A Combined Computational and Experimental Approach to Characterising the Adsorption of Hydrophilic Pollutants onto Oasis® HLB

A thesis presented in fulfilment of the Degree of Doctor of Philosophy

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Declaration

I, Christine Elizabeth Close, 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; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Melbourne, May 2018

Primary Supervisor: Assoc. Prof. Michelle Spencer, RMIT University
Secondary Supervisors: Assoc. Prof. Nichola Porter, RMIT University
Assoc. Prof. Oliver Jones, RMIT University
Abstract

Hydrophilic contaminants are regularly released into the aquatic environment from anthropogenic sources such as wastewater treatment plants. Whilst they are unlikely to bio-accumulate within the environment like their hydrophobic counterparts, due to their continual release, a number of these compounds have shown to have a detrimental impact on environmental health and potentially human health, even at trace levels.

Traditional environmental monitoring programmes involve the collection of multiple grab samples which can be both an expensive and a laborious process. Additionally, compounds under investigation are not always released into the environment at regular intervals. This therefore can lead to a hit and miss approach when it comes to monitoring their impact on the environment.

Passive sampling has the ability to overcome these issues by monitoring a body of water for a predetermined period of time at the end of which, a time-weighted average concentration for these compounds of interest can be generated. A number of different passive samplers have been developed over the last 20 years with the passive sampler – the polar organic chemical integrative sampler (POCIS) being developed specifically to sample for hydrophilic contaminants. Whilst the mechanisms of interaction between the sampler’s receiving phase and the contaminants have been fully elucidated for common hydrophobic samplers, the same cannot be said for the hydrophilic samplers such as POCIS.

The aim of the research presented in this dissertation was to elucidate potential mechanisms of interactions between three distinct classes of hydrophilic contaminants and a widely used POCIS matrix – Oasis HLB. In order to achieve this objective, both experimental and theoretical investigations were undertaken.
The experimental component of this work involved performing batch adsorption studies to determine the adsorption characteristics of the selected hydrophilic compounds and Oasis HLB. A sampling rate study was also conducted to determine the uptake rate value for one of the selected classes of compounds.

The theoretical investigations that were undertaken for this dissertation involved the use of hybrid-density functional theory (DFT) calculations in order to determine the type and strength of the interactions that can take place between the compounds of interest and the selected sorbent (Oasis HLB).

Using computational chemistry methods for the purposes stipulated above, is a novel approach and one that is expected to help further establish the validity of sorbent based passive samplers such as POCIS by providing useful information about how analytes and sorbents interact.

**Keywords:** Adsorption, Computational Chemistry, Emerging Contaminants, Hybrid DFT, Passive Sampling, POCIS, Solid Phase Extraction
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<tr>
<td>AM</td>
<td>Austin Model</td>
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<tr>
<td>BSSE</td>
<td>Basis set supposition error</td>
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<tr>
<td>DGT</td>
<td>Diffusive gradients in thin films</td>
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<tr>
<td>DFT</td>
<td>Density functional theory</td>
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<td>DIA</td>
<td>Desisopropyl atrazine</td>
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<td>DVB</td>
<td>Divinyl benzene</td>
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<tr>
<td>ECC</td>
<td>Emerging contaminants of concern</td>
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<tr>
<td>$E_{xc}$</td>
<td>Exchange correlation energy</td>
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<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>GGA</td>
<td>Generalised gradient approximation</td>
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<td>HF</td>
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<td>HLB</td>
<td>Hydrophilic/lipophilic balance</td>
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<td>High performance liquid chromatography</td>
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<td>Liquid/liquid extraction</td>
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<td>LOD</td>
<td>Limit of detection</td>
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<td>LOQ</td>
<td>Limit of quantification</td>
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<td>MAX</td>
<td>Mixed mode anionic exchange</td>
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<td>Polyethersulfone</td>
</tr>
<tr>
<td>P.E.S</td>
<td>Potential energy surface</td>
</tr>
<tr>
<td>POCIS</td>
<td>Polar organic chemical integrative sampler</td>
</tr>
<tr>
<td>PPCP</td>
<td>Pharmaceuticals and Personal Care Products</td>
</tr>
<tr>
<td>PRC</td>
<td>Performance reference compound</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SPMD</td>
<td>Semi permeable membrane device</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-chloroethyl)phosphate</td>
</tr>
<tr>
<td>TCPP</td>
<td>Tris(chloropropyl)phosphate</td>
</tr>
<tr>
<td>TDCPP</td>
<td>Tris(dichloropropyl)phosphate</td>
</tr>
<tr>
<td>TWA</td>
<td>Time-weighted average concentration</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>Ultraviolet/visible</td>
</tr>
<tr>
<td>VWN</td>
<td>Vosko, Wilk and Nusair (Functional)</td>
</tr>
<tr>
<td>WBL</td>
<td>Water boundary layer</td>
</tr>
<tr>
<td>WAX</td>
<td>Weak anion exchange</td>
</tr>
<tr>
<td>WCX</td>
<td>Weak cation exchange</td>
</tr>
<tr>
<td>ZPE</td>
<td>Zero point energy</td>
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Publications and Presentations

**Journal Articles**


**Poster Presentations**


Conference and Symposium Presentations


Christine Close, Oliver A.H. Jones, Michelle J.S. Spencer and Nichola Porter. Characterising the Molecular Interactions between Hydrophilic Contaminants and a Polymeric Sorbent with the Aid of Computational Chemistry. SETAC Australasian Conference, Gold Coast, Queensland, Australia, 4th -6th September, 2017
1 Introduction

1.1 Water Pollution Monitoring

Access to clean water for drinking, cooking, bathing and for recreational activities is a necessity for a happy, healthy, functioning society and is also a basic human right [1, 2]. However, both surface water and groundwater are prone to the effects of pollution from anthropogenic activity. Sources of pollution can arise from both point source (e.g. wastewater treatment plants) [3-6] and non-point source origins such as agricultural run-off [7, 8].

Within the last twenty years, there has been an increasing interest in a class of pollutants now commonly known as emerging contaminants of concern (ECCs). These substances are chemical compounds that have either a real or perceived potential threat to human and/or environmental health [9]. Additionally, a contaminant might also be considered to be emerging because of the discovery of a new source or pathway to humans [9].

ECCs contain a wide range of chemical classes including, but not limited to, pharmaceuticals and personal care products, pesticides and herbicides, hormones, steroids and a wide range of industrial chemicals [10-14]. Unlike legacy contaminants of the past such as heavy metals and polychlorinated biphenyls PCBs, many emerging contaminants are hydrophilic in nature [15, 16].

It is well known that traditional wastewater treatment processes are not designed to fully eradicate ECCs during normal operation [15-17] and therefore, a percentage of these compounds can make their way into receiving waters, where potentially they can have a detrimental impact on aquatic life, even at levels as low as parts per trillion [4, 18, 19]. It is therefore imperative that bodies of water that are impacted upon by both the
controlled and uncontrolled release of treated wastewater undergo continual monitoring by the governing water authorities to ensure their treatment technologies are fit for purpose.

1.2 Sampling Methods

Having a sensitive detection method for these compounds is necessary if we are to improve our wastewater treatment processes. The standard way of water testing is to take samples at a particular point in time which is commonly known as grab sampling.

One of the limitations of this method is that it only gives a snapshot of what is going on in the sampled environment at the time of sampling [20]. Another limitation to grab sampling is that it is reliant on the collection and processing of vast quantities of water in order to achieve quantifiable results with common instrumentation [21, 22].

An alternative method is to collect compounds of interest over a period of time using a passive sampler to give time-weighted average (TWA) concentrations of compounds of concern [23]. Examples of passive sampling used for water pollution monitoring studies include: Chemcatcher[24], semi permeable membrane device (SPMD) [25], diffusive gradients in thin films (DGT) [26], and POCIS [27].

This dissertation focuses on the use of a specific passive sampler known as the Polar Organic Chemical Integrative Sampler (POCIS) to investigate the underlying chemistry behind its function and operation when used for sampling trace contaminants in anthropogenically impacted water.

When a POCIS device is employed to sequester water pollutants, the analytes under investigation migrate through a rate limiting membrane before being adsorbed onto the sampler’s receiving phase, which is typically a commercially available solid phase
extraction (SPE) material. In order to provide a time-weighted average concentration of the contaminants under analysis, a number of POCIS devices (normally in triplicate) are left within the body of water being monitored, for time periods of a couple of days up to a couple of months [28, 29]. Once the sampler has been brought back to the laboratory, common organic solvents are then used to extract the analytes from the SPE sorbent so that they can then be quantified by common analytical instrumentation such as gas chromatography (GC) or high performance liquid chromatography (HPLC).

A number of different SPE materials are available for application with the POCIS device including the commercially available Oasis – HLB (Hydrophilic-Lipophilic Balance) [30], WAX (Weak Anion eXchange)[31] and MAX (Mix mode Anion eXchange) [31, 32] as well as a number of lesser known sorbents [32].

1.3 Sampling Method Validation

In order to be satisfied that the data that is generated in any environmental monitoring programme is robust and fit for purpose, the various methods that are utilised in these programmes must first go through the process of validation.

There are two types of errors that are commonly associated with sampling methods: random error and systematic error.

Random error arises from variations in sampling parameters which are outside the control of the analyst and as such these types of errors are considered to be unpredictable in nature. In contrast, systematic errors vary in a predictable manner and therefore cannot be reduced by increasing the number of samples taken.
Method validation aims to either eliminate these errors, or at the very least, control these errors so that they can be accounted for when it comes time to perform data analysis. The most difficult error to deal with is random error due to its unpredictable nature.

Calibration studies of POCIS can provide data to show that the sampler has the ability to reproducibly provide TWA concentration values of a wide range of analytes[27, 33, 34]. As such these studies can help validate the sampler for environmental monitoring purposes. However, as these studies are typically conducted under a controlled environment, they often do not have the ability to replicate what the sampler is exposed to when deployed in the environment.

Environmental factors such as temperature, salinity and water flow rate/turbulence are known to have a direct impact on the rate at which analytes accumulate in the sampler. These environmental factors mean that passive samplers such as POCIS are potentially susceptible to systematic error.

The performance reference compound (PRC) approach was developed in order to overcome this obstacle [35]. PRCs are compounds that are known to have poor affinity to the receiving matrix of the sampler. As such, they are known to desorb from the sampler during deployment. The rate at which they desorb from the sampler is said to be related to environmental factors such as water flow rate/turbulence (i.e. the greater the water flow rate/ turbulence/ the greater the amount of PRC that will desorb from the sampler) [36]. This phenomenon can be taken of advantage of. Samplers can be fortified with PRC prior to deployment and upon receipt into the laboratory, after deployment, the amount of PRC remaining in the sampler can be used to correct for differences in water flow rate that can occur between laboratory controlled calibration studies and deployment [36].
One of the drawbacks to this approach is that little is known about the mechanisms that govern the adsorption/desorption of these compounds onto and from the sampler which raises the question of how reliable this approach really is.

This deficiency in knowledge is believed to be a key reason why POCIS has not been accepted as an alternative sampling technique to traditional sampling techniques (grab sampling/automated sampling/biological sampling) [37].

1.4 Knowledge Gaps

Despite the fact that many of the aforementioned SPE sorbents have been in the market place for some time now, limited information is available about the mechanisms that govern analyte adsorption/desorption especially when used as the receiving phase of the passive sampler POCIS [38].

One of the key aims of this dissertation is to test the feasibility of using computational chemistry approaches such as hybrid density functional theory (DFT) for ascertaining analyte/sorbent interactions from which molecular interaction energies can be calculated and bond types can be deciphered [39-41]. It is anticipated that these methods will result in a better understanding of the chemical interactions taking place between analyte and sorbent and in doing so will allow for optimisation of the analyte/sorbent system much faster and cheaper than experimental studies currently allow.

1.5 Selected Sorbent

Oasis HLB has been selected as the sorbent for all investigations detailed in this dissertation as: 1) it is the most commonly used SPE material for the POCIS device and 2) it is considered to be sorbent of choice for the selected compounds (see next section), all of which are characterised as being both neutral and polar in nature.
Whilst Oasis HLB is constructed from a specific ratio of two monomers, the hydrophilic n-vinylpyrrolidone (NVP) and the hydrophobic divinylbenzene (DVB), the exact structural conformation of Oasis HLB is unknown. In order to simplify the calculations, computational studies were conducted between the individual monomers of the Oasis HLB sorbent and each of the selected compounds in turn.

1.6 Selected Compounds

The compounds that have been selected for this research have been chosen based on their physicochemical properties and structural features. Three categories of compounds have been selected: xanthines, chlorinated organophosphorus flame retardants (OPFRs) and triazine herbicides.

1.6.1 Xanthine Compounds

Three structurally similar xanthine compounds were selected for investigation: 1) caffeine (a ubiquitous water pollutant [42]), 2) theophylline (a compound that is commonly used in asthma medication [43] and 3) theobromine (a compound found in chocolate [44]). These three compounds have been selected because although they are all structurally similar to each other, they have varying degrees of solubility, with caffeine being the most soluble (21,600 mg L\(^{-1}\)) and theobromine being the least soluble (330 mg L\(^{-1}\)). Each of these three compounds contains an aromatic ring. One of the main aims of investigating these compounds is to determine if these compounds have the ability to bind onto the sorbent through the formation of π∙∙∙π interactions.

Another aim of investigating these three compounds is to determine the importance of hydrogen bonding to the adsorption process and to determine if this type of bonding is more or less influential when it comes to the ability of these compounds to bind to the
selected sorbent. Whilst caffeine is a hydrogen bond acceptor only, both theophylline and theobromine are both hydrogen bond acceptors and hydrogen bond donors.

1.6.2 Triazine Herbicides

Three structurally similar herbicides have been selected for investigation; 1) atrazine, 2) simazine and 3) des-isopropyl atrazine (DIA). Both atrazine and simazine are commonly used herbicides and des-isopropyl atrazine is a breakdown product of atrazine and to a lesser extent, simazine [45]. Due to their ubiquitous use in weed control, all three herbicides are frequently detected in the aquatic environment [46, 47]. Due to its poor affinity with Oasis HLB, a deuterated analogue of DIA is also used as performance reference compound for the POCIS device [48].

Each of the selected herbicides comprises a central triazine ring structure to which a single chlorine atom is attached to the top of the structure. Each compound also contains either one or more primary amine (NH) moieties. As with the other two compound categories, the selected triazines also have varying degrees of solubility in water with atrazine (34.7 mgL$^{-1}$) being more soluble than simazine (6.2 mgL$^{-1}$) and DIA being the most soluble (3,200 mgL$^{-1}$).

1.6.3 Chlorinated Organophosphorus Flame Retardants (OPFRs)

Three structurally similar chlorinated OPFRs were selected for investigation: 1) tris(chloroethyl) phosphate (TCEP), 2) tris(chloropropyl) phosphate (TCPP) and 3) tris(dichloropropyl) phosphate (TDCPP). These compounds are used to prevent the ignition and spread of fire in the material in which they are applied. Chlorinated OPFRs in particular, are commonly used in furniture foams automotive interiors and construction materials [49, 50]. They have replaced the poly-brominated flame retardants used in the past and are thought to be more environmental friendly, although
there is some debate about this in the literature in which a number of studies have shown these compounds to be ubiquitous in the aquatic environment of most industrialised areas [49-52].

All three chlorinated OPFRs selected contain a central ester group to which chlorinated hydrocarbon chains (of varying lengths and with varying number of chlorine atoms) are attached. These compounds also differ in their range of solubility with water with TCEP (7,820 mgL$^{-1}$) being the most soluble and TDCPP (7 mgL$^{-1}$) being the least soluble.

1.7 Aim

The aim of this dissertation is elucidate the mechanisms of adsorption between known hydrophilic contaminants (containing varying functionalities and of differing physicochemical properties) onto a common polymeric sorbent, Oasis HLB, using both empirical investigations (batch adsorption studies and a flow through calibration study) and computational calculations using the hybrid-DFT method. It is anticipated that molecular modelling will bring new insights into the interactions between the analyte and the sorbent in such systems which will generate new knowledge in this area and help to facilitate validating the use of POCIS devices for environmental monitoring purposes.

1.8 Hypothesis

As this dissertation has three main study areas (batch adsorption studies, molecular modelling studies, and chlorinated OPFR POCIS calibration studies) it also has three associated hypotheses. These hypotheses are detailed below.
1.8.1 Batch Adsorption Studies

The hypothesis for the batch adsorption studies conducted for this dissertation is that both the analytes physicochemical properties and structural features will have a significant impact on the ability of each analyte to bind onto the selected sorbent and that this impact will be reflected in the calculated adsorption characteristics of $C_{\text{max}}$, (adsorption maxima) $K_L$ (adsorption affinity), $K_D$ (adsorbent–water distribution coefficient) and $\log K_D$.

This hypothesis was tested by conducting a series of batch adsorption studies with three classes of hydrophilic analytes (xanthines, triazine herbicides and chlorinated OPFRs) and a commercial sorbent (Oasis HLB).

1.8.2 Molecular Modelling

The hypothesis for the molecular modelling studies detailed in this dissertation is that computational methods, in particular hybrid-DFT methods, can predict interactions taking place at the molecular level between analytes and SPE materials.

This hypothesis was tested by conducting a series of computational calculations using a carefully selected hybrid-DFT method. The results were then compared to experimental results in the form of batch adsorption studies. The results of these investigations are reported within this dissertation.

1.8.3 Chlorinated OPFR POCIS Calibration Studies

The hypothesis for the POCIS calibration study is that an established flow-through calibration system can be used to determine sampling rate data of the chlorinated OPFR flame retardant compounds investigated in chapter 7 of this dissertation (TCEP, TCPP, and TDCPP).
This hypothesis was tested by undertaking a POCIS calibration study in a flow-through calibration system using the standard POCIS device and using TCEP, TCPP and TDCPP as the test analytes.

1.9 Objectives

The objectives of this thesis are outlined in Table 1.1 below.

Table 1.1: Thesis Objectives

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>To review relevant literature</td>
<td>2</td>
</tr>
<tr>
<td>To discuss the theory that underpins computational chemistry and to select an appropriate computational method</td>
<td>3</td>
</tr>
<tr>
<td>To develop and investigate relevant experimental and instrumental methods for this dissertation</td>
<td>4</td>
</tr>
<tr>
<td>To determine adsorption characteristics of three xanthine compounds and Oasis HLB using batch adsorption methods</td>
<td>5</td>
</tr>
<tr>
<td>To determine molecular interactions of three xanthine compounds and Oasis HLB using computational methods</td>
<td>5</td>
</tr>
<tr>
<td>To determine the adsorption characteristics of three triazine compounds and Oasis HLB using batch adsorption methods</td>
<td>6</td>
</tr>
<tr>
<td>To determine molecular interactions of three triazine compounds and Oasis HLB using computational methods</td>
<td>6</td>
</tr>
<tr>
<td>To determine adsorption characteristics of three chlorinated OPFR compounds and Oasis HLB using batch adsorption methods</td>
<td>7</td>
</tr>
<tr>
<td>To determine molecular interactions of three chlorinated OPFR compounds and Oasis HLB using computational methods</td>
<td>7</td>
</tr>
<tr>
<td>To determine sampling rate data of COPFR compounds for POCIS device</td>
<td>8</td>
</tr>
</tbody>
</table>
1.10 Outline of Thesis

Chapter 1: Introduction

Chapter 1 provides a brief introduction into the area of research detailed in this thesis including aims and objectives of the studies conducted, and outlines the remaining chapters presented in the thesis.

Chapter 2: Literature Review

The literature review aims to put the research conducted for this thesis in context with previous research that has been conducted in this field of study including a detailed review of POCIS and adsorption characterisation methods.

Chapter 3: Experimental

Chapter 3 details the materials and methods that have been employed to conduct the experimental studies detailed in this dissertation.

Chapter 4: Computational Methods

Chapter 4 describes the computational methods (including key theoretical concepts) that were used for the work outlined in this dissertation.

Chapter 5: Xanthine Compounds

Chapter 5 discusses batch adsorption studies and molecular modelling studies that were conducted to ascertain potential intermolecular bonds that can take place between three xanthine compounds (caffeine, theophylline and theobromine) and the Oasis HLB monomers.
Chapter 6: Triazine Herbicides

Chapter 6 discusses batch adsorption studies and molecular modelling studies that were conducted to ascertain potential intermolecular bonds that can take place between three triazine compounds (atrazine, simazine and DIA) and the Oasis HLB monomers.

Chapter 7: Chlorinated OPFR Compounds

Chapter 7 discusses batch adsorption studies and molecular modelling studies that were conducted to ascertain potential intermolecular bonds that can take place between chlorinated OPFRs and the Oasis HLB monomers.

Chapter 8: Chlorinated OPFR POCIS Calibration Studies

Chapter 8 discusses the results of a POCIS calibration study that was conducted to determine the sampling rate of the chlorinated OPFR using a flow-through calibration set up that was purposely designed for this type of testing.

Chapter 9: Conclusions and Future Recommendations

Chapter 9 brings together the results from chapters 5-8 together to provide an overall discussion of the key findings from these chapters.

Chapter 9 also provides recommendations for future studies that could potentially be undertaken in order to gain greater insight into this area of analytical chemistry.
2 Literature Review

2.1 Water Pollution Monitoring

A standard water monitoring programme comprises a number of different phases, the first being sample collection. Several different sampling strategies currently exist including: grab sampling, automated sampling, biological sampling, and in more recent years, passive sampling. Once the samples have been collected, the analytes are then extracted before being identified and quantified using standard laboratory instrumentation (e.g. HPLC and GC). Each stage of the monitoring programme must be validated in order to ensure that all results generated provide an accurate snapshot of the body of water that is being monitored.

In this review, the pros and cons of each of the common traditional sampling technologies will be explored followed by a review of the most commonly used extraction methods. Following on from this, an extensive review of hydrophilic passive sampling technologies, in particular – POCIS is then provided.

Traditional sampling technologies are known to be time consuming, laborious and can be costly. It was hoped that with the development of passive sampling technologies, that many of the issues that plague traditional sampling technologies could be resolved. However, this review will show that passive sampling technologies are not without their own problems.
2.2 Traditional Sampling

Traditionally there are three main approaches that are used to collect samples from aquatic environments: 1) grab sampling, 2) automated sampling and 3) biological sampling. Each of these methods has their advantages and disadvantages.

2.2.1 Grab Sampling

Grab sampling is still the most commonly used sampling technique for the quantification and qualification of pollutants in aquatic environments. It involves the collection of samples at defined periods of time followed by extraction and pre-concentration in the laboratory [53].

As most hydrophilic contaminants are present in the environment at trace levels, large volumes of water may be required to ensure the compounds of interest are above the limit of detection of the analytical method employed to quantify and qualify these compounds. Collection of large quantities of water is can be costly and time consuming [54].

Due to the irregular influx of these pollutants into the aquatic environment, there is always the potential that pollutants of concern may be underestimated or missed altogether [55]. The analysis of grab samples provides for an instantaneous estimate of the analytes concentration at the time and point of sampling and as such is likely to miss peak inputs in a given aquatic system or the presence of contaminants at trace levels [56].
2.2.2 Automated Sampling

Automated sampling technologies can overcome problems associated with grab sampling as these devices collect samples at regularly timed intervals to produce a more representative sample. Examples of active sampling devices are shown in Figure 2.1.

![Examples of automated samplers](image)

Figure 2.1: Examples of automated samplers- a) a single-bottle composite sampler [57] b) a combination composite / sequential sampler [58]

Whilst these devices are deemed to be a convenient means by which multiple samples can be taken with minimum fuss, they are not without problems of their own. Automated systems can be both expensive to purchase and require experienced personnel to both maintain and operate [59]. Additionally, adsorption of analytes to system components can have a significant impact on the end result [60].

2.2.3 Biological Sampling

Biological sampling is another alternative to grab sampling. Biological sampling predominantly refers to the collection of aquatic organisms, to determine the concentration of selected compounds. A fundamental assumption of biological sampling is that the concentration of the contaminant found within the aquatic organism is indicative of the contaminant present in the aquatic environment [23].
Biological sampling programmes can suffer from a number of issues relating to the uptake of contaminants including the metabolism and clearance of compounds prior to analysis thus leading to an underestimation of contaminant concentration levels. Other potential problems can also arise when it comes to the analysis of biological materials including matrix effects [61]. In addition to this, the interpretation of the resulting data can be complicated by concentration capacity of the organism under investigation [23].

2.3 Sample Extraction

Once samples have been collected, they are then transported back to the laboratory where the analytes under investigation are extracted from the sample matrix and concentrated to a level that is suitable for instrumental analysis. The most commonly used extraction/concentration methods used are liquid/liquid extraction (LLE) and solid phase extraction (SPE).

2.3.1 Liquid/Liquid Extraction

LLE is a method that utilises the differing solubilities of two immiscible liquids (commonly water and an organic solvent) to extract the analytes from one phase into the other (i.e. to extract water pollutants from the sampled water into a common organic solvent). Once the analytes have been extracted, they are then ready for analysis using common laboratory instrumentation such as GC and HPLC.

LLE is most ideal for hydrophobic analytes where they will migrate from the water phase to the organic phase because of the analytes preference for the hydrophobic solvent. Some common organic solvents utilised in LLE are dichloromethane, methyl tert-butyl ether (MTBE) and hexane.
One of the major drawbacks to LLE is the usage of large quantities of organic solvent. Many of these organic solvents are either harmful to human health [62, 63] or a potential environmental pollutant [64], or both. Many commercial and research laboratories have moved away from using LLE as a standard extraction method towards safer and greener methods such as solid phase extraction (SPE) which requires the use of only a fraction of the amount of solvent compared to LLE [65, 66].

