder peaking at 56 Å develops in addition to the pore size peak at 38 Å in the templated polymer. In the non-templated polymer this shoulder is present even before the extraction process, but after extraction it develops to a relatively strong peak at 56 Å. This may be due to relaxation of the polymer chemical structure because of the extensive washing, or the removal of soluble materials such as the template and partially polymerized monomers. The cumulative pore volume in the pore size range 17 to 3000 Å was larger in the NIP (#32) than in the MIP (#33) as shown in Table 4.13. Comparison of the pore size distribution of the non-templated polymer before and after extraction (38 Å peak decrease and 56 Å peak increase) and the fact that the micropore volume remains the same for the non-templated polymer, supports the chemical structure relaxation process, which occurs during the washing step, as the primary reason for the observed changes. The micropore volume shows that the porosity of the non-templated and templated polymers approaches the same value (0.030 mL/g) after removal of the template from the MIP.

Table 4.13 Summary of active surface area analyses of EGDMA – MAA – DVB NIP, and pyrimethanil imprinted polymer (#32 and #33) prepared in 53% v/v CHCl₃ diluent before, and after, extraction process.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Before extraction</th>
<th>After extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#32</td>
<td>#33</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>300</td>
<td>121</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>291</td>
<td>115</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>186</td>
<td>106</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>253</td>
<td>155</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>73</td>
<td>6</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.298</td>
<td>0.166</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.261</td>
<td>0.174</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.270</td>
<td>0.183</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.030</td>
<td>0.001</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>40</td>
<td>55</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>56</td>
<td>66</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>43</td>
<td>47</td>
</tr>
</tbody>
</table>

To exploit aromatic π-π stacking and hydrophobic interactions between iprodione and the polymer, other FMs containing extended aromatic systems were selected, including 1-vinylimidazole (VI), 1-vinylcarbazole (VC), triallyl cyanurate (TAC) and triallyl isocyanurate (TAIC). The ratios of the polymer components are summarized in Table 4.14 and a 60% diluent ratio was employed.
Table 4.14 Non-templated, and iprodione imprinted, polymers used to study the template hydrophobic interaction and \( \pi-\pi \) stacking with various functional monomers (60% v/v solvent ratio was used).

<table>
<thead>
<tr>
<th>#</th>
<th>FM</th>
<th>Cross-linker</th>
<th>FM:Template:EGDMA</th>
<th>Solvent</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>VI</td>
<td>EGDMA</td>
<td>(2:0:6)</td>
<td>MeCN</td>
<td>Non-templated</td>
</tr>
<tr>
<td>35</td>
<td>VI</td>
<td>EGDMA</td>
<td>(2:1:6)</td>
<td>MeCN</td>
<td>Iprodione</td>
</tr>
<tr>
<td>36</td>
<td>VC</td>
<td>EGDMA</td>
<td>(1:0:6)</td>
<td>MeCN</td>
<td>Non-templated</td>
</tr>
<tr>
<td>37</td>
<td>VC</td>
<td>EGDMA</td>
<td>(1:1:6)</td>
<td>MeCN</td>
<td>Iprodione</td>
</tr>
<tr>
<td>38</td>
<td>TAC</td>
<td>EGDMA</td>
<td>(1:0:6)</td>
<td>MeCN</td>
<td>Non-templated</td>
</tr>
<tr>
<td>39</td>
<td>TAC</td>
<td>EGDMA</td>
<td>(1:1:6)</td>
<td>MeCN</td>
<td>Iprodione</td>
</tr>
<tr>
<td>40</td>
<td>TAIC</td>
<td>EGDMA</td>
<td>(1:0:6)</td>
<td>MeCN</td>
<td>Non-templated</td>
</tr>
<tr>
<td>41</td>
<td>TAIC</td>
<td>EGDMA</td>
<td>(1:1:6)</td>
<td>MeCN</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

These polymer systems were selected to utilize the heterocyclic \( \pi \)-system interaction between the template and the FM, assuming that the similarity between the electronic structures of the FM and the template would create the driving force to form an association between these two components.

The BET surface area and pore size distribution analysis of these systems may be used to estimate the effect of the template – FM interaction strength on the polymer chemical structure. Generally, higher surface areas for the templated polymers reflects the weakness of the interaction between the FM and template, as the template molecule in such systems acts as a bad solvating molecule to increase the porosity of the polymer structure. On the other hand, steric hindrance forces the template molecule to take a position in the polymer molecular structure that represents the minimum energy in the system. This is the reason for the changes in the porosity profile in the mesopore range observed in the BJH analysis of the desorption branch of the \( \text{N}_2 \) adsorption isotherm.

The high diluent ratio used in the synthesis of this set of polymers increased the pore volume in the upper values of the mesopore range (400 – 500 Å) and overshadowed any effects on the lower diameter pores as shown in Figure 4.22 to Figure 4.25 and Table 4.15.

The BET surface area increased by 2.5% when EGDMA – VI was imprinted with iprodione, most of this increase being due to the micropore area, which increased by 11.4%. This effect intensified when the larger aromatic system of VC was used to imprint iprodione. The BET surface area, and micropore area of the templated EGDMA – VC polymers (#37) increased by 4.3% and 17.5% respectively compared to the non-templated polymer (#36), while the BJH desorption average pore diameter decreased from 137 to 124 Å. That is, imprinting EGDMA – VC with iprodione resulted in a higher number of pores with smaller sizes compared to NIP. The templated EGDMA – TAC polymer (#39) showed a different imprinting effect, where the BET surface area and micropore area were increased by 5.1% and 8.6% respectively, compared to the NIP (#38). The BET surface area and micropore area of the templated EGDMA – TAIC polymer (#41) decreased by 3.7% and 43.9% respectively compared to the NIP (#40), this indicates that most of the micropores were lost during the imprinting process. The reduction of micropore volume by 60% confirms the loss of micropores in the EGDMA – TAIC MIP after imprinting with iprodione. The details of all the surface area analyses for these polymers are presented in Table 4.15.
Figure 4.22 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – VI non-templated and iprodione templated polymers (#34 and #35).
Figure 4.23 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – VC non-templated and iprodione templated polymers (#36 and #37), before and after extraction process.
Figure 4.24 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – TAC non-templated and iprodione templated polymers (#38 and #39), before and after extraction.
Figure 4.25 (A) $N_2$ adsorption isotherms and (B) pore size distribution, based on BJH analysis of desorption branch of EGDMA – TAIC non-templated and iprodione templated polymers (#40 and #41), before and after extraction.
Table 4.15 Summary of BET surface area analysis of EGDMA – VI, VC, TAC, or TAIC non-templated, and iprodione templated, polymers (#34 to #41).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#34</th>
<th>#35</th>
<th>#36</th>
<th>#37</th>
<th>#38</th>
<th>#39</th>
<th>#40</th>
<th>#41</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>399</td>
<td>409</td>
<td>371</td>
<td>387</td>
<td>415</td>
<td>436</td>
<td>324</td>
<td>312</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>387</td>
<td>398</td>
<td>358</td>
<td>374</td>
<td>402</td>
<td>422</td>
<td>312</td>
<td>298</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>305</td>
<td>308</td>
<td>281</td>
<td>289</td>
<td>317</td>
<td>333</td>
<td>272</td>
<td>276</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>309</td>
<td>307</td>
<td>279</td>
<td>291</td>
<td>315</td>
<td>335</td>
<td>270</td>
<td>284</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>79</td>
<td>88</td>
<td>57</td>
<td>67</td>
<td>70</td>
<td>76</td>
<td>41</td>
<td>23</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.924</td>
<td>0.984</td>
<td>0.886</td>
<td>0.877</td>
<td>0.968</td>
<td>0.963</td>
<td>0.883</td>
<td>0.755</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.901</td>
<td>0.959</td>
<td>0.934</td>
<td>0.909</td>
<td>1.069</td>
<td>1.045</td>
<td>0.915</td>
<td>0.810</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.894</td>
<td>0.951</td>
<td>0.952</td>
<td>0.899</td>
<td>1.078</td>
<td>1.075</td>
<td>0.904</td>
<td>0.807</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.032</td>
<td>0.037</td>
<td>0.022</td>
<td>0.026</td>
<td>0.028</td>
<td>0.030</td>
<td>0.015</td>
<td>0.006</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>93</td>
<td>96</td>
<td>96</td>
<td>91</td>
<td>93</td>
<td>88</td>
<td>109</td>
<td>97</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>118</td>
<td>125</td>
<td>133</td>
<td>126</td>
<td>135</td>
<td>126</td>
<td>135</td>
<td>117</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>116</td>
<td>124</td>
<td>137</td>
<td>124</td>
<td>137</td>
<td>128</td>
<td>134</td>
<td>114</td>
</tr>
</tbody>
</table>

By using the non-polar aromatic DVB (55%) as the FM to imprint iprodione at a low solvent ratio (37% v/v) the strength of the interaction between the FM and iprodione can be investigated.

Table 4.16 Non-templated and iprodione imprinted DVB – EGDMA polymers (MeCN was used as solvent with 37% v/v ratio).

<table>
<thead>
<tr>
<th>#</th>
<th>FM</th>
<th>Cross-linker</th>
<th>DVB:Template:EGDMA</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>DVB</td>
<td>EGDMA</td>
<td>(3:0:5)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>43</td>
<td>DVB</td>
<td>EGDMA</td>
<td>(3:1:5)</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

A comparison of the pore size distribution, in the mesopore range, between the non-templated and templated polymers (#42 and #43) shows the degree of interaction between the FM and the template, as can be seen in Figure 4.26. The pores in the mesopore range at about 172 Å for the NIP have shrunk to about 52 Å in the iprodione templated polymer, demonstrating the π-π stacking effect of the template on the polymer structure, creating a more structured and compact chemical structure for the polymer.
Figure 4.26 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – DVB NIP and iprodione templated polymers (#42 and #43).
A higher BET surface area was observed for the MIP despite the smaller pores in the mesopore range compared to the NIP, as detailed in Table 4.17. This indicates a secondary effect for the template molecule on the polymer structure, which arises when the imprinting is based on weak interactions between the FM and the template, such as hydrophobic interactions, and if the FM – FM interaction is weak too, the template molecule can act as a solvent molecule, increasing the volume of the solvating species in the pre-polymerization solution. This effect is prominent when the diluent ratio is small and the volume of the template molecule is significant. In this case, the template molecule increases the macro-porosity of the MIP causing the active surface area to increase dramatically.

Table 4.17 Summary of active surface area analysis of polymers #42 and #43.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#42</th>
<th>#43</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>35</td>
<td>94</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>33</td>
<td>89</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>28</td>
<td>90</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>50</td>
<td>139</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>3.5</td>
<td>2.5</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.097</td>
<td>0.167</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.099</td>
<td>0.178</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.098</td>
<td>0.172</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>111</td>
<td>71</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>141</td>
<td>79</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>79</td>
<td>49</td>
</tr>
</tbody>
</table>

Hydrophobic interactions such as π-π stacking are very weak (5 – 20 kJ/mol), but they can be maximized by employing an appropriate balance of FMs in a MIP. The molecular structure of iprodione consists of a chlorinated phenyl group and a heterocyclic conjugated π-system. Using DVB as both a hydrophobic FM and cross-linker, potentially adds both functionality and strength to the polymer structure. Similarly, 4VPy is a heterocyclic aromatic system that provides a polar π-system capable of interaction with the iprodione molecule through π-π stacking. In addition, the inclusion of 4VPy adds polarity to the polymer aromatic functionality, providing further possibilities for association to the iprodione template. Separate non-templated and imprinted polymers composed of EGDMA as cross-linker, with DVB and 4VPy as FMs, were synthesized to study the polymer morphology and iprodione imprinting effect, as detailed in Table 4.18. The use of 4VPy as FM provides a polar aromatic system with enhanced similarity to the iprodione conjugated system.
Table 4.18 Non-templated and iprodione imprinted EGDMA – DVB – 4VPy polymers (EGDMA was used as CL and MeCN 36% v/v as solvent).

<table>
<thead>
<tr>
<th>#</th>
<th>FM</th>
<th>4VPy: DVB:Template:EGDMA</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>4VPy, DVB</td>
<td>(2:3:0:4)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>45</td>
<td>4VPy, DVB</td>
<td>(2:3:1:4)</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

Figure 4.27 presents the \( \text{N}_2 \) adsorption isotherms and pore size distribution for polymers #44 and #45, where it can be seen there is little difference in morphology. The imprinting decreased the BJH desorption average pore diameter, but increased the active surface area of the polymer as tabulated in Table 4.19. This morphology change indicates that the iprodione effect on the chemical structure of the polymer occurs by increasing the number of smaller pores left by iprodione molecules, which increase the surface area and decrease the average pore diameter.

Table 4.19 Summary of BET surface area analysis of non-templated and iprodione imprinted EGDMA – DVB – 4VPy (#44 and #45) polymers.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#44</th>
<th>#45</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>305</td>
<td>327</td>
</tr>
<tr>
<td>Single point surface area at P/P₀ 0.2027 (m²/g)</td>
<td>296</td>
<td>316</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>198</td>
<td>220</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>207</td>
<td>234</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.492</td>
<td>0.486</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.461</td>
<td>0.455</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.445</td>
<td>0.442</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.027</td>
<td>0.026</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>64</td>
<td>59</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>93</td>
<td>83</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>86</td>
<td>75</td>
</tr>
</tbody>
</table>
Figure 4.27 (A) N₂ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch, and (C) SEM images of non-templated and iprodione imprinted EGDMA – DVB – 4VPy polymers (#44 and #45).
The EGDMA cross-linker provided a hydrophilic matrix for the FMs capable of interacting as separate entities with iprodione as the template or adsorbate in the rebinding tests. In this case, DVB (55%) was used as cross-linker, to provide a hydrophobic aromatic matrix which could cause the cross-linker to interact with iprodione as template, thereby participating more in the imprinting and rebinding processes. The impurity in DVB is ethylvinylbenzene isomers and with DVB cross-linker being the major component of the polymer structure, considering the impurity ratio, the polymer structure will be less cross-linked and less rigid compared to the EGDMA based polymers. This low rigidity makes these DVB-based polymers more susceptible to swelling in organic solvents.

Using a mixture of CHCl$_3$/MeOH as polymerization diluent provides poor solvency for both the monomers and template, which helps to drive the pre-polymerization association between the template and monomers in the solution, while at the same time forming a highly porous polymeric system with a highly active surface area.

The 4VPy FM is a polar aromatic system which may enhance the imprinting effect due its compatibility with the electron distribution within iprodione. In a further modification, cobalt (II) acetate was included in polymers #48, #49, #52 and #53 to investigate potential coordination of Co(II) to iprodione and 4VPy, as shown in Figure 4.28, which would enhance the association between template and polymer components in the pre-polymerization solution and lead to a more selective binding site.

Anhydrous MeOH/chloroform in a 1:3 volumetric ratio was used as solvent and diluent. The MeOH/chloroform mixture has good solvency for the cobalt salt, which provides an appropriate medium to form cobalt complexes in the pre-polymerization solution. Using a 60% (v/v) diluent to monomers ratio gave the porosity necessary for high surface area. Table 4.20 gives details of the polymers compositions.

As noted the DVB-based polymer morphology is significantly different to the EGDMA based polymers, especially in terms of their active surface area and pore size distribution. The DVB-based polymers also exhibited strong hydrophobic behaviour, showing very low wetting in water. When 4VPy and Co(II) were included in the DVB-based polymers, the morphology changed significantly as can be seen by comparing the pore size distributions in Figure 4.30 to Figure 4.33. As a general observation, these DVB-based polymers showed high levels of swelling in acetone and no wetting in water, so, when mixed with water, a layer of the polymer powder formed on the surface. Initial wetting with THF or acetone allowed wetting of the polymer to occur.
Table 4.20 Non-templated and iprodione templated DVB – 4VPy and DVB – 4VPy Co(II) polymers (DVB was used as CL and 3:1 CHCl₃/MeOH as solvent at a solvent to monomers ratio of 60% v/v).

<table>
<thead>
<tr>
<th>#</th>
<th>Monomers</th>
<th>4VPy:Co(II):DVB:Template</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>-</td>
<td>(0:0:20:0)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>47</td>
<td>4VPy</td>
<td>(2:0:20:0)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>48</td>
<td>(4VPy)₂ Co(II)</td>
<td>(2:1:20:0)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>49</td>
<td>4VPy Co(II)</td>
<td>(1:1:20:0)</td>
<td>Non-templated</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>(0:0:20:1)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>51</td>
<td>4VPy</td>
<td>(2:0:20:1)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>52</td>
<td>(4VPy)₂ Co(II)</td>
<td>(2:1:20:1)</td>
<td>Iprodione</td>
</tr>
<tr>
<td>53</td>
<td>4VPy Co(II)</td>
<td>(1:1:20:1)</td>
<td>Iprodione</td>
</tr>
</tbody>
</table>

Figure 4.29 SEM images of the DVB (#46), DVB – 4VPy (#47), DVB – 4VPy, Co(II) (#48) and DVB – 4VPy Co(II) (#49) non-templated polymers prepared in CHCl₃/MeOH diluent.

While not apparent from the SEM images of Figure 4.29, the BET surface area analysis shows major differences between the polymers of this set. The N₂ adsorption isotherms and BJH analysis of the desorption branch of these isotherms exhibit porosity differences which can be seen in Figure 4.30 to Figure 4.33. The BJH analysis indicated the polymer composed of DVB in the absence of any FM (#46 and #50) was a closely packed aggregate of particles, bringing the pore distribution in to the mesopore range (170 Å for #46 and 225 Å for #50). The wider distribution of pore sizes, and the shift to larger pores in the templated polymer (#50), indicates aggregation of larger particles in the templated polymer. This increase in the particle size shows the solvency enhancement by adding iprodione to the pre-polymerization solution, causing further growth of the polymer nucleus in the templated polymer. A comparison of the solvent effect between the NIP and MIP shows the attraction between the template and DVB monomers, which slightly increases the number of pores at 19 Å.
Figure 4.30 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of non-templated and iprodione templated DVB polymers ($\#46$ and $\#50$) prepared in CHCl$_3$/MeOH diluent.
Figure 4.31 (A) N$_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of non-templated and iprodione templated DVB – 4VPy polymers (#47 and #51) prepared in CHCl$_3$/MeOH diluent.
Figure 4.32 (A) N\textsubscript{2} adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of non-templated and iprodione templated DVB – (4VPy)\textsubscript{2} – Co(II) polymers (#48 and #52) prepared in CHCl\textsubscript{3}/MeOH diluent.
Figure 4.33 (A) $N_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of non-templated and iprodione templated DVB – 4VPy – Co(II) polymers (#49 and #53) prepared in CHCl$_3$/MeOH diluent.
Adding the 4VPy FM to the DVB polymer formulation (#47 and #51) had a significant effect on the pore size distribution, leading to a reduction of the pores sizes in the mesopore range of the DVB polymer to a single well defined pore size of 39 Å, indicating a fundamental change in the monomer’s distribution in the polymer chemical structure. The inclusion of 4VPy enhanced the polymers solubility in the MeOH/chloroform diluent, allowing the initial polymer particles to grow to much larger sizes, causing the pore diameter between the particles after phase separation, aggregation and fusion, to move out of the range of the BJH analysis. The templated DVB – 4VPy polymer (#51) exhibited a higher volume of pores of minimum diameter, showing that the relatively strong interaction between the iprodione and 4VPy has reduced the size of some of the larger pores.

Adding Co(II) to the DVB – 4VPy composition (#48 and #52) and (#49 and #53) forced the 4VPy FM to form complexed species in the pre-polymerization solution, as suggested in Figure 4.28. This strong association disturbs the random distribution of the monomers in the polymer matrix, causing the smallest pore to have a similar size to those in the DVB polymers (#46 and #50). The good solubility of the Co(II) – 4VPy complex in the diluent has enhanced the polymer solubility, increasing the pore volume due to the formation of larger polymer particles.

The 1:1 ratio of 4VPy:Co(II) in the non-templated polymer #49 reduces the concentration of 4VPy monomers around Co(II) and increases the number of MeOH ligands in the complexed species, but the nature of the ionic species consisting of 4VPy-FM causes the DVB monomer to polymerise in segregated regions, forcing the cobalt complex to be more concentrated in separate regions of the polymer matrix. The peak at 19 Å in the BJH pore size distribution analysis of these polymers, shown in Figure 4.33, indicates its similarity to polymers #47 and #51 where there are only DVB monomers. The reduction of the 4VPy:DVB ratio from 2:20 in some polymers in this set, to 1:20 in #49 and #53, reduces the impact of the FM on the polymer solubility during the polymerization process. The broad peak of pore sizes centred at 91 Å reappear due to the worsening solvency compared to polymers #48 and #52. The peak at 91 Å has shifted 62 Å and reduced in volume when the polymer was templated with iprodione (#53). Together with this shift, and the reduction in size of the 91 Å peak, the pores at 19 Å increased in volume. This shows the strong interaction between iprodione and the 4VPy-FM in the complex.

When the BET surface areas of the DVB based non-templated polymers are compared, it can be seen from Table 4.21 that including 4VPy in polymer #47 reduces the surface area by 40% compared to the DVB polymer (#46). By introducing polarity into the polymer structure, 4VPy enhances the solubility of the polymer in the diluent, and causes an increase in particle size of the polymer beads during polymer growth: larger particles produce larger pores and lower surface area. The presence of ionic species in the DVB – (4VPy)$_2$ – Co(II) polymer (#48) structure reduces its solvency in the organic medium compared to #47, and consequently the BET surface area increases, but it is still below that of #46 where a purely non-polar polymer forms in a mainly polar organic diluent. Lowering the ratio of 4VPy by 50% in polymer #49 compared to #48 further reduced the polymer solubility, and thus increased its BET surface area.
Templating these polymers increases the BET surface area of #50 by 9.8%, #51 by 7.6% and #52 by 5.5% compared to #46, #47 and #48 respectively, but decreases the BET surface area of #53 by 16.2% compared to its corresponding NIP.

The iprodione – DVB and 4VPy π-π stacking interaction is comparable to the solvation interaction, so the effect of iprodione as template on the polymer structure and morphology may be considered as diluent effect. The increase in the BET surface area is related to both an increase in the diluent volumetric ratio and the micro-pores created by the template molecule in the polymer structure. The average pore diameter derived from BJH desorption shows an increase due to templating of polymers #46, #47, and #48. The involvement of iprodione in the Co(II) complex species in #53 changed this trend, reducing the average pore diameter and BET surface area.

Table 4.21 Summary of active surface area analysis of polymers #46 to #53.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>#46</th>
<th>#47</th>
<th>#48</th>
<th>#49</th>
<th>#50</th>
<th>#51</th>
<th>#52</th>
<th>#53</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET surface area (m²/g)</td>
<td>460</td>
<td>276</td>
<td>417</td>
<td>512</td>
<td>505</td>
<td>297</td>
<td>440</td>
<td>429</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>442</td>
<td>266</td>
<td>399</td>
<td>488</td>
<td>482</td>
<td>285</td>
<td>422</td>
<td>410</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>337</td>
<td>169</td>
<td>294</td>
<td>390</td>
<td>388</td>
<td>194</td>
<td>313</td>
<td>313</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>361</td>
<td>229</td>
<td>361</td>
<td>444</td>
<td>420</td>
<td>255</td>
<td>390</td>
<td>390</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
<td>54</td>
<td>55</td>
<td>46</td>
<td>37</td>
<td>44</td>
<td>42</td>
<td>49</td>
<td>39</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.848</td>
<td>0.249</td>
<td>0.409</td>
<td>0.680</td>
<td>0.889</td>
<td>0.277</td>
<td>0.473</td>
<td>0.446</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.908</td>
<td>0.216</td>
<td>0.377</td>
<td>0.646</td>
<td>0.965</td>
<td>0.249</td>
<td>0.433</td>
<td>0.414</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.907</td>
<td>0.222</td>
<td>0.390</td>
<td>0.658</td>
<td>1.084</td>
<td>0.255</td>
<td>0.469</td>
<td>0.428</td>
</tr>
<tr>
<td>Micropore volume (mL/g)</td>
<td>0.018</td>
<td>0.021</td>
<td>0.015</td>
<td>0.009</td>
<td>0.012</td>
<td>0.015</td>
<td>0.016</td>
<td>0.012</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>74</td>
<td>36</td>
<td>39</td>
<td>53</td>
<td>70</td>
<td>37</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>108</td>
<td>51</td>
<td>51</td>
<td>66</td>
<td>100</td>
<td>51</td>
<td>58</td>
<td>53</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>101</td>
<td>39</td>
<td>43</td>
<td>59</td>
<td>103</td>
<td>40</td>
<td>48</td>
<td>44</td>
</tr>
</tbody>
</table>

4.5 Thermostability of the MIPs

The dimensional stability of the imprinted adsorption site in a MIP is determined by the stability of the chemical structure of the polymer. A NIP and a MIP of EGDMA – DVB – 4VPy (#44 and #45) were chosen to study the thermostability of the polymer morphology, especially in the microporosity range. The NIP (#44) was degassed at 30, 50, 80, 110, 150, 200, and 250 °C for 22 h.
at each temperature, and subsequently examined using the N\textsubscript{2} adsorption isotherm at liquid nitrogen temperature, the results of which are shown in Figure 4.34. The same process, starting from 40 to 250 °C, was performed on the corresponding MIP (#45), and the results of these measurements are shown in Figure 4.35 and summarized in Table 4.22. The surface area analysis results of this experiment show a gradual change in the pore size distribution with increasing temperature.

For each polymer, the variation of the BET surface area shows an initial slight increase when degassed at low temperature, then a steady decrease between 50 and 200 °C, and finally a steep drop in surface area at 250 °C, as shown in Figure 4.36 (A). The initial slight increase of surface area is due to further degassing of the polymer samples, while the consequent decrease of surface area as the degassing temperature increases, is ascribed to polymer structure relaxation, finally the large decrease of surface area, when the polymers were degassed at 250 °C, is attributed to the incipient collapse and decomposition of the polymer structure at such a high temperature.

The main parameter that is sensitive to temperature changes is the micropore area, this followed by ∆A in Figure 4.36 (A), which is the BET surface area when the micropore area is subtracted. Subtracting the micropore area from the BET area indicates that the non-templated polymer (#44) shows little change up to 200 °C, and that the main changes occur in the micropores, while the ∆A of the templated polymer (#45) demonstrate larger variations through the heating process, indicating a slightly higher resistance to structural change in the micropores with temperature.

Figure 4.36 (B) shows that the average pore diameter increases at higher temperatures, while the pore volume steadily decreases. This suggests that relaxation of the polymer structure leads to the destruction of some of the micropores via two possible routes; the collapse and eventual blockage of the pore, or the expansion of the pore walls causing the average pore diameter to increase.

Figure 4.37 (A and B) shows the pore volume comparison of the pore size distribution obtained after each step of heat treatment, and indicates the gradual disappearance of the original pores giving rise to the formation of other pores in different areas.

Figure 4.37 (A and B) illustrates the different patterns that are observed for the BJH analysis of the desorption branch for the pore volume distribution of the NIP and MIP polymers of EGDMA – DVD – 4VPy (#44 and #45). The heat treatment has caused shifts and broadening in some peaks and the gradual disappearance of some others as the polymer morphology is modified by the thermal treatment.

The FT-IR spectra of the polymers before and after the heat treatment described above confirmed that the composition of the polymers was stable to long term heating, up to 250 °C, as can be seen in Figure 4.38. Small changes appear in the finger-print region, and at 992, 1000, 1296 and 1319 cm\textsuperscript{-1} which support the notion of conformational relaxation of the molecular structure of the polymers. The intensity increase in the hydroxyl band area is a sign of decomposition and cleavage of the ester groups in the EGDMA segments of the polymer producing more carboxylic acid groups in the structure at 250 °C.
Figure 4.34 (A) N$_2$ adsorption isotherms and (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – DVB – 4VPy non-templated polymer (#44) after degassing for 22 h at stepwise increasing temperatures. The BJH pore size distributions were elevated by 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 units with increasing temperatures for clarity.
Figure 4.35 (A) N₂ adsorption isotherms, and, (B) pore size distribution based on BJH analysis of desorption branch of EGDMA – DVB – 4VPy templated polymer (#45) after degassing for 22 h at stepwise increasing temperatures. The BJH pore size distributions were elevated by 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 units with increasing temperatures for clarity.
Table 4.22 Summary of active surface area analysis of EGDMA – DVB – 4VPy NIP and iprodione templated polymers (#44 and #45) after degassing for 22 h at stepwise increasing temperatures.

