Highly permeable, anti-bacterial, gas selective membranes for the measurement of intra-ruminant gas production.

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

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July 2015
Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis/project 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.

Kyle James Berean

July, 2015
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To my beloved wife Sophia Tipping, who stuck by me,
supported and comforted me throughout the entire process.

I couldn’t have done this without you.
Abstract

In the recent past, researchers have turned to advancements in membrane technology for gas separation to help solve the enormous challenges faced by society in regards to limited resources and environmental sustainability. Compared with conventional gas separation methods, the membrane technology is environmentally benign and energy-efficient. Furthermore, membranes require less space and can be operated in a continuous mode at low cost. Ideal membrane attributes include high permeate flux, high gas selectivity and operational longevity.

The aim of this PhD project is to synthesise, optimise, characterise and evaluate the gas permeation properties of polydimethylsiloxane (PDMS) nanocomposites and finally to prolong their lifetime through the addition of antimicrobial properties. The author of this thesis thoroughly reviewed the fundamental properties and synthesis methods for gas permeable nanocomposite membranes. Based on the literature, the author recognised the lack in a standardised synthesis condition for the chosen base polymer PDMS. Additionally the author identified the significant enhancements that two dimensional (2D) nanomaterials had over other standard fillers when engineering the composites properties, due to an increased surface to volume ratio. Finally, in order to prolong the lifetime of membranes operating in aqueous or humid environments, bacterial growth needs to be controlled. The author explores novel experimental techniques to evaluate the antimicrobial effects of the developed material.
The first stage involved increasing the permeability of PDMS, which will be used as the base polymer throughout the thesis work. Due to the high intrinsic flux to a wide range of gas species, PDMS is an attractive base polymer for gas separating composites. Surprisingly, within the literature there is no reference to any standard crosslinking temperature during synthesis. As such, the author investigated a range of crosslinking temperatures evaluating the effects to the polymeric matrix and the resulting changes to the gas permeability. The author discovered that 75 °C is the optimal crosslinking temperature, where the structure of the PDMS chains is relaxed due to a reduction in crosslinking density and an increase in fractional free volume (FFV). This increase in FFV allowed for efficient diffusion of the gas molecules through the polymer matrix resulting in an increase in gas permeability. Nitrogen (N₂) increased from 360 to 590 Barrer, carbon dioxide (CO₂) from 3190 to 3970 Barrer, and methane (CH₄) from 850 to 1000 Barrer. The author demonstrated the advantages of this increase through incorporating the optimised membranes into a gas sensing system. This experiment illustrated the potential of the membranes for efficient, passive and low cost gas phase separation of selected gas species in pristine PDMS.

In the second stage, the author utilised 2D materials to alter the gas permeation properties of PDMS. The 2D materials investigated in this project are graphene and molybdenum disulphide (MoS₂). With most studies of graphene composites looking to exploit the impermeable gas barrier properties of this 2D material, the author however, focused on the interaction between the base polymer and flakes to create additional FFV at the interface of the two phases. The synthesised graphene-PDMS nanocomposite membranes were able to increase the gas
permeation of $N_2$, $CO_2$, argon (Ar) and $CH_4$ gas molecules. The creation of interfacial voids between the graphene flakes and polymer chains increased the FFV within the nanocomposites allowing for more efficient diffusion through the voids. A graphene loading of 0.25 wt% was found to be an optimum for gas permeation, where greater concentrations created agglomeration of the graphene flakes and hence reducing the effective surface area.

Research on 2D MoS$_2$ used as a filler in polymer composite materials is still in its early stages with no thorough investigation on the gas separation capabilities. In this thesis work, MoS$_2$-PDMS nanocomposite gas separating membranes were synthesised, characterised and the permeability was investigated for selected model gas species. Astonishingly, at extremely low loading concentrations of MoS$_2$ (~0.02 wt%), the nanocomposite membranes were able to almost completely block NO$_2$ gas molecule permeation at ppm levels. The high adsorption energy that NO$_2$ gas molecules have to 2D MoS$_2$ was identified as the key factor in the reduction of permeability. The photoluminescence (PL) of the embedded MoS$_2$ was assessed before and after exposure to NO$_2$, suggesting that 2D MoS$_2$-PDMS nanocomposite membranes could offer dual functionality, capable of not only separating NO$_2$ from gas streams but also monitoring the concentration of NO$_2$ if combined with a PL unit.

In the final stage, the author investigated increasing the lifespan and functionality of gas separating membranes through the addition of antimicrobial properties. Through a unique and novel in vivo investigations utilising the rumen of a cow as a ‘live lab’ the author evaluated the material. The author of this thesis exploited the well-known antimicrobial effects of silver (Ag) nanoparticles, and Ag$^+$ ions
and investigated these properties within the nanocomposite material. An optimal loading of 0.25 wt% Ag to PDMS was found to show the greatest antimicrobial activity and was observed through the \textit{in vivo} tests to reduce the microbial diversity colonising the surface.

Overall, the author strongly believes that the objectives achieved in this PhD research work, have contributed significantly to the advancement of gas separating membranes, antimicrobial materials and \textit{in vivo} experimental techniques regarding the investigation of microbial growth onto materials, thus creating new systems and adding significantly to the knowledge of the field.
List of symbols

\( \rho_p \) membrane density

\( M_A \) membrane weight in air

\( M_L \) membrane weight in the auxiliary liquid

\( \rho_0 \) density of the auxiliary liquid

\( M_{wet} \) membrane weight when dry

\( M_{dry} \) Membrane weight when wet

\( \sigma\text{-Ps} \) ortho-positronium

\( \tau_3 \) ortho-positronium lifetime

\( I_3 \) ortho-positronium intensity

\( \alpha \) gas separation value

\( \text{wt}\% \) weight loading concentration

\( \rho \) density

\( C \) Concentration

\( C_s \) Fixed concentration

\( D \) Diffusion constant

\( H' \) Shannon Weaver diversity
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>0D</td>
<td>zero dimensional</td>
</tr>
<tr>
<td>1D</td>
<td>one dimensional</td>
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<tr>
<td>2D</td>
<td>two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>three dimensional</td>
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<tr>
<td>A.E.</td>
<td>after exposure</td>
</tr>
<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
</tr>
<tr>
<td>Ag</td>
<td>silver</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>silver ions</td>
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<tr>
<td>Ar</td>
<td>argon</td>
</tr>
<tr>
<td>Av</td>
<td>absolute value</td>
</tr>
<tr>
<td>B.E.</td>
<td>before exposure</td>
</tr>
<tr>
<td>BPPOdp</td>
<td>poly(2,6-diphenyl-1,4-phenylene oxide)</td>
</tr>
<tr>
<td>CH₄</td>
<td>methane</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotubes</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CPVV</td>
<td>constant pressure variable volume</td>
</tr>
<tr>
<td>CVD</td>
<td>chemical vapour deposition</td>
</tr>
<tr>
<td>DGGE</td>
<td>denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>DI</td>
<td>deionized water</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
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<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FFV</td>
<td>fractional free volume</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>FTIR</td>
<td>fourier transform infrared</td>
</tr>
<tr>
<td>GADDS</td>
<td>general area detector diffraction system</td>
</tr>
<tr>
<td>H₂O</td>
<td>water</td>
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<tr>
<td>H₂S</td>
<td>hydrogen disulfide</td>
</tr>
<tr>
<td>HOMO</td>
<td>highest occupied molecular orbitals</td>
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<tr>
<td>HRTEM</td>
<td>high-resolution transmission electron microscopy</td>
</tr>
<tr>
<td>LB</td>
<td>luria-bertani</td>
</tr>
<tr>
<td>MoS₂</td>
<td>molybdenum disulfide</td>
</tr>
<tr>
<td>MSD</td>
<td>mass swelling degree</td>
</tr>
<tr>
<td>N₂</td>
<td>nitrogen</td>
</tr>
<tr>
<td>NDIR</td>
<td>non-dispersive infrared</td>
</tr>
<tr>
<td>NMP</td>
<td>n-methylpyrrolidinone</td>
</tr>
<tr>
<td>NO₂</td>
<td>nitrogen dioxide</td>
</tr>
<tr>
<td>PALS</td>
<td>positron anhilation lifetime spectroscopy</td>
</tr>
<tr>
<td>PAN</td>
<td>poliacronitrile</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCA</td>
<td>principal component analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>PL</td>
<td>photoluminescence</td>
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<tr>
<td>PRB</td>
<td>permeable reactive barrier</td>
</tr>
<tr>
<td>Psf</td>
<td>polysulfone</td>
</tr>
<tr>
<td>PVA</td>
<td>poly(vinyl alcohol)</td>
</tr>
<tr>
<td>PVP</td>
<td>polyvinylpyrrolidone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TMDC</td>
<td>transitional metal dichalcogenides</td>
</tr>
<tr>
<td>TZV</td>
<td>triple zeta valance</td>
</tr>
<tr>
<td>UPGMA</td>
<td>unweighted paired group method with mathematical averages</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>ultra violet to visible light</td>
</tr>
<tr>
<td>WAXS</td>
<td>wide angle x-ray spectroscopy</td>
</tr>
<tr>
<td>wt%</td>
<td>weight percentage</td>
</tr>
<tr>
<td>XPS</td>
<td>x-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>x-ray diffraction</td>
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Chapter 1: Introduction

1.1 Motivation and background

Currently, one area of intense research focus is on processes that can be implemented for different gas species using membrane technologies, due to the unique advantages that these technologies possess. Such advantages include stability, passive operation, low cost and mechanical robustness [1-4]. However, these membranes can only be viable in many procedures such as separation, filtration, purification as well as isolation of liquid and gas phases (gas permeable barrier), if they possess both high permeability and selectivity. The cost of many of these procedures is directly proportional to the flux of the membranes, which is related to the permeability.

This PhD research focuses on the development of novel gas separating membranes for biologically and industrially important gas species including methane (CH₄), carbon dioxide (CO₂) and nitrogen dioxide (NO₂).

The PhD candidate chooses to use the rubbery polymer polydimethylsiloxane (PDMS) as the base for the development of gas permeable membranes. PDMS is selected as the model polymer due its versatility and expansive usage in many fields of research and also a large number of industries.

To control the selectivity (the ratio of permeability between two gas species) and permeability of PDMS, nano materials are incorporated into the polymeric matrix. It is well-known that the addition of nano materials can result in the tuning of gas molecule tortuosity, adding nano gaps, catalytic and barrier properties, adsorption onto the surface and diffusion within the bulk of the membranes as well as many
other properties. Two dimensional (2D) materials are the target nano fillers in this PhD research. Graphene, as the first target, is chosen as the first 2D nano material of interest due to its large surface to volume ratio, functionalisation ability of the surface, low cost and known affinity too many gas species. 2D Molybdenum disulphide (MoS₂) nanoflakes are selected for the next stage of this PhD research, as it is known to have a great ability to adsorb NO₂ to potentially hinder the permeation of this gas through a composite membrane.

As the last stage of this PhD research, a study will be conducted to increase the longevity of the PDMS based membranes. The durability of PDMS based membranes is an important issue for many for gas sensing and separation applications, especially when they are used in a humid or aqueous environment. Silver (Ag) antimicrobial nanoparticles are investigated for enhancing the durability of PDMS mixtures.

A detail background and justification regarding the chosen research stages and the materials of interest in this PhD thesis are presented as follows.

### 1.1.1 Polydimethylsiloxane

PDMS is a commonly used material for many permeable gas membrane applications as it is known for showing a high permeability to a wide range of gas species, while being biocompatible, cheap and easy to use [5-9]. These properties make it an ideal polymer for gas permeable membranes or as a base polymer for nanocomposite membranes. PDMS has already been employed for gas separation or, in its intrinsic form, as a gas permeable barrier. It has also been employed in
mixed matrix and co-polymer membranes for gas separation, purification and effective permeable barriers [7, 10-18].

During the synthesis of PDMS membranes, there are two main factors in controlling the inter-chain bonding of the PDMS oligomers (Figure 1.1 (a)) and therefore dictating the fractional free volume (FFV) within the polymer matrix: the first is chemical curing agents and catalysts (Figure 1.1 (b)) and the second is the crosslinking temperature [19, 20]. While the investigations on curing agents have been extensive [21-23], there are only a few non-comprehensive reports on the crosslinking temperature and the physiochemical effect on PDMS. So far a common approach has been to allow the PDMS membranes to crosslink at room temperature [24-26], while some prefer to conduct the crosslinking beyond 150 °C [14, 27, 28]. There are also reports using a wide range crosslinking temperatures from 25 to 150 °C with little discussion regarding the choice and effect of those temperatures [13, 15, 18, 23, 29-33]. While it has been shown that the crosslinking temperature plays a role in the formation of the polymeric crosslinking structure [34], there is no report regarding the effect of crosslinking temperatures on the gas permeation of PDMS membranes.

Within a non-porous, rubbery polymer, such as PDMS, gas permeation is usually described by the solution-diffusion mechanism [35]. This mechanism involves three steps: (1) absorption at the upstream boundary, (2) diffusion through the membrane and (3) desorption on the downstream boundary. The ability to control the absorption, diffusion and desorption of the gas molecules, by engineering the structure of the polymer, can be a desirable property. Whether to increase the permeation rate through the membrane for gas sensing or separating applications
or to enhance the selectivity for industrial processes, the control of the free volume to optimise flux is a critical challenge to overcome [36].

![Structural formula of: (a) PDMS oligomer, and (b) common chemical curing agent for PDMS](image)

**Figure 1.1** Structural formula of: (a) PDMS oligomer, and (b) common chemical curing agent for PDMS

The adsorption, diffusion and desorption of gas molecules in a permeable polymeric membrane can be engineered through the incorporation of other materials in order to alter its permeability and selectivity. Critically, control over gas selectivity for industrial processes and increasing permeation for phase separations are significant areas of research for membrane applications [37-39]. Membranes for gas selectivity and phase separation have been implemented in a range of applications including food and beverage processing, greenhouse gas mitigation, wastewater treatment, pharmaceuticals production, and also applied in a range of separation technologies needed for manufacturing chemicals, electronics, fuels and many other products [40].

In addition to gas phase separation applications, PDMS is also a technologically important polymer for other industries. As a polymer, PDMS lends itself to many biomedical and biotechnological devices and systems. This is due to its many
interesting properties: non-toxicity, biocompatibility, optical transparency, durability, flexibility, hydrophobicity as well as being inexpensive yet easy to implement [5-8]. Pristine PDMS has been employed for a myriad of applications including implantable devices and biomedical devices as well as being employed extensively throughout many purification processes [13, 26, 41-44].

Motivation:

In this PhD research, the candidate will investigate the gas permeation properties of PDMS membranes synthesised at different crosslinking temperatures. Permeation properties for N₂, CO₂ and CH₄ gas species are studied. CO₂ and CH₄ gases are chosen as they are critical species for climate change scenarios, agricultural and industrial sectors as well as many different chemical and biochemical processes. N₂ is used as a reference while it is also a post-combustion flue gas by itself. The outcomes of this study will reveal the optimum conditions for the synthesis of PDMS base membranes. Such information has never presented before and will be invaluable for many industries and research sectors.

1.1.2 Nanocomposite membranes

As previously presented, composite membranes for gas permeation and phase separation, especially those including fillers, offer distinct advantages over pristine polymer membranes. The fillers used in the composites may function both physically and chemically to act as molecular or ionic sieves as well as catalytic/reactive materials [45, 46].

The background and justifications of nanomaterials of interests in this PhD thesis are presented as follows.
1.1.2.1 Graphene

Carbon-based materials including carbon nanotubes (CNTs), buckyballs and carbon black have been examined as fillers for gas permeation and phase separation membranes [26, 47, 48]. Carbon-based materials can be utilised to tune the polymeric matrix in order to engineer the polymer chain morphologies and the fractional free volume (FFV) of the membranes that has a direct effect on the gas permeation properties. On the contrary, the use of graphene, which is a two-dimensional (2D) sheet of carbon in a planar morphology, has been less frequently addressed for its effect on altering the gas permeation in polymeric membranes.

Graphene has many extraordinary physiochemical properties including a large Young's modulus and high specific surface area as well as high carrier charge mobility and thermal conductivity [49-51]. Moreover, graphene is able to offer outstanding qualities when used for developing polymeric nanocomposite materials that can be exploited in the chemical, electronic, optical and mechanical systems [52-56]. While defect free graphene itself is non-permeable to many gas species [57, 58], it can play an important role in creating membranes for gas permeation applications by exploiting the morphological and structural changes that it introduces to the composite. This trade-off of the barrier property of the graphene and the major alterations the flakes can have on the polymeric matrix may result in competing effects.

There have been several studies regarding gas separation using stand-alone graphene sheets with engineered defects as membranes [59-62]. However, such membranes are limited in what they can offer as their properties are highly
dependent upon the interactions between the graphene sheets and their orientations. Adding a polymer to make a nanocomposite membrane provides an extra degree of freedom for tuning the system permeation and selectivity towards desired gas species especially if that polymer shows intrinsically high gas permeability. Despite such a possibility, there has not been any study on using graphene together with such highly permeable polymers to create membranes with adjustable gas permeation and phase separation properties. This novel use can potentially exploit the 2D morphology of graphene, which can adjust the structure of the chains during polymerisation. At the same time, the presence of graphene in the nanocomposite provides a large surface area of gas impermeable carbon that polymer chains can spread onto with a specific distance between graphene and polymer, which is different from those between the polymer chains themselves. If such distances would occur in the nano to meso sizes then the nature of the effect they bestow upon the membrane is very different from those of angstrom size voids in conventional non-porous gas permeable membranes. However, the barrier qualities of the graphene may reduce any impact these changes have on permeability. The 2D morphology of graphene allows for even low weight percentage incorporation of this material to produce incredibly large surface areas that cannot be matched by nanofillers of other morphologies. The 2D structure of graphene can potentially create extended areas of large volume voids at the graphene-polymer interfaces. To produce the same volume of interfacial nano- and meso- sized voids with zero- or one-dimensional (0D and 1D) materials, a relatively large concentration of such morphologies would be needed to match the surface area of the 2D graphene. Such large quantities of 0D
and 1D materials can significantly increase the chances of establishing interlinking void structures within the membrane. If these interlinked pores are in the direction of the gas diffusion path in the membrane, molecular diffusion will occur with no selectivity through the connected voids. Similarly, incorporation of 2D materials such as graphene should also avoid the formation of paths normal to the surface of membranes.

**Motivation:**

In this PhD work, the candidate investigates the effects of graphene on the gas permeation properties of PDMS. The candidate explores the gas permeation mechanisms that are resulting from the incorporation of graphene to PDMS including additional tortuosity and the alteration of diffusion paths within the PDMS polymeric matrix. The hypothesis here is that graphene is naturally a gas barrier that should hinder the permeation of gas molecules. At the same time it can produce meso-/nanosized voids at the filler-polymer interface that can increase the permeation. The study considers these two competing effects to understand the how graphene incorporation at different concentrations effects the permeation of N₂, Ar, CH₄, and CO₂ gas species. The PhD candidate will use the developed membranes to test in actual gas sensing systems in order to assess the functionality of such membranes.

1.1.2.2 Molybdenum disulphide nanoflakes

Recent advances in the field of 2D transition metal dichalcogenides (TMDC) has attracted increasing attention due to their unique and tuneable properties for applications in electronic and optical devices, catalysts, energy storage units,
biological systems, and importantly for this study, gas sensors [63-65]. It has been shown that 2D MoS₂ has a high affinity to selected gas species including NO₂ and CO₂, while it shows no interactions with many other gases [66]. It is proposed that the key functional mechanism for the high specificity of 2D MoS₂ to NO₂ molecules is due to high adsorption energy of these gas molecules onto the basal surface of MoS₂ [64, 67]. The resulting interaction is non-reversible without the application of any external energy. As such, it is suggested that in addition to sensing applications, the interaction can be employed for other purposes including NO₂ separation, in which permanent removal of this gas is required. The removal of NO₂ gas molecules from an environment with other gas species is an important technological challenge for a number of applications as it is of major environmental concern and has hazardous effects to human health. The oxidation of fatty acids by NO₂ is a serious issue for packaged food [68]. Additionally, NO₂ is mainly an unwanted flue gas generated as a by-product during oil and gas refining [69].

Many methods for removing NO₂ are based on non-catalytic adsorption or catalytic processes [70, 71]. These methods can be more efficiently implemented through the use of permeable membranes that slow the gas molecules to allow a more effective catalytic or non-catalytic interaction take place [72]. 2D MoS₂ can be potentially incorporated as a NO₂ adsorbing material in a permeable membrane to make a functional permeable reactive barrier (PRB). However, the study of a NO₂ adsorbing PRB membrane containing 2D MoS₂ has yet to be carried out. There are investigations on 2D MoS₂ polymeric composites [73], although none focus on gas separation properties. The high surface adsorption energy and the
large basal plane that the 2D MoS$_2$ intrinsically possesses are the properties the author looks to exploit for the separation of NO$_2$.

