Synthesis, Characterisation and Evaluation of Biphenyl Monomers for Molecular Imprinting Applications

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Declaration

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HAZIT A. ZAYAS

Date Signed: Feb. 22, 2012
Dedication

For Reydick
Acknowledgements

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To my dearest Nanay, thank you for your love. You and Dido are my inspiration.

To Reydick, you are a picture of love. Thank you. I could not ask for more.
Abstract

This work was spearheaded with the synthesis of seven novel biphenyl monomers possessing a range of acidic and basic functional groups using Suzuki cross-coupling reactions. Application of microwave heating has significantly improved the reaction yield in a short period, i.e., 30.0 min, whilst suppressing the homocoupling and Heck-coupling side reactions. The optimised microwave conditions have been adapted to large-scale synthesis of the biphenyl monomers and routinely used throughout this research.

The aqueous acid dissociation constants of the biphenyl monomers were derived from the semi-aqueous potentiometric titration method developed in this research specifically for these sparingly water-soluble compounds. The first aqueous pKₐ values of these novel biphenyl monomers, ranging from pH 3-11, are reported herein.

The biphenyl monomers were first used as functional monomers in the preparation of molecularly imprinted polymers for test targets theophylline and phenylphosphonic acid (PPA) by thermal and microwave (MW) polymerisation. Results of the semi-empirical molecular modelling and ¹H and ³¹P NMR titrations for theophylline and PPA, respectively, identified 4′-vinyl-biphenyl-4-ol (M2) as the best monomer to use for the synthesis of both theophylline and PPA MIPs. Both thermal and MW theophylline and PPA MIP systems prepared using M2 showed significant imprinting effect as confirmed by Freundlich analysis of their binding properties but the microwave polymers exhibited higher template uptake than their thermal counterparts, attributed to its high microporosity and lower surface charge. Cross-reactivity and selectivity tests with caffeine applied to the theophylline MIP system also showed the MW MIP to be more selective to the template (theophylline) than the competing analyte (caffeine) than the thermal MIP.

The mode of polymerisation and nature of the porogen were observed to affect the physical properties of the polymers. MW polymers showed higher porosity, lower surface charge and lower surface area than their thermal equivalents. Polymers prepared in THF also exhibited higher porosity and higher surface area than those prepared in DMF.
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<table>
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<tr>
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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>$^{13}$C NMR</td>
<td>Carbon nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^1$H NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>$^{31}$P NMR</td>
<td>Phosphorus nuclear magnetic resonance</td>
</tr>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisbutyronitrile</td>
</tr>
<tr>
<td>ATR FTIR</td>
<td>Attenuated Total Reflectance Fourier Transformed Infrared</td>
</tr>
<tr>
<td>B</td>
<td>Amount of bound polymer</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer-Emmet-Teller</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated chloroform</td>
</tr>
<tr>
<td>CR</td>
<td>Cross reactivity</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Deuterated dimethyl sulfoxide</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>EGDMA</td>
<td>Ethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>F</td>
<td>Amount of free polymer</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GTP</td>
<td>Guanosine triphosphate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IF</td>
<td>Imprinting factor</td>
</tr>
<tr>
<td>$K_a$</td>
<td>Affinity constant</td>
</tr>
<tr>
<td>$K_d$</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>$m$</td>
<td>Measure of system heterogeneity</td>
</tr>
<tr>
<td>M</td>
<td>Monomer</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MIP</td>
<td>Molecularly imprinted polymer</td>
</tr>
<tr>
<td>MW</td>
<td>Microwave synthesised (mode of polymerisation)</td>
</tr>
</tbody>
</table>
NIP  Non-imprinted polymer
$N_T$  Number of binding sites
Pd(DIPHOS)$_2$  bis[1,2-bis(diphenylphosphino)ethane]palladium(0)
Pd(PPh$_3$)$_4$  Tetrakis(triphenylphosphine)palladium
Pd$_2$(dba)$_3$  tris(dibenzylideneacetone)dipalladium(0)
PdCl$_2$(dppf)  [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)
PdCl$_2$(PPh$_3$)$_2$  bis(triphenylphosphine)palladium(II) dichloride
Pd-EDTA  Palladium-ethylenediaminetetracetate
$pK_a$  Acid dissociation constant
$pK_w$  Autoprotolysis constant of water
PPA  Phenylphosphonic acid
$p_sK_a$  Apparent ionisation constant in organic solvent-water mixture
PT  Phosphotyrosine
S  Selectivity
SEM  Scanning electron microscopy
T  Thermally synthesised (mode of polymerisation)
TEA  Triethylamine
THF  Tetrahydrofuran
TLC  Thin layer chromatography
$\Delta E^o$  Energy of interaction
$\Delta H^o_f$  Heat of formation
$\epsilon$  Dielectric constant of the binary mixture
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Chapter 1

Introduction
Molecular recognition is the underlying mechanism that governs processes in biological systems.\textsuperscript{1} This phenomenon results in the highly specific and selective recognition displayed in substrate binding of an enzyme, antigen-antibody association, enzyme catalysis and DNA replication among many others. Such capability is attributed to the well-defined structure and spatial arrangement of molecules in the biological receptor, which is governed by intermolecular interactions such as hydrogen bonding, electrostatic interactions, or van der Waals forces. Therefore, it is of great interest to mimic this property into synthetic functionalised materials with molecular recognition capability.

\section{1.1 The Molecular Imprinting Technique}

The driving force in the formation of well-defined three-dimensional structures in biological receptors is molecular interaction\textsuperscript{1} which is the same principle applied in the fabrication of the artificial counterpart of biological receptors, the molecularly imprinted polymers (MIPs).\textsuperscript{2} In molecular imprinting, a group of polymerisable functional monomers forms a three-dimensional assembly around a template and the resulting adduct formed is maintained by copolymerisation with a crosslinker. Extraction of the template from the polymer generates a cavity with spatial arrangements and functional groups complementary to the template molecule (Figure 1.1).\textsuperscript{3-5}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematics of the imprinting process.}
\end{figure}
Currently, there are two approaches in the synthesis of molecularly imprinted polymers (MIPs) which are dependent on the molecular interaction involved during the formation of the template(T)–monomer(M) complex. These are the covalent approach developed by Wulff\(^6\) and the non-covalent approach developed by Mosbach.\(^7\) Covalent imprinting involves the formation of a predetermined complex of template and monomer produced by reversible covalent bonds.\(^6\) The complementary shape formed by these bonds is maintained during the imprinting process and results in a highly specific and selective cavity after extraction of the template.

On the other hand, the non-covalent approach makes use of the self-assembly of the template and monomers by non-covalent bonds such as hydrogen bonding or van der Waals forces followed by polymerisation.\(^7\) The simplicity of this approach makes it more popular than covalent imprinting because of the number of compounds that can be readily imprinted utilising non-covalent interactions.

### 1.2 Applications of Molecularly Imprinted Polymers (MIPs)

Molecularly imprinted polymers are easy to synthesise, cheap and robust therefore they offer great potential in various applications such as chromatography,\(^3,8-10\) drug screenings,\(^8,11\) binding assays,\(^10\) biosensors\(^8,9\) and enzyme-like catalysis.\(^9,12\) One of the primary objectives in the synthesis of MIPs is directed towards its use as stationary phase in liquid chromatography for compounds of clinical and environmental importance.\(^13\) Hence, there is a significant volume of work in the development of chromatographic separation using MIPs for small molecules, carbohydrates, peptides and proteins.\(^10,13\) Because of the enzyme-like recognition property of MIPs, which possess similar binding characteristics of the biological counterpart, these are also used as substituents for antibodies in immunoassays.\(^14\) MIPs have been used as substitute of antibodies in the immunoassay of drugs such as theophylline, diazepam, morphine, leu-enkephalin and propanolol.\(^10\) Although MIPs are gaining more applications in different fields of analytical and pharmaceutical chemistry, more studies are still needed for optimisation of MIPs for enhanced selectivity and affinity towards specific targets.
1.3 Optimisation of Molecularly Imprinted Polymers (MIPs)

Though the synthesis of MIPs may seem simple and straightforward, the selection and the optimisation of the number of variables such as functional monomers, crosslinked, porogen and polymerisation methods are complex and requires careful selection and optimisation of the parameters. Among the main factors influencing the outcome of molecular imprinting, the focus of this work is on functional monomers and polymerisation methods.

1.3.1 Functional Monomers

It has been long recognised that the formation of a well-defined cavity and highly specific binding sites are attributed to the strong interaction between template and functional monomers. The functional monomer provides the functional group necessary in forming interactions with the template.\textsuperscript{15,16} Commercially available addition monomers are commonly used but specialised monomers can also be tailor-made for a specific template.\textsuperscript{4}

In this work, novel biphenyl monomers with the generalised structure:

\[ \text{Functional Monomer Structure} \]

![Functional Monomer Structure](image)

Where

\[ \text{Functional Monomer Structures} \]

![Functional Monomer Structures](image)

were synthesised (Chapter 2) and evaluated as functional monomers for MIPs templated with theophylline (Chapter 4) and phosphate-bearing templates phosphotyrosine and
phosphonic acid (Chapter 5). The choice of biphenyl monomers and their application in molecular imprinting was influenced by the combinatorial engineering strategy of redesigning natural proteins to specifically create novel catalytic sites employing only 9 amino acids out of 20. The concept of redesigning natural proteins to obtain novel binding sites can be materialised by construction of “enzyme mimics” employing the biphenyl monomers. Enzyme mimics are used as screening tools for potential drug compounds; therefore, determination of the acid dissociation constants of the monomers is necessary. This is the focus of Chapter 3.

Aside from the choice of monomer, it is also necessary to control the stoichiometry of the T:M complex to promote their interactions (whilst minimising intermonomer interactions) and enhance MIP performance. Thus, pre-synthetic T:M interaction studies have also been conducted using a combination of semi-empirical molecular modelling and \(^1\)H and \(^{31}\)P NMR spectroscopies as discussed in Chapters 4 and 5.

### 1.3.2 Polymerisation Conditions

Polymers prepared in varying temperatures have shown to affect the selectivity of the imprinted polymer. Photoinitiated imprinted polymers at 0°C were found to be more enantioselective in rebinding \(L\)-phenylalanine anilide compared to thermally initiated polymerisation at higher temperature.\(^{18}\) The same result was observed by Sellergren\(^{19}\) in imprinting the same template resulting in a more selective polymer when prepared via initiated polymerisation at 15°C. However, the effect of varying temperatures in thermal polymerisation for \(3-L\)-phenylalanylaminopyridine suggests that the polymer prepared at 40°C is more enantioselective than the one prepared at 10°C.\(^{20}\) The result showed that the polymer prepared at low temperature via thermal polymerisation suffers incomplete polymerisation, which results in poor binding sites. The selectivity of the MIPs in thermally initiated polymerisation was observed to be not markedly affected by the temperature of polymerisation but by the degree of completeness of the reaction. Recently, our group reported microwave-induced polymerisation of MIPs specific for caffeine. Microwave polymerisation was shown to enhance the selectivity of the MIPs.
towards caffeine compared to thermally prepared MIPs. In this study, microwave polymerisation was also employed in the synthesis of both MIPs and biphenyl monomers.

1.4 Objectives of the Study

The synthesis of biphenyl monomers employing thermal and microwave induced Suzuki cross-coupling is described in Chapter 2. The goal of this chapter is to produce large scale amounts of biphenyl monomers in preparation for MIP synthesis. Problems encountered using the thermal Suzuki reaction were addressed by the use of microwave heating. The optimised and established procedure for the synthesis of biphenyl monomers was continually and successfully used for the whole duration of the research as to provide the necessary amount for characterisation and MIP synthesis.

Chapter 3 focuses on the characterisation of the biphenyl monomers in terms of acid dissociation constant, $pK_a$. Because of the biphenyl structure of the monomers, it presents restricted solubility in water. Therefore, determination of the $pK_a$ values of the monomers was done by potentiometric titration in THF-water mixtures and the aqueous $pK_a$ values extrapolated using Yasuda-Shedlovsly plots.

The first evaluation of the biphenyl monomers as functional monomers was applied in the synthesis of theophylline MIPs and is presented in detail in Chapter 4. Theophylline MIPs were prepared in two approaches: thermal polymerisation and microwave-induced polymerisation. The mode of polymerisation was found to affect the resulting selectivity and affinity of the polymers.

Lastly, Chapter 5 presents the potential use of biphenyl monomers in the development of phosphate-bearing templates, such as phosphotyrosine and phenylphosphonic acid. Specific interaction of the biphenyl monomers with the templates in the prepolymer complex was probed using $^{31}$P NMR.
1.5 References


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Chapter 2

Microwave-induced Synthesis of Functional Biphenyl Monomers by Suzuki Cross-coupling Reaction
2.1 Introduction

2.1.1 Suzuki Cross-coupling Reactions

Biphenyls are common backbones found in a number of biologically active drug candidate compounds\textsuperscript{1-4} and natural products.\textsuperscript{5-7} These compounds also provide the building blocks for the synthesis of complex and high molecular weight materials, which are used in the development of nanomaterials, sensing devices and catalysts.\textsuperscript{6,8} Although biphenyls appear to be simple in structure, the preparation of these compounds is far from simple. Of the numerous synthetic methodologies available for aryl-aryl bond formation, most common pathways include transition metal-catalysed reactions of organometallic aryl compounds and aryl halides, nucleophilic substitution of organometallic aryls and activated arenes, and oxidative coupling of electron rich aromatic rings.\textsuperscript{6}

The most exemplified approach among the methods available is the transition metal-catalysed reactions using copper, nickel and palladium.\textsuperscript{6} The Ullman reaction, a copper catalysed reaction for biaryl synthesis, is generally employed for the synthesis of symmetrical biaryls.\textsuperscript{9} Although recent advancements may extend its use towards the synthesis of unsymmetrical biaryls, this requires excess amount of arylhalides and stoichiometric amounts of copper.\textsuperscript{6} Later on, the successful coupling reaction of Grignard reagents with sp\textsuperscript{2} carbon halides using nickel as a catalyst paved the way for the synthesis of both symmetrical and unsymmetrical biaryls. This type of reaction only required milder conditions than the Ullman reaction, but with relatively higher yields.\textsuperscript{10} Further improvements on the product yields were realised when palladium was used as a catalyst instead of nickel.\textsuperscript{10}

The use of palladium over nickel allows the use of a wide range of organometallic reagents in cross coupling reactions with aryl halides.\textsuperscript{6,10} Organometallic reagents of mercury, silicon, germanium, lead, bismuth, antimony, copper, manganese, zirconium, tin and boron derivatives were successfully applied in various syntheses of biaryl compounds with palladium as a catalyst. Currently, a number of palladium catalysed cross coupling reactions for biaryls synthesis exist. These reactions are not only limited to biaryl
synthesis, but can also be used for carbon-carbon bond formation between alkene and aryl compounds. Some of these coupling reactions are the Heck, Stille and Suzuki reactions. For coupling with aryl/alkyl halides, Suzuki\textsuperscript{11} employs arylboronic acids (Scheme 2.1), whilst Heck\textsuperscript{12} uses olefins (Scheme 2.2) and Stille\textsuperscript{13} uses organotin compounds (Scheme 2.3). Among these three reactions, only Suzuki and Stille coupling are suitable for the synthesis of a wide range of substituted biaryls. The drawback for Stille coupling, however, is the toxicity of organotin compounds. On the other hand, Suzuki coupling employs non-toxic organoboron compounds\textsuperscript{14} but its downside is its long reaction time. Whilst some reactions may only take hours, others take days to complete.

\[
\begin{align*}
R'X + R\text{--B(OH)}_2 & \xrightleftharpoons[\text{base}]{\text{Pd}(0)} R'R \\
\text{Scheme 2.1 General reaction scheme for Suzuki coupling between alkyl halides and organoboron compound.}
\end{align*}
\]

\[
\begin{align*}
R'X + R\text{--} & \xrightleftharpoons[\text{base}]{\text{Pd}(0)} \text{Pd}(0) \quad R'R \\
\text{Scheme 2.2 General reaction scheme for Heck coupling between alkyl/aryl halides and olefins.}
\end{align*}
\]

\[
\begin{align*}
R'X + R\text{--Sn(R)}_3 & \xrightleftharpoons[\text{base}]{\text{Pd}(0)} R'R + R\text{--Sn(R)}_3 \\
\text{Scheme 2.3 General reaction scheme for Stille coupling between alkyl halides and organotin compound.}
\end{align*}
\]

The first thermal Suzuki reaction was reported in 1979.\textsuperscript{15} Akira Suzuki and Norio Miyaura successfully prepared conjugated dienes from 1-alkenylboranes and alkenyl halides catalysed by tetrakis(triphenylphosphine)palladium [Pd(PPh\textsubscript{3})\textsubscript{4}] in the presence of a base (Scheme 2.4).\textsuperscript{15} Later on, the success of the coupling reactions was extended to synthesis of biaryls from phenylboronic acids and arylhalides.\textsuperscript{16} In this study, for the thermal synthesis of biphenyl monomers, 4-vinylphenyl boronic acid and substituted aryl halides
were used for the coupling reaction (Scheme 2.5). It is noticeable, however, that the desired route for biphenyl monomer synthesis also falls in the Heck reaction scheme (Scheme 2.2), which is the reaction of aryl halides with olefins or alkenes. Thus, this makes the biphenyl synthesis tricky by the proposed route. This is because Heck coupling, a possible route for the reaction of the vinyl moiety of 4-vinylphenylboronic acid and aryl bromide, may compete with the desired reaction.

\[ \begin{align*}
\text{R}^1 & \quad \text{H} \\
\text{H} & \quad \text{BX}_2 \\
\text{R}^4 & \quad \text{Br} \\
\text{R}^3 & \quad \text{R}^2
\end{align*} \]

\[ + \quad \text{Pd(PPh}_3\text{)}_4 \quad \text{base} \]

\[ \text{R}^1 \quad \text{H} \\
\text{H} \quad \text{R}^3 \quad \text{R}^2 \\
\text{R}^4 \]

Where: \( X_2 = (\text{Sia})_2 \) or \( L = \text{PPh}_3 \)

**Scheme 2.4** The first Suzuki coupling prepared by Suzuki and Miyaura in the preparation of conjugated dienes from 1-alkenylboranes and alkenyl halides catalysed by tetrakis(triphenylphosphine)palladium \([\text{Pd(PPh}_3\text{)}_4]\) in the presence of a base.

\[ \begin{align*}
\text{Br} \\
\text{X}
\end{align*} \]

\[ + \quad \text{Pd} \quad \text{base} \]

\[ \text{HO} \quad \text{B} \quad \text{OH} \]

\[ \text{X} \]

**Scheme 2.5** General reaction scheme for the Suzuki cross coupling of aryl bromide with 4-vinylphenyl boronic acid for biphenyl synthesis.
A wide range of catalysts are commercially available, including the ligand-based Pd catalyst, Pd(PPh₃)₄. This catalyst is a popular choice for Suzuki reactions; however, Pd(PPh₃)₄ is light and air sensitive, forming undesired by-products as a result of the reaction of one of the phenyl rings of the ligand with the organoboron compound.

Pd(DIPHOS)₂ [bis[1,2-bis(diphenylphosphino)ethane]palladium(0)] is one catalyst reported to be air stable, in fact, reactions either under open air or nitrogen atmosphere were found to give identical yields. Other ligand-based Pd catalysts are Pd₂dba₃ [(tris(dibenzylideneacetone)dipalladium(0)), PdCl₂(dppf) [[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)] and PdCl₂(PPh₃)₂ [bis(triphenylphosphine)palladium(II) dichloride] and, over the past years, ligandless or phosphine free palladium catalysts have been reported to successfully form C-C bonds. Included among other modified Pd catalysts are: palladium chloride (PdCl₂), Pd-EDTA (palladium-ethelynediaminetetraacetate), palladium on carbon (Pd/C) and palladium hollow spheres (nanoparticles). Additionally, some of these ligand free catalysts were successfully used to catalyse Suzuki coupling in organic-aqueous or aqueous media towards a greener and cleaner chemistry.

Formation of the carbon-carbon bond between organoboranes and aryl halides is not a straightforward reaction. It entails a complicated general catalytic cycle that occurs in three main steps: oxidative addition, transmetalation and reductive elimination, which are depicted in Figure 2.1. The onset of the catalytic cycle commences from the oxidative addition of aryl halide to the palladium complex. It is the rate-limiting step of the catalytic cycle, which is dependent on the relative reactivity of aryl halides decreasing from I > Br > Triflates > Cl. This makes aryl iodides a popular reagent for the cross-coupling reaction, although aryl bromides and aryl chlorides are now commonly used. The second step of the reaction requires the addition of the base to generate the L₂Pd⁺ArOH complex that undergoes transmetallation via reaction with the boronate complex. Lastly, the Ar'-PdL₂-Ar complex formed from the second step easily undergoes reductive elimination to generate the biaryl and palladium(0) back to the catalytic cycle.
2.1.2 Microwave Synthesis

Over the past few years, the use of microwave synthesis has extensively improved reaction yields and productivity and reduced the reaction time for different organic reactions.\textsuperscript{31-33} Compared to traditional heating using an oil bath, which transfers heat by conductance, microwave chemistry produces internal heat from the interaction of microwave energy with polar and ionic materials (Figure 2.2).\textsuperscript{33-35} The efficient heating observed with microwaves compared to conventional heating is due to the dielectric heating effect.
The dielectric heating effect is dependent on the interaction between polar material (solvent or reagent) and microwave energy. Efficient heating is only observed if the materials absorb microwave energy and subsequently convert it to heat. However, recent reports by Gutmann et al. revealed that efficient microwave heating of nonpolar solvents like hexane was obtained using sintered silicon carbide microwave vessels. The use of silicon carbide in microwave reactions eliminates the influence of the dielectric heating effect in the reaction mixture because of the strong microwave absorptivity of the silicon carbide vessel. The high thermal conductivity of the vessel results in efficient heating via a heat transfer mechanism.