<table>
<thead>
<tr>
<th></th>
<th>#44</th>
<th>#45</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°C</td>
<td>50°C</td>
</tr>
<tr>
<td>BET surface area (m²/g)</td>
<td>277</td>
<td>284</td>
</tr>
<tr>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>268</td>
<td>276</td>
</tr>
<tr>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>190</td>
<td>188</td>
</tr>
<tr>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>198</td>
<td>195</td>
</tr>
<tr>
<td>Micropore area (m²/g)</td>
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<td>68</td>
</tr>
<tr>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>0.459</td>
<td>0.462</td>
</tr>
<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
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<td>0.433</td>
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<tr>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
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<td>0.417</td>
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<tr>
<td>Micropore volume (mL/g)</td>
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<td>0.028</td>
</tr>
<tr>
<td>Average pore diameter (4V/A by BET) (Å)</td>
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<td>65</td>
</tr>
<tr>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
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<td>92</td>
</tr>
<tr>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>85</td>
<td>86</td>
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</table>
Figure 4.36 (A) Surface area ($\Delta A$ is the BET surface area when the micropore area is deducted), and, (B) pore volume changes, with degassing temperature of EGDMA – DVB – 4VPy non-templated, and iprodione templated, polymers (#44 and #45).

Figure 4.37 Pore size distribution, based on pore volume desorption branch BJH analysis of heat treated EGDMA-DVB-4VPy: (A) non-templated (#44), and, (B) iprodione templated, (#45) polymers.
Figure 4.38 FTIR spectra of washed EGDMA – DVB – 4VPy NIP and iprodione templated polymers (#44 and #45), before and after degassing, and following stepwise elevation of temperature to 250 °C.
The TGA analysis, shown in Figure 4.39 (A and B), demonstrates different trends in the decomposition rate of the two polymers. The NIP left a carbonaceous residue that did not decompose completely up to 750 °C, while the templated polymer (#45) decomposed completely by 750 °C. The randomly distributed aromatic rings in the NIP can form a graphitic structure after releasing all the decomposable material. On the other hand, the MIP is believed to have a cluster of aromatic rings around the iprodione molecule, thus, unlike the NIP, the aromatic rings are localized in regions of the polymer matrix. These localised aromatic rings are separated from other localized groups, so they cannot join to form the extended graphite structure. The appearance of a second peak in the differential thermal analysis (\(dW/dT\)) curve for the MIP (B) - (#45) at about 650 °C provides evidence of a different molecular structure from the NIP (A) - (#44). The aromatic rings and conjugated π-system alignments in the templated polymer structure are believed to be responsible for the higher stability and delay of the decomposition process as the temperature is raised.

\[\text{Figure 4.39} \text{ TGA analysis of EGDMA – DVB – 4VPy (A) NIP (#44), and, (B) iprodione templated (#45) polymers.}\]
A similar trend was observed for the EGDMA – VC non-templated and templated polymers (#36 and #37). The templated polymer starts decomposing at a slightly higher temperature, as seen in Figure 4.40 (A). This behaviour is unlike the EGDMA – MAA, polymers because of the very weak interaction between the FM, MAA, and the template, iprodione. The TGA analysis shows almost identical decomposition rates for the NIP and MIP (#19 and #22), Figure 4.40 (B).

Figure 4.40 TGA analysis of (A) EGDMA – VC non-templated and templated polymers (#36 and #37); (B) EGDMA – MAA non-templated and templated polymers (#19 and #22).
4.6 Conclusions and discussion

In developing such adsorbing materials, an aim is to maximize the active surface area so as to obtain a high rebinding capacity. For a selective adsorbent membrane, a highly active surface area is essential to expose the highest population of selective sites to the targeted molecules in the adsorption medium.

Here the parameters affecting the porosity of the MIP and NIP materials are described, and the effect of the template molecule on the porosity of the polymeric system has been investigated throughout the imprinting studies.

A persistent feature of the EGDMA based polymers is a narrow peak of pore sizes centred around 38 Å, which can be assigned to channels composed of 10 or 12 membered EGDMA monomer rings. The appearance of these pores is a consistent feature of this polymer, and is not affected by the factors that further manipulate the polymeric system porosity.

The effects of solvent (also termed the diluent) ratio as well as the type and the choice of functional monomer all determine the resultant polymer porosity. Each one of these parameters was studied to separate and understand the contribution of the template molecule to the polymer chemical structure and porosity.

The templating effect was followed by comparing the porosity of the templated and non-templated polymers. Iprodione and pyrimethanil were chosen as template molecules with very different chemical functionalities and properties. Iprodione has strong hydrophobic properties while pyrimethanil is hydrophilic and acts as a base via its pyridyl and amine nitrogens. Both templates are aromatic organic compounds used as fungicides in viticulture. The solubility of these compounds was a major factor to consider in the pre-polymerization solution formulation. The choice of solvent and FM was limited by the combined solubility of template, FM, and initiator, to maximize the molecular interactions between the monomers and template prior to polymerization.

MAA, 4VPy, ACM, DVB, VI, VC, TAC, and TAIC were used as FMs and EGDMA and DVB were used as cross-linkers to investigate the effect of the interaction between template and FMs on the polymer porosity. In this study, strong interactions such as those between MAA and pyrimethanil, and weak π-π stacking interactions in VC – iprodione mixtures were investigated. A comparison of the pore size distribution of non-templated, and templated, EGDMA polymers in the absence of any FM demonstrated no imprinting effect by iprodione.

Iprodione, as a template, had a minimal effect on the EGDMA – MAA polymer system when compared to the effect of pyrimethanil. The stronger pyrimethanil – FM interaction forces the monomers to associate closely around the template in the pre-polymerization solution, and reduces the pore sizes of the EGDMA – MAA polymer to the minimum possible in the mesopore range. This strong initial association changes the polymerization kinetics of the templated polymer compared to when the template is absent, and the monomers are distributed more randomly, based on weaker interactions between the functional and cross-linker monomers. This change in the polymerization
mechanism may be related to the strength of interaction between the template and the FM, the stronger the interaction, the greater the change in the role of the FM in the polymerization reaction.

MAA and iprodione, which are hydrophilic and hydrophobic species respectively, prefer not to associate with each other in solution. In fact, MAA, although a weak acid, like most carboxylic acids, is capable of acting as an acceptor or donor in hydrogen-bonding. In the liquid state, it is assumed to exist only as a dimer. Less hydrophilic species are attracted to iprodione in the pre-polymerization solution. Solvent molecules, or the cross-linker monomers, which are less effective in the imprinting process, will solvate iprodione molecules. The opposite occurred when pyrimethanil was used as template in the imprinting process, as there is a very strong interaction between MAA and pyrimethanil.

In the preparation of NIP and MIP polymers of EGDMA – MAA the solvent ratio was increased from 36% (v/v) (in #28 and #29) to 60% (v/v) (in #30 and #31) to compare the solvent effect with the template effect in the imprinting process. In this instance, the template effect persisted, with the same trend, and to the same degree.

The degree of cross-linking was a major factor in the imprinted site stability, especially when a lengthy extraction was required to remove the template and any unreacted and soluble species from the polymer structure. The addition of DVB (55% purity) as both FM and cross-linker for the EGDMA – MAA polymer used to imprint pyrimethanil, reduced the association strength and increased the polymer flexibility. The active surface area analysis of EGDMA – DVB – MAA before and after, extraction showed considerable changes for both the templated and non-templated polymers in the mesopore range. The pores in the NIP with 38 Å diameter relaxed during the extraction process, and formed a wider distribution of larger pores around 56 Å. The MIP underwent a less dramatic transformation, forming a shoulder in the same pore size range. The smaller changes seen for the MIP were due to the initial higher stability of the 38 Å diameter pores because of a relatively strong pyrimethanil – MAA interaction in the pre-polymerization solution. This relaxation did not occur with EGDMA – MAA polymers, where the incorporation of DVB (55% purity) reduced the cross-linker ratio and introduced a weaker interaction in the polymer structure.

Incorporating less hydrophilic FMs such as ACM, and reducing the diluent ratio, made the imprinting effect of iprodione and pyrimethanil on pore size distribution more dominant. ACM provides both weak acid – base and dipole – dipole interactions for pyrimethanil and iprodione. Thus, a stronger interaction with iprodione can be observed, and compared with the weaker interaction with MAA. However, the ACM interaction with pyrimethanil was much weaker than the pyrimethanil interaction with MAA, as evidenced by the comparison of the pore size distribution of templated and non-templated polymers.

The EGDMA – ACM non-templated polymer shows two types of pore in the mesopore region, and in addition, two extra pore types appeared as a result of imprinting with iprodione and pyrimethanil. Larger pores for iprodione and smaller pores for pyrimethanil reveal the relative interaction strengths between the templates and ACM as the FM, with the smaller pores being indicative of the stronger interaction.
Focusing on the main target template in this study, iprodione, and taking into account the results of its imprinting effect using MAA, 4VPy, MAA – 4VPy, ACM and DVB FMs, it was concluded that \( \pi-\pi \) stacking was the major interaction that could be utilized to form an association in the pre-polymerization solution. Although such an association based on hydrophobic interactions is weak, it can align the evolving polymeric system to accommodate the template molecule in the energy wells in the intermediate stages of growing polymeric species, finally forming a highly ordered chemical structure. A selection of polar, heterocyclic aromatic systems VI, VC, TAC and TAIC were used as FMs to utilize the \( \pi-\pi \) stacking interaction to imprint iprodione in the EGDMA cross-linked matrix. However, this resulted in only slight changes in adsorption behaviour which were seen in the pore size distribution of the MIPs compared to the non-templated polymers.

In polymers #44 and #45, 4VPy and DVB were used as FMs to imprint iprodione in an EGDMA cross-linked matrix. Again the pore size distribution of the imprinted polymer showed some difference from the NIP. The pore size distribution of non-templated and templated polymers after heat treatment under vacuum at gradually increasing temperatures demonstrated the structural differences related to templating. The templated polymer pores showed greater thermal persistence before they deformed at 250 °C, while the non-templated polymer’s pores deformed and collapsed at the much lower temperature of 110 °C.

The TGA analysis of both EGDMA – DVB – 4VPy non-templated and templated polymers revealed another aspect of the molecular structure. The higher resistance of the templated polymer to decomposition at elevated temperatures suggests a more ordered structure due to \( \pi-\pi \) stacking interactions of the template and FM aromatic systems. The non-templated polymer carbonized at 500 °C while the templated polymer decomposed completely at 700 °C.

The TGA results for polymers composed of EGDMA – MAA and EGDMA – VC demonstrated the effect of imprinting using a FM that does not interact with the template, such as MAA – iprodione and the weak \( \pi-\pi \) interaction in VC – iprodione. The TGA analysis of EGDMA – MAA non-templated and iprodione templated polymers exhibit identical decomposition rates, while the EGDMA – VC MIP starts decomposing at a higher temperature than the NIP.

At low monomer concentrations, the diluent solvency strength, and the type of FM used in a MIP, determine the size of the polymer bead formed, and the type and porosity level of bulk MIP materials.

The evolving polymer passes through several growth stages during the formation of a bead or a porous system, as illustrated schematically in Figure 4.41. In radical polymerization, a radical fragment is formed during the initiation step using heat or UV irradiation, a seed particle forms initially from this fragment. The seed continues to grow to a bead till phase separation occurs, which is dependent upon the solvent strength of the diluent. The beads then start fusing to form larger beads which eventually precipitate from the solution. During this step, the polymer growth continues in spaces between the small beads fusing them together and reducing the porosity of the aggregated beads to a minimum. For EGDMA based polymers, the 38 Å pore diameter peak in the pore size distribution represents this family of pores. Larger pores are formed from the aggregation.
and fusion of these larger beads forming a second family of pores. The distribution of these pores is
dependent on the size distribution of the beads; one of the factors determining this distribution is
the template molecule.

Figure 4.41 Polymer bead formation steps

The presence of template molecules may affect the MIP porosity in two ways. When the template
interaction with the FMs is minimum or negligible, the template molecule can act as a diluent
species. In this case, the template molecule can be categorized as a good or bad solvent species, so,
the presence of the template will change the diluent ratio and the solvent strength compared to the
non-templated polymer. Alternatively, when the template forms a strong association with the FMs,
then it will change the behaviour of the FM and consequently change the polymerization kinetics
and mechanism so as to accommodate the template energy load. The diluent solvency of the
template species will determine the interaction strength between the template and the FMs. The
solvency of the species involved in a MIP system is the major factor to be optimized to design and
construct a selective adsorption site.

Considering the small ratio of the template species to the diluent, the solvency effect usually does
not have a significant influence on the porosity of the MIP system. The results of this present study
demonstrate that the strength of the association between template and FM is a major determinant
in modifying the pore size distribution, and causing porosity differences between the NIP and MIP.

Based on its adsorption properties, the EGDMA – DVB – 4VPy polymer was chosen for further
studies of iprodione adsorption and its application as a sensing film in the later stages of this
project.

4.7 References

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4. Toth, B.; Laszlo, K.; Horvai, G., Chromatographic behavior of silica-polymer composite
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5 Iprodion uptake studies from solution

5.1 Introduction

The present chapter describes the effectiveness of imprinting iprodion in MIP materials by the study of template rebinding isotherms.

Understanding adsorption phenomena in solid – liquid systems is a key element in analysing a gravimetric-based sensing event. Adsorbent swelling, structural deformation occurring during the adsorption process, the nature and energy of adsorbate – adsorbent interactions, heterogeneity of the adsorbent surface, adsorbate solubility and concentration, and the adsorbate – adsorbate interactions on the adsorbent surface, are all important parameters that affect the rebinding signal in such studies.

The condition for equilibrium between bulk and surface regions of an adsorbent is constancy of the chemical potential of each species on passing from locations adjacent to the solid surface into the bulk liquid phase. There are two boundaries to be considered when analysing the adsorption of a species from the liquid phase onto/into the solid phase; these boundaries are defined by the thickness of the “surface phase” which permits transfer between the liquid and solid phases in the adsorption process. One model considers the bulk solid adsorbent as an impenetrable material for the adsorbate species, which reduces the adsorption process to a surface phenomenon, and the “surface phase” to an interface between the liquid and adsorbent solid phases. The other model takes into account a thick surface phase and a high level of penetrability for the adsorbate species, which makes diffusion more prominent in the sorption process.

The other two important aspects of adsorption/sorption studies are an understanding of the mechanism and kinetics of the process. Solvent molecules, however, cannot be considered as inert species in a real system, since they, too, interact differently with adsorbent and adsorbate species. The presence of several adsorbate species in the liquid phase competing for adsorption on the surface, or sorbed by the bulk sorbent, complicates the data interpretation. The definition of surface excess of the adsorbate emphasizes the competitive nature of sorption phenomena of the solute – solvent system. Adsorbent surface solvation and bulk swelling are also major parameters that need to be considered in understanding the adsorption mechanism. These parameters become
more important when dealing with polymeric sorbents. The molecular structure of amorphous polymeric sorbents is more flexible than crystalline, or other rigid inorganic sorbents such as zeolites and graphite, thus allowing swelling and structural deformation during the sorption process.

5.2 Molecular modelling of iprodione

Analysing the inter- and intra-molecular interactions available to iprodione (1-isopropylcarbamoyl-3-(3,5-dichlorophenyl)hydantoin) - see Figure 5.1 - is essential to understanding its solubility and the possibility of FM – iprodione association in the pre-polymerization solution. The iprodione molecule consists of (1) a dichlorobenzene ring attached to (2) a five-membered imidazole ring with two carbonyl groups, and (3) a peptide linkage attached to (4) an isopropyl group, so that the electronic structure of the iprodione molecule contains 18 conjugated π-electrons forming an aromatic system extending over the entire molecule's structure. The aromatic system reduces the polarity of the molecule by redistributing the charge over the molecule. The internal hydrogen bond between the carbonyl group on the hydantoin ring and the N-H hydrogen of the carbamoyl functionality is an additional factor reducing the hydrophilic properties of iprodione.

Figure 5.1 The key functionalities of the iprodione molecule including the internal hydrogen-bond and the segments considered when conducting the conformational search.

Having a realistic model of the iprodione molecule based on a high level of modelling theory provides a better understanding of the properties and potential intermolecular interactions of the molecule. The first step in producing a molecular model is to generate the different conformations possible for the molecule and then optimize them using a suitable force field. The main torsion angles to be considered are those that change the orientation of the molecule with respect to the four entities shown in Figure 5.1. Different conformations were obtained by changing the torsion angles (dihedral angles) between these entities.

A complete conformational search was carried out using MacroModel, and each conformation was optimised by performing molecular mechanics calculations using the Merck Molecular Force-Field (MMFF) approach with the numbering scheme presented in Figure 5.2. The Monte Carlo Multiple Minimum (MCMM) algorithm was used, which efficiently searches the whole potential energy surface (PES), identifying low-lying minima. The total number of structures processed was 1000. Six unique conformations were found, of which 5 structures (A to E) minimized with good convergence, as described in Table 5.1.
Figure 5.2 The numbering used for the atoms in the iprodione molecule in the molecular simulation.

Table 5.1 Key dihedral angles of the different conformers of iprodione and their relative energies in kJ/mol at the B3LYP/6-31+G*/B3LYP/6-31+G* level of theory.

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<thead>
<tr>
<th>Torsion</th>
<th>Dihedral angles</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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</table>

The five geometries obtained by the molecular mechanics search were re-optimised by performing high level **ab initio** calculations using the Gaussian 03 program. Density Functional Theory (DFT) calculations produced the most accurate fit to the experimental data. Hence hybrid DFT functionals, specifically B3-LYP, were used in this instance. Geometries were optimised using the 6-31G basis set and adding polarization and diffuse functions. All the conformers were found to be true minima on the potential energy surface.

Partial atomic charges were derived using two methods and the results are summarized in Table 5.2. The first method was Mulliken charges, in which the electrons from each orbital are distributed between the parent atoms, and the second method, electrostatic fitting, determines the collection of charges that best reproduces the external field profile of the molecule. There is often a significant discrepancy between the two methods that estimate the partial atomic charges. The electrostatic method is more consistent with force field calculations. Electrostatic fitting uses the Atomic Polar Tensors (APT) which are calculated and analyzed with respect to the charge, charge flux, and atomic and homopolar dipole fluxes.
Table 5.2 Atomic charges on the atoms of the different conformers of iprodione calculated at the B3LYP/6-31+G*//B3LYP/6-31+G* level of theory using the numbering scheme in Figure 5.2.

<table>
<thead>
<tr>
<th>Method</th>
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<td>-0.092</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>0.617</td>
<td>0.602</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>-0.463</td>
<td>-0.462</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>0.489</td>
<td>0.478</td>
</tr>
<tr>
<td><strong>O</strong></td>
<td>-0.497</td>
<td>-0.498</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>-0.665</td>
<td>-0.667</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.213</td>
<td>0.213</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.220</td>
<td>0.220</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.215</td>
<td>0.215</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>-0.034</td>
<td>-0.038</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.225</td>
<td>0.224</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>-0.565</td>
<td>-0.560</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.225</td>
<td>0.225</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.206</td>
<td>0.206</td>
</tr>
<tr>
<td><strong>H</strong></td>
<td>0.211</td>
<td>0.210</td>
</tr>
</tbody>
</table>

115
The charge and orientation of H$_2$ (N–H group of carbamil) and O$_3$ (carbonyl group on the hydantoin ring) suggested the formation of an internal hydrogen bond in conformers A, B, C and D which reduces the hydrophobic properties of iprodione to a minimum. The absence of this orientation in conformer E increases its ground state energy dramatically, by 38.56 kJ/mol, rendering it less stable than the other four conformers. The very low solubility of iprodione in water (a value of 12.2 ppm at 20 °C is listed in Table 5.4) is one of the consequences of its hydrophobic character. As the comparison between templated and non-templated polymer morphologies demonstrated in Chapter 4, iprodione interacts weakly with hydrophilic FMs such as MAA and ACM, but does demonstrate better imprinting in polymers with aromatic FMs such as VI and VC, suggesting that π-π stacking is a predominant interaction between iprodione and the FM.

The small difference in energy between conformers A, B, and C shows that they can interchange among each other depending on the system temperature. Any one of these conformers can be preferentially stabilised in solution depending on the interaction energy between iprodione and other species in the solution. This similarity in energy of the different conformers of the iprodione molecule, and the effect of solvation on this energy, make imprinting the iprodione molecule a process that is vulnerable to several factors which are difficult to control during polymerization. For example, the conformational interchange of the iprodione molecule can leave unspecific imprinted sites in the MIP molecular structure. These modelling results predict reduced selectivity for iprodione in the uptake process due to a low affinity and capacity of the MIP compared to the non-imprinted polymer (NIP).

The presence of other compounds in the adsorption medium, or the pre-polymerization solution, can stabilize other conformers of the iprodione molecule not in the family studied in this section. This further reduces the imprinting efficiency.

5.3 Adsorption kinetics

Rough kinetic experiments of iprodione adsorption on polymers were performed to estimate the equilibrium time to produce the adsorption isotherms. The kinetics of iprodione uptake by the polymers prepared in this study varied slightly due to variations in the iprodione interaction with the polymer surface. Hydrophobic and π-π stacking interactions accelerated the iprodione partition equilibrium into the polymer as demonstrated by its ready uptake from 20% THF in water. Under these conditions iprodione uptake by the DVB – 4VPy non-templated and templated polymers (#47 and #51) reached equilibrium after 40 – 50 min, as shown in Figure 5.3 (A), but a slower adsorption rate was measured in EGDMA – DVB – 4VPy non-templated and templated polymers (#44 and #45), where adsorption only reached equilibrium after 50 – 60 min, as shown in Figure 5.3 (B). This was a consequence of using the more polar cross-linker (EGDMA) and a smaller ratio of hydrophobic monomers. Reducing the hydrophobic monomer ratio even further in the EGDMA – 4VPy non-templated and templated polymers (#20 and #23) increased the equilibrium time to 60 – 70 min, as shown in Figure 5.3 (C). These observations confirm that the polymer functionality determines the rate of adsorption of iprodione, since for these three polymers, decreasing the ratio of hydrophobic monomer in the polymer composition halved the migration rate of the hydrophobic
Iprodione molecule from the aqueous phase to the adsorbent polymer solid phase, see Table 5.3. The effect of the solvent mixture of the adsorption media on the kinetics of iprodione uptake by polymer film substrates is described further in Chapter 6.

Figure 5.3 Iprodione adsorption kinetics on DVB – 4VPy (#47 and #51) non-templated and imprinted polymer, EGDMA – DVB – 4VPy (#44 and #45) non-templated and imprinted, and EGDMA – 4VPy (#20 and #23) non-templated and imprinted polymers from 20% THF/water medium.

Table 5.3 Iprodione equilibration time with adsorbent non-imprinted polymers.

<table>
<thead>
<tr>
<th>Adsorbent polymer</th>
<th>Equilibrium time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DVB – 4VPy</td>
<td>40-50</td>
</tr>
<tr>
<td>EGDMA – DVB – 4VPy</td>
<td>50-60</td>
</tr>
<tr>
<td>EGDMA – 4VPy</td>
<td>60-70</td>
</tr>
</tbody>
</table>

5.4 Adsorption media

The results discussed in this section investigate the major role solvency plays in iprodione uptake by the adsorbent polymer.
A thermodynamic analysis of the solvent effect on solute–surface interactions can be gained by analysing the potential energies involved in the system. The thermodynamic state of a solution is determined by solute–solute, solute–solvent and solvent–solvent interaction potential energies. Thus, while two molecules may attract each other in free space, they may repel each other in a medium, for as two molecules move towards each other the work that must be done to displace other species may exceed that gained by the association of the two species, and in this case it becomes energetically more favourable for the two molecules to remain separated.

A similar explanation can be used when a surface is included in the system. The interaction energies between the solute–surface, solute–solvent and solvent–surface will determine the extent of solute–surface association, or adsorption.

\[
\Delta E_{\text{Solution}} = \sum_i \left( E_i^\Theta - E_i^{\text{Solvated}} \right) = q + w \tag{5-1}
\]

where \( \Delta E_{\text{Solution}} \) is the solution solvation energy and \( E_i^\Theta \) is the energy of formation of \( i \) in its pure state in standard conditions, \( E_i^{\text{Solvated}} \) is the energy of formation of \( i \) in its solvated state, \( q \) is the heat of solvation and \( w \) is the work done in the solvation process.

Most of the thermodynamic information regarding solvation was formulated using excess functions. For a thermodynamic property \( X \), the excess can be expressed as;

\[
X^{\text{ex}} = X^{\text{real}} - X^{\text{ideal}} \tag{5-2}
\]

For example the excess Gibbs energy for a non-electrolyte solution is;

\[
G^{\text{ex}} = G^{\text{real}} - G^{\text{ideal}} \tag{5-3}
\]

\[
G^{\text{ideal}} = G^{\text{ideal}}_{\text{Solvent}} + G^{\text{ideal}}_{\text{Solute}} = G^{\text{ideal}}_{\text{Solvent}} + m_{\text{Solute}} \mu_{\text{Solute}}^{\text{ideal}} \tag{5-4}
\]

where \( G^{\text{ideal}}_{\text{Solvent}} = G_i^\Theta - RT \ln m_{\text{Solute}} \), \( \mu_{\text{Solute}}^{\text{ideal}} = \mu_i^\Theta + RT \ln m_{\text{Solute}} \), \( m_{\text{Solute}} \) is the solute molality and \( \mu \) is the chemical potential.

Using a semi-empirical approach, and taking into consideration the interaction between solute species, the excess Gibbs energy is represented as a power series in solute(s) molality;

\[
G^{\text{ex}} = \sum_{i,j} g_{ij} m_i m_j + \sum_{i,j,k} g_{ijk} m_i m_j m_k + ... \tag{5-5}
\]

where the \( g \) terms represent, at least in a notational sense, the interactions between subscripted solvated species \( ^{10-12} \). The first term of this equation represents the binary interactions and the second term represents the ternary interactions between species in the solution.

In an adsorption process, the excess of component \( i \) adsorbed from solution is given by the Gibbs adsorption equation;

\[
\Gamma_i = -\frac{1}{RT} \frac{dy}{d \ln a_i} \tag{5-6}
\]
where $\Gamma_i$ is the surface excess, $\gamma$ is the interfacial tension, and $a_i$ is the activity of component $i$. Notably there is no direct method to measure $\gamma$.

In the case of water as solvent, if the non-polar solute is relatively small, it is possible for water molecules to pack around it without giving up any of their hydrogen bonding sites. Thus, the size and shape of the non-polar molecules are critical in determining the water structure adopted around them. Theoretical and experimental studies indicate that the orientation of water molecules in the presence of non-polar solutes is entropically very unfavourable, since their presence disrupts the existing water structure in the pure state and imposes a new, more ordered arrangement, on the water molecules around the solute in solution. The entropic contribution to the Gibbs free energy of the solution, $\Delta G_{\text{Solvation}}$, for hydrocarbons such as benzene, is close to 100%.

$$\Delta G_{\text{Solvation}} = \Delta H_{\text{Solvation}} - T\Delta S_{\text{Solvation}}$$

The hydrogen bonding network in water can be weakened and reorganized using a mixture of water and an organic solvent. For example, adding THF to water as an adsorption medium introduces an entropy advantage, giving rise to a favourable environment for a non-polar molecule such as iprodione in the bulk solution.

The solubility of iprodione varies greatly in various solvents, as shown in Table 5.4, and the very low aqueous solubility of iprodione requires a combination of water and organic solvents to optimize the medium for adsorption studies. This is not an ideal situation, as the increased solubility in the solvent medium partially negates adsorbent binding.

**Table 5.4** Solubility of iprodione in organic solvents (g/L at 20°C)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.0122</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.59</td>
</tr>
<tr>
<td>Octanol</td>
<td>10.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>147</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>225</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>168</td>
</tr>
<tr>
<td>Acetone</td>
<td>342</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>450</td>
</tr>
</tbody>
</table>

Indeed, there is negligible iprodione uptake by EGDMA – DVB – 4VPy polymers (#44 and #45) in a 100% MeCN medium. This demonstrates that iprodione molecules prefer to remain solvated with a higher degree of freedom (negative $\Delta S^{\text{Ads}}$) in solution and the solvent – iprodione interaction energy is similar to the surface, aromatic site – iprodione interaction, so that ($\Delta H^{\text{Ads}} = 0$).

$$\Delta G^{\text{Ads}} = \Delta H^{\text{Ads}} - T\Delta S^{\text{Ads}}$$

Generally there is an inverse relationship between the extent of adsorption of a species and its solubility in the adsorption medium, i.e., the less soluble the material in the adsorption medium, the more strongly will it tend to be adsorbed by the polymer. Hansen and Craig found that the
adsorption isotherms of a series of homologous fatty acids and alcohols are superimposable on each other if plotted as the adsorption values $B$ (g adsorbate/g adsorbent) vs. the reduced concentration $C_{eq}/C_{solubility}$, where $C_{eq}$ is the adsorbate concentration at equilibrium and $C_{solubility}$ is the solubility of the adsorbate in the adsorption medium. By analogy, the solubility can be varied by changing the solvent for the same adsorbate, and the adsorption isotherms of a particular adsorbate in different solvents should superimpose when the reduced concentration is considered.