**Motivation:**

In this PhD research, the candidate chose 2D MoS$_2$ nanoflakes as the second target incorporated nanomaterial to engineer gas selectivity to PDMS membranes. The 2D MoS$_2$ ideal for this study should be less than 5 layers with an average lateral dimension greater than 100nm. This is to maximize the surface area while still allowing the flakes to be easily distributed within the polymer matrix. This stage of the investigation is based on the well-known fact that 2D MoS$_2$ has a high affinity to NO$_2$ gas molecules. As such it should potentially adsorb and scrub the NO$_2$ gas molecules from the gas stream.

*1.1.2.3 Silver nanoparticles*

Ag and Ag$^+$ ions have long been known to exhibit high inhibitory and bactericidal effects. This property the author of this thesis looks to exploit within an Ag-PDMS nanocomposite. Currently Ag-PDMS nanocomposites have been investigated for many applications including biosensing, flexible displays and microfluidic systems [74-76]. However, the antibacterial effects of these nanocomposites have not been thoroughly investigated [77].

When employing membranes into many different applications the exposure within the aqueous or high humidity environment may result in microbial communities to grow on the surface of the membranes. Microbial colonisation and the
formation of biofilms can result in biofouling of the membranes used in biosensing and separation systems. This biofouling ultimately leads to a reduction in both the efficiency and the longevity of membranes used in such processes [78, 79]. Many studies on composite materials have shown that incorporating antibacterial nanomaterials, such as Ag nanoparticles are mainly used in implantable and other biomedical devices. In these studies it has been shown to dramatically reduce bacterial colonisation, reducing the incidence of such infections [77, 80-83]. One of the major risk factors for implantable and other medically relevant devices which are in contact with body fluid is the propensity for infection caused by microbial growth [84]. These nanocomposites have been suggested for potential use in implantable devices and medical aids such as catheters, prosthetics, bone adhesives, contact lenses, ureteral stents and pacemaker coatings [85-88]. Antimicrobial Ag nanocomposites have also been employed in applications other than the medical sector including water purification, treatment and filtration processes [38].

There are many standard in vivo and in vitro tests have been developed for studying bacterial growth evaluating the performance of materials for their antimicrobial viability [89, 90]. However, the in vitro laboratory tests are limited in their assessments that they provide regarding real life outcomes. On the other side, many in vivo experiments are costly, labour intensive, are invasive and require stringent health and safety protocols [91, 92].

Motivation:

This thesis work addresses the lack of research in maximising the efficiency, longevity and robustness of PDMS based gas separating membranes. In this PhD
research a novel experimental method is used by the PhD candidate for testing the antimicrobial effect of Ag nanoparticles incorporated into PDMS membranes. This method is based on using the environment of a rumen of a fistulated animal. A fistulated animal stomach provides a great live laboratory environment for *in vivo* tests providing a relative ease of operation and an environment with vibrant and diverse microbial community [93] that are also commonly found in human microbiome [94]. As such, fistulated animal rumen can potentially be implemented as viable live laboratory for tests of antimicrobial materials. Here, the concept is for Ag-PDMS membranes in the rumen environment as a model study that shows the viability of this approach for investigating materials for rumen gas permeation systems, implantable and other medical devices, which remain in contact with body fluid for a long time.

### 1.2 Objectives

This thesis is primarily focused on gas permeable PDMS membranes and nanocomposites involving PDMS and nanofillers for optimising these materials for many applications. In particular, to investigate the synthesis processes and gas permeation properties of these novel materials, in addition to this, the applications of these membranes in harsh biological environments. The properties of these materials had not been reported by any other researcher at the time that the PhD research started. The research work in this PhD dissertation can be briefly classified under the following objectives:

a) Optimise the permeation properties of pristine PDMS and characterise the changes to the polymeric matrix.

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b) Incorporate nanofillers into PDMS matrix to further enhance gas permeation properties. Specifically the investigation of 2D nanofillers to maximise the surface contact at low concentrations.

c) Investigate the application of these developed membranes in real world situations, such as phase separation membranes or permeable membranes for gas sensor protection.

d) Synthesis nanocomposite to prolong viability of membranes in harsh biological environments.

As research on nanocomposite membranes using PDMS as the base polymer for gas separation is still in progress, the author of this thesis starts his work by focusing on the optimisation of the polymer matrix for enhanced flux. Although the synthesis of PDMS is not new, the role of the crosslinking temperature on the polymeric matrix has not been investigated.

Next the author of this thesis focused his intention of the incorporation off 2D materials to alter the gas permeation properties of PDMS. The two 2D materials looked at for this thesis is graphene and MoS₂. The incorporation of graphene into rubbery polymers has not been carried out by many other studies due to the difficulties in controlling agglomeration of the flakes. With most other studies of graphene composites looking to exploit the gas impermeable barrier properties of the 2D material [95-99], the author focuses on the interaction between the base polymer and the flakes to create additional FFV at the interface of the two phases. Research on 2D MoS₂ as a filler in polymer composite materials is still in its infancy. The author focuses on utilising the high adsorption energy of 2D MoS₂ and specific gas molecules to create efficient gas separating membranes.
Specifically these nanocomposite membranes are targeting the reduction in permeation of NO\textsubscript{2} gas molecules. The photoluminescence (PL) of the embedded MoS\textsubscript{2} was assessed with exposure to NO\textsubscript{2} and the results suggest that these membranes could be implemented into a device capable of not only separating NO\textsubscript{2} from gas streams but also monitoring the concentration of NO\textsubscript{2} if combined with a PL unit, offering dual functionality.

To increase the lifespan and viability of gas permeable membranes in many real world situations, reducing the microbial growth on the surface of the membrane is critical. The author of this thesis exploits the well-known antimicrobial effects of Ag\textsuperscript{+} ions and has looked at the antimicrobial properties it adds to the composite material. Through unique \textit{in vivo} investigations utilising the rumen of a cow as a ‘live lab’ the author evaluates not only the material but the technique as well.

During the course of his PhD candidature, the author has focussed on realising permeable PDMS membranes and many different nanocomposites using PDMS as the base polymer. The author has also focussed on the applications of the synthesised nanocomposite membranes as well as overcoming the problems that these materials would face in the harsh environments that they might be deployed in. The research gaps in the current knowledge have been highlighted by the PhD candidate and as such the primary objectives of this PhD work are aimed at filling these gaps, as follows:

1. The author of this dissertation generated new knowledge through PDMS synthesis revealing an optimal temperature for crosslinking to either enhance or reduce flux within PDMS membranes. The in depth characterisation introduced the link between crosslinking temperature and
polymer chain length changing crosslinking density and therefore the FFV
within the polymeric matrix.

2. The author of this thesis demonstrated a new graphene PDMS
nanocomposite material. He then demonstrated the permeability of these
novel nanocomposites. For the first time it was shown to enhance flux
though a graphene nanocomposite material. Here, the author found that a
lack of interaction between the graphene flakes and PDMS polymer chains
creates an interfacial void between the two materials and therefore
increasing the FFV.

3. The author demonstrates the first use of 2D MoS$_2$ as a filler in a gas
separating membrane. He utilises the inherent high adsorption energy
between specific gas species and therefore making the membranes useful
for gas separation applications.

4. The author investigates the antimicrobial qualities of a proven H$_2$S
separating nanocomposite material [100]. These Ag-PDMS membranes
are not only investigated under lab strain bacteria but also examined for
the effectiveness when exposed to diverse microbial communities. He
utilises many of the ways in which Ag is known to be antimicrobial.

5. The author of this thesis creates a novel in vivo investigation technique
that offers superior ability to gauge a materials performance exposing it to
a diverse microbial community. This technique’s effectiveness has been
evaluated by comparing its validity to standard in vitro lab tests.
1.3 Thesis organisation

This thesis is primarily dedicated to the synthesis and characterisation of PDMS and PDMS nanocomposite membranes with a major focus on gas permeation with an emphasis on applications.

In chapter 2, the author presents his work on optimising gas permeation in pristine PDMS through an investigation of the crosslinking temperature. He describes the synthesis process and characterises the effect of the crosslinking has on the polymer matrix to define the optimal conditions of synthesis. In addition, the author presents the permeation properties and shows the enhancements in real world applications.

In chapter 3, the author delivers his research conducted on nanocomposite gas permeable membranes utilising 2D graphene as a filler. He describes the synthesis process and evaluates the effects that graphene provides to the composite material over a range of concentrations. Furthermore, the author characterises the interaction between the polymer chains and the graphene flakes and demonstrates the effect it has on the gas permeation properties.

In chapter 4, the author of this thesis covers his work on NO₂ separating membranes based on 2D MoS₂ adsorption properties. In detail he evaluates the influence of adsorption energy that 2D MoS₂ has on the separation properties on different gas species. The author presents the effect of solvent choice has on grinding-assist liquid phase exfoliation technique on the gas permeation properties of the resulting composites.
In chapter 5, the author presents his research on nanocomposites containing Ag nanoparticles for its antimicrobial properties. He describes the synthesis and characterises thoroughly the nanocomposite material. The author uses many techniques to quantify how Ag and its associated ions effect biofilm growth and microbial surface adherence. A thorough investigation reveals the full antimicrobial effects on both model bacteria with *in vitro* studies as well as diverse communities in a unique *in vivo* investigation.

Finally, in Chapter 6, the author presents the concluding remarks and the future outlook of the research work presented in this thesis.

**References**


Chapter 2: The effect of crosslinking temperature on the permeability of PDMS membranes: evidence of extraordinary CO$_2$ and CH$_4$ gas permeation

2.1 Introduction

It is important for gas permeable membranes, employed in many industrial applications, to have a high permeability as the cost of many processes such as gas separation and sensing directly depend on it. In the previous chapter, it was shown that a gas or phase separating membrane relies on high permeability to achieve high efficiencies to rival other more energy dependent processes. Therefore a necessity in fabrication of any such membranes and optimisation of the base polymer synthesis is required.

Polydimethylsiloxane (PDMS) has been a widely utilised polymer within permeable membranes as it possesses high intrinsic flux. However, little attention has been placed on the effect of crosslinking temperatures during synthesis. In this chapter, PDMS membranes were prepared using a range of crosslinking temperatures and evaluated for their gas permeation towards CO$_2$, N$_2$ and CH$_4$. The investigation of the effect of the crosslinking temperature on gas permeation of PDMS membranes revealed an optimum temperature of 75 °C at which the permeability increased (for N$_2$ from 360 to 590 Barrer, for CO$_2$ from 3190 to 3970 Barrer, and for CH$_4$ from 850 to 1000 Barrer). The vibrational and electron
beam spectroscopy studies show that at this optimum temperature the structure of polymer chains is relaxed due to the reduction of the crosslinking density and an enhancement in the fractional free volume (FFV) within the polymer matrix, allowing more efficient diffusion of the gas molecules. Eventually, the author will demonstrate the extraordinary capability of the membranes crosslinked at 75 °C by incorporating them in a sensing system. Remarkably the sensors’ response with and without the presence of the improved membrane are nearly the same for CO$_2$ and only show a 30% decrease for CH$_4$. This experiment depicts the strong potential of the developed membrane for efficient, passive and low cost gas phase separation of selected gas species.

The intent of this chapter is to provide an analysis of the crosslinking process on the gas permeation qualities of PDMS membranes. The author conducted this through changing the crosslinking temperature in the process of PDMS membrane synthesis and therefore controlling the dominant polymeric structure of the membranes. Subsequently, the PDMS membranes’ gas permeation qualities for N$_2$, CO$_2$ and CH$_4$ gas species are investigated. These gases are chosen as they are seen as critical gas species within the climate change scenarios [1]. For many agricultural and industrial sectors, control over the release of both CO$_2$ and CH$_4$ is seen as a key aspect in mitigating climate change. They also play important roles in various chemical and biochemical processes. N$_2$, represents the major gas present in post-combustion flue gases and so it is important to monitor the permeation of this gas relative to carbon dioxide. As the final step, a sensing system is used for demonstrating the efficiency of the permeation in an example
of a real world system. The contents of this chapter was published as a full article in the journal of *Separation and Purification Technology* [2].

### 2.2 Materials and Methods

#### 2.2.1 Materials and membrane preparation

Dense, unfilled PDMS (Sylgard 184, Dow Corning Corporation) membranes were prepared utilising the proprietary crosslinker at a 10 wt% ratio. The solution was degassed for 30 minutes before being spun onto a polyacrylonitrile (PAN) microporous support (SolSep BV, Netherlands). The membranes were then crosslinked at different temperatures in the oven for 45 minutes and left for up to 3 days at room temperature (25 °C). The crosslinking temperatures investigated included 25, 50, 75, 100 and 150 °C. The membranes were then used for the permeation measurements and material characterisation. The membrane thicknesses were determined using scanning electron microscopy to be in the order of 155 µm (±8 µm). The thickness was chosen because it lies within the suggested range in the report by Pinnau *et al.* [3]. A second series of membranes of thickness of 300 µm (±10 µm) was also prepared for comparison. These membranes however were not spun onto a PAN support but made as a free standing membrane.

#### 2.2.2 Membrane characterisation

Membrane characterisation was conducted using scanning electron microscopy, vibrational spectroscopy and wide-angle X-ray scattering (WAXS). FEI Nova NanoSEM scanning electron microscope (SEM) and Bruker MultiMode 8 with PF TUNA atomic force microscope were utilised to evaluate the level of defects
in the membranes. SEM was also used for assessing the membranes thicknesses. A Thermo Nicolet 6700 spectrophotometer was used for recording the Fourier transform infrared (FTIR) spectra of PDMS. Micro-Raman characterisation of the samples was performed using a Renishaw in via Raman spectrometer at 532 nm wavelength and 60 s exposure with a laser power of 4 mW and a spot size of 250 µm in diameter. Peak fitting was done fitting Gaussian peaks using Fytik software using the Levenberg-Marquardt method. X-ray diffraction (XRD) data was collected on a Bruker D4 ENDEAVOR diffractometer, fitted with a LYNX-EYE position sensitive detector, using graphite monochromated Cu Kα radiation (\( \lambda = 1.5406 \) Å) with all samples mounted onto a zero-background silicon plate using X-ray transparent Kapton tape.

Positron Annihilation Lifetime Spectroscopy (PALS) was used for determining the free volume of the membranes. The free standing 300 µm samples were measured at room temperature using a positron source of 30 µCi 22NaCl sealed in a Mylar film. The membranes were stacked to 4 mm thick bundles and the positron source was placed in the middle. The measurements were made using an automated EG&G Ortec fast-fast coincidence system with a timing resolution of 260 ps, collecting a minimum of 5 files each with 1x10^6 integrated counts per file. The data was analysed using LT (version 9.0) [4] using a source correction of 1.740 ns and 3.014%. The data was fitted to 3 components including a para-positronium component, fixed at 125 ps, a free positron annihilation component, ~400 ps and the ortho-positronium (o-Ps) component. The o-Ps component’s intensity was employed to determine the relative number of free volume elements and the lifetime was used for assessing the average size of the free volume.
elements within the sample. The Tao-Eldrup Equation [5, 6] was used for calculating the average size of the free volume elements.

2.2.3 Density and swelling experiments of PDMS membranes

Density measurements were determined using the hydrostatic weighing method utilising a Mettler Toledo balance (Model XS205, Switzerland) and density determination kit [7, 8]. The membrane density ($\rho_p$) was calculated by:

$$\rho_p = \frac{M_A}{M_A - M_L} \rho_0$$

(1)

where $M_A$ is the membrane weight in air, $M_L$ is the membrane weight in the auxiliary liquid and $\rho_0$ is the density of the auxiliary liquid. For the determination of density of PDMS, ethanol was used as the auxiliary liquid.

Solvent swelling measurements were carried out by using pre weighed dry membranes of the different crosslinking temperature ($M_{dry}$), immersing them in pure toluene for equilibrium swelling to be reached. The membranes were then dried and immediately weighed ($M_{wet}$). This was carried three times for each membrane to ensure repeatability. Then the swelling degree ($M_{SD}$) of the membranes was calculated by:

$$M_{SD} = \frac{M_{wet} - M_{dry}}{M_{dry}} * 100$$

(2)

2.2.4 Gas permeability measurements

The pure gas permeability of CH$_4$, N$_2$ and CO$_2$ (99.99%, Core Gas Australia) through each membrane was measured using a constant pressure variable volume
(CPVV) unit designed and built in-house as shown in Figure 2.1. The experimental set-up is modeled from Stern et al. [9] and has also demonstrated for PDMS permeability by Merkel et al.[10]. PDMS membranes under test were mounted within a permeation cell with a constant pressure of 400 kPa on the upstream boundary, while the downstream side was kept at atmospheric pressure. The surface area of the membrane was 13.85 cm$^2$. Prior to each experiment the upstream and downstream sides of the permeation cell were purged with the penetrant gas. The permeation cell was housed in an environmental chamber to control the temperature, with all measurements conducted at 35 °C based on the results found by Merkel et al. [10]. One PDMS membrane for each different crosslinking temperature was used for the three different gas species and conducted in the following order: N$_2$, CH$_4$, CO$_2$. When a steady state was achieved the flow rate was acquired every ten seconds for two minutes and the values were averaged and converted into permeability values given in Barrer (1 Barrer $= 1 \times 10^{-10} \text{cm}^3 \cdot \text{cm}^2 \cdot \text{s} \cdot \text{cmHg}$). Barrer, the permeability coefficient for gases in polymeric membranes represents the volume of gas permeating through a membrane of defined area and thickness per unit driving force per second.
2.2.5 Evaluation of the membranes permeability using a sensing system

For the evaluation of the membranes using a sensing system the experimental set-up demonstrated in Figure 2.2 is applied. Membranes were mounted within a permeation chamber in the vicinity of a commercial non-dispersive infrared (NDIR) (IR15TT-R, e2v technologies) sensor placed on the opposing side to the gas flow. The pure gas flow rate was controlled via a mass flow control unit to 10 sccm and pressure was maintained within the chamber with a back pressure regulator to 110 kPa. The permeation chamber and sensor were housed in an environmental chamber to control the temperature with all measurements conducted at 30 °C.
2.2.6 Evaluation of the membranes phase separation using a sensing system

The membranes were tested for their capabilities in the separation of dissolved gas from a liquid medium and for their durability in a mildly acidic environment. A similar experimental set-up to that demonstrated in Figure 2.2 is applied. Membranes were mounted within a permeation chamber in the vicinity of a commercial non-dispersive infrared (NDIR) (IR15TT-R, e2v technologies) sensor. The chamber on the other side of the membrane was filled with an ionic broth in DI water and dissolved gas at approximately 50% CO₂ and 50% CH₄ ensuring total immersion of the membrane under test. The gas and liquid was continuously/mechanically mixed during these measurements. The permeation chamber and sensor were housed in an environmental chamber to control the temperature with all measurements conducted at 37 °C.
2.3 Results and discussion

2.3.1 Vibrational spectroscopy

2.3.1.1 Micro-Raman spectroscopy

All membranes contained similar PDMS peaks regardless of the crosslinking temperature. These peaks are in agreement with typical signatures that are presented in previous works [11]. As such, only the whole Raman spectrum for the membrane formed at 75 °C is shown in Figure 2.3. The spectrum comprises of a Si–O–Si symmetric peak at 488 cm$^{-1}$. At 607 cm$^{-1}$ appears the Si–CH$_3$ symmetric rocking peak. The Si–C symmetric stretching appears at 708 cm$^{-1}$ and –CH$_3$ asymmetric rocking appears at 787 cm$^{-1}$. At 862 and 1262 cm$^{-1}$, –CH$_3$ symmetric rocking and symmetric bending appear, respectively. Finally each show the 2910 and 2965 cm$^{-1}$ Si–CH$_3$ symmetric stretching and Si–CH$_3$ asymmetric stretching bands [11-13]. The only change in spectra of membranes crosslinked at different temperatures appears in the Si–H stretching bonds (Figure 2.3 inset). Although weak, this peak represents the change in the intensity of the established crosslinking bond between the polymer chains formed from the use of a chemical crosslinker [14]. The membrane crosslinked at 150 °C almost lacks the formation of this bond due to the structure being formed from the oligomers themselves and not from the proprietary chemical crosslinking agent [14, 15], while the 75 °C membrane shows the strongest peak intensity, hence higher occurrence of this bond.
2.3.1.2 FTIR spectroscopy

The FTIR spectra for the different membranes shown in Figure 2.4 comply with those typically reported for PDMS. The peaks between 1400–1420 cm$^{-1}$ and between 1240–1280 cm$^{-1}$ correspond to –CH$_3$ deformation vibration in PDMS [16-18]. The Si–O–Si stretching multi-component peaks for PDMS is observed in the range between 930 to 1200 cm$^{-1}$. It has been previously reported [13, 16, 18-20] that Si–C bands and Si(CH$_3$)$_2$ rocking peaks appear in the region of 825–865 and 785–815 cm$^{-1}$, respectively, which are also seen in our FTIR spectra. As with the Raman spectra, the notable change occurs with the Si–H bonds, which is located at 910 cm$^{-1}$ [14, 18]. The decrease in the crosslinking Si–H bond from the membrane crosslinked at 150 °C can be clearly observed compared to the other membranes.