The two mechanisms for dielectric heating are dipolar polarisation and ionic conductance (Figure 2.3). The interaction of the polar materials with microwave energy is called the dipolar polarisation mechanism. This is commonly observed in the efficient microwave heating of systems containing polar solvents like DMF, methanol and water. Upon microwave irradiation at high frequency, polar molecules align themselves with the applied electric field. When the applied electric field oscillates, the polar molecules reorient themselves with alternating electric field. In this process, molecular agitation and friction occurs, generating heat due to dielectric heating.

Figure 2.2 Conventional (A) thermal heating compared to (B) microwave heating.
On the other hand, conduction mechanism involves the interaction of ions with the electric field. As with the polar molecules, the ions also align themselves with the applied electric field when irradiated with microwave energy. The induced mobility of ions in the solution in effect generates heat. This kind of interaction is much stronger and generates more heat than the dipolar mechanism.

This chapter details the syntheses of biphenyl functional monomers via microwave-assisted Suzuki cross-coupling reaction. This afforded us a more efficient (than the conventional thermal reaction) in-house method to produce functional biphenyl monomers in a large scale for use in subsequent synthesis of molecularly imprinted polymers.

2.2 Results and Discussion

2.2.1 Thermal Suzuki cross-coupling reaction

A typical Suzuki coupling reaction of equimolar amounts of organoboron compound and aryl halide is catalysed by catalytic amounts of Pd(PPh$_3$)$_4$ using K$_2$CO$_3$ as a base. As this catalyst has been reported to be air sensitive and can result in deactivation of the catalyst and formation of undesired side products,$^{17}$ we opted to use Pd(DIPHOS)$_2$ as this catalyst has been reported to be air and water tolerant.$^{18}$ This apparent tolerance presented the
opportunity to simplify the synthetic procedure by removing the requirement for an inert atmosphere and anhydrous chemistry approaches.

The Pd(DIPHOS)$_2$ catalyst was found to produce an almost identical yield from open air and under nitrogen atmosphere. In our trial biphenyl synthesis, the catalyst was first applied to the thermal reaction of 4-bromophenol and 4-vinylphenylboronic acid using the method of De et al.\textsuperscript{18} in neat THF instead of THF-MeOH mixture in an attempt to produce our biphenyl monomer M2 (as discussed in Section 2.4.2, also denoted as T-M2 for thermally heated).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\includegraphics[width=0.8\textwidth]{Scheme2}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 2.6} Thermal reaction of 4-bromophenol and phenylboronic acid catalysed by Pd(DIPHOS)$_2$ using the method of De et al.\textsuperscript{18} in neat THF.

The reaction of 4-vinylphenylboronic acid and 4-bromophenol using Pd(DIPHOS)$_2$ under the conditions reported, only afforded a homocoupled product (8) as shown in Scheme 2.6 and none of the desired product T-M2. Unfortunately, 8 was formed by the self-coupling of 4-vinylphenylboronic acid catalysed by palladium. From literature reports, the homocoupling product was found to be a persistent by-product often obtained in palladium catalysed reactions.\textsuperscript{38,39} This is obtained due to the reaction of the palladium catalyst with molecular oxygen, inducing the self-coupling of 4-vinylphenylboronic acid forming 8.\textsuperscript{40} In our biphenyl system, our findings suggest that the catalyst is not best used under an air atmosphere as it favours a reaction with air to form a homocoupling product. The result did
not conform with the reported air tolerance of the Pd(DIPHOS)$_2$ by De et al.$^{18}$ in their system, which produced an almost identical yield when performed in open air and under nitrogen. Catalyst deactivation in our biphenyl system may have produced the homocoupling product and, as such, an inert atmosphere may be necessary. Palladium catalysts in Suzuki cross-coupling reactions are air sensitive, with air causing oxidation and deactivation of the palladium catalyst.$^{17, 40}$

A detailed examination of the catalytic cycle for the self-coupling of arylboronic acids has fully elucidated the reaction mechanism and showed that molecular oxygen from air attacks the Pd$^0$ catalyst to form a peroxo complex ($\eta^2$-O$_2$)PdL$_2$ 9 (where $L = $ PPh$_3$) which subsequently reacts with the arylboronic acid to form an adduct 10 (Scheme 2.7).$^{41}$ The generated adduct reacts with another arylboronic acid to form the trans-ArPd(OH)L$_2$ complex (11), followed by transmetalation and reductive elimination to form the homocoupling product, in our case compound 8.

\[
\begin{align*}
\text{O}_2 \text{PdL}_2 + \text{ArB(OH)}_2 & \rightarrow \text{HO}_\text{B(OH)}_2 \text{PdL}_2 \\
9 & \rightarrow 10 \\
10 & \rightarrow \text{Ar-Pd-OH}_\text{L} \\
11 & 
\end{align*}
\]

**Scheme 2.7** Reaction scheme elucidating the transformation of the (9) deactivated Pd catalyst via (10) to produce the trans-ArPd(OH)L$_2$ complex (11) which then undergoes transmetalation and reductive elimination to form the homocoupling product.

Despite the unwanted presence of homocoupling products in the synthesis of biaryls, it opened a pathway useful for synthesis of symmetrical biaryls in even higher yields and faster reaction.$^{42}$ Compound 8 may be useful in polymer synthesis as it contains two polymerisable groups; however, it is not of major interest in this research. Considering the cause of the formation of homocoupling product 8, the reaction atmosphere was found to be an important factor in the Suzuki reactions. Therefore, in an effort to obtain the desired product, suppression of the self-coupling of 4-vinylphenylboronic acid in our method via
exclusion of oxygen in the reaction mixture was deemed necessary. Reports on the suppression of homocoupling involve exclusion of oxygen by subsurface sparging of the reaction mixture with nitrogen.\textsuperscript{39} In our method, the reaction system in Scheme 2.6 was modified to be performed under a nitrogen atmosphere as shown in Scheme 2.8. Reaction monitoring by TLC after an overnight reflux revealed no cross-coupling product. Therefore, the reaction was extended for a longer time and periodically checked for the presence of cross-coupling product T-M2. After a five-day reaction, the desired cross-coupling product T-M2 (GC yield, 13\%) was obtained along with homocoupling product 8 (GC yield, 5\%).

![Scheme 2.8](image)

**Scheme 2.8** Thermal reaction of 4-bromophenol and 4-vinylphenylboronic producing the desired cross-coupling product T-M2 after a 5-day reaction.
Figure 2.4 Structures of biphenyl monomers synthesised by Suzuki cross-coupling reaction. (M1) 4-(4-vinylphenyl)-pyridine, (M2) 4'-vinyl-biphenyl-4-ol, M(3) dimethyl-(4'-vinylbiphenyl-3yl)-amine, (M4) (4'-vinyl-biphenyl-4-yl)-methanol, (M5) dimethyl-(4'-vinyl-biphenyl-4yl)-amine, (M6) 4'-vinyl-biphenyl-4-carboxylic acid and (M7) 4-hydroxy-5-methyl-4'-vinyl-biphenyl-3-
carbaldehyde. M6 was obtained by the Suzuki cross-coupling reaction of 4’-vinyl-biphenyl-4-carboxylic acid benzyl ester (M6b) with 4-vinylphenyl boronic acid, after saponification.

Table 2.1 Summary of product yields (%) of thermally synthesised biphenyl monomers, T-Mx, by Suzuki cross-coupling reaction.

<table>
<thead>
<tr>
<th>Biphenyl Monomer</th>
<th>Yield(^{a,b}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-M1</td>
<td>78</td>
</tr>
<tr>
<td>T-M2</td>
<td>13</td>
</tr>
<tr>
<td>T-M3</td>
<td>30</td>
</tr>
<tr>
<td>T-M4</td>
<td>86</td>
</tr>
<tr>
<td>T-M5</td>
<td>41</td>
</tr>
<tr>
<td>T-M6(^c)</td>
<td>98</td>
</tr>
<tr>
<td>T-M7</td>
<td>28(^c)</td>
</tr>
</tbody>
</table>

\(^{a}\) THF reflux, \(N_2\), \(Pd(DIPHOS)_2\) catalyst, \(K_2CO_3\), 5 days. Heating attained by conventional thermal heating.

\(^{b}\) GC and \(^1\)H NMR yields (crude yields).

\(^{c}\) Used as precursor for M6; M6 obtained after saponification of M6b with isolated yield of 27%.

The successful synthesis of T-M2 after introduction of a nitrogen atmosphere in the reaction system was then applied to the synthesis of the rest of the biphenyl monomers (shown in Section 2.4.2) with the results of the thermally-synthesised monomers (T-Mx) presented in Table 2.1 and the structures shown in Figure 2.4. Nevertheless, even with the introduction of a nitrogen atmosphere in the system, the reaction was still very slow requiring five days to obtain the optimal cross-coupling yield of 13% for T-M2. T-M1 (yield, 78%) and T-M4 (yield, 86%) gave good yields but the T-M3 and T-M5 yields remain unsatisfactory for scale up. Even after exclusion of air and sparging with nitrogen, the synthesis remains difficult and slow. In addition, when the conditions were applied to 4-bromobenzoic acid, no cross-coupling product was obtained. However, in this instance, we believe the presence of the labile carboxylate proton was responsible. This was confirmed as protection of the carboxylate proton as the ester T-M6b allowed facile access.
to the desired T-M6 after saponification (Scheme 2.9). It required protection of 4-bromobenzoic acid before successfully producing T-M6b with excellent yield of >98%.

![Scheme 2.9 Reaction scheme of the synthesis of (6), a result of the saponification of T-M6b.](image)

The results of the thermal synthesis suggest that the Suzuki reaction was affected by the substituents in the aryl halide. The reactivity of the substituted aryl halides decreases from the most electron-withdrawing to the most electron donating groups.\(^{11,44}\) This makes the protected 4-bromobenzoic acid (T-M6a) susceptible to nucleophilic attack by palladium because of the deactivating effect of the carboxylate group. Thus, the highest thermal yield obtained was only after carboxylate protection. On the other hand, 4-bromophenol, the arylbromide used in the synthesis of T-M2, which contains a hydroxyl group (a strong electron-donating group), gave the lowest thermal yield. The hydroxyl functional group is directly attached to the ring with its electron readily available for donation (inductive effect), thereby making the ring less susceptible to attack by palladium. This also holds true for the other electron donating aryl bromides examined and used in the synthesis. The compounds 3-bromo-\(N,N\)-dimethylaniline and 4-bromo-\(N,N\)-dimethylaniline, which were
used to synthesise T-M3 and T-M5 respectively, gave poor yields. This is because the dimethylaniline functional group contains nitrogen that is directly attached to the ring, where electrons would be made available by an inductive effect. This stabilising effect made the ring less susceptible to nucleophilic attack from palladium. This differs from the starting materials 4-bromopyridine and 4-bromobenzylalcohol which gave moderate to good cross-coupling yields of 78% and 86%, respectively. In the case of 4-bromopyridine, the electron distribution in the ring is more concentrated towards nitrogen. The electron withdrawing effect of nitrogen makes the 4-position more susceptible to nucleophilic attack by palladium, thus favouring the cross coupling reaction in mild conditions. Similarly, the alkyl alcohol in 4-bromobenzyl alcohol promoted the susceptibility of the ring to nucleophilic attack. This is because the oxygen is not directly attached to the benzene ring and therefore cannot donate its electron to the ring unlike the oxygen in 4-bromophenol. This made 4-bromobenzyl alcohol more deactivated than bromophenol, and resulted in a higher T-M4 yield compared to T-M2.

T-M7 was synthesised via the procedure obtained from Baleizão et al. using Pd(PPh₃)₄ as a catalyst. The reaction was found to proceed smoothly and was directly used for upscale synthesis of T-M7 obtaining 1.8873 g (yield, 28%). The compound was not further synthesised using Pd(DIPHOS)₂ under thermal or microwave conditions, as with the other monomers, because of time limitation. Nevertheless, the reactivity of T-M7a, the arylbromide used in the synthesis of T-M7, was observed to follow the same trend in reactivity of aryl bromides.

### 2.2.2 Microwave Suzuki cross coupling reaction

The optimised conditions for the conventional thermal synthesis of biphenyl monomers, using Pd(DIPHOS)₂ as a catalyst under a nitrogen atmosphere and using THF as a solvent, has reduced the formation of homocoupling product 8 (yield, <5%). However, the optimised yield of the method is not sufficient for subsequent analysis. In an effort to increase the product yield and reduce reaction times, microwave heating was used for the
synthesis. The monomers synthesised using microwaves are designated as MW-Mx to discriminate them from thermally synthesised monomers (T-Mx).

Given the favourable results arising from the thermal synthesis of T-M6 from 4-bromobenzoic acid benzyl ester, Pd(DIPHOS)$_2$ and K$_2$CO$_3$ (Scheme 2.9), we deemed this the logical system to commence our explorations under microwave irradiation conditions. Our initial series of microwave syntheses were performed using pure THF as solvent at 100°C (100 W) for 30 min. However, the solvent evaporated leaving a clump of reactants with a low product yield. The 10 mL pressure vials were designed to vent only when the internal pressure was above 20 bar, and since the vapour pressure of THF at 100°C was only 2.8 bar, failure of the pressure seal may be attributed to the decomposition of organic peroxides. For safety purposes, the reaction was repeated, but using toluene as solvent due to its higher boiling point (~111°C) as compared to THF (66°C). Despite the higher boiling point of toluene, the reaction gave low yield of MW-M6b. We attribute this lower than anticipated yield to the inability to attain the desired reaction temperature of 100°C.

Toluene is known to exhibit low absorptivity of microwave irradiation causing poor heating when used in the absence of a microwave absorbing media.$^{33}$ To circumvent the poor microwave absorptivity associated with toluene, subsequent microwave experiments were conducted in a 1:1 toluene:H$_2$O mixed solvent system. This had the additional benefit of permitting an increase in reaction temperature to 150°C. Microwave irradiation of benzyl 4-bromobenzoate in toluene/water (1:1) with Pd(DIPHOS)$_2$ and K$_2$CO$_3$ at 150°C (100 W) for 30 min, resulted in a yield of > 98% MW-M6b (Table 2.2) similar to that obtained via thermal heating (Table 2.1). Our initial findings are consistent with previous reports in this area.$^{45-53}$

To further assess the applicability of this approach and examine the use of THF for microwave synthesis, a mixed solvent of 1:1 THF: H$_2$O was used at 100 °C (100 W) for 30 min. This mixed solvent approach eliminated the heating inefficiency (i.e., pure toluene) and possible safety hazards (i.e., pure THF). Even at a lower reaction temperature (100°C),
excellent yields (> 98%) as shown in Table 2.2 for all monomers were obtained when THF: H₂O was used. The effect of the mixed solvent composition and reaction temperature on the product yields are discussed in more detail below.

Table 2.2 Summary of product yields (%) of microwave synthesised biphenyl monomers, MW-Mx, by Suzuki cross-coupling reaction.

<table>
<thead>
<tr>
<th>Biphenyl Monomer</th>
<th>Yield(^a) (%)</th>
<th>Toluene:H₂O(^b)</th>
<th>THF:H₂O(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW-M1</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>MW-M2</td>
<td>33</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>MW-M3</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>MW-M4</td>
<td>85</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>MW-M5</td>
<td>80</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>MW-M6(^d)</td>
<td>&gt;98</td>
<td>&gt;98</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) GC and \(^1\)H NMR yields (crude yields)
\(^b\) PhCH₃/H₂O (1:1) at 150 °C for 30 min (100 W).
\(^c\) THF/H₂O (1:1) at 100 °C for 30 min (100 W).
\(^d\) Used as precursor for M6; M6 obtained after saponification of M6b with isolated yield of 27%.

The successful synthesis of MW-M6 in toluene: H₂O solvent affording an excellent yield was applied to the synthesis of M2. The thermal route (T-M2) only afforded very low levels of the desired product; however, with our microwave approach, we noted a significant improvement approaching 33% yield in just 30 min as compared to a yield of 13% only after 5 days of thermal heating. Whilst there was no evidence of the homocoupling product 8, the major species was the Heck product 12 (66%). The Heck product obtained was thought to be the cross coupling product MW-M2 as the TLC spot of 12 has the same \(R_f\) as the TLC spot for MW-M2. However, when checked in GCMS, two peaks were found with the same molar mass.
The Heck reaction is a cross-coupling reaction of aryl halide and olefins (alkenes) catalysed by palladium in the presence of a base to form aryl substituted olefins (Scheme 2.2).\textsuperscript{54, 55} Heck coupling reactions typically require elevated temperature conditions (110 - 180\degree C), thus, requiring the use of high boiling point solvents such as DMF, DMA and toluene.\textsuperscript{56} In the synthesis of the biphenyls, the starting materials comprise the required functional groups allowing for access to both the Suzuki and Heck coupling products, i.e., aryl bromide and alkene/boronic acid. Additionally, a palladium catalyst, a high boiling point solvent (i.e., toluene:H\textsubscript{2}O), an elevated reaction temperature (150\degree C), and a base (K\textsubscript{2}CO\textsubscript{3}) were all components of this reaction. Having satisfied the reaction conditions for both reactions, both the Suzuki cross-coupling MW-M2 (yield, 33%) and Heck cross-coupling product 12 (yield, 66%) were produced; however the undesired product (Heck) yield was not anticipated to be this high. Reducing the reaction temperature to 100\degree C did not suppress the formation of 12, resulting in similar yields as with the reaction at 150\degree C. The formation of 12 under these reaction conditions (toluene: H\textsubscript{2}O 1:1, 150 \degree C and 100 W) only applies to the synthesis of MW-M2, with all other monomers reaching very high yields. The percentage yields for the synthesis of the other biphenyls using these conditions are presented in Table 2.2.

\textbf{Scheme 2.10} Microwave reaction of 4-bromophenol and 4-vinylboronic acid using Pd(DIPHOS)\textsubscript{2} and K\textsubscript{2}CO\textsubscript{3} in toluene:water (1:1) producing MW-M2 and the Heck coupling by-product 12.
The effect of a mixed solvent composition on the suppression of 12 as an undesirable by-product in the synthesis of MW-M2 was explored whilst maintaining a reaction temperature of 150°C. There was no evidence for the Heck product 12 when reactions were performed with THF. The use of THF instead of toluene at 150°C and 30 min (100 W) was effective in suppressing the formation of the Heck product 12, thereby increasing the cross-coupling product yield of MW-M2 (> 98%). In addition, mixed solvent switching from toluene to THF and adjusting the reaction temperature from 150°C to 100°C further improved the yields of MW-M2 and other monomers to >98% respectively. THF is more miscible with H₂O and more polar, and as such resulted in a more homogeneous heating throughout the reaction mixture. Whereas toluene and H₂O form two distinct phases thus leading to less efficient microwave heating with respect to THF: H₂O system. Additional investigation on suppression of 12 by THF was not attempted in this study, as the compounds of interest are the Suzuki coupling products with the polymerisable vinyl biphenyls. Further, lowering the reaction temperature to 100°C would prevent any possible solvent evaporation should pressure vessel seal failure occur in the subsequent upscale syntheses.

The several modifications employed in the investigation of the optimised procedure for vinylbiphenyls were only applied to the synthesis of M2. Since M2 was the most difficult to synthesise, having the lowest yield in both thermal and microwave synthesis, any optimisation method was geared towards increasing the M2 yield. Optimisation of the synthesis of M2 led to the identification of mild reaction conditions that allowed facile access to the remaining biphenyl analogues required for our subsequent MIP synthesis efforts. The optimised procedure for M2 is shown in Scheme 2.11 and the results are presented in Table 2.2.
Scheme 2.11 The final microwave reaction conditions used in the scale-up synthesis of biphenyl compounds (THF/H₂O 1:1, 100 W, 100°C, N₂ and 30 min). M2 is used as a representative product monomer.

In this synthesis of functional biphenyl monomers and from literature reports, microwave-induced Suzuki cross-coupling offers ease of operation, increased reaction yield, suppression of undesired side-reactions, and most importantly reduced reaction time.

2.3 Conclusion

Suzuki cross-couplings reactions were successfully employed in the synthesis of polymerisable biphenyls using arylbromides and arylboronic acid. Production of the homocoupling product (8) of arylboronic acid was successfully suppressed by exclusion of air and purging with nitrogen gas in the microwave synthesis. The use of toluene and water as a mixed solvent system (toluene: H₂O) in the synthesis of M2 has produced the undesired a Heck product (12). Nevertheless, elimination of 12 was achieved by using a 1:1 ratio of THF: H₂O. Subsequent experimentation employing microwave irradiation with THF: H₂O as a solvent system has significantly improved the product yield at reduced reaction times. This optimised microwave-induced Suzuki coupling method has become the in-house routine method for the synthesis of the biphenyl monomers for use in this study.
2.4 Experimental

2.4.1 Chemicals and Reagents

All solvents and chemicals were of analytical grade. 4-Bromopyridine, 4-bromophenol, 3-bromo-N,N-dimethylaniline, 4-bromobenzyl alcohol, 4-bromo-N,N-dimethylaniline, 4-bromobenzoic acid, 2-hydroxy-3-methyl-benzaldehyde, 4-vinylphenyl boronic acid, K₂CO₃ and NaOH were purchased from Sigma Aldrich and were used without further purification. THF was obtained from Sigma Aldrich and was used as received.

2.4.2 Methodology

General procedure 1 (Thermal Suzuki cross-coupling). To a mixture of 4-vinylphenylboronic acid (1.00 mmol, 0.140 g) and 4-bromophenol (1.00 mmol, 0.173 g), Pd(DIPHOS)₂ (1 mol%), 2 M K₂CO₃ (2.4 mmol, 1.2 mL), distilled water (1.8 mL) and THF (3 mL) were added in a 100 mL round bottom flask, degassed and purged with nitrogen gas. The reaction mixture was heated to reflux for 5 days. The reaction mixture was allowed to cool and filtered using Celite before addition of ethyl acetate. The solvent was removed by rotary evaporation and the residue was dissolved in water and extracted with ethyl acetate (2 x 10 mL) before drying with Na₂SO₄ for column chromatography.