Since the MIPs studied in this work were to be used to bind iprodione in wine and grape juice media, a mixture of water – organic solvent was used for iprodione adsorption studies. Different ratios of THF in water from 10 – 35% (v/v) were used as adsorption media for iprodione on EGDMA – DVB – 4VPy polymers (#44 and #45). For each experiment 10, 20 or 30 mg of polymer was equilibrated with 10 mL of 100 ppm iprodione solution to obtain a three point adsorption isotherm; 50 ppm concentration was used in 10% THF/water medium due to the low aqueous solubility of iprodione. The slopes of the straight lines connecting the three point adsorption isotherms were equated to the Affinity of the polymers in the different media used. The results show a high dependency of the iprodione adsorption on the media used in adsorption process, as shown in Figure 5.4. This trend follows the solubility of iprodione in these media.

![Graph showing iprodione adsorption on NIP and MIP EGDMA – DVB – 4VPy (#44 and #45) polymers.](image)

**Figure 5.4** Iprodione adsorption on NIP and MIP EGDMA – DVB – 4VPy (#44 and #45) polymers at room temperature in various ratios of THF/water media.

The inclusion of EtOH (10% v/v) in the aqueous THF media while changing the THF ratio between 2% and 30% v/v produced similar results to those seen in Figure 5.4. The iprodione uptake was
reduced significantly at high ratios of organic solvent. Similar results were obtained by maintaining the THF ratio constant at 20% (v/v), and varying the EtOH ratio between 5% and 30% (v/v), clearly demonstrating that an increased organic fraction in the adsorption media decreases iprodione uptake by the polymers, as shown in Figure 5.5. The figures also show there is little difference between the discriminating abilities of the NIP and MIP polymers for iprodione when dissolved in such media. Clearly, the solubility effects dominate any subtle differences between the NIP and MIP binding affinities which may be present due to structural modification via templating in the MIP. In each experiment, 10mg of polymer sample was equilibrated with 10 mL of 20 ppm iprodione solution at room temperature for 2 hours and then the residual iprodione was quantified using HPLC.

Figure 5.6 illustrates schematically how an increased solubility of iprodione in organic rich aqueous solvents can occur. Organic species provide solvating molecules to accommodate the hydrophobic iprodione molecule in the mainly aqueous medium. This higher solubility reduces iprodione uptake by the adsorbent polymers when the adsorption media contain high ratios of organic solvents.

Figure 5.5 The iprodione uptake of NIP and MIP EGDMA – DVB – 4VPy (#44 and #45) in: (A) 10% (v/v) EtOH and various ratios of THF in water media, and (B) 20% (v/v) THF and various ratios of EtOH in water.
Figure 5.6 A schematic depiction of the mechanism by which iprodione solvency in water increases as a result of including an organic solvent such as THF in the solvent composition.

The thermodynamics of the adsorption of iprodione on polymers may be discussed in terms of its solubility in the adsorption medium. The iprodione uptake levels observed in Figure 5.4 and Figure 5.5 demonstrate a strong relationship between the adsorbate chemical potential

$$\mu_{\text{Solute}}^{\text{ideal}} = \mu_{\text{Solute}}^{\text{pure}} + RT \ln m_{\text{Solute}}$$

and the polarity of the medium used in the adsorption process.

The extent of the equilibrium between a solute ($A_{\text{Sol}}$) in solution, and its adsorbed form ($A_{\text{Ads}}$) on the surface, is determined by the chemical potential of the solutes when solvated at the equilibrium state.

$$A_{\text{Sol}} \xrightarrow{k_e} A_{\text{Ads}}$$

When the free energy changes between the solute/adsorbate species in the pure state $\mu_{\text{Solute}}^{\text{pure}}$, solvated state $\mu_{\text{Solute}}^{\text{Sol}}$, and adsorbed states $\mu_{\text{Solute}}^{\text{Ads}}$ are considered, the adsorption free energy $\Delta G_{\text{Ads}}$ can be divided into two parts; (i) the solvation free energy, $\Delta G_{\text{Sol}}$, and (ii) the free energy of changing the solvated species to an adsorbed species $\Delta G_{\text{Sol} \rightarrow \text{Ads}}$ as shown diagrammatically in Figure 5.7. Assuming that the solvent molecules do not compete with adsorbate species, $A$, for the adsorption sites, and that there is no interaction with the adsorbate, it may be concluded that the free energy difference between the pure adsorbate $A$ in the gaseous state and the adsorbed state of $A$, $\Delta G_{\text{Ads}}$, is constant, regardless of the adsorption media. Different adsorption media vary in solvency strength toward the adsorbate species, and this causes the solvation free energy ($\Delta G_{\text{Sol}}$) to vary and so affect $\Delta G_{\text{Sol} \rightarrow \text{Ads}}$, which controls the migration of the adsorbate species from the solution to the adsorbent surface. If $\Delta G_{\text{Sol}}$ is equal to $\Delta G_{\text{Ads}}$ there will be no excess adsorption by the adsorbent, and when $\Delta G_{\text{Sol}}$ is larger than the $\Delta G_{\text{Ads}}$ excess adsorption is negative, i.e. there will be no adsorption of solute.
The more soluble the adsorbate, the smaller $\Delta G^{Sol \rightarrow Ads}$ is, and so the lower the adsorption. This is consistent with the result that was observed, since changing the solubility of iprodione in water/organic media by varying the organic solvent ratio resulted in different levels of iprodione uptake by the adsorbent. Higher organic solvent ratios reduced the iprodione adsorption levels by increasing the solvation free energy with organic solvent molecules – iprodione in solution, as shown schematically in Figure 5.6.

In real systems, the adsorption medium is not an inert species in the adsorption process, as it has a mutual effect on both adsorbate and adsorbent by changing the adsorbate chemical potential in the bulk liquid phase, as well as changing the adsorbent nature by solvation or even swelling. The changes of adsorbent surface chemical potential throughout the adsorption process can be schematically depicted and analysed similar to that for the adsorbate species A in Figure 5.7, demonstrating the adsorbent contribution to $\Delta G^{Ads}$. The solvation effect will cause a deviation from the ideal mechanisms given above, and is depicted schematically in Figure 5.8.

Similar mechanisms apply to the FM – template association in the pre-polymerization solution during MIP synthesis. Solvents, cross-linkers and FMs must be selected to favour association before “freezing” the structure during the polymerization process. Of course, the aim of imprinting is to promote conditions that stabilize the strongest template association possible with the FMs to produce stable, selective and energetically favourable adsorption sites. In subsequent rebinding
studies, it is necessary to have solvency levels which favour the migration of adsorbate from the solution to the adsorbent material, preferably to a specific adsorption site in the case of imprinted polymers.

### 5.5 Adsorption Isothersms

Various polymers with different surface functionalities, synthesized from different monomer compositions and ratios, were examined to determine their effect on iprodione the adsorption affinity and capacity. The various FMs utilized to prepare non-imprinted, and iprodione imprinted, polymers are listed in Table 5.5. The physicochemical parameters and thermodynamic aspects of the adsorption process can be obtained from adsorption isotherm experiments. The imprinting effect on the polymer was investigated in comparative studies with non-templated polymers using iprodione adsorption isotherms and Scatchard plots.

<table>
<thead>
<tr>
<th>FM</th>
<th>Cross-linker</th>
<th>Possible interaction with the FM</th>
<th>Polymer designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAA</td>
<td>EGDMA</td>
<td>Hydrogen bonding</td>
<td>#19, #22</td>
</tr>
<tr>
<td>ACM</td>
<td>EGDMA</td>
<td>Hydrogen bonding</td>
<td>#25, #26, #27</td>
</tr>
<tr>
<td>4VPy</td>
<td>EGDMA, DVB</td>
<td>π-π stacking and dipole-dipole interaction</td>
<td>#20, #23, #44, #45, #47, #48, #49, #51, #52, #53</td>
</tr>
<tr>
<td>VI</td>
<td>EGDMA</td>
<td>π-π stacking and dipole-dipole interaction</td>
<td>#34, #35</td>
</tr>
<tr>
<td>VC</td>
<td>EGDMA</td>
<td>π-π stacking and dipole-dipole interaction</td>
<td>#36, #37</td>
</tr>
<tr>
<td>TAC</td>
<td>EGDMA</td>
<td>π-π stacking and dipole-dipole interaction</td>
<td>#38, #39</td>
</tr>
<tr>
<td>TAIC</td>
<td>EGDMA</td>
<td>π-π stacking and dipole-dipole interaction</td>
<td>#40, #41</td>
</tr>
<tr>
<td>DVB</td>
<td>EGDMA</td>
<td>π-π stacking</td>
<td>#42, #43</td>
</tr>
</tbody>
</table>
For homogeneous adsorbents, the Langmuir adsorption isotherm can be applied to the adsorption process;

\[ B = \frac{NKC_{eq}}{1 + KC_{eq}} \]  

where \( B \) is the adsorption, \( N \) is the total number of adsorption sites, \( C_{eq} \) is the equilibrium concentration of the adsorbate and \( K \) is a constant dependent on temperature. This equation can be rearranged so the graph of \( B \) against \( \frac{B}{C_{eq}} \) gives a straight line, which is known as a Scatchard plot \(^{18,19}\), and can be used to evaluate the adsorption isotherm deviation from the Langmuir isotherm;

\[ \frac{B}{C_{eq}} = KN - KB \]

Any curvature in the Scatchard plot shows that the adsorption sites are either not identical, or the adsorbed species are not independent of each other, that is, a co-operative effect may be occurring. This can arise when the initial uptake of adsorbed molecules affects the subsequent uptake of adsorbate molecules, for example, when adsorbed molecules affect the surface polarity, or when the distance between adsorbate molecules on the surface is not sufficient for their interaction to be ignored.

The choice of adsorption medium (solvent) for the present work was based on the results of binding medium studies discussed in the previous section. These results demonstrated there was no significant iprodione uptake by the polymers in pure MeCN and THF media, but by including water in the adsorption medium iprodione migration from the bulk solution to the adsorbent polymer was enhanced. Thus, in this chapter, adsorption studies were performed in 20% (v/v) THF in water.

In a comparative study of the adsorbent polymers, particular parameters must be considered. In this case, the active surface area of the polymer needs to be known, since the functionality of polymers cannot be compared based on their adsorption levels without considering similar adsorption surface areas. This factor becomes more complicated when it is recognized that the active surface area of an adsorbent is measured using a N\(_2\) adsorption isotherm but the adsorbent functionality toward a specific adsorbent is measured in the liquid phase. Nevertheless, logical estimates of differences can be derived from a comparison of the iprodione adsorption experiments.

The porosity and accessibility of pores in the adsorption medium towards the adsorbate species is another contributor to iprodione uptake. Pores with a bottle-neck shape may be accessible to the nitrogen molecule probe, but may not allow a medium size molecule such as iprodione to pass.

The effectiveness of the contact between the adsorbent and the solution, “wetting”, is another critical factor in the adsorption process. Such a process is important in forming the surface phase which allows adsorbate molecules to migrate from the bulk solution into the adsorbent matrix.
Wetting is dependant on the hydrophilic/hydrophobic properties of both the liquid and solid phases in contact with each other.

### 5.5.1 EGDMA – MAA and 4VPy polymers

Non-templated and iprodione templated polymers containing the EGDMA cross-linker were prepared to compare the iprodione adsorption affinity and capacity of the functionalized polymers. The iprodione adsorption isotherms of these two polymers are shown in Figure 5.9 (A), where it can be seen that no imprinting effect for iprodione is present, as both isotherms are superimposable. The non-templated and templated polymers show the same behaviour, and the Scatchard plot demonstrated identical trends, as shown in Figure 5.9 (B), verifying that the presence of the template in the polymerization solution did not influence the distribution or character of the adsorption sites.

![Figure 5.9](image)

**Figure 5.9** (A) Iprodione adsorption isotherms, and, (B) Scatchard plots on the non-templated and templated EGDMA polymers (#17, #18) in 20% THF/water at room temperature.

MAA and 4VPy FMs were used to imprint iprodione in an EGDMA cross-linked matrix. Identical linear adsorption isotherms for both non-templated and templated MAA – EGDMA polymers (#19 and #22) showed there was no imprinting effect; see Figure 5.10 (A). This result suggests that there was no interaction between the MAA FM and the template, iprodione, in the pre-polymerization solution and the polymer structure.

The 4VPy FM in the 4VPy – EGDMA polymers (#20 and #23) demonstrated a slightly stronger interaction with iprodione, as evidenced by the increasing adsorption levels compared to the MAA – EGDMA polymers (#19 and #22). Comparing the non-templated and imprinted 4VPy – EGDMA polymers in Figure 5.10 (B) only reveals a low level of imprinting.
Figure 5.10  Iprodione adsorption isotherms on, (A) non-templated and templated MAA – EGDMA (#19 and #22) and, (B) non-templated and templated 4VPy – EGDMA (#20 and #23) in 20% THF/water medium at room temperature.

Different intermolecular interactions are present when both MAA and 4VPy are incorporated as FMs into the EGDMA cross-linker matrix in the pre-polymerization solution. The MAA – 4VPy acid – base equilibrium produces charged species, which exert different interaction potentials on the template. The iprodione adsorption isotherms of non-templated and templated EGDMA – MAA – 4VPy polymers (#21 and #24) shown in Figure 5.11, exhibit very similar trends to the EGDMA non-templated and templated polymers (#17 and #18) adsorption isotherms of Figure 5.9 (A). This indicates that the FMs interact more strongly with each other than the template molecule, although slightly higher adsorption values can be assigned to the iprodione π-π stacking interaction with the neutral 4VPy and cationic 4VPyH⁺ species.

Figure 5.11 Iprodione adsorption isotherms on non-templated and templated EGDMA – MAA – 4VPy polymers (#21 and #24) in 20% THF/water medium at room temperature.
5.5.2 EGDMA – VI polymers

The hydantoin ring in the iprodione molecular structure is geometrically similar to the imidazole ring of the VI FM. The electron density distribution in the imidazole ring of VI is concentrated on the ring due to the presence of electronegative atoms in the heterocyclic aromatic system. On the other hand, in the hydantoin ring the charge is concentrated on the more electronegative oxygen atoms, and positive charges are distributed on the heterocyclic ring. \textit{AB initio} simulation using the minimal STO-3G set of core Hamiltonians for the interaction between iprodione and VI molecules showed \(\pi-\pi\) stacking of the five membered heterocyclic rings. The complementing charges on the rings are tabulated in Table 5.6. The complementary electron density distribution and similar structures of the hydantoin and imidazole rings makes VI a potentially suitable FM for designing a MIP for iprodione.

![Simulation showing \(\pi-\pi\) stacking interaction between VI and iprodione molecules.](image)

\textbf{Figure 5.12} The simulation showing the \(\pi-\pi\) stacking interaction between VI and iprodione molecules.

\textbf{Table 5.6} Mulliken atomic charges of the five membered heterocyclic rings of VI and iprodione; oppositely charged atoms interact with each other in the stacked ring conformation.

<table>
<thead>
<tr>
<th></th>
<th>Mulliken atomic charges</th>
<th></th>
<th>Mulliken atomic charges</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁</td>
<td>0.122</td>
<td>N₆</td>
<td>-0.305</td>
</tr>
<tr>
<td>N₂</td>
<td>-0.247</td>
<td>C₇</td>
<td>0.413</td>
</tr>
<tr>
<td>C₃</td>
<td>0.015</td>
<td>N₈</td>
<td>-0.314</td>
</tr>
<tr>
<td>C₄</td>
<td>-0.024</td>
<td>C₉</td>
<td>-0.027</td>
</tr>
<tr>
<td>N₅</td>
<td>-0.268</td>
<td>C₁₀</td>
<td>0.319</td>
</tr>
<tr>
<td>O₁₁</td>
<td></td>
<td>O₁₂</td>
<td>-0.290</td>
</tr>
</tbody>
</table>

However, the adsorption isotherms of the non-templated and templated VI functionalized polymers (#34, #35) showed little difference, as can be seen in Figure 5.13 (A). The Scatchard plots of these adsorption isotherms did reveal two different types of binding sites for the templated polymer, while the non-templated polymer gave a straight line over the entire iprodione concentration range. This result suggests there was some favourable association between the template and reactants in the pre-polymerization solution, but it was not of sufficient strength to create robust binding sites.
Figure 5.13 (A) The adsorption isotherms, and (B) Scatchard plots, for iprodione on the non-templated and templated EGDMA – VI polymers (#34, #35) from 20% THF/water at room temperature.

5.5.3 EGDMA – DVB polymers

The non-templated and templated EGDMA – DVB non-templated and templated polymers (#42 and #43) were prepared at a low diluent ratio, thus giving low active surface areas, of only 35 and 94 m$^2$/g respectively. The iprodione uptake of these polymers was significantly lower than the poly EGDMA polymers (#17 and #18). This is due to the much lower active surface area of these polymers, rather than a lower activity, as it was found that the DVB functionalized polymers have a higher affinity toward iprodione, due to the $\pi-\pi$ interaction between the aromatic systems of the adsorbent polymer and the adsorbate molecules.

The difference in adsorption between the non-templated (#42) and templated (#43) polymers was due to their active surface area difference, as seen in Figure 5.14. Despite the active surface area measured using $N_2$ adsorption isotherm of the templated polymer being about three times the non-templated polymer, the adsorption of iprodione from solution indicates the active surface area of the templated polymer is only about double that of the non-templated sample. This result suggests that much of the surface area measured using nitrogen was not accessible to iprodione in solution.

The Scatchard plot shows a linear trend for both polymers, indicating that the adsorption sites have uniform potential energies in the concentration range studied. The difference in the slopes of the Scatchard plots of the non-templated and templated polymers shows that the adsorption sites of the templated polymer possess a higher potential energy to rebind iprodione molecules, which is believed to arise from their alignment with the template molecule during the polymerization process.
**Figure 5.14** (A) The adsorption isotherms, and (B) Scatchard plots, for iprodione on the non-templated and templated EGDMA – DVB polymers (#42 and #43) in 20% THF/water at room temperature.

### 5.5.4 EGDMA – VC

When VC was used as FM, a higher affinity toward iprodione adsorption was observed, as seen in Figure 5.15. This confirmed the presence of a π-π stacking interaction between iprodione and VC. In fact, the presence of VC in the polymer structure increased the affinity and capacity of both the templated and non-templated polymers by the same amount compared with poly EGDMA (#17 and #18) (compare with Figure 5.9).

The best linear fit for the adsorption isotherms of polymers #36 and #37 above, demonstrates that they did not reach their binding capacity limit over the iprodione concentration range studied, and that binding saturation levels were not obtained.

**Figure 5.15** (A) The adsorption isotherms, and (B) Scatchard plots, for iprodione on the non-templated and templated EGDMA – VC polymers (#36 and #37) in 20% THF/water at room temperature.
The Scatchard plots of the adsorption isotherms show that although these polymers have a high affinity for iprodione, it varies with $C_{eq}$. The polymer network has a lower affinity at low $C_{eq}$, and a higher affinity at high $C_{eq}$, suggesting that increasing number of adsorption sites become exposed after adsorption of the initial iprodione molecules. This behaviour may be indicating that the initial potential energy barrier for iprodione association with the polymer surface arises from the need for the hydrophobic iprodione molecules to displace the solvent molecules (water and THF) from the surface and take their place. Energetically, the bond formed between the adsorbate and the adsorbent must provide sufficient energy to break the bond between the solvent molecules and the polymer for iprodione adsorption to occur.

Adsorption from a 25% THF/water medium shown in Figure 5.16 (A) provided more details about the interaction between the adsorbate, iprodione, and the polymer structure. Reducing the influence of the solvent on the adsorbate caused the adsorbate molecules to become more selective towards the adsorption sites on the polymers. The Scatchard plot of the adsorption isotherms in 25% THF/water showed more energetically uniform adsorption sites in the non-templated polymer than in the templated equivalent, as shown in Figure 5.16 (B). The adsorption sites with the higher potential to interact with iprodione were saturated at lower concentrations, and then the sites with lower potential started to rebind any additional iprodione molecules.

Changing the organic solvent ratio (THF) in the adsorption medium varied the adsorption levels dramatically, as can be clearly seen in Figure 5.17. In each case, 10, 20 and 30 mg of polymer was equilibrated with 10 mL of 50 ppm iprodione solution to obtain a three point adsorption isotherm. This demonstrated that despite the high affinity of the polymer toward iprodione, the extent of adsorption was still significantly governed by the adsorbate solubility in the medium.
Figure 5.17 Iprodione adsorption on non-templated and templated EGDMA – VC polymers (#36 and #37) at room temperature in various ratios of THF/water media.

5.5.5 EGDMA – TAC and TAIC polymers

TAC and TAIC can both act as FMs by $\pi-\pi$ stacking interactions, aligning with other aromatic systems in the polymer structure, and as cross-linkers through the multiple vinyl groups in their chemical structure.

Figure 5.18 (A) The adsorption isotherms, and (B) Scatchard plots for iprodione adsorption on the non-templated and templated EGDMA – TAC polymers (#38 and #39) in 20% THF/water at room temperature.

The iprodione adsorption isotherms on TAC – EGDMA non-templated and templated polymers (#38 and #39) shown in Figure 5.18 (A) did not show a significant difference in the adsorption levels between the non-templated and templated polymers. The Scatchard plots of the adsorption isotherms revealed that, in contrast with the NIP, the adsorption sites of the templated polymer are
not uniform. Figure 5.18 (B) exhibits two branches in the Scatchard plot for the templated polymer, indicating there are two types of adsorption sites present with different interaction energies for adsorbate.

In contrast, the polymers functionalized using TAIC (#40 and #41) demonstrated a different type of behaviour, see Figure 5.19. The iprodione adsorption isotherms on the templated and non-templated EGDMA – TAIC polymers are more divergent when compared to the TAC polymers (#38 and #39), indicating a stronger interaction between iprodione and the imprinted TAIC functionalized polymer.

The active surface area analysis, discussed in Chapter 4, provided surface areas of 324 and 312 m²/g for the non-templated and templated polymers respectively. Templating of the EGDMA – TAIC polymer reduced the BET surface area, the micropore area, the average pore diameter, and micropore volume, but, despite this reduction, the templated polymer demonstrated a higher capacity and affinity for iprodione adsorption. Thus, stronger specific interactions can be assigned to the imprinted sites compared to the randomly distributed monomer matrix in the non-templated polymer. The Scatchard plots show similar trends for both polymers #40 and #41, as shown in Figure 5.19, these trends are similar to the trends observed for EGDMA – VC polymers (#36 and #37) and can be justified using the same reasoning, namely that the surface has a lower affinity at low $C_{eq}$, and a higher number of adsorption sites become available, due either to surface modification by the adsorbed iprodione or a high number of sites being exposed after the adsorption of the initial iprodione molecules, increasing the surface affinity at higher $C_{eq}$.

![Figure 5.19](image)

**Figure 5.19** (A) The adsorption isotherms, and (B) Scatchard plots for iprodione on the non-templated and templated EGDMA – TAIC polymers (#40 and #41) in 20% THF/water at room temperature.

### 5.5.6 EGDMA – DVB – 4VPy polymers

Functionalizing EGDMA using DVB (#44 and #45) showed a relatively strong imprinting effect for iprodione as template. This is not unexpected, since adding 4VPy FM to this composition can provide the adsorbent polymer with a heterocyclic aromatic functionality that is similar to the
iprodione functionality. The DVB monomer contributes to the strength of the chemical structure as a cross-linker alongside its hydrophobic functionality, and can also play the role of a FM toward iprodione by utilizing π-π stacking interaction forces.

Using a combination of EGDMA and DVB as cross-linkers provides a polymer matrix that could interact with both hydrophobic and hydrophilic adsorption media, i.e., the polymer could be “wet” by a variety of mixed solvent compositions. This creates a more dynamic surface phase (interphase region) for the adsorption process. A slightly larger separation between the iprodione adsorption isotherms of the non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45) in 20% THF/water medium compared to the previously studied polymers was observed as seen in Figure 5.20 (A) and Figure 5.21 (A).

Bearing in mind the heterogeneity of the adsorbent polymers #44 and #45 due to the hydrophilic and hydrophobic nature of the monomers utilized in their synthesis, the surface phase formed during the adsorption process can be modified by changing the adsorption medium. The effect of varying THF/water and THF/EtOH/water ratios on the adsorption levels was studied in the “Adsorption media”, Section 5.4, but it must be remembered that the adsorption medium has a dual effect, both by changing the adsorbate solvency, and the adsorbent solvation and swelling. The effect of the solvent medium on the adsorbent, and consequently on the surface phase, can alter the mechanism of adsorption by changing the chemical potentials involved in the process.

![Figure 5.20](image)

**Figure 5.20** (A) The adsorption isotherms, and (B) Scatchard plots for iprodione on the non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45) in 20% THF/water at room temperature.

The addition of 10% EtOH to the adsorption medium further reduced iprodione adsorption, due to the enhanced iprodione solvency, and simultaneously, caused a greater divergence between the isotherms of the non-templated and templated polymers (#44 and #45) as shown in Figure 5.21 (A).
Figure 5.21 (A) The adsorption isotherms, and (B) Scatchard plots for iprodione on the non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45) in 10% EtOH/20% THF/70% water at room temperature.

The Scatchard plot of the adsorption isotherms in the 20% THF/water medium demonstrated a very similar trend for the affinity of the adsorption sites for templated and non-templated polymers (#44 and #45). However, the difference between the adsorption sites on polymers #44 and #45 was shown more distinctly. Increasing the adsorbate solubility in the adsorption medium, compare Figure 5.20 (B) and Figure 5.21 (B), thereby allows the adsorbate molecules to select the more energetically favourable site to associate with in the imprinted polymer structure. The Scatchard plot of the adsorption isotherm for the templated polymer #45 showed two branches, Figure 5.21 (B), indicative of two types of adsorption site with different affinities. On the other hand, the non-templated polymer exhibited an energetically more homogenous structure for the iprodione adsorption isotherm over the range of iprodione concentrations studied.

5.5.7 DVB as cross-linker

Using EGDMA as cross-linker produces a polar matrix, whilst various other functionalities were introduced by the FMs chosen in the previous polymers. Using DVB as a cross-linker provides another type of interaction for the adsorbent polymer matrices. Solvent molecules and adsorbate species, i.e. iprodione, have different interactions with aromatic and non-polar DVB cross-linked matrices compared to the EGDMA cross-linked matrices.

Using DVB as both the cross-linker and major monomer forming the adsorbent polymer matrix has two main consequences. The first is DVB causes the nature of the polymer to be hydrophobic, thus water molecules will not solvate the polymer surface or cause swelling of the polymer structure, and this reduces the energy barrier for iprodione adsorption. The second consequence is the ability of DVB to contribute to π-π stacking interactions and structural alignment, which can occur not only in adsorption sites where the FM resides, but also in the matrix of the polymer. These two factors combined to increase the iprodione uptake capacity of the polymers composed of DVB as cross-linker.
In non-templated (#47) and templated (#51) DVB – 4VPy polymers where a 1:10 4VPy : DVB ratio was used, the adsorption isotherms in 20% THF/water demonstrated unusual behaviour, as shown in Figure 5.22. For the first time, it was observed that the templated polymer showed lower levels of adsorption than the non-templated polymer. This is despite the higher active surface area measured for the templated polymer using the BET method (276 and 297 m²/g for #47 and #51 respectively). This suggests that the presence of iprodione in the polymerization solution has caused the monomers to reorganize in its vicinity, and produce a different molecular structure compared to the non-templated polymer, where the monomers are randomly distributed. Using CHCl₃/MeOH as diluent in the polymerization solution encouraged the association of iprodione and 4VPy to occur, due to the relatively low solubility of iprodione in this diluent. In this case, it is likely that imprinted sites constructed from the alignment of several pyridyl groups remained after template removal. These sites have a higher potential to adsorb iprodione in the rebinding process, compared to the single pyridyl group distributed randomly in the non-templated polymer. The lower capacity of the MIP compared to the NIP can then be attributed to a lower number of binding sites available in the polymer structure. This is based on the higher BET surface area obtained from the N₂ adsorption (276 m²/g for NIP and 297 m²/g for MIP), and the lower capacity for iprodione adsorption obtained for the MIP.