Figure 2.3 Raman spectra of PDMS membrane crosslinked at 75 °C. Inset: Gaussian peak fit of the Si–H bond for all the membranes crosslinked at different temperatures.
2.3.2 WAXS

X-ray diffraction data for all membranes are shown in Figure 2.5. In general, the diffraction peak positions for all membranes are similar, despite the varying temperature of the crosslinking process. The three key reflections occur at approximately $6.8^\circ 2\theta$ (equivalent $d$ spacing of 12.95 Å), $11.7^\circ 2\theta$ ($d$ spacing of 7.5 Å) and $14.4^\circ 2\theta$ ($d$ spacing of 6.1 Å). Due to the stability and relative dominance of the peak occurring at $6.8^\circ 2\theta$, all results were normalised to this feature. There are some minor variations in intensity of the peaks centered at $11.7^\circ 2\theta$ but this cannot be associated directly with major changes in the structure of the polymer chains.

However, one may observe that a key indicator for structural change occurs with the varying intensities of the peak at $14.4^\circ 2\theta$. At 25 °C this feature is the peak with the highest intensity, whilst the membranes crosslinked at higher...
temperatures all show the 6.8 °2θ peak as the dominant feature. Thus, a certain alteration in the structure of the polymer is observed with increasing the crosslinking temperature above 25 °C. The fluctuating nature of the intensity, or change in the degree of ordering, of the 14.4 °2θ peak, indicates that there may be two factors that influence the structure of the polymer chains. It is suggested that the chemical crosslinking agent plays a major role in dictating the polymeric structure and giving order at all crosslinking temperatures [21]. Whilst at elevated temperatures the heat energy also influences the structure [22]. Heat has the least influence on membranes established at 25 °C. The membranes crosslinked at 50 and 100 °C show a more disordered matrix indicated by the low intensity of the 14.4 °2θ peak. However, the membrane crosslinked at 75 °C appears to be within an ideal fabrication region due to the relatively higher degree of order in the matrix indicated by the increased intensity of the peak 14.4 °2θ. At this temperature, it seems that the heat and chemical crosslinking are optimised creating the most relaxed structure, reducing crosslinking density within the polymer matrix. Whilst after crosslinking the polymer at 100 °C, it is the heat energy that strongly influences the structure [22].
2.3.3 PALS

The PALS experiment is used in this study to characterise the free volume in the PDMS membranes crosslinked at different temperatures. It is known that PALS determines the o-Ps lifetime ($\tau_3$) and intensity ($I_3$) parameters [7, 8, 23]. The lifetime characterises the average size of the free volume elements in the sample, while the intensity can be correlated to the concentration, or relative number of free volume elements in the sample. The PALS data for the different membranes is presented in Figure 2.6. As can be seen from Figure 2.6(a) the o-Ps lifetime does not show any significant differences in the size of the free volume elements, with all variance between crosslinking temperatures lying within the range of uncertainty. With the average pore diameter found to be 0.808 nm. The intensity parameters, nevertheless do show an increase in the number of free volume elements as the crosslinking temperature increases. This increase does translate to

![Figure 2.5 WAXS curves of PDMS membranes crosslinked at different temperatures](image)
a small increase in FFV, although the increase lies well within the range of uncertainty for these measurements.

Figure 2.6 Influence of crosslinking temperature on (a) o-Ps lifetime ($\tau_3$) and (b) intensity ($I_3$)

2.3.4 Density and swelling experiments of PDMS membranes

The density of the membranes crosslinked at different temperatures is shown in Figure 2.7(a). It can be seen as an overall trend is that when the crosslinking temperature is increased the density of the membrane increases as well. This is to be expected as the increasing crosslinking temperature causes the polymerisation to form a tighter network and therefore increasing the density. However the membrane crosslinked at 75 °C shows a decrease in density compared to the membrane crosslinked at 50 °C. This change in density can be used to via the Bondi method to convert these density measurements into FFV [24] as shown in Figure 2.7(b).
Figure 2.7 The effect of crosslinking temperature on (a) the density and (b) the FFV of PDMS membranes.

The $M_{SD}$ of the PDMS membranes crosslinked at different temperatures was investigated. Figure 2.8 presents typical results that comply with other studies for PDMS [25-27]. As the crosslinking temperature increases the swelling degree increases dramatically from 100 to 195 % at a crosslinking temperature of 75 °C. The equilibrium swelling degree decreases just as dramatically to a final value of 83% for the membrane crosslinked at 150 °C. This substantial change in swelling degree is similar to that found by Stafie et al. [18] for swelling of PDMS membranes of varied crosslinker ratios in hexane. Such observations have been ascribed to a reduced chain length of the oligomers between cross-links [27]. This shortening of the chains between cross-links enhances the elastic resistance to the swelling stress and therefore lowers the degree of swelling [18, 28]. For the membrane crosslinked at 75 °C this enhanced swelling degree shows the largest length between cross-links and therefore creates the most relaxed polymer matrix [29].
2.3.5 Gas permeation properties

The gas permeation rate of the PDMS membranes of 155 μm thickness crosslinked at different temperatures was investigated under exposure to pure CO₂, N₂ and CH₄ (99.99%). The experimental setup was explained in Section 2.2.3.
Figure 2.9 Change in permeability for each gas species with respect to the change in crosslinking temperature.

Table 2.1 Summary of the permeability parameters of PDMS membranes crosslinked at different temperatures (Barrer)

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>Merkel et al. [30]</th>
<th>25°C</th>
<th>50°C</th>
<th>75°C</th>
<th>100°C</th>
<th>150°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$</td>
<td>850</td>
<td>850</td>
<td>940</td>
<td>1000</td>
<td>860</td>
<td>480</td>
</tr>
<tr>
<td>N$_2$</td>
<td>380</td>
<td>360</td>
<td>-</td>
<td>590</td>
<td>-</td>
<td>280</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>3200</td>
<td>3180</td>
<td>3430</td>
<td>3970</td>
<td>3190</td>
<td>1150</td>
</tr>
</tbody>
</table>

As can be seen in both Table 2.1 and Figure 2.9, the permeation of CO$_2$, N$_2$ and CH$_4$ through the 50 and 75 °C has been enhanced to provide greater flux than membranes crosslinked at other temperatures. It can be seen that a maximum permeability was reached when the PDMS membrane was crosslinked at a temperature of 75 °C. The permeability of the PDMS membranes crosslinked at 25 and 100 °C are similar and correspond to the permeability found by Merkel et al. [30], that also used a crosslinking temperature of 100 °C and a CPV V experimental set up, as well as other reports [31-33]. It can also be seen that the
membrane crosslinked at 150 °C has a far lower permeability than all other membranes.

**Table 2.2** Selectivity of the membranes crosslinked at different temperatures.

<table>
<thead>
<tr>
<th>Crosslinking Temperature</th>
<th>α (CO$_2$/CH$_4$)</th>
<th>α (CO$_2$/N$_2$)</th>
<th>α (CH$_4$/N$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>3.7</td>
<td>8.8</td>
<td>2.4</td>
</tr>
<tr>
<td>75°C</td>
<td>4</td>
<td>6.7</td>
<td>1.7</td>
</tr>
<tr>
<td>150°C</td>
<td>2.4</td>
<td>4.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

As can be seen in Table 2.2 the selectivity of the membranes crosslinked at different temperatures changes but not all the changes show a similar trend. The selectivity between CO$_2$ and CH$_4$ is at a maximum with the increased permeation of the 75 °C membrane, while the selectivity for CO$_2$ to N$_2$ decreases as the crosslinking temperature increases. It can also be seen that the selectivity between CH$_4$ and N$_2$ is at a minimum for the increased permeability of the membrane crosslinked at 75 °C. This is ascribed to the changes in the polymeric structure affecting the different sized penetrants in a different manner, which will be discussed at the end of this section.

**Table 2.3** Repeated permeability test comparing different thickness of membrane crosslinked at 75 °C.

<table>
<thead>
<tr>
<th>Penetrant</th>
<th>150 µm</th>
<th>300 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_4$</td>
<td>1000</td>
<td>1010</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>3970</td>
<td>3990</td>
</tr>
</tbody>
</table>

To provide some additional evidence that the increase in permeability of crosslinking PDMS at 75 °C is due to the change in physical properties of the polymer and not due to defects in the membrane and provide a comparison between the permeation of different membrane thicknesses, a second membrane
was prepared in the same manner but using a different thickness, 300 µm. The permeability of this membrane was also tested and the results are shown in Table 3. As can be seen, the permeability parameters are quite close for the 155 and 300 µm thick membranes. This also shows that the PAN support has no effect on the permeation results as the 300 µm membrane has no support.

Within the solution-diffusion model for gas permeation, the kinetic diameter of the penetrant has a direct effect on the diffusion coefficient [34]. N₂, CO₂ and CH₄ kinetic dimensions are 3.64, 3.3 and 3.8 Å, respectively, which are quite close. It is suggested that the solubility of these gases is generally associated with their thermodynamics, dictated by the interactions between these gases and the polymer [35, 36]. As such, any difference between the permeation of N₂, CO₂ and CH₄ molecules can be mainly due to the variance in their solubility in PDMS. However, from the observations, the optimum permeation occurred for the membrane formed at 75 °C for all gas species, so solubility of the species is not the reason for the difference in permeability as a function of crosslinking temperature. Instead, different gas diffusivity of N₂, CH₄ and CO₂ gas molecules into PDMS can describe the change. The gas diffusivity depends on the gas molecules capability to undertake diffusive jumps within the polymer, this is when the polymer dynamically forms momentary gaps, large enough to accommodate the penetrant, only in the immediate area of the penetrant gas molecule [37]. A change in diffusivity of a rubbery polymer is often attributed to an increase in FFV [38]. Two methods of characterising FFV have been used in this study, PALS experiments did not show a substantial difference in free volume that could be attributed to such a large increase in permeation between the
membranes. However the change in FFV from the density measurements shows a similar trend to that of the permeability and complies within the Fujita free volume theory as can be seen in Figure 2.10 [39].

![Figure 2.10 Permeability at 35 °C as a function of 1/FFV for PDMS membranes](image)

Crosslinking of the membranes at 75 °C, the permeability increases due to the increased free volume in conjunction with the decrease in crosslinking density. This is evident in the presence of additional stretching Si–H crosslinking bonds, as strongly evidenced from both the FTIR and Raman spectroscopy (Figures 2.3 and 2.4). The change in polymer matrix with the introduction of extra Si–H bonds that is a result of an increase in the length between cross-links therefore decreasing crosslinking density and increasing the FFV as was demonstrated with the density measurements (Figure 2.7(b)) and swelling tests (Figure 2.8). This
altering of the polymer matrix modifies the permeability in two separate ways. Firstly, the increase in FFV will increase the permeability of all components, and should affect CH$_4$>N$_2$>CO$_2$, so the largest change from this is due to molecule size. Secondly, there is a change in permeability in the response to the changing crosslinking density, which will affect the more condensable gas species the most CO$_2$>CH$_4$>N$_2$.

Crosslinking the membranes above 75 °C caused a decrease in Si–H bonds resulting in the polymer structure changing to become more rigid through an increased inter-chain bonding and therefore decreasing the diffusion of the penetrant gas molecules. This could also be confirmed by both the swelling test (Figure 2.8) and WAXS (Figure 2.7) analysis with the shifting of the dominant structure peak. Doo Sung Lee et al. [36] as well as others found similar results, for which crosslinking effects on the permeation characteristics of polyurethane-polystyrene were dependent on the nature of the crosslinking and therefore increasing free volume within the membrane.

2.3.6 Evaluation of the membranes using sensing systems

As presented in the introduction, in many real world applications, especially for those dealing with the separation of gaseous and liquid phases, reliable gas permeable barriers are required. Such membrane barriers should allow the permeation of the desired gas species at the highest possible flux. To show the capability of the developed PDMS membranes crosslinked at different temperatures, a sensing system setup presented in Section 2.2.4 used for the investigation of the passage of CO$_2$ and CH$_4$ (99.9%) with and without the presence of the membranes. Measurements were taken for 1 h and the steady flow
rate was controlled using the mass flow controller. All measurements were then normalised to the experimental setup response with no membrane present.

As can be seen in Figure 2.11, the permeation of both CO₂ and CH₄ through the 50 and 75 °C cross-linked membranes is much more rapid than the others. The membranes obtained at 25, 100 and 150 °C showed much slower permeation rates. As expected, the 75 °C performed the best in comparison to other membranes and had almost the same response as when there is no membrane for CO₂ (within 9 % of the response), indicating that even with the membrane being 155 µm thick it does little to inhibit gas permeation. In comparison, CH₄ permeation after 1 h only reaches 70% that of the without presence of any membrane.
2.3.7 Evaluation of the membranes phase separation using a sensing system

The 75 °C membrane was tested for its capabilities in the separation of dissolved gas from a liquid medium and for the durability. A chamber filled with approximately 50% CO₂ and 50% CH₄. The gas and liquid (ionic broth in DI water) were continuously/mechanically mixed during these measurements. Figure 2.12 shows the response of the sensor with incorporated 75 °C crosslinked PDMS membrane in response to the sensor after 2 h of gas being dissolved in the environment. As can be seen the CH₄/CO₂ selectivity is near 4, in great agreement
with our selectivity stated in Table 2.3, for the sensor before the saturation occurs providing good evidence that the sensors and membranes are defect free.

Figure 2.12 Sensor reading with 75 °C membrane for dissolved gas species (CO₂ and CH₄) in a liquid medium

The extraordinary results show that the membrane crosslinked at 75 °C are almost transparent to CO₂ gas molecules in gaseous media and also can be efficiently used for the separation of gases from liquid media. As such, the enhanced membrane can be used in a large number of applications concerning the efficient, rapid, passive, and low cost separation of CO₂ from other gas species and from liquid phase (as PDMS blocks the passage of liquid) [40]. These membranes can also be invaluable in in-situ monitoring of biosystems dealing with the generation of CO₂ and CH₄ as the by-product of many bio-processes.
2.4. Summary

In this chapter, the author of the thesis demonstrated the effect of the crosslinking temperature on CH₄, N₂ and CO₂ gas molecule permeation through defect free PDMS membranes. It was found that an optimal crosslinking temperature of 75 °C resulted in membranes with the highest gas permeation, significantly higher than those crosslinked at room or above 100 °C. This was ascribed to the structure of polymer chains that form the highest intensity of Si–H stretching bonds, as demonstrated by both Raman and FTIR spectroscopy at a crosslinking temperature of 75 °C, which was also confirmed by the WAXS analysis resulting in a decreased crosslinking density and a more relaxed polymer matrix and increasing the FFV as demonstrated by the density measurements and toluene swelling tests.

Referring to the solution-diffusion mechanism and the observations using vibrational and electron beam spectroscopy investigations, the optimised structure and extra free volume increases the diffusion rate, allowing the gas molecules to move more easily within the polymer matrix at this optimum temperature. The outcomes of this study show the significance of the crosslinking temperature in engineering membranes with enhanced flux, which can be readily implemented in many industrial, agricultural and biotechnology sectors that deal with sensing, purification and separation of N₂, CO₂ and CH₄ gas species.

In the next chapter, the author will demonstrate nanocomposite membranes with significantly increased permeability without a considerable decrease in mechanical strength or selectivity. Expanding on this chapter the author will use
the optimised synthesis of PDMS as the base polymer and incorporate 2D graphene as the filler.

2.5. Chapter Acknowledgments

I would like to acknowledge Dr. Majid Nour for helping with the FTIR, Dr. Cara Doherty for assisting with PALS and Dr. Kay Latham for contributing to the WAXS. I would also like to acknowledge the advice and guidance of Prof. Sandra Kentish, Dr. Anita Hill and Prof. Kourosh Kalantar-zadeh.

References


Chapter 3: Enhanced Gas Permeation through Graphene Nanocomposites

3.1 Introduction

In chapter 1, the author described the use of membranes for gas permeation and phase separation. Membranes can offer many distinct advantages over other more energy dependent processes. The operational efficiencies of these membranes rely heavily on high gas permeability. In chapter 2 the author significantly increased the intrinsic permeability of pristine PDMS through the synthesis parameters used. In particular, the addition of fractional free volume (FFV) into the polymeric matrix by optimising the crosslinking temperature.

In this chapter, the author reports membranes with significantly increased permeability without a considerable decrease in mechanical strength or selectivity, synthesised from a polymer nanocomposite that incorporates graphene and PDMS. These graphene-PDMS nanocomposite membranes were able to enhance the gas permeation of N₂, CO₂, Ar and CH₄ in reference to pristine PDMS membranes. This is achieved by creating interfacial voids between the graphene flakes and polymer chains, which increases the FFV within the nanocomposites giving rise to an increase in permeation. An optimal loading of graphene was found to be 0.25 wt%, while greater loading created agglomeration of the graphene flakes hence reducing the effective surface area. The author presents the enhancements that the membranes can provide to sensing and phase separation applications. The author shows that these nanocomposites are near
transparent to CO$_2$ gas molecules in sensing measurements. This chapter offers a new area of research for graphene-based nanocomposites.

The author investigates the effects and advantages that 2D graphene adds in terms of the intrinsic gas barrier properties as well as the interactions with the polymer matrix within a gas permeable membrane. Furthermore the author explores the two competing alterations to the gas permeation mechanisms that can arise from the addition of graphene to PDMS: firstly, the added tortuosity through the addition of non-permeable graphene; and, secondly, the decreased diffusion path within the polymer due gas molecules preference to travel through the permanent interfacial voids. The author of this thesis hypothesises that graphene as a gas barrier, together with the formation of meso-/nano-sized voids at the polymer interface establish a novel membrane for rapid permeation of gas molecules with maintained selectivity. This is based on maximizing the ‘sieve in a cage’ phenomenon through the enhanced interfacial area of 2D graphene to the polymeric matrix. This chapters investigation is conducted by varying the concentration of graphene, at low weight percentages, added to the PDMS matrix, altering the properties of the polymer and evaluating the permeation rates for N$_2$, Ar, CH$_4$, and CO$_2$. A demonstration of some applications for these membranes is shown, for phase separation where the membrane has to be defect free to separate liquid and gas phases determining that there are no micro defects in the polymer, and finally, the advantages of using such a membrane within a gas sensing systems is presented. This application of such a membrane is extremely advantageous for many reasons. Most importantly this novel method aims to
increase the lifespan of the sensor by blocking unwanted contaminants, while maintaining the gas interaction kinetics with the sensors.

The contents of this chapter was published as a full article in the Journal of Physical Chemistry C [1].

3.2 Materials and Methods

3.2.1 Nanocomposite preparation

Nanocomposite membranes were fabricated using PDMS and a proprietary cross-linker (Sylgard 184, Dow Corning Corporation) to provide the base polymer. Non-functionalised graphene flakes grown through chemical vapour deposition (CVD) (70160-100ML, Cheap Tubes Inc.), was added as the filler to make the nanocomposites. A reference PDMS membrane was made as well as multiple weight ratios of graphene-PDMS nanocomposites which included: 0.125 wt%; 0.25 wt%; 0.5 wt%; and a 1 wt%. The exfoliation of the graphene flakes occurred in 20 mL of p-xylene using an ultrasonic bath for 1 h. This allowed time for the π-π interaction of the aromatic ring in the solvent and in the carbon matrix to facilitate maximum dispersion of the flakes in solution. The mixture was then added to 20 g of the PDMS oligomer and stirred before being returned to the ultrasonic bath for 30 min.

This mixture was then mechanically stirred at 100 rpm on a hotplate at 120 °C for approximately 1 h. to allow for the evaporation of the majority of the solvent. After cooling to room temperature the proprietary crosslinking agent was added and thoroughly mixed in. All membranes were prepared utilising a 10% weight ratio of base PDMS to the crosslinker. The solution was degassed for 30 min.
before being spun onto a silicon wafer and crosslinked at 75 °C. As previously shown in chapter 2 this optimum temperature, the PDMS matrix forms with the greatest free volume [2]. The membranes were then carefully peeled from the wafer and used for permeation measurements as well as structural and spectroscopic characterisation. The membrane thickness as determined using scanning electron microscopy (SEM) imaging lies within 120 ± 4 µm range. This thickness was found to be unaffected by the quantity of graphene added.