2.3.2 Solid Phase Extraction

First developed in the early 1970s, SPE minimises many of the problems associated with LLE [67]. SPE is now one of the most commonly used pre-concentration and clean-up steps in environmental science. It has been theorised that SPE utilises distribution processes to chemically separate the different components of a liquid sample in the same manner as a high-performance liquid chromatography (HPLC) phase [68]. The analytes under investigation partition between the solid/stationary phase (sorbent) and the liquid/mobile phase (the sample). In order for SPE to be successful, the analyte must have a greater affinity for the sorbent than it does for the solvent (usually water) from which it is extracted [68]. The interaction between sorbent and analyte is largely dependent upon the physiochemical make-up of both the sorbent and the analyte. SPE takes advantage of the intermolecular forces at play between the analyte and the sorbent and the various matrices in which the analyte is dissolved. Adsorption of analytes onto sorbents are governed by one or more of the following intermolecular interactions: van der Waals forces (hydrophobic interactions), hydrogen bonding (polar interactions), dipole-dipole forces (polar interactions) and/or ion exchange interactions [69].
A diverse array of SPE sorbent materials exist in the marketplace. Traditional sorbents include reverse-phase sorbents (C8 and C18), normal phase sorbents (alumina and silica), ion exchange and mixed-mode sorbents (a combination of reverse phase sorbents and ion exchange material) as well as functionalised styrene-divinylbenzene resins [70] and hyper-crosslinked polymers. Newer SPE sorbent materials include graphitised carbon and molecularly imprinted polymers [71-73].

Although SPE is available in a number of different formats including cartridges, disks, pipette tips and multi-well plates, the most commonly used format is the SPE cartridge [66]. A schematic of a typical SPE cartridge is shown in Figure 2.2.

![Schematic of a SPE cartridge. a) SPE cartridge b) SEM image of sorbent beads (RMIT University)](image)

2.4 Passive Samplers

Passive sampling is defined as the free flow of analyte molecules from the sampled medium to a receiving phase as a result of differences in chemical potentials. It has applications for the determination of both organic and inorganic compounds in a wide range of matrices including air, water and soil [74].

First used in the 1970s for the monitoring of pollutants in air, in recent years passive sampling has shown promise as a tool for sequestering pollutants in aquatic environments [53]. Passive samplers are a convenient means by which trace
contaminants can be collected in-situ in aquatic environments, overcoming the time consuming process of collecting grab samples.

Passive sampling devices have the ability to provide data to estimate the time-weighted average concentrations as well as the ability to estimate the bio-concentration fraction of polar contaminants over time [23]. Examples of passive sampling used for water pollution monitoring studies include: Chemcatcher[24], semi permeable membrane device (SPMD) [25], diffusive gradients in thin films (DGT) [26], and POCIS [27].

2.5 Polar Organic Chemical Integrative Sampler

The polar organic chemical integrative sampler (POCIS) is a device that consists of a solid sequestration medium (typically Oasis HLB) enclosed within a polymer microporous membrane which is held together by two stainless steel compression rings. The primary purpose of this device is for the integrative sampling of hydrophilic organic chemicals [75].

Over the years, researchers have modified the POCIS device to suit their needs. Such modifications have included varying the type of membrane used (including membrane diameter and porosity) and varying the type and quantity of sorbent used [31, 76, 77].

An expanded view of a typical POCIS sampler is shown in Figure 2.3 and photographic image of a typical sampler/s is shown in Figure 2.4.
According to Alvarez et al. [27], POCIS was originally designed to ‘mimic respiratory exposure of aquatic organisms to dissolved chemicals without the inherent problems of dietary assimilation of chemicals, metabolism, clearance of chemicals, avoidance of contaminated areas, and mortalities of test organisms’. POCIS has since been used to monitor a wide range of contaminants in aquatic environments including pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds, pesticides and industrial chemicals [22].

2.6 Mechanism of Accumulation of Compounds (Theory and Modelling)

The mass of the analyte accumulated by the sampler reflects either the aqueous concentration with which the device is at equilibrium or the time-weighted average (TWA) concentration to which the sampler was exposed during deployment [53].
The accumulation of analytes by passive samplers will generally follow first order kinetics, characterised by an initial integrative phase, followed by a pseudo linear/curvilinear and equilibrium portioning phases [75]. A graphical representation of the uptake kinetics is depicted in Figure 2.5.

![Figure 2.5: The kinetic, pseudo-linear and equilibrium regimes of a POCIS as a function of time [79].](image)

The uptake mechanism of a passive sampling device can be described as a multistage mass transfer process [53]. The initial process is governed by convective processes. The second stage of the process takes place when the analytes diffuse through the aqueous boundary layer and the biofilm layer (if present). The final stage of the process is the movement of the analytes across the polymeric membrane layer onto the sorbent where they will accumulate over time [53].

The mathematical model governing the uptake mechanism of a standard passive sampler is described in equation 2.1 [80].

\[
C_{\text{POCIS}} = C_wK_{sw}(1 - e^{-ket})
\]  

(2.1)
In this equation, $C_{POCIS}$ is the concentration of the analyte in the sorbent (ug/g), $C_w$ is the TWA concentration of analyte in the water (ugL$^{-1}$), $K_{sw}$ is the membrane-water partition constant (Lg$^{-1}$) and $t$ is the exposure time (days).

The elimination rate constant $K_e$ (per day) is described in equation 2.2 [80].

$$K_e = \frac{R_s}{K_{sw}M_{POCIS}}$$ (2.2)

In this equation $M_{POCIS}$ (g) is the mass of the sorbent contained within the POCIS and $R_s$ (mLd$^{-1}$ or Ld$^{-1}$) the sampling rate.

Like other passive samplers, POCIS can operate in either an equilibrium mode or a kinetic mode.

### 2.6.1 Equilibrium Mode

When used in the equilibrium mode, the POCIS is submerged in the water phase until the analyte concentration remains constant [53].

To operate the sampler in the equilibrium mode, the exposure period must be sufficiently long enough to ensure a thermodynamic equilibrium has been achieved between the sampled water and the sampler [53].

If the sampler is used in the equilibrium mode then the concentration of the dissolved analyte fraction is estimated using the sorption phase water partition coefficient ($K_{sw}$) [23] and is determined using equation 2.3 [80]:

$$K_{sw} = \frac{C_{POCIS}}{C_w}$$ (2.3)
A key advantage of using a passive sampling device in the equilibrium mode is that the impact of environmental conditions (such as water temperature and turbulence/flow rate) on the uptake of compounds into the sampler is considered to be negligible [23]. Equilibrium mode is considered to be advantageous when used in aquatic environments that are considered stable (e.g. public swimming pools) or for use indoors (e.g. laboratory exposure experiments) [23].

2.6.2 Kinetic Mode

During the kinetic phase, the sampler acts as an infinite sink for the uptake of the analytes were they will be sequestered linearly relative to time as long as the concentration of the analytes remains constant over the course of the sampling period [75]. In the kinetic mode, the rate by which the analytes are eliminated from the sampler is negligible compared to the rate by which the analytes are sequestered [23]. Kinetic sampling is capable of producing TWA concentrations.

2.6.2.1 Time Weighted Average Concentrations

One of the advantages of a passive sampling device such as POCIS is its ability to provide meaningful data for the determination of time-weighted average (TWA) concentrations [74]. As previously mentioned, the main drawback to grab sampling, apart from cost and time, is that the result produced is a snapshot in time. This may mean that the contaminant of concern is either underestimated or is missed altogether [75].

2.7 Calculating Sampling Rates

In order to determine TWA concentrations, the sampling rate of the analyte under investigation must first be determined. Sampling rates represent the quantity of water
that has passed through the sampler over time, are compound specific and are dependent on environmental variables such as water flow rate/turbulence, temperature, pH, biofouling of the sampler and dissolved organic matter [81-83].

Sampling rates are normally derived by conducting from laboratory-controlled calibration experiments. Once the calibration experiments have been conducted, the analyte concentration in the water phase and the analyte concentration in the sorbent are then determined using equation 2.4 [27, 84].

\[
R_s = \frac{C_s M_s}{C_w t}
\]  

(2.4)

In this equation, \(R_s\) is the sampling rate of the compound (Ld\(^{-1}\)), \(C_s\) is the analyte concentration adsorbed to the sorbent (ng/g), \(C_w\) is the analyte concentration in the water (ngL\(^{-1}\)), and \(t\) is the sampling time (days) and \(M_s\) is the mass of the sorbent (g).

Sampling rates may also be derived by analysing the remaining in the water phase following post POCIS exposure as per equation 2.5 [79].

\[
R = \frac{C_i - C_t}{C_i} \times \frac{VT}{t}
\]  

(2.5)

In this equation \(C_i\) is the initial concentration of the analyte in the water (ngL\(^{-1}\) or µgL\(^{-1}\)), \(C_t\) is the concentration of the analyte in the water at time \(t\) (ngL\(^{-1}\) or µgL\(^{-1}\)). \(VT\) is the total volume in the calibration tank (L) and \(t\) is the sampling time (days).

The assumption made with this approach is that loss of analyte loss to degradation or adsorption is negligible.
2.8 The Calibration of POCIS

When the POCIS is operated in the equilibrium mode, calibration of the sampler is dependent on estimating the sampler/water partition of the analytes of concern which depending on the compound, maybe determined by evaluating the physiochemical properties of the analytes [85]. When the POCIS is operated in the kinetic regime, calibration of the sampler is dependent on determination of the sampling rate of the analyte. Compound specific sampling rates are determined via laboratory-controlled calibration studies. Several methods have been described in literature for the estimation of sampling rates for a wide range of compounds [85].

Calibration of the POCIS used in the kinetic mode involves the submersion of the POCIS device into water which is then spiked with compounds of interest at known concentrations. In general, most calibration studies are conducted under laboratory controlled conditions (temperature, agitation, flow rate, turbulence, and pH) [86].

The advantages of laboratory-controlled calibration studies is that the sampling rates obtained can be considered to be reliable as they are based on constant and controlled micro pollutant concentrations. [79].

2.9 Diffusion Pathways /Barriers

The rate at which analytes are sequestered by the sampler is in part controlled by the barriers contained within the sampler. The accumulation of analytes into the POCIS can be described using a first-order kinetic model. However, this model is only suitable if the accumulation kinetics are ruled by single transfer step from one compartment to another (e.g. polymeric membrane samplers) [48].
When it comes to the sequestration of analytes into the POCIS device, there are a number of different boundary layers which must be overcome in order for the analytes to accumulate in the sampler’s adsorbing phase. These barriers include the water boundary layer (WBL), the polymeric membrane and possible biofilm. A secondary WBL may also exist between the polymeric membrane and the sorbent [27]. These barriers act to retard the rate at which the analytes are transferred into the sorption phase. The diffusive limiting pathways are shown in Figure 2.6.

Figure 2.6: Diffusion limiting Pathways [87]

The first two barriers, the WBL, and the polymeric membrane are intrinsic components of the sampler. The third barrier, the biofilm layer, accumulates on the surface of the sampler during operation.

This section of the review will detail how these boundary layers can have an impact on the rate at which compounds are sequestered into the sampler and will discuss each barrier in turn.

2.9.1 Water Boundary Layer

The first boundary the analyte must cross is the WBL which is also known as the aqueous boundary layer (see Figure 2.6). The mechanism by which the analytes cross the WBL is via diffusion [86]. The WBL is a retarded layer of water that surrounds the
outer parameter of the passive sampling device. It has been hypothesised that water flow rate has a direct impact on the thickness of this boundary layer and thus the rate at which the analytes will accumulate into the sampler [86]. As the flow rate of the water increases, the WBL should decrease, thus resulting in an increase in analyte transfer into the sampling device [88]. Research has shown this to be the case in certain circumstances for the POCIS device. This topic will be further explored in section 2.10.1 Water Flow Rate / Turbulence.

2.9.2 Biofouling

The formation of biofilm on the polymeric membrane is also considered to be a rate limiting diffusion barrier. The nature and composition of the biofilm layer is dependent upon the environment into which the sampler is deployed and the length of deployment. Biofouling is the process by which unprotected submerged objects are colonised by various bacteria and flora and fauna which may ultimately form a biofilm across the surface of the object [53]. Environmental conditions can have an impact on the morphology (including thickness and density) of the film which means that the composition of the biofilm is dependent on the aquatic system being investigated [85]. The types of organisms that will colonise a submerged surface are dependent on the location of the aquatic system and can be endemic to that area.

Biofilm impacts the sequestering of compounds into the sampler by increasing resistance to mass transfer of the analyte across the polymeric membrane and therefore reduces the sampling uptake rates of these compounds [53].
2.9.3 Polymer Membrane

Transfer of analytes through the polymeric membrane is described as a biphasic process in which the analytes traverse the membrane either through the water wet pores or through the polymeric membrane itself [27]. The polymeric membrane performs as a semipermeable membrane between the sorbent and the environment, controlling the rate at which the analytes are sequestered by the sampler and protecting the sorbent from being fouled by particulate matter, micro-organisms and macromolecules greater than 0.1 µm in size. As such, typically, POCIS devices are constructed with membranes that have a porosity of 0.1 µm, although membranes with differing porosity to the standard POCIS device have also been investigated by researchers (e.g. 0.45 µm [89, 90] and 30 µm [76]). Additionally, most POCIS devices that have been constructed for research purposes have a typical surface area of 41 cm² however; some researchers have modified the original design and have thus opted for membranes with smaller surface areas such as 16 cm² [89]. Both porosity and surface area are expected to have an impact on the rate at which analytes accumulate into the sampler. It can reasonably be expected that as membrane porosity and surface area increases, analyte sampling rate should also increase.

As discussed in section 2.9.1.1, biofouling of the polymeric membrane can impact analyte sampling rate. During the development of the original sampler, Alvarez et al. [27] examined several different polymeric membranes including polyethylene, polyvinylidene fluoride (PVDF), regenerated cellulose, acrylic copolymer, nylon, hydrophilic polypropylene before settling on the polyethersulfone (PES) membrane due in part to its superior resistance to biofouling. However, as with porosity and surface area, some researchers have selected to deviate from the standard POCIS device with alternative membrane selections (e.g. nylon [76]).
2.9.4 Receiving Phase

The receiving phase of the sampler is where the analytes accumulate over time. It is also the medium from which the analytes are extracted from for analysis. As previously discussed, the receiving phase of the POCIS device is a SPE sorbent material. Whilst research has shown that certain analytes can accumulate in the polymeric membrane, due to biofouling, analytes are typically only ever extracted from the sorbent material. Some researchers have however, studied transfer kinetics of compounds across the polymeric membrane and have found that the compound physicochemical properties had the biggest impact on their transfer through and onto the rate limiting membrane[91].

Whilst a number of different SPE materials could potentially be used for the POCIS device, the sorbent that was selected for the investigations discussed in this dissertation was Oasis HLB.

Oasis HLB is a hyper-cross-linked polymer that was developed by Waters Corporation in the early 1990s to overcome the limitations that were experienced with early polymeric sorbents that contained only divinylbenzene (DVB) [92]. These early sorbents were not as efficient at binding with hydrophilic compounds as they were with hydrophobic compounds [92]. As the diagrams in Figure 2.7b and 2.7c show, Oasis HLB comprises both a hydrophobic monomer in the form of DVB and a hydrophilic monomer in the form of N-vinylpyrrolidone (NVP). The addition of the NVP monomer gives the new sorbent a ‘hydrophilic hook’ resulting in an improved extraction efficiency of water soluble compounds [92]. Furthermore, the addition of the NVP monomer, also ensures the sorbent does not accidently dry out during standard SPE operation, as water is able to hydrogen bond to the C=O region of the NVP monomer. Oasis HLB also has a relatively high surface area compared to the older style sorbents
due in part to the fact that as well as helping to facilitate the adsorption of compounds onto the sorbent matrix, DVB also acts as a crosslinking agent \[92\]. The properties of Oasis HLB are presented in Table 2.1.

As Oasis HLB is a commercial product that is registered by the Waters Corporation, the exact structure of Oasis HLB is not known. The main uncertainty with the structure of the Oasis HLB lies with the structural configuration of the DVB monomer.

DVB has three isomers where the divinyl moieties of the monomer can be attached to the benzene ring either in the para, ortho, or meta position. Initial optimisation calculations conducted for the DVB monomer showed that the meta-DVB molecule was the most energetically favourable of the three DVB structures. Therefore, all computational calculations detailed in this dissertation involving DVB have been conducted with DVB in the meta form. Meta-DVB is simply referred to as DVB from here on.

![Diagram](image)

Figure 2.7: Oasis HLB Sorbent: a) polymeric representation, b) NVP, c) meta-DVB
Table 2.1: Properties of Oasis HLB [92]

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Surface Area</td>
<td>800 m²·g⁻¹</td>
</tr>
<tr>
<td>Average Pore Diameter</td>
<td>80 Å</td>
</tr>
<tr>
<td>Specific Pore Volume</td>
<td>1.3 mL·g⁻¹</td>
</tr>
<tr>
<td>Average Particle Diameter</td>
<td>30 µm or 60 µm*</td>
</tr>
</tbody>
</table>

*In SPE cartridges that contain over 200 mg of sorbent

### 2.10 Factors Impacting Sampling Rate

A thorough review of the available research conducted on the standard POCIS device shows that compound specific sampling rates are affected by a number of different factors including environmental factors (water flow rate, temperature, salinity, pH) and compound physicochemical properties.

#### 2.10.1 Water Flow Rate / Turbulence

As previously discussed in section 2.9.1, it can be expected that water flow rate (or turbulence) should impact the degree to which compounds accumulated in the sampler and that there should be a direct correlation between water flow rate and compound sampling rate. A number of studies have been conducted to investigate the impact of water flow on the sampling rates of compounds by the POCIS using a wide range of laboratory controlled methods.

Studies conducted to determine the impact of water flow rate / turbulence on the sampling rate of POCIS have either been conducted under laboratory-controlled conditions either using static / static renewal methods or using flow through methods or in the field using in-situ techniques.
Li, Vermeirssen [83] studied the uptake of a variety pharmaceuticals and personal care products (PPCPs) in municipal wastewater. The study utilised channels to investigate four different flow rates regimes, ranging from 2.6 cm s\(^{-1}\) to 37 cm s\(^{-1}\). The study determined the concentration of compound accumulated into the samplers rather than provide sampling rate data for the compounds investigated. The results from the study showed that whilst an increase in flow increased the amount of compound accumulated in the sampler for most compounds investigated, an increase in flow rate had little impact for some of the compounds investigated. The authors postulated that a difference in compound physicochemical properties is the most likely reason why change in flow rate did not impact all compounds in the same manner. One of the limitations of this study was that the authors did not provide a detailed discussion explaining how and why they believed the physiochemical properties of the target analytes impacted the results of their study, they just stated that this is a possible reason for the variation seen in the results. Additionally, sampling rates were not calculated for the analytes investigated in this study rather the total amount of each compound that had accumulated into the sampler was measured instead. Whilst the results showed that flow rate did have an impact on the compound accumulation, the overall impact was minimal as there was less than two-fold difference between samplers exposed to the lowest flow rate compared to samplers expose to the highest flow rate.

Charlestra, Amirbahman [93] showed that the sampling rates generated in a stirred system produced similar results to sampling rates generated in a flow through system and that both studies generated higher sampling rates compared to a non-stirred system, although, like the study conducted by Li et al. [83], the difference was less than two-fold. From these results, the conclusion was made that it might not be necessary to
apply a correction factor to the sampling rate data to account for variability in water flow rate/turbulence.

2.10.2 Temperature

A limited number of studies have been undertaken in order to elucidate the impact that temperature may have on POCIS sampling rate. One such study was conducted by Soderstrom et al. [23].

It was theorised by Soderstrom, Lindberg [23] that an increase in temperature results in an increase in water solubility leading to a decrease in the amount of analyte that will partition to particulate matter. Therefore, as the POCIS measures the dissolved fraction of the analyte, and as temperature has an impact on how much of the analyte is freely available in the water phase, it can be predicted that temperature will have an impact on the rate at which the POCIS sequesters analytes from the environment. An increase in temperature should result in an increase in the rate in which the analyte accumulates in the sampler.

Temperatures in aquatic environments can fluctuate not only throughout the year (seasonal variation) but also throughout the day. As such, temperature is an important consideration when calibrating and operating a POCIS device.

Togola and Budzinski [94] studied the uptake of pharmaceuticals into a POCIS over the temperature range of 15 to 21 °C and found that as the temperature increased, the rate at which the compounds were sequestered into the sampler also increased. Li, Helm [95] also reported a similar result with a two-fold increase in sampling rates for 30 pharmaceutical and personal care products and endocrine disrupting compounds when sampled temperatures between 5 to 25 °C.
2.10.3 Salinity

An increase in salt concentration often leads to a decrease in water solubility of some analytes leading to an increase in the uptake of these compounds by the POCIS device due to an increase in adsorption efficiency [23]. This is known as the salting out effect; a phenomenon that is regularly taken advantage of when extracting analytes from SPE sorbents. Studies that have been undertaken to test the impact that salinity has on the sampling rates of the POCIS device have had varying results.

The studies detailed below show that the effect that salinity has on POCIS sampling rates is highly compound specific.

Togola and Budzinski [94] who tested the impact of salinity on the sampling rates of selected pharmaceuticals including both acidic and basic compounds found that whilst the sampling rates remained constant in both saline and non-saline environments, the basic compounds recorded up to 64% difference between the two environments.

In contrast, Zhang, Hibberd [77] who tested the effect of salinity (0.18 -35 PSU) on the sampling rates of endocrine disrupting compounds and pharmaceuticals (both acidic and basic) reported that the saline conditions had minimal impact on the sampling rates of the compounds tested.

Although it can be seen that environmental variables such as water flow rate, temperature and salinity can have an impact on the rate at which compounds are sequestered by the POCIS device, studies have also shown that compound physicochemical properties also directly impact the degree to which these environmental factors affect the uptake of these compounds into the sampler.
The next section of the review surmises additional research that has been completed in this specific area. That is, it examines research that has been conducted to specifically explore the impact that compound physicochemical properties has on sampling rates for POCIS.

2.10.4 Water pH and Compound pKa

It can be reasonably expected that compound water pKa will have a direct impact on the ability of all compounds (whether they be acidic, alkaline or neutral) to adsorb on to the receiving phase of the sampler.

Li, Helm [95] tested the effect that water pH (pH of 3, 7, and 9) has on POCIS sampling rate relative to compound pKa. As part of the study, three different POCIS sampling devices were evaluated: each containing a different type of sorbent. The sorbents that were trialled were; Oasis HLB, Oasis MAX (anion exchanger sorbent) and Oasis MCX (cation exchanger sorbent).

Overall, the authors found that sampling rates for acidic pharmaceuticals were higher at low pH (i.e. in their neutral form) than at high pH (in their ionised form) whilst basic pharmaceuticals were found to be higher at high pH (in their neutral form) than at low pH (in their ionised form). The sampling rates of neutral compounds exhibited minimal variation when tested across a range of pH values.

Additionally, the study also found that little was to be gained from using either Oasis MAX or Oasis MCX for charged compounds when the pH of the water being examined was neutral.
2.10.5 Compound Hydrophobicity

A number of researchers have attempted to correlate compound properties such as hydrophobicity or molecular weight with sampling rate so that the sampling rates of similar compounds derived theoretically without the need to conduct experimental investigations.

Theoretically, it is expected that there should be a positive correlation between sampling rate and increasing log $K_{ow}$ values. This is due to the fact that as hydrophobicity of the compound increases so will its tendency to adsorb onto solid material.

Togola and Budzinski [94] showed a positive correlation between sampling rate and log $K_{ow}$ when evaluating both basic and neutral analytes in freshwater this correlation was not evident with acidic compounds in saline water. Additionally, Li et al. [83] also described a positive correlation with log $K_{ow}$ sampling rate for some of the compounds they investigated but not for others.

2.11 Performance Reference Compounds

As PRCs compensate for potential discrepancies between laboratory derived sampling rates and field derived sampling rates and therefore can act as a quality control measure [22]. PRCs are deuterated analogues of the analytes under consideration, that are loaded into the sampler prior to deployment and that offload at a measurable rate [85]. Ideally the PRC is a compound that has moderate to high fugacity from the sampler and does not interfere with the analytical process [35].

The rate at which the PRC dissipates into the aquatic environment can be used to offset the impact of environmental variables such as water flow rate/turbulence, temperature and sampler biofouling if the rate at which the PRC is released into the environment and
the rate at which the analytes of interest are sequestered by the sampler operate isotopically [35]. The rate at which the PRC dissipates into the aquatic environment can be determined using equation 2.5. This is known as the elimination rate constant [35].

\[
K_{ePRC} = \frac{\ln \left[ \frac{C_{PRC}(0)}{C_{PRC}(t)} \right]}{t}
\]  

(2.6)

In this equation \(K_{ePRC}\) is the elimination rate constant, \(C_{PRC}(0)\) is the initial concentration of the PRC (μg g\(^{-1}\)) adsorbed on the sorbent, \(C_{PRC}(t)\) is the residual concentration of the PRC after time \(t\) (μg g\(^{-1}\)) and \(t\) is the exposure time (days).

The kinetics of analyte uptake versus PRC dissipation is depicted graphically in Figure 2.8.

![Figure 2.8: Analyte vs PRC Kinetics [96]](image)

The elimination rate of the PRC is first determined in laboratory calibration studies before being investigated in the field. Using these two values, the laboratory derived sampling rate can then be corrected to adjust for environmental conditions as shown in equation 2.6 [35].
\[ R_{\text{Scorr}} = R_{\text{Scal}} \times \left( \frac{k_{e\text{PRC}_{\text{insitu}}}}{k_{e\text{PRC}_{\text{cal}}}} \right) \] (2.7)

In this equation, \( R_{\text{Scorr}} \) is the corrected calibration rate, \( k_{e\text{PRC}_{\text{cal}}} \) is the elimination rate of the PRC (laboratory determined), \( k_{e\text{PRC}_{\text{insitu}}} \) is the elimination rate of PRC (field determined) and \( R_{\text{cal}} \) is the calibrated sampling rate (laboratory determined).