![Figure 5.22](image)

**Figure 5.22** (A) The adsorption isotherms, and (B) Scatchard plots for iprodione on the non-templated and templated DVB – 4VPy polymers (#47 and #51) in 20% THF/water at room temperature.

The participation of metal ions may strengthen the pre-polymerization association between the template and FMs, in this case iprodione and 4VPy. Metal ion complexes are based stronger ionic and covalent interactions compared to the π-π stacking which was considered in the prepolymerization associations in the previous polymers. Here polymers with Co(II) as a metal ion were tested in the template rebinding process.

The iprodione adsorption isotherms for the non-templated and templated polymers (#49 and #53), shown in Figure 5.23, exhibited greater separation when Co(II) was used to improve the imprinting effect through complexation with 4VPy and iprodione. The absence of iprodione in the non-
templated polymer preserved the association between 4VPy and Co(II) making the 4VPy less available for iprodione in the rebinding process.

![Graph](image)

**Figure 5.23** (A) The adsorption isotherms, and (B) Scatchard plots for iprodione on the non-templated and templated DVB – Co(II) 4VPy polymers (#49 and #53) in 20% THF/water at room temperature.

Utilizing CHCl₃/MeOH as diluent helps dissolve the Co(II) acetate salt in the pre-polymerization solution, allowing the proposed reaction in Figure 5.24 to occur.

The iprodione adsorption levels of the templated polymer were higher than those of the non-templated polymer. As shown in Figure 5.23 (A), the 1:1 ratio of 4VPy to Co(II) in the pre-polymerization solution prevented the 4VPy monomers from forming an association around iprodione, as they reacted to form a stronger bond with the Co(II). The 4VPy:Co(II) ratio did not provide the required number of ligands for Co(II), thereby encouraging iprodione to coordinate with Co(II) and share its available electrons to form an association in the pre-polymerization solution. One of the possible structures for the iprodione – Co(II) – 4VPy association is shown in Figure 5.24. The coordination can be completed with methanol solvent molecules in the pre-polymerization solution or water molecules in the adsorption medium.

![Chemical Structure](image)

**Figure 5.24** Proposed complexation reaction involving the 4VPy FM and iprodione in CHCl₃/MeOH solvent in the pre-polymerization solution.

The Scatchard plots of these adsorption isotherms (Figure 5.23 (B)) showed that it was more difficult for the initial iprodione molecules to adsorb on the polymer, but the adsorption becomes favourable at higher iprodione concentrations. This again demonstrates the dominant effect of
iprodione solvency in the adsorption medium on its uptake by the polymers. The curvature in the
Scatchard plots is attributed to the increased similarity in the hydrophobicity of the polymer
surface which occurs as it adsorbs more iprodione molecules, and sheds the water molecules
initially adsorbed from the surface, allowing further access for more iprodione molecules to be
adsorbed by the polymer.

5.6 The thermodynamics of iprodione adsorption

Iprodione adsorption isotherms were obtained at temperatures of 10, 20, 25, 30 and 40 °C to
determine thermodynamic values for iprodione adsorption on the templated and non-templated
EGDMA –DVB – 4VPy polymers (#44 and #45).

The equilibrium concentration range (C_{eq}) studied was between 1 and 10 µM iprodione in 20% (v/v)
THF/water medium. Under these conditions, the solution can be considered ideal, such that the
activity coefficient of iprodione is equal to unity at all temperatures and concentrations studied;

\[ a_i = \gamma_i x_i; \quad \text{and} \quad \gamma_i = 1; \quad \text{then} \quad a_i = x_i \]

where \( a_i, \ x_i \) and \( \gamma_i \) are the activity, molar fraction and activity coefficient respectively. The
adsorption system studied contains 3 components; iprodione, water and THF. Ideally the
adsorbent is not counted as a component, since thermodynamically it is assumed to be inert. The
two phases involved in the adsorption process are the liquid containing the adsorbate, and the
interface phase containing the adsorbate molecules on the surface. Based on the Gibbs phase
rule \[ 20, 21 \]

\[ \nu = c - p + 3 = 4 \]

where \( \nu, \ c, \) and \( p \) are the degrees of freedom, number of components and the number of phases
respectively, so in this three component system there are 4 degrees of freedom: \( T, \ x_1, \ x_2 \) and \( \gamma \),
the latter being the interfacial tension. The iprodione mole fraction, \( x_{iprodione} \), is the degree of
freedom changing in a single adsorption isotherm, while the solvent composition is kept constant.
Adsorption isotherms at different temperatures allows the calculation of thermodynamic functions
for the iprodione adsorption process, as defined by:

\[ \Delta G_{ads}^o = \Delta H_{ads}^o - T \Delta S_{ads}^o \]

Equation \[ 5-13 \] shows that the adsorption free energy is controlled by enthalpy and
entropy contributions to the adsorption process. The molar free energy change \( \Delta G_i \) of component
\( i \) during the adsorption process in a \( j \) component system can be calculated from \[ 5-14 \] :

\[ \Delta G_i = G_i(T, \gamma, x_1, x_2, ..., x_{j-1}) - G_i^o(T) = RT \ln x_i \]

where \( G_i^o(T) \) is the standard state molar Gibbs free energy of pure component \( i \), and
\( G_i(T, \gamma, x_1, x_2, ..., x_{j-1}) \) is the free energy of \( i \) adsorbed in the presence of the other components at
the temperature \( T \).
The enthalpy of adsorption under standard conditions, $\Delta H_{ads}^o$, can be obtained from

$$\left[ \frac{d(\Delta G_{ads}^o / T)}{d(1/T)} \right]_{x_i} = \Delta H_{ads}^o \quad \text{(5-15)}$$

The entropy contribution to the adsorption free energy $\Delta S_{ads}^o$, can be obtained from the following differential relationship:

$$\left( \frac{\partial G}{\partial T} \right)_p = -S; \quad \left( \frac{\partial \Delta G_{ads}^o}{\partial T} \right)_y = -\Delta S_{ads}^o \quad \text{(5-16)}$$

The adsorption of a solution on a polymer deviates from these thermodynamic relations for several reasons, the most important of these being:

(i) the changing nature of the adsorbent during the adsorption process due to polymer relaxation and swelling,

(ii) the solvents not only play a diluent role in the adsorption process, they also affect the nature of both the adsorbate and adsorbent,

(iii) the heterogeneous nature of the adsorbent surface and matrix.

Nevertheless, considering the process as an ideal system, and ignoring the above limitations, allows one to estimate the thermodynamic functions for iprodione adsorption on polymers #44 and #45, allowing a thermodynamic description of the adsorption process.

![Figure 5.25](image.png)

**Figure 5.25** Iprodione adsorption isotherms on, (A) non-templated #44, and (B) templated #45 EGDMA – DVB – 4VPy polymers at 10, 20, 25, 30 and 40 °C in 20% THF/water.

To obtain the enthalpy, entropy and Gibbs free energy of adsorption of iprodione on polymers #44 and #45, the adsorption isotherms were fitted to the best quadratic equation passing through the origin. Knowing $x_{iprodione}$ in solution at equilibrium, see Figure 5.25, the Gibbs free energy can be calculated from Equation 5-14, the adsorption entropy can be calculated from Equation 5-16, and
the constant surface tension $\gamma$ condition is satisfied by considering points on isotherms with constant $B_{\text{Iprodione}}$.

Figure 5.26 Iprodione Gibbs free energy of adsorption calculated at constant $B$, for (A) non-templated #44, and (B) templated #45 EGDMA – DVB – 4VPy polymers.

The entropy of adsorption was derived from the slope of the best linear fit of $\Delta G_{\text{ads}}^o$ versus $T$ as seen in Figure 5.26. The entropy values, shown in Figure 5.27, indicated that the process was not dominated by the entropic parameters. Moreover, the trend in $\Delta S_{\text{ads}}^o$ changes for the non-templated and templated polymers is demonstrating a higher degree of order (entropy decrease) for the iprodione uptake in the templated polymer producing a more ordered structure. This enhancement can be attributed to the degree of order produced by the adsorption of the molecule matching the adsorption site within the templated polymer, while in the non-templated polymer randomly distributed cross-linking and FMs have a lower degree of order, such that it does not effectively complement the iprodione molecular structure and functionality in the adsorption process.

Figure 5.27 Entropy of iprodione adsorption from 20% THF/water on non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45 respectively).
The enthalpy of adsorption $\Delta H_{ads}^o$ was obtained graphically from the slope of a plot of $\Delta G/T$ versus $1/T$ using Equation 5-15 as shown in Figure 5.28.

The values obtained for $\Delta H_{ads}^o$ were similar to the immersional energy values. The comparison between $\Delta H_{ads}^o$ and $\Delta S_{ads}^o$ showed that the energy term, enthalpy, is the dominant factor in the adsorption process. The changes in the differential adsorption enthalpy $\Delta H_{ads}^o$ with higher iprodione concentrations exhibit opposite trends for the non-templated and templated polymers, as can be seen in Figure 5.29.

**Figure 5.28** Graphical representation of Gibbs free energy of adsorption against temperature for, (A) non-templated #44, and (B) templated #45, EGDMA – DVB – 4VPy polymers having different series of constant B.

The enthalpy of iprodione adsorption on non-templated (#44) and templated (#45) EGDMA – DVB – 4VPy polymers versus the amount of bound iprodione.

**Figure 5.29** The enthalpy of iprodione adsorption on non-templated (#44) and templated (#45) EGDMA – DVB – 4VPy polymers versus the amount of bound iprodione.
The trend for the non-templated polymer uptake can be explained by the diversity in adsorption site energies in the polymer structure. Higher energy sites are first occupied by adsorbate molecules producing a higher heat of adsorption (\( \Delta H_{ad}^o = -q \) where \( q \) is the heat of adsorption). At higher concentrations of adsorbate, sites with lower potential energy are gradually occupied, producing lower heats of adsorption than the first sites.

Generally, the adsorption enthalpy was higher for the templated polymer compared to the non-templated polymer. This is to be expected, since the adsorption sites in the templated polymer have a higher potential energy toward iprodione than the non-templated polymer, due to the complementary arrangement of monomers structured around the adsorption sites.

The trend in the differential adsorption enthalpy of the templated polymer shows that the heat of adsorption increased as more of the adsorbate was adsorbed. Usually this occurs when there is a strong interaction between adsorbate molecules on the adsorbent surface (i.e. clustering). Assuming little deformation in the polymer structure during the adsorption process, the additional heat produced during the adsorption process can be assigned to bound iprodione – iprodione interactions on the polymer. The presence of iprodione as a hydrophobic template during the polymerisation process disturbs the random distribution of the monomers in the pre-polymerization solution allowing an association of the aromatic FMs to form around the template. This type of extended associations results in larger segregated hydrophobic regions compared to the non-templated polymer, as shown schematically in Figure 5.30. These large adsorption sites allow for the adsorption of multiple iprodione molecules in the vicinity of each other where their interaction can increase the heat of adsorption at higher concentration.

![Figure 5.30](image)

**Figure 5.30** The depiction of surface heterogeneity for, (A) Non-templated (#44), and (B) templated (#45), EGDMA – DVB – 4VPy polymers, and its effect on the iprodione adsorption process.

This is not the case for the non-templated polymer, where randomly distributed monomers in the pre-polymerization solution form small separated hydrophobic sites for iprodione adsorption. The
size of the adsorption sites in the non-templated polymer are generally too small to allow for the adsorption of more than one iprodione molecule, see Figure 5.30, where it is shown that the adsorption on small segregated hydrophobic sites prevents adsorbed iprodione molecules from interacting with each other on the polymer surface.

Considering the monomer ratio in the polymers studied (4VPy:DVB:EGDMA, 2:3:5) and assuming a similar size for the monomers, the ratio of hydrophilic to hydrophobic regions in the polymer structure is close to 1:1. Thus, the hydrophobic ratio of the surface is about 50% of the total BET surface area obtained by nitrogen adsorption (305 and 327 m$^2$/g for #44 and #45), as shown in Table 5.7. However, due to the larger size of the iprodione molecule compared to nitrogen, a smaller area is available to it for adsorption. Adsorption from liquid media further reduces the accessible surface area as a result of limitations imposed by solution diffusion into the adsorbent material.

**Table 5.7** Estimation of iprodione molecular coverage and the accessible absorbent surface area of non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45).

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<td>Iprodione Area/g</td>
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</tr>
<tr>
<td>BET surface area of #44 and #45</td>
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![Figure 5.31](image.jpg) Iprodione estimated dimensions for surface coverage calculation.

When the adsorption isotherm of iprodione in 10% EtOH/20% THF/70% water for EGDMA – DVB – 4VPy polymers, Figure 5.21, is converted to evaluate the surface coverage on the polymer due to iprodione adsorption Figure 5.32 is obtained, which can be used to provide information about the heterogeneity of the polymer surface.

The increased iprodione solubility in the EtOH/THF/water medium allows adsorbate molecules to approach the adsorbent surface with a higher degree of freedom, discriminating between the different binding sites in favour of those with the most favourable interaction energy for binding.
The adsorption isotherm in Figure 5.32 shows an initial rise and step for both the non-templated and templated polymers, saturating at iprodione concentration of 45 – 50 ppm, which correlates to 20 m²/g and 25 m²/g iprodione coverage respectively, corresponding to about 17% of the total hydrophobic surface. This fraction of the hydrophobic area provides more favourable adsorption sites for iprodione molecules than those utilized in the higher iprodione concentration range of the adsorption isotherm. These preferential sites may be explained by the higher potential energy toward iprodione, and a favourable geometrical arrangement of the FMs in the adsorption site. Such sites can originate where there are larger hydrophobic regions which reduce the solvation energy barrier to iprodione adsorption. Less initial water coverage on the hydrophobic regions of the adsorbent surface makes it easier for iprodione to reach the adsorption site without the need to break the water – polymer surface bond prior to uptake.

**Figure 5.32** Adsorption isotherm of iprodione in 10% EtOH/20% THF/70% water medium on non-templated (#44) and templated (#45) EGDMA – DVB – 4VPy polymers with B values representing surface coverage, and BET surface areas of the polymers.

### 5.7 The effect of key components in wine on iprodione adsorption

As shown in section 5.5.6, ethanol as a component in the wine medium had a significant effect on iprodione adsorption on EGDMA – DVB – 4VPy non-templated and templated polymers (#44 and #45) due to its solvency effect. Here other components present in wine and grape juice are evaluated. The main components of a model grape juice used by Ugiano et al. 25 for studying the fermentation process are listed in Table 5.8. The effect of the major components in this list on iprodione adsorption with EGDMA – DVB – 4VPy non-templated (#44) and templated (#45) polymers and DVB – 4VPy non-templated (#47) and templated (#51) polymers is described in this section.

The grape juice components undergo transformation during the fermentation process, and the mainly hydrophilic species of the grape juice turn to species with more hydrophobic properties. For
example, Figure 5.33 illustrates the formation of vinylphenols from hydroxycinnamoyl – and tartaric – acid esters during the winemaking process [A (enzyme preparation) and B (yeast)], that produce the high level of volatile phenols responsible for unpleasant phenolic off-flavours, and which decrease during the storage period [C] in Figure 5.33.

**Figure 5.33** The process of formation of vinylphenols from hydroxycinnamoyl acid – tartaric acid esters during winemaking, [A], [B] and [C] are enzyme preparation, yeast function and storage steps respectively.

**Table 5.8** Composition of the chemically defined grape juice-like medium used for model fermentations.

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<th>Class</th>
<th>Ingredients</th>
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<tbody>
<tr>
<td>Sugars</td>
<td>Glucose 100 g/L, Fructose 100 g/L</td>
</tr>
<tr>
<td>Acids</td>
<td>Potassium hydrogen tartrate 2.5 g/L, L-malic acid 3.0 g/L, Citric acid 0.2 g/L</td>
</tr>
<tr>
<td>Minerals</td>
<td>K₂HPO₄ 1.14 g/L, MgSO₄·7H₂O 1.23 g/L, CaCl₂·2H₂O 0.44 g/L</td>
</tr>
<tr>
<td>Nitrogen compounds</td>
<td>γ-aminobutyric acid 69.7 mg/L, Alanine 74.4 mg/L, Arginine 98.5 mg/L, Asparagine 14.9 mg/L, Aspartic acid 24.9 mg/L, Cysteine 1.4 mg/L, Glutamic acid 75.3 mg/L, Glutamine 111.9 mg/L, Glycine 4.7 mg/L, Histidine 19.6 mg/L, Isoleucine 11 mg/L, Leucine 11.2 mg/L, Lysine 52.4 mg/L, Methionine 3.7 mg/L, NH₃ (as NH₄Cl) 52 mg/L, Ornithine 1.1 mg/L, Proline 764.8 mg/L, Serine 50.8 mg/L, Threonine 48.6 mg/L, Tryptophan 10.9 mg/L, Tyrosine 18.7 mg/L, Valine 18.6 mg/L</td>
</tr>
<tr>
<td>Nitrogen compounds</td>
<td>γ-aminobutyric acid 69.7 mg/L, Alanine 74.4 mg/L, Arginine 98.5 mg/L, Asparagine 14.9 mg/L, Aspartic acid 24.9 mg/L, Cysteine 1.4 mg/L, Glutamic acid 75.3 mg/L, Glutamine 111.9 mg/L, Glycine 4.7 mg/L, Histidine 19.6 mg/L, Isoleucine 11 mg/L, Leucine 11.2 mg/L, Lysine 52.4 mg/L, Methionine 3.7 mg/L, NH₃ (as NH₄Cl) 52 mg/L, Ornithine 1.1 mg/L, Proline 764.8 mg/L, Serine 50.8 mg/L, Threonine 48.6 mg/L, Tryptophan 10.9 mg/L, Tyrosine 18.7 mg/L, Valine 18.6 mg/L</td>
</tr>
<tr>
<td>Trace elements</td>
<td>Co(NO₃)₂·6 H₂O 30 µg/L, CuCl₂ 15 µg/L, FeCl₃ 30 µg/L, H₂BO₃ 5 µg/L, KIO₃ 10 µg/L, MnCl₂·4H₂O 200 µg/L, Na₂MoO₄·2 H₂O 25 µg/L, ZnCl₂ 135 µg/L</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Biotin 0.125 mg/L, Calcium panthenolate 1 mg/L, Folic acid 0.2 mg/L, myo-inositol 100 mg/L, Nicotinic acid 2 mg/L, PABA.K 0.2 mg/L, Pyridoxine.HCl 2 mg/L, Riboflavin 0.2 mg/L, Thiamin.HCl 0.5 mg/L, Glycosidic extract 532 µM (glycosyl-glucose)</td>
</tr>
</tbody>
</table>

Volatile aromas such as hexanol, linalool, α-terpineol, citronellol, nerol, geraniol, benzyl alcohol, β-phenylethanol, 3,7-dimethyl-1,5-octadiene-3,7-diol, 3,7-dimethyl-1,7-octadiene-3,6-diol, trans-2,6-dimethyl-2,7-octadiene-2,7-diol, 8-acetoxy-linalool, 3-hydroxy-β-damascone and 3-oxo-α-ionol have been produced from sugar-bound aroma precursors (synthetic and grape glycosides) which are more hydrophilic and water soluble when they are attached to a saccharide.

Terpenic compounds, which are olefinic ethers, alcohols, aldehydes and acids, are mainly found linked to the glycoside group in grape juice and wine. Different techniques have been used to liberate these compounds during the fermentation process to produce the flavour of wine.

The main transformation undergone by grape juice during the fermentation process is the consumption of sugars and production of ethanol. The formation of ethanol modifies the solvent...
properties of the aqueous medium to accommodate more hydrophobic species. The increased solubility of iprodione in wine, and the presence of organic hydrophobic species, make the wine medium a competitive environment for iprodione adsorption to the polymer hydrophobic adsorption sites.

In this section, the effect of the major grape juice and wine components; maleic acid (1), malic acid (2) and tartaric acid (3), D-Glucose (4), D-fructose (5), and catechin (6) (as a model compound for polyphenols), and potassium chloride (to provide K\(^+\)) on iprodione uptake by the EGDMA – DVB – 4VPy polymers (#44 and #45) and DVB – 4VPy polymers (#47 and #51) in 20% (v/v) THF/water was studied.

5.7.1 Acids

Maleic acid (1), malic acid (2) and tartaric acid (3) were selected to study the effect of acidic species on iprodione uptake by EGDMA – DVB – 4VPy and DVB – 4VPy polymers.

Figure 5.34 indicates that maleic acid (1) affected the iprodione uptake on EGDMA – DVB – 4VPy polymers more than it did with DVB – 4VPy polymers. This is due to the lower adsorption levels of maleic acid on the non-polar DVB – 4VPy polymers, whereas the more polar functionalities on the EGDMA – DVB – 4VPy polymers change the nature of its surface and reduce the adsorption of iprodione to the less available hydrophobic adsorption sites.

Figure 5.34 The effect of 5 g/L maleic acid on iprodione uptake by, (A) templated (#45) and non-templated (#44) EGDMA – DVB – 4VPy polymers, and (B) templated (#51) and non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.
The presence of malic acid (2) did not affect iprodione uptake by either of the EGDMA – DVB – 4VPy and DVB – 4VPy polymers, within the errors of the measurement range, as indicated by the results in Figure 5.35.

Figure 5.35 The effect of 1, 5, and 10 g/L malic acid on iprodione uptake by, (A) templated (#45) and (B) non-templated (#44) EGDMA – DVB – 4VPy polymers and, (C) templated (#51) and (D) non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.

In a similar fashion, Figure 5.36 demonstrates that tartaric acid (3) has little effect on iprodione adsorption by both the non-templated and templated EGDMA – DVB – 4VPy and DVB – 4VPy polymers.
Figure 5.36 The effect of 1, 5, and 10 g/L tartaric acid on iprodione uptake by, (A) templated (#45) and (B) non-templated (#44) EGDMA – DVB – 4VPy polymers and, (C) templated (#51) and (D) non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.

5.7.2 Sugars

D-Glucose (4) and D-fructose (5) are the major sugar species present in grape juice and wine. The adsorption studies illustrated in Figure 5.37 and Figure 5.38 showed both these species did not reduce iprodione adsorption by the EGDMA – DVB – 4VPy and DVB – 4VPy polymers.
Figure 5.37 The effect of 5, 20 and 40 g/L D-glucose acid on iprodione uptake by, (A) templated (#45) and, (B) non-templated (#44) EGDMA – DVB – 4VPy polymers and, (C) templated (#51) and, (D) non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.

Figure 5.38 The effect of 20 g/L D-fructose acid on iprodione uptake by, (A) templated (#45) and non-templated (#44) EGDMA – DVB – 4VPy polymers and, (B) templated (#51) and non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.
5.7.3 Potassium cation

Potassium chloride was selected to study the effect of the K\(^+\) ion on iprodione uptake by the EGDMA – DVB – 4VPy and DVB – 4VPy polymers, and the results are illustrated in Figure 5.39, where it is clearly seen that there is no significant interference by K\(^+\) ions on the iprodione uptake.

![Figure 5.39](image)

**Figure 5.39** The effect of 2000 ppm K\(^+\) cation from KCl solution on iprodione uptake by, (A) templated (#45) and non-templated (#44) EGDMA – DVB – 4VPy polymers and, (B) templated (#51) and non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.

5.7.4 Catechin

Catechin (6) was chosen as a model for the polyphenoles in wine and grape juice, to investigate if such species were likely to have any effect on iprodione adsorption by EGDMA – DVB – 4VPy and DVB – 4VPy polymers.

![Figure 5.40](image)

**Figure 5.40** The effect of 2000 ppm 1 g/L catechin on iprodione uptake by, (A) templated (#45) and non-templated (#44) EGDMA – DVB – 4VPy polymers and, (B) templated (#51) and non-templated (#47) DVB – 4VPy polymers in 20% THF/water at room temperature.

The results suggest that catechin, and the polyphenols in wine and grape juice samples, have no significant affect on the adsorption of iprodione by polymers #44, #45, #47 and #51.
5.8 Competitive rebinding

Catechol was studied as a competing species in the iprodione adsorption process on EGDMA – DVB – 4VPy non-templated and templated polymers (#44 and #45) as it provides a simple model for the complex polyphenols present in wine and grape juice, which are responsible for colours and aromas. The adsorption study was conducted in 20% THF/water, with different ratios of catechol/iprodione prepared in 20% THF/water and equilibrated for 2 hours with the #44 and #45 polymers at room temperature. The residual iprodione and catechol were assayed using HPLC after the adsorption process had equilibrated (see Figure 5.41).

![Graph showing adsorption isotherms and preferential adsorption](image)

**Figure 5.41** (A) Adsorption isotherms of catechol and iprodione, and (B) the preferential adsorption from a mixture of catechol and iprodione on the non-templated (#44) and templated (#45) EGDMA – DVB – 4VPy polymers in 20% THF/water at room temperature.

The adsorption levels found for iprodione and catechol indicated a high preference of iprodione uptake by the polymers, with little interference, if any, from the catechol.

5.9 Rebinding of iprodione in wine and grape juice media

Iprodione adsorption by EGDMA – DVB – 4VPy polymers (#44 and #45) was studied in both 20% (v/v) THF in red and white wine, and 20% (v/v) THF in red and white grape juice, to estimate the cumulative effect of the species present on iprodione uptake. The results in Figure 5.42 and Table 5.9 show that the iprodione uptake was reduced in both media with the more significant reduction being in wine. This was attributed to the presence of EtOH, which confers a higher iprodione solvency in wine media, resulting in a decreased uptake by the EGDMA – DVB – 4VPy polymers.

There was no significant difference in the adsorption levels of iprodione uptake in red and white grape juice diluted with 20% (v/v) THF. This may, or may not, be significant as the samples of red grape juice were a similar color as the white grape juice, whereas it may be expected that the red grape juice would be more highly coloured due to polyphenols originally present in the berry skin and seeds.
Figure 5.42 Iprodione uptake of templated (#45), and non-templated (#44), EGDMA – DVB – 4VPy polymers in A(i) 20% THF/water, A(ii) 20% THF in white grape juice, A(iii) 20% THF in white wine; B(i) 20% THF/water, B(ii) 20% THF in red grape juice, B(iii) 20% THF in red wine.

Table 5.9 The slope of the iprodione adsorption isotherms on non-templated (#44), and templated (#45), EGDMA – DVB – 4VPy polymers in 20% (v/v) THF/water, red and white grape juice, and red and white wine, diluted with 20% THF.

<table>
<thead>
<tr>
<th>Media</th>
<th>Adsorption isotherm slope</th>
<th>The slope reduction compared to [*] (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#44</td>
<td>#45</td>
</tr>
<tr>
<td>20% THF/water [ * ]</td>
<td>0.00167</td>
<td>0.00205</td>
</tr>
<tr>
<td>20% THF in white grape juice</td>
<td>0.00064</td>
<td>0.00080</td>
</tr>
<tr>
<td>20% THF in red grape juice</td>
<td>0.00067</td>
<td>0.00077</td>
</tr>
<tr>
<td>20% THF in white wine</td>
<td>0.00028</td>
<td>0.00031</td>
</tr>
<tr>
<td>20% THF in red wine</td>
<td>0.00017</td>
<td>0.00021</td>
</tr>
</tbody>
</table>
The decrease in adsorption levels can be clearly seen by comparing the slope of the iprodione adsorption in each medium with the adsorption in 20% (v/v) THF/water as summarized in Table 5.9.

In each case a reduction in slope of the isotherm was observed, which indicates a reduction in sensitivity and selectivity for the iprodione analyte. The larger the percentage reduction, the poorer the sensitivity of the analysis.

5.10 Discussion

The driving force behind the process of adsorption of solution is the interaction between the components in solution, the interaction of solution components with the adsorbent matrix, and the interaction between components on the adsorbent surface. Assuming a constant effect for the solvent or mixture of solvents, the uptake of a particular adsorbate will be determined by its concentration in bulk solution, while the presence of a second solute affects (usually reducing) the first solute adsorption by covering a fraction of the adsorbent materials’ active surface in a dynamic equilibrium process. The solute’s solubility and the interaction of solvent molecules with the adsorbent matrix add more complexity to the adsorption mechanism and equilibrium state.