3.2.2 Nanocomposite characterisation

Membrane characterisations were conducted using SEM, Transmission electron microscopy (TEM), vibrational spectroscopy, X-ray photoelectron spectroscopy (XPS) as well as X-ray diffraction (XRD). FEI Nova NanoSEM imaging was utilised to evaluate the cross-sectional thickness of the membranes as well as the distribution and exfoliation of graphene flakes into the PDMS matrix. A JEOL 2010 TEM was used with a lacey formvar/carbon grid for determining the quality of the graphene. Atomic force microscopy (AFM) was used to determine the distribution graphene thickness using a Bruker D3100 in tapping mode. 200 flakes were assessed to obtain the distribution of graphene thickness. To understand the graphene lateral dimension and size distribution before being dispersed in PDMS an ALV fast dynamic light scattering (DLS) particle sizing spectrometer was employed. AFM was also used to determine the graphene flake size distribution after dispersion into the PDMS matrix using a Bruker D3100 in tapping mode on the surface of the material. 200 flakes were assessed on the surface to obtain the distribution.
A Thermo Nicolet 6700 spectrophotometer was used for recording the FTIR of the PDMS and nanocomposites. Micro-Raman characterisation of the samples was performed using a Renishaw Raman spectrometer at a wavelength of 514 nm. XRD data were collected on a D8 Advance Bruker AXS X-ray diffractometer with GADDS (General Area Detector Diffraction System). XPS was performed using a Thermo Scientific K-alpha instrument with a monochromated Al Kα source. Water contact angle measurements were performed using a KSV 101 system. The height of each drop was confirmed using a CCD camera prior to each measurement to ensure consistency in the drop volume. Drop volumes of approximately 8 μL were employed.

Solvent swelling measurements were carried out using pre weighed dry membranes of various weight concentrations of graphene and the PDMS membranes. The membranes were totally immersed in pure toluene until equilibrium swelling was reached. The membranes were then dried and immediately weighed. This was carried out four times for each membrane to ensure repeatability.

Tensile testing was carried out using an Instron 4467 Universal testing machine fitted with a 100N load cell. Bluehill software was used to control the tensile test and to calculate the various tensile properties reported. The testing speed was set to 10mm per min. The tests were performed using ASTM D638 as a guide. Tensile test specimens used were not in accordance with the requirements of the standard, but were a close approximation to a type IV specimen. As it was not possible to attach an extensometer to the specimens and as a result the modulus figure reported were calculated using the movement of the crosshead as the
extension measurement. This results in a lower than actual tensile modulus figure.

Density measurements were performed using a density determination kit employing the hydrostatic weighing method [3, 4]. For the determination of density of the membranes, ethanol was used as the auxiliary liquid. The theoretical densities of the composite membranes are determined based on simple consideration of the relevant densities:

$$\frac{m_{\text{composite}}}{\rho_{\text{theory}}} = \frac{m_{\text{graphene}}}{\rho_{\text{graphene}}} + \frac{m_{\text{PDM}}}{\rho_{\text{PDM}}}$$  \hspace{1cm} (3)

where $m$ is the mass of the relevant species, $\rho_{\text{graphene}}$ is taken as that of graphite (2.267 g/cm$^3$) and $\rho_{\text{PDM}}$ is 1.033 g/cm$^3$ [2]. The additional FFV and thus the total FFV could then be determined by a comparison between the theoretical and the experimentally determined densities:

$$FFV_{\text{Tot}} = FFV_{\text{PDM}} + \frac{1}{\rho_{\text{exp}}} - \frac{1}{\rho_{\text{theory}}}$$  \hspace{1cm} (4)

Positron annihilation lifetime spectroscopy (PALS) was performed using a positron source of 30 µCi $^{22}\text{Na}$Cl sealed in a Mylar film at room temperature. Free standing samples were stacked to 4 mm thick bundles with the positron source placed in the middle. A minimum of 5 measurements each with $1 \times 10^6$ integrated counts per measurement were made using an automated EG&G Ortec fast-fast coincidence system with a timing resolution of 260 ps. The data was analysed using LT (version 9.0) using a source correction of 1.740 ns and 3.014% [5]. The data was fitted to 3 components including a $para$-positronium
component, fixed at 125 ps, a free positron annihilation component, ~400 ps and the \( o \)-Ps component. The \( o \)-Ps component’s intensity and the lifetime were employed to determine the relative number of free volume elements and the average size of the free volume elements within the sample respectively. The Tao-Eldrup Equation was used for calculating the average size of the free volume elements [6].

### 3.2.3 Gas permeability measurements

A series of experiments were conducted to assess the gas permeability of the membranes. A constant pressure variable volume (CPVV) system (Figure 2.1) was used to measure the permeability of CH\(_4\), N\(_2\), Ar and CO\(_2\) (99.99%, Core Gas Australia) through the PDMS and graphene-PDMS nanocomposite membranes. The membranes for testing were mounted within a permeation cell with a constant pressure on the upstream boundary, while the downstream side was kept at the atmospheric pressure. The pressures tested to ensure repeatability were of 200, 300 and 400 kPa. Both sides of the permeation cell were purged with the penetrant gas prior to each experiment. To maintain a constant temperature the permeation cell was housed in an environmental chamber with all measurements conducted at 37 °C. The membranes’ permeability was measured in the following order: CH\(_4\), N\(_2\), Ar, CO\(_2\). The flow rate was acquired every ten seconds for two minutes once a steady-state was achieved and the values were averaged and converted into permeability values given in Barrer (1 Barrer = \(1\times10^{-10}\).cm\(^3\)./(STP).cm/cm\(^2\).s.cmHg).
3.2.4 Applications for graphene-PDMS nanocomposites

For the evaluation of the membranes using a sensing system and phase separation the experimental set-up demonstrated in Figure 2.2 is used. Membranes were mounted within a permeation chamber on the front of a commercial NDIR (IR15TT-R, e2v technologies) sensor placed on the opposing side to the gas flow. The pure gas flow rate was controlled to 10 sccm via a mass flow control unit and pressure was maintained within the chamber to 110 kPa. For phase separation experiments the chamber is filled with distilled water where the gases are dissolved slowly into it.

3.3 Results and Discussion

3.3.1 Graphene characterisation

To understand the properties of the graphene flakes, as well as their morphology (Figure 3.1), DLS, AFM and TEM were performed on the flakes after the exfoliation process but before being added into the nanocomposite. AFM revealed the thickness distribution of the graphene flakes, with the majority of flakes containing less than 10 layers (less than 4 nm based on Gupta et al. [7]).
Figure 3.1 Graphene characterisation. (a) AFM image of graphene flakes. (b) Corresponding height profile of graphene flake along the green line. (c) Distribution of graphene flake thickness taken from the analysis of 100 flakes with the AFM. (d) Distribution of graphene flake lateral dimensions obtained from the DLS. (e) and (f) Transmission electron microscopy (TEM) image of typical graphene sheets.

To understand the distribution of the flake dimensions, DLS was performed. This revealed that the lateral dimensions of the majority of flakes lie within 0.1 to 4 µm. This results in an average aspect ratio of 110 for the graphene flakes used in the composite membranes. TEM was used for assessing the morphology of the graphene flakes after the exfoliation process.

3.3.2 Nanocomposite characterisation

To understand and evaluate the alterations that graphene is providing to the nanocomposite different characterisation techniques were employed with an emphasis on studying the physiochemical properties that relate directly to gas
permeation. Graphene-PDMS composite membranes with the graphene concentration of 0.125 wt%, 0.25 wt%, 0.5 wt% and 1 wt% graphene were synthesised. Due to the formation of interconnected interfacial voids above 1 wt% graphene loading (which will be described later) thus creating defects within the membrane. This allows gas species to pass freely and hence providing no selectivity, rendering the membranes ineffective. A very important factor in dictating gas molecule permeation through rubbery polymers like PDMS is the FFV. This can fundamentally change the diffusion of the gas molecules through the membranes. To understand the impact that the addition of graphene has on FFV, both density measurements and PALS were carried out. The polymer density is commonly associated with FFV and it is known that PALS can be used to determine the average size of the free volume elements, and the concentration, or relative number of free volume elements in the sample [3, 4, 8].

The density of the various weight concentrations of graphene composite membranes are shown in Figure 3.2(a). It can be seen that adding graphene reduces the density of all of the composite membranes, relative to that of the predicted theoretical calculations. The lowest density for all of the different membranes is found at 0.25 wt% graphene in PDMS. This data can be used for assessing the additional free volume that possibly results from the weak interaction between the graphene flakes and the polymer (Figure 3.2(b)). Such increases in FFV are well known and are often referred to as the ‘sieve in a cage’ phenomenon [9, 10].

The PALS data for different membranes are presented in Figures 3.2(c) and (d). As can be seen from Figure 3.2(c), the o-Ps lifetime (τ₃) parameter does not show
any significant difference in the size of the free volume elements, revealing the average pore diameter to be approximately 0.8 nm, with all variance between the different nanocomposites lying within the range of uncertainty. With the addition of graphene into the polymeric matrix, the intensity parameter ($I_3$) (Figure 3.2(d)) nevertheless does demonstrate an initial decrease in the number of free volume elements (0.125 and 0.25 wt%). This number then increases with increasing graphene concentration (0.5 and 1 wt%). While the calculations from the density measurements show an increase in FFV in the nanocomposites, the PALS intensity parameter reveals only a minor decrease in free volume elements within the range of detection. As a result, there must be a formation of voids that lie outside the detection limit of PALS (approximately ≤20 nm) [11].

To investigate the formation of voids greater than 20 nm in dimension, SEM was employed. The membranes were prepared for SEM observations using liquid nitrogen fracturing to give an accurate representation of the morphology of the cross-sections and a 20 Å thick coating of platinum was employed to prevent charging of the non-conductive membranes. An important observation to make from the example SEM image in Figure 3.2(e) is the interface between the graphene and the PDMS. It can be seen that there is a creation of a void present at this interface. These nano- to meso-sized voids occur in the range of 25 to 250 nm, which are outside the detection limit of PALS and can be the reason for the discrepancy between the calculated FFV from density measurements and the PALS data. Another major observation from the SEM images is that the PDMS does not make strong bonds with the surface of the flakes during the polymerisation process, reinforcing the lack of interaction between the materials
that is driving the formation of these voids. Such an effect is likely due to graphene’s surface energy and intrinsic low wettability of individual flakes [12].

Figure 3.2 Density and FFV study of the membranes. The effect of various weight amounts of graphene on (a) the density, relative to that calculated from simple mixing of the respective components (b) the resulting total FFV based on density measurements (c) PALS o-Ps lifetime ($\tau_3$) parameters (d) PALS o-Ps intensity ($I_3$) parameters of the composite membranes. (e) Example SEM image of the 0.25 wt% nanocomposite membrane showing the formation of nano- to meso-sized voids around the flakes and that the polymer does not efficiently make strong bonds with the surface of the flakes.

From example SEM images in Figures 3.3(a) to (d) it can be seen that the best graphene flake dispersion is attained at 0.25 wt% graphene-PDMS (Figure 3.3(b)). It appears that with the addition of graphene beyond this concentration, an increased agglomeration is found with multiple flakes forming bundles. This has the ability to create large voids between flakes with excessive aggregation.
Another key aspect to gain from the sample SEM images is understanding the orientation of the flakes within the membranes, which can greatly affect the diffusion path and therefore the permeation of the gas molecules. From Figure 3.3(b), it can be seen that the majority of flakes lie close to perpendicular to the diffusion path (parallel to the membrane’s surface) for 0.25 wt% graphene-PDMS samples (highlighted by the dotted lines). However, such an effect is not seen for other concentrations (Figures 3.3(c) and (d)).

AFM at the surface of the membranes gives insight into the surface roughness and therefore relative surface artifact size distribution on the membranes (Figure 3.3(e)). With the graphene samples being prepared using the same method before adding into the polymer, it can be assumed that any increase in the size of surface artifacts is due to agglomeration of flakes. At 0.25 wt% the majority of surface artifacts’ dimensions lie below 300 nm and increasing the graphene loading causes agglomerates to form larger artifacts where at 0.5 wt% the majority lie between 400 and 800 nm.
Figure 3.3 Morphology of graphene nanocomposite membranes. Cross-sectional scanning electron microscopy (SEM) images of (a) pristine polydimethylsiloxane (PDMS) (b) 0.25 wt% graphene-PDMS (c) 0.5 wt% graphene-PDMS and (d) 1 wt% graphene-PDMS nanocomposite (e) Graphene flake size distribution, as a function of the artifacts seen on the surface, based from AFM analysis of membrane surface.

Equilibrium swelling measurements of the composite membranes was investigated and compared to a pristine PDMS reference to evaluate any changes in crosslinking density [13]. The solvent uptake, or MSD, is presented in Figure 3.4(a). An increase in the chain length between the cross-links reduces the elastic resistance to the swelling stress and therefore increases the degree of swelling
The pristine PDMS complies with other studies for PDMS [13, 15]. It can be seen that all of the composites have a higher degree of swelling than that of pristine PDMS. Even with the addition of 1 wt% of graphene an increase in swelling of approximately 10% is observed, with the 0.25 wt% graphene to PDMS showing the lowest crosslinking density with a maximum chain length between crosslinks within the polymer [16]. This minimum crosslinking density could represent an optimal dispersion of graphene flakes, allowing maximum interaction with the PDMS matrix and interfering with the normal crosslinking structure of the polymeric matrix.

The decrease of crosslinking density, shown in the MSD results, is also confirmed through tensile strength measurements (Figure 3.4(b)). The reduction in elastic resistance and tensile strength are seen with the same trend as the swelling measurements, with most variance lying within the range of uncertainty. The graphene flakes affect the polymerisation process, inhibiting the crosslinking bonds created and therefore producing a looser polymeric matrix with less tensile strength.

An investigation into the different bonds formed by the pristine PDMS and nanocomposite membranes was undertaken using micro-Raman spectroscopy. All membranes contained similar PDMS peaks regardless of the concentration of graphene added, with all the peaks in agreement with typical signatures reported in previous work [17]. The composite materials however, also show the addition of Raman shift peaks representative of graphene. These include a clear G band and a 2D band that is almost non distinguishable in our samples. These peaks have been widely studied and occur at approximately 1570 and 2700 cm$^{-1}$. 

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respectively [18, 19]. The lack of a substantial D band, usually found at \(~1350 \text{ cm}^{-1}\), in the nanocomposites that is associated with defect density in graphene, indicates a low level of oxidation in graphene flakes [20]. The whole Raman spectra for the pristine PDMS and the 0.125 \text{ wt}\% graphene-PDMS membranes are shown in Figure 3.4(c).

Two major changes to the overall spectra of the composite materials are observed with the addition of graphene into the PDMS matrix. The first and most obvious is the addition of the sharp and distinctive graphene associated G graphitic band shown in Figure 3.4(e). This peak is formed from the fundamental resonance of the $sp^2$ carbon hybridisation, which is not present in PDMS, and increases as the amount of graphene within the polymeric matrix is increased. However, the addition of graphene also has an influence on the alteration of the polymeric matrix, which can be seen when studying the second order Si–O peak occurring at approximately 1090 cm$^{-1}$ (Figure 3.4(d)). This weak, broad peak that occurs in pristine PDMS reduces in intensity as the amount of graphene in the polymer increases, vanishing at 1 \text{ wt}\% of graphene in PDMS. This represents a reduction in rigidity of the Si–O bond and probably the formation of irregular chains and decreased crosslinking density, which establishes the backbone of the PDMS polymer chain. No apparent change is seen for the first order Si–O bond peak at 488 cm$^{-1}$, which hints at the fact that the number of these bonds has not been reduced.
Figure 3.4 The effect of various weight amounts of graphene on the nanocomposites. (a) Crosslinking density study of the nanocomposites based on MSD. (b) Maximum tensile strength of the nanocomposite membranes. (c) Raman spectra of pristine PDMS and 0.125 wt% graphene-PDMS composite membranes. Inset: (d) comparison of the second order Si–O bond and (e) comparison of the G band.

The FTIR spectra for the graphene-PDMS nanocomposites and the reference membranes (Figure 3.5) comply with those typically reported for PDMS. It has been previously reported that Si–H bonds, which is located at 910 cm\(^{-1}\) and Si(CH\(_3\))\(_2\) rocking peaks appear in the region of and 785–815 cm\(^{-1}\). The Si–O–Si stretching multi-component peaks for PDMS is observed in the range between 930 to 1200 cm\(^{-1}\). The peaks between 1400–1420 cm\(^{-1}\) and between 1240–1280 cm\(^{-1}\) corresponds to –CH\(_3\) deformation vibration in PDMS.
Figure 3.5 FTIR spectra of pristine PDMS and the graphene-PDMS nanocomposite membranes.

XRD was utilised to understand the changes in the polymeric structure of PDMS as a function of the concentration of graphene impregnated within the nanocomposite membranes (Figure 3.6). XRD data shows that graphene loading affected three key diffraction features of pristine PDMS film, observed at 7.1°, 8.5° and 14.12° that correspond to a \(d\)-spacing of 14.45, 12.07 and 7.29 Å, respectively. These \(d\)-spacing’s may be considered as large, medium and small chain spacing’s, respectively, within the PDMS matrix. The change in the size of these polymeric structures can be directly associated with the changes in the structure of the polymer chains due to graphene impregnation.

It is evident that even with the smallest amount of graphene loading in PDMS, the distribution of the chain spacing shifts remarkably. In particular the average chain spacing in the PDMS polymer appears to shift from 7.3 Å in the pristine polymer to 7.8 Å at 0.125 wt% graphene and 8.1 Å at 1 wt% graphene, suggesting that the
PDMS crosslinks formed in the presence of the graphene flakes are extended, relative to the pristine polymer. A further interesting change is also observed for the large spacing of 14.45 Å, where at 0.25 wt% graphene-PDMS there is no occurrence of this spacing. This is likely due to an increased dispersion where there are fewer larger agglomerates that are able to produce this larger spacing and therefore alter the polymer chain stacking.

Figure 3.6 XRD patterns for PDMS and graphene-PDMS nanocomposites with $x$ axis converted from 2$\theta$ to $d$ spacing using Bragg's law.

3.3.3 Gas permeation properties

The pure gas permeation rates of the pristine PDMS and composite graphene-PDMS membranes were investigated under exposure to CO$_2$, N$_2$, Ar and CH$_4$ (99.99%) using the CPVV experimental setup.
As can be seen in both Table 3.1 and Figure 3.7, the permeation of all gas species significantly increases with the addition of graphene as a filler to the PDMS matrix. The permeability of the pristine PDMS membrane is similar and corresponds to the permeability found by Merkel et al. that also used a CPVV experimental set up [21].

A maximum permeability for Ar, N₂ and CH₄ was found at 0.25 wt% graphene loading, providing a greatly enhanced flux of over 60% in the case of N₂ for the composite membranes. A maximum permeability for Ar, N₂ and CH₄ was found at 0.25 wt% graphene loading, providing a greatly enhanced flux of over 60% in the case of N₂ for the composite membranes. However, at this condition, there is some minor loss of selectivity, consistent with the Robeson trend of falling selectivity when permeability increases [22]. Interestingly for CO₂, while the 0.25 wt% graphene-PDMS membrane showed an increase in permeation, it was the 0.5 wt% membrane that provided the greatest flux. This difference in the behavior of CO₂, with greater permeation at 0.5 wt%, whilst the other gas species maximum

Figure 3.7 (a) Change in permeability for each gas species with respect to the change graphene concentration. (b) Comparison of experimental data, Maxwell model and Nielson model for CO₂ permeation through 0.25 wt% graphene-PDMS
permeation occurs at 0.25 wt%, may be ascribed to the high affinity of graphene towards CO\textsubscript{2} [23]. Importantly, this increase in permeability at 0.5 wt% is achieved with no loss of CO\textsubscript{2}/N\textsubscript{2} selectivity. It appears that CO\textsubscript{2}/CH\textsubscript{4} selectivity increase slightly rising from 3.6 for pristine PDMS to 4.2 for the 0.5 wt% composite. In general, it can be seen that most of the changes in selectivity lie within the error range, revealing that the increase in permeability has come without major loss in selectivity. This is an important factor in the application of these membranes for gas separation.

Table 3.1 Gas separation performance of PDMS membranes with different graphene concentrations.