When sampling for a range of analytes with varying physiochemical properties, it is expected that a number of different PRCs will be required as the assumption is made that the kinetics of the PRC and the analyte will correlate closely to each other [35].

### 2.11.1 Challenges with the PRC Approach

Whilst the PRC approach has been successfully applied to hydrophobic passive samplers such as the semi-permeable device (SPMD) [97-99], this is not the case with hydrophilic passive samplers such as POCIS. Although several studies have been conducted evaluating the use of various PRCs with POCIS [3, 48, 100], unlike the SPMD, the mechanism by which these compounds dissipate into the aquatic environment have not been fully elucidated as yet. Harman et al. [38] state that further research is required not only to determine the mechanisms by which PRCs dissipate into the environment but also the manner by which analytes are sequestered in the first place.

The next section of the review focuses on sorption mechanisms that control the interactions between analytes and SPE material.

### 2.12 Sorption Mechanisms

Sorption is a dynamic process between adsorption and desorption. When in solution, if an analyte and sorbent are in contact with each other for a sufficient period of time,
equilibrium will form between the amount of analyte that has been adsorbed onto the sorbent and the amount of analyte that remains in solution. The equilibrium constant, \( K_D \) as shown in equation 2.8 mathematically describes the interactions between analytes, sorbents and solution [101].

\[
K_D = \frac{[A]_{\text{Sorbent}}}{[A]_{\text{Solution}}}
\]  

(2.8)

In this equation \([A]_{\text{Sorbent}}\) is the concentration of the analyte adsorbed onto the sorbent and \([A]_{\text{Solution}}\) is the concentration of the analyte that remains in the solution. The magnitude of the \( K_D \) value indicates the degree to which the analyte has interacted with the sorbent. During the adsorption phase of SPE, \( K_D \) values are typically large when the analyte preferentially interacts with the sorbent and are typically small (close to zero) during the process of desorption when the analyte preferentially desorbs from the sorbent into the extracting solvent [101].

If the reverse is true (that is the value of \( K_D \) is small during the adsorption stage and/or large during the desorption stage), then the analytes will be poorly extracted from the solution in which they are dissolved. In terms of environmental monitoring, this means that the derived concentration values will not truly represent what is in the aquatic environment.

The same conditions that are required for producing a reliable data with the SPE method, also apply to passive sampling. That is, the analytes of interest must have a greater affinity for the receiving phase of the sampler than it does to the aquatic environment.

Another factor that we should keep in mind when evaluating the robustness of the POCIS device, is its ability to hold on to the sequestered compounds for the duration of
the sampling period. When the bond between the analyte and the sorbent is weak, there is always the possibility that the analyte could desorb from the sampler. This would therefore potentially make the POCIS device an unreliable sampling method for these analytes.

2.12.1 Sorption Mechanisms

Mechanisms of adsorption/desorption are governed by intermolecular forces that occur between the analyte, the solution in which the analyte is dissolved and the surface chemistry of the sorbent [67]. Two fundamental processes control adsorption – chemisorption and physisorption.

Chemisorption takes place when a compound adsorbs strongly onto the surface of a solid material resulting in the formation of strong and irreversible bonds between the analyte/s and sorbent (i.e. covalent bonds) and the desorption process previously mentioned, is minimised.

Retention of analyte/s onto SPE polymeric material is normally achieved by the formation of reversible interactions between the analyte and the surface of the sorbent [69]. This type of adsorption is known as physisorption. These interactions are characterised by the formation of London dispersion forces, polar (hydrogen bonding and dipole-dipole forces), electrostatic/ion exchange interactions and π···π interactions [69, 70] all of which vary in strength. Typical binding strengths that are associated with each of these intermolecular interactions along with examples of sorbents that are dominated by these interactions are detailed in Table 2.2.
Table 2.2: Binding energy values of interactions in SPE [70]

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Sorbents</th>
<th>Energy of interaction [kcalmol(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>London Dispersion forces</td>
<td>Octadecyl, octyl, ethyl, phenyl, cyclohexyl, styrene-divinyl benzene</td>
<td>1-10</td>
</tr>
<tr>
<td>Polar / dipole-dipole</td>
<td>Cyani, silica, alumina, Florisil</td>
<td>1-10</td>
</tr>
<tr>
<td>Hydrogen bonding</td>
<td>Amino, diol</td>
<td>5-10</td>
</tr>
<tr>
<td>Electrostatic bonding</td>
<td>Cation exchange, anion exchange, graphitised carbon</td>
<td>50-200</td>
</tr>
<tr>
<td>(\pi\cdots\pi) interaction</td>
<td>Styrene divinylbenzene, porous graphitized carbon</td>
<td>1-5</td>
</tr>
</tbody>
</table>

2.12.2 The Sorption Mechanisms of Oasis HLB

Whilst a number of studies have been undertaken to validate Oasis HLB as a SPE medium [102, 103], only a limited number of studies have been conducted to determine its sorption characteristics with common environmental contaminants. One such study was performed by Dias and Poole [104] who investigated the mechanistic interactions between an array of compounds and Oasis HLB via an online SPE method.

In this study, Oasis HLB was treated as a packing material in a HPLC column. Dias and Poole measured the retention factors for their selected analytes before applying the solvation model approach in order to characterise sorption characteristics with their selected analytes and Oasis HLB.

Dias and Poole determined that when used in a HPLC system, Oasis HLB prefers to interact with water resulting in lower retention values for the selected analytes compared with other sorbents tested. Additionally, the authors also postulated that Oasis HLB is best suited to low molecular weight polar compounds.
Bäuerlein et al. [105] also conducted an investigation with the intent to elucidate the mechanism of interaction between a select group of compounds and the Oasis HLB sorbent. In contrast to the study conducted by Dias and Poole[104] who conducted their investigations using online methods, Bäuerlein et al. utilised the batch adsorption method. The aim of their investigation was to explore the role in which compound structure (in particular functional groups) affects the ability of the selected compounds to bind onto Oasis HLB (among other sorbent materials). By analysing the batch adsorption results, Bäuerlein et al. were able to generate a number of general rules of thumb which outline how the analytes functional groups affect the ability of Oasis HLB to sequester compounds from water. Bäuerlein et al. also raised the idea that the mechanism, by which an analyte is adsorbed onto Oasis HLB or to any sorbent, in general, is largely governed by the structural characteristics of the analyte itself. The results from this study showed that neutral analytes had the highest adsorption capacity and affinity with Oasis HLB sorbent compared to the other sorbents tested (MCX, WCX, WAX and MAX).

A key finding from the research conducted by Bäuerlein et al. was that apolar components of the analytes investigated, in particular aromatic rings, are important for the sorption process for two fundamental reasons. Firstly, the more pronounced the apolar part is in the analyte, the greater the adsorption maxima and adsorption affinity with Oasis HLB. Secondly, that the aromatic ring structure of the sorbent (the DVB monomer) can interact with the aromatic moieties of the analyte to form \( \pi \cdots \pi \) interactions.

Another key finding from the research conducted by Bäuerlein et al. is that Van der Waals forces instead of hydrogen bonding seem to be the dominant intermolecular interaction governing the sorption of neutral analytes onto Oasis HLB. Bäuerlein et al.
hypothesised that analytes are unlikely to hydrogen bond onto the sorbent matrix as a stronger and more energetically favourable interaction is likely to take place with water and the sorbent instead.

Following on from Bäuerlein et al., Hofman-Caris et al. [106], conducted a similar investigation with a select group of analytes and Oasis HLB (among other sorbents). The aim of the research reported by Hofman-Caris et al. was to determine the feasibility of using polymeric sorbents as part of a novel wastewater treatment process. The adsorption studies conducted by Hofman-Caris et al. applied the same batch adsorption methods as used by Bäuerlein et al. in their investigations. As expected, the assumed interactions were theorised to be a combination of hydrogen bonding and π⋯π interactions. The results of Hofman-Caris et al. studies indicated that hydrophobic compounds interacted well with Oasis HLB when adsorbed from ultrapure water which is what Bäuerlein et al had discovered in their findings.

In addition to this, it was found that in contrast to the findings of Dias and Poole [104], these hydrophobic interactions were more effective with analytes that had a relatively high molecular weight. The rationale behind this observation is that higher molecular weight compounds also have a large surface area which would therefore result in a higher adsorption affinity/capacity with Oasis HLB in contrast with lower molecular weight compounds.

A reason for the discrepancy in the results achieved by Dias and the results achieved by Poole and Hofman-Caris et al. is that whilst Dias and Poole conducted their study using a HPLC method, Hofman-Caris et al. studies were conducted with a batch adsorption method. Whilst it is commonly believed that analytes behave in the same manner in a HPLC system as they would in a SPE system [68], a recent review conducted by Andrade-Eiroa et al. [107]. theorised that this is not the case. It was postulated by
Andrade-Eiroa et al. that the mechanisms of interaction differ in a system that is operating at high pressure (HPLC) compared to a system that is operating at low pressure (SPE). Andrade-Eiroa et al. asserted that in a HPLC system, interactions between analytes and the sorbent matrix is likely to be controlled by the polarizability of the analyte. In contrast, in a SPE system, interactions between analytes and the sorbent matrix is likely to be controlled by the solubility of the analyte. Whilst the studies reported by Dias and Poole might not mimic the exact conditions under which analytes interact with the sorbent matrix in a SPE system and as an extension of that - sorbent passive samplers, the reported results still provide important information about how the Oasis HLB sorbent functions, especially in relation to how it interacts with water.

A more recent study by Jeong et al. [108] determined the equilibrium constant (K_D) of environmentally relevant organic compounds with Oasis HLB. Like Bäuerlein et al. [105] and Hofman-Caris et al. [106], Jeong et al. [108] also employed the batch adsorption method in order to determine adsorption characteristics of their selected analytes and Oasis HLB sorbent. However unlike Bäuerlein et al. and Hofman-Caris et al., Jeong et al. used smaller sorbent masses (1 mg for all analytes), smaller solute volumes (studies were conducted in HPLC vials which have a typical volume of 2 mL) and a lower analyte concentration range. Additionally, whilst Bäuerlein et al. and Hofman-Caris et al. [106] investigated each analyte independently of each other, Jeong et al. conducted their studies with all analytes contained in a single mixture.

The results from the investigations conducted by Jeong et al. showed that the sorption of the selected analytes were affected by their physicochemical properties such as log D (water distribution coefficient) and pKa and environmental factors such as temperature, solution pH. All analytes investigated were found to be affected by temperature with the ability of the analytes to partition to Oasis HLB decreasing with increasing solute
temperature. Solution pH was shown to impact the ability of charged analytes to partition to Oasis HLB but had no impact on neutral analytes. Whilst the ability of anionic analytes to partition onto Oasis HLB decreased with increasing solution pH, the ability of cationic analytes to partition to Oasis HLB actually increased with solution pH. The results of the study also showed that the primary driving force behind the interaction between Oasis HLB and the selected compounds were through apolar interactions. These results concur with the results that were derived from both Bäuerlein et al. [105] and Hofman-Caris et al. [108] investigations into analyte/Oasis HLB interactions.

In summary, the results of above mentioned sorption studies show that both physicochemical properties of the analytes under investigation and environmental factors such as temperature, solution pH and salinity as well as analyte concentration all impact the ability of the selected analytes to interact with Oasis HLB.

However, there are still gaps in the knowledge relating to how compound structure, and compound physicochemical properties affect bonding interactions with the sorbent material contained within the POCIS device. Experimental data can only tell you so much.

Additionally, experimental studies that are conducted in order to characterise interactions between sorbent and analyte/s are often laborious and time consuming. One of the aims of this dissertation was to investigate a means by which adsorption phenomena could be investigated using computational means so as to reduce the amount of experimental studies required.

The next section of the literature review, examines how computational chemical methods have already been successfully applied to advance our understanding of
customised sorbent materials, otherwise known as molecular imprinted polymers or MIPS. Conversely, this area of the literature review explores how this approach can be used to help explore interactions taking place between hydrophilic compounds and Oasis HLB sorbent (the most commonly used receiving phase for the Oasis HLB sampler).

2.1.2.3 A Computational Chemistry Approach

Computational chemistry is a division in chemistry that utilises both theoretical physics and modern computer software in order to solve often complex chemical problems. This dissertation will show that computational chemistry has the ability to detail molecular level interactions taking place between analytes and sorbent. One area of research in which computational chemistry is commonly employed, is in the design of MIPs.

MIPs are a class of highly cross-linked polymers that have the ability to bind to target analytes with a high level of specificity. In other words, MIPs differ from traditional SPE materials in that they can be custom designed so that selected analyte adsorbs onto the MIP sorbent in preference to all other compounds.

MIPs are prepared in the laboratory using the target analyte as a template. In order to create MIPs, functional monomers that have a high affinity for the target analyte must first be selected. Once the target monomers have been chosen, they are then mixed with the template monomer in order to generate a cavity within the MIP in which can later be used to sequester the target analyte from the fluid that is being investigated. A thorough review by Takeuchi et al. [109] written in 2016 provides a comprehensive analysis of the nature and development of MIPs up until its publication date.

In this field of study, computational methods are often used to determine the interactions between the functional monomers and the compounds of interest [73, 110].
Investigations involving computational chemistry approaches help to expedite the selection process for the MIP’s functional monomers as these studies provide useful information about the types of bonds that can form between the selected functional monomers and the target analyte and helps to determine the strength of these interactions. This information can then be used to ensure the most efficient functional monomer/s are selected during the design phase of the MIP.

A thorough review conducted by Nicholls et al. [111] examines cases in literature up until its publication date in 2009, where computational approaches had been used to develop MIPs for a range of analytes. Specifically, the review explores how each of the above mentioned computational methods had been utilised in order to 1) design MIPs and 2) to evaluate MIPs.

Nicholls et al [111] discusses two commonly used computational chemistry approaches used in the characterisation and evaluation of MIPs – 1) the quantum mechanical approach and 2) the molecular dynamics approach.

The quantum mechanical approach can be further subdivided into two methods: ab initio methods and semi-empirical methods. Each of these computational methods can be used to describe the chemical properties of two or more interacting molecules through the approximation of the electron distribution of the molecules under investigation. This approach is known to be computationally expensive [111, 112].

The second approach explored is the molecular dynamics approach. This approach is based on classical Newtonian physics which means that it is not as computationally as expensive as the first approach[112]. This approach is commonly used to investigate the possible formation of non-covalent bonds between two or more molecules over time.
This approach also allows for the investigation of large systems but without the accuracy that is afforded by the use of quantum chemistry approaches.

Newer methods include a combination of the first approach (the quantum mechanical approach) and the second approach (the molecular dynamics approach).[111] This allows for the application of quantum mechanical techniques in targeted areas (e.g. bond formation of the template molecule and the active site of the MIP polymer) whilst applying a molecular dynamic approach to the remaining portion of the MIP polymer which is not partaking in the bond formation between template and MIP polymer and which would not require the same level of investigation as the active site would.

Whilst a number of computational approaches are available, each computational approach has its advantages and disadvantages. Further exploration of this area will be undertaken in chapter 4 of this dissertation.

2.13 Conclusion

This literature review shows that the POCIS passive sampler has the potential to be a viable means by which trace level environmental contaminants can be sampled in aquatic environments. This review also shows that the mechanisms by which compounds accumulate into the receiving phase of the sampler are not fully understood. Whilst some research has been undertaken in this area, there is still scope for further exploration.

One of the main objectives of this dissertation was to use a similar computational approach as commonly used in MIP studies so as to investigate the potential molecular interactions at play between Oasis HLB and three classes of environmental contaminants (xanthine compounds, triazine herbicides and chlorinated flame retardants) so as to close some of the knowledge gaps explored in this review.
3 Experimental

3.1 Introduction

Robust data is reliant on both sound experimental methods and reliable and validated instrumental systems. Appropriate selection of both experimental and instrumental methods is contingent on the operator having knowledge of the limitations that each method possesses.

Experimental data presented in this dissertation primarily consist of results from batch adsorption studies that were conducted to determine adsorption characteristics of the selected compounds with Oasis HLB sorbent. Additionally, a POCIS calibration was also conducted to determine sampling rate data for chlorinated OPFRs under laboratory controlled conditions.

Instrumental techniques that were selected for this dissertation were selected due to their ability to adequately determine the concentrations of the investigated compounds post exposure to Oasis HLB sorbent.

The aim of this chapter is to describe the materials, methods and analytical techniques that were used to gather data for this dissertation.

3.2 Materials

All chemical reagents were used as received and were of an analytical grade or higher. All standards, excluding deuterated versions were purchased from Sigma Aldrich (Castle Hill, NSW, Australia). HPLC grade methanol was obtained from Merck Australia (Frenchs Forest, NSW, Australia). Deuterated chlorinated OPFRs were purchased from Novachem (Collingwood, Vic, Australia). SPE cartridges (Oasis HLB...
200 mg and Oasis HLB 1 g) were purchased from Waters Corporation (Rydalmere, NSW, Australia). Supor® PES, 0.2 µm syringe filters and 90 mm Supor® PES, 0.1 µm membrane filters were obtained from Pall Corporation (Cheltenham, VIC, Australia). Purified water was collected in-house from a Milli-Q water purification system (Merck-Millipore, Bayswater, VIC, Australia) with a resistivity of 18 mega-ohms.

3.3 Batch Adsorption Studies

Batch adsorption studies are conducted in order to elucidate adsorption characteristics such as maximum adsorption value ($C_{\text{max}}$), the adsorption distribution coefficient ($K_D$) and the adsorption affinity value ($K_L$) and the equilibrium constant ($K_D$). All of these values give insight into the potential mechanisms at play when analyte/s are adsorbed onto sorbent.

The batch adsorption method involves the preparation of a series of solutions of the analyte under investigation at known concentrations followed by the addition of a known quantity of sorbent. The analyte/sorbent mixture is then agitated for a specific period of time, after which the sorbent is removed from the mixture either by filtration or centrifugation. It is expected that during the mixing phase of the experiment that a certain percentage of the compound/s will have been adsorbed onto the sorbent and that a certain percentage of the compound/s will still be present in the aqueous phase. By analysing the concentration of the compound/s that remain in the aqueous phase, we can then deduce how much of the compound/s has/have been adsorbed onto the sorbent. It is also necessary to ensure that the compound/s have actually been adsorbed onto the sorbent and not onto the materials (e.g. glassware) used in the batch adsorption study or if a filter was used to remove the sorbent, onto the filter.
The batch adsorption studies conducted for this dissertation were modelled on a study conducted by Bäuerlein et al [105] for comparative purposes.

### 3.3.1 Single Compound Batch Adsorption Studies

A series of single compound batch adsorption studies were conducted in order to determine the adsorption characteristics of the selected compounds binding to Oasis HLB sorbent. The experimental method that was utilised for each adsorption study conducted is detailed below.

### 3.3.2 Stock and Standard Preparation

The method by which stock solutions and analytical standards were prepared for each compound class investigated is discussed below. All solutions were prepared and stored in glass. All standards were prepared from the initial stock solution and were kept at 4 °C (±2 °C) and were analysed within 24 hours of being prepared. All stock solutions were stored at 4 °C (±2 °C) for a maximum time period of 1 month, after which fresh stock solution was prepared.

#### 3.3.2.1 Xanthine Compounds

A stock solution of each xanthine compound was prepared by transferring 100 mg of each compound to separate 500 mL flasks which were then filled up to mark with ultrapure water to produce a concentration of 200 mgL⁻¹. Seven samples with concentrations ranging from 5 mgL⁻¹ to 100 mgL⁻¹ were then prepared from this initial solution via serial dilution with ultrapure water.

#### 3.3.2.2 Triazine Compounds

A stock solution of atrazine was prepared by transferring 5 mg of atrazine to a 1L volumetric flask which was then filled up to the mark with ultrapure water to produce a
concentration of 10 mgL⁻¹. Six samples with concentrations ranging from 0.5 mgL⁻¹ to 5 mgL⁻¹ were then prepared from this initial solution via serial dilution with ultrapure water. A stock solution of both simazine and DIA were prepared by transferring a 5 mg of each compound to separate 1 L volumetric flasks which were then filled up to the mark with ultrapure water to produce a concentration of 5 mgL⁻¹. Six samples with concentrations ranging from 0.5 mgL⁻¹ to 4 mgL⁻¹ were then prepared from this initial solution via serial dilution with ultrapure water.

3.3.2.3 Chlorinated OPFR Compounds

A stock solution of TCEP and TCPP was prepared by transferring 5 mg of each compound to separate 5 mL volumetric flask which was then filled up to the mark with ultrapure water to produce a concentration of 1000 mgL⁻¹. Six samples with concentrations ranging from 0.5 mgL⁻¹ to 80 mgL⁻¹ were then prepared from this initial solution via serial dilution with ultrapure water. A stock solution of TDCPP was prepared by transferring a 7 mg of TDCPP to a 1L volumetric flask which was then filled up to the mark with ultrapure water to produce a concentration of 7 mgL⁻¹. Six samples with concentrations ranging from 1 mgL⁻¹ to 7 mgL⁻¹ were then prepared from the original stock.

3.3.3 Batch Adsorption Method

The batch adsorption studies were conducted in appropriately sized Erlenmeyer flasks that had been previously baked in a laboratory oven at minimum temperature of 500° C for a minimum of 12 hours to ensure the removal of all organic compounds that may interfere with the results of the study.

As preliminary investigations showed that the compounds investigated varied in the level of affinity they had with Oasis HLB, solute volumes and sorbent masses used
varied from study to study. A summary of the solute volumes and sorbent masses used for each study are detailed in Table 3.1.

Once the solutes had been added to the flasks, they were then placed into the temperature controlled, shaking incubator and allowed to equilibrate at 15 °C for 1 hour. At the conclusion of the equilibration period, appropriate masses of Oasis HLB sorbent (as shown in Table 3.1) were weighed and added to each flask.

Table 3.1: Batch adsorption method details

<table>
<thead>
<tr>
<th>Compound Class</th>
<th>Compound</th>
<th>Volume (mL)</th>
<th>Sorbent Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>xanthine</td>
<td>caffeine</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>theophylline</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>theobromine</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>triazine</td>
<td>atrazine</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>simazine</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>DIA</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>chlorinated OPFR</td>
<td>TCEP</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>TCPP</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TDCPP</td>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

The flasks were then returned to the shaking incubator and the solutions mixed at 200 rpm at 15 °C for 16 hours. The adsorption time of 16 hours deviates from that used by Bäuerlein et al (who used a batch adsorption time of 15 hours) as laboratory investigations could only be conducted during the hours in which the laboratory operates due to safety reasons. Another deviation in the method is the means by which the solute/sorbent mixture was agitated. Whilst Bäuerlein et al. used a stuart roller that used end over end mixing, in the studies described in this dissertation a temperature controlled shaking incubator was used.
Additionally, although Bäuerlein et al. had previously conducted batch adsorption studies for the xanthine compounds, in order to validate the method and to determine potential impacts deviations to the method would have on the end results, these studies were repeated for this dissertation.

After 16 hr, the Erlenmeyer flasks were removed from the incubator and the solutions were filtered through 0.2 µm, Supor® PES syringe filters into new, clean 20 mL glass vials. A 2 mL aliquot of each sample was then transferred into 2 mL glass chromatography vials (Agilent, Mulgrave, VIC, Australia). All batch adsorption studies were conducted in duplicate.

### 3.4 Competitive Adsorption

Environmental samples contain complex mixtures of compounds that can differ markedly in their structure and in their physicochemical properties. It can therefore be expected that there may be some process of competition taking place between the various analytes (analytes) during the adsorption phase where each compound class potentially competes for available sites on the sorbent polymer. As water samples under investigation often contain analytes that are present in the water matrix at trace levels competitive adsorption should not be a major issue with typical SPE analysis. However, this cannot said to be the same for sorbent based passive samplers such as POCIS.

During deployment, compounds migrate through the rate limiting membrane and onto the receiving phase (sorbent). As the samplers are maintained within the water body under analysis from a few days up to a few months, there is always the possibility that compounds that are only tentatively adsorbed onto the sorbent may be ‘knocked off’ by compounds that have a greater affinity for the sorbent. This is one of the potential drawbacks to using an in-situ sampling device such as POCIS.
3.4.1 Multiple Component Batch Adsorption Study – Xanthine Compounds

A multiple batch adsorption study was conducted with the xanthine compounds and the Oasis HLB sorbent in order to determine what impact a mixture has on the adsorption characteristics as previously determined by the single component batch adsorption method.

3.4.1.1 Xanthine Stock Preparation

A mixed stock solution of xanthine compounds was prepared by transferring a known mass of each compound into a single 500 mL flask which was then filled up to mark with ultrapure water to produce a solution of 200 mmol. Dilutions ranging from 5 mmol to 200 mmol of solution were then prepared from this initial solution via the process of serial dilution with ultrapure water. Seven samples were prepared in total. In this instance, the concentrations of the mixed solutions were prepared in mmol so as to ensure the concentration of each analyte in the mixture was the same.

3.4.1.2 Multiple Component Batch Adsorption Method

For each experiment, 10 mL aliquots of each dilution were transferred into 50 mL Erlenmeyer flasks. The flasks were then placed into the temperature controlled shaking incubator for 1 hour in order for the solutions to equilibrate. At the end of the equilibration period, 10mg of sorbent was placed into each flask before being returned to the temperature controlled shaking incubator. The multiple batch adsorption study was then conducted using the same method as detailed in section 3.3.2. As with the single component batch adsorption studies, the multiple component batch adsorption study was conducted in duplicate.
3.5 Adsorption Isotherm Modelling

A number of adsorption isotherm models are in existence. Adsorption isotherm models that will be explored in this dissertation are as follows: the Langmuir isotherm, the double Langmuir isotherm, the competitive Langmuir isotherm and the Freundlich isotherm.

3.5.1 Single-Langmuir Isotherm Model

The single-Langmuir equation assumes that when an analyte is adsorbed onto the surface of the sorbent, the surface of the sorbent is homogenous in nature (i.e. that the energy of adsorption is constant for all adsorption sites) [113].