General procedure 2 (Microwave Suzuki cross-coupling). A mixture of 4-vinylphenylboronic acid (1.00 mmol, 0.140 g), 4-bromophenol (1.00 mmol, 0.173 g), Pd(DIPHOS)₂ (1 mol%), 2 M K₂CO₃ (2.4 mmol, 1.2 mL), distilled water (1.8 mL) and THF (3 mL) in a 10 mL microwave test tube with a magnetic stirrer bar was degassed and purged with nitrogen gas prior to microwave irradiation. The 10 mL pressure vessel was placed in the microwave cavity of CEM Discoverer Benchmate and sealed with a pressure lock. The microwave source was set to 100°C using 100 W of power to heat the reaction for 30 min. After microwave irradiation, the reaction mixture was allowed to cool and filtered using Celite before the addition of ethyl acetate. The combined filtrate was evaporated using the rotary evaporator. The residue was dissolved in H₂O, extracted with
ethyl acetate (2 x 10 mL) and dried with anhydrous Na$_2$SO$_4$ before drying in rotary evaporator for column chromatography.

General procedures (1) and (2) were used for the synthesis of the following compounds:

**4-(4’-Vinylphenyl)-pyridine (M1).** Biphienyl monomer 1 (M1) was synthesised using equimolar amounts of 4-vinylphenylboronic acid (1.00 mmol, 0.148 g) and 4-bromopyridine hydrochloride (1.00 mmol, 0.1940 g) neutralised with NaOH. The yellow crude product was purified by column chromatography (90:10 DCM:EtOAc) to obtain a pale yellow powder (isolated yield, 50%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ ppm 5.33 (dd, $J =$ 10.9, 0.7 Hz, 1H, vinyl H); 5.83 (dd, $J =$ 17.6, 0.7 Hz, 1H; vinyl H); 6.77 (dd, $J =$ 17.6, 10.9 Hz, 1H; vinyl H); 7.50-7.54 (m, 4H, Arom. H); 7.62 (d, $J =$ 8.4 Hz, 2H; arom H); 8.66 (dd, $J =$ 4.6, 1.5 Hz, 2H; arom H); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$ ppm 115.0, 121.4, 127.0, 127.2, 136.1, 137.4, 138.5, 147.9, 150.4 ppm.

**4’-Vinylbiphenyl-4-ol (M2).** Biphenyl monomer 2 (M2) was synthesised using equimolar amounts (1.00 mmol, 0.148 g) of 4-vinylphenylboronic acid and 4-bromophenol (1.00 mmol, 0.1730 g). The crude yellow product was purified by column chromatography (60:40 Hex:EtOAc) to afford a light brown powder. (Isolated yield, 50.53%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 4.82 (s, 1H, OH); 5.25 (dd, $J =$ 10.9, 0.9 Hz, 1H, vinyl H); 5.77 (dd, $J =$ 17.6, 0.9 Hz, 1H, vinyl H); 6.75 (dd, $J =$ 17.8, 10.7 Hz, 1H, vinyl H); 6.90 (d, $J =$ 8.8 Hz, 2H, Arom H); 7.26 (d, $J =$ 0.9 Hz, 1H, CDCl$_3$); 7.44-7.53 (m, 6H, Arom. H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ ppm 112.6, 114.7, 125.6, 125.8, 127.2, 132.6, 135.1, 135.5, 139.2, 154.2 ppm.
**Dimethyl-(4′-vinylbiphenyl-3-yl)amine (M3).**

Biphenyl monomer 3 (M3) was synthesised using equimolar amounts of 4-vinylphenylboronic acid (1.00 mmol, 0.148 g) and 3-bromo-N,N-dimethylaniline (1.00 mmol, 0.2000 g). The crude product was purified by column chromatography (20:1 Hex:EtOAc) to afford a yellow oil. (Isolated yield, 54%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) ppm 2.96 (s, 6H, N(CH\(_3\))\(_2\)), 5.23 (d, \(J = 10.9\) Hz, 1H, vinyl H); 5.75 (d, \(J = 17.6\) Hz, 1H, vinyl H); 6.72 (ddd, \(J = 9.3, 6.6, 4.6\) Hz, 2H, vinyl H and Arom. H); 6.93 (dd, \(J = 8.7, 1.4\) Hz, 1H, Arom. H); 7.28 (t, \(J = 7.8\) Hz, 2H, Arom H); 7.44 (d, \(J = 8.4\) Hz, 2H, Arom H); 7.55 (d, \(J = 8.2\) Hz, 2H, Arom H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) ppm 40.4, 113.0, 113.8, 125.9, 127.0, 127.3, 130.5, 135.1, 136.7, 140.1, 150.3. Melting point 44-47°C.

**4′-Vinylbiphenyl-4-yl) methanol (M4).**

Biphenyl monomer 4 (M4) was synthesised using equimolar amounts of 4-vinylphenylboronic (1.00 mmol, 0.148 g) acid and 4-bromobenzyl alcohol vinylphenylboronic (1.00 mmol, 0.18703 g). The crude product was purified by column chromatography (60:40 Hex:EtOAc) to afford a yellow powder (Isolated yield, 59%). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) ppm 1.62 (s, 1H, OH); 4.74 (s, 2H, CH\(_2\)); 5.27 (d, \(J = 10.9\) Hz, 1H, vinyl H); 5.78 (d, \(J = 17.6\) Hz, 1H, vinyl H); 6.76 (dd, \(J = 17.6, 10.9\) Hz, 1H, vinyl H); 7.25 (s, 1H), 7.45 (dd, \(J = 12.2, 8.3\) Hz, 4H, Arom H); 7.58 (dd, \(J = 10.7, 8.3\) Hz, 4H, Arom H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) ppm 64.6, 113.4, 126.1, 126.6 (2C), 126.9, 135.8, 136.2, 139.4, 139.6 (2C) ppm.

**Dimethyl-(4′-vinylbiphenyl-3-yl)amine (M5).**

Biphenyl monomer 5 (M5) was synthesised using equimolar amounts of 4-vinylphenylboronic acid (1.00 mmol, 0.140 g) and 4-bromo-N,N-dimethylaniline (1.00 mmol, 0.2003 g). The crude product was purified by column chromatography (20:1 Hex:EtOAc) to afford a light brown flaky product.
(Isolated yield, 40%) $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ ppm 2.94 (s, 6H, N(CH$_3$)$_2$); 5.23 (d, $J = 10.9$ Hz, 1H, vinyl H); 5.80 (d, $J = 17.7$ Hz, 1H, vinyl H); 6.74 (dd, $J = 15.2$, 8.5 Hz, 1H, vinyl H); 6.80 (d, $J = 8.9$ Hz, 2H, Arom. H); 7.46–7.58 (m, 6H, Arom H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ ppm 41.2, 111.8, 112.3, 114.1, 116.2, 127.0, 127.9, 129.9, 137.1, 142.2, 142.3 (2C), 151.5 ppm. Melting point 170°C.

4-Bromo-benzoic acid benzyl ester (6a).

Compound 6a was synthesised utilising the procedure of Wakasugi et al. using 4-bromobenzoic acid (1.00 mmol, 0.201 g) and benzyl alcohol (1.00 mmol, 0.1081 g). The crude product was purified by column chromatography (60:40 Hex:EtOAc) to obtain a white crystalline product. (Isolated yield, 79%) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ ppm 5.28 (s, 2H, CH$_2$); 7.27–39 (m, 5H, Arom. H); 7.50 (d, $J = 8.7$ Hz, 2H, Arom. H); 7.86 (d, $J = 8.6$ Hz, 2H, Arom. H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ ppm 67.4, 128.6, 128.7, 128.8, 129.1, 129.4, 131.6, 132.1, 136.2, 166.1 ppm.

4'-Vinyl-biphenyl-4-carboxylic acid benzyl ester (6b). Biphenyl monomer 6b was synthesised using equimolar amounts of 4-vinylphenylboronic acid (1.00 mmol, 0.147 g) and compound 6a (1.00 mmol, 0.3144 g). The crude product was purified by column chromatography (60:40) to afford an off white powder (Isolated yield, 30%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ ppm 5.22 (d, $J = 10.7$ Hz, 1H, vinyl H); 5.31 (s, 1H, CH$_2$); 5.73 (d, $J = 18.2$ Hz, 1H, vinyl H); 6.68 (dd, $J = 28.4$, 10.7 Hz, 1H, vinyl H); 7.26–7.35 (m, 5H, Arom H); 7.42 (d, $J = 8.3$, 2H, Arom. H); 7.51 (d, $J = 8.4$, 2H, Arom H); 7.58 (d, $J = 8.6$, 2H, Arom. H); 8.06 (d, $J = 8.6$ H, ArH); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ ppm 66.1, 113.9, 126.2 (2C), 126.3 (2C), 126.8, 127.6, 127.7, 127.8, 128.0, 129.7, 137.0, 138.7, 144.7, 165.7 ppm.
**4'-Vinyl-biphenyl-4-carboxylic acid (M6).**

Biphenyl monomer 6 (M6) was synthesised by saponification of compound 6b. To a solution of compound 6b (0.27 mmol, 0.0861 g) in methanol, an excess amount of NaOH (1.37 mmol, 0.548 g) was added and stirred for overnight at room temperature. The reaction mixture was washed with water and the organic layer discarded. The aqueous solution was then acidified to low pH using HCl and the organic product extracted with ethyl acetate. Purification of the crude product was via column chromatography (60:40 Hex:EtOAc) to afford a white powder. (Isolated yield, 27%) $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$ ppm 3.42 (s, 1H, OH); 5.32 (d, $J = 11.3$ Hz, 1H, vinyl H); 5.91 (d, $J = 17.70$ Hz, 1H, vinyl H); 6.80 (dd, $J = 28.33$ Hz, 1H, vinyl H); 7.60 (d, $J = 8.43$ Hz, 2H, Arom. H); 7.74 (d, $J = 16.19$ Hz, 2H, Arom H); 7.81 (d, $J = 24.38$ Hz, 2H, Arom. H); 8.02 (d, $J = 8.52$ Hz, 2H, Arom H); $^{13}$C NMR (75 MHz, DMSO-d$_6$): $\delta$ ppm 115.3, 126.9, 127.2, 127.5, 130.3, 132.0, 136.4, 137.4, 144.0, 167.5 ppm.

**5-Bromo-2-hydroxy-3-methylbenzaldehyde (7a).**

Compound 7a was synthesised using the procedure reported by Baleizão et al. and afforded a light brown powder. (Isolated yield is 77.30 %.) $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ ppm 2.19 (s, 3H, CH$_3$); 7.42 (d, $J = 2.2$ Hz, 1H, Arom. H); 7.44 (d, 1H, $J = 2.1$ Hz, Arom. H); 9.75 (s, 1H, OH), 11.12 (s, 1H, CHO); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ ppm 14.4, 110.3, 120.4, 129.1, 132.5, 139.6, 158.5, 195.1 ppm.
4-Hydroxy-5-methyl-4′-vinylbiphenyl-3-carbaldehyde (M7). Biphenyl monomer 7 (M7) was synthesised by using general procedure 1 using equimolar amounts of 4-vinylphenylboronic acid (28.38 mmol, 4.200 g) and compound 7a (28.38 mmol, 6.1036 g). The crude product was purified using column chromatography (20:1 Hex:EtOAc) to afford a bright yellow powder. (Isolated yield, 28%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ ppm 2.33 (s, 3H, -CH$_3$); 5.27 (d, $J$ = 10.94 Hz, 1H, vinyl H); 5.78 (d, $J$ = 17.6 Hz, 1H, vinyl H); 6.74 (d, $J$=18.04, 10.94 Hz, 1H, vinyl H); 7.47 (d, $J$ = 8.58 Hz, 1H, Arom. H); 7.51 (d, $J$ = 8.6 Hz, 1H, Arom. H); 9.93 (s, 1H, OH); 11.24 (s, 1H, CHO); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$ ppm 14.7, 113.5, 119.6, 126.1, 126.2, 126.9, 128.8, 131.7, 135.7, 135.8, 136.0, 138.3, 158.9, 196.2 ppm.
2.5 References


Chapter 3

Determination of Acid Dissociation Constants (pK$_a$s) of Functional Biphenyl Monomers by Potentiometric Titration
3.1 Introduction

The biphenyl monomers synthesised in Chapter 2 are designed to be used as functional monomers for the synthesis of molecularly imprinted polymers (MIPs). The choice of functional monomers in the synthesis of MIPs is vital because the functional monomers provide the site of contact for MIP-template interaction, similar to enzyme-substrate recognition. The biphenyl monomers synthesised in this work are a new class of functional monomers which have the potential to be used for imprinting pH sensitive molecules. Knowledge of the acid dissociation constant (pKₐ) values of these monomers will be useful in the design and optimisation of molecularly imprinted polymers with strong interaction towards a template via pH variation.

The acid dissociation constant, pKₐ, is a physico-chemical parameter that indicates the protonation state of ionisable groups at certain pH.¹ ² The pKₐ is a crucial parameter in the characterisation of a compound for its solubility, stability, lipophilicity and its fate in reaction pathways.³ ⁴ Of the various analytical techniques employed to determine pKₐ, which include capillary electrophoresis, liquid-liquid partitioning, potentiometry and spectrophotometry, potentiometry is the most commonly used, primarily due to its relatively inexpensive and simple operation.³ ⁵ Although the determination of pKₐ for water-soluble compounds by potentiometry is relatively easy and straightforward, this is not the case with organic compounds because of their limited solubility in aqueous solution. However, this problem, can be circumvented by the use of binary or even ternary mixtures of organic solvents and water in order to increase the solubility of organic compounds in water, enabling potentiometric titrations to be performed.⁶

In semi-aqueous potentiometric titrations, the choice of an organic co-solvent is crucial. It must readily dissolve organic compounds as well as be completely miscible with water. A commonly used solvent in semi-aqueous titrations of water-insoluble organic compounds is a binary mixture of water and methanol.⁶ ⁷ Methanol is commonly used as a co-solvent due to its strong ability to dissolve organic compounds and high polar nature similar to water.² For organic compounds that are not soluble in methanol, acetonitrile, dimethylformamide, dioxane, ethylene glycol, dimethyl sulfoxide and tetrahydrofuran (THF) have also been
used. In this study, semi-aqueous potentiometric titration of the biphenyl monomers were performed in binary mixtures of THF and water.

This chapter deals with the determination of the acid dissociation constants (pK\textsubscript{a}s) of the synthesised biphenyl monomers 1-7 (Figure 3.1) using potentiometric titrations and represents the first reported pK\textsubscript{a} values of these biphenyl monomers. Potentiometric titrations were conducted in binary mixtures of THF and water and extrapolated to zero organic content to obtain the aqueous pK\textsubscript{a} values.
Figure 3.1  Structures of the compounds tested using potentiometric titration to determine pK\textsubscript{a} values. (M1) 4-(4-vinylphenyl)-pyridine, (M2) 4'-vinylbiphenyl-4-ol, M(3) dimethyl-(4'-vinylbiphenyl-3yl)-amine, (M4) (4'-vinylbiphenyl-4-yl)-methanol, (M5) dimethyl-(4'-vinylbiphenyl-4yl)-amine, (M6) 4'-vinyl-biphenyl-4-carboxylic acid and (M7) 4-hydroxy-5-methyl-4'-vinyl-biphenyl-3-carbaldehyde.
3.2 Results and Discussion

3.2.1 Solubility tests

The solubility of 0.050 mmol and 0.100 mmol biphenyl monomers 1-7 in 10.00 mL of various organic solvent-water systems to form 0.005 M and 0.010 M solutions, respectively, was tested to identify the appropriate co-solvent for potentiometric titration. Among the three co-solvents tested, THF was found more suitable than methanol and acetonitrile in that it is capable of dissolving the test compounds even at < 50% (v/v) THF. Monomer 1 exhibited complete dissolution at ≥ 35 % (v/v) THF in water at a concentration of 0.010 M whilst the solubility of monomers 2-4 was limited to ≥ 40 % (v/v) THF in water at the same concentration. Monomers 5-7 exhibited a more limited solubility at ≥ 40 % (v/v) THF in water at 0.005 M. Whilst methanol is the most common solvent for potentiometric titration in binary solvent mixtures, it was not capable of dissolving some analytes at < 50% (v/v) MeOH; hence, it was not used in this study.

The solubility of the titrant NaOH in the THF-water mixture was also investigated. A solution of 0.150 M NaOH was prepared in THF-water compositions of ≥ 35% (v/v) THF based on the results of the solubility tests for the monomers. The 0.150 M NaOH solution was found soluble only up to 55 % (v/v) THF.

Overall, THF-water mixtures with 35-55 % (v/v) THF were chosen as the binary solvent systems for the potentiometric titration of monomer 1, whilst 40-55 % (v/v) THF was used for monomers 2-4 (0.010 M) and monomers 5-7 (0.005 M). NaOH solution (0.150 M) was also prepared in 35-55 % (v/v) THF in water.

3.2.2 Dielectric and autoprotolysis constants of THF-H₂O mixtures

The calculation of the pKₐ of each monomer at various THF-water mixtures (Section 3.2.4 and Equation 3.1) requires the dielectric (ε) and autoprotolysis (pKₐ) constants of water at specific organic solvent-water mixture as input parameters. Whilst aqueous titrations only require the dielectric and autoprotolysis constants of water (78.48 and 14, respectively), semi-aqueous titrations involve organic solvents which change the ε and pKₐ of water.
In this study, the dielectric constants of THF-water mixtures used for the titrations were interpolated using reported values of dielectric constants in a range of THF-water mixtures measured at 25°C as shown in Figure 3.2 and summarised in Table 3.1. Semi-aqueous potentiometric titrations using binary mixtures with dielectric constants greater than 50 have been reported to give relatively accurate pKₐ results. In this work, however, the highest ε obtained is only 53.7 (35% v/v THF) whilst the rest fall below 50 due to limitations imposed by the monomer solubility at < 35% (v/v) THF. Hence, to establish the validity of use of dielectric constants below 50, two test model analytes with known literature aqueous pKₐ values were titrated in 35-55 % (v/v) THF compositions. The results will be discussed in detail in the next section.

The ionisation constants of water at various THF-water mixtures at 25°C were also taken from the literature and the polynomial (4th degree) regression correlation established only for these values as shown on Figure 3.3 and Table 3.1 to obtain the ionisation constant of water from 35-55 % v/v THF.

Figure 3.2  Linear plot of experimentally determined dielectric constants of water in various THF-water mixtures at 25 °C.
Figure 3.3 Autoprotolysis constant of H$_2$O at various THF-water mixtures measured at 25 °C.\textsuperscript{12}

The $\varepsilon$ and p$K_w$ of water interpolated from the 35-55 % (v/v) THF content summarised in Table 3.1 have been observed to decrease with an increasing amount of THF in the solvent system. THF reduces the polarity of water, thus reducing the ionisation capacity of compounds dissolved in the binary solvent mixture.

<table>
<thead>
<tr>
<th>THF, % (v/v)</th>
<th>$\varepsilon$</th>
<th>p$K_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>53.7</td>
<td>14.6</td>
</tr>
<tr>
<td>40</td>
<td>50.5</td>
<td>14.7</td>
</tr>
<tr>
<td>45</td>
<td>46.3</td>
<td>14.9</td>
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<tr>
<td>50</td>
<td>42.3</td>
<td>15.0</td>
</tr>
<tr>
<td>55</td>
<td>38.9</td>
<td>15.2</td>
</tr>
</tbody>
</table>
3.2.3 Potentiometric Titrations

3.2.3.1 Test Titration using Model Analytes

Preliminary potentiometric titrations in THF-water solvent systems were conducted with commercially available analogues of known pKₐs in order to establish the validity of the methodology for the determination of the pKₐs of biphenyl monomers 1-7. In semi-aqueous titrations, a validation compound is usually tested to benchmark the methodology. For example, in the case of diphacinone and chlorophacinone, which are water insoluble compounds, ibuprofen was used as a standard compound to validate the potentiometric titration methodology for pKₐ determination in a dioxane-water solvent system. The validated method was subsequently applied to determine the pKₐs of diphacinone and chlorophacinone after a good correlation of experimental (pKₐ 4.47) and literature values (pKₐ 4.31-4.91) for ibuprofen was obtained. In this study, two model analytes, basic 2-aminobiphenyl (8) and acidic 4-biphenylcarboxylic acid (9) illustrated in Figure 3.4 were used to validate and benchmark the semi-aqueous titration of biphenyl monomers.

![Figure 3.4 Structures of commercially available analogues (8) 2-aminobiphenyl and (9) 4-biphenyl carboxylic acid used in method validation of THF-water mixtures.](image)

Preliminary titrations of the two model analytes involved four titrations of 0.010 M analyte solution prepared in a series of THF-water mixtures containing 40, 45, 50 and 55 % (v/v) THF. The two model analytes were found to be soluble at the chosen range of THF-water mixtures. Prior to titration with a standard basic solution (0.150 M NaOH), the analyte solution was pre-acidified to low pH (~2.5). Thus, whilst 9 remains in its acidic form, basic 8 was protonated and converted to its conjugate acid.
The potentiometric titration data was then fitted using the global minimisation algorithm (Globpot)\(^9\) developed in our laboratories at the University of Newcastle. This Matlab-based program simultaneously fits the complex and free ligand species concentration at equilibrium. The fitting routine accounts for the NaOH-HCl equivalence point, and is therefore able to resolve and predict the analyte pK\(_a\). An example of the titration data and corresponding species concentrations for conjugate acid of 8 and analyte 9 are shown in Figure 3.5.