<table>
<thead>
<tr>
<th>Percentage weight of graphene in the composite membrane</th>
<th>Penetrant</th>
<th>PDMS</th>
<th>0.125 wt%</th>
<th>0.25 wt%</th>
<th>0.5 wt%</th>
<th>1 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability (Barrer)</td>
<td>CH\textsubscript{4}</td>
<td>850 ± 20</td>
<td>930 ± 15</td>
<td>1120 ± 18</td>
<td>1070 ± 17</td>
<td>920 ± 19</td>
</tr>
<tr>
<td></td>
<td>N\textsubscript{2}</td>
<td>380 ± 15</td>
<td>400 ± 18</td>
<td>610 ± 12</td>
<td>550 ± 14</td>
<td>400 ± 22</td>
</tr>
<tr>
<td></td>
<td>Ar</td>
<td>710 ± 17</td>
<td>830 ± 19</td>
<td>1030 ± 16</td>
<td>910 ± 17</td>
<td>840 ± 20</td>
</tr>
<tr>
<td></td>
<td>CO\textsubscript{2}</td>
<td>3020 ± 16</td>
<td>3450 ± 21</td>
<td>3790 ± 12</td>
<td>4460 ± 18</td>
<td>3360 ± 18</td>
</tr>
<tr>
<td>Selectivity</td>
<td>CO\textsubscript{2}/N\textsubscript{2}</td>
<td>7.9 ± 0.37</td>
<td>8.6 ± 0.46</td>
<td>6.2 ± 0.14</td>
<td>8.1 ± 0.24</td>
<td>8.4 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>CO\textsubscript{2}/CH\textsubscript{4}</td>
<td>3.6 ± 0.10</td>
<td>3.7 ± 0.08</td>
<td>3.4 ± 0.06</td>
<td>4.2 ± 0.08</td>
<td>3.7 ± 0.10</td>
</tr>
</tbody>
</table>

Gas permeation through a rubbery polymer is dictated by the solution-diffusion mechanism [24]. This mechanism comprises three steps: (1) adsorption at the upstream boundary, (2) diffusion through the membrane and (3) desorption on the downstream boundary. The increase in permeation for all gases is due to the
change in diffusion of the gas molecules through the composite material. When referring back to the solution-diffusion model for gas permeation, the kinetic diameter of the penetrant and the FFV have direct effects on the diffusion coefficient [25]. Ar, N₂, CO₂ and CH₄ kinetic diameters are 3.4, 3.64, 3.3 and 3.8 Å, respectively. The diffusion of gas molecules through the membranes depends on the molecules’ ability to undertake diffusive jumps within the polymer, the smaller the molecule or the larger the free volume, the more rapid these diffusive jumps will occur. These diffusive jumps take place when the polymer chains dynamically form temporary voids, large enough to accommodate the penetrant, only in the immediate area of the gas molecule [26]. A change in diffusivity and therefore permeation in a rubbery polymer is often attributed to an increase in FFV [27].

The presence of graphene in the PDMS matrix has the ability to create permanent voids at these interfaces facilitating diffusion, where the distance between the oligomers and the graphene flakes is different than the distance between the oligomers themselves under normal crosslinking conditions. Therefore the introduction of graphene into the PDMS matrix increases the amount of free volume within the polymer and thus giving rise to an increase in permeation.

The gas permeation mechanisms through the graphene-PDMS nanocomposite membranes in this paper differ from other carbon nanomaterial composites previously reported. In reality, the surface energies of other forms of carbon are very different from those of graphene with no dangling bonds. Carbon fillers, other than graphene, have been used for making permeable composite membranes, generally they have been shown to reduce permeability [28, 29].
Carbon black has been generally shown to reduce the gas permeability [30]. While nanocomposites containing CNT’s have been demonstrated to either decrease or increase the permeation base on the type of gas species, the surface treatment used and also the orientation of the CNTs in the composite [28, 31, 32]. In many of these reports the increase in permeation has been ascribed to a tunneling effect and the reduction in permeation to a sieving effect bestowed upon by the added CNTs. However, most of these works have ignored the possible effect of the interfacial voids formation. The lack of interaction between the graphene flakes, which was presented in our work, and PDMS has not been reported in CNT-PDMS composite materials.

3D carbon nanoparticle fillers, such as buckyballs, have been shown to increase FFV within the composite membrane thus increasing gas permeation through the composite [33]. However, such increases has only been seen in functionalised buckyballs, which are well distributed within the polymer matrix and disrupting the polymer chains by making bonds with them. This is the opposite of what is presented in this work.

Graphene has been employed to prepare polymer nanocomposite membranes for various separation processes [34-36]. However they have only reported decreases in gas permeation, this is most likely due to the relatively large quantity of graphene used in their studies, causing a greater increase in the diffusion path length from the non-permeable graphene flakes. With most studies choosing to functionalise the graphene flakes to maximise interaction and compatibility between the base polymer and the flakes, therefore not allowing the formation of any interfacial void.
There are several reports on the incorporation of functionalised graphene flakes into PDMS at different aspect ratios and various concentrations of the flakes. For example, Adamson et al. and Ozbas et al.\cite{37, 38} demonstrated a decrease in the overall gas permeation of the graphene-PDMS nanocomposites at high concentrations of functionalised graphene flakes and also showed that a larger aspect ratio (aspect ratio of 1000 in comparison to 400 at the same concentration of 1 wt%) resulted in a decrease of \(\sim 20\%\) in gas permeation. At 1 wt% of functionalised graphene loading they observed a \(\sim 50\%\) reduction in gas permeation compared to pristine PDMS. The outcomes however, are in contrast with the increase in the gas permeability of our non-functionalised graphene-PDMS nanocomposites presented in this work. Of recent, there have been studies of graphene-polymer nanocomposites that have observed interfacial voids. For example, ion exchange membranes synthesised from sulfonated polyimide–graphene exhibited higher ion conductivity than pure polyimide membrane \cite{39}. Polysulfone (PSf)–graphene ultrafiltration membrane observed water permeation flux increase by \(>20\) times compared to that of the pure PSf membranes \cite{40}. These studies attributed the increases with the addition of porosity or transport channels constructed by graphene within the polymer matrix. Furthermore, poly(vinyl alcohol) (PVA)–graphene nanocomposite membranes demonstrated increased separation of aromatic/aliphatic mixture and was ascribed to a high affinity between the graphene and target penetrant as well as the compatibility at the polymer and graphene flake interface \cite{41}.

Some studies of PDMS and PDMS nanocomposite membranes have also reported an increase in gas permeability, although moderate. An ideal crosslinking
temperature was found to increase the FFV in the polymeric matrix and therefore enhancing the permeation of all gas species tested against [2]. Silicatite particles have also been shown to enhance CO₂’s permeation through PDMS, increasing the permeability from the base 3000 to 3835 Barrer [42]. However, the permeability of all other gases tested was found to decrease due to a molecular sieving effect. Of these membranes none show an ability to improve gas permeation to the degree demonstrated in this study.

There have been many models established in the past in order to predict the effect that loaded particles would have on the permeability of the composite material. The two most common and traditional approaches are the Maxwell model [43] and Nielsen model [44]. These describe the effects of the filler as barrier properties assuming the regular and perfectly spaced arrays of aligned impermeable particles or flakes of ideal spherical geometry for Maxwell’s model or aspect ratio associated with their cross section for Nielsen’s. There have been updates to these models by many groups allowing for the accountability of particle geometry and size distribution and particle orientation [45, 46]. However, these models are still lacking the effect of the interaction between the filler materials and the polymer matrix. The limitations of these models are clearly seen in Figure 3.7(b) as the predictions of these models both indicate a decrease in permeability when the experimental shows the opposite. The Maxwell model only shows a very slight decrease in permeability, due to the low volume of graphene added and the Nielsen model shows a major decrease in permeability due to the aspect ratio of graphene used.
For describing the permeation performance of the composite membranes two competing factors should be considered. The introduction of extra FFV, through interfacial voids, drives an increase in permeability. In contrast, gas transport across the added impermeable graphene flakes is harder, which naturally increases the diffusion path length for the gas molecules (Figure 3.8(a)). This interfacial void created between the polymer and the graphene is similar to that was found by Cong et al. [47] where it was observed that gas permeation through poly(2,6-diphenyl-1,4-phenylene oxide) (BPPOdp) increased when silica nanoparticles were dispersed through the polymer. This increase in permeation was attributed to the introduction of what they referred to as a nanogap and has been observed through many studies incorporating nanomaterials as fillers [48, 49]. Where, the interaction between the nano-filler and the polymer caused the introduction of permanent voids at the filler-polymer interface, thus increasing FFV and permeability.

The 2D planar morphology of the graphene with its intrinsically high specific surface area results in an alteration of the polymeric matrix. As shown in the Raman spectra (Figure 3.4(d)) a decrease in the second order vibration of the Si–O bonds due to the reduction in crosslinking density, resulting in a more relaxed polymeric matrix as shown with tensile strength (Figure 3.4(b)) and swelling tests (Figure 3.4(a)) as well as increasing the FFV through the formation of nano- and meso-sized voids (Figures 3.2(b) and (e)). The change in polymeric matrix acts to alter the oligomer chains in relation to the graphene flakes changing the regular crosslinking structure within PDMS. The PALS intensity data (Figure 3.2(d)) therefore strengthens the case that higher graphene content is not as well
dispersed within the PDMS matrix due to agglomeration resulting in less inhibition with the PDMS crosslinking therefore improved mechanical properties (increased tensile strength and reduced swelling).

Figure 3.8 (a) A schematic of the diffusion paths for PDMS and graphene-PDMS nanocomposites of thickness $l$. The total path length for pristine PDMS membrane is $l$. In the graphene-PDMS nanocomposite membrane, the diffusion path through PDMS ($D_α$) shown in red and diffusion path through the interfacial void ($D_β$) shown in green, where, $\sum D_α < l$ but $\sum D_α + D_β > l$. (b) A schematic depicting the difference between passive surface and bulk diffusion of the gas molecules in pristine PDMS and graphene-PDMS nanocomposite membranes.

The maximum formation of these voids was found to occur at 0.25 wt\% graphene-PDMS which was ascribed to an increased dispersion of graphene flakes found at this concentration. This could effectively increase the number of
interfaces between the graphene and PDMS where at concentrations higher than 0.25 \textit{wt}\% the graphene flakes start to agglomerate and therefore reducing the number of interfaces. The introduction of additional interfaces after the graphene loading can directly impact upon the gas permeability by producing nano- to meso-sized along the surface of the flakes, facilitating the gas transport. The total gas molecules diffusion path length increases by adding graphene flakes. However, as these molecules spend most of their time in the interfacial voids, their diffusion rate is much higher, which results in a higher permeability than the pristine PDMS.

The maintaining selectivity with the increase in permeability implies the interfacial voids do not create an interconnected pore through the membrane and thus remain defect free. All of these elements combine to provide high flux gas selective membranes that are ideally suited for phase separation and sensing applications demonstrated in the following sections.

3.3.4 Applications for graphene-PDMS nanocomposites

Within gas sensing systems there are many environmental conditions that can affect the operation, accuracy and longevity of sensors. A unique way to overcome this is to protect the sensor from the environment is by covering it with a permeable membrane. However the very coating that protects the sensor also deteriorates its performance by hindering the gas molecules, unless the targeted gas species can permeate through the membrane effortlessly. Therefore, a sensing system was established to evaluate the use of the membranes effect using a commercial gas sensor (Figure 2.2).
The membranes were placed in between the gas flow and the sensor directly at the sensor head. The performance of the graphene membranes in comparison with PDMS and no membrane present is shown in Figures 3.9(a) and (b). As can be seen, the sensor response for the graphene-PDMS nanocomposite membrane is almost the same as that of not having a membrane present at all. There is a slight attenuation and a delay of approximately 20 seconds. However, when comparing it to the PDMS reference membrane the composite material offers a far superior performance with less attenuation and a far quicker response. This effectively means the membranes are almost transparent to gas molecules and can offer effective solutions when monitoring gases while keeping the sensor isolated from the surrounding environmental contaminants.

Interestingly, the Barrer number for CO$_2$ permeation (Table 3.1) reveals an increase of 50% but in the sensing experimental setup, the results show a response that appears greater than this number. It is important to consider that the measurements to obtain the Barrer number were performed using a high, fixed pressure difference across the membranes. However, the sensor measurements are conducted with negligible pressure difference across the membranes. As a result, an effective passive gas diffusion that is not necessarily driven by pressure is the reason for the enhanced performance of the membranes for sensing applications. It is known that such passive diffusion follows Ficks second law of diffusion, where near the surface the diffusion of gas molecules occurs more facile and can be approximated using [50]:

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\[ C(x, t) = C_s \left( 1 - \frac{2}{\sqrt{\pi}} \text{erf}\left( \frac{x}{2\sqrt{Dt}} \right) \right) \]  

where \( C \) is the concentration at distance \( x \) from the surface at time \( t \), \( C_s \) is the fixed concentration of gas molecules at the surface, \( D \) is the diffusion constant and \( \text{erf} \) is the error function. This approximation is only valid near the surface boundary while the diffusion far from the surface, in the bulk, reduces to a constant rate. By producing voids using the graphene flakes, more of this surface effect is produced and the passive surface diffusion, which has a much higher rate, according to the Fick’s second law becomes the dominant effect of the gas molecule diffusion. While in only PDMS membranes the constant rate which is equal to that one obtained by the Barrer measurements dominate the system (Figure 3.8 (b)).

To evaluate the membranes’ capability for effective phase separation, \( \text{CO}_2 \) was dissolved into DI water with the membranes placed in between the liquid and the sensor directly at the sensor head (Figure 2.2). As can be seen from comparing Figures 3.9(c) and (d) as well as Table 3.2, the addition of graphene into the PDMS matrix has made the membrane more hydrophobic. PDMS itself is known as a hydrophobic material and so is graphene [51, 52].

Through this added hydrophobicity and increased gas permeability, the graphene nanocomposites act as a high throughput phase separating membranes, as can be clearly seen in Figure 3.9(e). This allows for efficient transfer of gas molecules, while stopping the liquid from permeating.
Table 3.2 Water contact angles for pristine PDMS and the PDMS-graphene nanocomposite membranes.

<table>
<thead>
<tr>
<th>Material</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>102</td>
</tr>
<tr>
<td>0.125 (\text{wt})% graphene-PDMS</td>
<td>118</td>
</tr>
<tr>
<td>0.25 (\text{wt})% graphene-PDMS</td>
<td>116</td>
</tr>
<tr>
<td>0.5 (\text{wt})% graphene-PDMS</td>
<td>115</td>
</tr>
<tr>
<td>1 (\text{wt})% graphene-PDMS</td>
<td>115</td>
</tr>
</tbody>
</table>

Recent work by Hu et al. shows proton transport through single layer graphene membranes surrounded by proton exchange polymers [53]. Our study can ideally be further expanded via similar templates to look at fundamentals for continuing this area of research in the future.

Figure 3.9 Applications of nanocomposite membranes. Sensor reading for (a) \(\text{CH}_4\) and (b) \(\text{CO}_2\) permeation. (c) Water droplets contact angle for PDMS. (d) Water droplets contact angle for 0.5 \(\text{wt}\)% graphene-PDMS. (e) Sensor reading for dissolved \(\text{CO}_2\) in deionized water (DI), phase separation through PDMS and 0.5 \(\text{wt}\)% graphene-PDMS.
3.4. Summary

In this chapter, the author has demonstrated that the addition of graphene to PDMS, to make a new nanocomposite material, has a large effect on the gas permeation properties. The addition of graphene results in an increase in permeation for all gas species tested without significantly altering the selectivity or reducing the mechanical strength of the membranes. An optimal loading of 0.25 wt% of graphene to PDMS was found to provide the highest gas permeation for the investigated gas species except for CO₂, which has the highest permeation at an optimal loading of 0.5 wt% graphene. The intrinsic affinity of graphene for CO₂ led to this increased permeation at the higher loading than other gas species. The high specific surface area of the 2D graphene flakes played a key role in the creation of the highly permeable membranes. The incorporation of graphene resulted in the creation of interfacial voids between the graphene flakes and the polymer chains. This caused an increase in the amount of free volume within the polymer, enhancing the permeation governed by the surface effect according to the Fick’s second law, thus gives rise to an overall increase in the permeation of the membrane. At 0.25 wt% graphene-PDMS shows the lowest degree of agglomeration while the flakes were mostly placed in parallel to the surface of the membranes. Therefore maximising the effective surface area between the graphene flakes and polymer matrix and creating the highest FFV within the nanocomposites. This unique material can be efficiently used for many applications such as phase separation and enhancing gas sensing capabilities and lifetime as well as in a plethora of systems in many energy sectors.
In the next chapter, the author will investigate novel nanocomposite membranes for NO₂ gas separation. These membranes will be synthesised from a polymer nanocomposite that incorporates 2D MoS₂ and PDMS as the base polymer.

3.5. Chapter Acknowledgments

I would like to acknowledge Dr. Majid Nour for helping with the FTIR, Dr. Cara Doherty for assisting with PALS Dr. Rajesh Ramanathan for assisting with XRD. I would also like to acknowledge the advice and guidance of Prof. Sandra Kentish, Dr. Anita Hill and Prof. Kourosh Kalantar-zadeh.

References


Chapter 4: 2D MoS\textsubscript{2} PDMS nanocomposites for NO\textsubscript{2} separation

4.1 Introduction

In the previous chapter, it was shown that the addition of 2D graphene to PDMS greatly impacted the permeability of gas molecules with little effect on gas selectivity. The large surface area and high surface to volume ratio that 2D materials possess enhances any effect the filler provides to the gas permeable membrane. This was indicated by the major enhancement in gas permeability shown in chapter 3. However, it is not only an increase in permeability that is desirable in gas permeable membranes, high gas selectivity is fundamentally required for many different gas separation applications and processes.

There is a continuous quest for discovering new applications for 2D TMDC. In this study, composite gas separating membranes are synthesised using PDMS and 2D MoS\textsubscript{2} and investigated for selected model gas species. Specifically, it is found that even at a relatively low 2D MoS\textsubscript{2} loading concentration (~0.02 wt\%), the composite membrane was able to almost completely block NO\textsubscript{2} gas permeation at ppm levels. This major reduction is ascribed to strong adsorption energy of NO\textsubscript{2} gas molecules to 2D MoS\textsubscript{2}. This chapter establishes a novel area of research for 2D MoS\textsubscript{2} based composite materials as the separation of NO\textsubscript{2} gas is critical in many industrial and farming processes.

The author investigates different solvents during the exfoliation of 2D MoS\textsubscript{2} and the subsequent effect on both the intrinsic gas adsorption properties as well as the
interaction with the polymer matrix. This study is conducted by looking at two of the most reported solvents for exfoliating MoS$_2$. The 2D MoS$_2$ is dispersed in the PDMS matrix and the alterations to the properties are characterised and the evaluation of the permeability of N$_2$, CH$_4$, and CO$_2$ gas molecules are examined. Finally a thorough investigation of the NO$_2$ gas separating properties is explored. As a byproduct from many industries, NO$_2$ removal is a significant field of research as it is hazardous to human health and environmentally destructive. The work in this chapter has been compiled and submitted to the journal Small [1].

4.2 Materials and Methods

4.2.1 Synthesis of 2D MoS$_2$ nanoflakes

One gram of MoS$_2$ powder (99% purity, Sigma Aldrich) was added to 0.5 mL of solvent: N-methylpyrrolidinone (NMP, 99% anhydrous, Sigma Aldrich) or 0.25 mL H$_2$O and 0.25 mL ethanol solution, in a mortar and ground with a pestle for 30 min. The mixture was then dispersed into 10 mL of the appropriate solvent (NMP or H$_2$O/ethanol solution). The slurry was then probe-sonicated (Ultrasonic Processor GEX500) for 120 min at 125 W sonication power and finally centrifuged for 45 min at 4000 rpm. The supernatant containing 2D MoS$_2$ nanoflakes was collected.

4.2.2 Synthesis of composite membranes

Nanocomposite membranes were fabricated using PDMS and a proprietary cross-linker (Sylgard 184, Dow Corning Corporation) to provide the base polymer. A reference PDMS membrane was made as well as two weight ratios of the two solvent exfoliated MoS$_2$ flakes. The supernatant containing the MoS$_2$ was dried in a vacuum oven for 72 h to remove the solvent. Para-xylene, was then added to the
dried flakes and the mixture was then probe-sonicated for another 90 minutes. The suspension was next added to 20 g and 40 g of the PDMS oligomer to create the two separate concentrations. This mixture was then mechanically stirred at 100 rpm on a hotplate at 120 °C for approximately 1 h to allow for the evaporation of the majority of the solvent. After cooling to room temperature the proprietary crosslinking agent was added and thoroughly mixed in. All membranes were prepared utilising a 10 wt% ratio of base PDMS to the crosslinker. The solution was degassed for 30 minutes before being spun onto a porous PAN support and cured at 75 °C. This method was chosen as it has been shown to maximise the gas permeability in pristine PDMS [2]. The membranes were then used for permeation measurements as well as structural and spectroscopic characterisation. The membrane thickness, as determined using scanning electron microscopy (SEM) imaging, lies within the 20 ± 3 µm range.