When the adsorbent surface is heterogeneous and the adsorbent species have different polarities, the adsorption of each species will be on different regions of the adsorbent surface. This allows independent adsorption of the species involved, and the presence of one species will not significantly affect the adsorption level of the other.

Thermodynamically, it is not easy to separate the parameters involved in a multi-component adsorption on a heterogeneous polymeric system such as EGDMA – DVB – 4VPy. Factors such as adsorbate (in this case iprodione) solubility, the effect of the solvent on the adsorbent, the deviation from ideal solution behaviour, the deformation and swelling of the polymeric adsorbent, etc. simultaneously affect the adsorption of iprodione in THF – water medium. The measured adsorption level of iprodione is the result of all these processes combined.

Solvency was a major determinant of the extent of iprodione adsorption on the polymers studied in this work. This arose because of the weak interaction between iprodione and the adsorption sites. Changing the ratio of organic/aqueous species in the adsorption medium clearly demonstrated that iprodione adsorption was critically dependent on the solubility of iprodione, such that increasing the organic solvent ratio increased the solubility of iprodione, and hence, reduced its adsorption on the polymers’ studied.

Although swelling can be an important factor for some adsorbents, the high degree of cross-linking in the present polymers minimizes the swelling process and reduces the flexibility of the polymer structure, keeping the adsorbent properties relatively constant during the adsorption process.

The functionality of the adsorbent surface is another factor affecting iprodione adsorption. Using the EGDMA polymer (without any FM) as an adsorbent allowed an evaluation of the non-specific adsorption of iprodione by the hydrophilic adsorbent matrix. When FMs were incorporated into the EGDMA matrix, the difference in iprodione adsorption could be related to the polymer
functionality, taking into consideration the accessible active surface area of the adsorbent. Surface area analysis of the iprodione adsorption isotherm on EGDMA – DVB – 4VPy in a 20% THF, 10% EtOH, 70% water mixture showed two steps. This shed some light on the heterogeneity and accessibility of adsorption sites in the polymers. The first step of the adsorption isotherms indicated a different energy interaction between the surface and iprodione. This can be related to the hydrophobic/hydrophilic regions of the polymer surface. The small difference between the adsorption levels of the non-templated and templated polymers was attributed to the imprinting effect, creating more accessible, geometrically complementing, energetically favourable sites.

Figure 5.43 shows a comparison of the iprodione adsorption isotherms for the non-templated functionalized polymers in 20% THF/water. Polymers functionalized with heterocyclic aromatic monomers such as 4VPy (#20) and VC (#36) demonstrated a stronger interaction with iprodione, while the iprodione adsorption isotherm on an MAA functionalized polymer (#19) was similar to that of the unfunctionalized poly EGDMA (#17), demonstrating a lack of interaction between iprodione and the MAA monomer, as may have been expected for a hydrophobic adsorbate interacting with a hydrophilic polymer. The BET surface area of the DVB functionalized polymer (#42) is about 10% of the other polymers studied in this comparison, and when this is taken into account, the polymer demonstrates a high capacity toward iprodione adsorption (on a surface area basis). The VI functionalized polymer (#34) showed a lower capacity than the simple EGDMA polymer, see Figure 5.43. This is a surprising result in spite of the similar surface area of EGDMA – VI to the other polymers in this comparison. This may be due to the monomer – monomer interaction in the polymerization process. The association of similar monomers in the pre-polymerization solution, and formation of unfavourable structures in the polymer chemical structure, may cause lower iprodione adsorption in the adsorption process.

**Figure 5.43** A comparison of the non-templated functional polymers toward iprodione adsorption.
The trend of change in Scatchard plot shows the variation of the affinity \( \frac{B}{C_{eq}} \) of the adsorbent vs. the surface coverage or adsorption \( B \). Two main categories of Scatchard plots were observed for the adsorption isotherms of iprodione to the different adsorbent polymers studied here. In this case, several parameters including the hydrophobic nature of the adsorbate, the nature of the adsorbent surface, the polarity of the adsorption medium and the imprinting effect on the adsorbent polymer determine the shape of the Scatchard plots.

Part \( a \) of the Scatchard plot type I in Figure 5.44 shows little initial affinity for iprodione at low concentration, the affinity increases as more iprodione absorbed by the surface, this is similar to the water clustering on hydrophobic surfaces. In this case it is the opposite, iprodione, a hydrophobic compound, is absorbed on hydrophilic surface. This behaviour in Scatchard plot can be explained by the wetting properties of the adsorption medium toward the adsorbent surface as well, adsorbent surface shows more affinity for the adsorbate when there is considerable surface phase region where the adsorbate migrates from the bulk solution to the surface. When the adsorption changes the surface nature so it can be wet by the adsorption medium the affinity increases with more adsorption. In part \( b \) of type I the hydrophilic/hydrophobic properties of the surface becomes similar to iprodione, this makes the adsorption take its normal path and the adsorbate chooses the higher energy sites first and then the lower energy sites.

![Scatchard plot categories observed in the iprodione adsorption isotherms on imprinted (---) and non-imprinted polymers (-----).](image)

**Figure 5.44** The schematic representation of Scatchard plot categories observed in the iprodione adsorption isotherms on imprinted (- - - - - -) and non-imprinted polymers (-----).

When there are two types of adsorption sites in the adsorbent material with two different energies, the Scatchard plot shows two legs as demonstrated in type II in Figure 5.44. When the adsorbent surface is energetically uniform it shows a straight line Scatchard plot for the adsorption isotherm as in the non-imprinted polymer in II. The iprodione adsorption isotherms studied in this chapter, Table 5.10, depend on the range of the equilibrium concentrations, may show both \( a \) and \( b \) regions or stop short only in \( a \) region. Some of the studied polymers showed both types of Scatchard plot depend on the adsorption medium used in the process, this demonstrate the effect of solvent and wetting of the adsorbent surface.
Table 5.10 The Scatchard plot type representing the adsorption isotherms of iprodione on the polymers studied in this chapter.

<table>
<thead>
<tr>
<th>Scatchard plot type</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>EGDMA-VI, EGDMA-VC, EGDMA-TAC, EGDMA-DVB-4VPy, EGDMA-DVB</td>
</tr>
<tr>
<td>II</td>
<td>EGDMA-VC, EGDMA-TAIC, EGDMA-DVB-4VPy, DVB-4VPy, DVB-Co(II)4VPy</td>
</tr>
</tbody>
</table>

The rebinding of the template molecule (iprodione) in terms of its adsorbent capacity and affinity revealed another aspect of the imprinting effect of the template. Low energy interactions between the template and FMs, such as π-π stacking, had no significant imprinting effect on the templated polymer in comparison with the non-templated polymer. The effect of the template on the capacity and affinity of the polymer originates from the change in the polymerization mechanism due to diluent type and solvent ratio variation. In the case of a very weak template – FM interaction energy, which is close to the solvation energy value, weak associations may form but they are not the dominant factor affecting the heterogeneity of the polymer matrix. This is the main reason for the low iprodione recognition observed in this study. The formation of strong segregated adsorption sites in the polymer structure generally only occurs when interaction between the template and the FM is sufficiently strong to form a significant association, altering the heterogeneity of the polymer structure compared to the randomly distributed monomers in the non-templated polymer.

The adsorptive strength of a site in a MIP is dependent on the accumulation of template – FM interaction energy in a geometrically designated space within the polymer matrix. Therefore, the aim of the imprinting process is to concentrate the interaction energy into one site, by having more than one FM in the correct position to form an optimal geometrical space to rebind the template molecule in the rebinding process. When the template – FM interaction energy is small, the multi-step equilibrium to form the template – FM association in the pre-polymerization solution does not proceed to form species with more than one FM associated with the template molecule, and so produces mainly binary associated species:

\[ \text{FM} + \text{T} \leftarrow \rightarrow \text{FM} ...... \text{T} \]

Further association steps, as shown below, are therefore less likely to occur;

\[ \text{FM} ...... \text{T} + \text{FM} \leftarrow \rightarrow \text{FM} ...... \text{T} ...... \text{FM} \]

This reduces the imprinting effect when templates such as iprodione are the target, and a hydrophobic interaction such as π-π stacking is the only interaction energy utilized in the imprinting process.

Despite the low imprinting effect left by iprodione in the polymers investigated in this study, the thermodynamic analysis of the iprodione adsorption isotherms on EGDMA – DVB – 4VPy polymers at different temperatures did demonstrate fundamental differences between the templated and non-templated polymers.
Iprodione adsorption by the templated EGDMA – DVB – 4VPy polymers produced a more ordered configuration during the adsorption process by having a lower $\Delta S_{ads}^{o}$ than the iprodione adsorption to its non-templated counterpart, which indicated there was low level specific adsorption by the MIP compared to the lack of specific adsorption by the NIP.

The adsorption enthalpy $\Delta H_{ads}^{o}$ was found to be low for the iprodione rebinding process. However, the enhanced iprodione adsorption at higher concentrations showed the opposite trend for $\Delta H_{ads}^{o}$ variation. The NIP showed a lower $\Delta H_{ads}^{o}$ at higher adsorption levels, indicating that the iprodione occupied the energetically more favourable sites in the initial stage of adsorption. On the contrary, the MIP showed increasing $\Delta H_{ads}^{o}$ at higher adsorption levels, which can be interpreted in two ways; the first is that the adsorbent surface becomes more hydrophobic due to the accumulation of adsorbed iprodione molecules, this lowers the energy barrier to subsequent binding of iprodione molecules in the vicinity of the initially adsorbed iprodione molecules, by removing the solvating water molecules and clusters from the polymer surface. The other explanation is the occurrence of multi-molecule adsorption, considering the low interaction energy of iprodione with other species, and its low solubility in protic solvents, especially water, it is possible that in the pre-polymerization solution a few iprodione molecules associate with each other and are then solvated by the FMs and other species in the solution. After the association fixed during polymerization and subsequently template removed, the site left behind is tailored to accommodate more than one iprodione molecule. Both these factors are believed to contribute to the low $\Delta H_{ads}^{o}$ observed for the initial rebinding of iprodione molecules, compared to that for the subsequent adsorbed iprodione molecule(s).

The rebinding of iprodione to these large sites is depicted in Figure 5.45, which shows schematically that the second iprodione molecule adsorbed can produce more energy, because of interaction with both the functional groups at the adsorption site and the previously adsorbed iprodione molecules.

**Figure 5.45** A schematic depiction of the rebinding of iprodione to an adsorption site imprinted by multiple template molecules; [A] represents the adsorption of the initial iprodione molecule, and [B] represents the rebinding of the second iprodione molecule to the same adsorption site.

Competitive iprodione adsorption studies in the presence of species which are major components of wine and grape juice, were conducted to assess the adsorbent polymers performance in the medium of interest. The results showed there was generally a slight effect on iprodione adsorption in the
presence of high levels of the individual components, on both templated, and non-templated, EGDMA – DVB – 4VPy (#44 and #45) and DVB – 4VPy (#47 and #51) polymers.

A more important observation was that iprodione adsorption decreased significantly in 20% (v/v) THF in red and white wine, primarily due to its higher solubility in such media because of ethanol content. This drop was more significant in red wine than white wine due to the presence of the polyphenols competing with iprodione in the adsorption process. Iprodione uptake in red and white grape juice has lower reductions compared to wine medium; the reduction in grape juice medium is due to the presence of high sugar content in the medium and the possibility of adsorption of this component on the hydrophilic regions of the polymer. Despite the decrease in iprodione adsorption in real media, as listed in Table 5.9, the remaining adsorption of iprodione on an EGDMA – DVB – 4VPy polymer film was considered to be sufficient to study iprodione adsorption using a QCM, and this is described in the following chapter.

5.11 References


6 Quartz Crystal Microbalance (QCM) sensing

In this chapter the QCM sensing system and the signal produced by a QCM – MIP sensor with iprodione are analysed and discussed, together with a study of several other factors which influence the QCM response.

The use of the QCM is based on its fundamental frequency shift during a physical or chemical change. Several variables, including mass and viscosity of the surrounding medium, temperature and pressure, or a combination of these factors, can cause a frequency shift to occur. The Sauerbrey equation, 6-1 (see Appendix A.1) describes the relationship between the mass of a rigidly adhered film $\Delta m$ and the resonance frequency shift of the quartz crystal $\Delta f$:

$$\Delta f_{\text{Mass}} = -\left( \frac{2 f_o^2}{\sqrt{\rho_q \mu_q}} \right) \Delta m \quad 6-1$$

where $f_o$ is the resonance frequency of the unloaded crystal, and $\rho_q$ and $\mu_q$ are the density and elastic shear modulus of quartz respectively. The viscosity of the adsorbed material and the surrounding medium may cause viscous coupling and high energy dissipation when shear waves propagate through the medium; this can be quantified by measuring the crystal resistance $1$;

$$R = \frac{\pi}{8K^2C_o} \left( \frac{\rho \eta}{\pi \rho_q \mu_q} \right)^{1/2} \quad 6-2$$

where $\rho$ and $\eta$ are the liquid density and viscosity respectively, $K^2$ is the quartz electromechanical coupling coefficient and $C_o$ is the static capacitance of the device. At the same time, viscous coupling can cause a shift in the QCM resonance frequency $2$ according to;

$$\Delta f_{\text{Viscous coupling}} = -\left( \frac{\rho \eta^3}{\pi \rho_q \mu_q} \right)^{1/2} \quad 6-3$$
A complete description of the QCM surface load can be obtained from impedance and phase analysis over the frequency range, including the crystal resonance frequency, using a network analyser.

6.1 Imprinted and non-imprinted films

The EGDMA – DVB – 4VPy imprinted and non-imprinted polymers were selected to be applied as film on QCM for a detailed study of the QCM-NIP/MIP response to iprodione in solution. This explored their capacity, selectivity as well as the effect of other factors on iprodione rebinding. The choice of EGDMA – DVB – 4VPy polymers was based on the prior studies of batch re-binding of iprodione on these polymer systems in previous chapters.

6.1.1 Film uniformity and thickness

The importance of the polymer film properties, and the techniques used to cast the sensing materials on the QCM device, have been reviewed and discussed in Chapter 2, section 2.3.2. In the present study, the sandwich method was used to deposit the MIP and NIP films. Figure 6.1 shows an EGDMA – DVB – 4VPy polymer film prepared using this sandwich method, after removing the cover-slip and rinsing in acetone for 12 h and drying at 70°C.

![Figure 6.1 SEM images of the cross section of an EGDMA – DVB – 4VPy polymer film cast on a QCM.](image)

If the impedance and phase analysis of the crystal before and after film deposition are compared, they can provide information about the deposited film mass, elasticity, and surface roughness.

The crystal impedance, $Z$, is a minimum at its resonance frequency, thus it is convenient to characterize a crystal in terms of its admittance, that is, the inverse of impedance, $Y = \frac{1}{Z}$, because $Y$ is a maximum at the resonance frequency. Generally, impedance is proportional to the voltage developed across a device after applying a current, and admittance is proportional to the applied current. The admittance of a quartz crystal at any frequency is a complex value that can be expressed in terms of magnitude and phase. A phase diagram is the polar representation of an impedance or admittance function using the phase as an implicit parameter, as shown Figure 6.2.

The operation of a phase lock oscillator or phase lock loop (PLL) is based on a technique that traces the maximum admittance where the phase is zero in the phase diagram.
Loading the crystal increases its resistance and shifts its zero phase in the polar plot. For the crystal to resonate in the PLL, this resistance is cancelled using a compensating capacitance. A tolerance range in zero phase lock must be considered to keep the loaded crystal resonating. Thus, higher crystal resistance requires a broader band width to cover the tolerance margin in the zero phase lock.

![Polar plot of resonating crystal admittance.](image)

**Figure 6.2** Polar plot of resonating crystal admittance.

Heavily loaded crystals, such as those loaded with MIP films prepared using the sandwich method, usually have high resistance, and thus demonstrate difficulty locking to the PLL. This is made more difficult when operating in liquid media and when the deposited film has a rough surface, since this increases the resistance of the resonating crystal and causes damping of its admittance.

There are three significant regions on the QCM crystal, the area between the electrodes, the edges, and the area between these two regions. The region between the oscillator electrodes is driven into oscillations of the largest amplitude. This makes the mass sensitivity a maximum at the centre of the plate. The oscillation amplitudes decrease toward the crystal edges. The oscillation is not permitted on the crystal edges which makes it possible to clamp the QCM to a rigid support. The electrodes of a QCM used in liquid have a special design to allow only one electrode to be in contact with the solution. The QCM used in the present study are polished AT-cut crystals from Maxtek Inc (USA). The electrodes possess an evaporated chromium adhesion film and an evaporated gold film coating the chromium layer. The upper electrode (also called the face electrode or liquid side) is larger than the lower (air side) electrode. The liquid side electrode extends around the edge of the crystal to the air side, where both electrodes are contacted with spring loaded contacts.
Using MeCN as a diluent in film preparation produces a ring of glassy film on the edges of the film due to rapid solvent evaporation, where the solution surface is exposed. This leads to inhomogeneity in the polymer film prepared. Using the high boiling point (b.p. 210 – 230°C) and low vapour pressure (120 Pa at 20°C) solvent triglyme (TG) in the preparation of the polymer film reduces the probability of forming bubbles in the film during the polymerization process. In addition, triglyme produced a film with an overall enhanced uniformity with regards to morphology.

The polymer film was deposited on the central region of the liquid side electrode. After thiolizing the gold electrode surface to enhance the polymer adhesion to the electrode, 10, 5, and 1 µL volumes of the solution of monomers were sandwiched between the cover-slip and the gold electrode of the QCM, as shown in Figure 6.4. The polymerization was conducted in an oven at 70°C for 10 h. Figure 6.5 shows the output from network analyser studies on the polymer films. The network analyser was used to monitor the frequency shift and the damping of admittance at the crystal resonance frequency after film deposition. The film quality can be quantified by calculating the $Q$ factor of the crystal, as defined in equation 6-4. The $Q$ factor represents the rate at which an oscillating system dissipates its energy;

$$Q = \frac{f_R}{\Delta f}$$  \hspace{1cm} 6-4

where $f_R$ is the system resonance frequency and $\Delta f$ is its bandwidth.
Figure 6.5 Network analyser studies of the 4VPy-DVB-EGDMA polymer film produced using the sandwich technique on QCM. Data shown corresponds to varying volumes of the pre-polymerization solution where (A) 10 µL, (B) 5 µL, and (C) 1 µL. The magnitude and phase characteristics of the QCM before film deposition is shown at the right (higher frequencies), and the characteristics of the QCMs with polymer film cast is shifted to the left (lower frequencies).

The higher the $Q$ factor, the better the quality of the QCM resonator, and the lower the dissipation of oscillation energy in the resonating system.

It is easier to couple a high $Q$ oscillating system to its resonance frequency due to the higher magnitude of the oscillation energy at that frequency, but it is more difficult to tune the system.
because of the narrower bandwidth. For a resonating acoustic system, the bandwidth $\Delta f$ in the range of reflected power is within 3 dB (a reduction to $\frac{1}{e^{\pi^2}}$ of the original oscillation energy) of its minimum value reached at $f_R$.

**Figure 6.6** The $Q$ factor calculation for a QCM which was coated with a polymer film using 10 µL of the pre-polymerization solution. The network analyser response of this crystal before, and after, film deposition is shown in Figure 6.5 (A).

**Table 6.1** The $Q$ factor of the bare QCM crystals and the crystals after polymer film deposition, calculated from the network analyser output in Figure 6.5.

<table>
<thead>
<tr>
<th>Pre-polymerization solution volume</th>
<th>After film deposition</th>
<th>Bare crystal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$f_R$ (Hz)</td>
<td>$\Delta f$ (Hz)</td>
</tr>
<tr>
<td>10 µL (A)</td>
<td>4,953,530</td>
<td>1,959</td>
</tr>
<tr>
<td>5 µL (B)</td>
<td>4,973,141</td>
<td>965</td>
</tr>
<tr>
<td>1 µL (C)</td>
<td>4,986,141</td>
<td>380</td>
</tr>
</tbody>
</table>

There are three factors that must be optimized in order to produce a polymer coated QCM with a high $Q$ factor, suitable for use in the PLL oscillator system in a liquid medium. They are: (i) the
mass load, (ii) the film uniformity (mass distribution and uniform morphology), and (iii) the surface roughness. Mass loading can be controlled by the volume and concentration of the pre-polymerization solution used to prepare the film on the QCM resonator. When optimizing the mass load, additional parameters must be considered, including:

(i) the variation in morphology of the polymer film when the monomer concentration is changed, and

(ii) the non-uniform polymer morphology across the film profile. When the film thickness is about the same or less than the pore sizes produced in the bulk synthesis, a nonporous coating is formed. This causes a wide variation in the film morphology as a function of its thickness.

Achieving a uniform thickness and mass distribution of the film deposited on the QCM is a technical challenge that must be addressed by careful selection of the most suitable film deposition technique and appropriate materials to reduce bubble formation, so as to produce a uniform morphology across the polymer film.

When the sandwich method is used to cast a film on the QCM, the roughness of the outer side of the polymer film is determined by the pre-treatment of the cover-slip. This pre-treatment can affect the adhesion characteristics of the polymer film surface in contact with the cover-slip. When the cover-slip is treated so that the polymer film does not adhere, a smooth non-porous surface will be exposed after cover-slip removal, but when the film adheres to the cover-slip, the smooth skin of the film is peeled off during cover-slip removal, and a rough surface with open pores is exposed, as shown schematically in Figure 6.7.

Another weakness of the sandwich method when used to cast the polymer film is that it does not allow adequate control of the thickness and evenness of the polymer film, as the top layer of the film in contact with the cover-slip is removed, as shown in Figure 6.7. A lower mass load, and uniform film thickness, improves the coating quality and causes less damping of the QCM oscillation energy, whilst maintaining a sufficiently high $Q$ factor of the resonating crystal.

![Figure 6.7](image.jpg)

**Figure 6.7** A schematic representation of surface roughening cover-slip removal after polymerization using the sandwich technique.

The QCM resonator system can be considered as an electric or electromechanical system. The $Q$ factor of the QCM as an electric resonating system represents the resistance of the system, but as an electromechanical resonator, the $Q$ factor represents the level of mechanical friction that causes
the decay of the oscillation energy. A rough surface causes a higher mechanical friction and thus damping of the oscillation energy \( z, 6, 7 \).

An increase of the QCM surface roughness causes a higher frequency shift and signal damping. Viscous coupling with a smooth surface affects the frequency of the QCM by a factor of \( \left( \frac{\rho \eta}{\rho \eta} \right)^{1/2} \) due to the laminar flow of the liquid across the surface, as shown earlier in equation 6-2. For rough surfaces an additional \( \rho \) term arises because of the trapped liquid and compressional wave generation \( 7 \).

An additional way of describing the frequency shift in terms of the surface roughness of the QCM device, beside the trapped liquid model, is by considering the hydrodynamics of the fluid in contact with the rough surface, or trapped fluid in the crevice of an open pore. This approach has been used as an auxiliary theory to solve the complication of trapped fluid by some workers, while others have used it to describe the problem purely in hydrodynamic terms \( 6, 8, 9 \).

There is a compromise between the available surface area and the signal damping, to obtain the appropriate degree of surface roughness of the polymer film. While it is necessary to maximise the available surface area for adsorption of the targeted analyte, the polymer coated QCM surface roughness needs to be kept low, to produce a sufficiently strong signal when used in contact with a liquid in a PLL oscillator system. Thinner films contain less pore volume, and thus a lower volume of rigidly coupled liquid will be trapped in the film pores. This leads to a smaller shift in the fundamental frequency, and thus a reduced damping in the oscillation energy of the resonating crystal: Equation 6-5 expresses the relationship between the associated frequency decrease \( \Delta f_{\text{crevice}} \) and the total crevice volume \( V \);

\[
\Delta f_{\text{crevice}} = -\frac{2f_0^2 V}{A(\rho_\eta \mu_q)} \rho_L = -\alpha \rho_L \quad 6-5
\]

where \( \rho_L \) is the fluid density \( 6 \).

The polymer film mass deposited on the QCM resonator need to be sufficiently large to produce a reasonable frequency shift during the adsorption of analyte to create a detectable signal. In the present work, the QCM prepared with polymer films using more than 1 µL of pre-polymerization solution did not lock to the PLL system in liquid media. Thus, in this study, 0.5 – 1 µL pre-polymerization solution volumes were used to synthesize the polymer coatings on the QCM, and these coatings caused an approximately 2 kHz shift in the QCM resonator frequency.

### 6.1.2 Film porosity

A batch of bulk EGDMA – DVB – 4VPy polymer was prepared using the same formulation as that used to study its active surface area and pore size distribution. The polymer was crushed, and then the soluble materials extracted for 72 h in a Soxhlet extractor using acetone. The active surface area was analysed after drying the polymer at 70°C.
The nitrogen adsorption isotherm shown in Figure 6.8 (A) does not show significant hysteresis, indicating that the morphology of the polymer includes macropores which are too large for capillary condensation to occur during the adsorption process. The large pore volume and high surface area suggest a highly macro-porous morphology for this polymer, as shown in the SEM images in Figure 6.8 (C). Table 6.2 lists the surface area analysis results.

Table 6.2 Summary of active surface area analysis of the EGDMA – DVB – 4VPy non-templated polymer.

<table>
<thead>
<tr>
<th>Polymer label</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4VPy : DVB : EGDMA in triglyme diluent (80%)</td>
<td>BET surface area (m²/g)</td>
<td>483</td>
</tr>
<tr>
<td></td>
<td>Single point surface area at P/Po 0.2027 (m²/g)</td>
<td>467</td>
</tr>
<tr>
<td></td>
<td>BJH cumulative adsorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>BJH cumulative desorption surface area of pores between 17 and 3000 Å diameter (m²/g)</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>Micropore area (m²/g)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>BJH cumulative adsorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>1.0128</td>
</tr>
<tr>
<td></td>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>1.1844</td>
</tr>
<tr>
<td></td>
<td>BJH cumulative desorption pore volume of pores between 17 and 3000 Å diameter (mL/g)</td>
<td>1.4660</td>
</tr>
<tr>
<td></td>
<td>Micropore volume (mL/g)</td>
<td>0.0332</td>
</tr>
<tr>
<td></td>
<td>Average pore diameter (4V/A by BET) (Å)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>BJH adsorption average pore diameter (4V/A) (Å)</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>BJH desorption average pore diameter (4V/A) (Å)</td>
<td>169</td>
</tr>
</tbody>
</table>

The pore size distribution obtained by BJH analysis (Figure 6.8 (B)) shows that the main pore volume occurs between 400-1000 Å (40 – 100 nm), which can be seen in the SEM images of Figure 6.8 (C). This morphology provides the necessary open macro-porosity and high surface area to maximize the surface in contact with the adsorbate medium. The drawback to this morphology is the fragility of the polymer structure, which necessitates careful handling of the polymer film used for the sensing device.

This morphology imparts sufficient flexibility to the polymer film to avoid cracking and peeling of the surface due to internal stresses produced during drying and swelling. These types of stresses are distributed globally in the highly porous structure without becoming localised in fatigue points, and thus preventing the production of cracks and fractures in the polymer structure, or causing the film to peel from the QCM device surface.
Figure 6.8 (A) N$_2$ Ads/Des isotherms, (B) pore size distribution based on BJH analysis of desorption branch, and, (C) SEM images of the non-templated EGDMA – DVB – 4VPy polymer prepared in 80% v/v TG.
6.2 MIP – QCM response to different media

The behaviour of water adjacent to any surface is based on the balance between long-range\textsuperscript{††} interactions, the short-range water – surface atom interactions and the driving forces for water molecules to keep their hydrogen-bonded network intact\textsuperscript{10}.

Molecular simulation shows that the hydrogen-bonding network is insensitive to the morphology of the surface\textsuperscript{11}. Rudich et al.\textsuperscript{10} studied the interaction of water molecules on a hydrophobic surface using a QCM and a MOCSER (Molecular Controlled Semiconductor Resistor). The same interactions were simulated and studied theoretically. The results show that water adsorption occurs mainly on surface defects, with the model suggesting that water adsorbs as small droplets on the imperfect areas of the surface.

Lee et al.\textsuperscript{12} studied the effect of ionic strength on β-casein protein adsorption using a QCM. They found that entrapped solvent molecules contribute to the QCM response and complicate the measurement, making it difficult to quantify the adsorbed protein mass.

In this section, the response of the QCM to the media in contact with the polymer film (EGDMA – DVB – 4VPy) was investigated. Iprodione adsorption on the polymer was studied with solutions containing varying ratios of water, THF and EtOH. The interaction of these solvents with the polymer films was studied using the QCM prior to iprodione uptake experiments.