**Figure 3.5 (a)** Titration curves for conjugate acid of 8 and analyte 9 in 50:50 THF:H\(_2\)O mixtures and their (b) corresponding species equilibrium concentrations. The species equilibrium plots show the equivalence point of the species involved in the titration, including the monomer [L], acid [H], protonated monomer [LH] and base [OH].
Table 3.2 shows the apparent ionisation constants ($pK_a$) and $p_{sK_a} + \log [H_2O]$ values for conjugate acid of 8 and analyte 9 showing correlation values ($R^2$) of 0.91 and 0.99, respectively. The Yasuda-Shedlovsky plots (discussed in Section 3.2.4 and defined by Equation 3.1) on Figure 3.6 show a linear relationship in the potentiometric titration of the model analytes in the THF-water system. 2-Aminobiphenyl (8), a weak base, and 4-biphenyl carboxylic acid (9), a weak acid, are characterised by negative and positive slopes, respectively. The trend of the slopes for acidic and basic compounds in semi-aqueous titrations was also observed in other solvent systems.4 The negative slope in the titration of the conjugate acid of 8 indicates a reduced basicity whilst the positive slope indicates a reduced acidity of 9 with increasing THF content. This result is expected since an increase in THF content decreases the dielectric constant value of the binary mixture (Figure 3.2). Higher organic content promotes deprotonation of the conjugate acid of 8 while suppresses the ionisation of compound 9 in THF-water mixtures, thus a decrease in basicity and acidity was observed with increasing THF content.

Table 3.2 Summary of $psK_a$ and $psK_a + \log [H_2O]$ value for conjugate acid of 8 and analyte 9 in THF-Water mixtures.

<table>
<thead>
<tr>
<th>THF, % (v/v)</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td></td>
<td>$pK_a$</td>
<td>$p_{sK_a} + \log [H_2O]$</td>
</tr>
<tr>
<td>40</td>
<td>3.19 ± 0.09</td>
<td>4.72</td>
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<tr>
<td>45</td>
<td>2.67 ± 0.05</td>
<td>4.15</td>
</tr>
<tr>
<td>50</td>
<td>2.42 ± 0.07</td>
<td>3.86</td>
</tr>
<tr>
<td>55</td>
<td>2.32 ± 0.08</td>
<td>3.72</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.91</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Figure 3.6  Yasuda-Shedlovsky plot of conjugate acid of 8 and analyte 9 showing $p_{K_a} + \log [H_2O]$ against the inverse of $\varepsilon$ in 35, 40, 45, 50 and 55 % (v/v) THF in THF-water mixtures. The X-intercept where x equals the inverse of the dielectric constant of water is the $p_{K_a}$ at zero THF content.

The extrapolated aqueous $p_{K_a}$s of conjugate acid of 8 and analyte 9 are shown in Table 3.3 and compared to literature $p_{K_a}$ values. These two test model analytes are a weak acid 8 and a weak base 9; despite this, the method was able to determine their $p_{K_a}$ values which are in close agreement with literature $p_{K_a}$ values. The extrapolated $p_{K_a}$ value of 9 is found to be 0.71 pH units off from the literature value, but its regression value is 0.99 ($R^2$) suggesting a highly linear relationship of $p_{K_a}$ and % THF content in the solvent mixture. The Yasuda–Shedlovsky plots of the conjugate acid of 8 and analyte 9 were found to follow the expected trends of acidity and basicity in non-aqueous titrations of water insoluble compounds. The extrapolated $p_{K_a}$ values are in good agreement with literature values,13 having a difference ($\Delta p_{K_a} = |p_{K_a\text{ extrapolated}} - p_{K_a\text{ literature}}|$) of 0.15 for the conjugate acid of 8 and 0.71 for analyte 9. In the spectrophotometric titration of ibuprofen in THF-water mixtures, the $p_{K_a}$ difference obtained was in the range of 0.55-0.85. In the case of the conjugate acid of 8 and analyte 9, the acceptable $p_{K_a}$ difference observed indicates the applicability of the method for $p_{K_a}$ determination of biphenyl systems.
**Table 3.3** Literature aqueous $pK_a$ and extrapolated aqueous $pK_a$ values of the conjugate acid of 8 and analyte 9.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$pK_a$ (literature value)</th>
<th>$pK_a$ (this study)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>3.83$^{13}$</td>
<td>3.98</td>
</tr>
<tr>
<td>9</td>
<td>4.19$^{14}$</td>
<td>4.90</td>
</tr>
</tbody>
</table>

### 3.2.3.2 Titration of Monomers 1-7

Potentiometric titrations (performed in triplicates) in THF-water mixtures with THF content of 35-55% (v/v) were carried out for monomer 1 at 0.010 M, whilst THF content of 40-55% (v/v) was employed for monomers 2-7 at 0.010 M for monomers 2-4 and at 0.005 M for monomers 5-7 (Tables 3.4 and 3.5). The plots in Figures 3.7 and 3.8 show examples of the titration curves and their corresponding equilibrium concentrations of all species in solution including the monomer $[L]$ at 50:50 v/v THF-H$_2$O mixtures.

The apparent ionisation constant ($p_aK_a$) and $p_aK_a + \log [\text{H}_2\text{O}]$ values of the basic and acidic monomers are summarised in Tables 3.4 and 3.5, respectively. The linear plots of $p_aK_a + \log [\text{H}_2\text{O}]$ values against the inverse of dielectric constants show correlation values, $R^2$, above 0.90 for all monomers. The aqueous $pK_a$s were then extrapolated from the Yasuda-Shedlovsky plot as shown in Figures 3.9 and 3.10.
Figure 3.7 Titration curves (a) and corresponding species equilibrium concentration plots (b) of the conjugate acids of 1, 3, and 5 at 50:50 THF:H₂O mixture. The species equilibrium plots show the equivalence point of the species involved in the titration, including the monomer [L], acid [H], protonated monomer [LH] and base [OH].
Figure 3.8 Titration curves (a) and their corresponding species equilibrium concentrations (b) for acidic monomers M2, M4, M6 and M7 in 50:50 v/v THF:H₂O mixtures. The species equilibrium plots show the equivalence point of the species involved in the titration, including the monomer [L], acid [H], protonated monomer [LH] and base [OH].
### Table 3.4 Summary of the $p_K_a$ and $p_K_a + \log [H_2O]$ values for the conjugate acids of basic monomers M1, M3 and M5 in THF-water mixtures.

<table>
<thead>
<tr>
<th>% THF (v/v)</th>
<th>M1 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
<th>M3 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
<th>M5 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>4.24 ± 0.02</td>
<td>5.79</td>
<td>4.38 ± 0.09</td>
<td>4.90</td>
<td>2.84 ± 0.07</td>
<td>4.23</td>
</tr>
<tr>
<td>40</td>
<td>4.22 ± 0.02</td>
<td>5.74</td>
<td>3.30 ± 0.05</td>
<td>4.78</td>
<td>2.82 ± 0.04</td>
<td>4.27</td>
</tr>
<tr>
<td>45</td>
<td>4.08 ± 0.03</td>
<td>5.57</td>
<td>2.76 ± 0.02</td>
<td>4.20</td>
<td>2.89 ± 0.03</td>
<td>4.38</td>
</tr>
<tr>
<td>50</td>
<td>3.62 ± 0.01</td>
<td>5.07</td>
<td>2.69 ± 0.05</td>
<td>4.08</td>
<td>3.30 ± 0.05</td>
<td>4.90</td>
</tr>
<tr>
<td>55</td>
<td>3.62 ± 0.02</td>
<td>5.02</td>
<td>2.69 ± 0.05</td>
<td>4.08</td>
<td>3.30 ± 0.05</td>
<td>4.90</td>
</tr>
</tbody>
</table>

$R^2$ = 0.93  0.92  0.91

*Blank entries mean the monomer is insoluble at this composition, thus no titration was done.*

### Table 3.5 $p_K_a$ and $p_K_a + \log [H_2O]$ values for acidic monomers M2, M4, M6 and M7 in THF-water mixtures.

<table>
<thead>
<tr>
<th>% THF (v/v)</th>
<th>M2 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
<th>M4 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>10.07 ± 0.09</td>
<td>11.60</td>
<td>10.33 ± 0.13</td>
<td>11.85</td>
</tr>
<tr>
<td>45</td>
<td>10.25 ± 0.20</td>
<td>11.72</td>
<td>10.29 ± 0.11</td>
<td>11.77</td>
</tr>
<tr>
<td>50</td>
<td>10.39 ± 0.13</td>
<td>11.84</td>
<td>10.05 ± 0.12</td>
<td>11.49</td>
</tr>
<tr>
<td>55</td>
<td>10.48 ± 0.15</td>
<td>11.88</td>
<td>9.54 ± 0.18</td>
<td>10.94</td>
</tr>
</tbody>
</table>

$R^2$ = 0.94  0.91

<table>
<thead>
<tr>
<th>% THF (v/v)</th>
<th>M6 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
<th>M7 $p_K_a$</th>
<th>$p_K_a + \log [H_2O]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>5.43 ± 0.03</td>
<td>6.96</td>
<td>8.79 ± 0.03</td>
<td>10.31</td>
</tr>
<tr>
<td>45</td>
<td>5.70 ± 0.03</td>
<td>7.18</td>
<td>8.86 ± 0.01</td>
<td>10.35</td>
</tr>
<tr>
<td>50</td>
<td>5.80 ± 0.05</td>
<td>7.25</td>
<td>9.03 ± 0.02</td>
<td>10.47</td>
</tr>
<tr>
<td>55</td>
<td>6.05 ± 0.03</td>
<td>7.45</td>
<td>9.22 ± 0.02</td>
<td>10.61</td>
</tr>
</tbody>
</table>

$R^2$ = 0.96  0.95
Figure 3.9 Yasuda-Shedlovsky plots of the conjugate acids of the basic monomers M1, M3 and M5 in THF-water mixtures. The x-intercept where x equals the inverse of the dielectric constant of water is the apparent ionisation constant ($p_K_a$) at zero THF content.

Figure 3.10 Yasuda-Shedlovsky plots for acidic monomers M2, M4, M6 and M7 in THF-water mixtures. The X-intercept where x equals the inverse of dielectric constant of water is the $p_K_a$ at zero THF content.
The experimental \( pK_a \) values obtained for the basic monomers (M1, M3 and M5) are for their conjugate acids. In general, the Yasuda-Shedlovsky plots of the conjugate acids of the basic monomers (Figure 3.9) are characterised by negative slopes. The negative slope indicates reduced basicity with increasing organic content. The increased amount of THF in the mixture decreases the dielectric constant value of the THF-water mixture, which promotes deprotonation of the conjugate acids of the basic compounds in the mixture. The \( pK_a \) (aqueous) values of basic monomers are expectedly higher than their corresponding \( pK_a \) values as shown in the Yasuda-Shedlovsky plot of the monomers in Figure 3.9.

Compounds of similar molecular weight and ionisable functional group were reported to have similar close slope values. This was observed for compounds chlorpromazine and imipramine, both containing a tertiary amine functional group attached at the same position. In this study, M3 and M5 (Figure 3.9) which have the same functional group and molecular weight, show different slope values of -1.53 and -0.35, respectively (Table 3.6). The difference in their slope values is attributed to the position of the amine functional group. The slope of the Yasuda-Shedlovsky plot is inversely proportional to the average ionic diameter of the solvated molecule. It is affected by the ionic diameter and the polar functional group of the compound that interacts with the solvent in the solvation process. The functional group in M3 is attached in the meta position whilst that in M5 is in the para position (Figure 3.1). An interaction of the functional group in M5 with the solvent makes the ionic diameter bigger than the ionic diameter of M3, resulting in the observed more negative slope value for M3.
Table 3.6  Slopes for the acidic and basic monomers from the Yasuda-Shedlovsky plots.

<table>
<thead>
<tr>
<th>Basic compounds</th>
<th>Slope</th>
<th>Acidic compounds</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>-1.24</td>
<td>M2</td>
<td>0.49</td>
</tr>
<tr>
<td>M3</td>
<td>-1.53</td>
<td>M4</td>
<td>-1.53</td>
</tr>
<tr>
<td>M5</td>
<td>-0.35</td>
<td>M6</td>
<td>0.79</td>
</tr>
<tr>
<td>8</td>
<td>-1.63</td>
<td>M7</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
</tbody>
</table>

Of the three basic monomers tested, relative basicity decreases in the order of $M_1 < M_3 < M_5$ according to the result of extrapolated $pK_a$ values. $M_1$ with the pyridine functional group is the most basic among the basic monomers. Although pyridine is an aromatic system, the lone pair of electrons in nitrogen is not part of the aromatic system and is available and accessible for sharing. Moreover, the lone pair in nitrogen is also not sterically hindered which makes it more available compared to sterically hindered dimethylaniline in $M_3$ and $M_5$. The difference in basicity between $M_3$ and $M_5$ with the same functional group could be attributed to the difference in the position of the basic dimethylaniline group in the benzene ring. The meta position of the dimethylaniline group in $M_3$ makes it more basic than $M_5$ where dimethylaniline occupies the para position. Both $M_3$ and $M_5$ are basic by virtue of the nitrogen, with an available lone pair, in the dimethylaniline substituent which is capable of accepting a proton. Both monomers also exhibit resonance. In the case of $M_5$, delocalisation of the lone pair from nitrogen extends from the first ring through to the second ring as illustrated in Scheme 3.1. This makes the electron pair on nitrogen for $M_5$ less available to a proton, thus a reduced basicity. On the other hand, whilst electron delocalisation can also happen in $M_3$, the meta position of the dimethylaniline group limits the movement of the lone pair from nitrogen to the first ring as illustrated in Scheme 3.2. The electron pair on nitrogen in $M_3$ is more available for protonation than for $M_5$, hence, it is more basic than $M_5$. 
Scheme 3.1 Resonance structures of M5 showing delocalisation of the lone pair from nitrogen from the first ring through to the second ring, making the lone pair electrons less available for sharing, thus a reduced basicity.

Scheme 3.2 Resonance structures of M3 showing delocalisation of the lone pair from nitrogen on the first ring only makes the lone pair electrons more available for sharing, thus an increased basicity.

Figure 3.10 shows the Yasuda-Shedlovsky plots of acidic monomers M2, M4, M6 and M7 for which all, bar M4, displayed positive slopes. A positive slope indicates that the acidity of the monomers decreases with increasing organic content, therefore extrapolation of the aqueous $pK_a$ from the Yasuda-Shedlovsky plot would result to a lower $pK_a$. Because the dielectric constant value of THF-water mixtures is lower compared to water, the extent of ionisation of the monomers in THF-water mixtures is also lower — thus, the monomers exhibit lower acidity in the binary mixtures. The same trend was observed in the titration of
common acids (e.g. citric acid, phthalic acid, boric acid and acetic acid) in THF-water mixtures with an increasing pKa value obtained in increasing THF content.\textsuperscript{14,15}

The trend of acidity among the acidic monomers tested decreased from M6 > M7 > M2 > M4. Upon ionisation, all of the monomers except M4 underwent resonance stabilisation, the extent of which was dependent upon the effects of the substituents which influenced acidity accordingly. M6 which has the carboxylic acid as functional group is the most acidic. This is due to the inductive effect of oxygen from the carbonyl that withdraws electrons from the oxygen of the hydroxyl group (Scheme 3.3). Between M7 and M2, M7 is more acidic because of the presence of the carbonyl group ortho to phenol. Whilst M2 only has resonance stabilisation of negative charge through to the second ring (Scheme 3.4), M7’s carbonyl oxygen withdraws electrons via the inductive effect of the ring. This results to the delocalisation of electrons away from the hydroxyl oxygen of M7 (Scheme 3.5). M4 is a very weak acid and acts as an amphoteric compound as indicated by its negative slope.

\begin{center}

\textbf{Scheme 3.3} Resonance structures of M6. Delocalisation of lone pair electrons on the oxygen from the hydroxyl by inductive effect of the carbonyl group, a stronger effect than resonance stabilisation, makes M6 the most acidic among the monomers.

\end{center}
Scheme 3.4  Resonance structures of M2. Delocalisation of electrons from the oxygen by resonance stabilisation, results in a weak effect on the acidity of M2.

Scheme 3.5  Resonance structures of M7. Delocalisation of electrons by inductive effect of the carbonyl group ortho to the hydroxyl group makes a strong effect on the acidity of M7.
The slopes of the Yasuda-Shedlovsky plot (Table 3.6) of compounds having the same functional groups were observed to be comparable and could be due to the similar interaction types during the solvation process.\textsuperscript{13} This was observed for monomers M2 (0.49) and M7 (0.52), for which a phenol functionality is shared, and monomer M6 (0.79) and model compound 9 (0.86) for which a carboxylic acid functionality is shared.

Among the acidic monomers, M4 is the only one with a positive slope which indicates that the monomer is amphoteric. M4 is a weaker acid with a pK\textsubscript{a} value of 11.28 compared to M2 with a pK\textsubscript{a} of 9.53 (Table 3.7), as it bears an alkyl alcohol group and not a phenol. The –CH\textsubscript{2}– group acts as an insulator to possible resonance stabilisation of lone pair electrons from the oxygen and even donates electrons making oxygen more electron rich, thus suppressing the ionisation of the hydroxyl group. Upon acidification of M4 prior to titration, the presence of a strong acid (Scheme 3.6) makes it a base by accepting the hydrogen ion from the acid.

\textbf{Scheme 3.6} Effect of the –CH\textsubscript{2}– group on the acidity of M4. The presence of the –CH\textsubscript{2}– group in M4 prevents electron delocalisation through to the ring and even donates electron to oxygen making it more electron rich and a very weak acid.

\textbf{3.2.4 Comparison with pK\textsubscript{a} Prediction Tools}

Monomers 1-7 and test model compounds 8 and 9 were titrated in various THF-water mixture compositions and the aqueous pK\textsubscript{a} values extrapolated via Yasuda-Shedlovsky plots. The extrapolated pK\textsubscript{a} values obtained were compared to values from pK\textsubscript{a} prediction tools available online. The low solubility of the monomers in higher water content media makes it difficult to obtain more than four data points for the Yasuda-Shedlovsky extrapolation. Although the Yasuda-Shedlovsky plots showed correlation values (R\textsuperscript{2}) of more than 0.90, good agreement with predicted pK\textsubscript{a} values will establish the validity of the potentiometric titration method.
pKa prediction tools use algorithms to predict pKa values based on the molecular structure of the compound without the need for experimental data, thus it is easy and practical to use. The pKa prediction programs used for this study included SPARC pKa\textsuperscript{16}, VCC pKa\textsuperscript{17} and Marvin sketch\textsuperscript{18} which are available online. In a comparative evaluation of the pKa predictive tools available commercially and online, VCC pKa was found to be the most predictive.\textsuperscript{19} The evaluation was done using the Gold Standard dataset, a database of drug-like molecules compiled by Alex Avdeef\textsuperscript{20} with pK\textsubscript{a} values determined mostly by potentiometric titration. Direct comparison of the prediction tool pK\textsubscript{a} values against the standard dataset pK\textsubscript{a} values was made as well as analysis of common outliers in terms of functional group moieties. On the other hand, SPARC has also been validated exhaustively against more than 5000 pK\textsubscript{a}s in various organic solvents and is capable of calculating not only pK\textsubscript{a} but various physical and chemical properties of compounds.\textsuperscript{21}

Table 3.7 presents the comparison of the experimental pK\textsubscript{a} values against predicted pK\textsubscript{a} values for monomers 1-7 and test model analytes 8 and 9. Most of the pK\textsubscript{a} experimental values fall within the range of the pK\textsubscript{a} prediction models, except for M4 and M5. It is to be noted that these monomers are the weakest in the series, i.e., M4 is the weakest (in fact, it is amphoteric) among the acidic monomers tested and M5 is the weakest among the basic monomers analysed. Monomer 5 was only titrated using three THF:water mixtures because of the insolubility of M5 in the other THF:water mixtures studied. The pK\textsubscript{a} prediction tools on the other hand, calculate the pKa’s of the compounds based solely on the molecular structure. Therefore, differences in the pK\textsubscript{a} values can be expected for this modelling.
Table 3.7 Literature aqueous pK\(_a\), Marvin pK\(_a\), VCC pK\(_a\) and extrapolated aqueous pK\(_a\) values of tested compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Literature</th>
<th>SPARC*</th>
<th>Marvin**</th>
<th>VCC ***</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>N/A</td>
<td>5.16</td>
<td>5.10</td>
<td>5.10</td>
<td>4.82</td>
</tr>
<tr>
<td>M2</td>
<td>N/A</td>
<td>9.36</td>
<td>9.90</td>
<td>9.20</td>
<td>9.53</td>
</tr>
<tr>
<td>M3</td>
<td>N/A</td>
<td>5.39</td>
<td>5.20</td>
<td>4.20</td>
<td>4.26</td>
</tr>
<tr>
<td>M4</td>
<td>N/A</td>
<td>14.9</td>
<td>15.00</td>
<td>N/A</td>
<td>11.28</td>
</tr>
<tr>
<td>M5</td>
<td>N/A</td>
<td>4.84</td>
<td>4.83</td>
<td>4.40</td>
<td>2.93</td>
</tr>
<tr>
<td>M6</td>
<td>N/A</td>
<td>4.08</td>
<td>4.07</td>
<td>4.10</td>
<td>4.69</td>
</tr>
<tr>
<td>M7</td>
<td>N/A</td>
<td>8.22</td>
<td>8.69</td>
<td>7.10</td>
<td>8.17</td>
</tr>
<tr>
<td>8</td>
<td>3.83(^{13})</td>
<td>3.88</td>
<td>4.08</td>
<td>3.70</td>
<td>3.98</td>
</tr>
<tr>
<td>9</td>
<td>4.19(^{14})</td>
<td>4.06</td>
<td>4.07</td>
<td>4.10</td>
<td>4.90</td>
</tr>
</tbody>
</table>

*SPARC pK\(_a\) (SPARC Performs Automated Reasoning in Chemistry) uses a mechanistic perturbation method to estimate pK\(_a\) value based on a number of models that account for electronic effects, solvation effects, hydrogen bonding effects and the influence of temperature.

**Marvin pK\(_a\) uses Chemaxon’s pK\(_a\) prediction tool applying partial charge increment, polarizability increment and structure specific increment from ionisation site-specific regression equations to form estimated pK\(_a\) as the sum of the above increments.

***VCC pK\(_a\) is online pK\(_a\) predictor that uses the prediction tool of Pharma Algorithms.
3.3 Conclusion

The pKₐ values of the biphenyl monomers 1-7 were determined by potentiometric titrations in THF-water mixtures. Aqueous pKₐ values for the monomers were obtained from extrapolation via the Yasuda-Shedlovsky method. The Yasuda-Shedlovsky plot of the titration shows decreasing acidity and basicity in increasing THF content for the acidic and basic monomers respectively. The dielectric constant of the THF-water mixture is lower than pure water causing relatively weaker proton ionisation for acidic compounds and promoted deprotonation of the conjugate acids of the basic compounds, hence lowering their acidity and basicity respectively.