4.2.3 Characterisation methods

FEI Nova NanoSEM imaging was utilised to evaluate the cross-sectional thickness of the membranes as well as the distribution and morphology of the MoS₂ flakes into the PDMS matrix. Crystal structures were characterised using high resolution transmission electron microscopy (HRTEM) (JEOL 2100F) and Raman microscopy (Renishaw InVia Micro-Raman, 514 nm laser). The absorbance spectra of the nanocomposites were examined using a spectrophotometric system consisting of a Micropack DH-2000 UV-vis-NIR light source and an Ocean Optics HR4000 spectrometer.
4.2.4 Pure gas permeation experiments

A series of experiments was conducted to assess the gas permeability of the membranes. A constant pressure variable volume (CPVV) system was used to measure the permeability of CH₄, N₂, and CO₂ (99.99%, Core Gas Australia) through the PDMS and graphene-PDMS nanocomposite membranes. The membranes for testing were mounted within a permeation cell with a constant pressure on the upstream boundary, while the downstream side was kept at the atmospheric pressure. The pressures were tested at 200, 300 and 400 kPa to ensure repeatability. Both sides of the permeation cell were purged with the penetrant gas prior to each experiment. To maintain a constant temperature the permeation cell was housed in an environmental chamber with all measurements conducted at 37 °C. The membranes’ permeability was measured in the following order: CH₄, N₂, CO₂. The flow rate was acquired every ten seconds for two minutes once a steady-state was achieved and the values were averaged and converted into permeability values given in Barrers (1 Barrer = 1×10⁻¹⁰.cm³.(STP).cm/cm².s.cm.Hg).

4.2.5 NO₂ separation experiments

Spin-dependent Hybrid Density Functional Theory calculations were performed using Gaussian basis set ab initio package CRYSTAL14 [3]. The B3LYP hybrid exchange-correlation functional was used augmented with an empirical London-type correction to the energy to include dispersion contributions to the total energy [4]. The correction term is the one proposed by Grimme et al. [5] and has been successfully used with B3LYP to calculate cohesive energies in dispersion bonded molecular crystals [6]. For all atoms (other than Mo) a triple zeta valence (TZV) basis set, with polarisation functions, was used to model the electrons [7].
For Mo, a Hay-Wadt type effective core pseudopotential was used to account for the 28 core electrons (1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10}) and a 311-31G basis set for the valence electrons [8]. A periodic 5×5×1 slab of MoS₂ was used representing the MoS₂ surface. The NO₂ molecule was initially placed approximately 2.4 Å from the sulphur surface layer of MoS₂ and the molecule/slab configuration was optimised prior to calculating the molecule/slab binding energy. The molecule-surface binding energy of NO₂ on 5×5×1 MoS₂ was calculated using DFT (B3LYP) and the method described by Grimme to calculate the dispersion forces [5, 6]. The value of the binding energy of NO₂ onto the MoS₂ surface was −231 meV and the minimum separation distance between the NO₂ and the surface was 2.696 Å. The weak binding energy and large separation distance indicate that physisorption had occurred.

For the evaluation of the membranes using a sensing system, the experimental set-up shown in Figure 2.2 was used. Membranes were mounted within a permeation chamber on the front of a commercial electrochemical NO₂ (EC4-250-NO, e2v Technologies) sensor placed on the opposing side to the gas flow. The gas flow rate was controlled to 10 sccm of 10 ppm NO₂ in zero air balance via a mass flow control unit and pressure was maintained within the chamber at 110 kPa. Care was taken in the handling and use of NO₂ to avoid exposure to the toxic gas. The sensor results were converted into permeance values (Barrer) using the ppm/s gradient of the second phase of permeation. In order to test the electrical characteristics of exfoliated 2D MoS₂ nanoflakes upon their exposure to NO₂ gas, 10 µL of 2D MoS₂ nanoflake suspension was first drop-cast on to a LiNbO₃ substrate (10 × 10 mm) with pre-patterned platinum interdigitated electrodes (IDTs with 20 µm line/space). Each sample was then dried at 70°C for 24 h in the
ambient air in order to minimise the residual solvent content. After naturally cooling down to room temperature, each sample was placed into the Linkam gas testing chamber and its electrical resistance was measured using Keithley 2001 multimeter in situ before and after exposure of 10 ppm NO2 in zero air balance at the regulated flow rate of 200 sccm. The photoluminescence (PL) spectra of the membranes were obtained using a custom built unit with a 532 nm monochromatic CW laser at 200 µW of power and a 10× objective. A single sample of 10 × 10 mm was cut in two pieces. One half was exposed to 10 ppm of NO2 in zero air balance in a small permeation unit for 24 h and the PL spectra was obtained from 8 locations on the membrane and averaged, immediately after removal from the permeation cell. The other half of the sample was left in an ambient environment for 24 h, where the PL spectra was obtained from 8 locations on the membrane and averaged. This experiment was then repeated replacing the NO2 exposure with zero air.

4.3 Results and Discussion

4.3.3 2D MoS2 nanoflake and nanocomposite characterisation

In this chapter the 2D MoS2 flakes were prepared from MoS2 bulk powder using a grinding-assisted liquid phase exfoliation technique with the composites synthesised via in situ polymerisation method using N-methyl-2-pyrrolidone (NMP) and ethanol/water (EtOH/H2O) solution (details are presented in 4.2.1) [9]. For the MoS2 flakes exfoliated in NMP, the average lateral dimensions are approximately 53 nm and the average thickness ranges below 3 layers. For the flakes exfoliated in EtOH/H2O, the average lateral dimensions are approximately 55 nm and the average thickness ranges below 4 layers (Figure 4.1). High-resolution transmission electron microscopy (HRTEM) shown in Figure 4.2(a)
reveals the crystal structure with a lattice spacing of 0.27 nm assigned to the (100) set of planes [9].

Figure 4.1 2D MoS₂ nanoflakes characterisation (a) Distribution of MoS₂ nanoflakes exfoliated in NMP based on dynamic light scattering (DLS) assessment. (b) Distribution of MoS₂ nanoflakes exfoliated in NMP thickness taken from the analysis of 100 flakes using atomic force microscopy (AFM). (c) Distribution of MoS₂ nanoflakes exfoliated in EtOH/H₂O using DLS. (d) Distribution of MoS₂ nanoflakes exfoliated in EtOH/H₂O thickness taken from the analysis of 100 flakes using AFM.

Raman spectroscopy has been utilised to further investigate the crystal structure and thickness of the 2D MoS₂ flakes. From Figure 4.2(c), two distinct Raman shift peaks can be found at ~381 and ~408 cm⁻¹ for the MoS₂ bulk powder, corresponding to in-plane ($E_{2g}^i$) and vertical plane ($A_{1g}$) vibrations of Mo-S bonds in MoS₂, respectively. By normalising both the Raman spectra taken from the bulk powder and flakes with the $E_{2g}^i$ mode, it is found that the 2D flakes have a smaller Raman shift difference between $E_{2g}^i$ and $A_{1g}$ modes ($\Delta = \sim 20$ cm⁻¹ ) in
comparison with $\Delta = \sim 27 \text{ cm}^{-1}$ from their bulk counterpart. Using information provided by Li et al. [10], the Raman spectra indicate that the thicknesses of 2D MoS$_2$ flakes lie in between one to two layers, where this is further supported through AFM (Figure 4.1(b) and (d)).

Figure 4.2 (a) TEM image of an MoS$_2$ flake. (b) The Raman spectra of (x) MoS$_2$ exfoliated in NMP (y) MoS$_2$ exfoliated in EtOH/H$_2$O. (z) Bulk MoS$_2$. (c) Images of the polymer composites and (d) The effect of various solvents used for exfoliation of MoS$_2$ on the UV-Vis spectra of the nanocomposites showing the relative loading concentration of the different membranes. (i) MoS$_2$ exfoliated in NMP at a high concentration, (ii) MoS$_2$ exfoliated in NMP at a low concentration, (iii) MoS$_2$ exfoliated in EtOH/H$_2$O at a high concentration, (iv) MoS$_2$ exfoliated in EtOH/H$_2$O at a low concentration, (v) Pristine PDMS. (e) SEM image of MoS$_2$ dispersion and morphology in PDMS exfoliated in NMP. (f) SEM image of MoS$_2$ dispersion and morphology in PDMS exfoliated in EtOH/H$_2$O.

The UV-Vis absorbance spectra from the composite materials were used to analyse yield and concentration of distributed MoS$_2$ within the composite material (Figure 4.2(b)). The concentration of the membranes using the absorbance measurements as described by O’Neill et al.[11] indicate that the MoS$_2$-PDMS
composites where the flakes were exfoliated in NMP were approximately 0.011 and 0.021 wt%, while the composites where the flakes were exfoliated in an ethanol and water solution were 0.0051 and 0.01 wt% for the low and high concentrations respectively. This technique reveals that the low concentration of MoS$_2$ exfoliated in NMP shows almost the same as the high concentration of MoS$_2$ exfoliated in ethanol water solution. The peak at 490 nm is characteristic of PDMS, where the MoS$_2$ absorbance peaks are not present due to the small concentrations within the composites. However, the enhancement of this peak in the composites is due to the presence of 2D MoS$_2$. The inherent PDMS bonds are not affected by the low concentrations of MoS$_2$ added within the composite where only van der Waals forces are present between the oligomers and flakes themselves based on FTIR and Raman spectra of the composites (Figure 4.3).

![FTIR spectra](image1.png)
![Raman spectra](image2.png)

**Figure 4.3** (a) FTIR spectra comparing pristine PDMS with the MoS$_2$-PDMS nanocomposites (b) Raman comparing pristine PDMS with the MoS$_2$-PDMS nanocomposites
The pure gas permeation rates of the pristine PDMS and composite MoS$_2$-PDMS membranes were investigated under exposure to CO$_2$, N$_2$, and CH$_4$ (99.99%) using the CPVV experimental setup described in the Materials and Methods section.

**Figure 4.4** (a) Pure gas steady state permeation results for composites with MoS$_2$ exfoliated in NMP. (b) NO$_2$ steady state permeation for PDMS and MoS$_2$-PDMS nanocomposites, where: i-PDMS membrane; ii-MoS$_2$-PDMS (exf. EtOH/H$_2$O low concentration); iii-MoS$_2$-PDMS (exf. EtOH/H$_2$O high concentration); iv-MoS$_2$-PDMS (exf. NMP low concentration); v-MoS$_2$-PDMS (exf. NMP high concentration) (c) NO$_2$ permeation sensor results: PDMS and MoS$_2$-PDMS nanocomposites (d) Long NO$_2$ permeation sensor results through MoS$_2$-PDMS (exf. NMP high concentration) (e) MoS$_2$ resistance before, during and after NO$_2$ exposure.

As can be seen in Figure 4.4(a), the permeability of the pristine PDMS membrane is similar and correspond to the permeability found by Merkel et al. that also used
a CPVV experimental set up [12]. A key aspect of these results shows that the addition of MoS\(_2\) at low concentrations yields no significant penalty to the permeation of CH\(_4\) and N\(_2\). However, permeation of CO\(_2\) is significantly decreased with the addition of MoS\(_2\) into the PDMS matrix with permeability being essentially inversely proportional to concentration. While the concentration of MoS\(_2\) flakes within the MoS\(_2\)-PDMS composite membranes did not affect the permeation of CH\(_4\) or N\(_2\) it did significantly decrease the permeation of CO\(_2\) gas molecules likely due to its calculated higher adsorption energy to single layer MoS\(_2\) [13].

The experimental method demonstrated by Nour et al. for H\(_2\)S separation was employed for the NO\(_2\) separation experiments (see Materials and Methods Section) [14]. As seen in the state and dynamic responses of the NO\(_2\) permeation shown in Figure 4.4(b-d) there was a major effect on the NO\(_2\) gas permeation through the addition of 2D MoS\(_2\) flakes. The change in NO\(_2\) permeation kinetics through analysis of the dynamic response curves can be divided into three phases. The first phase, represented by the delay in the sensor response curve reflects the gas molecules solubility; this is often referred to as the time lag method. Pristine PDMS shows a much faster NO\(_2\) sensor response with a delay of less than 100 s compared to approximately 400 s for the lower concentration composite membranes. This indicates a decreasing NO\(_2\) solubility for the nanocomposite membranes. It was noted that as the concentration of MoS\(_2\) flakes increased, the sensor delay time was prolonged, indicating a decrease in NO\(_2\) solubility.

In the second phase, the gradient of the sensor response is used to give an indication of the NO\(_2\) gas molecules diffusivity through the composite membranes. It can be seen that the NO\(_2\) molecules permeation kinetics, at this
stage, were strongly dependent on the MoS$_2$ concentration in the membrane, in which higher MoS$_2$ concentrations results in lower diffusion. At the highest concentration of MoS$_2$ the permeation of NO$_2$ gas molecules is almost totally prevented. Although it has been shown that NMP residue is retained on the surface of the flakes after exfoliation if the drying process does not exceed 200 °C [9, 15]. However, it appears that NO$_2$ adsorption is not affected by NMP present on the flake surface and therefore diffusion is not dependent on the exfoliation solvent (Figure 4.4(b,c)).

Finally, the third phase is the membrane’s operation once the MoS$_2$ surface is saturated with NO$_2$ molecules. For the highest concentration of MoS$_2$ in PDMS, this stage is reached after ~12 h (Figure 4.4(d)). Calculations can be made using the permeation of NO$_2$ through pristine PDMS to assess the number of NO$_2$ molecules that have been adsorbed onto MoS$_2$ flakes in the nanocomposite membrane during this experiment. Using the average flake dimensions (3 layers and 53 nm lateral dimensions) it can be calculated that on average one NO$_2$ molecule is adsorbed to every ~110 Mo atoms assuming that NO$_2$ can intercalate and adsorb in the interlayer, or every ~75 Mo atoms assuming that NO$_2$ can only adsorb onto the basal surfaces of MoS$_2$.

It may also be possible that NO$_2$ intercalates into MoS$_2$ layers at room temperature [16]. NO$_2$ gas molecule has a relatively low highest occupied molecular orbitals (HOMO) energy of $-7.6$ eV [17], which lies under that of MoS$_2$ potentially promoting the insertion of this gas in between MoS$_2$ layers. Weiss and Phillips have calculated the interlayer binding energy in MoS$_2$ to be approximately $-520$ erg/cm$^2$, which corresponds to $-0.0324$ eV/Å$^2$. This is
associated to \(-0.28\) eV per NO\(_2\) molecule adsorption site [18]. We calculate the physisorption binding energy for NO\(_2\) onto MoS\(_2\) surface to be approximately \(-0.23\) eV per NO\(_2\) molecule (see Materials and Methods for detail), therefore we believe it is energetically feasible for NO\(_2\) to insert and immobilise between the van der Waals bonded MoS\(_2\) layers at near room temperature.

Since higher adsorption energy gives rise to a stronger binding between the adsorbate and the host, we can see stronger interaction between NO\(_2\) (absolute value (av) of 230-245 meV) and CO\(_2\) (av 205 meV) gas molecules with MoS\(_2\) monolayers compared to N\(_2\) (av 137 meV) and CH\(_4\) (av 140 meV) [13]. This is especially true when a van der Waals interaction for the weakly bonded gas adsorption system is considered. In reality, 2D MoS\(_2\) does not show any response to the largely inert CO\(_2\) gas (our measurements – not shown). As such, it can be inferred that even in the presence of such gas species, NO\(_2\) will be the dominant gas molecule to adsorb onto the surface of MoS\(_2\). It is important to consider that NH\(_3\) and SO\(_2\) are the other two gas species which show some physisorption response to MoS\(_2\) at lower adsorption energies hence less strong surface adsorption [13, 19]. However, there have been no thorough experimental studies regarding measurements of the adsorption energies of gas molecules on MoS\(_2\) monolayers, thus the majority of discussions on such energies are based on first principle calculations [13, 19].

\[
E_{ad} = E_{Gas+MoS_2} - E_{MoS_2} - E_{Gas} \tag{6}
\]
Zhao *et al.* calculated that the highest adsorption energy to MoS$_2$ was held by NO$_2$ (Equation 6) in correspondence with our results [13]. This strong adsorption energy is apparent in Figure 4.4(e), during exposure to NO$_2$ gas molecules which act as electron acceptors from the MoS$_2$ causing the resistance of the flake to increase. However, after exposure, there is no sign of recovery indicating that the NO$_2$ gas molecules are still adsorbed on the surface. It is suggested that the NO$_2$ gas molecules adsorbed (as schematically shown in Figure 4.5(a) on the surface of the MoS$_2$ act as p-type dopants (electron acceptors) [20, 21]. Theoretically this should cause an increase in the PL intensity; however, as can be seen in Figure 4.5(b) and (c), PL quenching is observed after NO$_2$ adsorption. This could possibly be related to the effects seen by nonuniform doping profiles due to the induced defects during the exfoliation process of the flakes that may play a role in suppressing exciton formation and/or its radiative recombination [22, 23]. It is interesting to see that the intensity decreases by over 60% for the composite containing MoS$_2$ exfoliated in EtOH/H$_2$O, while the quenching of PL intensity only occurs by 35% for composites containing MoS$_2$ exfoliated in NMP. This is most likely associated with the residual NMP on the surface as previously discussed. The same experiment was run exposing the composites to ‘dry air’ as a control where a minor decrease in PL intensity is seen, approximately 15% (Figure 4.6).
Remarkably, at a relatively low loading concentration of 0.021 wt% the MoS$_2$-PDMS composite membrane was able to completely block NO$_2$ gas permeation likely due to strong adsorption energy as depicted in Figure 4.5(a). The PL results suggest that these composite membranes could be implemented into a device capable of not only separating NO$_2$ from gas streams but also monitoring the concentration of NO$_2$ if combined with a PL unit, offering dual functionality.
Figure 4.6 Photoluminescence spectra of: (a) MoS$_2$-PDMS composites before and after exposure to ‘zero air’ and (b) MoS$_2$-PDMS composites before and after exposure to NO$_2$.

4.4 Summary

In this chapter, the author has demonstrated that the addition of 2D MoS$_2$ to PDMS, to make a novel nanocomposite material, has a dramatic effect on the gas permeation properties for some gas species while not affecting others. This is due the varied adsorption energy that the different gas species have to 2D MoS$_2$ nanoflakes.

The author of this thesis successfully demonstrated NO$_2$ separation through MoS$_2$-PDMS nanocomposite membranes. The adsorption of NO$_2$ molecules onto the surface of the embedded 2D MoS$_2$ flakes allows for efficient separation of low concentrations of NO$_2$ from gas streams. This chapter has shown that the permeation of NO$_2$ is almost completely stopped at ppm concentrations as well as a 60% decrease in pure CO$_2$ permeability. Furthermore, the author demonstrated that the solvent the flakes are exfoliated in is of minor consequence and yield is
the driving factor for solvent choice. As such, the presented investigation shows a
unique capability for 2D MoS$_2$ with potential research and industrial applications.
It is important to note that while other 2D materials, such as graphene (shown in
chapter 3) [24-26], have been extensively investigated for their gas separation
properties, 2D MoS$_2$ with its multifaceted characteristics, originating from its
basal surfaces and prismatic edges, can potentially offer significantly different
possibilities for gas separation research.

In the next chapter, the author will look to increase longevity of PDMS
nanocomposites used in aqueous and humid environments by exploring the
antimicrobial qualities that Ag nanoparticles can provide. Furthermore as
established in chapter 1 the many standard $in$ vivo and $in$ vitro tests developed for
studying bacterial growth are limited and often ineffective. The author will
establish and evaluate a new $in$ vivo experimental technique for evaluating
microbial growth and biofilm formation. This technique involves utilising a
fistulated ruminant and using the rumen as a ‘live laboratory’ for growing diverse
communities of microorganisms.

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References


Chapter 5: A unique *in vivo* approach for investigating antimicrobial materials utilising fistulated animals

5.1 Introduction

In chapters 2, 3 and 4 membranes were synthesised and evaluated for gas permeability and gas selectivity. However, generally the use of these membranes for many applications occurs in aqueous and humid environments containing biological contaminants. Therefore the ability of these membranes to resist microbial colonisation is essential for operational longevity. In this chapter, the author looks to employ Ag nanoparticles as the antimicrobial filler within PDMS based nanocomposites.

The antimicrobial effects of Ag nanoparticles have been well established [1]. However, no in depth investigations have been carried out on the antimicrobial effects of Ag-PDMS nanocomposites. Furthermore the author, with the contribution of microbiologists and agricultural biologists, establishes a novel *in vivo* experimental technique that has many advantages over more traditional methods of testing the antimicrobial materials.