The mathematical equation that governs the Langmuir adsorption model is as follows (equation 3.1).

\[ C_s = \frac{(C_0 - C_e) \cdot V}{W} \]  

(3.1)

In this equation, \( C_s \) is the amount of analyte adsorbed onto the sorbent; \( C_0 \) is the initial concentration of compound (mgL\(^{-1}\)); \( C_e \) is the equilibrium concentration of the individual xanthine compound after 16 hours (mgL\(^{-1}\)); \( V \) is the volume of the solution (L); and \( W \) is the mass of the dry sorbent used (g). In this instance, adsorption characteristics of \( C_{\text{max}} \) and \( K_L \) were determined using the Langmuir adsorption model as shown in equation 3.2.

\[ C_s = \frac{K_L \cdot C_{\text{max}} \cdot C_w}{1 + K_L \cdot C_w} \]  

(3.2)
Where $C_w$ is the concentration of the analyte retained in the aqueous phase, $C_{\text{max}}$ (mmol kg$^{-1}$) is the maximum concentration of analyte that can be adsorbed onto the sorbent and $K_L$ (L mmol$^{-1}$) is the adsorption constant.

In order to obtain the adsorption characteristics for the compound/Oasis HLB complexes, the Langmuir equation was transformed into its linear expression (equation 3.3) from which adsorption maxima ($C_{\text{max}}$) and adsorption affinity ($K_L$) were derived.

$$\frac{1}{C_s} = \frac{1}{C_{\text{max}}} + \frac{1}{K_L \cdot C_{\text{max}}} \cdot \frac{1}{C_w}$$

(3.3)

The adsorption characteristic (adsorption coefficient) $K_D$ and Log$K_D$ were determined using equation 3.4 and 3.5 respectively.

$$K_D = (K_L \cdot C_{\text{max}})$$

(3.4)

$$\log K_D = \log(K_D)$$

(3.5)

3.6 Instrumentation

The following analytical instruments were used for the experimental work conducted for this dissertation:

- HPLC UV-Vis (Hewitt Packard 1100)
- HPLC MS/MS (Agilent 1200 Infinity HPLC coupled with a 4610 MS/MS)

3.6.1 HPLC Systems

High performance liquid chromatography (HPLC) is an analytical technique that is commonly used to separate, identify and to quantify components in a mixture of
chemical compounds. In a standard HPLC, a solvent containing a small portion of the sample mixture is pumped through a HPLC column that contains a solid sorbent material.

The rate at which each component of the sample mixture moves through the column is dependent upon whether the compound prefers to interact with the solvent (mobile phase) or the receiving phase (contained within the HPLC column). Compounds that preferentially interact with the receiving phase take longer to move towards the detector in comparison to compounds that prefer to stay dissolved in the mobile phase. The degree to which the mixture is separated out into its individual compound is dependent upon both selected column and mobile phase mixture.

HPLC systems can either be normal phase (where the column is polar and the mobile phase is non-polar) or reverse phase (where the column is non-polar and the mobile phase is polar). Currently, reverse phase systems are more widely used than normal phase systems.

A typical reverse phase mobile phase consists of an organic component and an aqueous component. Common organic mobile phases include acetonitrile, methanol and isopropanol.

A HPLC system can be operated in either an isocratic mode where the solvent remains at the same concentration throughout the run or in a gradient mode where the solvent mix changes throughout the run. The choice of whether to run the system in the isocratic mode or the gradient mode is made based on the physicochemical properties of the chemical compounds that are to be analysed.
3.6.2 HPLC Detector

Once the chemical components of the sample have been separated out, they then move onto the detector for qualification and quantification. There are number of detectors that are currently available. The detectors that were used to generate data for this dissertation were ultraviolet-visible spectroscopy (UV/Vis) and the mass spectroscopy (MS); both of these detectors are briefly discussed below.

3.6.2.1 UV-VIS Detector

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. A schematic of a UV-Vis detector is shown in Figure 3.1. In this instance, the absorption spectrum was used.

![Figure 3.1: Schematic of a UV-Vis detector](image-url)
Detection and quantitation of the target analyte/s using a HPLC UV-Vis system are heavily reliant on two factors: 1) time taken for the analyte to migrate along the chromatographic column (i.e. retention time), and 2) measurement of the absorption of electromagnetic radiation at a wavelength that is optimal for the analyte/s of interest (i.e. chromatographic peak area). In this type of system, retention time is used to identify an analyte/s and peak area is used to quantify the analyte/s.

For this dissertation, a Hewitt Packard 1100 HPLC coupled with a UV/Vis detector was used for all xanthine adsorption studies discussed in Chapter 5 (see Figure 3.1). The operating parameters used for the HPLC UV-Vis for these experiments are detailed in Table 3.2.

![Hewitt Packard HPLC UV/V](image)

Figure 3.2: Hewitt Packard HPLC UV/V
Table 3.2: HPLC UV/Vis details for xanthine compound analysis

<table>
<thead>
<tr>
<th></th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column</strong></td>
<td>Agilent Reverse Phase Poroshell 120 EC-C18, (2.1×100 mm, 2.7 µm)</td>
</tr>
<tr>
<td><strong>Mobile Phase</strong></td>
<td>Ultrapure water with 4.1% v/v acetic acid (A) / methanol (B)</td>
</tr>
<tr>
<td><strong>Elution Mode</strong></td>
<td>Isocratic (72% A and 28% B)</td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>20 µL</td>
</tr>
<tr>
<td><strong>Flow Rate</strong></td>
<td>2 mL min⁻¹</td>
</tr>
<tr>
<td><strong>Column Temperature</strong></td>
<td>45°C</td>
</tr>
<tr>
<td><strong>Detector Wavelength</strong></td>
<td>275 nm</td>
</tr>
<tr>
<td><strong>Retention Times</strong></td>
<td>theophylline (0.975 min), theobromine (1.187 min) and caffeine (1.455 min)</td>
</tr>
</tbody>
</table>
3.6.2.2 Mass Spectrophotometry

Mass spectrometry is a sensitive technique that operates by ionising chemical species into corresponding ions. These resulting ions are then separated by their mass-to-charge ratio, a process that takes place in both an electric and magnetic field. Although both positive and negative ions are produced in the ion source (along with uncharged and neutral species) only one polarity is detected at a time.

Typically, mass spectrometers are available as either a single quadrupole configuration or a triple quadrupole configuration. Whilst single quadrupole mass spectrometry relies on the analysis of the parent mass alone, triple quadrupole mass spectrometry uses the unique combination of the specific parent mass (parent ion) and the unique fragment ion (product ion) in order to selectively monitor and therefore measure specific analyte/s. The schematic of a typical triple quadrupole mass spectrometer is shown in Figure 3.3.

Triple quadrupole mass spectrometers can be operated in either the selected reaction monitoring mode (SRM) or the multiple reaction monitoring mode (MRM). When operated in the SRM mode, the first and third quadrupole are tuned to allow for the for a distinct fragment ion that has been generated from a specific precursor ion to be detected. By operating the mass spectrometer in SRM mode, the selectivity of the instrument is increased as the probability of producing a false positive identification is greatly reduced. This is because although the mass spectrometer may initially generate more than one ion with the same m/z ratio in the first quadrupole, due to structural differences, the product ions that are generated in the third quadrupole are likely to be unique to the selected analyte, resulting in the quantification of the selected analyte alone [114].
The operation of a triple quadrupole mass spectrometer in MRM mode means that quadrupole 1 and 3 have been configured to detect more than a single mass at a time which also means that multiple analytes can be analysed at one time [114].

![Schematic of a typical triple quadrupole mass spectrophotometer](image)

Figure 3.3: Schematic of a typical triple quadrupole mass spectrophotometer

An Agilent 1200 Infinity HPLC (Figure 3.4a) coupled with an Agilent 4610 triple quadrupole mass spectrometer (Figure 3.4b) was used for all analytical investigations conducted for both the chlorinated OPFR and triazine herbicides.

![Images](image)

Figure 3.4: Images of a) an Agilent 1200 Infinity HPLC and b) an Agilent 4610 triple quadrupole mass spectrometer
3.6.3 HPLC MS/MS Analysis – Chlorinated OPFRs

HPLC parameters utilised for the analysis of both chlorinated OPFRs and deuterated OPFRs are detailed in Table 3.3.

Table 3.3: HPLC details - chlorinated OPFRs

<table>
<thead>
<tr>
<th></th>
<th>Agilent Reverse phase Zorbax Eclipse C18 column (2.1 x 50 mm, 1.8μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Ultrapure water with 0.1% formic acid (A) / methanol (B)</td>
</tr>
<tr>
<td>Elution Mode</td>
<td>Isocratic (30% A, 70% B)</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>2 μL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.35 mL min⁻¹</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>45°C</td>
</tr>
</tbody>
</table>

A 1 µg mL⁻¹ solution of each of the three chlorinated OPFR compounds and each of the deuterated chlorinated OPFRs were prepared in methanol. Optimisation of the mass spectrometer parameters were conducted in scan mode with the assistance of the Agilent Optimizer Automated MS Method Development software. A collision energy was optimised for each of the three chlorinated OPFR compounds. Mass spectrometry details are presented in Table 3.4 and selected characteristic ions and retention time used for the quantification for the chlorinated OPFRs are presented in Table 3.5.
Table 3.4: Mass Spectrometry Parameters

<table>
<thead>
<tr>
<th>Ionisation</th>
<th>Positive ion electrospray</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition</td>
<td>MRM mode</td>
</tr>
<tr>
<td>Nebulizer Gas</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>Gas Temperature</td>
<td>350°C</td>
</tr>
<tr>
<td>Gas Flow</td>
<td>12 L min$^{-1}$</td>
</tr>
<tr>
<td>Nebulizer Pressure</td>
<td>50 psi</td>
</tr>
<tr>
<td>Capillary Voltage</td>
<td>4000 v</td>
</tr>
</tbody>
</table>

Table 3.5: Selected characteristic ions and retention time windows used for the quantification for the chlorinated OPFRs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Fragmentor Voltage (V)</th>
<th>Collision Energy (V)</th>
<th>Precursor Ion(s)</th>
<th>Product Ion(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP</td>
<td>0.490</td>
<td>108</td>
<td>21</td>
<td>285</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>285</td>
<td>63.1</td>
</tr>
<tr>
<td>TCEP-d$_{12}$</td>
<td>0.494</td>
<td>108</td>
<td>29</td>
<td>285</td>
<td>99</td>
</tr>
<tr>
<td>TCPP</td>
<td>0.830</td>
<td>80</td>
<td>25</td>
<td>329</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>327</td>
<td>99</td>
</tr>
<tr>
<td>TCPP-d$_{18}$</td>
<td>0.804</td>
<td>80</td>
<td>25</td>
<td>345</td>
<td>99</td>
</tr>
<tr>
<td>TDCPP</td>
<td>1.425</td>
<td>136</td>
<td>29</td>
<td>432.9</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>430.9</td>
<td>99</td>
</tr>
<tr>
<td>TDCPP-d$_{15}$</td>
<td>1.388</td>
<td>136</td>
<td>29</td>
<td>446</td>
<td>102</td>
</tr>
</tbody>
</table>
### 3.6.4 HPLC MS/MS Analysis – Triazine Herbicides

HPLC parameters utilised for the analysis of the triazine herbicides are detailed in Table 3.6.

Table 3.6: HPLC details – triazine herbicides

<table>
<thead>
<tr>
<th>Column</th>
<th>Reverse phase Zorbax Eclipse C18 (2.1 x 50 mm, 1.8μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Ultrapure water with 0.1% acetic acid (A) / acetonitrile (B)</td>
</tr>
<tr>
<td>Elution Mode</td>
<td>Isocratic (50% A, 50% B)</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>2 μL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.35 mL/min</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>45°C</td>
</tr>
</tbody>
</table>

A 1 μg mL$^{-1}$ solution of each of the triazine herbicides was prepared in ultrapure water. Optimisation of the mass spectrometer parameters were conducted in scan mode with the assistance of the Agilent Optimizer Automated MS Method Development software. A collision energy was optimised for each of the three triazine herbicides. Mass spectrometry details are presented in Table 3.4 and selected characteristic ions and retention times used for the quantification of the triazine herbicides are presented in Table 3.7.
Table 3.7: Selected characteristic ions and retention time windows used for the quantification for the triazine herbicides

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Fragmentor Voltage (V)</th>
<th>Collision Energy (V)</th>
<th>Precursor Ion(s)</th>
<th>Product Ion(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrazine</td>
<td>1.167</td>
<td>108</td>
<td>17</td>
<td>216.1</td>
<td>174.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>216.1</td>
<td>68.2</td>
</tr>
<tr>
<td>simazine</td>
<td>0.853</td>
<td>122</td>
<td>17</td>
<td>202.1</td>
<td>124.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>202.1</td>
<td>132.2</td>
</tr>
<tr>
<td>DIA</td>
<td>0.513</td>
<td>80</td>
<td>25</td>
<td>174.1</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29</td>
<td>174.1</td>
<td>68.1</td>
</tr>
</tbody>
</table>

3.6.5 Attenuated Total Reflectance Fourier-transform Infrared Spectroscopy

Attenuated Total Reflectance Fourier-transform Infrared Spectroscopy (ATR/FTIR) Spectroscopy was used in Chapter 5 with the aim of deducing the molecular interactions taking place between the xanthine compounds and the Oasis HLB sorbent.

FTIR is an analytical technique that uses infrared light to obtain an absorption spectrum of a sample in order to determine a materials molecular composition or structure. FTIR is known to be very effective at determining the types of functional groups that may be present in material under examination.

ATR is a sampling technique that is used in conjunction with FTIR so that the sample can be examined directly (either in a liquid state or a solid state) without the need for further treatment or preparation. ATR operates by passing a beam of light through a crystal which sits below the sample. The beam of light passes through the crystal in such a way that it reflects off the internal surface that is contact with the sample. The beam of light is collected by the detector once it has exited the crystal. A simple schematic diagram of an ATR showing how the light moves through both the sample and the crystal is shown in Figure 3.5.
Figure 3.5: A simple schematic diagram of an ATR-FTIR. The IR beam protrudes into the sample and once it has moved through the crystal and the sample before making its way to the detector.

In performing this experiment, it was anticipated that the mechanism by which the xanthine compounds bind onto the Oasis HLB sorbent would result spectra that differed slightly to that of the Oasis HLB and thus provide useful insight into the interactions that are taking place between the sorbent and the xanthine compounds. Further experimental details can be found in section 5.3.3 in chapter 5.
4 Computational Chemistry

4.1 Introduction

Computational chemistry is a broad division of chemistry that encompasses the application of a wide range of computer simulations to calculate the structures and properties of molecules. The field of computational chemistry is divided largely into two main methods, namely molecular mechanics and quantum mechanics.

Molecular mechanical methods are based on classical mechanics theory where atoms are treated as spheres and bonds are treated as springs[115]. The primary objective of molecular mechanical methods is to predict the energy that is associated with a particular molecular conformer. Energies that are predicted with molecular mechanical methods have no meaning when studied in isolation. Only differences between two or more confirmations can provide any useful information.

Quantum mechanical methods include both semi-empirical methods and the ab initio methods. These methods are grounded in quantum mechanical theory that was originally developed to counteract classical physics inability to describe interactions that occur at the microscopic level including but not limited to: subatomic particles, atoms and molecules, their structures and associated interactions.

The Hartree-Fock (HF) theory [116] is the simplest quantum mechanical method available. The approximation that is used by the HF method assumes that the Columbic electron-electron repulsion can be averaged rather than having to explicitly calculate repulsion interactions. As such, a weakness of the HF method is that it excludes electron correlation which is the energy contributions arising from electrons interacting with one
another. This can often result in sizable deviations from experimental results occurring when using this method.

Semi-empirical methods use a combination of HF theory and empirical data in order to model large molecules in a cost-effective manner. With semi-empirical based methods, the orbits that are selected are restricted to those that are involved in bond formation.

*Ab initio* methods are reliant solely on the laws that govern quantum mechanics and do not rely on experimental parameters. As such, *ab initio* methods are in general, more computationally expensive but can calculate electronic properties such as interaction energies, bond lengths and spectroscopic properties.

The aim of this chapter is to give a description of both the theory that underpins the calculations that were conducted for this dissertation and to outline the steps that were taken to perform these calculations.

### 4.2 Model Chemistry Framework

The model chemistry framework that is utilised by computational chemists comprises two parts: 1) a theoretical method (section 4.2.1) and 2) an appropriate basis set (section 4.2.2). In order to compare properties of different molecular systems, both the theoretical method and the basis set must be the same.

#### 4.2.1 Theoretical Methods

A number of different theoretical methods are available, each with their own resource requirements, associated accuracy and computational cost. The most commonly utilised theoretical methods are detailed in Table 4.1.
Table 4.1: Common theoretical methods

<table>
<thead>
<tr>
<th>Theoretical Method</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Mechanics</td>
<td>Molecular mechanical (MM) methods are based on classical mechanical theory (i.e Newtonian mechanics)</td>
<td>MM1 [117]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MM2 [118]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MM3 [119]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MM4 [120]</td>
</tr>
<tr>
<td>Hartree-Fock</td>
<td>The HF method is the least computationally expensive method of all the electron structure methods. This method suffers from the fact that it does not include the effects of electron correlation (the energy contributions arising from electrons interacting with one another).</td>
<td>HF [116]</td>
</tr>
<tr>
<td>Semi-Empirical</td>
<td>Semi-empirical methods such as the Austin Model (AM) and the Parameterized Model (PM) use a combination of HF theory and empirical data in order to model large molecules in a cost-effective manner. These methods are ideal for large molecules were the utilisation of more accurate methods would be cost prohibitive.</td>
<td>AM1 [121]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM3 [122]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PM6 [123]</td>
</tr>
<tr>
<td>Moller-Plesset Perturbation Theory</td>
<td>The Moller-Plesset perturbation (MP) theory treats electron correlation by incorporating the Rayleigh–Schrödinger perturbation theory into its methods. Whilst this makes the MP methods more computationally accurate than HF methods it also makes them more computationally demanding.</td>
<td>MP2 [124]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MP4 [125]</td>
</tr>
<tr>
<td>Density Functional Theory (DFT)</td>
<td>DFT methods are based on solutions to the Kohn-Sham equations which relate the energy of a system to the electron density instead of the wavefunction. A drawback to DFT methods is that exact exchange correlation functionals are not incorporated into their methods.</td>
<td>PW91 [126]</td>
</tr>
<tr>
<td>Hybrid Density Functional Theory</td>
<td>Hybrid DFT methods improve upon DFT methods as they incorporate a portion of the exact exchange correlation from the HF theory into its methods. DFT methods are generally only fractionally more expensive to run in comparison to the HF methods. As such, these methods are routinely used in the field of computational chemistry. Long range forces, such as van der Waals interactions can also be accounted for in some of these methods, and are usually represented by the addition of a ‘ω’ at the beginning of the method name.</td>
<td>B3LYP [127]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PBE0 [128]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSE [129]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B97XD [130]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC-PBE [131]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oB97XD [132]</td>
</tr>
<tr>
<td>Coupled Cluster Methods</td>
<td>Coupled cluster methods take the HF molecular orbital method and construct multi-electron wavefunctions using the exponential cluster operator to account for electron correlation. Whilst the results are very accurate, they are also computationally demanding.</td>
<td>CCSD(T) [133]</td>
</tr>
</tbody>
</table>
4.3 Schrodinger Equation

Quantum mechanical methods rely on solving Schrodinger’s equation to determine the ground state energy of system. The development of the Schrodinger equation (equation 4.1) by Erwin Schrödinger in 1925 and the subsequent publication of these findings in 1926 [134], led to a revolution in quantum chemistry. Rather than having to rely solely on empirical evidence to correlate different chemical properties (e.g. chemical reactivity, thermodynamic and structural properties), the application of the Schrodinger equation led to the generation of generalised rules that could be used to underpin these data [135].

\[ \hat{H}\Psi = E\Psi \] (4.1)

The Schrödinger equation is a function of all the fundamental particles (electrons and nuclei) in the system where \( \hat{H} \) represents the Hamiltonian operator which is associated with the observable energy and \( E \) represents the total energy of the system [136].

Furthermore, the Hamiltonian operator can be calculated using the following equation:

\[ \hat{H} = \hat{T}_{ne} + \hat{V}_{ee} + \hat{V}_{ne} \] (4.2)

Where \( \hat{T} \) is the kinetic energy operator, \( \hat{V}_{ne} \) is the electron-nucleus attraction energy operator and \( \hat{V}_{ee} \) is the electron-electron repulsion energy operator.

In a limited number of occasions, the Hamiltonian operator has a relatively simple form which means it can be solved exactly, for example the particle in the box equation and the hydrogen atom. However, as most calculations involve multiple electrons that have the ability to interact with multiple nuclei, calculations are typically much more complicated than the above examples.
In order to simplify the Schrodinger equation, physicists Max Born and Julius Robert Oppenheimer developed what has become known as the Born-Oppenheimer approximation [137]. The theory that underpins the Born-Oppenheimer approximation is that the motion of electrons and atomic nuclei can be separated, thus allowing for the wave function of a molecule to be broken into its electronic and nuclear (vibrational and rotational) and electronic components. The Born-Oppenheimer approximation simplifies the computation of both the energy and the wave function of an average sized molecule.

### 4.3.1.1 Hartree-Fock Theory

The Hartree-Fock (HF) method originated at the end of the 1920s (not long after the discovery of the Schrodinger equation) when Douglass Hartree presented a process that he called the self-consistent field method [138]. The purpose of the new process was to provide a method for which chemists were able to calculate the approximate wave functions and energies for a many body system in a stationary phase. A typical application for the HF method is the solution of the Schrodinger equation for atoms, molecules and nano-structures.

### 4.3.1.2 Density Functional Theory

DFT was established by Walter Kohn and Pierre Hohenberg in the 1960s with the development of two key mathematical theorems [139, 140]. The first theorem states that the ground state energy from Schrodinger’s equation is a unique functional of the electron density.

The electron density functional \( n(\vec{r}) \) is obtained by integrating \( \Psi^*\Psi \) over the coordinates of all the electrons except one. A normalized \( \Psi \) is given by the following equation:
\[ n(\vec{r}) = N \int d\vec{r}_2 \cdots \int d\vec{r}_N \Psi(\vec{r}, \vec{r}_2, \ldots, \vec{r}_N) \Psi(\vec{r}, \vec{r}_2, \ldots, \vec{r}_N) \]  

(4.3)

In contrast to the wave function, the electron density is an observable function as it can be measured experimentally using techniques such as X-ray diffraction. When it is integrated over all space it will give the total number of electrons, \( N \) as shown in the equation below:

\[ \int n(\vec{r}) d\vec{r} = N \]  

(4.4)

One of the key advantages of using the electron density in preference to the wave function method is that it reduces the many-body problem of \( N \) electrons with \( 3N \) spatial coordinates, to only three coordinates, irrespective of the number of electrons in the system.

One of the drawbacks to this theorem is that although it proved that a functional exists it did not provide that functional. The development of the second theorem by Hohenberg, Kohn and Sham would lead to the discovery of an important property of that functional. The second theorem states that the electron density that reduces the energy of the overall functional is the true electron density corresponding to the full solution of the Schrodinger equation. In essence, by varying the electron density until the energy from the functional is at a minimum, the correct ground-state electron density for that system can be determined.

The theorems that were derived by Kohn and Sham allow us to approximate \( \Psi \) as a product of the one electron wave function. The Kohn-Sham equation is as follows:

\[ \left[ -\frac{\hbar^2}{2m} \nabla^2 + V(\vec{r}) + V_H(\vec{r}) + V_{xc}(\vec{r}) \right] \Psi_1(\vec{r}) = \varepsilon_i \Psi_1(\vec{r}) \]  

(4.5)
The solution of the Kohn-Sham equations provides us with a single electron wave function \( \psi (\vec{r}) \), that is dependent on three spatial coordinates: \( V(\vec{r}) \), \( V_H(\vec{r}) \) and \( V_{XC}(\vec{r}) \).

In this equation \( \nabla^2 \) is the Laplacian operator, \( V \) is the external potential, \( V_H \) is the Hartree potential, and \( V_{XC} \) is the exchange correlation potential and \( \varepsilon_i \) is the eigenvalue of the mono-electronic equation.

These spatial coordinates describe interactions between the electron and all the nuclei, the repulsion between the electron and the total electron density (the Hartree potential) and the exchange and correlation contributions respectively.

\[
V(\vec{r}) = - \sum_{k}^{\text{nuclei}} \frac{Z_k}{|\vec{r} - \vec{r}_k|} 
\]

Where \( \vec{r}_k \) denotes the positions of the nuclei, \( Z_k \) is their atomic number of the k-th nucleus.

\[
V_H(\vec{r}) = e^2 \int \frac{n(\vec{r}')}{|\vec{r} - \vec{r}'|} \, d\vec{r}'
\]

The Hartree potential \( (V_H(\vec{r})) \) provides us with the potential energy being experienced by the nuclei.

\[
V_{XC}(\vec{r}) = \frac{\delta E_{XC} (\vec{r})}{\delta n(\vec{r})}
\]

As previously mentioned, \( V_{XC} \) is the exchange correlation potential.

The conundrum that arises from the above equations is that in order to solve equations 4.9 and 4.10, the value of the electron density, \( n(\vec{r}) \) must first be calculated. However, in
order to calculate the electron density, the single electron wave functions, $\Psi(\mathbf{r})$ must also be calculated, which cannot be calculated without first solving the Kohn-Sham equations. This creates a cyclical problem that has no obvious solution. Therefore, in order to solve these equations and henceforth, break the cycle, the solution must initially be resolved iteratively using the steps listed in Figure 4.1.