3.4 Experimental

3.4.1 Chemicals and Reagents

Monomers 1-7 (Figure 3.1) were synthesised and purified as described in Chapter 2. Sodium hydroxide (NaOH), hydrochloric acid (HCl), 2-aminobiphenyl and 4-biphenyl carboxylic acid were purchased from Sigma-Aldrich and used as received. HPLC grade tetrahydrofuran (THF) was used as received. Carbon dioxide free deionized water was used freshly prepared by heating deionized water to 95°C.

3.4.2 Solubility Tests

The organic co-solvent for the titration was chosen based on the solubility of 0.050 and 0.10 mmol test compounds in 10.00 mL (0.0050 and 0.010 M, respectively) of binary mixtures of water and organic solvents at various volume ratios. The organic solvents tested were methanol, acetonitrile and THF.

3.4.3 Potentiometric titration

Potentiometric titrations were performed using a Metrohm 665 Dosimat autotitrator carried out at constant temperature (25°C ± 0.50) under a nitrogen atmosphere. In a typical titration, 10.00 mL of a 0.0100 M or 0.0050 M analyte solution was pre-acidified to low pH.
(~2.5) with 12 M HCl and titrated with 0.15 M NaOH to high pH (~11). Titrations were conducted in triplicate at different THF-water mixtures ranging from 35-55 % (v/v) THF.

### 3.4.4 Determination of $p_sK_a$ and $pK_a$

The $p_sK_a$ value in each THF-water mixture was calculated using the Global Minimisation algorithm for fitting potentiometric data developed in Matlab by Maeder et. al.\(^8\) The apparent acid dissociation constant ($p_sK_a$) and the log $[\text{H}_2\text{O}]$ were plotted against the inverse of dielectric constant of the binary mixture. Aqueous $pK_a$ was then extrapolated using the Yasuda-Shedlovsky method\(^9\) from the x-intercept of the linear plot as shown in Equation 3.1.

$$p_sK_a + \log [\text{H}_2\text{O}] = \frac{a}{\varepsilon} + b \quad \text{Equation 3.1}$$

where: $p_sK_a = $ apparent ionization constant in organic solvent-water mixture

$\varepsilon = $ dielectric constant of binary mixture

$a = $ slope

$b = $ intercept
3.5 References


Chapter 4

Evaluation of Vinyl Biphenyl Monomers as Functional Monomers for the Synthesis of Theophylline-Specific Molecularly Imprinted Polymers
4.1 Introduction

A number of variables, including but not limited to functional monomers, crosslinker, porogen, and polymerisation temperature, affect the efficiency of molecularly imprinted polymers (MIPs). However, it is the functional monomer which drives the formation of molecule specific cavities and is subsequently one of the dominant influences on MIP selectivity and affinity for the target molecule(s). Hence, selection and evaluation of appropriate functional monomers for a specific target is essentially the very basic step in the design and development of highly selective molecularly imprinted polymers.

This chapter deals with the evaluation of the performance of the synthesised biphenyl compounds as functional monomers for the preparation of MIPs selective to theophylline by thermal and microwave-induced polymerisation. Since the MIP synthetic method only utilises existing non-covalent interactions between the target/template and biphenyl monomer to form the molecule specific cavity, pre-synthetic template (T) - monomer (M) interaction studies were conducted via combined semi-empirical molecular modelling and NMR titrations. Both these methods are being used by several groups to assess T-M interactions prior to MIP preparation.

As with the synthesis of the biphenyl monomers, microwave-induced polymerisation was also employed. Microwave-induced polymerisation has been reported to be an efficient method for polymer preparation. A recent report from our group also showed successful preparation of MIPs selective for caffeine, an analogue of theophylline, by microwave induced polymerisation.

Theophylline was chosen as the model target/template molecule. Theophylline is a widely used drug for treatment of asthma; however, its use has continually declined over the years due to its adverse health effects and toxicity. For this reason, the use of theophylline has been limited and applied based on carefully regulated dosages. This requires regular monitoring of theophylline levels in the blood stream or urine, usually determined and quantified via conventional chromatography. As an alternative, several theophylline-imprinted polymers have been shown to be successful in the determination of theophylline
from human serum samples. These theophylline imprinted polymers were used as a solid phase sorbent in solid phase extraction or micro columns and as a sorbent in ligand binding assays. So far, these theophylline selective MIPs were prepared using methacrylic acid (MAA) as the functional monomer, taking advantage of its strong interactions with theophylline (via hydrogen bonding) favouring the formation of stable T:M clusters and, subsequently, the formation of stable target-specific cavities. The biphenyl monomers utilised in this study also possess functional groups capable of interaction with theophylline, and their potential as functional monomers, i.e., formation of stable T:M clusters, will be tested for the first time.

4.2 Results and Discussion

4.2.1 Template-monomer interaction studies

Semi-empirical molecular modelling using AM1 forcefield was conducted in order to predict the interaction of the target/template theophylline with the various biphenyl monomers, and possibly identify the T:M ratios where optimal T:M interactions are observed. The net interaction energy \( \Delta E^\circ_{\text{cluster}} \) values estimated from Equation 4.1 are shown in Table 4.1. These \( \Delta E^\circ_{\text{cluster}} \) values obtained for all T:M clusters are negative and indicate favourable cluster formation at T:M ratios from 1:1 to 1:5. It is to be noted, however, that the stability of the clusters arose from a combination of favourable template-monomer interaction and inter-monomer interactions as shown from the computer-generated images. Thus, whilst a strong hydrogen bond interaction (< 2.5 Å) between the template and all monomers has been observed, the presence of pi-pi interactions (Figure 4.1) between the monomer and the template or between monomers can affect the strength of the hydrogen bond. These interactions, which are prevalent in aromatic compounds, could affect the stability of the T:M cluster by either promoting or destabilising the hydrogen bonding sites. This type of interaction can be observed from the computer generated images of the clusters.

\[
\Delta E^\circ_{\text{cluster}} = \Delta H^\circ_{f(T-M \text{ cluster})} - (\Delta H^\circ_{f(M-M \text{ cluster})} + \Delta H^\circ_{f(T)})
\]

Equation 4.1
Table 4.1 Calculated $\Delta E^\circ$ (kcal/mol) values for template-monomer clusters from molecular modelling experiments for theophylline and monomers 1-7.

<table>
<thead>
<tr>
<th>Monomer Units</th>
<th>$\Delta E^\circ_{(cluster)}$ (kcal/mol) for Theophylline-Monomer Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>1</td>
<td>-6.3</td>
</tr>
<tr>
<td>2</td>
<td>-4.4</td>
</tr>
<tr>
<td>3</td>
<td>-7.9</td>
</tr>
<tr>
<td>4</td>
<td>-6.8</td>
</tr>
<tr>
<td>5</td>
<td>-3.1</td>
</tr>
</tbody>
</table>

Figure 4.1 Geometries of aromatic interactions as (A) edge-face, (B) offset stacked and (C) face-to-face stacked. Image taken from Waters.¹⁶

¹H NMR titration experiments were employed to further assess the interaction of theophylline and the biphenyl monomers, and to determine the stoichiometry of the predominant T:M complexes (from Job’s plots). To probe the interaction of each monomer with theophylline, the signal of the -NH proton, depicted as proton 1 (shown inset of Figure 4.2 for the structural formula of theophylline), was monitored for changes in chemical shifts as this proton is capable of forming hydrogen bonds. The signal of the vinylic proton, designated as proton 2, was also monitored as the interaction of the neighbouring
nitrogen atoms with the biphenyl monomers can also cause movement of its chemical shift.

The $^1$H NMR spectrum of theophylline in deuterated DMSO is shown in Figure 4.2, signals at 13.50 ppm and 8.01 ppm are attributed to protons 1 (–NH) and 2 (=CH), respectively, and are consistent with the spectrum obtained by Dong et al.\textsuperscript{17}

![Figure 4.2](image)

**Figure 4.2** $^1$H NMR spectrum of theophylline. Inset is the structural formula of theophylline showing the NH and vinylic CH protons designated as proton 1 (13.50 ppm) and proton 2 (8.01 ppm) in the $^1$H NMR spectrum. Protons 3 (3.45 ppm) and 4 (3.24 ppm) are from the two –NCH$_3$ groups in theophylline. Signals at 3.30 ppm and 2.50 ppm are from water and DMSO, respectively.

Modelling images of theophylline and monomer 1 (M1) clusters (Figure 4.3) revealed only a single point of interaction for hydrogen bonding, that is, between the basic –NH proton from theophylline and the nitrogen from the pyridine ring of M1. Addition of a second monomer unit (Figure 4.3 A) does not seem to enhance the interaction as the second monomer does not show any interaction with the template. However, addition of a third monomer unit was observed to result in a cluster formation that promotes interactions not only between monomers but also with theophylline whilst keeping the hydrogen bond between the nitrogen from one monomer unit with the –NH proton of the theophylline
intact (Figure 4.3 B). The favourable formation of the 1:3 T:M cluster was not sustained upon addition of more monomer units (Figure 4.3 C) as inter-monomer interactions predominate.

The $^1$H NMR titration results for theophylline and M1 are shown in Figure 4.4A. The titration, repeated twice, revealed a gradual increase in the movement of the chemical shift of proton 1 upon addition of up to three equivalents of M1 followed by a gradual decrease and plateauing after the addition of six equivalents of M1. This result is consistent with the modelling studies showing the hydrogen bond between proton 1 of theophylline and the nitrogen of M1 becoming stronger going from 1:2 (1.876 Å) to 1:3 T:M cluster (1.852 Å), and becoming weaker at higher T:M ratios, i.e., 1.899 Å at 1:5 (Figure 4.3). Nevertheless, the chemical shift of proton 2 was observed to become constant after the addition of two equivalents of M1 suggesting that optimum T-M interaction has already been reached. The Job’s plots of theophylline in Figure 4.4B suggest the existence of a mixture of T:M complexes showing a maximum at 1:2.3, i.e., 0.3 mol fraction theophylline. The existence of a maximum when theophylline > M1 could be attributed to inter-template interactions.
Figure 4.3  Molecular modelling images of theophylline-M1 (A) 1:2, (B) 1:3 and (C) 1:5 clusters. Labels T and M1 stand for theophylline and monomer1, respectively. The $\Delta E^\circ_{(\text{cluster})}$ values for these T-M clusters are -4.4 and -7.9 and -3.1 kcal/mol, respectively.
Figure 4.4  $^1$H NMR titration results for theophylline with M1. (A) Titration plot showing the change in chemical shifts of protons 1 and 2 of theophylline against the amount of equivalent monomer added. (B) Job's plots showing the complex induced shift (CIS) of protons 1 and 2 of theophylline against its mol fraction.
The molecular modelling results of theophylline and monomer 2 (M2) indicate a strong interaction between the two. The formation of favourable clusters between theophylline and M2 can be attributed to the number of hydrogen bonding interactions between them as seen in Figure 4.5. Being a hydrogen bond donor and acceptor, M2 is capable of forming hydrogen bonds with the basic –NH, the oxygens and the other nitrogen of theophylline. Thus, even in the presence of four monomer units, T:M interactions still predominate with minimal inter-monomer interactions observed.

The titration curve of theophylline with M2 (illustrated in Figure 4.6A) showed a gradual rise in the movement of the chemical shift of proton 1 upon the addition of incremental amounts of M2 consistent with the modelling results, showing an increase in T:M interactions in the presence of more monomer units. Interaction of M2 with theophylline has caused a downfield chemical shift of protons 1 and 2. As with M1, the movement of the chemical shift of proton 2 of theophylline was already minimal after the addition of two equivalents of M2. Whilst the Job’s plots in Figure 4.6B suggest the existence of a mixture of T:M complexes, it also shows that T:M stoichiometries between 1:2.3 and 1:5 are slightly favoured. The predominance of the T:M clusters in favour of theophylline, i.e., mol fraction of theophylline ≥ 0.50, could be discounted in the preparation of MIPs as the stoichiometric ratio of the T to the M always favours the monomer. The greater complex induced shifts for both protons in this region, indicating the presence of strong inter-template interactions.
Figure 4.5 Molecular modelling images of theophylline-M2 (A) 1:2 and (B) 1:4 clusters. Labels T and M2 stand for theophylline and monomer 2, respectively. The $\Delta E^\circ_{\text{cluster}}$ values for these T:M clusters are -15.1 and -13.1 kcal/mol, respectively.
Figure 4.6 $^1$H NMR titration (A) of theophylline and M2 plotting the change in chemical shifts of protons 1 and 2 against the amount of equivalent monomer added. Job’s plots (B) of theophylline and monomer 2 plotting the complex induced shift (CIS) of protons 1 and 2 against the mol fraction of theophylline.
The theophylline-monomer 3 (M3) clusters gave one of the smallest $\Delta E^\circ_{\text{(cluster)}}$ values (4-5 kcal/mol). Like M1, it is only a hydrogen bond acceptor and only shows hydrogen bonding with the basic $-\text{NH}$ from the nitrogen of its dimethylamino group. Nevertheless, the presence of more than one monomer unit seems to promote interaction between monomers, as shown by the overlapping of electron clouds in Figure 4.7B, enhancing the T:M interaction. This effect is also observed in the NMR titration shown in Figure 4.8A where incremental addition of M3 to theophylline has produced a significant change in the chemical shift of protons 1 and 2. This indicates a considerable change in electron density in the theophylline ring and the neighbouring $-\text{NH}$ proton for proton 2 to feel the effect. The Job’s plots for the system suggest favourable formation of a 1:1.5 (i.e., 0.4 theophylline mol fraction) T:M complex when the amount of monomer is higher than that of theophylline.
Figure 4.7 Molecular modelling images of theophylline-M3 (A) 1:2 and (B) 1:4 clusters. Labels T and M3 stand for theophylline and monomer 3, respectively. The $\Delta E^\circ_{\text{(cluster)}}$ value for both T:M clusters is -4.9 kcal/mol.
Figure 4.8 $^1$H NMR titration (A) of theophylline and M3 plotting the change in chemical shifts of proton 1 and 2 against the amount of equivalent monomer added. Job's plots (B) of theophylline and M3 plotting the complex induced shift (CIS) of protons 1 and 2 against the mol fraction of theophylline.
The Δ$E^{\circ\text{(cluster)}}$ values for the theophylline and monomer 4 (M4) clusters show comparable results to that of M2; however, unlike M2, molecular modelling (Figure 4.9) shows that, whilst T:M ratios higher than 1:1 allows more hydrogen bonding interactions between theophylline and M4 to form, the interaction between monomers also became predominant.

The result of the $^1$H NMR titration and Job’s plots of theophylline with M4 are presented in Figure 4.10. The $^1$H NMR titration with respect to proton 2 of theophylline indicates that a highly favourable T-M4 interaction is achieved after the addition of three equivalents of M4. However, based on hydrogen bonding effects, the $^1$H NMR titration revealed strong interaction of M4 with the basic -NH proton (proton 1) of theophylline only with the first two additions of monomer equivalents. The succeeding additions resulted in a decrease in the change in chemical shift of proton 1; in fact, the chemical shift of proton 1 no longer changed upon the addition of 8 equivalents of M4. This is in contrast to expectations that, with theophylline having four interaction sites available for hydrogen bonding and M4 being both a hydrogen bonding donor and acceptor, addition of increasing amounts of M4 should have promoted more hydrogen bonding interactions between theophylline and the monomer, and would have resulted in a gradual increase in the movement of the chemical shift of proton 1 as was observed with proton 2. This observation suggests that inter-monomer interactions prevailed at higher T:M ratios (i.e., >1:2) such that hydrogen bonding with proton 1 was weakened.

The Jobs’ plots shown in Figure 4.10 B point to maxima at 1:1-1:2 T:M stoichiometries, i.e., 0.3 – 0.4 mol fraction of theophylline, which is expected when inter-monomer interactions predominate at higher T:M ratios.
Figure 4.9 Molecular modelling images of theophylline-M4 (A) 1:2 and (B) 1:3 clusters. Labels T and M4 stand for theophylline and monomer 4, respectively. The $\Delta E^{\circ}_{\text{cluster}}$ values for these T-M clusters are -4.8 and -9.7 kcal/mol, respectively.
Figure 4.10 $^1$H NMR titration (A) of theophylline and M4 plotting the change in chemical shifts of protons 1 and 2 against the amount of equivalent monomer added. Job's plots (B) of theophylline and monomer 4 plotting the complex induced shift (CIS) of protons 1 and 2 against the mol fraction of theophylline.
Among the monomers modelled, monomer 6 (M6) gave the highest $\Delta E^\circ_{(\text{cluster})}$ values (Table 4.1). Figure 4.11 shows the molecular modelling images of some T:M clusters of M6 and theophylline. As with M4, M6 is also a hydrogen bond donor and acceptor capable of forming multiple hydrogen bonds with theophylline.

The $^1$H NMR titration and Job’s plots of theophylline and M6 are presented in Figure 4.12. The titration result shows a strong interaction of theophylline with M6 indicated by a steep rise in change in chemical shift upon addition of four equivalents of M6. There is a significant change in the electron density of the theophylline ring as the change in chemical shift of proton 2 also shows a steep rise like proton 1. Addition of M6 to theophylline caused a significant downfield shift of protons 1 and 2, indicating that the strong interaction of the carboxylic group of M6 with theophylline reduced the electron density in the ring and caused the downfield shift. However, the change in chemical shift starts to level off for protons 1 and 2 upon the addition of five equivalents of M6. A further addition of M6 no longer changed the chemical shift of protons 1 and 2 suggesting that, at this point, significant inter-monomer interactions had already occurred.

On the other hand, the Job’s plots indicate a mixture of T:M complexes in solution with a ratio of 1:1.5, i.e., 0.4 mol fraction of theophylline, favourably forming over other stoichiometries when the amount of M6 is higher than that of theophylline. The favourable formation of T:M complexes in favour of theophylline is due to inter-theophylline interactions.
Figure 4.11 Molecular modelling images of theophylline-M6 (A) 1:3 and (B) 1:4 clusters. Labels T and M6 stand for theophylline and monomer 6, respectively. The $\Delta E^{\circ}_{\text{(cluster)}}$ values for these T-M clusters are -10.8 and -17.7 kcal/mol, respectively.
Figure 4.12 $^1$H NMR titration (A) of theophylline and M6 plotting the change in chemical shifts of protons 1 and 2 against the amount of equivalent monomer added. Job's plots (B) of theophylline and M6 plotting the complex induced shift (CIS) of protons 1 and 2 against the mol fraction of theophylline.
The modelling images in Figure 4.13 show intramolecular hydrogen bonding occurring between the carbaldehyde oxygen and hydroxyl proton of monomer 7 (M7) competing with the hydrogen bonding between the hydroxyl proton of M7 and the oxygen of theophylline. This effect is observable in the \(^1\)HNMR titration curve in Figure 4.14 A which shows a steady downfield chemical shift of proton 1 and 2 after the first addition of M7, although the shift is not as steep as that of M6. This could be attributed to the presence of two functional groups in the ring (carbaldehyde and hydroxyl group) ortho to each other. The electron withdrawing effect of the carbaldehyde group caused delocalisation of the lone pairs of electron from the hydroxyl oxygen by inductive effect promoting this intramolecular hydrogen bonding and limiting hydrogen bonding between M7 and theophylline.

Although there is an observed interaction of M7 with the N-H from the titration curve and molecular modelling, these interactions did not seem to promote the formation of a stable T:M complex. This is evident in the Job’s plots in Figure 4.14 B showing no dominating T:M complex. Again, this could be attributed to the dominant effect of the intramolecular hydrogen bonding in M7 disrupting the monomer’s interaction with theophylline. Nevertheless, from the \(^1\)H NMR titration based on proton 2, optimum T:M interaction is already evident at a T:M ratio of 1:2.
Figure 4.13 Molecular modelling images of theophylline-M7 (A) 1:2 and (B) 1:4 clusters. Labels T and M7 stand for theophylline and monomer 7, respectively. The $\Delta E^{\circ}_{\text{cluster}}$ values for these T:M clusters are -9.8 and -5.0 kcal/mol, respectively.
Figure 4.14 $^1$H NMR titration (A) of theophylline and M7 plotting the change in chemical shifts of protons 1 and 2 against the amount of equivalent monomer added. Job's plots (B) of theophylline and M7 plotting the complex induced shift (CIS) of protons 1 and 2 against the mol fraction of theophylline.
In summary, results of the T:M interaction studies demonstrate that all biphenyl monomers 1-7 are capable of interacting favourably with theophylline via hydrogen bonding. Both molecular modelling and \(^1\)H NMR spectroscopy approaches suggest that favourable T:M interaction with minimal inter-monomer association can be achieved at T:M ratios \( \leq 1:4 \). Among the seven monomers studies, M2 and M6 gave the highest complex induced shifts, as obtained from Job’s plots at theophylline mol fraction < 0.5, and suggest that the interaction of M2 and M6 with theophylline is stronger than with the other monomers.

Monomer 5 was not included in this study because of the difficulty encountered in purification during large-scale preparation.

**4.2.2 Synthesis and Characterisation of Theophylline MIPs**

The performance of the synthesised biphenyl monomers as functional monomers for the synthesis of molecularly imprinted polymers was evaluated using theophylline as the template. Previously reported MIPs specific for theophylline commonly utilise ethylene glycol dimethacrylate (EGDMA) as the crosslinker and methacrylic acid (MAA) as the functional monomer.\(^{10,12,13,18-21}\) For this study, EGDMA was also employed as the crosslinker but MMA was substituted with six (except for monomer 5) of the seven synthesised biphenyl monomers as functional monomers. Because the biphenyl functional monomers have limited solubility in most porogens used for molecular imprinting, dimethyl formamide (DMF) was used as the porogen. Acetonitrile was used as a rebinding solvent as with most literature reports. All polymers obtained after polymerisation were bulk monoliths and were ground and sieved to obtain a uniform particle size (\(< 38 \mu m\)) for subsequent rebinding studies.