Unique *in vivo* tests are conducted through the use of a fistulated ruminant, providing an ideal environment with a diverse and vibrant microbial community. Utilising such a procedure can be especially invaluable for investigating the performance of antimicrobial materials related to human and animal related infections. In this chapter, it is shown that the rumen of a fistualted animal provides an excellent live laboratory for assessing the properties of antimicrobial
materials. In this chapter the author of the thesis investigated microbial colonisation onto model nanocomposites based on Ag nanoparticles at different concentrations into PDMS. With implantable devices posing a major risk for hospital-acquired infections, the present chapter provides a viable solution to understand microbial colonisation with the potential to reduce the incidence of infection through the introduction of Ag nanoparticles at the optimum concentrations. *In vitro* measurements are also conducted to show the validity of the approach. An optimal loading of 0.25 wt% Ag is found to show the greatest antimicrobial activity and observed through the *in vivo* tests to reduce the microbial diversity colonising the surface.

The aim of this chapter is to test the concept of *in vivo* testing of the model Ag-PDMS nanocomposite materials for their antimicrobial viability. This material is subjected to an *in vivo* biological environment in the rumen of a fistulated steer in order to evaluate the growth and diversity of the microbial community able to colonise on its surface. The antimicrobial effects and mechanisms that come from the introduction of Ag nanoparticles into the PDMS matrix are tested *in vitro* to validate the results from the unique *in vivo* experiment. The content of this chapter was published as a full article in the journal Scientific Reports [2].

5.2 Materials and Methods

5.2.1 Materials and Nanocomposite Preparation

The Ag-PDMS nanocomposite material was fabricated from the base polymer of PDMS (Sylgard 184, Dow Corning corporation) and with the addition of an Ag nanopowder (Sigma-Aldrich Pty Ltd) with a particle size of less than 100 nm, containing polyvinylpyrrolidone (PVP) as dispersant. A pristine PDMS membrane (control) and three different Ag-PDMS nanocomposites with Ag
nanoparticle weight percentages of 0.25%, 0.5% and 1%, were synthesised. To facilitate the homogenous dispersion of Ag nanoparticles within the polymer, p-xylene was added to the PDMS elastomer while the mixture was mechanically stirred before sonication using a high intensity ultrasonic probe at 100 W for 1 h. To prevent the agglomeration of the nanoparticles, rapid precipitation was performed in a methanol bath while being magnetically stirred. The proprietary PDMS crosslinking agent was then mixed into the nanocomposite at a ratio of 10:1 (base: crosslinking agent) and then placed in a vacuum for 30 min to de-gas. Finally, the composite mixture was spun onto silicon wafers and placed in a 75 °C oven to crosslink for 40 minutes to provide smooth, defect free films.

5.2.2 Nanocomposite Characterisation

The control PDMS and Ag-PDMS nanocomposite materials were characterised through vibrational spectroscopy. The Micro-Raman spectra of the nanocomposites were obtained utilising a Renishaw Raman spectrometer at 633 nm wavelength. The Fourier transform infrared (FTIR) spectroscopy of the PDMS and Ag-PDMS nanocomposites was recorded using a Thermo Nicolet 6700 spectrophotometer. Atomic force microscopy (AFM) was used to determine the Ag nanoparticle size distribution after dispersion into the PDMS matrix using a Bruker D3100 in tapping mode on the surface of the material. 200 particles present at the surface were assessed to obtain the distribution. A hydrostatic weighing method was used for determining the density of the PDMS and nanocomposites. The samples were weighed in air ($M_A$) and then in an auxiliary liquid ($M_L$) (ethanol in this case) and finally the PDMS and nanocomposite membrane density ($\rho_p$) was calculated using Equation (1).
Considering the relevant densities, the composites theoretical density ($\rho_{\text{theory}}$) can be calculated using Equation (3):

### 5.2.3 In Vitro Measurements

#### 5.2.3.1 Ag$^+$ Ion Leaching

Leaching of Ag$^+$ ions from Ag-PDMS was determined by immersing the films in deionized water (pH 6.2) for 1 month at room temperature, and analysing the supernatant using an inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer Optima 4300 DV).

#### 5.2.3.2 Catalysis

The catalytic ability of the Ag nanoparticles embedded within the PDMS films were performed by studying the model reaction involving metal-induced reduction of ferricyanide by thiosulfate ions. The catalysis experiments were performed by immersing equally sized Ag-PDMS films in an aqueous solution (10 mL) containing 0.1 M thiosulphate and 1 mM potassium ferricyanide. The reaction was held at 20 ± 2 °C with continuous stirring at 200 rpm. The conversion of ferricyanide to ferrocyanide was analysed by UV-vis absorbance spectroscopy (Cary 50 Bio spectrophotometer) in a cell of 1 cm path length by taking aliquots from the reaction.

X-ray diffraction (XRD) data was collected on a D8 Advance Bruker AXS X-ray diffractometer with GADDS. X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Scientific K-alpha instrument with an Al Kα source.

#### 5.2.3.3 Hydrophobicity

Water contact angle measurements were performed using a KSV 101 system. The height of each drop was confirmed using a CCD camera prior to each
measurement to ensure consistency in the drop volume. Drop volumes of approximately 8 μL were employed.

5.2.4 In Vitro Bacterial Growth Assay

5.2.4.1 Culturing Procedure

Luria-Bertani (LB) broth powder (US Biological) (100 mL) was prepared as instructed and sterilised by autoclaving at 121°C for 40 min. An aliquot (10 mL) of the LB broth was decanted and used to culture a stock solution of *E. coli* (ATCC strain, Sigma Aldrich), where the culture was incubated for 12h at 37 °C with rotation and stored at 4 °C. The control PDMS and the nanocomposites with varying Ag concentrations of 0.25, 0.5 and 1 wt%, were first sterilised using UV light, for 30 mins on each side in a glass Petri dish. Each membrane was then transferred into a vial containing 5 mL of LB broth to give 5 vials with membranes and 1 vial without, as a control. Each vial was then seeded with 0.5 μL of the stock *E. coli* solution and incubated with rotation at 37 °C for 5 h.

5.2.4.2 Measurement Procedure

UV-vis (600 nm) was performed on 4 mL aliquots of the broth after incubation on a Varian Cary 50 Bio UV-Vis Spectrophotometer. Quantitative fluorescence microscopy was performed on the pristine PDMS (control), 0.25 and 1 wt% nanocomposites. Two fluorescent dyes were used in combination: SYTO9 (Invitrogen AG, Basel, Switzerland), and PI (Invitrogen). Stock solutions from the LIVE/DEAD BacLight kit (Invitrogen) were prepared as instructed by the manufacturer. Samples were incubated in the dark at room temperature for 25 min before analysis. Cell counts and area coverage were calculated using ImageJ software.
5.2.5 In Vivo Bacterial Growth Assay

5.2.5.1 Experimental Procedure

All experiments involving ruminants were undertaken within the procedure outlined and approved by the CSIRO FD McMaster Laboratory Chiswick Animal Ethics Committee. The control PDMS and the Ag-PDMS nanocomposites that were synthesised were cut into $2 \times 2$ cm squares. Twenty squares of each type of material, PDMS control, 0.25 wt% Ag-PDMS, 0.5 wt% Ag-PDMS and 1 wt% Ag-PDMS nanocomposites, were made and labeled.

One of each Ag concentration square was placed into a nylon mesh bag, measuring $10 \times 24$ cm, and each sewn into place using a 6 pound KATO fishing braid and superfine needle to keep them separated during the experiment and prevent occlusion of the surface area to the rumen fluid. Once all 20 bags were finished (80 membranes in total), 4 bags were selected at random and tied together. Each group of bags was bound together with 0.8mm fishing line, with a 200g brass weight attached to approximately 20 cm length of line. There were 5 groups of 4 bags created in total, each weighted, tied together and placed into the rumen of a fistulated 3 year old Jersey steer. Each group of bags was retrieved from the steer at successive time intervals. The first was removed 4, 7, 14, 21 and 28 days. The steer grazed on native pasture throughout the experiment, plus ~500 g of lucerne pellets during retrieval of each bag.

Once a randomly selected group of 4 bags was removed from the steer the PDMS and nanocomposite material was rinsed before each being cut into two. Half of each square was placed into jars, one containing glutaraldehyde for electron microscopy studies and the other into a phosphate buffered saline (PBS) solution for denaturing gradient gel electrophoresis (DGGE) and polymerase chain
reaction (PCR) analysis. The glutaraldehyde jars were stored at room temperature while the PBS Jars were stored in a freezer at -20 °C.

5.2.5.2 Electron Microscopy

Once removed from the glutaraldehyde, each square went through a series of ethanol rinses slowly increasing the concentration of ethanol up to 100%. The samples were then dried in a critical point dryer before being coated with a thin layer of gold (2.5 nm). An FEI Nova NanoSEM was employed to evaluate the morphologies and relative surface growth on the control PDMS and nanocomposite materials.

5.2.5.3 DNA Extraction and Purification

DNA was extracted from duplicate control (pristine PDMS, 0 wt% Ag nanoparticle content) and test membranes (0.25 wt%, 0.5 wt% and 1 wt% Ag nanoparticle content) retrieved from the rumen of fistulated animals over 28 days using a phenol-chloroform-isoamyl alcohol bead beating method. Each membrane was added to a 2 mL Eppendorf tube which contained 0.5 mL of Tris equilibrated phenol-chloroform-isoamyl alcohol (24:24:1), 0.5 mL of sterile phosphate buffer (100 mM, pH 8) and 0.5 g of sterile glass beads (150-212 µm; Sigma-Aldrich, Castle Hill, NSW, Australia). The mixture was then subjected to bead beating using a mini-bead beater K9, (Biospec, USA) for 30 s twice, stored on ice in-between the bead beating sessions before being centrifuged at 10,000 × g for 10 min. The aqueous layer was then aseptically removed into a fresh sterile Eppendorf tube and an equal volume of phenol-chloroform-isoamyl alcohol added to it, followed by a quick vortex and centrifuging at 10,000 × g for 10 min. This process was repeated twice to obtain the crude DNA of microorganisms. The
crude DNA was purified using GENECLEAN<sup>R</sup> Turbo kit (MP Biomedicals, USA). Three hundred microliters of crude DNA was added to a sterile Eppendorf tube to which 5 × volume of Gmomic Turbo solution was added. The mixture was vortexed for 5 s after which 600 µL of this mixture was added to GENECLEAN filter and centrifuged at 10,000 × g for 5 s and the flow through discarded. The remaining manufacturer protocol was then followed.

5.2.5.4 PCR and DGGE

Universal eubacterial primers 341FGC and 518R (Muyzer <i>et al.</i>, 1993) were used for PCR assay of purified DNA obtained from control and Ag nanoparticle impregnated membranes. The thermocycling program used was: 1 cycle at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s and a final extension at 72 °C for 10 min. PCR amplicons were analysed on a Universal Mutation Detection System D-Code apparatus (BioRad, CA, USA) with a 9% acrylamide gel. The denaturing gradient used was 40-60% and the DGGE gel was run for 20 h at 60 V and at 60 °C. The DGGE gels were Ag stained (Girvan <i>et al.</i>, 2003), scanned and saved as TIFF files. For ease of analysis, cumulative DGGE gels were prepared and loaded with an equal mixture of duplicate samples per DGGE lane.

5.2.5.5 Statistical Analysis

The digitised images were analysed with TL 120 D advance analysis package (Totallab, U.K.) for similarity relationships and diversity values. The relatedness of the microbial community on the control and Ag nanoparticle impregnated membranes was expressed as similarity clusters using the unweighted paired group method with mathematical averages (UPGMA). The microbial community
diversity of the membrane samples was evaluated with Shannon Weaver diversity (\(H'\)) index using the equation [3]:

\[
H' = -\sum p_i \times \ln p_i
\]  

(7)

where \(p_i\) is the proportion of the community that is made of species \(i\) (intensity of the band \(i\)/total intensity of all bands in the lane) and \(\ln p_i\) is the natural log of \(p_i\).

Principal component analyses were carried out on the matrix data obtained from DGGE profiles using SPSS version 21 software.

5.3 Results

5.3.1 Nanocomposite Characterisation

The Ag-PDMS nanocomposite synthesis process is presented in Materials and Methods section. Leaching of Ag\(^+\) out of the polymeric matrix, the catalytic properties of the Ag nanoparticles in the nanocomposites and the overall antibacterial properties of the nanocomposites using a fluorescence assay are assessed. Ag\(^+\) ions have long been known to exhibit high inhibitory and bactericidal effects as well as showing broad antibacterial activity while exhibiting low toxicity towards mammalian cells making Ag an ideal antimicrobial material for biomedical and implantable devices[4-6]. However, it is not only the Ag\(^+\) ions that have shown to have an antibacterial effect. The Ag nanoparticles themselves use multiple approaches, acting as catalysts for effective antibacterial activity; lysing of the bacterial membrane, denaturing proteins, terminating metabolic enzymes and disrupting bacterial division and proliferation [5, 7].

XRD assessments and vibrational spectroscopy was performed to understand the physiochemical changes to the nanocomposite and presented in. AFM of the membranes demonstrate that the best dispersions are obtained for 0.25 wt% Ag-
PDMS nanocomposite with some agglomerations are seen for higher concentrations of Ag (Figure 5.1(b) to (d)).

![Figure 5.1 In Vivo experiment (a) Fistulated ruminant showing the cannula into the rumen. Particle size distribution of (b) 0.125 wt% Ag-PDMS, (c) 0.25 wt% Ag-PDMS, (d) 0.5 wt% Ag-PDMS and (e) 1 wt% Ag-PDMS based from AFM analysis of material surface.](image)

Figure 5.1 In Vivo experiment (a) Fistulated ruminant showing the cannula into the rumen. Particle size distribution of (b) 0.125 wt% Ag-PDMS, (c) 0.25 wt% Ag-PDMS, (d) 0.5 wt% Ag-PDMS and (e) 1 wt% Ag-PDMS based from AFM analysis of material surface.

XRD patterns of the pristine PDMS and the Ag-PDMS nanocomposites shown in Figure 5.2 demonstrate very little to no change in the polymeric structure with the addition of Ag nanoparticles. This was also observed in the Raman and FTIR (Figure 5.3) spectra, where the only significant difference seen in material bonding characterisation methods is the addition of PVP, the dispersant coating of the Ag nanoparticles.
This was also seen when looking at the surface hydrophobicity of the nanocomposites, where no observable change was detected with the addition Ag nanoparticles (Table 5.1). As a result, the change in hydrophobicity does not play a significant role in the adherence of bacteria to the surface of the control or nanocomposites in the *in vitro* and *in vivo* characterisations that will be presented later.

Vibrational spectroscopy was employed to investigate the changes in the chemical bonding formed in pristine PDMS compared to the nanocomposite material. This study was performed utilising both micro-Raman and Fourier transform infrared (FTIR) spectroscopy.
Table 5.1 Water droplet contact angle of pristine PDMS and Ag-PDMS nanocomposites

<table>
<thead>
<tr>
<th>Material</th>
<th>Contact Angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>114.2 ± 2.6</td>
</tr>
<tr>
<td>0.125 wt% Ag-PDMS</td>
<td>113.3 ± 3.5</td>
</tr>
<tr>
<td>0.25 wt% Ag-PDMS</td>
<td>112.7 ± 4.2</td>
</tr>
<tr>
<td>0.5 wt% Ag-PDMS</td>
<td>112.3 ± 1.4</td>
</tr>
<tr>
<td>1 wt% Ag-PDMS</td>
<td>113.3 ± 2.1</td>
</tr>
</tbody>
</table>

The micro-Raman spectra of pristine PDMS and the Ag-PDMS nanocomposites are presented in Figure 5.3(a) with all PDMS peaks complying with previous studies implementing this material [8-10]. The addition of Ag nanoparticles into the PDMS polymeric matrix has resulted in a decrease in the intensity of all the standard PDMS peaks. This diminishing nature is to be expected with the ‘darkening’ of the material as the Ag concentration increases. A key change found in the Raman spectra occurs at 1567 cm\(^{-1}\), which can be ascribed to the C–C stretching bond that does not occur in the pristine PDMS spectrum. This bond can be attributed to the PVP coating that surrounds the Ag nanoparticles. There are no other major chemical bonding changes that occur within the PDMS matrix with the addition of Ag nanoparticles. This is also confirmed though XRD analysis (Figure 5.2).

The FTIR spectra of pristine PDMS and the Ag-PDMS nanocomposites materials are shown in Figure 5.2(b) with PDMS peaks complying with those typically reported [9-11]. The notable difference between pristine PDMS and the Ag-PDMS nanocomposites’ spectra is apparent at 1414 and 1450 cm\(^{-1}\), which can be
ascribed to C=O and C–C ring stretching bonds respectively [12, 13]. These bond intensity changes can be associated with the increase in the interaction between the dispersant PVP coating with the PDMS oligomer that can be associated with an altering the crosslinking structure [14]. Interestingly, both peaks are the most prominent at the concentration of 0.25 wt% Ag-PDMS. This can be attributed to an optimal dispersion found at 0.25 wt% where larger concentrations of Ag nanoparticles result in agglomeration, effectively reducing the interaction between the PVP and PDMS.

Figure 5.3 Changes in structural characteristics with the addition of Ag nanoparticles through vibrational spectroscopy (a) Raman spectra of the pristine PDMS and Ag-PDMS nanocomposites. Inset: Comparison of C–C stretching bond. (b) FTIR spectra of the pristine PDMS and Ag-PDMS nanocomposites. Inset: Comparison of C=O and C–C ring stretching bonds.
5.3.2 In Vitro Measurements

A series of *in vitro* characterisations were conducted to assess the antibacterial performance of the nanoscomposite membranes.

5.3.2.1 Ag⁺ Ion Leaching

Assessing the leaching of Ag⁺ ions from the nanocomposites can give an indication of their relative antimicrobial properties as they are known to be responsible for reducing microbial colonisation. Figure 5.4(a) shows the release of Ag⁺ ions from the different nanocomposite materials. As can be seen, the 0.25 wt% Ag-PDMS releases the highest concentration of Ag⁺ ions from the material and leaches approximately 60% more Ag⁺ ions than the 1 wt% nanocomposite containing four times more Ag nanoparticles within the polymeric matrix. To understand the source of leaching difference, FFV within the polymeric matrices were assessed. A change in density (Figure 5.4(b)) as a function of the Ag concentration and comparison to theoretical density in the nanocomposites was employed for evaluating the FFV within the polymer. As can be seen in Figure 5.4(b) the 0.25 wt% Ag-PDMS has the lowest density. The lowest relative density and therefore highest relative FFV allowing for more efficient transportation of Ag⁺ ions through the polymer matrix at this concentration.

It is known that a relatively high change in FFV is an indication of better nanoparticle dispersion when nanoparticles are incorporated without any significant surface modification. An increase in agglomeration of particles causes a lower surface to volume ratio, forming fewer voids between the polymer chains and particles [15]. When comparing to the theoretical density (Figure 5.4(b)), it can be seen that this case is applicable for indicating the agglomeration effect of Ag nanoparticles for concentrations above 0.25 wt%. More agglomeration means
a less relaxed and homogenously porous polymeric matrix, inhibiting ionic release from the nanoparticle cores through the polymeric matrix and into the surrounding environment. At 0.25 wt% an optimal loading of Ag nanoparticles within the polymer matrix is found to give rise to the most successful dispersion of the particles.

**Figure 5.4** Leaching, density and catalysis experiments (a) Leached Ag\(^+\) ion concentration after one month and (b) change in density from different concentrations of Ag-PDMS nanocomposites. (c) Catalysis reaction data of 0.125 wt% Ag-PDMS (d) Catalysis reaction data of 0.25 wt% Ag-PDMS (e) Catalysis reaction data of 0.5 wt% Ag-PDMS (f) Catalysis reaction data of 1 wt% Ag-PDMS (g) Catalysis reaction Ln plots showing rate kinetics in presence of different Ag-PDMS nanocomposites.
5.3.2.2 Catalysis

Evaluation of the catalytic activities of different concentrations of Ag loading within the polymer matrix highlights two separate time frames of interest (Figure 5.4(c) to (f)). The first lies at the steady state value in the linear range between 60 and 160 min where 0.25 wt% Ag-PDMS shows the highest reaction rate, whereas after this time the 0.5 and 1 wt% show a sudden increase in reaction rate. This suggests that in 0.25 wt% Ag-PDMS nanocomposite, more Ag nanoparticles are initially accessible to the reaction solution due to their uniform distribution within the matrix and the better porosity of the membranes, allowing the reaction to occur at a steady rate. This steady rate is maintained over longer time periods, most likely due to a sustained Ag leaching profile. Conversely, in higher Ag-loaded nanocomposites reaction rates are initially lower than that in 0.25 wt% Ag-PDMS nanocomposite, followed by a notable sharp increase in catalytic activity after 160 min. This suggests that in 0.5 and 1 wt%, Ag nanoparticles are originally present as inaccessible clustered aggregates leading to lower activity initially, however once these clusters start to become more accessible they are able to react strongly to the penetrate. The overall initial lower reaction rate of the 1 wt% Ag-PDMS compared to the 0.5 wt% Ag-PDMS further affirms the influence of nanoparticle clustering on reaction rates. The increased dispersion coupled with an increase in Ag⁺ ion leaching from the 0.25 wt% Ag-PDMS causes this major difference in activity between the 0.25 wt% and the higher concentrations of Ag-PDMS nanocomposites.