![Flowchart of solving the Kohn-Sham Equations](image)

**Figure 4.1: Solving the Kohn-Sham Equations**

In order to reach a solution for the Kohn-Sham equations, these steps need to be repeated until such time as convergence has been met.
4.3.1.3 Exchange-Correlation Energy Approximations

It can be seen from the previous section that in order to solve the Kohn-Sham equations, the exchange-correlation energy ($E_{XC}$) must first be determined (as per equation 4.11). However, as the $E_{XC}$ is not known, the use of approximate functionals that are based on the electron density are required. The two most commonly used approximations for this purpose are the local density approximation (LDA) and the generalised gradient approximation (GGA). The LDA is the simpler of the two approximations as it makes the assumption that the correlation energy (at point $\mathbf{r}$) is equal to the exchange energy. Examples of where LDA approach is applied is the Perdew-Zunger (PZ81) functional [141] and the Vosko, Wilk, and Nusair (VWN) functional [142].

However, one of the shortcomings of the LDA is it fails in situations where the density is experiencing rapid changes, for instance as it would in molecular systems. The GGA improves upon LDA as these functionals allow for corrections to the electron density to be made as it moves away from the source. One of the most commonly used GGA approaches is the Perdew-Wang functional (PW91) functional [126].

4.3.1.4 Hybrid DFT

Whilst the HF method is often considered to be both a practical and cost-effective computational approach, especially when it comes to describing large systems, it is by no means a perfect method.

A fundamental problem with the HF method is that it does not take electronic correlation (which is a measure of how much the movement one electron is affected by the presence of all other electrons) into consideration. As such, the resulting energies calculated using the HF method tend to be overestimated.
Subsequently, a number of Post-HF techniques, that do incorporate electron correlation, have been developed in order to overcome this particular limitation of the HF method. Post-HF methods include DFT and MP theory amongst others.

One of the primary advantages of DFT is that it overcomes the limitations experienced by the HF method in a much more cost effective manner than other computational methods such as MP theory which can be very expensive to run and often fails to operate well with large systems.

However, one of the drawbacks to pure DFT methods is that its coulomb term does not cancel with its exchange term, which leads to the self-interaction error. By incorporating the HF exchange term in the functional to form the hybrid functional, the self-interaction error can be reduced, leading to more accurate energy values. Examples of standard hybrid methods include the Becke, three-parameter, Lee-Yang-Parr (B3LYP) functional [127], Perdew–Burke-Ernzerhof (PBE0) functional [128], and Heyd-Scuseria-Ernzerhof (HSE) functional [129].

Another drawback of pure DFT methods is that they have tendency to overestimate local contributions (covalent forces) and to underestimate non-local or long-range contributions (non-covalent forces). Self-interaction errors like these can be addressed through the use of the long-range corrected (LC) hybrid density functionals. These methods overcome the issues faced by pure DFT methods by portioning the exchange correlation functional where the HF term is employed for long range electron-electron distances and pure DFT exchange is used at short electron-electron distances. The long - ranged corrected version of Perdew, Burke, and Ernzerhof (LC-PBE) functional [131] is an example of this type of functional.
Additionally, both pure DFT functionals and most hybrid DFT functionals fail to take into consideration dispersion forces (i.e. van der Waals forces). These are the weak interactions that result from the uneven distribution of charge around a molecule. Whilst dispersion forces are often considered inconsequential when forces such as covalent and dipole-dipole interactions are present, they can be of importance in systems that are not dominated by stronger forces. The Grimmes dispersion corrected (B97XD) functional [130] was one the first functionals of its kind to correct for dispersion forces.

The Head-Gordon dispersion corrected (ωB97XD) functional [132] improves upon the B97XD method with the addition of the ω parameter which indicates that a portion of the HF exchange has also be added to the short-range part of the function. It has been established that when the short-ranged portion of the functional incorporates no to very low levels of the HF exchange correlation it might result in an overcorrection of the long range forces [143].

4.3.2 Basis Sets

Basis sets are defined as functions that describe atomic orbitals and are used in a linear combination to create molecular orbitals. The basis set restricts the electrons to a particular region of space. The larger the basis set, the fewer constraints on the electron, resulting in a more accurate approximation of the real molecular orbitals or electron density.

Examples of commonly used basis sets are detailed in Table 4.2 starting with the simplest of basis sets (the minimal basis sets) before progressing to more computationally demanding basis sets.
Table 4.2: Commonly used Basis Set

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal</td>
<td>Minimal basis sets use only one function for each atomic orbital. Minimal basis sets generate coarse results</td>
<td>STO-3G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STO-4G</td>
</tr>
<tr>
<td>Split-valance (Pople)</td>
<td>Split-valence basis sets are ones where the valence orbitals are represented by two or more basis functions</td>
<td>3-21G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-31G</td>
</tr>
<tr>
<td>Polarised</td>
<td>Polarised basis sets are modified split-valence basis sets. In contrast to split-valence basis sets, polarised valence sets allow orbitals to not only change size but to also change shape. Polarised basis sets that have an * or a d means that a d type functional has been added on to all atoms other than hydrogen and that a f type orbital has been added to transition metals. Polarised basis sets that have either a ** or a dp means that p type functions have been added to hydrogen, d type functions have been added to all other atoms excluding hydrogen and that f type functions have been added to transition metals</td>
<td>3-21G*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-21G (d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-31G**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-31G (d,p)</td>
</tr>
<tr>
<td>Diffused Functions</td>
<td>Diffused functions are also modified split-valence basis sets. Diffused functions allow orbitals to occupy a larger region of space. These functions are required for systems that contain atoms/molecules that are located a good distance from the nuclei. Diffuse functions contain either a + or a ++ in front of the G. A split valence set with a + means that a diffuse function has been added on to atoms other than the hydrogens, whilst a split valence set with ++ means that diffuse functions have been added onto all atoms.</td>
<td>3-21 +G (d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-31 ++G (dp)</td>
</tr>
<tr>
<td>Correlation-Consistent</td>
<td>The above mentioned basis sets are all optimised at the HF level, as such it was deemed that a new set of basis sets was needed for correlated wavefunctions. These basis sets are denoted as C-pVXZ. A aug prefix can be added to these basis sets in order to add a diffuse function.</td>
<td>cc-pVDZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cc-pVTZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aug-cc-pVTZ</td>
</tr>
</tbody>
</table>
4.4 Calculated Properties

A number of different properties of molecules can be calculated, including their optimised geometry (or preferred configuration), and vibrational frequencies. Details of the properties calculated in this thesis are described below.

4.4.1 Geometry Optimisation Calculations

Geometry optimisation is the process undertaken to find the arrangement by which the net inter-atomic forces on each atom within a system is as close to zero as possible. When a geometry calculation is performed, the wave function and the energy of the initial molecule are determined. Following on from this, the position of the atoms within a system are then changed until the energy of the system reaches a minimum. Additionally, a structure is considered to be fully optimised when it reaches a stationary point on the potential energy surface (PES) (see example in Figure 4.2).

![Figure 4.2: An example of a PES curve](144)
A number of different computational algorithms have been developed to determine if the energy of a system has reached a minimum value. These methods can be identified as either being derivative or non-derivative. Derivative methods such as steepest descents, Newton Raphson, conjugate gradients, variable matrices and the Berny algorithm calculate the slope of a potential surface from the potential energy function.

Whilst non-derivative methods do not require the derivative of the potential energy surface to be calculated in order to determine if a minimum energy state has been achieved, these methods are in general considered to be less efficient.

In this work, geometry optimisation calculations were performed for each xanthine (chapter 5), triazine (chapter 6) and chlorinated OPFR (chapter 7) molecule, the two Oasis HLB monomers) comprising the sorbent, and each compound-monomer complex, in order to determine their lowest energy structure or configuration. The geometry optimisation algorithm that was used was the Berny algorithm using the GEDIIS/GDIIS optimizer.

4.4.2 Vibrational Frequency

Vibrational frequency calculations are performed to calculate the vibrational frequencies of a system, as well as to determine whether the structure is a true minimum on the PES. As seen from Figure 4.2, reaching a stationary point on the PES curve does not necessarily mean that a minimum energy configuration of a system has been obtained.

Vibrational frequency calculations involve determining the second derivatives of the energy with respect to the atomic coordinates. As such, these calculations are conducted after the geometry optimisation calculation has been completed. The aim of the vibrational frequency calculations is to obtain frequency values that are all positive (i.e.
real). Only then can it be concluded that the system is at minimum. A single negative frequency value indicates that the structure is a transition state and multiple negative frequency values indicate that the structure is a higher order saddle point, and therefore is unstable.

4.4.3 Solvation

Standard computational chemistry calculations are conducted in a ‘vacuum’. In order to take into account solvation of a system, a solvated method is employed. There are two primary methods for determining solvation, the first being the implicit method and the second being the explicit method.

In the implicit method, a charged field is place around the molecule/s to mimic the presence of the solvent molecules. The polarised continuum model (PCM) is an example of an implicit method, where solvent effects are modelled by generating a solvent cavity around the atoms of the participating molecules which is then surrounded by a dielectric medium (see Figure 4.3 a). Whilst the implicit solvation method can produce a reasonable description of the behaviour of the solvent molecules, this method often fails to take into account local fluctuations in solvent density around the solute molecules.

In the explicit solvation model, solvent molecules are placed in key locations around the molecule/s that are being studied. These solvent molecules are added to regions of the molecule where they would most likely interact i.e. places where hydrogen bonding is most likely to occur when the selected solvent is water. A disadvantage of this approach is that the method can at times fail to replicate experimental data often due to certain fitting methods and parameterisation. The explicit solvation method is more computationally demanding than the implicit solvation method and thus is more
expensive to run. In order to overcome failures in both methods, hybrid solvation methods now exist. However, these newer methods are not as intuitive as either the implicit solvation method or the explicit solvation methods and they can also often need the addition of post calculation correction factors to be included in the calculations in order to improve their accuracy.

In this work, the implicit solvation method was employed, primarily due to the increased computational cost required to use the explicit method.

4.4.4 Basis Set Supposition Error

For the calculations described here, where the binding energy between two molecules is calculated, the binding energy value can be liable to what is known as basis set superposition error or BSSE. This occurs when the atoms of interacting molecules approach each other, their basis functions will overlap and as a result, each monomer ‘borrows’ functions from other near-by components. This results in an increase in its basis sets and thus an artificial increase in its calculated properties (e.g. energy). In order to calculate the BSSE using Gaussian 09 [145] software, each molecular fragment must first be identified using visualisation software such as GausView [146]. The next step is to conduct a single point energy calculation for the complex. When these calculations are conducted in the gas phase, the keyword counterpoise is incorporated into the job script. In order to run the BSSE calculation, the number of fragments must also be identified i.e. counterpoise=2 (symbolising that there are two fragments to be taken into consideration). Once the BSSE has been determined, this value is then added to the optimised energy of the complex under investigation, thus resulting in a counterpoise corrected energy.
As the key word *counterpoise* is only supported for gas phase calculations the method required to calculate the BSSE for a solvated system is as follows:

As per the ‘automated’ BSSE calculation, the first step of the manual calculation is to define each molecular fragment using visualisation software (i.e. GausView) The next step is to calculate the energy of the dimer complex, replacing the atoms in molecule A with ghost orbitals. This is also referred to as a dimer-centred basis set calculation. Following on from this, the energy of molecule A is then calculated with the atom groups of the monomer defined. The whole process is then repeated for molecule B.

Once these energy values have been obtained, the BSSE for the complex is then calculated using equation 4.11.
Figure 4.3: Counterpoise correction method with polarizable continuum model (PCM). a) dimer calculation. b) dimer-centred basis set calculation. c) monomer-centred basis set calculation (modified from [147]).
4.4.5 Zero Point Energy Correction

The zero-point energy (ZPE) is defined as the vibrational energy of a molecule at 0 K (absolute zero). It is defined as the lowest possible energy that a quantum mechanical system may have. The zero-point energy correctional factor (ΔZPE) is applied to the total energy of the complex in order to correct for these vibrations that occur at 0K. The change in ZPE, ΔZPE, calculates the vibrational energy of the complex relative to the vibrational energies of each of the participating monomers.

4.5 Computational Details

4.5.1 Selection of Computational Method

The computational method that was selected for this dissertation was the Head-Gordon dispersion corrected density functional method (ωB97XD)[132]. The rationale behind selecting this method is the fact that the ωB97XD method has the ability to adequately describe weak dispersion forces which are the type of forces that are said to dominant analyte/SPE interactions.

Prior to selecting the ωB97XD method, the more commonly used B3LYP and MP2 methods were trialled for the xanthine-Oasis HLB calculations. However, as these calculations were computationally demanding and as they do not best describe weak dispersion forces, it was decided that all computational calculations would use the ωB97XD method.
4.5.2 Computational Calculations

The molecules and the two monomers were optimised separately using the Head-Gordon dispersion corrected density functional method ($\omega$B97XD) [132] and the 6-31G (d,p) basis set as implemented in Gaussian 09. A vibrational frequency calculation was then performed to confirm that the optimised structure was a local minimum.

To model the interaction of each compound with the Oasis HLB sorbent, a series of calculations were performed where each of the selected molecules was allowed to interact with the two structural monomers comprising the sorbent, namely NVP and DVB.

Calculations of the molecule-monomer dimers were then performed to determine the preferred conformation of each of the investigated molecules (xanthines, triazines and the chlorinated OPFRs) with each sorbent monomer (NVP and DVB). Different initial starting geometries were considered, with each of molecules being placed in a variety of locations and orientations around each monomer, and were initially located where possible H-bonds might form. In all cases, the two molecules in each complex were initially located at least 1.7 Å away from each other (as measured from the closest atoms on each molecule).

A geometry optimisation of each complex was performed using the $\omega$B97XD/6-31G(d,p) method in Gaussian 09. A vibrational frequency calculation was then used to verify that the complex was a local minimum.

The interaction energies of each dimer, corrected for the $\Delta$ZPVE were calculated according to the following equation (equation 4.9):
\[ \Delta E = E_{\text{complex}} - [E_{\text{molecule}} + E_{\text{monomer}}] + \Delta \text{ZPVE} + \text{BSSE} \quad (4.9) \]

where \( \Delta E_{\text{complex}} \) is the energy of the molecule-monomer complex, \( E_{\text{molecule}} \) is the energy of the molecule and \( E_{\text{monomer}} \) is the energy of the Oasis HLB monomer. A more negative \( E \) value indicates a stronger compound-monomer interaction.

The \( \Delta \text{ZPVE} \) correction was calculated as the difference in the ZPVE energies between the complex and the isolated molecules as shown in equation 4.10:

\[ \Delta \text{ZPVE} = \text{ZPVE}_{\text{complex}} - [\text{ZPVE}_{\text{molecule}} + \text{ZPVE}_{\text{monomer}}] \quad (4.10) \]

The BSSE was determined manually using the CP approach (section 4.4.4) using the following equation:

\[ \text{BSSE} = [\text{MCBS1+MCBS2}] - [\text{DCBS1+DCBS2}] \quad (4.11) \]

where MCBS1 and MCBS2 are the monomer-centered basis sets and DCBS1 and DCBS2 are the dimer centered basis sets as per Figure 4.3 [147].

In order to determine the effect that the solvent (in this case, water) has on the resulting calculations, the polarizable continuum method using the integral equation formalism [148] (IEFPCM) was utilised.

All molecules were visualised and the reported bond angles and bond lengths were determined using the freeware software Avogadro [149].
5 Xanthine Compounds

5.1 Introduction

In this chapter, a simple model system using three structurally similar xanthine compounds (caffeine, theophylline and theobromine) and Oasis HLB was investigated using hybrid DFT calculations and the results compared to batch adsorption studies of the same setup. The aim of the research presented in this chapter was to use both experimental and computational investigations to help further our understanding of the interactions that are taking place at the molecular level between the three selected xanthine compounds and Oasis HLB so as to ascertain if it is possible to make predictions on analyte/sorbent behaviour.

5.1.1 Xanthine Compounds

Caffeine is as well-known ubiquitous micro-pollutant with concentrations of up to 1100 μgL⁻¹ having been detected in surface water in Costa Rica [137] and up to 11μgL⁻¹ in the coastal water of Darwin, Australia [138]. Caffeine is also a common environmental tracer [28, 136]. In comparison, theophylline is a pharmaceutical preparation that is primarily used as treatment for respiratory diseases [29] and theobromine is commonly found in chocolate and cocoa [30]. Additionally, both theophylline and theobromine are breakdown products of caffeine. As such it is therefore would be expected that both theophylline and theobromine would be present in aquatic environments in which caffeine has been detected.
5.1.2 Molecular Structures and Physiochemical Properties of the Selected Xanthine Compounds

Xanthine compounds are characterised by the presence of two aromatic ring structures: a 6-membered imidazole ring and a 5-membered pyrimidinedione ring. When comparing the structures of the three selected xanthine compounds (see Table 5.1), it can be seen that whilst caffeine contains three attached methyl groups, both theophylline and theobromine contain only two attached methyl groups. In addition to this, both theophylline and theobromine also contain an N-H moiety that is lacking in the caffeine molecule. Whilst the N-H moiety contained within the theophylline molecule is located in the pyrimidinedione ring, the N-H moiety contained within the theobromine compound is located within the imidazole ring.

These slight structural differences result in all three compounds having differing physiochemical properties. It is expected that these differing physiochemical properties will have an impact upon the ability of these compounds to bind onto the Oasis HLB sorbent.

All three xanthine compounds vary in solubility in water with caffeine having the highest solubility and theobromine having the lowest solubility (Table 5.1). The log $K_{ow}$ value of a compound characterises the propensity of a compound to partition itself between an aqueous phase and an organic phase (eg soil, biological matter). Chemical compounds that have a low $K_{ow}$ value (less than 4) are considered to be relatively hydrophilic. Following on from this definition, all three xanthine compounds are considered to be hydrophilic. Of the three compounds investigated, theobromine (with the lowest log $K_{ow}$ value), is considered to be the most hydrophilic.
All three xanthine compounds are considered to be polar in nature as shown by their electric dipole moments. Of the three xanthine compounds, theobromine is the most polar. Both the structures and physiochemical properties of the compounds investigated are detailed in Table 5.1.

### Table 5.1: Chemical structure and physiochemical properties of the xanthine compounds [150]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Aqueous Solubility gL$^{-1}$ at 25°C</th>
<th>Log $K_{ow}$</th>
<th>Dipole* Moment (debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td><img src="image" alt="Structure" /></td>
<td>21.60</td>
<td>-0.07</td>
<td>3.80</td>
</tr>
<tr>
<td>Theophylline</td>
<td><img src="image" alt="Structure" /></td>
<td>7.36</td>
<td>-0.02</td>
<td>3.51</td>
</tr>
<tr>
<td>Theobromine</td>
<td><img src="image" alt="Structure" /></td>
<td>0.33</td>
<td>-0.78</td>
<td>4.30</td>
</tr>
</tbody>
</table>

*Calculated with Gaussian 09

#### 5.1.3 Potential Intermolecular Interactions

Retention of the xanthine compounds onto the Oasis HLB sorbent is likely to be established through the formation of two different types of reversible bonds; hydrogen bonds, and π···π interactions.

Hydrogen bonding is typically characterised by the formation of a dipole-dipole interaction between an electronegative atom and an acidic hydrogen. Classically, hydrogen bonds are classified as being highly electrostatic and at times, even slightly
covalent in nature. Strong hydrogen bonds typically include interactions between N-H, O-H, and either O or N where the N-H and O-H moieties are hydrogen bond donors and O and N are hydrogen bond acceptors. In more recent years the definition of what constitutes a hydrogen bond has relaxed somewhat with weaker interactions now being considered as being hydrogen bonds. Weaker hydrogen bonds include interactions between C-H and either O or N. Additionally, in certain circumstances, an aromatic ring can act as a hydrogen bond acceptor. This type of molecular interaction is also considered to be relatively weak.

The strength of the hydrogen bonds formed in the complexes is based on their bond lengths and are classified as very strong (1.2-1.5 Å), strong (1.5-2.2 Å) or weak (2.0 - 3.0 Å) [153].

All three xanthine molecules have the potential to form weak hydrogen bonds between the xanthine molecules C=O moiety and hydrogen atoms on both the NVP and DVB monomers and weak hydrogen bonds with the C=O moiety contained within the NVP monomer and hydrogen atoms contained within the xanthine compounds. Both theophylline and theobromine also have the ability to form relatively strong hydrogen bonds between their N-H moieties and the C=O moiety that is contained within in the NVP monomer. π···π Interactions.

The aromatic ring structures that are present in all three xanthine compounds should also be able to form π···π interactions with the DVB monomer. The π-orbital electron cloud contained within an aromatic ring conveys a partial negative charge above and below the ring structure whilst the hydrogen atoms continue to be positively charged. Thus, when two aromatic ring structures interact, they have the ability to form dipole-
dipole interactions. These dipole-dipole interactions are considered to be relatively weak in strength.

5.2 Materials and Methods

5.2.1 Xanthine-Oasis HLB Batch Adsorption Studies

The adsorption equilibria of xanthine compounds onto Oasis HLB were determined using the batch adsorption method [151] and is based on previous work conducted by Bäuerlein et al. [105] who evaluated the adsorption characteristics of Oasis HLB an array of analytes (including caffeine, theobromine and theophylline). Batch adsorption studies were conducted according to section 3.3 in chapter 3.

5.2.2 ATR FTIR Spectroscopic Analysis

Spectroscopic studies were conducted on sorbent that had been exposed to each xanthine compound to determine if these methods could be of assistance in determining the primary mechanism/s of adsorption taking place between the analyte (xanthine compounds) and the Oasis HLB sorbent.

Mid Infrared (MIR) analysis was performed using a Perkin Elmer Spectrum (Mulgrave, VIC Australia) 100 spectrometer with a single bounce diamond attenuated total reflectance (ATR) attachment. The scan range was set to 550 - 3550 cm\(^{-1}\) with a resolution of 4.00 cm\(^{-1}\). Approximately 0.5 g of sorbent, or sorbent with xanthine compound (caffeine, theophylline or theobromine) attached was used for each sample and a scan of 5 s was repeated 4 times across the sample with the mean of these results taken as the final value. A force gauge was used to ensure all samples had a transmittance of 70%.
5.2.3 Xanthine-Oasis HLB Molecular Modelling Studies

To model the interaction of each xanthine compound with the Oasis HLB sorbent, a series of hybrid-DFT calculations were performed using Gaussian 09 software, where each of the xanthine molecules was allowed to interact with the two structural monomers of the sorbent, namely NVP and DVB. The computational details are provided in section 4.7 in chapter 4.

5.3 Results and Discussion

5.3.1 Xanthine-Oasis HLB Batch Adsorption Studies

The results of the single component batch adsorption studies that were conducted for this chapter are shown in Table 5.2 and the adsorption isotherms are shown in Figures 5.1 -5.3. All correlation coefficients (R² values) were greater than 0.97 which suggests that the Langmuir model was able provide a favourable model of the data.

Batch adsorption investigations show that the order of the maximum adsorption (from highest to lowest) is as follows: caffeine > theophylline > theobromine. The results also show that whilst the maximum adsorption capacity value for caffeine is more than twice that of theophylline and theobromine, the latter two compounds are in close proximity to each other in value. The batch adsorption data also shows that the order of adsorption affinity for the selected xanthine compounds with Oasis HLB is as follows: caffeine > theophylline > theobromine.
Table 5.2: Batch adsorption results of caffeine, theophylline and theobromine with Oasis HLB

<table>
<thead>
<tr>
<th>Adsorption Characteristic</th>
<th>caffeine (±SD)</th>
<th>theophylline (±SD)</th>
<th>theobromine (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption Capacity</td>
<td>271 (5)</td>
<td>159 (14)</td>
<td>73 (1)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mmolkg$^{-1}$)</td>
<td>[96 (4)]</td>
<td>[47 (13)]</td>
<td>[n.q]</td>
</tr>
<tr>
<td>Adsorption Affinity</td>
<td>11 (1)</td>
<td>10 (1)</td>
<td>5 (0)</td>
</tr>
<tr>
<td>$K_L$ (Lmmol$^{-1}$)</td>
<td>[47 (4)]</td>
<td>[12 (5)]</td>
<td>[n.q]</td>
</tr>
<tr>
<td>$K_D$ (Lkg$^{-1}$)</td>
<td>3,057 (53)</td>
<td>1,597 (0)</td>
<td>392 (5)</td>
</tr>
<tr>
<td>Log$K_D$</td>
<td>3.48</td>
<td>3.20</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>[3.65]</td>
<td>[2.75]</td>
<td>[n.q]</td>
</tr>
</tbody>
</table>

n.q – not quantifiable
Figure 5.1: Caffeine-Oasis HLB batch adsorption isotherm.

Figure 5.2: Theophylline-Oasis HLB batch adsorption isotherm

Figure 5.3: Theobromine-Oasis HLB batch adsorption isotherm

Error bars shown in Figures 5.1, 5.2 and 5.3 represent the standard deviation of the mean.
5.3.1.1 Comparison of Batch Adsorption Results with Published Data

The batch adsorption method that was utilised for this chapter and subsequent chapters, was taken from a journal article written by Bäuerlein et al. [105]. In order to validate the method, the batch adsorption study was repeated for the three xanthine compounds that were previously investigated by Bäuerlein et al. One point of difference between the two studies was the means by which the samples were agitated. Whilst Bäuerlein et al. used an end over end mixing device which was kept refrigerated at 16 °C for the duration of their study, all batch adsorption studies that were reported in this dissertation were conducted in a temperature controlled, shaking incubator (also kept at 16 °C) which was operated at 200 rpm.

The results show that the adsorption characteristics as determined in the current study, whilst consistent between duplicates, differ from those determined by Bäuerlein et al. As seen in Table 5.2, two of the three adsorption characteristics (adsorption capacity and adsorption affinity) were found to be greater in value in our experiment than the results reported by Bäuerlein et al.

The discrepancy in the xanthine-Oasis HLB batch adsorption results presented in this chapter and those presented by Bäuerlein et al. maybe due to a number of reasons including the speed in which the samplers were mixed. It is reasonable to expect that a greater amount of analyte could be adsorbed to sorbent material when the analyte/sorbent samples are mixed at high speed compared with low speed.