An EGDMA polymer was synthesised to determine the degree of theophylline binding to EGDMA alone. The crosslinker commonly makes up 80% of the polymer with each unit containing four oxygen atoms available for hydrogen bonding. Although previous reports\(^{12, 18,21}\) showed favourable binding of theophylline with the imprinted polymer over the non-imprinted polymer, indicating minimal crosslinker binding effect, the functional monomers
used in this study had not been used as functional monomers for theophylline MIPs. Thus, an assessment of any possible competitive interaction between EGDMA and theophylline is warranted.

Microwave polymerisation has been reported to be an efficient method for polymer preparation.\textsuperscript{6-8} A recent report from our group showed successful preparation of MIPs selective for caffeine, an analogue of theophylline, using this method.\textsuperscript{8} Thus, microwave polymerisation was also employed in the preparation of theophylline MIPs.

\textbf{4.2.3 Thermal Polymerisation}

Thermal polymerisation was conducted at a temperature of 60\textdegree{}C for 18 h using AIBN as an initiator. All thermal MIPs and NIPs (T-MIP and T-NIP) exhibited modest swelling in the binding solvent acetonitrile and the results are summarised in Table 4.3. The swelling factor, which is simply the ratio of the volume of the wet polymer to its dry volume, ranged from a low of 2.0 (T-MIP 4) to a high of 5.0 (T-MIP 6). These polymers, as shown in the SEM micrographs in Figure 4.15 and 4.16, possess highly porous surfaces as imparted by the DMF solvent.

\textbf{Table 4.3} Swelling factor of thermal MIPs and NIPs of monomers 1-4 and 6-7.

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
Polymer & Swelling factor\textsuperscript{a} & \\
& T-MIP & T-NIP \\
\hline
1 & 2.2 & 2.2 \\
2 & 3.5 & 3.5 \\
3 & 2.2 & 2.0 \\
4 & 2.0 & 2.0 \\
6 & 5.0 & 4.3 \\
7 & 3.2 & 3.3 \\
\hline
\end{tabular}
\textsuperscript{a} ratio of the volume of the wet polymer to its dry volume
\end{table}
Figure 4.15  Scanning electron micrographs of (A) T-MIP 1 and (B) T-NIP 1, (C) T-MIP 2 and (D) T-NIP 2 and (E) T-MIP 3 and (F) T-NIP 3 at 20000x magnification.
Figure 4.166  Scanning electron micrographs of (G) T-MIP 4 and (H) T-NIP 4, (I) T-MIP 6 and (J) T-NIP 6 and (K) T-MIP 7 and (L) T-NIP 7 at 20000x magnification.
4.2.3.1 Sorption study

A sorption study is a rebinding experiment employed to evaluate the imprinting effect of molecularly imprinted polymers. The imprinting factor, $IF^{22}$, was evaluated by comparing the binding of the template theophylline to the MIP and to the equivalent non-imprinted polymers (NIPs). A high imprinting factor indicates high affinity of the target molecule towards the MIP over its NIP counterpart, which is expected due to the presence of target/template-specific cavities formed during imprinting.

Varying amounts of MIPs and NIPs for each set of polymers were used to bind a constant amount of template over a constant binding period using 1.00 mL of 0.0800 mM theophylline in acetonitrile. The samples were shaken during the entire duration to provide maximum contact between theophylline and the polymers. A binding time of 18 h was chosen to ensure equilibrium binding in all cases. In order to determine the amount of bound theophylline, the concentration of free (unbound) theophylline was measured by HPLC and subtracted from the initial concentration of the theophylline binding solution.

The results of the sorption experiments are presented in Figures 4.17. Each graph shows the amount of theophylline using varying amounts of MIPs and NIPs compared against the EGDMA polymer. The sorption of theophylline by the EGDMA polymer (black columns) was observed to increase with increasing amount of the polymer and most likely the result of non-specific interactions of theophylline with EGDMA. Nevertheless, the amount of theophylline sorbed was less than 5% of the initial theophylline concentration in all cases.
Figure 4.17 Plot of bound theophylline against increasing amounts of thermally-prepared EGDMA polymer with no functional monomer (T-EGDMA) and (1) T-MIP 1 and T-NIP 1 prepared using monomer 1, (2) T-MIP 2 and T-NIP 2 prepared using monomer 2, (3) T-MIP 3 and T-NIP 3 prepared using monomer 3, (4) T-MIP4 and T-NIP4 prepared using monomer 4, (6) T-MIP 6 and T-NIP 6 prepared using monomer 6 and (7) T-MIP 7 and T-NIP 7 prepared using monomer 7. The sorption studies used the following conditions: 1.00 mL of 0.0800 mM theophylline in acetonitrile, with a binding time of 18 h.
The amount of theophylline bound by T-MIP 3, T-MIP 4 and T-MIP 7 are, within errors, comparable to the theophylline sorption exhibited by the EGDMA polymer. No binding discrimination can be observed between the MIPs and NIPs, indicating the absence of well-defined cavities for theophylline within the MIPs. Therefore, the affinity of theophylline to the MIP is comparable to that of the non-imprinted polymer. The result for T-MIP 3 is not surprising since M3 contains a bulky dimethylamine functionality that is found by modelling studies to be only capable of one-site interaction (H-bonding with proton 1) with theophylline as shown in Figure 4.7. On the other hand, while M7 of T-MIP 7 is capable of interacting with theophylline at multiple sites, it also shows intramolecular hydrogen bonding due to its carbonyl and hydroxy substituents ortho to each other (Figure 4.13). This possibly inhibited the formation of specific cavities for theophylline. Both modelling and NMR results showed favourable interactions between M4 and theophylline but failed to produce an effective MIP. A possible explanation is the preferential formation of monomer dimers², suppressing T:M interactions and the formation of specific cavities.

Both T-MIP 1 and T-MIP 6 showed enhanced the non-selective uptake of theophylline. However, there is no imprinting effect obtained for the two MIPs even though it exhibited a higher theophylline uptake over T-MIP 3, T-MIP 4 and T-MIP 7. As with M3, M1 is a hydrogen bonding acceptor that is only capable of one-site interaction with theophylline, which, because of its rigid structure, would possibly not be able to form a stable T:M cluster required for imprinting. The carboxylic acid functionality of M6, as with M4, was shown by modelling and NMR studies to favourably interact with theophylline, but under the synthetic conditions employed, it could undergo preferential dimerisation² minimising its interaction with the template.

Among all the polymers, only T-MIP 2 exhibited an imprinting effect and theophylline binding discrimination between the MIP and its NIP. It would appear that the phenolic structure of M2 enhanced its capability to form a stable T:M cluster for successful imprinting of theophylline. It has a rigid structure with a more flexible functional group (-OH) than M1 but less flexible than monomer 4 and 6 to be favourable for the formation of dimers. Molecular modelling shows the dimerisation of M6 and M4 to be energetically
more favourable than for M2, with $\Delta H^\circ_f$ values of -44.9 kcal/mol for M6 dimer, 19.6 kcal/mol for M4 dimer and 43.2 kcal/mol for M2 dimer.

Overall, this screening stage for the best MIP identified M2 as the most effective for imprinting theophylline with high selectivity, i.e., imprinting factors greater than 2, and the highest among the polymers investigated.

4.2.4 Microwave-induced polymerisation

Polymers prepared under conditions of varying temperatures and modes of polymerisation have been shown to vary in the selectivity of the imprinted polymer. For example, MIPs prepared at sub-ambient temperatures were found to be more enantioselective to L-phenylalanine anilide compared to thermally initiated polymerisation. Another study has shown that MIPs prepared at 40ºC demonstrated higher enantioselectivity to 3-L-phenylalanylaminopyridine than the one prepared at 10ºC which was not due to temperature effects but to incomplete polymerisation at lower temperature resulting in poor binding sites. Just recently, our group has reported the effect of microwave irradiation on the selectivity of a caffeine-imprinted polymer. The result shows that molecular imprinting via microwave polymerisation can enhance selectivity in the imprinted polymer which was attributed to a faster rate of polymerisation and ‘snap-freezing’ of the imprints. In line with this area of investigation, microwave polymerisation was also applied to the study of theophylline imprinted polymers.

The sorption experiment was used to screen MIPs prepared using M2 towards their ability to selectively bind the template theophylline. Of all the polymers tested, only T-MIP 2 displayed an IF greater than 2 in varying amounts of polymer used and bound the highest amount of theophylline (0.10 mg/mg) among the polymers investigated. Thus, MIP 2 was subsequently synthesised via microwave irradiation (MW-MIP 2) to investigate the effect of microwave polymerisation on the specificity and affinity towards theophylline using the same amount of monomer, crosslinker, initiator and solvent as used in the thermal synthesis. This mixture was sealed in a 10.0 mL microwave vial and polymerised at 100 W
for 15 min. A non-imprinted polymer (MW-NIP 2) was also synthesised under the same conditions. The effects of microwave power and polymerisation time were not investigated in this study.

4.2.5 Comparison between Thermal and Microwave Polymers

4.2.5.1 Physical Characteristics

The resulting polymer from microwave polymerisation was a less rigid polymer, i.e., less grinding was required, compared to that of the thermal polymer, which may be due to incomplete polymerisation, thus leading to reduced cross-linking as a direct consequence of the reduced polymerisation time (15 mins vs 18 hours thermally). The infrared spectra of the thermal and microwave polymers presented in Figure 4.18 show no significant difference between MIP 2’s and NIP 2’s of both polymers. However, there is a slight difference between thermal and microwave polymers, particularly the peak at ~1605 cm\(^{-1}\) attributed to the stretching of unreacted \(>\text{C}=\text{C}<\) of EGDMA. The relative ratio of the intensity of this peak to that of the intensity of the carbonyl group (\(>\text{C}=\text{O}\)) at 1760 cm\(^{-1}\) for microwave polymers was slightly higher than that of the thermal polymers suggesting a slightly higher degree of crosslinking in thermal polymers than for the microwave polymers.

Examination of the degree of crosslinking in the polymer is important because the selectivity of the resulting polymer is also influenced by the choice and amount of crosslinker used in the imprinted polymer.\(^{22}\) The crosslinker provides the scaffold that maintains the complex formed by the functional monomer and template to form a rigid polymer network.\(^{24}\) Therefore, an optimum amount of crosslinker increases the rigidity of a polymer required to maintain its cavity, whilst improving its selectivity.\(^{24}\)
Figure 4.18 shows the scanning electron micrographs of the thermal and microwave polymers. The polymers produced in both modes of polymerisation are porous with the MIP 2 and NIP 2 of each set exhibiting similar gross surface morphology. There seems to be a slight difference in surface morphology between the thermal and microwave polymers, with both MW-MIP 2 and MW-NIP 2 show rougher surfaces. This is not surprising considering that thermal polymerisation produced polymer monoliths that are more compact and harder than the microwave produced polymers.
Swelling measurements for both microwave and thermal polymers were obtained. Although both thermal and microwave polymers exhibited similar swelling, the microwave polymers are found to bind more theophylline than the thermal polymers. This difference in sorption might come from the difference in the mode of polymerisation between the two polymers.
4.2.5.2 Surface Area and Porosity (Brunauer, Emmett, Teller) Measurements

Surface area measurements via the Brunauer, Emmett, Teller (BET) gas adsorption method were conducted using carbon dioxide (CO$_2$) gas as adsorbent as previous attempts to use nitrogen gas as adsorbent were unsuccessful. The use of nitrogen gas as adsorbent would have allowed measurements of the pore size, pore volume and pore distribution of larger pores, meso- and macroporosity, because N$_2$ is a condensing gas capable of forming multilayers on adsorption. However, the use of CO$_2$ as an adsorbent only gave us information on the specific surface area and pore size distribution of micropores because CO$_2$ is a non-condensing gas only capable of forming monolayer adsorption.

Figure 4.20 shows the gas adsorption isotherms for T-MIP 2 and its corresponding NIP 2, and MW-MIP 2 and its corresponding NIP 2. It is interesting that whilst the difference in gas adsorption between T-MIP 2 and T-NIP 2 is minimal, a striking difference is noticeable between the adsorption isotherm of MW-MIP 2 and that of MW-NIP 2, neither of which resembles the adsorption isotherms of the thermal polymers.

At a relatively low pressure, there is a rapid increase in the volume of gas adsorbed by the MW-MIP 2 as indicated by the “knee” in the isotherm and this represents rapid monolayer adsorption at the micropore region of the MW-MIP 2 polymer. On the other hand, the MW-NIP 2 displayed a lower volume of gas adsorbed at the same relative pressure which indicates that MW-MIP 2 has a higher microporosity than MW-NIP 2. This difference in microporosity between MW-MIP 2 and MW-NIP 2 could be attributed to the template-effect which seems to impart a significant influence on the microporosity of MW-MIP 2 but not that of T-MIP 2. The pore size distribution covering the micropore region (< 10 Å) shown in Figure 4.20 confirmed the high distribution of pore size in the range 1-2 Å for MW-MIP 2 which is not observed with the rest of the polymers, including MW-NIP 2.

Figure 4.20 also suggests that both T-MIP 2 and T-NIP 2 have bigger pores than the microwave polymers as indicated by the higher volume of gas required to fill the pores at higher pressures. There is also evidence of a template effect as T-MIP 2 shows a higher gas volume at higher pressure compared to T-NIP 2.
From the BET measurements, it can be deduced that the template as well as the mode of polymerisation has influenced the formation of micropores in MW-MIP 2. As further investigation is necessary to understand this effect, we can only speculate that the faster rate of polymerisation under microwave conditions (15 mins versus 18 hours under thermal conditions) seems to induce the formation of micropores, and in the presence of a M2 “snap freezing” the template within the polymeric network resulting in a higher microporosity. It is speculated that the template is not fully enclosed during the “snap freezing”, which results in a micropore size of around 2 Å, which is less than the diameter of theophylline which is around 7 Å.

Figure 4.20 Gas adsorption isotherms of T-MIP 2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2.
The distribution of the pore size in thermal and microwave polymers is shown in Figure 4.21, which only covers the micropore regions of the polymers (< 10 Å). The presence of micropores in MW-MIP 2 is confirmed by the high distribution of pore size in the range between 1-2 Å. The rest of the polymers, including MW-NIP 2, show similar pore size distributions. It can be deduced from this experiment that the template as well as the mode of polymerisation have influenced the formation of micropores in MW-MIP. The induced polarisation caused by microwave irradiation has probably forced the alignment and dipole interaction of the hydroxyl group of M2 and the NH group of theophylline, with this being “snap frozen” during polymerisation and retained in the polymer network.

![Figure 4.19 Pore size distribution for T-MIP 2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2.](image)

The macroporosity of polymers has been explained by Albright to be due to the formation of clusters of microgel that are fused together at their interface. The meso and macroporosities are imparted by the voids within the clusters and the spaces between the microgels. A phase separation occurring rapidly during polymerisation, as in the case of microwave-induced polymerisation, will result in more fused microgels, and consequently
in lower surface areas than for a phase separation occurring later as in the case of thermal polymerisation. The specific surface areas of the T-MIP 2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2 derived from the BET gas adsorption measurements presented in Table 4.4 are consistent with Albright’s model. The thermal polymers exhibited higher specific surface areas than the microwave-prepared polymers. Both T-MIP 2 and MW-MIP 2 display higher surface areas (1.1 times higher) than their NIP 2 counterpart, which can be attributed to the presence of the template. The effect of the mode of polymerisation is also evident in the surface area as the thermal polymers exhibited higher surface areas than the MW polymers.

**Table 4.4** Specific surface area of T-MIP2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2 derived from BET measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-MIP 2</td>
<td>240</td>
</tr>
<tr>
<td>T-NIP 2</td>
<td>228</td>
</tr>
<tr>
<td>MW-MIP 2</td>
<td>185</td>
</tr>
<tr>
<td>MW-NIP 2</td>
<td>162</td>
</tr>
</tbody>
</table>
4.2.5.3 Sorption Study

MW-MIP 2 was subjected to sorption studies under the same conditions as for T-MIP 2. The results are presented in Figure 4.22 together with the sorption results of T-MIP 2.

![Figure 4.20](image-url)

**Figure 4.20** Plot of bound theophylline against increasing amounts of microwave and thermal MIP 2 and NIP 2.

The MW-prepared polymers displayed higher (~2 times) theophylline binding compared to thermally-prepared polymers in all polymer masses tested. In particular, MW-NIP 2 exhibited a higher uptake of theophylline than T-NIP 2, which is not consistent with BET measurements showing MW-NIP 2 to possess a lower surface area (Section 4.3.5.2) and pore volume (Figure 4.21) than T-NIP 2. However, measurement of their zeta potentials (Table 4.5) revealed the surface charge of MW-NIP 2 to be 40% lower than the surface charge of T-NIP 2 which means that adsorption of polar theophylline at the surface of MW-NIP 2 will meet less repulsion compared to binding at the surface of T-NIP 2.
Table 4.5 Zeta potential values of thermal and microwave polymers measured at pH 7.

<table>
<thead>
<tr>
<th>Polymer 2</th>
<th>Zeta Potential (mV)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIP 2</td>
<td>NIP 2</td>
</tr>
<tr>
<td>Thermal</td>
<td>-31.4 ± 4.84</td>
<td>-22.7 ± 2.88</td>
</tr>
<tr>
<td>Microwave</td>
<td>-13.1 ± 8.22</td>
<td>-8.91 ± 3.43</td>
</tr>
</tbody>
</table>

Although T-MIP 2 exhibited a higher imprinting factor than MW-MIP 2 in all masses tested, the difference in theophylline sorption ($\Delta B_{\text{theophylline}}$) between MW-MIP 2 and the corresponding NIP in all mass range is still higher (ranging from 0.008 to 0.024 mM) than those between T-MIP 2 and its corresponding NIP. This demonstrates the effect of the mode of polymerisation, in this case, the use of microwave irradiation, on the selectivity of MIP 2’s. This result is consistent with the finding of Turner et al.\textsuperscript{8} which also shows higher delta bound in microwave polymers than its thermal counterparts.

As with the thermal polymers, MW-MIP 2 also attained binding saturation with 30 mg of polymer. Thus, 30 mg of polymer was used in subsequent experiments.

4.2.5.4 Time-binding Study

Thirty milligrams of MW-MIP and NIP 2 and T-MIP and NIP 2 were incubated with 1.00 mL of 0.0800 mM theophylline in acetonitrile for various periods of time. The results of the time binding experiments are shown in Figure 4.23.
Figure 4.213 (A) Amount of bound theophylline (mM) as a function of time for T-MIP 2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2. (B) Imprinting factor (IF) of T-MIP 2 and MW-MIP as a function of time.
The trend in adsorption, as exhibited both by MW-MIP 2 and MW-NIP 2, in the time binding experiments is consistent with the adsorption study, where more theophylline was bound compared to their thermal counterpart. MW-MIP 2 already showed saturation at 2.0 h of binding, which also gave the highest imprinting factor among the various time studied. The saturation point of T-MIP 2 is also noticeable at 2.0 h and all imprinting factor values for T-MIP 2 are above 2 over the period of incubation studied. The imprinting factor of T-MIP 2 remains higher than MW-MIP 2 in the time-binding experiments. For the succeeding experiment, 2.0 h was used as the time of contact of polymers for saturation binding.

### 4.2.5.5 Saturation Binding

The sorption and time binding results for the thermal and microwave polymers were used to determine the optimum mass and time for saturation binding studies in order to characterise the nature of the binding sites found in thermal and microwave generated MIP 2 and NIP 2. Thirty milligrams of each polymer was incubated with increasing concentrations of theophylline in acetonitrile for a period of 2 hours. The amount of theophylline bound (mg/g polymer) was then plotted against the free concentration (mM) of theophylline, generating the binding isotherm curve. From the binding isotherm, the key parameters for characterisation of the affinity of the polymers towards theophylline, $B_{\text{max}}$ and the dissociation constant ($K_d$) were obtained.

The binding isotherms for the thermal and microwave generated NIP 2 and MIP 2 are presented in Figure 4.24. These binding isotherms were analysed using the Freundlich model and Graphpad PRISM 5.0 software and a summary of the binding parameters are presented in Table 4.6.
Figure 4.224 Binding isotherms generated for microwave (A) and thermal (B) MIP 2 and NIP 2. Thirty (30) mg of each polymer were incubated with increasing concentrations of theophylline ranging from 0.04 mM to 1.0 mM in acetonitrile for 2.0 h.
Table 4.6 $K_d$, $N_T$ and $m$ values obtained from Freundlich plots of the binding isotherms of thermal and microwave MIP 2 and NIP 2.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Binding Parameters</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_d$</td>
<td>$N_T$ (mg/g)</td>
<td>$R^2$</td>
<td>$m$</td>
</tr>
<tr>
<td>MW-MIP 2</td>
<td>1.38</td>
<td>0.72</td>
<td>0.98</td>
<td>0.71</td>
</tr>
<tr>
<td>MW-NIP 2</td>
<td>2.43</td>
<td>0.41</td>
<td>0.81</td>
<td>0.59</td>
</tr>
<tr>
<td>T-MIP 2</td>
<td>2.31</td>
<td>0.43</td>
<td>0.96</td>
<td>0.88</td>
</tr>
<tr>
<td>T-NIP 2</td>
<td>3.38</td>
<td>0.30</td>
<td>0.94</td>
<td>0.93</td>
</tr>
</tbody>
</table>

The Freundlich isotherm is a good measurement of the heterogeneity of noncovalent MIPs as investigated by Umpleby et al.\textsuperscript{28} Eleven out of twelve noncovalent MIPs from a literature survey were found to obey the Freundlich isotherm, obtaining regression correlation coefficients of greater than 0.95 in the log B and log F format.