5.3.3 In Vitro Bacterial Growth Fluorescence Assay

In order to assess the antibacterial properties of the Ag-PDMS nanocomposites, as a combination of both catalytic and ion leaching activities, an in vitro bacterial
growth test was carried out. This was done to evaluate cell adherence and viability on the surface of the nanocomposite materials. A fluorescence assay was utilised to quantify cell adherence and assess cell viability. As can be seen from both Figure 5.5 and Table 5.2 the addition of Ag into the polymeric matrix significantly reduced cell adherence to the surface of the material, with four to five times the number of cells found on the surface of the control PDMS compared to the surface of the nanocomposite. The well-known Ag bactericidal effect appears to have a substantial adverse effect on the viability of the cells that have attached to the surface, with the addition of Ag significantly reducing the ratio of live to dead cells. It is very interesting to note that the 0.25 \( \text{wt}\% \) Ag-PDMS nanocomposite (Figure 5.5(b)) shows a higher percentage of dead cells to live cells than the 1 \( \text{wt}\% \) Ag-PDMS (Figure 5.5(c)).

Table 5.2 Quantitative fluorescence study of the surface of the nanocomposites.

<table>
<thead>
<tr>
<th>Material</th>
<th>Live surface area coverage (%)</th>
<th>Dead surface area coverage (%)</th>
<th>Live/Dead cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMS</td>
<td>22.5 ± 2.1</td>
<td>1 ± 0.6</td>
<td>6.2</td>
</tr>
<tr>
<td>0.25 ( \text{wt}% )</td>
<td>2.1 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>1 ( \text{wt}% )</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

UV-Vis was performed on the media used in the growth tests to understand any inhibiting factors for proliferation and growth within the medium surrounding the material (Figure 5.5(d)). The addition of Ag in the nanocomposites has significantly reduced the overall growth of \( E. \ coli \) from the surrounding medium implying that the inhibiting effect of Ag not only influences those in direct contact with the surface of the material but all cells within close proximity. This suggests the leaching of \( \text{Ag}^+ \) ion leaching is the major antimicrobial mechanism.
Figure 5.5 *In vitro* study. Fluorescent images of bacterial surface growth with live cells (green) and dead cells (red) on the surface of: (a) PDMS; (b) 0.25 wt% Ag-PDMS; (c) 1 wt% Ag-PDMS. (d) UV-Vis absorbance measurements of *E. coli* growth in broth containing the different concentration of Ag-PDMS nanocomposites.

5.3.4 *In Vivo* Microbial Growth and Diversity Assays

The effects of different wt% concentrations of Ag nanoparticle impregnation on the microbial communities growing on the surface of the intra-ruminal materials were assessed over 28 days with a SEM study and DGGE analysis. The SEM images shown in Figure 5.6 were taken to evaluate the relative surface coverage as well as colony morphology for the different wt% concentrations of Ag impregnation. The images shown are representative of the average coverage seen in the many images taken. Any Ag nanoparticle impregnation has visually reduced the surface coverage and the size of the microbial colonies, with the images of the control PDMS showing much larger colonies than the others. The
images suggest an inhibition of the microbial growth rate and therefore the size of colonies that are formed. Total surface coverage is achieved by the microbial growth on the reference PDMS after 21 days (Figure 5.6(g)). When comparing surface coverage and colony size of microorganisms on membranes with Ag nanoparticles, a substantial difference can be seen between the two concentrations shown. The 0.25 wt% Ag-PDMS shows far less microbial surface coverage than the material that contains four times more Ag implying an optimal loading of Ag in the PDMS matrix that allows for the maximum antimicrobial effect.

**Figure 5.6** SEM images of microbial surface growth from *in vivo* study: (a) PDMS at 4 days; (b) 0.25 wt% Ag-PDMS at 4 days; (c) 1 wt% Ag-PDMS at 4 days; (d) PDMS at 14 days; (e) 0.25 wt% Ag-PDMS at 14 days; (f) 1 wt% Ag-PDMS at 14 days; (g) PDMS at 21 days; (h) 0.25 wt% Ag-PDMS at 21 days and (i) 1 wt% Ag-PDMS at 21 days.
A cumulative DGGE gel was prepared and analysed to assess the overall effects on the bacterial community growing on these membranes. Figure 5.7(a) suggests that the bacterial community growing on the membranes with 0.25 wt% Ag nanoparticles impregnation were more similar to one another (65-77% similarity) than the bacterial groups on the control PDMS without Ag nanoparticles (0%) or those with higher Ag nanoparticle concentration (~50% similarity).

**Figure 5.7** *In vivo* bacterial growth assay. (a) UPGMA dendrogrand derived from cumulative DGGE profiles of Ag nanoparticle impregnated PDMS membranes retrieved from the rumen of fistulated steer over 28 days. 0, 0.125, 0.25, 0.5 and 1 wt% refer to the level of Ag nanoparticle impregnation while D refers to day. Scale is indicative of similarity levels; (b) Principal component analysis of microbial communities on Ag nanoparticle impregnated PDMS membranes retrieved from the rumen of fistulated steer over 28 days. T0.125, T0.25, T0.50 and T1.00 refer to 0.125, 0.25, 0.50 and 1 wt% Ag nanoparticles impregnation respectively while D refers to day. C refers to controls of pristine PDMS.
Day 7 to Day 28 bacterial communities on 0.25 \text{wt}\% Ag nanoparticle impregnated nanocomposite also formed largely different clusters compared to the control PDMS and 1 \text{wt}\% Ag-PDMS. PCA of the data also validated this trend showing two distinct groups. Group 1 consisted of 0.25 \text{wt}\% Ag nanoparticles samples while group 2 consists of all other samples (Figure 5.7(b))

The differences between PDMS membranes with 0 \text{wt}\% and 0.25 \text{wt}\% Ag nanoparticles were further investigated by using DGGE to compare the community profiles of these respective materials. The UPGMA dendrogram generated from these profiles showed that two distinct clusters were formed.

\textbf{Figure 5.8} In vivo bacterial growth assay. (a) UPGMA dendrogram derived from cumulative DGGE profiles of Ag nanoparticle impregnated PDMS membranes retrieved from the rumen of fistulated steer over 28 days. 0, and 0.25 \text{wt}\% refer to the level of Ag nanoparticle impregnation while D refers to day. Scale is indicative of similarity levels; (b) Principal component analysis of microbial communities on Ag nanoparticle impregnated PDMS membranes retrieved from the rumen of fistulated steer over 28 days. T refers to 0.25 \text{wt}\% Ag nanoparticles impregnation while D refers to day. C refers to controls of pristine PDMS
These clusters were based on the presence or absence of Ag nanoparticles (Figure 5.8(a)). The difference between the controls and nanocomposites with 0.25 \text{ wt\%} Ag nanoparticle impregnation was further validated with PCA plots (Figure 5.8(b)) which also showed two distinct groups based on presence and absence of Ag nanoparticles.

DGGE-based Shannon Weaver bacterial community diversity analysis indicated that the presence of 0.25 \text{ wt\%} Ag nanoparticles caused a reduction in bacterial diversity compared to the control over 28 days (Table 5.3). At each time point, the bacterial community diversity ($H'$) in membranes with Ag nanoparticles was lower than the diversity on the control PDMS membranes. For example, on Day 7 and Day 28, the $H'$ for control samples (without Ag nanoparticles) were 3.05 and 3.01 compared to 2.43 and 2.70 for samples with 0.25 \text{ wt\%} of Ag nanoparticles (Table 5.3).

**Table 5.3** The effects of Ag concentration on bacterial community diversity on nanocomposite membranes placed in the rumen of a fistulated steer for up to 28 days.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>0 \text{ wt%} Ag ($H'$)</th>
<th>0.25 \text{ wt%} Ag ($H'$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>3.05 ± 0.11</td>
<td>2.43 ± 0.10</td>
</tr>
<tr>
<td>14</td>
<td>2.90 ± 0.35</td>
<td>2.52 ± 0.13</td>
</tr>
<tr>
<td>21</td>
<td>2.92 ± 0.26</td>
<td>2.89 ± 0.18</td>
</tr>
<tr>
<td>28</td>
<td>3.01 ± 0.13</td>
<td>2.70 ± 0.07</td>
</tr>
</tbody>
</table>

The Ag-PDMS nanocomposite material has shown very interesting antibacterial properties with Ag nanoparticle loading within the PDMS matrix, appearing to have significantly reduced the amount of bacteria that adheres to the surface (Figures 5.5 and 5.6) and has decreased the diversity of bacteria growing on the
material (Table 5.3). Interestingly, the 0.25 wt% Ag-PDMS nanocomposite showed the least surface coverage or fewest bacterial colonies. This can be ascribed to the maximum concentration of Ag\textsuperscript{+} ions leaching (Figure 5.4(a)) from the nanocomposite which not only affects cells in contact with the surface but those within the surrounding media as well (Figure 5.5(d)). The greatest performance at 0.25 wt% is due to an optimal loading of Ag nanoparticles where dispersion is at a maximum. Higher concentrations of Ag loading resulted in agglomeration of nanoparticles, reducing the number of active particles within the polymer matrix, which could interact with the outside environment. This effect of difference in nanoparticle dispersion with loading concentrations was also validated through the catalysis data (Figure 5.4(f)).

This optimal concentration of 0.25 wt% Ag-PDMS nanocomposite possesses an enhanced \textit{in vivo} antimicrobial property which at low Ag concentrations is of potential interest to many medical, agricultural and purification technologies and applications. With such low concentrations of Ag impregnation showing efficient antimicrobial properties, these nanocomposites can have many benefits in regards to production cost and any potential harmful effects from high quantities of Ag.

A proof of concept, \textit{in vivo} measurement using a fistulated steer was demonstrated in this chapter. There are many parameters that can be adjusted and tuned with this experimental method including the type of animals, their feed and diet types, substrates that can promote or hinder specific microorganisms as well as the environmental parameters of measurements. There also might be subtle differences between individual animals. Although there are still many unknowns to be explored, the potential of such usable live \textit{in vivo} laboratories are significant.
5.4 Summary

The Ag-PDMS nanocomposites were subjected to both in vitro laboratory and in vivo animal testings to investigate their performance. The in vitro tests found that the 0.25 wt% nanocomposite was able to reduce the number of E. coli cells adhering to the surface by approximately 80% with three times the number of dead cells after 5h growth. The in vivo tests revealed a lowering in the diversity (H') of bacteria that adhered to the surface from 2.9 for the control PDMS to 2.5 for the 0.25 wt% nanocomposite after 14 days in the rumen of a fistulated steer. The in vitro tests suggested that these improvements were associated to the antibacterial mechanisms that catalytic Ag and leaching Ag⁺ ions are known to exhibit. The in vitro fluorescence study suggests that the leaching Ag⁺ ions are the dominant antimicrobial mechanism.

Both in vivo and in vitro tests proved that Ag-PDMS nanocomposites, even at relatively low Ag concentrations, show significant antimicrobial properties making it advantageous for biomedical implantable devices. The agreement between the in vivo and in vitro tests validates the possibility of implementing fistulated animals for similar investigations. Additionally, the fistulated animal in vivo tests also provide information about the bacterial community and their diversity that could not be readily seen using any in vitro tests.

This chapter clearly shows the possibility and some of the potential in using fistulated animals as live laboratories for testing antimicrobial properties of nanocomposites. The procedure can be adopted for many other applications, providing a new route that provides a large scope for understanding microbial behaviour and diversity that cannot be replicated in vitro.
In the next chapter the author will present a summary of his PhD thesis and discusses future work related to the PhD research project.

5.5 Chapter Acknowledgements

I would like to acknowledge Dr. Eric Adetutu for assisting with the DGGE, UPGMA and PCA analysis, Dr. David Paull and Dr. Jess Mcleod for their work with the fistulated steer, Dr. Rajesh Ramanathan and Dr. Vipul Bansal for their assistance with the catalysis. I would also like to acknowledge the advice and guidance of Dr. Chris McSweeney, Dr. Greg Bishop-Hurley, Prof. Andy Ball and Prof. Kourosh Kalantar-zadeh.

References


with regard to their anti-microbial properties," *Surface and Coatings Technology*, vol. 192, pp. 252-256, 2005.


Chapter 6: Conclusions and future works

6.1 Concluding remarks

The author’s objectives in this PhD research were to investigate synthesis and characterise the gas permeability selectivity and antimicrobial effects of pristine PDMS and PDMS nanocomposite membranes. These permeable membranes with tuneable properties were incorporated into sensing and phase separating applications. As such, the author’s research was organised and pursued in three major stages in order to achieve the proposed research outcomes and to target the gaps in the current knowledge.

In this PhD research, the author thoroughly investigated the literature on gas permeation though PDMS and other polymer nanocomposites containing 2D material. At the time of this PhD research started, the synthesis of PDMS was not standardised for the temperature during crosslinking. Some studies would use room temperature while others chose 100 °C up to 150 °C without giving any explanation as to why this temperature was chosen or the resulting effects that this choice would have on the eventuating PDMS matrix. Therefore the first stage the author conducted an extensive investigation on the controlling and altering the synthesis crosslinking temperature and thoroughly characterise the alterations to the polymeric matrix through both traditional and modern methods and finally demonstrating the resulting effect on the gas permeation.

In the second stage of this research work, the author investigated the use of 2D filler materials to enhance any alterations that may come about from the incorporation of these fillers into the polymeric composite due to the enhanced
surface to volume ratio and high surface area to maximise the resulting interaction. This stage is split into two sections to focusing on the following outcomes. Firstly, to enhance flux increasing gas permeability and secondly, to add selectivity, where the combination of these leads to enhanced separation membranes.

In the third stage, the author of this thesis demonstrated the use of Ag nanoparticles to reduce the biofilm formation to enhance the lifetime of membranes used in biological environments. In pursuing this goal the author established a current lack in outcomes for both in vitro and current in vivo testing models. Therefore, the author with the help of collaborators developed a novel in vivo test to assess microbial activity in a diverse community.

As such, major achievements in each stage of this research are summarised as follows:

### 6.1.1 Stage 1

- As presented in chapter 2, at the time of this work there was no consensus during synthesis of PDMS as to what crosslinking temperature should be chosen and what effect this had on the overall polymeric structure. The author’s effort to overcome this lack of knowledge resulted in a thorough investigation of a wide range of crosslinking temperatures looking at the most commonly used temperatures.

- The investigation revealed an ‘optimum’ temperature of 75 °C resulting in an increase of 25% in CO₂ permeation from the most commonly used temperatures.
This increase in permeation was ascribed to a decrease crosslinking density and an increase in FFV within the polymeric matrix. The material was characterised in depth and this was revealed through Raman spectroscopy, density and mass swelling tests.

6.1.2 Stage 2

In this stage, the use of 2D fillers was explored for the enhanced physiochemical properties that these materials have compared to their bulk counterparts. The 2D materials investigated were graphene and MoS$_2$. These two were identified as potential fillers for different reasons.

Firstly, graphene was chosen as the enhanced surface energy and the lack of interaction between carbon and PDMS was hypothesised to increase flux and with the large surface area this increase would happen at low weight loadings. Secondly, MoS$_2$ was chosen as it has been both calculated and shown that the 2D form of this material has high adsorption energy to specific gas species including NO$_2$ and CO$_2$.

As presented in chapter 3, graphene-PDMS nanocomposite membranes were developed at low loading concentrations below 1 wt%. It was found that all loadings resulted in an increase in gas permeation this was ascribed the creation of an interfacial void, increasing the FFV and lowering the crosslinking density. An optimal loading concentration of 0.25 wt% was found to yield the highest gas permeation for most gas species tested while 0.5 wt% was found to give the highest permeation for CO$_2$. This difference is likely due to the known affinity graphene has to
CO₂. It was found that agglomeration occurs above 0.25 wt%, reducing the surface area creating this interfacial void.

- Nanocomposite membranes containing MoS₂ presented in chapter 4, at the time of this thesis work, was the first investigation of the use of 2D MoS₂ used as a filler material for gas separation applications. The developed nanocomposites were effective in separating NO₂, almost completely blocking NO₂ permeation and reducing CO₂ permeation by 60% at extremely low loading concentrations (~0.02 wt%) while not effecting N₂ or CH₄ permeation.

6.1.3 Stage 3

- In the final stage presented in chapter 5, the author developed Ag-PDMS nanocomposites to investigate the antimicrobial properties with the intent of reducing surface colonisation and biofilm formation. The author demonstrated the development of Ag-PDMS with Ag concentrations of up to 1 wt%. Ag was identified by the PhD candidate due to its well-known strong antimicrobial activity.

- During the investigation it was identified that both in vitro and modern in vivo assays were unable to thoroughly examine the operation of these materials in the environment with diverse antimicrobial communities. So with the help of collaborators a novel in vivo technique utilising a fistulated ruminant.

- The Ag-PDMS nanocomposites were subjected to in vitro and in vivo investigations revealing an optimal loading percentage of 0.25 wt%. At this loading the Ag⁺ ion leaching was higher than nanocomposites.
containing four times more Ag nanoparticles. This has been ascribed to both an increase in FFV at this concentration and higher degree of agglomeration at higher loading concentrations.

- Through *in vitro* investigations, this optimal loading concentration was found to reduce cell coverage after 24 h. growth, from 22 % for pristine PDMS to 2 % for the Ag-PDMS nanocomposite.
- Through novel *in vivo* assays a unique understanding of the how the antimicrobial effects changed the communities of bacteria that are able to adhere to the surface. Through DGGE and UPGMA analysis the bacterial community diversity ($H'$) was found to be reduced from 2.9 for the control PDMS to 2.5 for the 0.25 wt% nanocomposite after 14 days in the rumen of a fistulated steer.

In conclusion, this research project has successfully brought new ideas and knowledge to the field of gas separating membranes and antimicrobial materials. As such, the outcomes of this PhD research have been published in peer reviewed scientific journals. A complete list of publications by the author since the beginning of his PhD research project, are as follows:

### 6.2 Journal publications

The work conducted by the author of this dissertation during his PhD candidature, resulted in 9 journal publications (four as the first author). The list of author’s scientific manuscripts is as follows:

effect of crosslinking temperature on the permeability of PDMS membranes: evidence of extraordinary CO\textsubscript{2} and CH\textsubscript{4} gas permeation," *Separation and Purification Technology*, vol. 122, pp. 96-104, 2014.


### 6.3 Conference presentations and publications

In addition to the journal publications, the author also had the opportunity to present his work in the international conference of Materials Research Society (MRS) Fall Meeting 2014 Boston, USA and the European Materials Research Society (E-MRS) Spring Meeting 2014 Lille, France.


As well as presenting the author had the opportunity to publish the following conference publication.


### 6.4 Recommendations for future work

Recently significant advances have been made in the application and research of nanocomposite membranes in gas separation and biomedical systems. For this purpose, many studies, as presented in this PhD thesis, have been conducted using a variety of polymers and nanoparticles for the development of such membranes. However, the use of 2D materials is still an emerging prospect in the membrane field. As there are still ample opportunities for expanding the body of research in alignment with the outcomes presented in this thesis, the author of this thesis presents the following as the future outlook of this dissertation:

- A critical issue in the embedding of nanofillers into nanocomposites is the homogeneity, dispersion and orientation of nanofillers that can play very
important roles in the gas permeation and selectivity of the resulting membranes. For instance graphene, considerable challenges with regards to their design, alignment and dispersion are still remaining. Therefore, devising different methods of dispersions and taking into account the considerations of polymerisation in the presence of nanofillers and then applying novel methods for orienting the nanofillers is crucial.

- Another issue to consider that has not been given much attention is the practical robustness of nanocomposite membranes. Industrial and biomedical conditions are often harsh leading to decreases in efficiency and longevity. There are still very few studies regarding nanocomposite membranes’ durability within such harsh conditions. It therefore follows logic that significant emphasis should be placed on these investigations, that can address not just the materials but the methods of assessing the robustness of nanocomposite membranes for gas separation and removal in biologically rich environments.

- The use of 2D TMDC’s as a filler in composite materials has yet to be investigated in depth. There are many inherent properties that the TMDC’s possess that have many possibilities that can be advantageous to numerous applications including gas separating membranes.

- The capping or encapsulation of nanofillers in an intermediate polymer can completely change the interaction that these fillers have on the polymeric matrix and therefore alter the dynamics of gas molecules diffusing through it. Whether this capping is more or less porous than the polymer itself, or
whether the capping increases or decreases the interaction to the polymer matrix or altering the chain stacking a model for if and how it affects the dynamics of the system are the issues to be addressed in nanocomposite membrane designs.