Additionally, whilst a sorbent mass of 10 mg was used for each adsorption study presented in this chapter, Bäuerlein et al. reported that they used varying quantities of sorbent for each of the analytes they investigated. As the exact mass of the sorbent utilised was not reported, Bäuerlein et al study could not be replicated in its entirety.
Although the results for the adsorption study reported in this chapter do differ significantly from those previously reported by Bäuerlein et al, the information that can be inferred from these studies do not. Both sets of results show that the analyte that has the highest adsorption maxima for Oasis HLB as determined by adsorption capacity is caffeine followed by theophylline and then theobromine.

### 5.3.2 Xanthine-Oasis HLB Multi-Component Batch Adsorption Study

A competitive adsorption study was conducted to determine the impact that the presence of more than one xanthine compound has on the ability of Oasis HLB to adsorb the xanthine compounds under investigation. The results of the competitive adsorption study are presented in Figure 5.4.

From the competitive batch adsorption results it can be seen that the adsorption isotherm for all three xanthine compounds investigated differ from the adsorption isotherms depicted in the single component isotherm (as shown in Figures 5.1-5.3). The results show that the adsorption maxima for all three xanthine compounds were reduced in the multi-component batch adsorption study in comparison to the single batch adsorption studies. This is because unlike the single component batch adsorption study, the selected compounds must compete for available adsorption sites in a multi-component batch adsorption study with other analytes which may bind preferentially to the surface of the sorbent. Additionally, whilst all three xanthine’s reach a maxima, they the upward trend in the data indicates that once the xanthine molecules have adsorbed onto all available sites on the sorbent polymer, they then most likely proceed to interact with each other, almost certainly through the formation of \( \pi \cdots \pi \) bonds between the aromatic rings contained within the xanthine compounds.
5.3.3 ATR FTIR Spectroscopic Results

ATR-FTIR analysis was conducted on Oasis HLB sorbent both before and after exposure to the xanthine compounds to determine if insight could be gained into the intermolecular interactions that take place when the selected compounds bind onto the polymeric sorbent. Unfortunately, the ATR-FTIR spectra as shown in Figure 5.5 showed no major differences between loaded and unload sorbent samples. ATR-FTIR results as presented here are consistent with results that were achieved by Sailia et al. [152] who also saw only minor changes to the spectra of polymeric sorbent before and after exposure to caffeine. It is possible that this technique is not sensitive enough to be able to provide useful insight into the interactions taking place between analyte and sorbent.

Figure 5.4: Xanthine-Oasis HLB competitive batch adsorption isotherms. Error bars shown in Figure 5.4 represent the standard deviation of the mean.
Figure 5.5: ATR-FTIR spectra of Oasis HLB with and without adsorbed xanthine compounds (caffeine, theophylline and theobromine)

5.3.4 Molecular Modelling

The results of the molecular modelling studies that were conducted for this chapter are presented in the next section of this chapter. Prior to conducting these studies, both the xanthine compounds and the Oasis HLB monomers were studied to determine potential sites of interaction. The result of this analysis is also presented below.

In Figures 5.6 to 5.13 oxygen is red, nitrogen is blue, carbon is grey, and hydrogen is white.
5.3.5 Optimised Compounds

The optimised geometries of caffeine, theophylline, and theobromine are presented in Figure 5.6 and the optimised geometries of the Oasis HLB monomers (NVP and DVB) are presented in Figure 5.7.

Figure 5.6: Optimised xanthine molecules. a) caffeine, b) theophylline, c) theobromine.

Figure 5.7: Optimised Oasis HLB monomers. a) NVP, b) DVB
5.3.6 Caffeine-Monomer Complexes

After geometry optimisation, 7 stable caffeine-monomer complexes were found, including 3 caffeine-NVP complexes and 4 caffeine-DVB complexes. The optimised geometries of the caffeine-monomer complexes can be found in Figure 5.8 and 5.9 and the binding energies, bond lengths and bond angles of the theophylline-monomer complexes are detailed in Tables 5.3 and 5.4.

The binding energies of the caffeine-monomer complexes ranged from -9.47 kcal mol\(^{-1}\) to -0.29 kcal mol\(^{-1}\). The strongest interaction was shown to occur with the DVB monomer (Figure 5.8a) with the primary interaction between the two compounds being governed by \(\pi\cdots\pi\) interactions. The next most stable caffeine complex was also with the DVB monomer with a binding energy of -8.94 kcal mol\(^{-1}\) (Figure 5.8b) The next three strongest interactions are between caffeine and the NVP monomers (Figure 5.9a, b & c) with the formation of weak hydrogen bonds with bond lengths ranging from 2.463 to 2.937 Å and bond angles of between 118.6 and 157.7\(^\circ\). The remaining two complexes are between caffeine and the DVB monomer (Figure 5.8c & d) which are weaker in strength with binding energies of -1.40 kcal mol\(^{-1}\) and -0.29 kcal mol\(^{-1}\) respectively. The caffeine-DVB3 complex is bound together through the formation of a single weak hydrogen bond the caffeine-DVB4 complex is bound together through the formation of \(\pi\cdots\pi\) interactions.
Figure 5.8: The optimised complexes of caffeine-NVP. a) caffeine-NVP1, b) caffeine-NVP2, c) caffeine-NVP3

Figure 5.9: The optimised complexes of caffeine-DVB. a) caffeine-DVB1, b) caffeine-DVB2
Table 5.3: Calculated binding energies of caffeine with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>caffeine-NVP1</td>
<td>-7.53</td>
<td>C2-H11⋯N27</td>
<td>2.586</td>
<td>157.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1-H10⋯N20</td>
<td>2.937</td>
<td>123.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C31-H32⋯O6</td>
<td>2.709</td>
<td>109.8</td>
</tr>
<tr>
<td>caffeine-NVP2</td>
<td>-5.03</td>
<td>C3-H14⋯O26</td>
<td>2.463</td>
<td>154.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1-H9⋯N36</td>
<td>2.685</td>
<td>143.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C21-H22⋯O6</td>
<td>2.675</td>
<td>118.6</td>
</tr>
<tr>
<td>caffeine-NVP3</td>
<td>-5.57</td>
<td>C2-H12⋯N36</td>
<td>2.474</td>
<td>173.1</td>
</tr>
</tbody>
</table>

Table 5.4: Calculated binding energies of caffeine with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>caffeine-DVB1</td>
<td>-9.47</td>
<td>π⋯π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>caffeine-DVB2</td>
<td>-8.94</td>
<td>π⋯π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>caffeine-DVB3</td>
<td>-1.40</td>
<td>C31-H38⋯N19</td>
<td>2.484</td>
<td>158.2</td>
</tr>
<tr>
<td>caffeine-DVB4</td>
<td>-0.29</td>
<td>π⋯π</td>
<td>~3.3</td>
<td></td>
</tr>
</tbody>
</table>
5.3.7 Theophylline-Monomer Complexes

After geometry optimisation, 10 stable theophylline-monomer complexes were found, including 5 theophylline-NVP complexes and 5 theophylline-DVB complexes. The optimised geometries of the theophylline-monomer complexes are shown in Figures 5.10 and 5.11 and their calculated properties are detailed in Tables 5.5 and 5.6.

The binding energies of the theophylline-monomer complexes ranged from -9.20 kcal mol\(^{-1}\) to -1.80 kcal mol\(^{-1}\). The two strongest interactions are between theophylline and the NVP monomer (Figures 5.10a & b) with each complex each forming two weak hydrogen bonds. The next four most stable complexes are between theophylline and the DVB monomer (Figures 5.11a, b, c, & d) with binding energies ranging from -9.01 to -8.31 with the formation of \(\pi\cdots\pi\) bonds between the aromatic ring on the DVB monomer and the aromatic ring on the theophylline molecule. The least favourable theophylline complex was theophylline-NVP5 (Figure 5.10e) with a binding energy of -1.80 kcal mol\(^{-1}\) with the formation of two relatively weak hydrogen bonds.
Figure 5.10: The optimised complexes of theophylline-NVP. a) theophylline-NVP1, b) theophylline-NVP2, c) theophylline-NVP3, d) theophylline-NVP4, e) theophylline-NVP5

Figure 5.11: The optimised complexes of theophylline-DVB. a) theophylline-DVB1, b) theophylline-DVB2, c) theophylline-DVB3, d) theophylline-DVB4, e) theophylline-DVB5
Table 5.5: Calculated binding energies of theophylline with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>theophylline-NVP1</td>
<td>-9.20</td>
<td>C3-H13…O19</td>
<td>1.729</td>
<td>175.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N30-H13…O6</td>
<td>2.314</td>
<td>162.5</td>
</tr>
<tr>
<td>theophylline-NVP2</td>
<td>-9.10</td>
<td>N30-H38…O6</td>
<td>1.723</td>
<td>176.1</td>
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<td></td>
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<td>C3-H14…O19</td>
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<td>145.5</td>
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<tr>
<td>theophylline-NVP3</td>
<td>-8.12</td>
<td>C3-H14…O26</td>
<td>2.521</td>
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<tr>
<td></td>
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<td>C1-H19…N32</td>
<td>2.656</td>
<td>147.2</td>
</tr>
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<td></td>
<td></td>
<td>C21-H22…O6</td>
<td>2.609</td>
<td>121.8</td>
</tr>
<tr>
<td>theophylline-NVP4</td>
<td>-7.74</td>
<td>C1-H9…N30</td>
<td>2.591</td>
<td>161.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H14…N32</td>
<td>2.563</td>
<td>136.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N30-H38…O6</td>
<td>2.591</td>
<td>135.9</td>
</tr>
<tr>
<td>theophylline-NVP5</td>
<td>-1.80</td>
<td>C3-H13…O26</td>
<td>2.332</td>
<td>173.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C21-H22…O6</td>
<td>2.343</td>
<td>171.5</td>
</tr>
</tbody>
</table>

Table 5.6: Calculated binding energies of theophylline with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>theophylline-DVB1</td>
<td>-9.01</td>
<td>π…π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>theophylline-DVB2</td>
<td>-8.75</td>
<td>π…π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>theophylline-DVB3</td>
<td>-8.41</td>
<td>π…π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>theophylline-DVB4</td>
<td>-8.31</td>
<td>π…π</td>
<td>~3.3</td>
<td>-</td>
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<tr>
<td>theophylline-DVB5</td>
<td>-7.97</td>
<td>π…π</td>
<td>~3.3</td>
<td>-</td>
</tr>
</tbody>
</table>
5.3.8 Theobromine-Monomer Complexes

After geometry optimisation, 7 stable theobromine-monomer complexes were found in total, including 4 theobromine-NVP complexes and 3 theobromine-DVB complexes. The optimised geometries of the theobromine-monomer complexes are shown in Figures 5.12 and 5.13 and the optimised energies, bond lengths and bond angles are detailed in Tables 5.7 and 5.8.

The binding energies of the theobromine-monomer complexes ranged from -8.80 kcal mol\(^{-1}\) to -3.96 kcal mol\(^{-1}\). The strongest complex formed was with the DVB monomer through the formation of π∙∙∙π bonds (Figure 5.12a). The next two strongest interactions are also with the DVB monomer (Figure 5.12b and c) which also contain π∙∙∙π bonds.

Theobromine also has the ability to bind with the NVP monomer via hydrogen bonding with binding energies ranging from -7.20 kcal mol\(^{-1}\) to -3.96 kcal mol\(^{-1}\). As with the theophylline- complexes, the intermolecular forces at play in these complexes range from strong hydrogen bonding (N-H⋅⋅⋅O) to relatively weak hydrogen bonding (C-H⋅⋅⋅O). The strongest of these interactions contained three weak hydrogen bonds (Figure 5.12a) whilst the weakest of these interactions contained two weak hydrogen bonds (Figure 5.12c &d).
Figure 5.12: The optimised complexes of theobromine-NVP. a) theobromine-NVP1, b) theobromine-NVP2, c) theobromine-NVP3, d) theobromine-NVP4

Figure 5.13: The optimised complexes of theobromine-DVB. a) theobromine-DVB1, b) theobromine-DVB2, c) theobromine-DVB3
Table 5.7: Calculated binding energies of theobromine with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>theobromine-NVP1</td>
<td>-7.20</td>
<td>C1-H9···O22</td>
<td>2.667</td>
<td>156.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1-H9···N23</td>
<td>2.721</td>
<td>129.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H14···N32</td>
<td>2.543</td>
<td>136.4</td>
</tr>
<tr>
<td>theobromine-NVP2</td>
<td>-7.09</td>
<td>N20-H38···O6</td>
<td>1.810</td>
<td>178.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H13···O22</td>
<td>2.349</td>
<td>153.8</td>
</tr>
<tr>
<td>theobromine-NVP3</td>
<td>-6.74</td>
<td>N20-H38···O6</td>
<td>1.805</td>
<td>175.4</td>
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<tr>
<td></td>
<td></td>
<td>C3-H14···O22</td>
<td>2.470</td>
<td>135.3</td>
</tr>
<tr>
<td>theobromine-NVP4</td>
<td>-3.96</td>
<td>C27-H30···O6</td>
<td>2.329</td>
<td>139.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H14···N32</td>
<td>2.757</td>
<td>146.8</td>
</tr>
</tbody>
</table>

Table 5.8: Calculated binding energies of theobromine with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>theobromine-DVB1</td>
<td>-8.80</td>
<td>π···π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>theobromine-DVB2</td>
<td>-8.59</td>
<td>π···π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>theobromine-DVB3</td>
<td>-8.20</td>
<td>π···π</td>
<td>~3.3</td>
<td>-</td>
</tr>
</tbody>
</table>
5.3.9 Comparison of: Caffeine vs Theophylline vs Theobromine

The hybrid-DFT calculations show that all three compounds can be adsorbed onto the Oasis HLB sorbent, and that as expected, the interactions are weak with binding energies no greater than -9.47 kcalmol\(^{-1}\).

Further, whilst the \textit{ab-initio} calculations show the xanthine compounds can interact with both monomers that comprise the sorbent polymer, the molecular modelling studies show that both caffeine and theobromine prefer to bind to the DVB monomer through the formation of π···π interactions, and that theophylline prefers to bind to the NVP monomer Oasis HLB to be with the DVB monomer through the formation of weak hydrogen bonds.

These properties explain why Oasis HLB is a good sorbent for solid phase extraction of caffeine and its associated compounds as they can be adsorbed in multiple locations on the sorbent material as well as can be removed relatively easily due to their weak interactions.

The order of binding strength determined from the hybrid DFT calculations showed that caffeine binds the strongest, followed by theophylline and then theobromine. This trend is consistent with the results detailed in the adsorption studies reported both in this chapter and by Bäuerlein \textit{et al.} [105] which show that Oasis HLB has a greater adsorption capacity for caffeine than it does for either theophylline or theobromine.

5.4 Conclusion

Batch adsorption investigations show that the order of the maximum adsorption (from highest to lowest) is as follows: caffeine > theophylline > theobromine. The batch
adsorption data also shows that the order of adsorption affinity for the selected xanthine compounds with Oasis HLB is as follows: caffeine > theophylline > theobromine.

The molecular modelling results presented in this chapter show that the primary mode of interaction between caffeine and the Oasis HLB monomers is most likely through the formation of $\pi \cdot \cdot \cdot \pi$ interactions that results in caffeine being physisorbed onto the Oasis HLB sorbent. Caffeine is able to form 7 energetically favourable configurations with the Oasis HLB monomers and has a greater number of energetically favourable configurations with the NVP monomer. The theophylline-monomer complexes produced 10 energetically favourable configurations favourable of which the NVP configurations were found to have the greatest binding energy for theophylline due to the formation of strong hydrogen bonds between the N-H moieties and hydrogen on the NVP monomer. The theobromine-monomer complexes produced 7 energetically favourable configurations of which the DVB configurations were found to have the greatest binding energy with the formation of $\pi \cdot \cdot \cdot \pi$ interactions between the theobromine molecule and the DVB monomer.
6 Triazine Herbicides

6.1 Introduction

Three structurally similar herbicides have been selected for investigation. These compounds are atrazine, simazine and DIA. Both atrazine and simazine are commonly used herbicides and DIA is breakdown product of atrazine and to a lesser extent, simazine [45]. Due to their ubiquitous use in weed control, their low affinity to soil, their moderate solubility in water and as they are considered to be stable in the environment, these compounds are frequently detected in both surface and ground water [46, 47]. As these compounds have been found to have adverse effects to both human and environmental health, over the years, a number of different extraction and analytical methods have been developed for the continual monitoring of these compounds [153-155] including the development of passive sampling methods [33, 156, 157].

During the development of the passive sampling device, the polar organic chemical integrative sampler (POCIS), it was discovered that one triazine compound in particular, DIA had poor affinity for the sampler and that the desorption of this particular compound had good correlation with water turbulence leading to DIA being considered as a suitable performance reference compound for the POCIS device [36].

The aim of this study was to elucidate the mechanisms of interaction at play between Oasis HLB and three structurally similar triazine herbicides (atrazine, simazine and DIA) and a polymeric sorbent (Oasis HLB).
6.1.1 Molecular Structures and Physiochemical Properties of the Selected Triazine Compounds

All three triazine compounds investigated are classified as nitrogen containing heterocyclic compounds. Comparison of all three compounds shows that whilst the central aromatic-ring with attached N-H moieties is the same for all three compounds, the attached functional groups varying between compounds.

Atrazine is an asymmetrical molecular with an isopropyl group attached to one end of the molecule and a methyl group attached to the other end of the molecule. In contrast, simazine is a symmetrical molecule that contains a methyl group attached to either end of the molecule. Like atrazine, DIA is also a non-symmetrical molecule with a methyl group attached to one end of the molecule and a N-H2 moiety attached to the other end of the molecule. The structure and physicochemical properties of the triazine compounds investigated in this chapter are presented in Table 6.1

Table 6.1: Structures and physicochemical properties of atrazine, simazine and DIA [150, 158]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Aqueous Solubility mgL$^{-1}$ at 25°C</th>
<th>Log $K_{ow}$</th>
<th>Dipole Moment (debye)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td><img src="image" alt="Atrazine Structure" /></td>
<td>34.7</td>
<td>2.61</td>
<td>4.02</td>
</tr>
<tr>
<td>Simazine</td>
<td><img src="image" alt="Simazine Structure" /></td>
<td>6.2</td>
<td>2.10</td>
<td>4.40</td>
</tr>
<tr>
<td>DIA</td>
<td><img src="image" alt="DIA Structure" /></td>
<td>3,200</td>
<td>1.20</td>
<td>4.25</td>
</tr>
</tbody>
</table>

*Calculated with Gaussian 09
6.1.2 Potential Intermolecular Interactions

Each triazine compound has the potential to interact with Oasis HLB sorbent through the formation of two distinct and reversible intermolecular interactions. The first is hydrogen bonding and the second is π···π interactions.

All three triazine molecules have the potential to form weak hydrogen bonds between the hydrogen atoms contained within the triazine compounds and the C=O moiety within the NVP monomer. All three compounds also have the potential to form relatively strong hydrogen bonds between their N-H moieties of the triazine compounds and the hydrogen atoms on both the NVP and DVB monomers. The strength of the hydrogen bonds formed in the complexes is based on their bond lengths and are classified as very strong (1.2-1.5 Å), strong (1.5-2.2 Å) or weak (2.0-3.0 Å) [159]. Each triazine molecule also contains an aromatic ring giving them the potential to form π···π interactions with the DVB monomer. All potential molecular interactions described above were investigated in the molecular modelling studies presented in this chapter.
6.2 Materials and Methods

6.2.1 Triazine-Oasis HLB Batch Adsorption Studies

The adsorption isotherms of triazine compounds and Oasis HLB were determined using the batch adsorption method [151] and is based on previous work conducted by Bäuerlein et al. [105] who evaluated the adsorption characteristics of Oasis HLB an array of analytes (including caffeine, theobromine and theophylline). Batch adsorption studies were conducted according to section 3.3 in chapter 3.

6.2.2 Computational Details

To model the interaction of each triazine compound with the Oasis HLB sorbent, a series of calculations were performed where each of the triazine molecules was allowed to interact with the two structural monomers of the sorbent (NVP and DVB). The computational methods utilised for this study are discussed in section 4.7 of chapter 4.

6.3 Results and Discussion

6.3.1 Triazine-Oasis HLB Batch Adsorption Studies

The results of the single component batch adsorption studies conducted for this chapter are shown in Table 6.2 and the batch adsorption isotherms are shown in Figures 6.1.-6.3. All correlation coefficients (R^2 values) were greater than 0.98 which suggests that the Langmuir model was able to provide a favourable model of the data.

Batch adsorption investigations show that the order of maximum adsorption capacity of the selected triazine compounds with Oasis HLB (from highest to lowest) is as follows: atrazine > simazine > DIA. The results also show that the maximum adsorption capacities of both atrazine and simazine are similar to the maximum adsorption
capacities of the xanthine compounds investigated in chapter 5, and that in comparison; DIA has a significantly lower maximum adsorption capacity value. Also, although the batch adsorption data for DIA showed a good fit for the Langmuir model, an inflection point in the isotherm indicates the possibility that there could be more than one adsorption mechanism taking place when DIA is adsorbed onto Oasis HLB. However, as the concentration of DIA that was investigated for this study far exceeds the concentration levels that are predict to be in the environment, this phenomenon is not expected to model how environmental relevant concentrations of DIA would behave with either SPE cartridges or POCIS devices.

The batch adsorption data also shows that that the order of the adsorption affinity for the select triazine compounds with Oasis HLB is as follows: atrazine > simazine > DIA. Additionally, the calculated adsorption affinities of the triazine compounds are all significantly greater than the adsorption affinities of the xanthine compounds investigated.

Table 6.2: Batch adsorption results for atrazine, simazine and DIA with Oasis HLB

<table>
<thead>
<tr>
<th>Adsorption Characteristics</th>
<th>atrazine (±SD)</th>
<th>simazine (±SD)</th>
<th>DIA (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption Capacity $C_{\text{max}}$ (mmolkg$^{-1}$)</td>
<td>176.7 (7.7)</td>
<td>152.8 (3.0)</td>
<td>16.1 (1.3)</td>
</tr>
<tr>
<td>Adsorption Affinity $K_L$ (Lmmol$^{-1}$)</td>
<td>5,695 (247)</td>
<td>1,175 (100)</td>
<td>94 (10)</td>
</tr>
<tr>
<td>$K_D$ (Lkg$^{-1}$)</td>
<td>1,005,927 (222)</td>
<td>179,783 (18,783)</td>
<td>1,505 (39)</td>
</tr>
<tr>
<td>Log $K_D$</td>
<td>6.0 (0)</td>
<td>5.3 (0)</td>
<td>3.2 (0)</td>
</tr>
</tbody>
</table>
Figure 6.1: Adsorption isotherm of atrazine and Oasis HLB

Figure 6.2: Adsorption isotherm of simazine and Oasis HLB

Figure 6.3: Adsorption isotherm of DIA and Oasis HLB. Error bars shown in Figures 6.1, 6.2 and 6.3 represent the standard deviation of the mean.
6.3.2 Triazine-Oasis HLB Molecular Modelling Studies

The results of the molecular modelling studies that were conducted for this chapter are presented below. Prior to conducting the molecular modelling studies, both the triazine compounds and the Oasis HLB monomers were studied to determine potential sites of interaction. The results of this analysis are also presented below.

6.3.2.1 Optimised Compounds

The optimised geometries of atrazine, simazine, and DIA are presented in Figure 6.5 and the optimised geometries of the Oasis HLB monomers (NVP and DVB) are presented in Figure 6.6.

In Figures 6.4 to 6.11, oxygen is red, nitrogen is blue, carbon is grey, chlorine is green and hydrogen is white.

Figure 6.4: Optimised triazine molecules. a) atrazine, b) simazine, c) DIA

Figure 6.5: Optimised Oasis HLB monomers. a) NVP, b) DVB
6.3.2.2 Optimised Atrazine-Monomer Complexes

After geometry optimisation, 7 stable atrazine-monomer complexes were found, including 3 atrazine-NVP complexes and 4 atrazine-DVB complexes. The optimised complexes of atrazine-monomer complexes can be found in Figures 6.5 and 6.6 and the optimised energies, bond lengths and bond angles of the atrazine-monomer complexes are detailed in Tables 6.4 and 6.5.

The binding energies of the atrazine-monomer complexes ranged from -7.61 kcal mol\(^{-1}\) to -3.14 kcal mol\(^{-1}\). The strongest interaction was shown to occur with the DVB monomer with the formation of π···π interactions between the aromatic rings (Figure 6.5a). The next strongest interaction was between atrazine and the NVP monomer with the formation of a strong hydrogen bond between the N-H moiety contained within the atrazine molecule and the C=O moiety contained within the NVP monomer (Figure 6.4a and b). Similarly, the next two strongest interactions were also between the atrazine molecule and the NVP with the formation of strong hydrogen bonds between the N-H moiety on the atrazine molecule and the C=O contained within the NVP monomer (Figure 6.4c). The weakest interaction was between atrazine and the DVB monomer with the formation of a weak hydrogen bond formed between nitrogen contained within the aromatic ring of the atrazine molecule and the C-H moiety contained within the DVB monomer (Figure 6.5e).
Figure 6.6: The optimised complexes of atrazine with NVP. a) atrazine-NVP1, b) atrazine-NVP2, atrazine-NVP3

Figure 6.7: The optimised complexes of atrazine with DVB. a) atrazine-DVB1, b) atrazine-DVB2, c) atrazine-DVB3, d) atrazine-DVB4, e) atrazine-DVB5
Table 6.3: Calculated binding energies and molecular interactions of atrazine with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding Energy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrazine-NVP1</td>
<td>-7.17</td>
<td>N25-H45···O6</td>
<td>1.944</td>
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<td>atrazine-NVP2</td>
<td>-7.15</td>
<td>N25-H45···O6</td>
<td>1.880</td>
<td>169.2</td>
</tr>
<tr>
<td>atrazine-NVP3</td>
<td>-6.90</td>
<td>N24-H27···O6</td>
<td>1.927</td>
<td>175.8</td>
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</tbody>
</table>

Table 6.4: Calculated binding energies and molecular interactions of atrazine with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding Energy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>atrazine-DVB1</td>
<td>-7.61</td>
<td>π···π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>atrazine-DVB2</td>
<td>-6.57</td>
<td>N22-H42···π</td>
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</tr>
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<td>-5.41</td>
<td>N27-H30···π</td>
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</tr>
<tr>
<td>atrazine-DVB4</td>
<td>-3.53</td>
<td>C7-H11···N20</td>
<td>2.475</td>
<td>166.0</td>
</tr>
<tr>
<td>atrazine-DVB5</td>
<td>-3.14</td>
<td>C7-H11···N22</td>
<td>2.451</td>
<td>161.7</td>
</tr>
</tbody>
</table>
6.3.2.3 Optimised Simazine-Monomer Complexes

After geometry optimisation, 5 stable simazine-monomer complexes were found, including 2 simazine-NVP complexes and 3 simazine-DVB complexes. The optimised complexes of simazine-monomer complexes are described in Figures 6.7 and 6.8 and the optimised energies, bond lengths and bond angles of the simazine-monomer complexes are detailed in Tables 6.6 and 6.7.