6.2.1 THF – water media

The interaction of different ratios of THF/water media with templated EGDMA – DVB – 4VPy polymer film was investigated in this section using a flow-through cell arrangement. The QCM response was baselined with water, and then the input stream was switched sequentially to 10, 20, 30, 50, and 80% (v/v) THF/water media. After the last medium, 100% water was introduced to the polymer film again. Figure 6.9 shows the data obtained from this experiment, where it can be seen that the QCM response exhibits a steady decrease in frequency up to 80% THF/water. When the film was in contact with 80% THF/water the frequency shifted positively in the opposite direction, but, with the re-introduction of pure water, the frequency shift $\Delta f$ dropped sharply, and then increased to return close to the original water baseline, due to the dynamic change of the solvent ratio in the flow-cell chamber.

The QCM response for the solvation process of the polymer film may be explained by taking into account the solvation of polymer chains in the cross-linked network and the polymer swelling.

The molar ratio of the monomers used in the polymer synthesis determines the hydrophilic – hydrophobic ratio of the polymer surface and matrix. The association of the monomers with similar polarity species in the pre-polymerization solution leaves a heterogeneous system within polymer structure. This heterogeneity results in regions with different activities in the polymer chemical structure. Hydrophobic regions of the network of the cross-linked polymer chains have a stronger

\textsuperscript{††} Structural hexagonal lattice of the hydrogen bonded water molecules.
interaction with organic species and solvents, and hydrophilic regions interact more strongly with water and highly polar species, that are able to form hydrogen bonds.

When the polymer film is equilibrated with water, the hydrophilic regions become saturated with water molecules, and water clusters are connected to the surface via these hydrogen-bonds. Due to the unfavourable chemical potential, water molecules are unable to bond to hydrophobic regions as shown schematically in part (A) of Figure 6.10.

Adding a small fraction of organic solvent to the water medium in equilibrium with the polymer film, provides solvent molecules that are able to interact with hydrophobic regions of the polymer network. For example, the 10% THF/water media lowered the QCM resonance frequency by 213 Hz. Assuming ideal conditions for the application of the Sauerbrey equation (6-1), and taking into account the mass of the polymer film (\( \Delta f = 2301 \text{ Hz}, \) equivalent to 0.0462 mg), the mass load measured by the QCM showed an increase of 0.09 mg/mg polymer at equilibrium. The observed mass change in the polymer film is due to the solvation and swelling of the hydrophobic regions of the polymer and the adsorption of THF molecules on to the hydrophobic segments of the polymer chain network as shown in Figure 6.10 (B). Increasing the THF ratio to 50% (v/v), further decreased the resonance frequency by 764 Hz. The trend in mass change (\( \Delta m \)) reaches a maximum at 50% THF, as seen in Figure 6.9 (B), and summarized in Table 6.3.

**Figure 6.9** (A) The QCM response of the porous EGDMA – DVB – 4VPy polymer film in contact with varying ratios of THF/water media, and, (B) the mass change detected by the QCM due to an increasing THF ratio in the THF/water medium.

Increasing the THF ratio to 80% (v/v) changed the direction of the frequency shift, indicating a mass decrease for the polymer film in equilibrium with this medium that suggests the following mechanism. In this case, the ready availability of THF molecules reduces the size of water clusters.
due to the limited hydrogen-bonding that can occur with THF molecules causing the polymer chain solvation to be dominated by THF molecules. In the hydrophilic regions the participation of THF in water clusters solvating the polymer chains reduces the water clusters to a smaller size and thus also reduces the load sensed by the QCM. Strong bonding to the sensing surface is necessary to detect a response using such a QCM device. This necessary strength cannot be provided by THF molecules, because they do not form clusters and the interaction between THF molecules is much weaker than the extended hydrogen-bonding in water, as illustrated schematically in Figure 6.10 (C). These results emphasize the importance of solvent composition in the mechanism of adsorption on the polymer film.

![Schematic representation of solvation](image)

**Figure 6.10** A schematic representation of the solvation of the network of the polymer chains in, (A) water, (B) a low ratio of THF/water, and (C) a high ratio of THF/water.

**Table 6.3** The QCM response for the EGDMA – DVB – 4VPy polymer film in contact with various ratios of THF/water.

<table>
<thead>
<tr>
<th>THF % (v/v) in water</th>
<th>Δf (Hz)</th>
<th>Δm (mg)</th>
<th>Δm/m_{polymer film} (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-213</td>
<td>0.0043</td>
<td>0.0926</td>
</tr>
<tr>
<td>20</td>
<td>-517</td>
<td>0.0104</td>
<td>0.2247</td>
</tr>
<tr>
<td>30</td>
<td>-736</td>
<td>0.0148</td>
<td>0.3199</td>
</tr>
<tr>
<td>50</td>
<td>-765</td>
<td>0.0154</td>
<td>0.3325</td>
</tr>
<tr>
<td>80</td>
<td>-193</td>
<td>0.0039</td>
<td>0.0839</td>
</tr>
</tbody>
</table>

Switching from 80% THF/water to pure water caused an initial drop to the minimum $\Delta f$, then an increase to the initial water baseline value of $\Delta f$. This change can be rationalized by considering the dynamic change of the THF ratio according to the flow rate inside the flow cell chamber. The
medium above the film is initially 80% THF/water. After switching the flow to 100% water, the THF content gradually decreases; at about 50% THF the frequency reaches a minimum and then returns to the water baseline frequency values.

6.2.2 Ethanol – water media

A similar study in EtOH/water mixtures was conducted to confirm the role of the solvent in the polymer film behaviour. This was an important consideration, as the polymer film was to be used as a sensor in wine. Ethanol, which is capable of forming a hydrogen bond, was added to water and introduced to EGDMA – DVB – 4VPy polymer film on the QCM in incremental steps while the QCM response was recorded. Figure 6.11 and Table 6.4 show the recorded response and the calculated mass load changes detected by the QCM.

![Figure 6.11](image)

**Figure 6.11** (A) The QCM response of the porous EGDMA – DVB – 4VPy polymer film in contact with varying ratios of EtOH/water media, and, (B) the mass change detected by the QCM due to increasing EtOH ratios in the EtOH /water medium.

**Table 6.4** The QCM response for the EGDMA – DVB – 4VPy polymer film in contact with various ratios of EtOH/water.

<table>
<thead>
<tr>
<th>EtOH % (v/v) in water</th>
<th>Δf (Hz)</th>
<th>Δm (mg)</th>
<th>Δm/m_{polymer film} (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-58</td>
<td>0.0012</td>
<td>0.0252</td>
</tr>
<tr>
<td>20</td>
<td>-130</td>
<td>0.0026</td>
<td>0.0565</td>
</tr>
<tr>
<td>30</td>
<td>-187</td>
<td>0.0038</td>
<td>0.0813</td>
</tr>
<tr>
<td>40</td>
<td>-175</td>
<td>0.0035</td>
<td>0.0761</td>
</tr>
<tr>
<td>50</td>
<td>-73</td>
<td>0.0015</td>
<td>0.0317</td>
</tr>
</tbody>
</table>
In this instance, EtOH molecules, unlike THF, are able to form hydrogen-bonds with each other and with water molecules. This allows EtOH molecules to participate in water clusters, and potentially, form their own clusters in the hydrophobic regions.

Hydrophobic regions of the polymer film swell less in the presence of EtOH than THF, due to the greater hydrogen bonding ability of EtOH molecules, as shown in Figure 6.12. For this reason, and because of the lower molecular weight of EtOH compared to THF, lower $\Delta f$ changes for 10, 20, 30% (v/v) EtOH/water ratios are observed, compared to similar ratios of THF/water.

The EtOH molecules can solvate both the hydrophobic and hydrophilic segments of the polymer chains. The replacement of the water clusters with very limited in size EtOH molecules associations is another reason for lower response for the change of EtOH/water compared to the response THF/water.

Similar to the polymer film behaviour at 80% THF/water the frequency shift starts taking the opposite direction at 40% EtOH/water. A small rise in $\Delta f$ with EtOH ratio occurs at 40% EtOH/water, followed by a sharp rise at 50% EtOH/water relatively comparable to a similar change in 80% THF/water. This ratio indicates a critical point in the solvent exchange process in the solvating molecules on the hydrophilic and hydrophobic segments of the cross-linked polymer chains. At ratios higher than 40% EtOH/water the load sensed by the QCM becomes lighter. This critical point appears for similar reasons to those mentioned for the polymer film response in contact with THF/water media. But the reasons for the appearance of this point at a lower ratio than that of THF/water are: (i) the higher polarity and hydrophilic characteristics of EtOH compared to THF, this allows EtOH molecules to preferentially associate with the aqueous medium and hydrophilic regions in the polymer; (ii) the lower molecular weight of EtOH (relative to THF) reduces the $\Delta f$ for similar molar solvent exchange on the adsorbent, and so reduces the EtOH/water ratio at which the critical point occurs compared to THF; (iii) the weaker hydrogen-bonds with the hydrophilic segments which are geometrically hindered and so produce a less ordered cluster of water and EtOH molecules; this reduces the density, and makes these clusters smaller because they are attached with weaker hydrogen-bonds.

![Figure 6.12](image) A schematic representation of the different segments in the network of the polymer chains solvation in EtOH/water.
6.3 NIP response to iprodione

Iprodione adsorption on the polymer film can be considered as adsorption from a three component system. Water, THF and iprodione molecules compete for sites in the polymer structure. Prior to the iprodione adsorption studies, the film was equilibrated with the appropriate solvent, 20% (v/v) THF/water, until a stable baseline (±1 Hz/min) was achieved, indicating the polymer network had reached the maximum solvation and matrix swelling possible in the medium to be used. Introducing an iprodione solution prepared with 20% (v/v) THF/water, and taking into consideration the adsorption levels of iprodione found from batch adsorption studies, iprodione molecules will be adsorbed to the polymer film. Assuming that the conditions are appropriate to apply the Sauerbrey equation, the QCM response for iprodione uptake by the polymer film is equivalent to the net mass change on the sensor surface, as expressed in equation 6-6;

$$\Delta f \propto \sum_i \Delta m_i$$  \hspace{1cm} 6-6

where $i$ denotes the species participating in the process.

The QCM response for concentrations of 100, 50, 25 ppm iprodione was monitored in 20% THF/water medium using the NIP film as a sensing adsorbent polymer film. The iprodione adsorption caused an increase in the resonance frequency, that is, the total loaded on the QCM was reduced in the process of iprodione uptake by the polymer film, as shown in Figure 6.13.

![Figure 6.13](image)

**Figure 6.13** The QCM response for iprodione adsorption on the non-templated EGDMA – DVB – 4VPy polymer film in 20% THF/water.
The QCM response indicated that the mass change was not dominated by iprodione uptake by the polymer film, suggesting that the signal for iprodione was determined by other components involved in the equilibrium with the polymer film. The signal magnitude was affected by the stage of equilibrium between THF and water absorbed by the polymer film. This explains why different $\Delta f$ values were obtained for the same iprodione concentrations (100 ppm), since the baseline was drifting and the THF/water – polymer film equilibrium was not fully established for the first pulse shown in Figure 6.13.

The previous studies of solvent exchange (THF/water and EtOH/water) on the heterogenous polymer film showed that the QCM response for adsorption of solution is a cumulative dynamic response to the mass changes of all the species involved. The response for iprodione adsorption may be explained on the same basis. The very low solubility of iprodione in water (13 ppm) indicates that it is quite hydrophobic. Thus, it is expected that the hydrophobic regions of the polymer are energetically favourable to provide adsorption sites for iprodione molecules. However, iprodione adsorption on the hydrophobic regions has to be preceded by the removal of organic solvent molecules already solvating polymer segments in these regions. Iprodione adsorption on the hydrophobic regions changes the nature of the polymer network, by making it increasingly hydrophobic. This reduces the affinity of the polymer for water molecules causing the break-up and loss of water clusters close to the adsorbed iprodione molecules. Shedding the water clusters from the polymer structure is the dominant factor causing the positive $\Delta f$ response (loss of mass) produced by the QCM for the iprodione adsorption process. The macroscopic aspect of this phenomenon is the reduction of the viscous coupling between the aqueous bulk solution and the polymer film, due to hydrophobic iprodione adsorption, causing a positive $\Delta f$ according to the Equation 6.3. Figure 6.14 shows a schematic representation of the exchange of the species on the polymer film during the iprodione adsorption process.

Figure 6.14 A schematic representation of the, (A) solvated, and, (B) iprodione adsorbed on different segments of network of the polymer chains in equilibrium with THF/ water solutions.

A linear QCM response was observed for iprodione in 20% (v/v) THF/water over a wide range of iprodione concentrations, as shown in the insets of Figure 6.13 and Figure 6.15. The insets of these figures show different calibration curves were obtained for similar measurements, this is due the
swelling state of the polymer film and the equilibrium stage with the solvent prior producing the iprodione signal.

**Figure 6.15** The QCM response for iprodione adsorption on the non-templated EGDMA – DVB – 4VPy polymer film in 20% THF/water.

**Figure 6.16** The reproducibility of the QCM-NIP signal for 10 ppm iprodione in 20% (v/v) THF/water, an average $\Delta f$ of $31 \pm 3$ Hz was obtained in four measurements.
The reproducibility of the signal was examined in four pulses of 10 ppm iprodione in 20% (v/v) THF/water as shown in Figure 6.16. An average signal of $31 \pm 3$ Hz was obtained for four replicate injections of 10 ppm iprodione.

Low levels of reproducibility of the signal magnitude was obtained for iprodione in different equilibrium conditions. This makes the need for a strict procedure for quantitative measurement a necessity to operate an iprodione sensing system based on MIP-QCM.

This indirect response to iprodione in partially aqueous media makes it difficult to assay the absolute iprodione taken up by the film using Sauerbrey equation, but, measuring the response for various concentrations of iprodione in the same medium shows that the response can be calibrated.

### 6.4 MIP response to iprodione

Previous chapters have described the effect of templating on polymer morphology and its affinity toward the templated molecule. In this section the effect of templating on the QCM response is investigated. Templating EGDMA – DVB – 4VPy with the iprodione molecule can change the heterogeneity of the polymer, as shown in the TGA results on the NIP and MIP of this polymer in Chapter 4. This difference in the degree of heterogeneity may affect the QCM response for the iprodione uptake process.

#### 6.4.1 Iprodione sensing in THF – water medium

The MIP – QCM response for iprodione is similar to that observed with the NIP film, as can be seen from Figure 6.17. The signal produced for iprodione adsorption by the MIP film is weaker compared to the NIP film. This indicates that the NIP is more heterogeneous than the MIP as shown by the thermodynamic analysis of iprodione on these polymers in bulk studies, see section 5.6 in the previous Chapter, which suggests that the presence of the template molecule in the monomer solution during the polymerization process caused the hydrophobic regions to be larger due to the association of the hydrophobic monomers around the template molecule (iprodione). The adsorption of iprodione on such large hydrophobic regions in the MIP film is less disturbing for the water clusters solvating the hydrophilic regions.

Concentrations below 5 ppm iprodione in 20% THF/water gave a steady QCM response, as shown in Figure 6.18. The kinetics of the mass change detected by the QCM during the iprodione adsorption process reveals some aspects of the adsorption mechanism.

The sharp frequency increases observed after the iprodione solution enters the chamber of the flow-cell showed that the polymer film commences uptake of the first iprodione molecules. This initial pattern of QCM response for iprodione adsorption at low concentrations is noisy in comparison to the signal observed for higher concentrations. In the adsorption process, the first iprodione molecules approaching the polymer network need to dislodge solvent molecules, especially water clusters, to reach the adsorption sites. Figure 6.18 demonstrates that this causes an initial frequency spike for the 1, 2 and 3 ppm iprodione solutions, but this spike is diminished in the 5 ppm concentration response, and absent at the higher concentrations shown in Figure 6.17.
Figure 6.17 The QCM response for iprodione adsorption on the templated EGDMA – DVB – 4VPy polymer film in 20% THF/water.

Figure 6.18 The QCM response for iprodione adsorption on the EGDMA – DVB – 4VPy MIP polymer film for 1, 2, 3 and 5 ppm in 20% THF/water.

As equilibration proceeds, iprodione molecules may relocate to more stable positions in the polymer network, where the hydrophobic regions are larger and geometrically favoured, via the
dynamic nature of physical adsorption. This allows some of the water molecules to return and solvate polymer chains in the network, causing a frequency decrease after the initial sharp increase. Further adsorption of iprodione molecules later causes steady solvent shedding from the polymer, and a further frequency increase, until equilibration of the QCM response.

The results of iprodione sensing at low concentrations in 20% THF/water demonstrate some aspects of the interaction of the three component system with segments of different hydrophobic/hydrophilic nature in the polymer structure during the equilibration process. These results emphasise the dynamic nature of the exchange, involving all three species during iprodione adsorption, and shows that there is more information in the QCM signal analysis than only the mass change due to adsorption of a particular species.

6.4.2 Iprodione sensing in EtOH – water media

The solvent effect on iprodione adsorption was studied in bulk adsorption experiments discussed in the previous chapter; further studies were conducted by varying the EtOH/water ratio of the adsorption medium in QCM measurements. It is now clear that the formation of water clusters as well as the hydrophobic and hydrophilic properties of the surface are the dominant factors determining the QCM response.

Changing the EtOH/water ratio reveals the relationship between the iprodione solvency and adsorption to the polymer film. Increasing the organic component of the adsorption medium increases iprodione solvency and reduces the adsorption levels of iprodione.

As before, the QCM signal indicates the dominant process to be the removal of solvating water-cluster from the polymer film, due to hydrophobic iprodione adsorption. Higher ratios of water in the adsorption medium allow the water-clusters to be the main solvating feature of the polymer network. Iprodione competition for adsorption sites on the polymer leads to the removal of water clusters and an increase of QCM resonance frequency. Higher EtOH ratios reduce the population and size of the water-clusters solvating the polymer chains (e.g. small alcohols are H-bond breakers), so there are less water clusters available to be removed upon iprodione adsorption. Furthermore, the higher solvency of iprodione in high EtOH/water ratios reduces the $\Delta G^{\text{Sol} \rightarrow \text{Ad}}$ of iprodione adsorption on the polymer film from the bulk solution. The key aspects of the $\Delta G^{\text{Sol} \rightarrow \text{Ad}}$ process have been discussed in the previous Chapter. Both of these factors reduce the size of the signal produced by the QCM in media containing a higher THF or EtOH ratio.

Figure 6.19 (A) shows that $\Delta f$ decreased dramatically from 262 Hz to 162 Hz for 50 ppm iprodione when the adsorption medium was changed from 10% to 20% (v/v) EtOH in water, and the $\Delta f$ steps continued to decrease further when a higher ratio of EtOH was included in the adsorption medium. The $\Delta f$ response isotherms for iprodione adsorption in EtOH/water are shown in the inset of Figure 6.19 (B). Clearly, the degrading influence of EtOH on the iprodione signal is an impediment to the application of such a MIP films for the assay of ethanolic media such as wine.
Figure 6.19 (A) QCM response for iprodione adsorption on the EGDMA – DVB – 4VPy MIP polymer film in 10, 20, 30 and 40% (v/v) EtOH/water media, and (B) the $\Delta f$ isotherms for iprodione adsorption at room temperature.
6.4.3 White grape juice and white wine

Iprodione uptake studies by the non-templated and templated EGDMA – DVB – 4VPy polymers (#44 and #45) in batch experiments showed considerable adsorption from wine and grape juice. However, the previous studies in this Chapter on iprodione sensing using a QCM in both THF/water and EtOH/water media showed that the resulting sensor signal was a cumulative effect of all species present in the adsorption medium. While significant iprodione uptake by the polymer film is necessary, it may not be sufficient, to produce a detectable signal. Thus, iprodione uptake measurements using the QCM – MIP sensor in real viticulture media (wine and grape juice) is necessary in order to establish whether the adsorption can be transformed into a detectable signal.

The effect of white grape juice (WGJ) and white wine (WW) on the MIP polymer film was investigated by initially introducing 10% EtOH/water. The WGJ and WW samples were filtered using a 0.45 µm nylon syringe filter before the studies were conducted. In the next step, 10% EtOH/WGJ was introduced to the QCM – MIP to investigate the effect of other components present in WGJ. This step evaluates the effect of EtOH present in a typical mid-fermentation grape juice sample on the polymer film. In the case of WW, after a baseline with 10% EtOH/water was established, WW was introduced followed by 4% EtOH/WW. The later medium was included to study the effect of different EtOH contents in WW on the QCM – MIP baseline. Figure 6.20 and Figure 6.21 show the response of the QCM – MIP in contact with the WGJ and WW media during this evaluation.

![Graph showing MIP-QCM response to white grape juice and 10% EtOH in white grape juice after a baseline in 10% EtOH/water.](image)

**Figure 6.20** MIP-QCM response to white grape juice and 10% EtOH in white grape juice after a base line in 10% EtOH/water.
Figure 6.20 shows that hydrophilic interactions dominate the QCM – MIP response in WGJ. WGJ contains a high ratio of hydrophilic species such as sugars, acids, polyphenols, proteins, nucleic acids and other components of living organisms. The significant frequency decrease of 626 Hz observed after switching the flow from 10% EtOH/water to WGJ indicates that the high adsorption level of grape juice components by the polymer network may not affect the water cluster population interacting with the polymer film. Sugar molecules, the main component of the WGJ, see Table 5.8 in Chapter 5, with their multiple hydroxyl groups interact strongly with both water molecules in the solution and the hydrophilic regions of the polymer structure. Thus, water molecules can solvate already adsorbed sugar molecules after association with the hydrophilic regions in the polymer structure.

The initial reduction in frequency can be assigned mainly to the adsorption of sugar and acid species on the hydrophilic regions of the polymer structure, followed by subsequent adsorption and rearrangement of hydrophobic species from the grape juice medium on the hydrophobic regions of the polymer. This gives a slight increase in $\Delta f$ from a minimum at -659 to -626 Hz (Figure 6.20).

When the WGJ spiked with 10% (v/v) EtOH was introduced, it did not significantly affect the QCM response, as shown in the inset of Figure 6.20. The frequency was reduced by only about 7 Hz due to the presence of EtOH. This is similar to the effect of adding EtOH to an aqueous stream which has previously been equilibrated on a QCM – MIP (Figure 6.11 in Section 6.2.2).

In the fermentation process, most of the sugars are transformed to ethanol, while other hydrophilic species are transformed to more hydrophobic volatile organic compounds to produce wine from grape juice. Studying the MIP film response to WW using the QCM also indicates a change in the ratio of hydrophilic to hydrophobic species during the fermentation of WGJ (Figure 6.21). A frequency increase of +325 Hz was observed when WW was introduced following 10% (v/v) EtOH/water, as shown in Figure 6.21. The 10% (v/v) EtOH/water response was used as the baseline due to the similar EtOH content in WW. The observed frequency shift was accounted for by the additional adsorption of hydrophobic polyphenols, terpenes and other components from the wine onto the polymer network that replaced the water clusters. This explains the reduction in total mass load on the QCM surface, compared to the state of the polymer surface at equilibrium with 10% (v/v) EtOH/water.

As shown in the inset of Figure 6.21 adding 4% EtOH to WW reduced the frequency only slightly when compared to the considerable shift with WW alone. This result confirms that the frequency increase due to WW occurs because of adsorption of some species from WW onto the film, rather than simply a response to the introduction of ethanol alone.

The hydrophobic/hydrophilic heterogeneity of the polymer film surface must be considered in the iprodione adsorption process in grape juice or wine media. The adsorbent properties of the film in these media differ from the solvated film in “model” THF/water or EtOH/water media. The QCM – MIP response to iprodione uptake can be analysed by considering the solvent components and the nature of the many other species present in real media.
Figure 6.21 MIP – QCM response to white wine and 4% EtOH in white wine after establishing the baseline in 10% EtOH/water.

Iprodione adsorption from wine or grape juice is a competitive process involving other species with a significant range of hydrophobic/hydrophilic properties. 

Batch studies of iprodione adsorption from wine and grape juice described in the previous Chapter, have shown reduced levels of uptake compared to the adsorption from 20% THF/water. The next section describes studies of iprodione adsorption to a MIP film using a QCM operating in wine and grape juice media.

6.4.4 Iprodione in white grape juice

The signal produced for iprodione sensing using the QCM – MIP in white grape juice (WGJ) had a different pattern compared to the signal observed in the model THF/water and EtOH/water media. When iprodione was adsorbed on the polymer film, the resonance frequency of the crystal decreased, indicating that there was a higher mass loading on the QCM – MIP than when the film was equilibrated with the unspiked medium. This behaviour is the opposite to that observed previously, and indicates that processes of water cluster removal and viscous decoupling of liquid and QCM – MIP when hydrophobic species are adsorbed, are not dominant here.

Iprodione sensing in WGJ is shown in Figure 6.22. The QCM – MIP response was initially equilibrated with 20% THF/water, followed by 20% THF/WGJ, and then 100 ppm iprodione in 20% THF/WGJ. Finally the adsorbed species were removed from the polymer film using 20% THF/water to restore the original equilibrium baseline.
The initial 637 Hz drop in frequency after the QCM – MIP was exposed to 20% THF/WGJ demonstrated the uptake of various species from the medium used in this study. The solvation of the polymer film surface with 20% THF/water produces the equilibrium water cluster coverage on the surface. The subsequent treatment with 20% THF/WGJ establishes a new equilibrium state with the other components in WGJ. The new equilibrium introduces different mainly hydrophilic species to the polymer network, including acids and sugars, being the other main components besides water and THF present in WGJ. Sugar and acid molecules anchor to the hydrophilic regions of the polymer network using multiple hydroxyl and carboxyl groups. Due to their hydrophilic properties these species do not change the hydrophilic/hydrophobic nature of the regions in the polymer network and thus do not disrupt the solvating water molecules loading the polymer film, as illustrated schematically in Figure 6.23.

When 20% THF/WGJ spiked with 100 ppm iprodione was introduced to the polymer film, a further 56 Hz frequency decrease was observed (increasing mass loading). This result indicates that iprodione molecules cannot provide the activation energy necessary to remove strongly adsorbed sugar and acid molecules, or the water clusters that are hydrogen-bonded to them, from the polymer surface as shown schematically in Figure 6.23. Iprodione molecules therefore only adsorb on the hydrophobic regions that are not covered with high affinity hydrophobic species. Subsequent washing of the polymer film with 20% THF/water returns the response to its original frequency.

**Figure 6.22** The MIP (EGDMA – DVB – 4VPy) QCM response for 100 ppm iprodione in 20% THF in white grape juice after equilibrating the response with 20% THF in white grape juice.
Repeating this study with a different procedure is shown in Figure 6.24. Here the QCM – MIP response was equilibrated with water first and then 20% THF/water. After equilibration 20% THF/WGJ was added then finally a solution of 100 ppm iprodione in 20% THF/WGJ. A similar frequency shift $\Delta f$ (62 Hz) for 100 ppm iprodione was observed in the same media. The adsorbed iprodione was removed from the polymer surface using 20% THF/WGJ to regenerate the original response to 20% THF/WGJ.

Figure 6.24 The MIP (EGDMA – DVB – 4VPy) QCM response for 100 ppm iprodione in 20% THF/WGJ after equilibrating the response with 20% THF/WGJ; the adsorbed iprodione was removed using 20% THF/WGJ.
Figure 6.25 The MIP (EGDMA – DVB – 4VPy) QCM response for 50 ppm iprodione in 20% THF/WGJ after equilibrating the response with 20% THF/WGJ; the adsorbed iprodione was removed using 20% THF/WGJ.

Figure 6.25 shows the QCM – MIP response observed for a 50 ppm iprodione sample in 20% THF/WGJ. The observed response behaviour in this experiment was similar to the response to the 100 ppm iprodione. A similar frequency shift $\Delta f$ was observed between the 20% THF/water and the 20% THF/WGJ equilibrium frequency values. A frequency shift of 30 Hz was observed for 50 ppm iprodione in 20% THF/WGJ as shown in the inset in Figure 6.25. As this value is about half the frequency shift observed for 100 ppm iprodione, this indicates that the QCM – MIP response is linearly proportional to the iprodione concentration.

6.4.5 Iprodione in white wine

White wine (WW) contains about 12.5% EtOH and this increases the solubility of iprodione in this medium, therefore only 5% (v/v) THF was added to the WW for the QCM – MIP sensing of iprodione. This lower ratio of THF ensured the adsorption level, and thus the signal produced by the QCM – MIP in response to iprodione adsorption would be of sufficient magnitude for detection at low iprodione concentrations. The signal was much lower for similar concentrations in 10% THF/WW medium. Figure 6.26 shows the response of the QCM – MIP for a 20 ppm iprodione in 5% THF/WW after base-lining with 10% EtOH/water.