The Freundlich isotherm defines the amount bound ($B$) as a power function to the free concentration ($F$) defined in Equation 4.2. The binding parameter $a$ is a measure of the number of binding sites ($N_T$) in the system and also equals the value of the affinity constant ($K_a$) when $m=1$. On the other hand, $m$ measures the heterogeneity of the system, with value ranges from 0 to 1, and it also measures the ratio of high affinity sites in the polymer. A heterogeneous system obtains a value of $m$ close to 0 whilst an $m$ value approaching 1 indicates increasing homogeneity. To obtain the binding parameters, $a$ and $m$, the Freundlich isotherm is transformed to its linear form as shown in Equation 4.3.

\[
B = aF^m \quad \text{Equation 4.2}
\]
\[
\log B = m \log F + \log a \quad \text{Equation 4.3}
\]
The Freundlich isotherm is best applied at lower concentration regions where the adsorption has not yet reached saturation level and, unlike Scatchard analysis, only requires limited experimental data for fitting and obtains binding parameters in high accuracy.

The Freundlich plot of microwave and thermal polymers, and each of their corresponding affinity distributions in semi log and log format of $N_T$ and $K_d (K_a^{-1})$ are shown in Figure 4.25. From the calculated binding parameters in Table 4.6, the MW-MIP and both thermal polymers obey the Freundlich isotherm with $R^2 \geq 0.94$ except for the MW-NIP which has an $R^2$ of 0.81. The differences in the nature of the binding sites in each polymer can then be assessed by the heterogeneity index, m, obtained as the slope in the linear plot of the Freundlich isotherms. MW-MIP 2 displayed an m value of 0.71 compared to 0.59 for MW-NIP 2. This suggests that MW-MIP 2 has a slightly more homogenous mix of affinity sites. The $K_d$ value of MW-NIP 2, on the other hand, is 1.8 times higher than MW-MIP 2 (2.43 vs 1.38) which indicates that the binding sites in MW-MIP 2 have a higher affinity for theophylline than the binding sites in MW-NIP 2, consistent with the expected imprinting effect. In addition, MW-MIP 2 also exhibits a higher number of binding sites ($N_T$) compared to MW-NIP 2 (0.72 and 0.41 mg/g, respectively).

The Freundlich isotherm (Figure 4.25A) of the thermal polymers reveals that T-MIP 2 and T-NIP 2 have similar m values of 0.88 and 0.93, respectively. These values are closer to 1, which indicates that the binding sites of these polymers are more homogenous than for the MW-polymers. The higher m value also indicates a more discrete distribution of affinity sites within the polymers than for the microwave polymers. The $K_d$ value for T-NIP 2 is 1.5 times higher than that of T-MIP 2 confirming the presence of high affinity sites in T-MIP 2 most likely introduced during the imprinting process. Interestingly, the ratios of the $K_d$ of the MIP to the NIP of the thermal and microwave polymers are comparable and suggest, that whilst the mode of polymerisation seems to influence the surface porosity and binding sites, as seen from the results of the BET measurements and the sorption studies, it does not have an apparent impact on the nature of the template-specific cavities formed during imprinting.
As with the MW polymers, T-MIP 2 has a higher number of binding sites \( (N_T) \) than T-NIP 2 however, MW-MIP 2 displayed a higher \( N_T \) than T-MIP 2. This is in agreement with the observed sorption behaviour of the MIPs towards theophylline where MW-MIP 2 bound more theophylline than T-MIP 2. Similarly, MW-NIP 2 displayed higher \( N_T \) than T-MIP 2. In addition to the surface being more favourable for theophylline binding because of a low surface charge, i.e., zeta potential, it can also be speculated that the “snap freezing” effect of microwave polymerisation probably created a “functional group” specific cavity for theophylline. As mentioned earlier, microwave induced polymerisation has caused an alignment of polar groups in the solution, the –NH from theophylline and the –OH from M2 in this case. Upon rapid microwave heating, which caused a faster polymerisation rate, this aligned –NH and OH functional groups could be “snap frozen” in the sub-surface of the polymer, creating a functional group specific cavity that can fit the –NH group of theophylline leaving the rest of the molecule exposed at the surface. This also explains the presence of a high distribution of micropore sizes in MW-MIP 2 (≤7Å), smaller than the diameter of theophylline.
Figure 4.235 Freundlich isotherms for (A) thermal and (D) microwave MIP 2 and NIP 2. The corresponding affinity distribution in N vs log K format of (C) thermal (E) microwave MIP 2 and NIP 2. The log N vs log K format is also shown in (C) for thermal and (F) microwave MIP 2 and NIP 2.
4.2.5.6 Cross Reactivity and Selectivity Study

Molecularly imprinted polymers are designed to be target specific and capable of selective determination of the template from a pool of analogues\textsuperscript{29} to be useful in their intended application. Hence, it is important to evaluate their selectivity and specificity towards a template in the presence of its analogues or other compounds by conducting competitive binding (selectivity study) or non-competitive binding (cross reactivity study). Competitive binding assays involve the rebinding of the template in the presence of analogues in the same environment, whereas non-competitive binding tests require a separate environment for each analyte.

In this study, caffeine was used as the competing analogue to theophylline with MIP 2. The structures of theophylline and caffeine are shown in Figure 4.26. Results of the cross reactivity (CR) and selectivity studies (S) are presented in Figure 4.27.

![Figure 4.26](image_url) Structures of theophylline and caffeine used in the cross reactivity and selectivity studies for T-MIP 2 and T-NIP 2 and MW-MIP 2 and MW-NIP 2.
Under a non-competitive binding situation, both T-MIP 2 and MW-MIP 2 show a preference for theophylline over caffeine which indicates that theophylline-specific cavities were formed during imprinting. Interestingly, the uptake of caffeine by T-MIP 2 is similar to that of T-NIP 2 which suggests that caffeine was only superficially bound to T-MIP 2 and that the surface of T-NIP 2 can accommodate both theophylline and caffeine non-preferentially. MW-NIP 2, on the other hand, exhibited higher theophylline uptake than caffeine suggesting that the surface of MW-NIP 2 also contains binding sites that can accommodate theophylline but not caffeine. This is probably caused by the functional group’s specific cavity induced by microwave polymerisation, in which MW-MIP highly adsorbs theophylline because of its interaction with the NH group to the “functional group” specific cavity. These results are consistent with the BET adsorption measurements. Figure 4.21 showed that the thermal polymers possessed larger pores that can accommodate both theophylline and caffeine hence the non-preferential binding of the two analytes. The
microwave polymers, on the other hand, possess a high volume of micropores that could fit and interact with the –NH moiety of the theophylline molecule in addition to the larger pores than can accommodate both theophylline and caffeine.

The competitive binding assay of theophylline and caffeine showed a reduction in the uptake of theophylline and caffeine by both thermal and microwave polymers although preferential binding of theophylline over caffeine was still evident as with the cross reactivity study. Whilst MW-MIP 2 retained its selectivity to theophylline such that the uptake of theophylline by MW-MIP 2 was still higher than that of MW-NIP 2, no apparent difference in analyte binding can be observed between T-MIP 2 and T-NIP 2. The reason for this change of binding behaviour is unclear but can most likely be influenced by the concentration of the analytes in the binding solution, which, effectively, is twice the concentration used in non-competitive binding, i.e., 0.20 mM of theophylline and 0.20 mM of caffeine. A speculation is that the surface charge (zeta potential) of the thermal polymers (Table 4.5), which is higher than that of the microwave polymers, prevents the analytes from accessing the binding sites within the polymer or it could be that the analyte molecules form a secondary surface layer blocking access to the binding sites. In addition, a possible formation of dimers between theophylline and caffeine can also cause the reduced uptake of caffeine in the competitive binding study. Callahan et al.\textsuperscript{30} reported the formation of dimers by caffeine, detected as a strong signal from mass spectroscopy, whilst surprisingly theophylline did not. However, it may be reasonable to assume that theophylline and caffeine may also interact in a similar fashion (to caffeine:caffeine) due to their structural similarity. Theophylline and caffeine may form a dimer in solution, thereby competing with T-MIP and thus resulting in reduced theophylline recognition without any otherwise obvious competition from caffeine.
4.3 Conclusion

The T-M interaction studies demonstrated that biphenyl monomers 1-4, 6 and 7 are capable of interacting favourably with theophylline via hydrogen bonding. Both molecular modelling and \(^1\)H NMR spectroscopy approaches suggest that favourable T-M interaction with minimal inter-monomer association can be achieved at T:M ratios \(\leq 1:4\). Among the monomers studied, M2 and M6 gave the highest complex induced shifts, obtained from Job’s plots at a theophylline mol fraction < 0.5, and suggest that the interaction of M2 and M6 with theophylline is stronger than with the other monomers.

The biphenyl monomers were evaluated as functional monomers for thermally prepared theophylline imprinted polymers. From the six monomers evaluated via sorption experiments, T-MIP 2 synthesised from monomer 2 showed higher affinity and selectivity for theophylline. The same polymer synthesised using monomer 2 was prepared employing microwave polymerisation to investigate the effect of the mode of polymerisation in the imprinted polymer. The microwave prepared polymers exhibited high affinity towards theophylline and greater selectivity to the template (theophylline) than the competing analyte (caffeine) compared to the thermal MIP.

The nature of binding sites obtained in the polymers is influenced by the mode of polymerisation. Thermal polymers obtained more homogenous binding sites because of the equilibrium driven thermal polymerisation, whilst microwave polymers resulted in a greater distribution of heterogeneous binding sites which was attributed to the “snap freeze” polymerisation. However the effect of microwave polymerisation leads to formation of micropores in the MW-MIP 2 which is not evident in MW-NIP 2 and the thermal polymers.
4.4 Experimental

4.4.1 Chemicals and Reagents

All monomers used were synthesised as previously described in Chapter 2. Theophylline (anhydrous, ≥99%) and caffeine were obtained from Sigma-Aldrich and were used as received. Ethylene glycol dimethacrylate (EGDMA), from Sigma Aldrich, was purified using aluminum oxide (activated, basic) prior to use. Azobisbutyronitrile (AIBN, Dupont) was recrystallised from acetone prior to use. HPLC grade (Fluka) dimethylformamide (DMF), acetonitrile (ACN) and methanol (MeOH) were used as received. Glacial acetic acid, triethylamine (TEA) and deuterated dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich and used as received.

4.4.2 Molecular Modelling

Template-monomer molecular interactions were modelled using Spartan '04 software implementing the semi-empirical Austin Model 1 (AM1) using equilibrium geometry calculations. This molecular orbital computational method predicts the stable configuration of the template (T), monomer (M), M-M clusters and T-M clusters and calculates their standard heats of formation (ΔH_f). The molecules were randomly positioned and the T-M clusters were modelled with respect to increasing the template-monomer ratio from 1 to 4. To account for the M-M interaction, the M-M clusters of up to five molecules were also surveyed. The energy of interaction of the T-M clusters, ΔE^{(cluster)}(cluster), at different molecular ratios were then calculated using Equation 4.1 (as presented in Section 4.2.1).^{14}

4.4.3 1H NMR Study

1H NMR titration. Typically, from a stock solution of 500.0 mM in deuterated DMSO, incremental amounts of 50.0 µL of the biphenyl monomer was added to a 0.50 mL of 50.00 mM solution of theophylline in deuterated DMSO. The proton signal from the NH and vinylic protons of theophylline (Figure 4.2) were monitored as incremental amounts of monomers were added. The titration curve was then constructed from the plot of change in chemical shift, δ (ppm), of the NH and vinylic protons of theophylline against increasing
amount of monomers added. All $^1$H NMR spectra were recorded on a Bruker 300 MHz spectrometer.

**Job’s Plots.** Appropriate volumes of theophylline (51.5 mM) and monomer (51.5 mM) stock solutions prepared in deuterated DMSO were combined in an NMR tube to make up 0.50 mL solution with the resulting theophylline:monomer ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. The proton signals from the NH and vinylic protons of theophylline (Figure 4.2) were followed and recorded. A Job’s plot was then constructed from the plot of theophylline:monomer complex-induced shift (ppm) of the NH and vinylic protons of theophylline against the mole fraction of theophylline.

### 4.4.4 Polymer Synthesis

Typically, molecularly imprinted polymers were prepared as follows. Pre-determined quantities of the template theophylline (0.50 mmol, 90.08 mg), biphenyl monomer M1 (1.00 mmol, 271.8 mg), EGDMA (10.00 mmol, 1.888 mL) and AIBN (1 mmol %, 35.3 mg) were dissolved in DMF (4.00 mL) in a 10-mL vial, purged with nitrogen gas for 5 min, sealed and polymerised at 60 ± 1.0°C in a Thermoline oven for overnight. The polymers were then ground and sieved to a particle size <38 µm. The template was extracted via Soxhlet extraction in methanol:acetic acid (90:10 v/v) for 24 h and further washed in methanol via Soxhlet extraction for another 24 h. In some cases, a blank is run to check leaching of the template form the polymer. But according to Phillip et al., Soxhlet extraction of theophylline with methanol-acetic acid (8:2, v/v) from EGDMA-crosslinked polymer for 2 h followed by extraction with ethanol and chloroform, was sufficient enough to remove theophylline. The polymers were then dried at 40°C in a vacuum oven. A similar procedure was applied to non-imprinted polymers with the exception that no template was added.
Table 4.2 Preparation of T-MIPs and T-NIPs and poly-EGDMA.

<table>
<thead>
<tr>
<th>Polymer a</th>
<th>monomer (mmol)</th>
<th>theophylline (mmol)</th>
<th>EGDMA (mmol)</th>
<th>DMF (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-MIP 1</td>
<td>1.500</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>T-MIP 2 b</td>
<td>1.000</td>
<td>0.250</td>
<td>5.00</td>
<td>2.00</td>
</tr>
<tr>
<td>T-MIP 3</td>
<td>1.500</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>T-MIP 4</td>
<td>1.000</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>T-MIP 6 b</td>
<td>1.000</td>
<td>0.250</td>
<td>5.00</td>
<td>2.00</td>
</tr>
<tr>
<td>T-MIP 7</td>
<td>1.000</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>poly-EGDMA</td>
<td>-</td>
<td>-</td>
<td>10.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

aPolymerisation was conducted at 60°C for 18 h. The same formulation was used for the preparation of T-NIPs except in the addition of theophylline.
bThe polymers were prepared in smaller amount due to limited amount of monomers.

For the microwave synthesis, a similar procedure was used as for the thermal synthesis, except that polymers were polymerised in a 10 mL pressure vessel via microwave heating using CEM Discoverer Benchmate at 60ºC using 100W for 15 min.

4.4.5 Sorption Study

A sorption study was performed using batch binding experiments where various amounts of polymers ranging from 10.0 to 50.0 mg were placed into a 5 mL vial to which was added 1.00 mL of 0.0800 mM theophylline in acetonitrile. The mixture was shaken for 18 h, filtered and the filtrate analysed directly by HPLC. The amount of free theophylline was subtracted from the initial binding solution concentration to obtain the amount of theophylline bound in the polymer. All binding experiments for this study were done in triplicate to ensure reproducibility. A sorption isotherm was generated from the plot of theophylline bound (mM) versus amount of polymer (mg).

4.4.6 Time-Binding Study

The optimal weight obtained from the adsorption study was 30.0 mg and this was the quantity used for determining the optimum time of template binding. To a set of triplicate of 30.0 mg of polymer, 1.00 mL of 0.0800 mM theophylline was added and the mixture shaken for a designated time of contact. The binding times investigated were 0.5, 2.0, 4.0,
7.0 and 18 hours. After binding, the mixtures were filtered and the filtrates analysed by HPLC. The amount of bound theophylline was then obtained by subtracting the amount of theophylline left in solution from the initial concentration. A plot of the amount of theophylline bound versus time of contact was generated to determine the optimum time of contact for binding theophylline.

4.4.7 Saturation Binding

The optimum weight and time of contact obtained from sorption and time-binding studies were used for the saturation binding experiments. A series of 30.0 mg of polymers were incubated with different concentrations of theophylline for 2 h, after which, the mixtures were filtered and the filtrates analysed directly by HPLC. The amount of bound theophylline was then obtained by subtracting the amount of theophylline left in solution from the initial concentration. A plot of bound template against free theophylline concentration was then generated to visualise the saturation binding isotherm of the polymers.

4.4.8 Binding measurements

Theophylline rebinding was measured using a Shimadzu Prominence HPLC equipped with SPD-20A/M20A lamp and LC-20AD pump. Analyses were performed on an Econosphere C18 5µ column (150 x 4.6 mm) using isocratic elution of MeOH:water (80:20, v/v, with 0.10 % TEA) at 1.00mL/min at 40ºC. The injection volume used was 10 µL and the chromatograms were recorded at 254 nm and the peak at 1.25 min attributed to theophylline was measured.

A five-point calibration curve from five standard concentrations of theophylline with linear regression values ($R^2$) of 0.9996 was used to determine the concentrations of theophylline after binding measurements.

4.4.9 Scanning Electron Microscopy

Morphology of the polymers was examined using a Phillips XL30 scanning electron microscope. Each polymers was deposited on a sticky carbon tab and coated with gold
using a SPI gold spotter coating unit. SEM micrographs of the polymers were obtained at 20000x magnification at 15.0 kV.

**4.4.10 Swelling Measurements**

Thirty milligrams of each polymer were packed into an NMR tube and the height of the dry polymer measured. A solution of theophylline (1.00 mL of 0.0800 mM) in acetonitrile was added and allowed to soak for 24 h. Polymers were allowed to settle and the bed height of the swollen polymers was measured. The swelling factor was calculated from the ratio of the bed height of the swollen polymer to the dry polymer.

**4.4.11 Zeta Potential**

Zeta potential measurements were performed using a Malvern Nanosizer S fitted with a maintenance-free folded capillary cell (DTS 1060). Very dilute suspensions of polymers were prepared using ~ 0.75 mL deoxygenated distilled deionized water (non-equilibrated in air, 18.2 MΩ cm⁻¹). Measurements were performed at 25.0 °C, pH 7.0 in 5 replicates.

**4.4.12 Specific Surface Area and Porosity (Brunauer-Emmett-Teller)**

Gas adsorption analysis was carried out using a Micrometrics ASAP 2020 Accelerated Surface Area and Porosity instrument (Norcross, GA, USA). The analysis was carried out using 100 mg of sample and degassed at 110°C under vacuum for 12 h to remove any adsorbed solvent and water. The adsorption isotherm of this degassed sample was then measured using carbon dioxide as the adsorbate at a temperature of 500°C covering the partial pressure \((P/P_0)\) range 1x10⁻⁶ to 0.03. The specific surface area of each sample was determined from the adsorption data using the linearised BET equation ¹, whilst the pore size distribution was calculated using the BJH (Barrett, Joyner & Halenda) model.
4.5 References


Chapter 5

Evaluation of Vinyl Biphenyl Monomers as Functional Monomers for the Synthesis of Molecularly Imprinted Polymers for Phosphate-containing Templates
5.1 Introduction

One of the impetuses for this project was the potential for these biphenyl monomers to be used for the development of MIPs for phosphate-bearing compounds. The long term aim is the development of guanosine triphosphate (GTP) MIPs that mimic the GTPase domain of dynamin and can then be used to screen for potential lead compounds for dynamin inhibitors.

Dynamin\(^1\) is a 100kDa GTPase that mediates endocytosis,\(^2\) a mechanism of internalization of extracellular materials and fluids by the cell that requires GTP hydrolysis. However, pathogens and viruses can invade this pathway to gain entry and spread infection. Entry of these unwanted extracellular materials can be prevented by dynamin inhibitors. Dynamin inhibitors\(^3\) are compounds that interfere with the activity of dynamin’s GTPase to stop endocytosis by competitive binding with GTP.

The choice of biphenyl monomers and its application in molecular imprinting was influenced by the combinatorial engineering strategy of redesigning natural proteins to specifically create novel catalytic sites employing a limited number of amino acids but yet able to perform the required function.\(^4\) Inspired by this previous work, it is envisaged that it is possible to construct a ‘plastic’ (polymeric) GTPase mimic, i.e., MIPs, using polymerisable ‘amino acid mimics’ (biphenyl monomers) that complement the pK\(_a\)s (acidity/basicity) and functional characteristics of the amino acids comprising the active site of Dynamin GTPase.

The biphenyl monomers chosen for this study have favourable characteristics that warrant their evaluation as potential functional monomers for a polymeric GTPase mimic. These biphenyl compounds possess various functional groups that cover a wide range of pK\(_a\)s, from an acidic 4 to a basic 9 (Chapter 3), whilst the biphenyl moiety can impart strength to the bulk polymers, a characteristic that promotes the creation of robust molecular imprints even at lower crosslinker concentration.
The aim of this part of the project was to evaluate the potential of these polymerisable biphenyl monomers as functional monomers for the imprinting of simpler (than GTP) phosphate-bearing templates which can then form the basis for the development of GTP MIPs. Two single phosphate-containing compounds, phosphotyrosine (PT), a phosphorylated tyrosine, and phenylphosphonic acid (PPA) shown in Figure 5.1 were investigated as potential templates.

Phosphotyrosine, a phosphorylated tyrosine, was of interest because of its potential as a biomarker for diseases. Reversible phosphorylation of proteins, e.g. tyrosine, plays a significant role in cellular processes because an imbalance of protein kinases and protein phosphatases that control the level of phosphorylation in proteins is implicated in several diseases. Phenylophosphonic acid (PPA) was chosen because it is more readily soluble than PT and is commonly used as model template for the development of phosphate specific MIPs.
Previous reports on the preparation of phosphate specific MIPs have focused on the use of urea based\textsuperscript{7,8} or thiourea based monomers.\textsuperscript{6,9} In this study, we propose to use biphenyl monomers for the preparation of phosphate-specific MIPs.

5.2 Results and Discussion

As with the theophylline systems discussed in Chapter 4, initial work involved the evaluation of the degree of interaction between the phosphate-containing template and the biphenyl monomers utilising semi-empirical modelling (Spartan ’04, AM1 field) and $^{31}$P NMR spectroscopy. $^{31}$P NMR spectroscopy was used for titration because it can directly probe any specific interaction of the phosphate group with the functional monomer, as changes in chemical shift of the phosphorous in the solution implies an electronic interaction of the phosphate group with the functional monomer. The solubility of phosphotyrosine (PT) in organic solvents was problematic. As it was only found soluble to be soluble at 2.0 mM in DMSO, detection of the proton peaks by $^1$H NMR spectroscopy proved difficult and unreliable. However, $^{31}$P NMR was found sensitive to 2 mM PT and was therefore used in the titration experiments.