The binding energies of the simazine-monomer complexes ranged from -8.86 k cal mol\(^{-1}\) to -3.66 k cal mol\(^{-1}\). The strongest interaction was shown to occur with the DVB monomer (Figure 6.9a) with the primary mode of interaction being the formation of a \(\pi\cdots\pi\) bond. The next strongest interaction was between simazine and the NVP monomer (Figure 6.8a) with the formation of a strong hydrogen bond between the N-H moiety contained within the simazine molecule and the C=O moiety contained within the NVP monomer. The weakest interaction was between simazine and the DVB monomer (Figure 6.7c) with the formation of a hydrogen bond between the N-H moiety contained within the simazine molecule and the aromatic ring of the DVB monomer.
Figure 6.8: The optimised complexes of simazine with NVP. a) simazine-NVP1, b) simazine-NVP2

Figure 6.9: The optimised complexes of simazine with DVB. a) simazine-DVB1, b) simazine-DVB2, c) simazine-DVB3
Table 6.5: Calculated binding energies and molecular interactions of simazine with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ∆Energy (kcalmol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>simazine-NVP1</td>
<td>-7.58</td>
<td>N25-H32···O6</td>
<td>1.931</td>
<td>174.3</td>
</tr>
<tr>
<td>simazine-NVP2</td>
<td>-6.59</td>
<td>C26-H34···O6</td>
<td>2.886</td>
<td>120.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2-H11···N24</td>
<td>2.587</td>
<td>132.9</td>
</tr>
</tbody>
</table>

Table 6.6: Calculated binding energies and molecular interactions of simazine with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ∆Energy (kcalmol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>simazine-DVB1</td>
<td>-8.86</td>
<td>π···π</td>
<td>~3.3</td>
<td>-</td>
</tr>
<tr>
<td>simazine-DVB2</td>
<td>4.89</td>
<td>N27-H30···π</td>
<td>2.853</td>
<td>-</td>
</tr>
<tr>
<td>simazine-DVB3</td>
<td>-3.66</td>
<td>C2-H11···N22</td>
<td>2.460</td>
<td>170.8</td>
</tr>
</tbody>
</table>
6.3.2.4 Optimised DIA-Monomer Complexes

After geometry optimisation, 8 stable DIA-monomer complexes were found, including 4 DIA-NVP complexes and 4 DIA-DVB complexes. The optimised DIA-monomer complexes are described in Figures 6.9 and 6.10 and the optimised energies, bond lengths and bond angles are detailed in Tables 6.8 and 6.9.

The binding energies of the DIA-monomer complexes ranged from -7.43 kcal mol\(^{-1}\) to 1.83 kcal mol\(^{-1}\). The strongest interaction was shown to occur with the DVB monomer (Figure 6.11a) with the primary mode of interaction being the formation of a π···π bond. The next 4 strongest interactions were between DIA and the NVP monomer (Figures 6.10 a-d) with the formation of hydrogen bonds between the NVP monomer and the DIA molecule. The weakest interaction was between the DIA molecule and the DVB monomer (Figure 6.11 d) through the formation of a weak hydrogen bond.
Figure 6.10: The optimised complexes of DIA with NVP. a) DIA-NVP1, b) DIA-NVP2, c) DIA-NVP3, d) DIA-NVP4

Figure 6.11: The optimised complexes of DIA with DVB. a) DIA-DVB1, b) DIA-DVB2, c) DIA-DVB3, d) DIA-DVB4.
Table 6.7: Calculated binding energies and molecular interactions of DIA with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ∆Energy (kcal mol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
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<tbody>
<tr>
<td>DIA-NVP1</td>
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<tr>
<td></td>
<td></td>
<td>C3-H13⋯N21</td>
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<td>150.9</td>
</tr>
<tr>
<td>DIA-NVP2</td>
<td>-7.39</td>
<td>C2-H11⋯N19</td>
<td>2.865</td>
<td>156.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C27-H30⋯O6</td>
<td>2.974</td>
<td>119.2</td>
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<td></td>
<td></td>
<td>C27-H34⋯O6</td>
<td>2.635</td>
<td>112.6</td>
</tr>
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<td>DIA-NVP3</td>
<td>-7.16</td>
<td>N24-H35⋯O6</td>
<td>1.974</td>
<td>170.3</td>
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<td>C3-H14⋯N19</td>
<td>2.703</td>
<td>120.6</td>
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<td>DIA-NVP4</td>
<td>-6.45</td>
<td>C7-H11⋯N22</td>
<td>1.922</td>
<td>173.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C27-H35⋯π</td>
<td>~3.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.8: Calculated binding energies and molecular interactions of DIA with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ∆Energy (kcal mol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
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<td>DIA-DVB1</td>
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<td>π⋯π</td>
<td>~3.3</td>
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<tr>
<td>DIA-DVB2</td>
<td>-5.37</td>
<td>N28-H35⋯π</td>
<td>~3.0</td>
<td></td>
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<tr>
<td>DIA-DVB3</td>
<td>-2.84</td>
<td>C7-H11⋯N26</td>
<td>2.476</td>
<td>162.3</td>
</tr>
<tr>
<td>DIA-DVB4</td>
<td>-1.83</td>
<td>C7-H11⋯N22</td>
<td>2.428</td>
<td>175.5</td>
</tr>
</tbody>
</table>
6.3.3 DIA as a PRC – Examining the Underlying Mechanisms

The results of the molecular modelling studies reported in this chapter show that the primary interaction between all three triazine compounds and the Oasis HLB polymer is through the formation of π···π bonds with the DVB monomer. The reason the binding energies are lower for the DIA complexes than they are for either the atrazine complexes or the simazine complexes, may be because DIA has an NH2 moiety, which would be increase its attraction to the water and hence it would be expected that DIA would prefer to partition into water rather than stay bound to the polymeric sorbent when the POCIS device is placed into an aqueous environment.

6.4 Conclusion

Batch adsorption investigations show that the order of maximum adsorption capacity of the selected triazine compounds with Oasis HLB (from highest to lowest) is as follows: atrazine > simazine > DIA. The hybrid-DFT calculations show that the order of binding strength of the 3 compounds with the Oasis-HLB monomers is the same.

Hybrid-DFT calculations show that all three compounds can be adsorbed onto the Oasis HLB sorbent, and that as expected, the interactions are weak with binding energies no greater than -8.99 kcalmol⁻¹. Furthermore, whilst the modelling studies show the triazine compounds can interact with either monomer, atrazine and simazine both prefer to bind to the DVB monomer through the formation of π···π bonds, DIA prefers to bind to the NVP monomer through the formation of relatively strong hydrogen bonds.
7 Chlorinated Organophosphorous Flame Retardants

7.1 Introduction

Chlorinated OPFRs have been in widespread use since the 1970s being employed in a number of different materials from clothing to furniture to various building materials.

Due to the large scale use, combined with the fact that they are not chemically bound to the materials they are employed, diffusion of these compounds into the surrounding environment, through water, air, and particulate matter is highly likely.

Recent studies have shown that these compounds are only slightly biodegradable, potentially toxic to humans and aquatic life, and are frequently detected in all environmental compartments, and in particular in wastewater treatment plant effluent [160, 161].

As their name suggests, chlorinated OPFRs are phosphorus-based compounds that incorporate chlorine into their structure. They are produced through the reaction of alkylene oxides and phosphorus chlorides in the presence of catalysts [162].

The chlorinated OPFRs that will be evaluated in this study are as follows: tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl)phosphate (TCPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCPP). Traditionally, these compounds have been used as flame retardants in a wide range of polymeric materials including: plastic foams, resins, and latexes and in the production of liquid unsaturated polyester resins, respectively (World Health Organisation, 1998).
Chlorinated OPFRs are considered to be a high-volume product. In 2005, 85,000 tonnes of OPFRs was used in Europe alone, of which 46 000 tonnes, or just over half of all OPFRs used, were chlorinated. By 2007, this number had increased to 51,000 tonnes [163]. Although it is expected that the quantity of chlorinated OPFRs has increased in recent years, current manufacturing quantities for these compounds could not be located despite an extensive internet search being conducted.

Due to the large scale use, combined with the fact that they are not chemically bound to the materials they are employed, diffusion of these compounds into the surrounding environment, through water, air, and particulate matter is highly likely[49]. Chlorinated OPFRs have been detected in aquatic environments in many countries, including Sweden [164, 165], Austria [166], Germany[52, 167], Spain [168], and the United Kingdom [169] amongst others. They have been detected in waste-water [170, 171], drinking water [172] and surface water [173]. Research has shown that these compounds exist in aquatic environment at concentrations as low as ngL\(^{-1}\) [174] and as high as ugL\(^{-1}\) [175].

### 7.1.1 TCEP

TCEP is a clear, colourless liquid additive halogenated flame retardant and is used as a flame retardant in plastics, especially in flexible foams used in automobiles and furniture, and in rigid foams used for building insulation [176].

### 7.1.2 TCPP

TCPP is a clear, colourless liquid additive halogenated flame retardant [176]. The trade product consists of a mixture of four halogenated phosphoric acid esters of which the main components are tris(chloroiso-propyl) phosphate (75%), and bis(1-chloro-2-propyl)-2-chloropropyl-phosphate (15%-30%) [176].
7.1.3 TDCPP

TDCPP is a viscous colourless liquid additive flame retardant used in resins, latexes, and foams [176]. TDCPP is used in similar products to TCPP but because of the higher cost of TDCPP, it is often used in products where more effective flame retardants are required. TDCPP is often used in foams utilised by the automotive industry and can be found in foams used in furniture [176].

7.1.4 Molecular Structures and Physiochemical Properties of the Selected COPFR Compounds

All three chlorinated OPFR compounds investigated in this chapter contain a central phosphate ester moiety to which varying length chlorinated-alky chains are attached. The number of chlorine atoms and the length of the attached alkyl chain appear to have a direct impact on the ability of the select chlorinated OPFRs to dissolve into water. TCEP which has the shortest attached alkyl chains has the highest solubility value of the three compounds. In contrast, TDCPP which has the highest number of attached chlorine atoms has the lowest water solubility of the three compounds. It is expected that these structural features and physicochemical properties will have a fairly significant impact on the ability of the selected flame retardants to bind to the Oasis HLB sorbent. The structures and physicochemical structures of the selected chlorinated OPFRs investigated in this chapter are detailed in Table 7.1.
Table 7.1: Structures and Physicochemical properties of TCEP, TCPP and TDCPP [150].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Aqueous Solubility mgL$^{-1}$</th>
<th>Log $K_{ow}$</th>
<th>Dipole Moment (debye)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP</td>
<td><img src="image1" alt="Structure" /></td>
<td>7,820</td>
<td>1.78</td>
<td>3.8602</td>
</tr>
<tr>
<td>TCPP</td>
<td><img src="image2" alt="Structure" /></td>
<td>1,600</td>
<td>2.59</td>
<td>5.5198</td>
</tr>
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<td>TDCPP</td>
<td><img src="image3" alt="Structure" /></td>
<td>7</td>
<td>3.65</td>
<td>3.3092</td>
</tr>
</tbody>
</table>

7.1.5 Potential Intermolecular Interactions

It is expected that all three chlorinated OPFR molecules should be able to form weak hydrogen bonds between the hydrogen atoms within these compounds and the C=O moieties on the NVP monomer. It is also expected that all three chlorinated OPFR compounds can potentially form hydrogen bonds with the DVB monomer with the aromatic ring contained within the DVB monomer acting as a hydrogen bond acceptor. What is unknown is the impact in which the distribution of the chlorine atoms within the three flame retardant molecules will have on the formation of these bonds. Although the chlorine atom is considered to be highly electronegative, as it is a relatively large atom, the strength of the associated electronegativity is dampened somewhat. In contrast, although nitrogen and oxygen have similar electronegative values to chlorine as they are much smaller in size, they have the ability to take part in hydrogen bond formation, something the chlorine atom is not recognised in being able to do.
As mentioned previously, the strength of the hydrogen bonds formed in these complexes is based on their bond lengths and are classified as very strong (1.2-1.5 Å), strong (1.5-2.2 Å) or weak (2.0 -3.0 Å) [153].

7.2 Materials and Methods

7.2.1 Batch Adsorption Study

The adsorption isotherms of the selected chlorinated OPFR compounds and Oasis HLB were determined using the batch adsorption method [151] and is based on previous work conducted by Bäuerlein et al. [105]. Batch adsorption studies were conducted according to section 3.3 chapter 3.

7.2.2 Computational Details

To model the interaction of each of the chlorinated OPFR compounds with the Oasis HLB sorbent, a series of calculations were performed where each of the chlorinated OPFR molecules was allowed to interact with the two structural monomers of the sorbent (NVP and DVB). The computational methods that were utilised for this study are discussed in section 4.7, chapter 4.

7.3 Results and Discussion

7.3.1 Batch Adsorption Results

The results of the single component batch adsorption studies conducted for this chapter are shown in Table 7.2 and the batch adsorption isotherms are shown in Figures 7.1.-7.3. All correlation coefficients (R² values) were greater than 0.98 which suggests that the Langmuir model was able to provide a favourable model of the data.
Batch adsorption investigations show that the order of maximum adsorption capacity of the selected triazine compounds with Oasis HLB (from highest to lowest) is as follows: TCPP > TDCPP > TCEP.

The batch adsorption data also shows that the order of adsorption affinity for the select chlorinated OPFR compounds with Oasis HLB (from highest to lowest) is as follows: TCPP > TCEP > TDCPP.

Table 7.2: Batch adsorption results for TCEP, TCPP and TDCPP with Oasis HLB.

<table>
<thead>
<tr>
<th>Adsorption Characteristic</th>
<th>TCEP (±SD)</th>
<th>TCPP (±SD)</th>
<th>TDCPP (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption Capacity (C_{\text{max}}) (mmolkg(^{-1}))</td>
<td>131 (7)</td>
<td>638 (20)</td>
<td>435 (26)</td>
</tr>
<tr>
<td>Adsorption Affinity (K_L) (Lmmol(^{-1}))</td>
<td>511 (100)</td>
<td>4,708 (3,043)</td>
<td>349 (78)</td>
</tr>
<tr>
<td>(K_D) (Lkg(^{-1}))</td>
<td>66,889 (9,762)</td>
<td>3,004,890 (2,302,023)</td>
<td>151,561 (21,405)</td>
</tr>
<tr>
<td>Log (K_D)</td>
<td>4.82 (0)</td>
<td>6.48 (1)</td>
<td>5.20 (0)</td>
</tr>
</tbody>
</table>
Figure 7.1: Adsorption isotherm – TCEP and Oasis HLB

Figure 7.2: Adsorption isotherm – TCPP and Oasis HLB

Figure 7.3: Adsorption isotherm – TDCPP and Oasis HLB. Error bars shown in Figures 7.1, 7.2 and 7.3 represent the standard deviation of the mean.
7.4 Molecular Modelling Results

7.4.1 Optimised Compounds

The optimised geometries of TCEP, TCPP, and TDCPP are presented in Figure. 7.4 and the optimised geometries of the Oasis HLB monomers (NVP and DVB) are presented in Figure. 7.5.

In Figures 7.5 to 7.12 oxygen is red, nitrogen is blue, carbon is grey, chlorine is green and hydrogen is white.

![Optimised chlorinated OPFR molecules. a) TCEP, b) TCPP, c) TDCPP](image1)

![Optimised Oasis HLB monomers. a) NVP, b) DVB](image2)

Figure 7.4: Optimised chlorinated OPFR molecules. a) TCEP, b) TCPP, c) TDCPP

Figure 7.5: Optimised Oasis HLB monomers. a) NVP, b) DVB
7.4.2 TCEP-Monomer Complexes

After geometry optimisation, 7 stable TCEP-monomer complexes were found in total, including 4 TCEP-NVP complexes and 3 TCEP-DVB complexes. The optimised geometries of the TCEP-monomer complexes can be found in Figures 7.6 and 7.7 and the binding energies, bond lengths and bond angles of the TCEP-monomer complexes are detailed in Tables 7.2 and 7.3.

The binding energies of the TCEP-monomer complexes ranged from -6.94 kcal mol\(^{-1}\) to -5.47 kcal mol\(^{-1}\). The strongest interaction were shown to occur with the DVB monomer (Figure 7.7a and b) with the primary interaction within the two complexes being governed by the formation of hydrogen bonds with the aromatic ring contained within the DVB monomer. In this instance, the aromatic ring is acting as a hydrogen bond acceptor.

The next most stable TCEP-monomer complexes were shown to be with the NVP monomer all of which resulted from the formation of hydrogen bonding between the NVP monomer and the TCEP molecule (Figures 7.6a-d). In all of these interactions, the NVP monomer acted as the hydrogen bond acceptor and the TCEP molecule acted as the hydrogen bond donor.
Figure 7.6: The optimised complexes of TCEP-NVP. a) TCEP-NVP1, b) TCEP-NVP2, c) TCEP-NVP3, d) TCEP-NVP4

Figure 7.7: The optimised complexes of TCEP-DVB. a) TCEP-DVB1, b) TCEP-DVB2, c) TCEP-DVB3
Table 7.3: Calculated binding energies of TCEP with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ∆Energy (kcalmol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP-NVP1</td>
<td>-6.94</td>
<td>C29-H40⋯O2</td>
<td>2.249</td>
<td>152.4</td>
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<td>C8-H20⋯O32</td>
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<td>C27-H36⋯O2</td>
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<td>128.4</td>
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<td></td>
<td>C29-H39⋯O3</td>
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<td>109.7</td>
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<td>TCEP-NVP4</td>
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<td>164.3</td>
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<td>C27-H35⋯O2</td>
<td>2.393</td>
<td>143.7</td>
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<td></td>
<td>C6-H13⋯O32</td>
<td>2.812</td>
<td>116.6</td>
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Table 7.4: Calculated binding energies of TCEP with DVB

<table>
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<th>Binding ∆Energy (kcalmol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP-DVB1</td>
<td>-6.77</td>
<td>C7-H14⋯π</td>
<td>2.868</td>
<td>136.8</td>
</tr>
<tr>
<td>TCEP-DVB2</td>
<td>-6.73</td>
<td>C6-H13⋯π</td>
<td>2.894</td>
<td>139.9</td>
</tr>
<tr>
<td>TCEP-DVB2</td>
<td>-6.73</td>
<td>C11-H24⋯π</td>
<td>2.901</td>
<td>131.6</td>
</tr>
</tbody>
</table>
7.4.3 TCPP-Monomer Complexes

After geometry optimisation, 9 stable TCPP-monomer complexes were found in total including 4 TCPP-NVP complexes and 5 TCPP-DVB complexes. The optimised geometries of the TCPP-monomer complexes are shown in Figures 7.8 and 7.9 and their calculated properties are detailed in Tables 7.5 and 7.6.

The binding energies of the TCPP-monomer complexes ranged from -10.50 kcal mol$^{-1}$ to -5.04 kcal mol$^{-1}$. The strongest interactions were found to be between TCPP and the DVB monomer (Figure 7.7a). As with the TCEP-DVB complexes, TCPP was shown to hydrogen bond on to the DVB monomer with the TCPP acting as a hydrogen donor and the DVB monomer acting as a hydrogen bond acceptor. The next strongest interaction was TCPP-NVP1 (Figure 7.8a) with the formation of several weak hydrogen bonds. The weakest interaction was TCPP-NVP4 (Figure 7.9d). Like TCPP-NVP1, the dominant intermolecular interactions in the TCPP-NVP4 complex where weak hydrogen bonds. However, as only 2 weak hydrogen bonds were apparent in this complex, the TCPP-NVP1 (with 4 weak hydrogen bonds) had a stronger binding energy.
Figure 7.8: The optimised complexes of TCPP-NVP. a) TCPP-NVP1, b) TCPP-NVP2, c) TCPP-NVP3, d) TCPP-NVP4

Figure 7.9: The optimised complexes of TCPP-DVB. a) TCPP-DVB1, b) TCPP-DVB2, c) TCPP-DVB3, d) TCPP-DVB4
Table 7.5: Calculated binding energies of TCPP with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
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<tbody>
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</tr>
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<td></td>
<td>C2-H11⋯O21</td>
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<td>143.7</td>
</tr>
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<td>C26-H28⋯O6</td>
<td>2.620</td>
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<td></td>
<td></td>
<td>C3-H14⋯O19</td>
<td>2.868</td>
<td>128.8</td>
</tr>
<tr>
<td>TCPP-NVP4</td>
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<td>2.622</td>
<td>132.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2-H12⋯O21</td>
<td>2.677</td>
<td>116.3</td>
</tr>
</tbody>
</table>

Table 7.6: Calculated binding energies of TCPP with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol(^{-1}))</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCPP-DVB1</td>
<td>-10.50</td>
<td>C26-H35⋯π</td>
<td>2.820</td>
<td>142.1</td>
</tr>
<tr>
<td>TCPP-DVB2</td>
<td>-8.55</td>
<td>C32-H43⋯π</td>
<td>2.772</td>
<td>144.1</td>
</tr>
<tr>
<td>TCPP-DVB3</td>
<td>-6.51</td>
<td>C32-H43⋯π</td>
<td>2.866</td>
<td>138.7</td>
</tr>
<tr>
<td>TCPP-DVB4</td>
<td>-5.94</td>
<td>C42-H45⋯π</td>
<td>2.921</td>
<td>139.0</td>
</tr>
<tr>
<td>TCPP-DVB5</td>
<td>-5.80</td>
<td>C6-H17⋯O24</td>
<td>2.392</td>
<td>140.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C9-H18⋯O25</td>
<td>2.841</td>
<td>136.2</td>
</tr>
</tbody>
</table>
7.4.4 TDCPP-Monomer Complexes

After geometry optimisation, 7 stable TDCPP-complexes were found in total, including 3 TDCPP-NVP monomer complexes and 4 TDCPP-DVB complexes. The optimised geometries of the TDCPP-monomer complexes are shown in Figures 7.10 and 7.11 and their calculated properties are detailed in Tables 7.7 and 7.8.

The binding energies of the TDCPP-monomer complexes ranged from -10.05 kcalmol\(^{-1}\) to -4.18 kcalmol\(^{-1}\). The strongest interaction was shown to occur between TDCPP and the NVP monomer (Figure 7.8a) with the formation of multiple weak hydrogen bonds. The next strongest interaction was between TDCPP and the DVB monomer (Figure 7.9a). As with the TCEP and TCPP compounds, the TDCPP compound interacted with the DVB monomer through the formation of a hydrogen bond between with the aromatic ring in the DVB monomer. The weakest interaction was between TDCPP and the DVB monomer (Figure 7.9d).
Figure 7.10: The optimised complexes of TDCPP-NVP. a) TDCPP-NVP1, b) TDCPP-NVP2, c) TDCPP-NVP3, d) TDCPP-NVP4

Figure 7.11: The optimised complexes of TDCPP-DVB. a) TDCPP-DVB1, b) TDCPP-DVB2, c) TDCPP-DVB3, TDCPP-DVB4
Table 7.7: Calculated binding energies of theobromine with NVP

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDCPP-NVP1</td>
<td>-10.05</td>
<td>C1-H9⋯O19</td>
<td>2.520</td>
<td>153.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C30-H46⋯O6</td>
<td>2.315</td>
<td>142.5</td>
</tr>
<tr>
<td>TDCPP-NVP2</td>
<td>-8.49</td>
<td>C3-H14⋯O18</td>
<td>2.518</td>
<td>141.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C24-H37⋯O6</td>
<td>2.396</td>
<td>140.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H14⋯O2O2</td>
<td>2.839</td>
<td>116.1</td>
</tr>
<tr>
<td>TDCPP-NVP3</td>
<td>-5.01</td>
<td>C22-H49⋯O6</td>
<td>2.175</td>
<td>173.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C23-H31⋯O6</td>
<td>2.324</td>
<td>158.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3-H13⋯O32</td>
<td>2.550</td>
<td>119.4</td>
</tr>
</tbody>
</table>

Table 7.8 Calculated binding energies of TDCPP with DVB

<table>
<thead>
<tr>
<th>Complex</th>
<th>Binding ΔEnergy (kcal mol⁻¹)</th>
<th>Molecular Interaction</th>
<th>Bond Length (Å)</th>
<th>Bond Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDCPP-DVB1</td>
<td>-8.50</td>
<td>C13-H29⋯π</td>
<td>2.809</td>
<td>142.3</td>
</tr>
<tr>
<td>TDCPP-DVB2</td>
<td>-8.04</td>
<td>C31-H46⋯π</td>
<td>2.802</td>
<td>140.0</td>
</tr>
<tr>
<td>TDCPP-DVB3</td>
<td>-6.39</td>
<td>C29-H32⋯π</td>
<td>2.977</td>
<td>146.5</td>
</tr>
<tr>
<td>TDCPP-DVB4</td>
<td>-4.18</td>
<td>C5-H32⋯π</td>
<td>2.966</td>
<td>121.2</td>
</tr>
</tbody>
</table>
7.4.5 Comparison of TCEP vs TCPP vs TDCPP

The hybrid-DFT calculations show that all three compounds can be adsorbed onto the Oasis HLB sorbent, and that as expected, the interactions are weak with binding energies no greater than -10.50 kcalmol\(^{-1}\).