The signal for iprodione in this medium is similar to the response in 20% THF/WGJ. Figure 6.26 shows a frequency decrease with iprodione uptake. Like the sensing in 20% THF/WGJ, water cluster disruption in the interface area between the bulk solution and the adsorbent solid polymer
does not explain the phenomenon. The polymer film adsorbs other species present in wine such as polyphenols, anthocyanins and tannins, as shown schematically in Figure 6.27. Additionally, hydrophobic species such as terpenes, may compete with iprodione for the hydrophobic adsorption sites. The adsorption of iprodione on the polymer film causes an increase in the mass loading on the QCM device which is signalled by a decrease in the resonance frequency.

Figure 6.26 The MIP (EGDMA – DVB – 4VPy) – QCM response for 20 ppm iprodione in 5% THF/WW after equilibrating the response with 10% EtOH/water, and then 5% THF/WW, and then the 20 ppm iprodione in 5% THF/WW followed by iprodione removal using 5% THF/WW.

Figure 6.27 A schematic representation of iprodione adsorbed in the vicinity of a catechin molecule (as a model for polyphenols) on the polymer surface.
Polyphenols and other wine components responsible for wine aroma, taste and color will also be adsorbed on the hydrophobic and hydrophilic regions of the polymer film. The degree of their adsorption will be based on their polarity. The adsorption of these species will effectively reduce the water cluster coverage of hydrophilic regions of the polymer network. This behaviour was described in the study of baseline shift between 10% EtOH/water and WW in Figure 6.21 and Figure 6.26. In that study, the QCM resonance frequency shifted to higher values when WW was introduced, indicating a reduced mass loading, despite the adsorption of wine components onto the polymer film. The shift showed that the overall film became lighter by adsorbing wine components, which can be explained by water cluster removal from the polymer network. The adsorption of the hydrophobic aromatic polyphenols, terpenes, anthocyanins and tannins components affects the polymer network polarity so it is unable to couple with the aqueous solution. This causes an increase in the crystal resonance frequency due to the viscous uncoupling shift according to Equation 6-3. Figure 6.28 shows the response of a QCM – MIP for different concentrations of iprodione after equilibration with 5% (v/v) THF/WW.

![Image of QCM response](image)

**Figure 6.28** The QCM – MIP (EGDMA – DVB – 4VPy) response for different concentrations of iprodione in 5% THF/WW. After equilibrating the film with the adsorption medium, 5% THF/WW, to generate a baseline, and then obtaining the signal in contact with the iprodione solution; the adsorbed iprodione was washed off using, 5% THF/WW to regenerate the baseline.

These studies of QCM – MIP response to iprodione in 5% THF/WW, demonstrated that, in principle, the detection of low concentrations of iprodione in this complex medium is possible. To produce an iprodione measuring QCM – MIP based device it is necessary to develop a method to enhance the reproducibility of the deposited MIP film morphology on the QCM sensor and a
protocol for establishing a QCM – MIP response before iprodione measurement in order to improve the signal reproducibility.

The compositional differences from one WW or WGJ sample to the other are still a challenge for this system. This is mainly due to the small signal produced for iprodione. Future work should focus on improving the sensitivity, and synthesising more specific regions of hydrophobic/hydrophilic properties in the heterogeneous sensing polymer film. The highly hydrophilic regions in the polymer film will adsorb the WW components, regardless of their concentration in the medium, and the extensive hydrophobic regions should provide for efficient uptake of the iprodione.

### 6.4.6 Red wine and red grape juice

Batch studies on the iprodione adsorption from red wine (RW), discussed in the previous chapter, have shown that iprodione uptake by polymers #44 and #45 was reduced even further in RW compared to WW. This reduction arises from the adsorption of polyphenols, in particular tannins present in the medium, competing with iprodione for the hydrophobic adsorption sites, as well as adsorption on the hydrophilic polymer matrix.

The polymer film showed a permanent color change after establishment of the baseline with RW. Rinsing the QCM – MIP device in various solvents such acetone, EtOH, MeOH, ethylacetate and methylethylketone failed to regenerate the film. Iprodione adsorption on the MIP film did not produce a detectable signal using QCM, which indicates that iprodione adsorption does not cause a change in the mass loading on the QCM – MIP system in RW medium. This suggests that iprodione only displaces similar compounds with similar molecular weights on the polymer network during the adsorption process. Hence, as no detectable signal was observed for iprodione in 5% (v/v) THF/RW (10 – 50 ppm concentration range) no further work was explored.

### 6.5 Discussion

X-ray diffraction, IR spectroscopy and thermodynamic data have provided evidence of short range order for water (also referred to as cluster, [H$_2$O]$_n$, or ice-like structure) in the liquid form due to the strong hydrogen-bonding between molecules. $^{21-23}$ Many models have been proposed to predict the properties of liquid water and the behaviour of water molecules in the liquid. These models are inadequate for defining the structural characteristics of liquid water or precisely predicting the structural changes that occur when hydrophilic, hydrophobic or amphiphilic species are dissolved in the water. $^{21}$ Experimental data of the effect of hydrophobic species and alcohols on water structure is reported in the literature. $^{24-35}$ These species are of special importance to this study because of the presence of iprodione, ethanol, sugars, and THF in the aqueous adsorption medium. The terms “structure breaker” and “structure maker” or “enhancer” arise from the various effects solutes may have on water clusters or the short- and long-range order of water molecules in solution. The enhanced or broken structure of water in solution affects its density, viscosity, surface tension, diffusion and the kinetics of the processes conducted in aqueous solutions. $^{22, 27, 31, 32, 34, 35}$
Based on this, water cannot be considered a homogeneous solvent system with a negligible contribution to the adsorption mechanisms involved.

An overview of the structure of water molecules in the solution is necessary to explain QCM responses to the analyte (in this case iprodione and other species) studied in aqueous media. The presence of other species in aqueous solution affects the water short-range order (cluster) by either (i) rearranging the hydrogen-bonds between water molecules around the solute species, or (ii) by the participation of the solute species in the water cluster structure. The solute has a disruptive effect on the regional structure in aqueous solution when the solute – water interaction does not have a similar strength to the water – water interaction, and when the geometrical size and structure of the solute does not fit the geometry of water clusters.

The phenomenon of adsorption from solution onto a solid adsorbent is a competitive process among all species in the solution, including solvents and solutes. Adsorption from an aqueous solution is a special case due to the strong bonds between solvent molecules and resulting properties in solution, and in the interfacial region, between the adsorbent material and the bulk solution. This is especially important to consider when the adsorption process is correlated to a gravimetric sensing based device such as the QCM.

Taking in to account Equations 6-1 and 6-3 when applied to liquid media, the QCM frequency shift mainly occurs due to mass loading and viscous coupling when other parameters such as temperature are constant. The adsorption process provides a mass loading that can be sensed by the QCM based on the Sauerbrey Equation 6-1, and when the mass loading arises from several components a sum of the masses must be considered to evaluate $\Delta f$, as expressed in equation 6-6.

The adsorption of structure breaking species such as iprodione and polyphenols on a hydrophilic polymer film deposited on QCM device may change the nature of the film. Hydrophobic films have less affinity for water cluster binding and wetting. This causes lower viscous coupling between the bulk solution and the polymer network, which was expressed in the previous section as water cluster removal from the polymer network. This behaviour causes a positive frequency shift (increase in resonance frequency) due to the loss of adsorbed water. The total load on the QCM can be represented by the following equation:

$$\Delta f = \Delta f_{\text{Mass}} + \Delta f_{\text{Viscous coupling}}$$  

Considering the species involved in this study, low molecular weight alcohols are structure making species, but when the non-polar (hydrophobic) alkyl group of the alcohol becomes larger it creates disruption in the water cluster structure. Parmar et al. 37 reported the weakening of solute–solute/ion–ion interactions, which were relatively strong in water, on the addition of aprotic THF. These interactions are further weakened with further increase of THF in water. An increase in solvation of oxalic acid and its salts was observed upon addition of THF to water. 37 It is expected that this effect is strengthened in the case of iprodione in the THF/water mixture because of more efficient interaction between the hydrophobic hydrocarbon portion of the THF molecule and the hydrophobic iprodione molecule.
The strong hydrogen-bond between water molecules in pure water clusters and the strong hydrophobic interaction between iprodione molecules in the solid minimises the solubility of iprodione in pure water. The solubility of iprodione increases by “loosening” the water local structure. The addition of EtOH (structure maker) or THF (structure breaker) reduces the number and extent of available hydrogen-bonds in the mixture clusters. This reduces the cluster size and further weakens the solvent – solvent interactions thereby enhancing the iprodione – solvent interactions. The resultant effect is an increase in the solubility of iprodione.

Sugar molecules possess correct geometry and chemical functionality (hydroxyl groups) and reactivity (hydrogen-bonds) to make them a good fit within the water clusters and thus enhance the local water structure. 34

Nose et al. 26 studied the effect of phenolic compounds on the water local structure in EtOH/water mixtures. They demonstrated that acids or phenolic components could strengthen the EtOH/water structure, and also promote proton exchange between water and EtOH in EtOH/water solutions. With the assistance of stronger hydrogen-bonding forces provided by acids or phenols, ethanol molecules are presumed to be incorporated into the “water network” to form tight associated water molecules. In a process where phenolic compounds are adsorbed on the surface, such as in the current study, the enhancement of the water structure in the bulk aqueous solution is due to the enhanced hydrogen bonding arises from the presence of H⁺(aq). H⁺(aq) adversely affects the water structure in the interfacial region where the large water structure breaker anions are adsorbed. The increase of $\Delta f$ when the EGDMA – DVB – 4VPy film was equilibrated with WW (after equilibration with 10% EtOH/water) showed that there is a reduced mass load on the film after the adsorption of WW components. This response can be explained by the less extensive water cluster structure on the polymer due to the adsorption of phenolics.

The adsorbed water clusters on the polymer network inherit the structure characteristics of the water cluster in bulk solution. In addition, the hydrophobic/hydrophilic properties and geometrical conformation of the polymer segments in the polymer network impose further restraints on the adsorbed cluster. The matter of the local structure of the aqueous solution in the interface between two phases in terms of the interfacial free energy between bulk liquids and the hydrophobic effect has been discussed by Tanford. 22 In the case of adsorption of solution on polymer network, the hydrophobic/hydrophilic regional heterogeneity of the EGDMA – DVB – 4VPy polymer network contains hydrophilic structure enhancer EGDMA based segments and aromatic hydrophobic DVB and 4VPy structure breaker segments.

Due to the difficulty in tracking the precise macroscopic implications of the short range structure of water and aqueous solutions on factors such as density, viscosity and surface tension in the bulk liquid and interfacial regions, the molecular aspects of the adsorption process have been the focus of this chapter. The molecular interpretation of the QCM response suggested that it could be used as a tool to investigate simultaneous events of solvent exchange, adsorption and water cluster structuring in aqueous solutions, and adsorbed on the polymer network. The analysis of the response of the QCM – MIP and QCM – NIP in contact with various solvents and solvent mixtures,
and in the iprodione adsorption process as detailed in this Chapter, has revealed a new way to study and discuss the solvent exchange and adsorption of a particular species from a specific medium on a heterogeneous polymer network.

Solvent exchange and clustering were studied by changing the THF – water and EtOH – water ratios in contact with a heterogeneous EGDMA – DVB – 4VPy polymer film (NIP). The investigation of this basic phenomenon thus provided insights into the parameters affecting the signal produced using a mass sensing device.

Water cannot be adsorbed by the hydrophobic regions when water alone is equilibrated with the polymer film. By introducing THF or EtOH, polymer network hydrophobic regions will interact with the organic species causing the film to have an increased mass loading and the frequency to shift to lower values. At lower ratios of THF or EtOH the effect of the THF and EtOH on the water clusters is less significant compared to the interaction with the hydrophobic regions. The introduction of THF shows a higher mass loading compared to EtOH at the same volumetric ratio, as shown in Figure 6.29. This is possibly due to the adsorption of protic solvent molecules such as EtOH on the hydrophilic segments of the polymer network. The aprotic THF cannot form such a hydrogen bond with polymer hydrophilic segments. As such, THF can be taken up only by hydrophobic regions within the polymer network while EtOH may be adsorbed on both hydrophilic and hydrophobic regions as it can replace some of the heavy water clusters. This replacement causes the polymer film to be lighter in comparison, see Figure 6.29. The $\Delta f$ increase at high ratios of THF and EtOH shows the disappearance of the water clusters in the regional structure of the solution arising from much weaker interactions between the solvent molecules.

![Figure 6.29](image.png)

**Figure 6.29** The NIP – QCM response, $\Delta f$ (Hz), for variable ratios of THF/water and EtOH/water media.

The QCM response to iprodione in THF/water and EtOH/water media reflect the hydrophobic nature of the molecule, its effect on the water cluster structure, and the coverage of hydrophilic/hydrophobic regions within the polymer network. The $\Delta f$ increase due to the
adsorption of iprodione on the polymer film indicates displacement of the THF or EtOH molecules on the hydrophobic regions by iprodione and the removal of some water clusters from the hydrophilic regions. However, the increase of mass loading on the MIP film due to iprodione adsorption is relatively small compared to the mass loading reduction resulting from solvent cluster removal from the polymer network.

Figure 6.30 A comparison of the MIP-QCM and NIP-QCM response for 10, 20 and 30 ppm iprodione in 20% THF/water medium.

The lower response of MIP – QCM compared to the NIP – QCM for iprodione in 20% THF/water may be due to the difference in the heterogeneity of the polymer structure (Figure 6.30). Larger hydrophobic regions are formed from the association of hydrophobic monomers around the template iprodione. This causes less water cluster removal or viscous decoupling due to the adsorption of iprodione, as shown in Figure 5.30 in the previous Chapter.

Studying the QCM – MIP response for iprodione in real wine and grape juice samples demonstrates how its detection is affected by changing the adsorption medium. Grape juice with different sugar concentrations, wines with different colour shades, aromas and sweetness, all affect the response for a specific analyte such as iprodione. Sugars, as water structure enhancers, have a significant effect on the response of the QCM – MIP for iprodione. A frequency decrease for the adsorption of iprodione from white grape juice is observed. This indicates extensive sugar molecule coverage of the hydrophilic regions of polymer network due to their multiple hydroxyl groups. Adsorption of iprodione only increases the mass loading on the QCM without water cluster removal (Figure 6.23). A similar response was observed for iprodione in white wine where adsorption of polyphenols, anthocyanins and tannins to the polymer network caused a change in the hydrophobic/hydrophilic
properties of the film resulting in a frequency decrease for iprodione adsorption from this medium. This response indicates that the frequency shift is not dominated by water cluster removal from the polymer network.

Figure 6.31 The QCM-MIP response for iprodione in 5% and 10% THF/WW. The error bars are calculated as $\Delta f \pm 2\sigma$, where $\sigma$ is the standard deviation and $\Delta f$ is the average of the signal magnitude for 2 or 3 measurements.

Figure 6.31 shows a comparison of the QCM – MIP response for iprodione in 5% and 10% THF/WW. A weaker signal was observed in 10% THF/WW due to the higher solubility of iprodione. This is in agreement with results obtained from the solvent effect on the iprodione adsorption in batch studies described in the previous Chapter. Good reproducibility of the signal was achieved under similar baseline conditions.

The development and practical application of polymer-QCM sensors in the laboratory and industry remains a complex task. The work in this thesis shows that a fundamental understanding of polymer morphology, sensing protocols, media composition, and the integration of these factors are the key to their successful development and utilisation.

6.6 References


7 Conclusions and future work

7.1 Summary and conclusions

In this work a film of MIP was utilized as a sensing membrane in combination with a QCM. It was confirmed here that the porosity, microstructure and stability of adsorbent membrane are important factors that determine the material affinity and capacity in the adsorption process, thus these parameters were investigated to design an applicable membrane for iprodione detection using QCM – MIP system in liquid media.

It was shown that the presence of a template in the polymerization solution, the solvent type and the ratio affect the porosity and the microstructure of highly cross-linked EGDMA and DVB based polymers. In this study iprodione, a hydrophobic species, and pyrimethanil, a polar basic species, were used as templates to imprint a wide range of acidic, basic and aromatic functional monomers and cross-linkers. The polymerization solvent (diluent) had a significant effect on the polymer morphology and porosity. Porogenic solvents produced mesoporous polymers with high surface areas of up to 510 m²/g as measured using the BET method. Non-porogen solvents have produced macroporous polymers with much lower surface areas between 50 – 150 m²/g. It was shown that low solvent ratios (~5%) produce glassy polymers with very low surface areas. In contrast high solvent ratios (~95%) produce polymer microspheres. Between the these limits, porous polymeric systems are produced.

The inclusion of a template in the pre-polymerization solution affected the polymerization process in two ways. When the template – FM association is very weak, the template acted as a diluent species and had a minimal effect on the microstructure of the polymer. When there was a strong interaction between the FM and the template, the formation of an association, affected the spatial distribution of the monomers in the polymer chains and consequently the microstructure in the resultant polymer. Nitrogen BET, SEM and TGA were used to characterize the morphology and microstructure of the polymers. It was found that the strong interaction between pyrimethanil and MAA had a significant effect on the pore size distribution obtained using BJH theory, while the weak hydrophobic \( \pi-\pi \) interactions of iprodione with aromatic FMs had only small effect on the polymers pore size distribution.
Comparison of TGA patterns of the templated and non-templated polymers showed the effect that a weak template – FM π-π interaction can have on the polymer structure. Identical TGA decomposition profiles were obtained for the iprodione templated and non-templated EGDMA – MAA polymers, where no interaction was expected between the hydrophobic iprodione and the acidic, polar MAA FM. Higher thermostability was observed for the iprodione templated polymers where aromatic FMs such as VC, 4VPy and DVB were used in the polymer composition, suggesting the production of a higher order in the polymers microstructure due to the template presence.

Polymer functionality, surface area and the adsorption medium had significant effect on iprodione adsorption affinity and capacity.

Iprodione adsorption on the polymers showed high dependency on the adsorption medium. Iprodione solubility in the adsorption medium was the dominant factor affecting its uptake by the polymers. Increasing solubility in the adsorption medium reduced the iprodione adsorption levels on the polymers. Iprodione solubility was controlled by varying the aqueous/organic solvent ratio of mixtures of water, THF and EtOH. Iprodione uptake dropped dramatically in highly organic solvents and no adsorption was detected in 100% organic solvents such as acetonitrile and THF.

The affinity for template rebinding and its capacity showed another aspect of the templating effect on the adsorbent polymer properties. The comparison of the adsorption isotherms of the iprodione templated and non-templated polymers has shown higher adsorption capacities and affinities for the templated polymers that depend on the interaction strength between the FM and iprodione. Iprodione templated polymers demonstrated a higher imprinting effect when aromatic FMs were used. This effect showed an increase in the templated polymer capacity and affinity for iprodione compared to the non-templated polymer. Comparing the iprodione uptake of non-templated polymers functionalized with VI, 4VPy, and VC shows that an increase of the aromatic system size corresponds with an increase in the polymer binding capacity and affinity toward iprodione (VI<4VPy<VC).

The adsorption data was also used to examine the thermodynamics of iprodione uptake by the templated and non-templated EGDMA – DVB – 4VPy polymers. The results showed there were fundamental differences in the adsorption process between the polymers. A higher heat of adsorption (\(q\)) and lower entropy of adsorption (\(\Delta S_{\text{ads}}^o\)) were obtained for the MIP relative to the NIP. The small difference in \(\Delta S_{\text{ads}}^o\) showed there was only a low level of specific adsorption by the MIP, compared to the total lack of specific adsorption by the NIP. This study indicated that the adsorption sites in the MIP have a higher chemical potential for iprodione, and when iprodione is adsorbed on these sites, a more organized conformation is formed compared to the NIP. The trend in enthalpy (\(\Delta H_{\text{ads}}^o\)) variation versus surface coverage revealed a major difference in hydrophilic/hydrophobic heterogeneity between the NIP and MIP. The low iprodione adsorption enthalpy, \(\Delta H_{\text{ads}}^o\), on both the NIP and MIP, indicated that there was only weak interactions between the adsorption sites and the adsorbate molecule. However, the presence of iprodione in the pre-polymerization solution changed the distribution of monomers in the solution due to the
template – FM association. This led to the conclusion that large hydrophobic adsorption sites allowed adsorbed iprodione molecules to interact with each other on the polymer network, producing a higher heat of adsorption at higher coverage.

The uptake of iprodione on both a MIP and NIP composed of EGDMA – DVB – 4VPy was studied in the presence of wine and grape juice components to evaluate the selectivity of rebinding and any interference arising from the major grape juice and wine components, including maleic acid, malic acid and tartaric acid, D-Glucose, D-fructose, and catechin (as a model compound for polyphenols), and potassium chloride (K\textsuperscript{+} source). The results showed there was generally only a slight effect on iprodione adsorption in the presence of high levels of the individual components.

The adsorption of iprodione from wine and grape juice media was smaller than that from the model solution, 20% THF/water. However, the remaining capacity of the polymer towards iprodione was sufficient to produce a usable signal using a mass-change based sensor, such as a QCM.

Using polymerizable thiolizing agent and a low vapour pressure, high boiling point solvent MIP and NIP films with good adhesion and proper porosity were prepared on QCM gold electrode. The film thickness was adjusted and monitored using a network analyser. It was shown that porous polymer films with a 2000 Hz shift response are appropriate for application on a 5 MHz QCM that can be used for sensing in liquid media.

A thin film of EGDMA – DVB – 4VPy polymer was then used to examine the sensor response for solvent mixtures of THF/water and EtOH/water. The results revealed evidence for water clustering in aqueous solutions and in the interfacial region between the bulk solution and the heterogeneous polymer microstructure.

The sensor response for iprodione in THF/water and EtOH/water suggested that the adsorption of iprodione disrupted the water clusters solvating the polymer chains in the cross-linked network causing lower viscous coupling and thus a positive resonance frequency shift of the QCM. The sensor response for iprodione adsorption was dependant on the adsorption medium with lower detection limits and longer response times observed in highly aqueous media. Concentrations as low as 1 ppm iprodione were detected in 10 and 20% EtOH/water over 4 and 3 hours respectively.

The sensor response for iprodione in grape juice and white wine demonstrated that iprodione adsorption led to an increase in the mass loading on the polymer film. This suggested a different mechanism for the adsorption of iprodione in these media compared to THF/water and EtOH/water media. Concentrations as low as 5 ppm in 5% THF/white wine were measured with good reproducibility giving the possibility of designing a portable, easy to use, low cost and on-site sensor for viticulture and the wine-making industry.

The implication of the results obtained in this research is important for the agricultural industry and food quality monitoring. This technology can provide efficient, low cost and easy to use devices for the on-site evaluation of targeted analytes. For example in this research iprodione detection and quantification in wine and grape juice was demonstrated. These devices may potentially have an improvement in performance in simpler media such as water based samples. This may lead to
increased international market competitiveness, improved food and water safety and further utilization in the agrochemicals sector.

7.2 Future work

The synthesis of an efficient FM to allow for the targeted recognition of a specific analyte has been a key consideration since the early stages of MIP technology. This is especially so for template molecules which require the exploitation of the weak hydrophobic interactions between the FM and template. Further investigations are necessary to design specific FMs to improve the imprinting efficiency of templates with these weak interactions. When the imprinting process is dependent on weak interactions such as π-π stacking and dipole – dipole interactions, the template molecule cannot engage with multiple functional monomers in the pre-polymerization association to maximize the specificity of the constructed adsorption site in the MIP matrix. Thus, to help the assembly of a more efficient adsorption site, the interaction energy of the template – FM association should be maximised using a pre-designed FM. The role of the MIP cross-linked matrix will then be to provide the specific geometry for the adsorption site, and generate a stable matrix to preserve and protect the adsorption site.

In the case of iprodione imprinting, polymers showed higher affinity as the FM aromatic system increased in size (VI<4VPy<VC), which maximized the interaction energy between a large hydrophobic aromatic system such as the one in iprodione and the aromatic FM. The FM can then be designed to envelope the template, as shown in Figure 7.1, forming a strong 1:1 association in the pre-polymerization solution.

![Figure 7.1](image-url)  
**Figure 7.1** A design for a functional monomer, maximizing the π-π stacking interactions.

Following the detailed investigation of the micro-, meso-, and macro-porosity of molecularly imprinted and non-imprinted polymeric systems (MIP and NIP) in this work, it has been possible to identify the parameters that determine the resultant polymer microstructure and morphology. A further study of these parameters in specifically designed experiments is required to provide even greater detail regarding the polymerization process and how the porosity, active surface area in porous bulk polymers, the bead size distribution, and the average bead size in the polymer may be precisely controlled during synthesis.

In addition to the effect of diluents such as H₂O, MeOH or EtOH on polymer morphology, their use in the MIP preparation may encourage association between a non-polar template and FM. Studying this effect may allow more efficient imprinting of a poorly functional template, so as to maximize the formation of weak associations between template and FMs.
The physical chemistry of template rebinding to the MIP was highlighted in Umpleby’s work, although it did not provide the required full thermodynamic analysis of the rebinding process. The studies presented here point to fundamental differences between the thermodynamic behaviour of template rebinding to templated and non-templated polymers, such as the interaction between the adsorbed molecules on the adsorbent polymer, and the energetic and geometrical order resulting from the rebinding to the imprinted polymer. Further detailed thermodynamic analysis of template rebinding in designed experiments is required to assist to broaden our understanding of these processes. A MIP system based on strong template-FM interactions is needed to study the thermodynamic properties of the MIP microstructure and thermodynamic functions of the adsorption process.

Measurements of the energy and entropy functions, such as \( \Delta H_{\text{ads}}^{\circ} \) and \( \Delta G_{\text{ads}}^{\circ} \) for the rebinding process, are vital for understanding the mechanism governing MIP synthesis and template rebinding to the MIP adsorption sites. The value of \( \Delta S_{\text{ads}}^{\circ} \) for the template rebinding process is a good indicator of the imprinting efficiency of the template molecule, and the specificity of the imprinted adsorption sites.

The study of the response of a QCM–polymer film (EGDMA – DVB – 4VPy) in contact with different THF/water and EtOH/water ratios provided a novel means to monitor clustering in aqueous solutions and solvent exchange occurring on the heterogeneous highly cross-linked polymer network. This technique can be utilized for similar investigations on other QCM coatings and many other solvent systems. Future studies would provide fundamental information about the kinetics of solvent exchange on surfaces, as well as an evaluation of surface hydrophobic/hydrophilic heterogeneity.

Direction in the future development of QCM-MIP sensors for pesticides in viticulture would focus on arrays type sensing in combination with partially selective adsorbent polymer coatings for targeted species and other components in the medium. An array of sensors will efficiently compensate for the effect and the presence of other species on analyte detection. The QCM array would produce a potentially sample “finger print” that may give a more accurate result for the analysis. The response of the QCM array would then be analysed using neural network, chemometrics or pattern recognition techniques to identify the chemical being detected and determine its concentration. An improvement in the selectivity and the capacity of the sensing materials i.e. MIPs in the QCM–MIP array would improve the response quality and accuracy.

7.3 References


Appendix A

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A Appendix

This appendix summarizes the principles of the sensing acoustic devices operation and the models available to formulate their characteristics. The effect of different media and types of mass loadings are also discussed. The application of a rough MIP film to the QCM device and the application of this combination in a liquid medium are special cases which are also reviewed.

A.1 Piezoelectricity

Piezoelectricity is the ability of certain crystals to couple mechanical strain to electrical polarization, and for this behavior such a crystal must lack a center of inversion. Hence, applying a time-varying electric field to the piezoelectric material will cause a synchronous mechanical deformation of the substrate with the coincident generation of an acoustic wave in the material and vice versa. The relationships between the interchanging forces involved in a functioning piezoelectric material are shown schematically in Figure A.1.

![Figure A.1](image-url) The relationships between the interchanging forces when a piezoelectric solid is oscillating.
An acoustic wave can propagate in either a longitudinal (compressional) or transverse (shear) form in an elastic medium, see Figure A.2. By choosing the proper orientation of the piezoelectric single crystal, it is possible to define the type and direction of the wave propagation. 

![Bulk transverse or shear wave moving at 2,000-6,000 m/s.](A)

![Bulk longitudinal or compressional wave moving at 4,000-12,000 m/s.](B)

**Figure A.2** Pictorial representation of bulk elastic, (A) transverse, and, (B) longitudinal, waves in a solid, from Ref. 3.