Additionally, whilst the modelling studies show the chlorinated OPFR compounds can interact with both monomers that comprise the sorbent polymer, the molecular modelling studies show that both TCEP and TDCPP prefer to bind to the NVP monomer through the formation of weak hydrogen bond and TCPP prefers to bind to the DVB monomer through the formation of hydrogen bonds with the aromatic ring of the DVB monomer.

The order of binding strength determined from the hybrid DFT calculations showed that TCPP binds the strongest, followed by TDCPP and then TCEP. This trend is consistent with the results detailed in the adsorption studies reported in this chapter which show that Oasis HLB has a greater adsorption capacity for TCPP than it does for either TDCPP or TCEP.

7.5 Conclusion

Batch adsorption investigations show that the order of maximum adsorption capacity of the selected triazine compounds with Oasis HLB (from highest to lowest) is as follows: TCPP > TDCPP > TCEP.

The batch adsorption data also shows that the order of the adsorption affinity for the select chlorinated OPFR compounds with Oasis HLB (from highest to lowest) is as follows: TCPP > TCEP > TDCPP.
The results of the molecular modelling studies show that the primary mode of interaction between the chlorinated OPFRs and the Oasis HLB monomers is most likely through the formation of weak hydrogen bonds resulting in all three chlorinated OPFR compounds being able to physisorb onto the Oasis HLB sorbent.

The TCEP-monomer complexes produced 7 energetically favourable configurations with the Oasis HLB monomers it has a greater number of energetically favourable configurations with the DVB monomer. The TCPP-monomer complexes produced 9 energetically favourable configurations favourable configurations with the Oasis HLB monomers, of which the DVB configurations were found to have the greatest binding energy for TCPP due to the formation of weak hydrogen bonds between the hydrogen atom on the TCPP and the aromatic ring in the DVB monomer. The TDCPP-monomer complexes produced 7 energetically favourable configurations of which the DVB configurations were found to have the greatest binding energy with the formation of a hydrogen bond between the hydrogen atom contained within TDCPP and the aromatic ring in the DVB monomer.
8 Chlorinated OPFR-POCIS Calibration Study

8.1 Introduction

As detailed in the introduction of chapter 7, the chlorinated OPFRs investigated in this dissertation (TCEP and TCPP, TDCPP) are ubiquitous pollutants that are commonly detected in anthropogenically affected water. Chlorinated OPFRs are also known to be transient in wastewater effluent.

Thus, this class of contaminant is ideal for monitoring by the POCIS device. To date, sampling rate data has only been determined for two types of passive sampling devices. The first is a modified POCIS device and the second is a ceramic dosimeter (using Oasis HLB as the receiving phase). Therefore, the aim of the work reported in this chapter was to perform a flow-through calibration study so as to ascertain sampling rate data for a typical commercially available POCIS device.

8.2 Methods

8.2.1 Determination of Limit of Detection and Limit of Quantification

An Agilent 1200 Infinity HPLC coupled with an Agilent 4610 triple quadrupole mass spectrophotometer was used for all analytical investigations conducted with both the chlorinated OPFR. Details are provided in Tables 3.3 - 3.5 (chapter 3).

Both the instrumental limit of detection (LOD) and limit of quantification (LOQ) were determined for each chlorinated OPFR compound by running a series of calibration standards at concentration levels between 0.1 – 100 ngmL⁻¹. The limit of detection and
limit of quantification were defined as the concentration in which the signal to noise ratio was 3 and 10 respectively.

8.2.2 SPE Extraction and Recovery Studies - Chlorinated OPFRs

SPE extraction and recovery studies were conducted in order to validate the solvent types and solvent volumes used in the extraction of the chlorinated OPFR compounds from the SPE matrix.

The SPE method that was validated for this investigation is a modification of a published method [102] and is described in Figure 8.1.
8.3 POCIS Calibration Studies

POCIS calibration studies were conducted in order to determine the rate at which the selected chlorinated OPFRs are sequestered into the passive sampler – POCIS. The sampling rate for each of the selected compounds was then compared to both the batch adsorption studies and to the molecular modelling studies that were conducted for these compounds in chapter 7.
The method that was selected to determine the rate at which the chlorinated OPFRs are sequestered into the POCIS device was the ‘Flow-through’ calibration method.

### 8.3.1 Preparation of POCIS Devices

POCIS devices were prepared in the laboratory using sorbent taken from Oasis HLB SPE extraction cartridges and 90. mm, 0.1 μm PES membranes. Both the sorbent and the PES membranes were used as is without further treatment. Each of the POCIS devices were prepared by weighing out 200 ± 5 mg of sorbent into the centre of a PES membrane. A second PES membrane was then carefully placed over the top of the first PES membrane before being enclosed between two stainless steel o-rings (of the same diameter as the PES membranes). The stainless steel o-rings were then fixed together with stainless steel bolts. Once prepared, the POCIS devices were wrapped in aluminium foil that had previously been cleaned with analytical grade ethanol. The wrapped samplers were then placed into separate zip lock bags before being stored at 4 °C until they were required for use.

### 8.3.2 Preparation of Chlorinated OPFR Concentrate

Stock solutions containing 4000 ngL⁻¹ (4 μgL⁻¹) of all three solutions were prepared in ultrapure water. The spiking solution was fed into a mixing tank (containing baffles) were it was diluted with tap water. The flow rate of the water used to dilute the spiking solution was controlled using a flow meter. The flow rate of the spiked solution was 0.3 mLmin⁻¹ and the flow rate of the feed water was 4 mLmin⁻¹ resulting in a nominal concentration of 280 ngL⁻¹ in the mixing tank.
8.3.3 POCIS Calibration Method

Ten POCIS devices (5 samplers in duplicate), each containing 200 mg of Oasis HLB sorbent were exposed to a continual stream of water containing trace quantities of each of the three chlorinated OPFR compounds under investigation for up to 10 days. Over the course of the calibration study, 2 samplers were removed from the system every two days, wrapped in clean aluminium foil, and kept at 4 ±2°C until the compounds were extracted from the sorbent matrix. Additionally, 500 mL water samples (4 samples in duplicate) were taken from the calibration tanks over the course of the study in order to determine the concentration of each of the chlorinated OPFRs in which the POCIS device was exposed to during the calibration study.

8.3.4 Calibration Microcosom

The calibration microcosm (as shown in Figures 8.2 and 8.3) was located at the Victorian Marine Science Consortium in Queenscliff, Victoria. The system was originally set up by Associate Professor Graeme Allison for determination of sampling rates for the Chemcatcher passive sampler with triazine herbicides [177].

As can be seen in Figure 8.2, the flow rate of the water that was fed into the mixing tank was controlled by a rotameter. Initial investigations of the system found that the rotameter was unable to adequately control the flow of water into the system, which can fluctuate over the course of both the day and the week, depending on frequency of use by others occupants of the building. In order to try and control the flow of water into the rotameter and hence the system, a pressure regulator was placed upstream of the rotameter as seen in Figure 8.3a
Figure 8.2: Calibration Set-Up
Figure 8.3: Photographs of the calibration set up – a) pressure regulator to control water flow, b) mixing tank, c) mixing tanks and calibration tanks
8.3.5 Compound Extraction from POCIS

Each POCIS device was opened and the sorbent was transferred from the POCIS device into empty 6 mL SPE cartridges containing polyethylene frits using no more than 20 mL of ultrapure water to aid in the transfer of the sorbent to the empty SPE cartridge. The sorbent was dried under vacuum for 30 minutes before the chlorinated OPFR compounds were extracted as per Figure 8.1.

8.3.6 Compound Extraction from Water

Each of the 500 mL water samples collected throughout the calibration study was initially filtered through a 1 μm glass-fibre filter. The chlorinated OPFR compounds were extracted using 200 mg Oasis HLB cartridges as per Figure 8.1.

8.3.7 Sampling Rate Calculations

Sampling rates for all three chlorinated OPFR compounds were determined using the following equation:

\[
R_s = \frac{C_s M_s}{C_w t}
\]

Where \( R_s \) is the sampling rate of the compound (Ld\(^{-1}\)), \( C_s \) is the analyte concentration adsorbed to the sorbent (ngg\(^{-1}\)), \( C_w \) is the analyte concentration in the water (ngL\(^{-1}\)), and \( t \) is the sampling time (days) and \( M_s \) is the mass of the sorbent (g).

8.3.8 Quality Control

Procedural blanks containing all components of the standards and extracted samples bar the analytes themselves were analysed alongside the calibration standards and the
samples so as to ensure the validity of the data being produced by the HPLC MS/MS and to ensure the purity of all solvents used to generate both standards and samples.

All POCIS devices were prepared under a laminar flow of air in a biological safety cabinet so as to minimise contamination of the samplers with the chlorinated OPFR compounds (which have been shown to be ubiquitous in dust [178]).

Two additional POCIS devices were prepared at the same time as the calibration POCIS devices. These samplers were kept in the laboratory in which the calibration study was conducted for the duration of the study so as to assess potential contamination caused during sampler preparation, compound extraction and analysis. Passive sampling devices that are created for this purpose are commonly known as a fabrication blanks.

At the end of the calibration study, the same protocol that was used to determine the concentration of the chlorinated OPFR analytes in the calibration POCIS devices was also employed for the fabrication blanks.

8.4 Results and Discussions

8.4.1 Limits of Detection and Quantification

The chlorinated OPFRs investigated in this dissertation were evaluated using an Agilent HPLC MS/MS. The limits of detection (LOD) and limit of quantification (LOQ) as well as the associated calibration linear regression values ($R^2$) that were obtained for these analytes using this instrumental method are shown below in Table 8.1.
Table 8.1: LOD and LOQ for TCEP, TCPP and TDCPP

<table>
<thead>
<tr>
<th>Compound</th>
<th>R²</th>
<th>LOD (ngmL⁻¹)</th>
<th>LOQ (ngmL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP</td>
<td>0.9816</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>TCPP</td>
<td>0.9855</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>TDCPP</td>
<td>0.9979</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

8.4.2 SPE Extraction and Recovery Results

Extraction and recovery studies were conducted to validate the SPE procedure that was used for this chapter. As shown in Table 8.2, whilst the recovery levels decreased with increasing concentrations, good levels of recovery were still achieved with minimum recovery levels being greater than 80% for all three compounds. Additionally, standard deviations between replicates (n=3) were found to be low indicating that the extraction methods employed are suitable for the extraction of chlorinated OPFRs from Oasis HLB.

Table 8.2: Extraction and Recovery Studies –TCEP, TCPP and TDCPP

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ultrapure water spiked with 20 ngmL⁻¹</th>
<th>Ultrapure water spiked with 100 ngmL⁻¹</th>
<th>Ultrapure water spiked with 250 ngmL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Recovery (± SD)</td>
<td>% Recovery (± SD)</td>
<td>% Recovery (± SD)</td>
</tr>
<tr>
<td>TCEP</td>
<td>89 % ± 6</td>
<td>87 % ± 2</td>
<td>82 % ± 3</td>
</tr>
<tr>
<td>TCPP</td>
<td>95 % ± 5</td>
<td>88 % ± 2</td>
<td>83 % ± 3</td>
</tr>
<tr>
<td>TDCPP</td>
<td>107 % ± 10</td>
<td>102 % ± 2</td>
<td>96 % ± 4</td>
</tr>
</tbody>
</table>
8.4.3 POCIS Calibration Data

The average concentration of each chlorinated OPFR in the calibration tanks over the course of the calibration study is shown in Figure 8.4. Although the concentration of two of the three analytes (TCEP and TDCPP) were determined to be lower than anticipated concentration of 300 ngL\(^{-1}\) possibly due to adsorption of these compounds onto the system components such as the tubing that fed the stock solution into the mixing chamber or the tubing that fed the diluted compounds into the calibration tanks. As the flow-through calibration system was able to maintain a relatively constant concentration of the analyte for the duration of the study this deviation from the expected result should have no impact on the validity of the sampling rate data derived for these compounds.

Figure 8.4: Average concentration of each analyte in the calibration tanks Error bars shown in Figures 8.4 represents the standard deviation of the mean.
The average concentration of each chlorinated OPFR accumulated in the POCIS devices is shown in Figures 8.5, 8.6 and 8.7. The results show a steady uptake of the analytes into the POCIS device over the course of the study with $R^2$ values ranging from 0.9131 (TCEP) to 0.9767 (TDCPP).

The POCIS calibration study showed that for the Oasis HLB POCIS device that both TCEP and TDCPP had not reached equilibrium after an exposure period of 10 days as the amount of each these compounds accumulated into the sampler continued to increase in a linear fashion for the entire exposure period (see Figures 8.5 and 8.7). In contrast, Figure 8.6 suggests that TCPP may have reached equilibrium after an exposure period of 10 days as the data seems to flatten out at day 10. However, this assumption is based off only one data point. Further calibration studies are required to determine possible impacts to sampling rate kinetics for longer time periods and for concentrations greater than those investigated in this study.
Figure 8.5: Average concentration of TCEP accumulated in the POCIS devices over time.

\[ y = 15.748x + 28.818 \]
\[ R^2 = 0.9767 \]

Figure 8.6: Average concentration of TCPP accumulated in the POCIS devices over time.

\[ y = 27.281x + 56.995 \]
\[ R^2 = 0.9131 \]

Figure 8.7: Average concentration of TDCPP accumulated in the POCIS devices over time. Error bars shown in Figures 8.5, 8.6 and 8.7 represent the standard deviation of the mean.

\[ y = 12.291x + 51.823 \]
\[ R^2 = 0.9365 \]
### 8.4.4 Quality Control

As detailed in section 8.3.8, both procedural blanks and fabrication blanks were used as quality control measures for this study.

Procedural blanks were used to determine the likelihood that any of the solvents used to extract the chlorinated OPFRs from the sorbent material were contaminated with chlorinated OPFRs. Procedural blanks for this study were found to be below the LOQ in this instance.

Fabrication blanks were constructed in duplicate in order to determine potential contamination of the samplers during construction, transport, and analysis. The concentration of each chlorinated OPFR that was detected in the fabrication blanks are detailed in Table 8.4.

#### Table 8.4: Concentration of chlorinated OPFRs in Fabrication Blanks

<table>
<thead>
<tr>
<th>Compound</th>
<th>POCIS Concentration (ng/sampler±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP</td>
<td>13.95 (0.24)</td>
</tr>
<tr>
<td>TCPP</td>
<td>3.00 (0.39)</td>
</tr>
<tr>
<td>TDCPP</td>
<td>0.36 (0.12)</td>
</tr>
</tbody>
</table>

Whilst TCPP and TDCPP were below the LOQ, TCEP was found to be marginally above the LOQ. Whilst care was taken to avoid the possibility of cross contaminating the POCIS samplers during preparation, transport, and subsequent analysis, these results highlight how important quality control procedures are in environmental processes.

Although the source of TCEP in the fabrication blanks are unknown, as the accumulated concentration of TCEP in each of the POCIS devices used for the calibration study was
at least 4 times the concentration that was detected in the fabrication blanks, it is safe to say that the potential contamination of the POCIS devices has in this instance not impacted the validity of the data that was generated for this study. However, it must be noted that 10 \text{ugL}^{-1} is still a substantial concentration, especially in terms of environmental concentration levels, where parts per trillion concentrations (ngL$^{-1}$) are known to have environmental effects. Therefore, additional studies are needed in order to: 1) determine the source of the contamination, 2) the likelihood that future samplers will be contaminated if the same method was used, 3) to develop methods and protocols to ensure any future POCIS devices are not contaminated as the fabrication samplers (and potentially, the calibration samplers) were in this instance.

8.4.5 Sampling Rates

Sampling rates for each compound was determined using equation 8.1 and are shown in Table 8.5.

$$R_s = \frac{C_s M_s}{C_w t}$$

(8.1)

Where $R_s$ is the sampling rate, $C_s$ (Ld$^{-1}$) is the concentration of analyte that has accumulated in the sampler (blank corrected values), $M_s$ (g) is the mass of the sorbent, $C_w$ (ngL$^{-1}$) is the average concentration of analyte in the water and $t$ (days) is sampling time.
Table 8.5: Sampling rate data for all three chlorinated OPFRs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sampling Rate Ld$^{-1}$ (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCEP</td>
<td>0.14 (0.02)</td>
</tr>
<tr>
<td>TCPP</td>
<td>0.13 (0.01)</td>
</tr>
<tr>
<td>TDCPP</td>
<td>0.10 (0.02)</td>
</tr>
</tbody>
</table>

As sampling rate data for all three chlorinated OPFRs were disproportionally higher on day 2 compared to all other sampling days, the average sampling rate has been calculated from results generated from days 4 -10 only. Higher than expected sampling rate values on day 2 are most likely due to rapid uptake of the analytes upon initial exposure of the samplers to the spiked water (contained within the calibration microcosm).

8.4.6 Sampling Rate Data Compared to Literature

Whilst chlorinated OPFRs are considered to be ubiquitous in most environmental waters, few studies have been conducted in order to calibrate the POCIS device for the passive sampling of these compounds. The studies that have been conducted, to the best of our knowledge, none have involved the standard POCIS device.

Yang et al. [179] conducted a calibration study that included two out of the three chlorinated OPFRs discussed in this dissertation (TCEP and TDCPP), using a modified POCIS device. In contrast to the standard POCIS device whose rate limiting membrane has a porosity of 0.1 µm, the modified POCIS device contained a rate limiting membrane of 0.45 µm. As such, it is expected that the sampling rate values for the modified POCIS device should be greater than that of the standard POCIS device. The sampling rate values derived by Yang et al. are as follows: TCEP - 0.327 Ld$^{-1}$, and
TDCPP - 1.037 Ld\(^{-1}\). These results show that whilst the sampling rate for TCEP calculated in the calibration study of the modified POCIS was more than twice that of the sampling rate of the standard POCIS device, the TDCPP sampling rates was approximately 10 times the rate in the modified POCIS compared to the standard POCIS device. In order to verify these results additional studies using a rate limiting membranes containing differing porosity values would need to be conducted.

Additionally, Cristale et al. [180] also performed a calibration study for an alternative sorbent passive sampling device – the ceramic dosimeter. Whilst this alternative device also contained Oasis HLB sorbent, it has a different configuration to the POCIS device. The ceramic dosimeter utilised in the study conducted by Cristale et al. was comprised of a ceramic tube of 5 cm in length and 1 cm in diameter, a wall thickness of 1.5 mm and a porosity of 5nm. Each ceramic dosimeter used contained 400 mg of Oasis HLB sorbent which is twice the amount of sorbent that is typically used in a standard POCIS device.

As the external walls of the ceramic dosimeter is thicker than the rate limiting membrane used in the standard POCIS device, it can be expected that the sampling rates for the dosimeter will be lower than that of the standard POCIS device. Results achieved by Cristale et al. showed this to be true as the sampling rates for the ceramic device was a factor of 10 lower than the result achieved by the sampling rate study conducted for this dissertation with Cristale et al. achieving the following sampling rates: TCEP - 0.0037 L d\(^{-1}\), TCPP – 0.0027 Ld\(^{-1}\) and TDCPP -0.0026 Ld\(^{-1}\).
8.4.7 Sampling Rate Data Compared to Batch Adsorption Studies and Molecular Modelling Studies

Whilst both the batch adsorption studies and the molecular modelling studies show that TCPP had the greatest affinity for Oasis HLB compared to the other two chlorinated OPFRs, surprisingly, as previously mentioned, the sampling rate values of TCEP and TCPP are remarkably similar to each other. Comparatively, TDCPP which had the lowest affinity for Oasis HLB as shown in the batch adsorption studies and the molecular modelling studies also had the lowest sampling rate value as well.

8.5 Conclusions

The results presented in this chapter show that the standard POCIS device can be applied to chlorinated OPFRs. Sampling rates for the three chlorinated OPFRs were found to be: TCEP - 0.14 Ld⁻¹, TCPP - 0.13 Ld⁻¹ and TDCPP - 0.10 Ld⁻¹.
9 Conclusions and Future Recommendations

9.1 Conclusions

9.1.1 Batch Adsorption Studies

From the results gained from the batch adsorption studies, it can be seen that adsorption characteristics such as adsorption maxima ($C_{\text{max}}$) and adsorption affinity ($K_L$) and the adsorption equilibrium constant ($K_D$) are heavily dependent on both compound structure and compound physicochemical properties. Small changes in structure can result in noticeable differences in physicochemical properties which can influence the ability of the analyte to bind to the sorbent matrix.

9.1.2 Molecular Modelling Studies

The results from the hybrid-DFT studies showed that all analytes investigated interact with the Oasis HLB sorbent through the formation of weak bonds that typically occur when a compound physisorbs to the surface of a solid. All analytes investigated can interact with both the NVP monomer and the DVB monomer. The analytes under investigation interact with Oasis HLB through the formation of weak to moderately strengthened hydrogen bonds between the selected analytes and both monomers.

Both the xanthine compounds and the triazine compounds are able to form $\pi\cdots\pi$ interactions with the DVB monomer. In comparison, the chlorinated OPFR compounds are able to form hydrogen bonds with the DVB monomer with the flame retardant
molecule acting as the hydrogen bond donor and the aromatic ring of the DVB monomer acting as the hydrogen bond acceptor.

The strength of the interactions between the analytes and Oasis HLB is dependent upon two factors: 1) the number of bonds that can form between the analyte and the monomer and 2) bond length and bond angle. This is especially evident where hydrogen bond formation occurs. As would be expected, analytes that were able to form moderately strong hydrogen bonds were found to have a greater interaction energy than analytes that have weak hydrogen bonds. However, if an analyte was found to form multiple weak hydrogen bonds, then the interaction energy was found to be comparable to the analyte-monomer complexes that interacted with the sorbent via the formation of only one or two moderately strong hydrogen bonds.

As evident with the triazine-Oasis HLB investigations, it can be seen that the interactions that take place between analyte and sorbents are not 100% governed by surface interactions (i.e. formation of bonds between the analyte and the sorbent). From the batch adsorption results, it can be seen that although DIA prefers to remain in solution rather than adsorb onto the sorbent, the hybrid-DFT results show that the interaction energy between DIA and either sorbent monomer is only marginally less than that of simazine or atrazine. These results suggest that other phenomena also impact the ability of the analytes to 1) bind to the sorbent and 2) stay bound to the sorbent.

9.1.3 Chlorinated POCIS Calibration Studies

From the flow-through calibration study conducted it was found that the standard POCIS device can be used to detect and potentially quantify chlorinated OPFRs in
anthropogenically affected water. The sampling rate that was determined for each chlorinated OPFR was found to be comparable to values found in literature.

One drawback to the system however, is that whilst it is feasible for use for determination of sampling rate data at low flow rates/velocities, the same cannot be said at high flow rates. This is due to the economic cost that comes about with using greater amounts of analyte. As the analyte is flushed through the system, a continual flow of analyte into the sampling tanks means that at high flow rates greater amounts of analyte must be used resulting in a very costly experiment.

However, what the system can provide is a starting point for the determination of sampling rate data for analytes that have not been previously investigated. From there, more cost effective and more environmentally relevant methods can be employed for the generation of sampling rate data as in-situ calibration techniques.

9.2 Future Recommendations

9.2.1 Batch Adsorption Studies

The batch adsorption studies discussed in this dissertation were conducted at concentrations levels that are well in excess of expected environmental levels. These concentration levels were selected in order to determine adsorption maxima ($C_{\text{max}}$) for the selected compounds with Oasis HLB. Batch adsorption studies conducted with analytes at expected environmental concentrations may provide further information regarding the interaction of the selected analytes with the sorbent. It should be noted, however, that the molecular modelling work still confirms adsorption of the analytes on the sorbent can occur at low concentrations.
Additionally, it is recommended that further batch adsorption studies be conducted, both with a broader range of analytes and sorbents so as to determine what impact other analyte and sorbent functionalities have on the analyte/sorbent interactions.

Ideally, adsorption studies would be conducted using analytes with a diverse array of functional groups and physicochemical properties; first as single batch adsorption studies followed by multiple component batch adsorption studies. As the POCIS device is likely to encounter a number of different compounds when deployed in atypical aquatic environment, it would be of interest to know if compounds that have a higher affinity for the sorbent (as determined by the initial batch adsorption study) preferentially interacts with the sorbent during the multi-component batch adsorption study.

9.2.2 Molecular Modelling

Whilst the molecular modelling studies detailed in this dissertation provided some useful insights into the interactions between the selected analytes and the sorbent, a degree of automation would greatly improve the process of identifying different possible interaction geometries. This process could also include initial low level optimisation of the resulting complexes (e.g. semi empirical methods) so as to keep computational costs down. Once the initial optimisation process had taken place, energetically favourable complexes could then be selected for further optimisation at a higher level (e.g. hybrid DFT methods such as utilised in this work).

Along with conducting further batch adsorption studies with differing analytes and sorbent types, it is also recommended that corresponding hybrid-DFT studies should also be conducted in order to elucidate the molecular interactions taking place between these additional analytes and sorbents.
9.2.3 Chlorinated OPFR POCIS Calibration Studies

As the calibration study presented in this dissertation is limited to a single flow through calibration study, future studies involving the use of different water flow rates and water temperatures and salinity levels are required in order to determine the impact that these environmental factors have on the uptake of the chlorinated OPFRs into the standard POCIS.

Furthermore, ideally an in-situ calibration should be conducted to determine the sampling rate of chlorinated OPFR compounds under environmentally relevant conditions. This would help to further validate the use of the standard POCIS as a sampling device for these compounds.
References