In the interaction with an oscillating inertial force, an elastic medium behaves similar to a network of springs. Similarly, the propagation of a wave in a solid depends on the material’s properties and its boundaries.

When a force is applied to a material surface it produces stress, which causes a strain, or displacement in the position of the components of the material network. Similarly, a propagating oscillatory wave generates a continuum of displacements in the solid in its propagation direction.

The displacement vector in a three dimensional system can be expressed as:

\[
u(x,y,z,t) = \left[ u_x \hat{x} + u_y \hat{y} + u_z \hat{z} \right] e^{i(\omega t - kx)}
\]

in which \(u_1\), \(u_2\) and \(u_3\) represent particle displacement in the \(x\), \(y\) and \(z\) directions, \(\hat{x}\), \(\hat{y}\) and \(\hat{z}\) are unit vectors in their respective directions, \(j = \sqrt{-1}\), \(\omega\) is the angular frequency of the wave \(\omega = 2\pi f\), \(k\) is the wavenumber, \(\lambda\) is the wavelength \(k = 2\pi / \lambda\), and \(f\) is the oscillation frequency.

The local deformation of the solid is the gradient of the displacement vector which can be represented by a second rank tensor \([\nabla \nu] = [\partial u_i / \partial x_j]\):

\[
\nabla \nu = \begin{bmatrix}
\partial u_1 / \partial x_1 & \partial u_1 / \partial y_2 & \partial u_1 / \partial z_3 \\
\partial u_2 / \partial x_1 & \partial u_2 / \partial y_2 & \partial u_2 / \partial z_3 \\
\partial u_3 / \partial x_1 & \partial u_3 / \partial y_2 & \partial u_3 / \partial z_3 
\end{bmatrix}
\]

A-2

Adding the displacement gradient tensor to its transpose produces the strain matrix \(S\):

\[
S_{ij} = \frac{1}{2} \left( \partial u_i / \partial x_j + \partial u_j / \partial x_i \right)
\]

A-3

---

* Stress defined as the ratio of the force to the cross section area of the stressed material.

† Strain is the dimensionless ratio of the length of the stressed solid material to its unstressed length.
The equation of wave motion in a particular direction can be derived from the definition of the stress and strain. The equation of motion of the elastic deformation of a solid relates inertial forces to the stress gradient:

\[ \sum_{j=1}^{3} \frac{\partial T_{ij}}{\partial x_j} = \rho \frac{\partial^2 u_i}{\partial t^2} \]  

A-4

The relationship between strain and stress enables the solid motion to be characterized. The constitutive relation of the stress \( T_{ij} \) and strain \( S_{kl} \) described by Hook’s law can be generalized to three dimensions:

\[ T_{ij} = \sum_{k,l=1}^{3} c_{ijkl} S_{kl} \]  

A-5

where \( c_{ijkl} \) is the elastic stiffness constant which characterizes the elastic behavior of the solid in the small deformation limit. The four indices of the stiffness constant produce \( 3^4 = 81 \) elements in the stiffness tensor, but this number can be reduced by considering the symmetry of the strain and stress tensors.

\[ T_I = \sum_{J=1}^{6} c_{IJ} S_J \]  

A-6

The coupling between the electric field and strain (i.e., between Hook’s law \( S = sT \) and the electrical behavior of the material \( D = \varepsilon E \)) modifies the elastic constitutive relation (A-6) and gives rise to the electromagnetic constitutive relations;

\[ T_I = c_{IJ}^E S_J - \varepsilon_{ij}^E E_j \]  

A-7

\[ D_I = \varepsilon_{ij}^E E_j + \varepsilon_{ij} S_J \]  

A-8

where \( \varepsilon_{ij} \) (charge/length²) are the piezoelectric stress constants, \( E_j \) are the electric field components, \( D_j \) are the electrical displacement components, and \( \varepsilon_{ij}^E \) are the permittivity constants. Thus, equations A-7 and A-8, the piezoelectric constitutive relations, completely describe the interplay of stress, strain and the electric field in a piezoelectric solid.

A.2 Quartz crystal microbalance (QCM)

Acoustic wave devices are sensitive to both external and internal perturbations. Monitoring of mass uptake, density, viscosity, elasticity, conductivity, and temperature changes or a combination of these parameters, has been studied, and a wide range of applications appear to be possible.

Quartz is silicon dioxide (SiO₂) of triclinic crystal symmetry. It has a high melting point, with a phase transition point at 573°C, limiting the useful temperature span for both applications and processes. Quartz is an anisotropic material, i.e. its materials properties coefficients change depending on the different angles or directions considered in the crystal. Therefore, quartz has
different resonance frequencies and vibration modes based on the cutting angle of the crystal plate. To make a shear mode device from a quartz crystal and produce a QCM, the crystal plate must be cut in a specific orientation relative to the crystal axes as shown in Figure A.3.

The AT-cut (35°15') and BT-cut (-49°), which are commercially available, belong to the Y-cut family. A QCM is a shear mode device in which the acoustic wave propagates in a direction perpendicular to the crystal surface sensing a compression wave. In general, an AT-cut quartz crystal is suitable for the substrate in a QCM due to its low temperature coefficient at room temperature, with only minimal frequency changes and a higher resonance frequency than other kinds of quartz crystals. An AT-cut quartz crystal is cut at 35°15' with respect to the optical axis (z-axis), and Figure A.3 shows the various cutting directions of a quartz crystal producing QCM plates with different properties.

The QCM widely referred to as a thickness-shear mode (TSM) resonator, typically consists of a thin disk of AT-cut quartz with patterned electrodes on each side.

**Figure A.3** The different cuts used to produce QCM plates for a quartz crystal, from Ref. 13, 14.

An equivalent circuit can be devised to represent a piezoelectric mechanical resonator such as a QCM device so as to analyse its electrical properties, and an appropriate version is presented in Figure A.4. The equivalent circuit contains two branches, a series inductor, capacitor and resistor \((L_m, C_m, R_m)\) of the motion branch in which \(R_m\) corresponds to the dissipation of the oscillation energy due to the viscous effect and internal friction, \(C_m\) corresponds to the stored energy in the oscillation due to the elastic properties of the system, and \(L_m\) corresponds to the inertial mass displacement of the oscillation. The second branch contains a shunt of capacitance \(C_0\), associated with the piezoelectric element because of the dielectric properties of the piezoelectric material \(C_0\) and the electrodes in the device \(C_p\), see Figure A.4 15. Martin et al. 5 have obtained the elements of
this circuit as follows; \( C_0' = C_0 + C_p, \) \( C_0 = \frac{\varepsilon_g A}{\eta_q}, \) \( C_m = \frac{8K^2C_0}{(N\pi)^2}, \) \( L_m = \frac{1}{\omega_s^2C_m}, \) \( R_m = \frac{\eta_q}{\mu_qC_m}, \) where \( \varepsilon_g \) is the dielectric permittivity of quartz, \( K \) is the electromechanical coupling coefficient, \( N \) is the resonator harmonic number and, \( \omega_s = 2\pi f_s, \) where \( f_s \) is the series resonant frequency for the unperturbed QCM resonator.

Figure A.4 The so called Butterworth-van Dyke (BVD) model equivalent electrical circuit of a piezoelectric resonator, also called the lumped-element model, from Ref. 15.

The resonance frequency, \( f_r, \) occurs within the \( L_m - C_m - R_m \) part of the circuit, and the anti-resonance frequency, \( f_a, \) occurs within the parallel circuit \( C_0'. \) The circuit behaves as a capacitor at frequencies below \( f_r, \) and above \( f_a, \) and as an inductor between \( f_r \) and \( f_a, \) see Figure A.5. The \( f_r \) and \( f_a \) resonance frequencies can be calculated from; \( f_r = \frac{1}{(2\pi\sqrt{LC_m})} \) and \( f_a = \frac{(1/2\pi)\sqrt{(C_m + C_0')/LC_mC_0'}}{\mu_q}. \)

Figure A.5 Typical frequency scan of the magnitude of the impedance of a 5 MHz QCM.

The QCM crystal can be excited mechanically to produce a shear deformation in the crystal by using an oscillating electrical voltage. As shown in Figure A.6, the displacement maxima occur at the crystal face, which make the device sensitive to surface perturbations.
Figure A.6 Shear displacement profiles across the resonator thickness for the fundamental and the 3rd harmonic resonances, from Ref. 3.

The fundamental resonant frequency and the other harmonics of the crystal are determined by the thickness of the plate;

$$h_s = N\left(\frac{\lambda}{2}\right)$$  \hspace{1cm} (A-9)

where $N$ is an integer indicative of the harmonic resonance, $h_s$ is the thickness of the crystal and $\lambda$ is the wave length, using the relation between the frequency and wave length, $\lambda = \frac{V_s}{f_N}$, equation A-9 transforms to;

$$f_N = \frac{NV_s}{2h_s}$$  \hspace{1cm} (A-10)

where $V_s$ is the shear wave velocity and $f_N$ is the resonance frequency of the $N^{th}$ harmonic. The shear wave phase velocity $V_s$ in the substrate is given by,

$$V_s = \left(\frac{\mu_q}{\rho_q}\right)^{1/2}$$  \hspace{1cm} (A-11)

where $\mu_q$ is the shear stiffness and $\rho_q$ is the mass density of the crystal.

For an infinite height liquid over-layer, the frequency shift of the device will depend on the viscosity $\eta$, and density of the liquid $\rho_{\text{liquid}}$ as follows:

$$\Delta f_\eta = -\left(\frac{\rho_{\text{liquid}}\eta V_s}{8\pi\rho_s^2 h_s}\right)^{1/2} = -f_o^{3/2}\left(\frac{\rho_{\text{liquid}}\eta}{\pi\mu_q\rho_q}\right)^{1/2}$$  \hspace{1cm} (A-12)

QCM devices exhibit sensitivity to the conductivity and permittivity of a liquid medium typically when immersed in an effectively infinite layer of the liquid according to:

---

1 Surface electrodes can only excite the crystal to odd harmonics; $N=1, 3, 5, ...$

2 $\mu_q = 2.947 \times 10^9 \text{g cm}^{-1} \text{s}^{-2}$

3 $\rho_q = 2.648 \text{g cm}^{-3}$
\[ \Delta f = \left[ \frac{\rho_{\text{liquid}} \eta v}{8 \pi^2 h_x} \right]^{\frac{1}{2}} - \frac{\nu \delta h^2 \sigma_{\text{liquid}} + \pi \nu^2 \epsilon_{\text{liquid}} (\epsilon_{\text{liquid}} + \epsilon_{22})}{2h \pi^2 h^2 \sigma_{\text{liquid}} + \pi \nu^2 (\epsilon_{\text{liquid}} + \epsilon_{22})} \]  

where \( \sigma_{\text{liquid}} \) is the conductance, and \( \epsilon_{\text{liquid}} \) is the permittivity, of the liquid, and \( \epsilon_{22} \) is the permittivity of the quartz cut. The first term in the above relation is due solely to the liquid viscosity and density. The second term depends on the conductance of the film, and the permittivity of both the liquid and the quartz cut.  

The frequency–temperature characteristics of an AT-cut crystal are usually described by a third order power series;

\[ \Delta f_T = a_0 + a_1 T + a_2 T^2 + a_3 T^3 \]  

where \( T \) is the temperature and \( a_0, ..., a_3 \) are the temperature coefficients, which are reported to be dependent on the angle of cut, ratio of crystal dimensions, order of overtone, shape of plate and type of mounting.  

The pressure effect on the resonance frequency is formulated in an empirical relation,

\[ \Delta f_p = C_P P = 1.9 \times 10^{-7} f_o P \]  

where \( f_o \) is the QCM fundamental resonance frequency in vacuum, and the pressure is expressed in Pa. The coefficient \( C_P \) is independent of the nature of the gas.  

Thus, the resonance frequency shift \( \Delta f_{\text{total}} \) of the QCM occurs for several different reasons. It is important in the sensing event to monitor only one of the parameters to obtain meaningful results.

\[ \Delta f_{\text{total}} = \Delta f_p + \Delta f_T + \Delta f_m + \Delta f_r + \Delta f_{\eta} \]  

The frequency shift caused by mass accumulation on the device surface, \( \Delta f_m \), is the main parameter reported in the literature that has been used in sensing processes and is discussed in the next section.  

**A.2.1 QCM resonator mass sensitivity**  
Mass loading on the crystal surface perturbs the frequency of its resonance with the mass that is rigidly adhered to the crystal surface moving synchronously with the surface. The presence of a shear wave mode displacement maxima on the crystal surface makes the device more sensitive to this perturbation. The QCM, originally used to measure metal deposition rates in vacuo, has subsequently been modified to operate in contact with a liquid phase, enabling the device to act as a mass sensor in solutions.  

Based on Rayleigh’s principle, resonance in a mechanical system occurs at frequencies at which the peak of kinetic energy exactly matches the peak of potential energy. This hypothesis gives the
relationship between the resonant frequency $\omega$, and surface mass density $\rho_s = \frac{m}{A}$ (where $A$ is the adsorbed or deposited film area) expressed as:

$$\left(\frac{\omega_0}{\omega}\right)^2 = 1 + \frac{2\rho_s}{h_s\rho_q} \tag{A-17}$$

where $\omega_0 = (N\pi / h_s)\left(\mu_q / \rho_q\right)^{1/2}$ is the unperturbed resonant frequency. This relationship is approximately linear when $\rho_s \ll h_s\rho_q$, and can be expressed as:

$$\frac{\Delta f}{f_0} = -\frac{\rho_s}{h_s\rho_q} \tag{A-18}$$

which relates the fractional resonant frequency shift to the fractional mass change $\Delta m$. The combination of equations (A-9, A-10, A-11, A-19) gives the Sauerbrey equation $\Delta f = \frac{2f_0^2}{\left(\mu_q\rho_q\right)^{1/2}} \tag{A-19}$, which is used to relate changes in QCM resonant frequency to surface mass density.

Using equation (A-10), $f_N = \frac{NV_s}{2h_s}$, and assuming that the distribution of the adsorbed material is uniform, $\rho_{Film} = \frac{\Delta m}{A\rho_{Film}} = \frac{\rho_s}{h_{Film}}$, then the $\Delta f$ can be related to the mass of the adsorbed film, $\Delta m$, on the QCM device via this relation $\Delta f = \frac{V_s}{2h_s^2 \rho_s A} \tag{A-20}$.

Denison $^{23}$ demonstrated that a heavily loaded QCM exhibits a linear response $\Delta f$, for a mass deposition $\Delta m$ in the gas phase. This is in agreement with the theory that postulates no elastic loss within the deposited film.

### A.2.2 Liquid media and roughness effects

The surface roughness of such a sensing device $^{8}$ is one of the more important parameters when measurements are conducted in the liquid phase $^{5,6,24-29}$. Two different approaches have been taken to study the influence of this parameter on QCM measurements. The first is via equivalent circuit models $^{27}$ of the system, and the second, is analysing the real data using mathematical models $^{30}$. Figure A.7 provides a schematic diagram illustrating the surface roughness and parameters used in its theoretical treatment. The surface roughness length scale can be defined by the Navier-Stokes equation for liquid velocity, and by the wave equation for the elastic displacement in the crystal $^{31}$. The decay length of liquid velocities is given by:

$$\delta = (2\eta / \omega \rho)^{1/2} \tag{A-21}$$
where \( \eta \) is the liquid viscosity, and \( \rho \) is the liquid density. The wavelength of the shear-mode oscillations in the quartz crystal is given by:

\[
\lambda = \frac{2\pi (\mu_q / \rho_q)^{1/2}}{\omega} \quad A-22
\]

where \( \mu_q \) and \( \rho_q \) are the shear modulus and density of the quartz crystal respectively, and \( \omega = 2\pi f \) is the angular excitation frequency of oscillations.

The \( \lambda \) and \( \delta \) lengths are of the order of 0.161 \( \mu \text{m} \) and 0.1 cm respectively. The roughness scale can vary widely. Urbakh et al. have reported that the empirical representation of the QCM response in a liquid, as a linear combination of \((\rho \eta)^{1/2}\) and \(\rho\) terms, can be applied to the description of the shift of the resonance frequency, \( \Delta f \), in the case of a thin rough layer where \( L < \delta \), with \( L \) being the thickness of the thin rough layer.

Martin et al. showed that the surface treatments of a polished (hydrodynamically smooth) crystal had no significant effect on the surface mechanical impedance \( Z_s \), while for rougher surfaces, laminar contributions increase linearly with average surface roughness, due to an increase in solid/liquid contact surface area. For rough surfaces, nonlaminar contributions also arise due to the liquid trapped in the rough surface microstructure behaving as a rigidly adhered mass to the surface.

Some of the interferences on the mass sensing by a QCM device, such as the temperature, density and viscosity of the medium, can be minimized by utilizing a dual QCM system.

A.2.3 QCM resonator application

When an alternating electric field is applied perpendicular to the QCM crystal surface, it induces an oscillating deformation in the crystal structure with the same frequency. The resonance of this electromechanical system occurs at frequencies where the crystal thickness is equal to an odd multiple of half the induced wavelength. Bandey et al. have developed a general equivalent circuit model that describes the electrical response of a QCM resonator subject to a variety of surface conditions.

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\(^{11}\) Laminar flow occurs when a fluid flows in parallel layers.
If the QCM is considered as a single port resonating system, it can be characterized using the input impedance, $Z$, over a range of frequencies. In this case, the resonator is excited with a controlled amplitude incident voltage and the reflected signal is analyzed using an impedance analyzer over a range of frequencies around the crystal resonance frequency. The ratio of the reflected to incident voltages is denoted by the scattering parameter, which is a complex quantity, representing both the magnitude of the ratio of the reflected to incident signals, and the phase relation between them $^{35}$.

A transmission line model (the Mason $^{36, 37}$ model) is the most accurate electrical representation of a piezoelectric bulk-wave resonator. Two acoustic ports, representing the crystal faces, are connected by a transmission line that represents the phase shift and loss experienced by an acoustic wave propagating across the quartz plate. A transformer coupled between the applied voltage and quartz shear displacement, represents the coupling between the acoustic ports and electrical ports $^{38}$. Figure A.8 demonstrates the transmission line model circuit, in which $Z_s$ is the surface loading, $h_q$ is the quartz plate thickness, and $j = \sqrt{-1}$.

Indirect frequency shifts are a consequence of parasitic reactance, which arise as a function of the circuit's active and passive components. They can be minimized by maintaining the highest possible crystal quality $Q$ $^{39}$. The mechanical reactance $X$ is zero when the transducer assembly is operating at its natural resonance frequency. However, if the resonant frequencies of the overall system and the transducer assembly are different, the mechanical reactance, $X$, of the transducer assembly will not be zero.

![Figure A.8](image)

**Figure A.8** Transmission-line model representation of a QCM resonator with one stress free, and one loaded surface, from Ref. $^{38}$.

The mechanical impedance,

$$Z_s = \left. \frac{T_{xy}}{V_x} \right|_{y=0}$$

represents the mechanical loading of the resonator, which is the ratio of surface stress $T_{xy}$ to particle velocity $V_x$ at the device surface, $y = 0$ $^{34}$. Using this model it can be shown that an unperturbed resonator can be described by the motional impedance of the equivalent circuit in a BVD model $^{38}$, which can be modified to accommodate the surface load as mechanical impedance, see Figure A.9.
In the unloaded resonator, the parasitic capacitance $C_0$ dominates the admittance shift from the resonance frequency, while the motional contribution dominates near resonance.

Three primary types of load can be applied to a QCM surface: an ideal mass layer, a fluid, or a viscoelastic medium. The first two types are special cases of the latter. An ideal mass layer is thin and rigid, so there is negligible acoustic phase shift across the film, and the entire layer moves synchronously with the quartz surface. The load on the QCM surface can be represented by $Z_s$ or $Z^1_m$ according to the two models discussed above. $Z_s$ is a complex quantity in which the real part, $\text{Re}(Z_s)$, corresponds to the component of surface stress in phase with the surface particle velocity or the mechanical power dissipation at the surface, and the imaginary part, $\text{Im}(Z_s)$, corresponds to the stress component, 90° out of phase with the particle velocity, or mechanical energy storage at the surface. $R_2$ can be related to $\text{Re}(Z_s)$ and $L_2$ to $\text{Im}(Z_s)$ using the relation 8:

$$Z^1_m = R_2 + j\omega L_2$$  \hspace{1cm} A-24

In an unloaded QCM the motional impedance is given by:

$$Z_m = R_1 + j\omega L_1 + \frac{1}{j\omega C_1}$$  \hspace{1cm} A-25

This equation can be modified to include the load on the QCM surface as:

$$Z_m = (R_1 + R_2) + j\omega (L_1 + L_2) + \frac{1}{j\omega C_1}$$  \hspace{1cm} A-26

The series resonant frequency, $f_s$, is obtained when the motional reactance is zero, i.e.,

$$j\omega L_1 + \frac{1}{j\omega C_1} = 0,$$  giving

$$f_s = \frac{1}{2\pi \sqrt{L_1 C_1}}$$  \hspace{1cm} A-27
For the perturbed resonator with an ideal mass load,

$$\Delta f_s \equiv \frac{L_s f_s}{2L_f} = \frac{2f_s^2 \rho_s}{N \sqrt{\mu_s \rho_q}}$$  \hspace{1cm} \text{A-28}$$

which is equivalent to the Sauerbrey equation when \( N = 1 \).

In the case of a semi-infinite fluid loading on the QCM surface, the shear motion of the acoustic wave in the surface entrains the fluid close to the surface. Solving the Navier-Stokes equation for a one dimensional plane parallel flow gives the decay length \( \delta \) of the shear wave in the fluid loading:

$$\delta = \left( \frac{2 \eta_l}{\rho_l} \right)^{1/2}$$  \hspace{1cm} \text{A-29}$$

where \( \eta_l \) and \( \rho_l \) are the liquid shear viscosity, and density respectively; \( \frac{\eta_l}{\rho_l} \) is the kinematic viscosity of the liquid. Newtonian liquid loading leads to an equal component of energy storage \( L_2 \), and power dissipation \( R_2 \). In expressions A-24 and A-26:

$$R_2 = \omega L_2 = \frac{N \pi}{4K^* \omega C_0 Z_y} \left( \frac{\rho_l \eta_l}{2} \right)^{1/2}$$  \hspace{1cm} \text{A-30}$$

\( \omega L_2 \) represents the kinetic energy of the entrained liquid layer, resulting in a decrease in the oscillation frequency, and \( R_2 \) represents the power applied on the contacting liquid layer by the oscillating surface resulting in resonance damping. Viscoelastic Maxwell fluids exhibit first order relaxation process, where the viscosity is a function of shear stress or shear wave frequency; which is given by:

$$\eta(\omega) = \frac{\eta_0}{1 + j \omega \tau}$$  \hspace{1cm} \text{A-31}$$

in which \( \tau \) is the relaxation time ( \( \tau = \eta_0 / G_\infty \) where \( G_\infty \) is the high frequency rigidity modulus).

When the strain rate is low \( \omega \tau \ll 1 \) the Maxwellian fluid behaves as a simple Newtonian fluid. When \( \omega \tau = 1 \), the elastic energy cannot be totally dissipated in viscous flow, and some is stored elastically.

The mechanical impedance applied by a finite (several \( \delta \)) thickness viscoelastic layer on a QCM surface depends on the phase shift and the attenuation of the wave propagating across the film, that is, the nature of the interference between the waves generated at the lower film surface and those reflected from the upper surface. Shear displacement in a viscoelastic layer can be described by the equation of motion:

$$G \frac{\partial^2 u_s}{\partial y^2} = \rho_f \ddot{u}_s$$  \hspace{1cm} \text{A-32}$$

where \( G \) is the complex shear modulus ( \( G = G' + jG' \), where \( G' \) is the storage modulus and \( G' \) is the loss modulus) and \( \rho_f \) is the film density.

The acoustic wave propagation, and decay length, in the mass layers applied on top of a QCM surface depends on the properties of the mass layers. Figure A.10 shows the propagation and decay
of the acoustic wave in various mass loadings. Accordingly, the QCM response as a sensor is determined by the properties of the sensing film material and the working media in which the targeted analyte is present.

![Cross sectional view of a QCM resonator with; (a) a layer behaving as an ideal mass layer, the acoustic phase shift across the layer is negligible; (b) Newtonian fluid on the upper surface; (c) ideal mass layer plus semi-infinite Newtonian fluid on the upper surface; (d) a finite viscoelastic layer plus semi-infinite Newtonian fluid on the upper surface, from Ref. 40.](image)

**Figure A.10** Cross sectional view of a QCM resonator with; (a) a layer behaving as an ideal mass layer, the acoustic phase shift across the layer is negligible; (b) Newtonian fluid on the upper surface; (c) ideal mass layer plus semi-infinite Newtonian fluid on the upper surface; (d) a finite viscoelastic layer plus semi-infinite Newtonian fluid on the upper surface, from Ref. 40.

The application of a QCM and other acoustic wave devices in gas sensing is well established and documented 41-45, on the other hand, its use in the liquid phase is still a growing research field.

Considering the BVD crystal as a model to describe a QCM device, an Automatic Gain Control amplifier (AGC) system may sustain the assembly functioning during the sensing process. If the crystal load is denoted as $R_L$, by feeding back the voltage on $R_L$ as an input to the AGC amplifier the crystal oscillation frequency will be the circuit resonance frequency at which the phase shift is $0^\circ$. To keep the circuit locked to the resonance frequency it is necessary to have sufficient gain of voltage from $R_L$. The system used to operate the QCM device is called a phase lock oscillator or phase lock loop (PLL) show simplistically in Figure A.11.

![Schematic illustration of the phase lock loop (PLL) system to operate the QCM.](image)

**Figure A.11** Schematic illustration of the phase lock loop (PLL) system to operate the QCM.

The presence of $C_o^*$, a parasitic capacitance, injects a leading current into $R_L$ which reduces the gain of the resonating circuit. The $C_o^*$ must be cancelled to obtain the $0^\circ$ phase difference between the AGC and the crystal, this is called capacity cancellation. Thus, capacity cancellation is essential
for accurate measurements in liquids and soft films, using phase-lock oscillating frequency counters. The anti-resonant $f_a$ caused by the parasitic capacitance $C_o$ is decoupled by capacity cancellation and only the crystal oscillation frequency $f_r$ will be measured in a PLL system. The anti-resonant frequencies of each mode are given by $f_m = M \frac{v_m}{2h}$, where $m$ denotes the mode, $v_m$ the mode velocity, $h$ is the plate thickness, and $M$ is an odd integer.

### A.3 Sensing surfaces

Using sensing films or coatings on a QCM device introduces other factors to the signal measurement. In the case of polymer films; polymer modulus, polymer elasticity, film thickness, etc. have to be considered. In the case of porous films and uneven surfaces, the viscosity of the working media will severely affect the signal.

Working in the liquid phase introduces a whole new set of factors to consider in the measurement, some of the more important being: flow rate, liquid phase density and viscosity, the geometry of the flow cell, the crystal design, and the liquid phase thickness on top of the sensing device.

There is no significant signal damping when the sensing surface operates in the gas phase, as seen in Figure A.12 (A). In this case, loading the crystal with a heavy layer of the sensing material causes less difficulty in locking the AGC to the QCM in the PLL circuit. However, working in a liquid medium causes significant signal damping in the crystal response, Figure A.12 (B). This acoustic wave energy damping has been studied using Fast Fourier Transform Admittance Analysis of the oscillatory waveform of the entrained acoustic wave for the phase in contact with the QCM device. Figure A.12 shows the acoustic waveform damping in contact with (A) air, (B) water, and (C) a rigid polymer film.

![Figure A.12](image)

**Figure A.12** The acoustic waveform propagating in, (A) air, (B) a drop of water, and, (C) with one side of the crystal in contact with a rigid polymer film, from Ref. 30.

Various techniques have been used to introduce selectivity into the sensing materials applied to acoustic wave devices. These techniques depend on the working media, the type of targeted species and other species present in the sample.

When a combination of MIP and QCM is used as a sensing system in liquid medium, the rigidity of the adhered polymer film, the liquid medium loading and the adsorbed species must all be
considered in the data analysis. In the case of the BVD model, the additional mass load can be accommodated by adding an $L$ element to the circuit, and $R$ elements represent viscous loss terms of the adhered layer. A schematic representation of the BVD model for a loaded QCM-MIP system is shown in Figure A.13.

![BVD model schematic](image)

Figure A.13 Modified BVD equivalent circuit for a QCM-MIP loaded with liquid medium in the presence of adsorbed species.

A.4 References