5.2.1 Phosphotyrosine: Initial Investigations

The possible interactions of phosphotyrosine (Figure 5.1) with the six biphenyl functional monomers were modelled using Spartan ’04, AM1. Varying ratios of phosphotyrosine and each functional monomer were modelled to predict the optimum T:M complex for MIP synthesis. The same calculation for $\Delta E^{\circ}_{\text{cluster}}$ from Chapter 4 was applied to each T:M complex of phosphotyrosine and functional monomer. The favourable interaction of the biphenyl monomers with phosphotyrosine is denoted by the high negative values obtained in the modelling as summarised in Table 5.1. These high negative values are not surprising because phosphotyrosine is capable of multiple hydrogen bonding via its phosphate, amino and carboxylic acid functionalities as was observed with its 1:4 T:M cluster with M2 (Figure 5.1 B).
The modelling image of the 1:2 cluster of PT and M2 (Figure 5.2 A) shows preferential interaction of the monomer at the phosphate group of PT forming multiple hydrogen bonds. The addition of four monomers resulted in crowding, with only one monomer unit interacting with the carboxylic group of PT.

Table 5.1 Calculated $\Delta E^\circ_{\text{(cluster)}}$ (kcal/mol) values for T:M clusters from molecular modelling experiments of phosphotyrosine and biphenyl monomers.

<table>
<thead>
<tr>
<th>Monomer Units</th>
<th>$\Delta E^\circ_{\text{cluster}}$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>1</td>
<td>-8.8</td>
</tr>
<tr>
<td>2</td>
<td>-7.1</td>
</tr>
<tr>
<td>3</td>
<td>-10.7</td>
</tr>
<tr>
<td>4</td>
<td>-16.4</td>
</tr>
<tr>
<td>5</td>
<td>-15.1</td>
</tr>
</tbody>
</table>

Determination of the optimum T:M complex ratio prior to polymerisation was also conducted using $^{31}$P NMR titration. The change in chemical shift of phosphate contributed by possible electronic interaction with the solvent was ruled out by blank titration with deuterated DMSO. However, any interaction of the monomer with the carboxyl and amide group could not be monitored in the $^{31}$P NMR titration.
Figure 5.2 Molecular modelling images of PT:M2 (A) 1:2 and (B) 1:4 clusters. Labels T and M2 stand for theophylline and monomer 2, respectively. The $\Delta E^0_{\text{cluster}}$ values for these T:M clusters are -20.5 and 15.1 kcal/mol, respectively.

Figure 5.3 shows the results of the titrations of PT with the six biphenyl monomers, including a blank titration with DMSO. The blank titration of PT with DMSO resulted in a negligible change in chemical shift of phosphorous compared to the change observed with the monomers. Among the six monomers investigated, the titration of PT with M2 produced the highest change in chemical shift of phosphorous whilst M6 produced the
lowest. The titration caused an upfield shift of the phosphorous signal, suggesting that an interaction of the neighbouring oxygen atoms of phosphorous with M2. The first three additions of M2 caused a gradual increase in the chemical shift of phosphorous which then started to plateau after 4 additions (Figure 5.3). The observed large shift (Figure 5.4) of the phosphorous signal can be attributed to multiple bonds being formed between the three monomer units and the phosphate group. The result of the titration with M6 was unexpected, as the carboxyl group is also a strong proton donor and acceptor. We speculate that M6 preferentially binds to the carboxyl group of PT to form a stable 6-membered dimer.

Figure 5.3 $^{31}$P NMR titrations of phosphotyrosine with the biphenyl monomers showing the change in chemical shift of phosphorous against the amount of equivalent monomer added.
Figure 5.4 $^{31}$P NMR titration of PT with M2 showing the movement of the phosphorous peak with incremental addition of M2 from a-f. The peak at -4.48 ppm is attributed to the phosphorous peak of PT.

From molecular modelling and $^{31}$P NMR results, M2 emerged as the most favourable monomer for PT. M2 gave one of the highest $\Delta E^o_{\text{(cluster)}}$ values and its molecular modelling images show preferential interaction of M2 with the phosphate group. This is further confirmed by the $^{31}$P NMR titration, with M2 showing the highest change in chemical shifts among the monomers investigated.

$$\text{4-Vinyl-biphenyl-4-ol (M2)}$$
The preparation of a PT MIP using M2 was attempted, however the solubility of PT is limited to water and 2.00 mM in DMSO. As a solution to this solubility problem, phosphotyrosine could have been protected according to Emgenbroich et al.’s\textsuperscript{8} approach. Instead, we opted to use a more soluble phosphate containing molecule. One such molecule is phenylphosphonic acid (PPA), a smaller molecule than PT with no other functional groups present except the phosphate group. Although PPA differs in conformational geometry with PT (PPA lacks the oxygen atom linking the phenyl to phosphate), it has been successfully used as a test template for the development of phosphate specific MIPs.\textsuperscript{6}

5.2.2 Phenylphosphonic acid

Phenylphosphonic acid was used in the preparation of a MIP because it was found more soluble in organic solvents compared to phosphotyrosine. The choice of M2 as the monomer to take forward was based on the molecular modelling and \textsuperscript{31}P NMR studies presented in the previous section. Out of the six monomers, it was M2 that showed evidence of the most favourable interaction with the phosphate group of PT. Therefore, PPA was also modelled and titrated with M2 for which the calculated $\Delta E^\circ$\textsubscript{(cluster)} values are summarised in Table 5.2 together with the molecular modelling result of PT with M2. As with PT, the clusters formed with PPA are all favourable as indicated by their negative $\Delta E^\circ$\textsubscript{(cluster)} values.

**Table 5.2** Calculated $\Delta E^\circ$\textsubscript{(cluster)} (kcal/mol) values for T:M clusters from molecular modelling experiments of PPA and M2. The PT-M2 results are included for comparison.

<table>
<thead>
<tr>
<th>Monomer Units</th>
<th>$\Delta E^\circ$\textsubscript{(cluster)} (kcal/mol)</th>
<th>PT-M2</th>
<th>PPA-M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-10.1</td>
<td></td>
<td>-9.1</td>
</tr>
<tr>
<td>2</td>
<td>-20.5</td>
<td></td>
<td>-18.1</td>
</tr>
<tr>
<td>3</td>
<td>-14.3</td>
<td></td>
<td>-13.4</td>
</tr>
<tr>
<td>4</td>
<td>-15.8</td>
<td></td>
<td>-15.4</td>
</tr>
<tr>
<td>5</td>
<td>-15.0</td>
<td></td>
<td>-8.8</td>
</tr>
</tbody>
</table>
The molecular modelling images of PPA and M2 (Figure 5.5) show that multiple hydrogen bonds are formed between PPA and M2 in the 1:2 T:M cluster with no evidence of inter-monomer interaction. In the presence of 4 or more monomers (Figure 5.5 C), monomer-monomer interactions occur due to overcrowding and limited hydrogen bonding sites in PPA. On the other hand, the monomer-monomer interaction that can be observed with the 1:3 cluster (Figure 5.5 B) seems to promote the T:M interaction as shown by the decrease in the H-bond lengths compared to the 1:2 cluster.

The $^{31}$P NMR titration results and Job’s plots for PT and PPA with M2 are presented in Figure 5.6. Compared to the PPA titration, the change in chemical shift of phosphorous in PT is larger than that in PPA. This is expected because of the presence of the carboxyl and amino group in PT providing more sites for hydrogen bonding in the template, whilst PPA is only limited to the phosphate group only. Nevertheless, M2 was still able to form strong interaction with phenylphosphonic acid.

On the other hand, the broad Job’s plots for both compounds suggest the presence of a mixture of T:M complexes in the solution although PPA displays higher complex induced shifts at 0.4-0.5 mol fraction PPA equivalent for T:M clusters ≤ 1:2. The maximum at 0.10 mol fraction PPA could be due to the compounded effect of the monomers surrounding the PPA causing a large shift in the phosphorous signal.

Thus, based on the molecular modelling and $^{31}$P NMR results for PPA and M2, it would appear that the optimum T:M ratio is 1:2 cluster. This was the PPA:M2 ratio used in subsequent experiments.
Figure 5.5 Molecular modelling of PPA:M2 clusters: (A) 1:2 and (B) 1:3 and (C) 1:4. The electron cloud distribution was not included in B and C to highlight inter-monomer interactions.
Figure 5.6 Plot of (A) $^{31}$P NMR titration of PT (-●-) and PPA (-●-) against increasing equivalents of M2; and (B) Job's plots showing the complex induced shift of phosphorous for PT (-●-) and PPA (-●-) as a function of monomer M2 mole fraction.
5.2.3 Thermal polymerisation

The most commonly used functional monomers for phosphate containing templates are the urea based functional monomers, whilst EGDMA is commonly employed as a crosslinker.\textsuperscript{6-10} This study, on the other hand, used a novel biphenyl monomer M2 as a functional monomer, confirmed by pre-synthetic interaction studies to favourably interact with PPA forming a 1:2 T:M cluster. Previous attempts to use PT as a template had been thwarted by the limited solubility of PT (2 mM in DMSO) in most solvents. As with the theophylline MIPs, EGDMA was also used as the crosslinker. Both PPA and monomer 2 were found soluble in THF, therefore THF was used as the porogen and acetonitrile as the rebinding solvent.

A pure polyEGDMA polymer was synthesised to determine the degree of PPA binding to EGDMA. The crosslinker commonly makes up 80\% of the polymer with each unit containing four oxygen atoms available for hydrogen bonding. Although previous reports\textsuperscript{11-13} showed favourable binding of phenylphosphonic acid with the imprinted polymer compared to the non-imprinted polymer, indicating a minimal crosslinker binding effect,\textsuperscript{6} the functional monomers used in this study have not been previously used as functional monomers for phosphate MIPs. Thus, an assessment of any possible competitive interaction between EGDMA and phenylphosphonic acid was warranted.

5.2.4 Polymer Characterisation

The surface morphology of the polymers was examined using scanning electron microscopy. The scanning electron micrographs of the thermal MIP (T-PPA-MIP2) and NIP (T-PPA-NIP2) shown in Figure 5.7 both display porous surfaces imparted by the THF porogen.
Figure 5.7 Electron micrographs of T-PPAMIP2 (A) and T-PPANIP2 (B) at 12500x magnification.

The differential scanning calorimetry (DSC) curves of the polymers (Figure 5.8) were also obtained to examine the difference of crosslinking between the two polymers compared to pure EGDMA. The polymer with the highest degree of crosslinking is expected to exhibit the highest melting point; conversely, the one with the lowest degree of crosslinking is expected to show the lowest melting point. As expected, the pure EGDMA polymer exhibited the highest meting point (~92°C). Addition of the functional monomer in T-PPANIP2 resulted in a lowering of the melting point (~84°C) and addition of PPA in T-PPA-MIP2 further lowered the melting point (~80°C) confirming that both the functional monomer and template lowers the degree of crosslinking in the polymer.
5.2.5 Evaluation of T-PPA-MIP2: Rebinding Studies

5.2.5.1 Sorption study

The performance of T-PPA-MIP2 was evaluated by comparison of its binding performance against that of the non-imprinted polymer and polyEGDMA. The sorption experiments were conducted using batch binding experiments, where varying amounts of polymer were incubated with a constant amount of PPA over a period of time.

The results of the sorption experiments are presented in Figure 5.9. All polymers were observed to bind more than 50% of the PPA concentration (0.0800 mM), with T-PPA-MIP2 rebinding the highest amount as expected. Despite the high PPA uptake of both T-PPA-NIP2 and polyEGDMA, there is still a clear binding discrimination between the imprinted and non-imprinted polymers, which indicate that binding sites specific to PPA have been formed during imprinting. Saturation point was attained at 20 mg of polymer, with no significant increase in adsorption beyond this mass.

Figure 5.8 DSC thermograms of T-PPA-MIP2, T-PPA-NIP2 and polyEGDMA showing the melting point peaks.
**Figure 5.9** Sorption of PPA on T-PPA MIP2, T-PPA NIP2 and polyEGDMA. 1.00 mL of 0.0800 mM PPA in acetonitrile was used for binding for 18 hours.

### 5.2.5.2 Time binding Study

Time-binding tests were used to assess the optimum time of contact between the template and the polymer. The experiments were performed using the optimum polymer amount obtained from the sorption experiment (30.0 mg) and incubated with 1.00 mL of 0.0800 mM PPA in acetonitrile at various time. Figure 5.10 shows the results of the time-binding experiments. The amount of polymer used was 30.0 mg instead of 20.0 mg as there is no significant change in the uptake of T-MIP 2 and T-NIP 2 from 20.0 mg to 30.0 mg, however, the uptake of theophylline by poly-EGDMA has only shown a plateau at 30.0 mg of polymers.
Figure 5.10 Rebinding of PPA on 30.0 mg of T-PPA-MIP2 and T-PPA-NIP2 as a function of time using 1.00 mL of 0.0800 mM PPA in acetonitrile.

The sorption capacity of the polymers remained high in this experiment, binding more than 50% of the PPA at all times studied. T-PPA-MIP2 showed a rapid uptake of PPA binding more than half of the PPA in the solution in 30 min. There is an observable small increase in sorption after 2.0 h; however, beyond this time, the saturation was already achieved with no further increase in sorption observed. Although a decrease in T-NIP binding was observed after 15 h, this is within the range of errors associated with the binding measurements.

5.2.5.3 Saturation binding Study

The binding isotherms and Freundlich plots for T-PPA-MIP2 and T-PPA-NIP2, with the corresponding affinity distributions in semi log and log format of $N_I$ and $K_d$, are shown in Figure 5.11. The binding isotherms of the polymers were analysed using the Freundlich model and Graphpad PRISM 5.0 software, and a summary of the binding parameters is presented in Table 5.3.
Figure 5.2 (A) Binding isotherms for T-PPA-MIP2 and T-PPA-NIP2. Thirty (30) mg of each polymer were incubated with an increasing concentration of PPA ranging from 0.04 mM to 0.28 mM in acetonitrile for 2.0 h. (B) The Freundlich plot of the binding isotherms and the corresponding affinity distributions in $N$ vs log $K$ format (C) and (D) the log $N$ vs log $K$ format.
Table 5.3 \( K_d, N_T \) and \( m \) values obtained from the Freundlich plots of the binding isotherms of T-PPA-MIP2 and T-PPA-NIP2.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Binding Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_d )</td>
</tr>
<tr>
<td>T-PPA-MIP2</td>
<td>1.16</td>
</tr>
<tr>
<td>T-PPA-NIP2</td>
<td>1.76</td>
</tr>
</tbody>
</table>

As with the theophylline imprinted polymers, the binding isotherms for the PPA imprinted polymer was fitted to the Freundlich model – the most appropriate for heterogenous systems (Section 4.2.5.5).\(^{14, 15}\) Both T-PPA-MIP2 and T-PPA-NIP2 have a comparable heterogeneity index \( (m = 0.5) \), suggesting similarity in their binding sites and consistent with the high PPA binding observed in T-PPA-NIP2. However, T-PPA-MIP2 still has a higher affinity for PPA as shown in all sorption experiments and a higher number of binding sites \( (N_T = 0.86 \text{ mg/g}) \) than T-PPA-NIP2 \( (N_T = 0.57 \text{ mg/g}) \), which could be attributed to the imprinting effect of PPA. In addition, the \( K_d \) (1.76) for T-PPA-NIP2 is 1.5 times larger than the \( K_d \) (1.16) for T-PPA-MIP2, confirming that the imprinted polymer has a stronger affinity for PPA than its non-imprinted equivalent.
5.2.6 Comparison of Thermal and Microwave PPA Imprinted Polymers

As the microwave method was observed to influence the sorption capability of the imprinted polymers as observed in Chapter 4, it is of interest to see if the same effect can be observed in imprinting another template utilising the same monomer M2. The PPA microwave polymers were prepared using an identical reaction mixture as for the thermal PPA monomers. The microwave condition used was the same as for the theophylline polymers, a temperature of 60ºC, 100 W power and 15 min reaction time.

The resulting microwave PPA polymers prepared from M2 (MW-PPA-MIP2 and MW-PPA-NIP2) were ‘softer’, requiring less grinding, than the thermal polymers, just like MW-MIP2 obtained for theophylline in Chapter 4. This is most likely due to the shorter polymerisation time as the MW-PPA polymers were polymerised for 15 mins compared to 18 h for the thermal PPA polymers.

The MW-PPA polymers were subjected to sorption tests under the same conditions as for the T-PPA polymers. However, 30 mg of both MW-PPA-MIP2 and NIP2 were observed to bind all of the PPA in 1.00 mL of 0.0800 mM, solution equivalent to a rebinding of 0.42 mg PPA/g polymer. Whilst the imprinting efficiency was not determined, this amount is still very small (1%) compared to the theoretical amount of PPA that would have been imprinted assuming 100% imprinting efficiency. Therefore, a higher concentration of 1.00 mM was used in the sorption experiments and this was applied to both thermal and microwave PPA polymers for 18.0 h. The results of these rebinding experiments are shown in Figure 5.12.
The sorption capacity of the MW-PPA polymers is higher than that of the T-PPA polymers, which is similar to what was observed with the MW theophylline polymers (as discussed in Chapter 4, Section 4.3.5.3). Unlike the thermal polymers, which already attained saturation above 20.0 mg of polymer used, the microwave polymers displayed a continual increase in PPA rebinding with increasing amounts of polymer. On the other hand, the differences in binding between the MIP and the NIP of both the thermal and MW polymers in each amount of polymer tested, attributed to cavity specific PPA binding, are comparable except for the 10 mg polymer experiment. These results suggest that the MW-PPA polymers are more prone to superficial binding than the thermal polymers, most likely caused by the mode of polymerisation.

**Figure 5.3** Plot of bound PPA against increasing mass of polymers using 1.00 mL of 1.00 mM PPA in acetonitrile and a binding time of 18 h.
The superficial binding of PPA to the MW-PPA polymers is further confirmed by the zeta potential obtained as shown in Table 5.4 for both MW-PPA polymers. Because the zeta potential values for the MW-PPA polymers are considerably low, it means that PPA binding at the surface will meet less repulsion from the surface charge of the polymers. Thus, an increased in sorption capacity of the microwave polymers was observed.

<table>
<thead>
<tr>
<th>Polymer 2</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIP</td>
</tr>
<tr>
<td>thermal</td>
<td>-21.1</td>
</tr>
<tr>
<td>microwave</td>
<td>-1.52</td>
</tr>
</tbody>
</table>

The MW-PPA polymers and T-PPA polymers were also subjected to surface area and porosity measurements. The same experiment was applied from the theophylline polymers in Chapter 4. Since CO₂ gas was used in the sorption experiment, only the micropore size distribution is covered in the isotherm. The CO₂ adsorption isotherm and pore size distributions for the T-PPA and MW-PPA polymers are shown in Figures 5.13 and 5.14, respectively.
Figure 5.4 Gas adsorption isotherms for the T-PPA and MW-PPA polymers. The low pressure region is also shown as an inset graph to highlight the adsorption behaviour of the polymers at low pressures.

The adsorption isotherms of the MW-PPA and T-PPA polymers differ significantly in the lower pressure region as shown in the inset graph. The MW-PPA polymers display “knees” at lower pressures, indicating rapid monolayer adsorption in the micropores of the polymers, not observed with their thermal equivalents but was observed with the MW theophylline MIP2 (as discussed in Section 4.3.5.2). Interestingly, this was also observed in MW-PPA-NIP2 but not observed with the MW theophylline NIP and could be attributed to solvent effects as the MW theophylline polymers were prepared in DMF whilst the MW-PPA polymers were prepared in THF.

The inset graph in Figure 5.13 displays the ‘knees’ for MW-PPA-MIP2 and MW-PPA-NIP2. The “knee” observed in the MW-PPA-MIP2 plot could be attributed to the micropores formed from “snap freezing” of the aligned functional groups of the OH of PPA and the OH of M2 during microwave polymerisation. Since the presence of the “knee”
in MW-PPA-MIP2 also showed an increase in adsorption of PPA and the same trend was also observed with theophylline MW-MIP2, the effect can be attributed to the presence of the template.

![Figure 5.5 Pore size distribution of thermal and microwave PPA MIP 2 and NIP 2.](image)

The resulting surface areas of the PPA thermal and microwave polymers are summarised in Table 5.5. There is no significant difference observed between the thermal and microwave MIPs and their corresponding NIPs. However, the surface area of the T-PPA polymers is 1.3 times larger than for the MW-PPA polymers. This difference can be attributed to the mode of polymerisation. Consistent with Albright’s theory, the microwave method induces early phase separation during polymerisation, resulting in the aggregation of the polymer microgels lowering the surface area of the polymers. On the other hand, thermal polymerisation results in more stable microgels that are more separated from each other, giving larger surface areas. Interestingly, the difference between the surface areas between the thermal and microwave theophylline MIPs and NIPs (thermal 1.1 times larger) is
comparable to the observed difference between the thermal and microwave PPA polymers (thermal 1.3x larger) and supports the effect of the mode of polymerisation on the surface area of the resulting polymers.

The surface areas obtained for all PPA MIPs and NIPs are larger than those obtained for all theophylline MIPs and NIPs and could only be attributed to the effect of the solvent on the rate of polymerisation and the onset of phase separation.

**Table 5.5** Specific surface areas for the thermal and microwave PPA polymers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Specific Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-MIP</td>
<td>283</td>
</tr>
<tr>
<td>T-NIP</td>
<td>289</td>
</tr>
<tr>
<td>MW-MIP</td>
<td>213</td>
</tr>
<tr>
<td>MW-NIP</td>
<td>210</td>
</tr>
</tbody>
</table>
5.3 Conclusion

The biphenyl monomers were evaluated for their potential as functional monomers for phosphotyrosine (PT) and phenylphosphonic acid (PPA). The interaction of these biphenyl monomers with the phosphate groups of PT and PPA were assessed via semi-empirical molecular modelling and $^{31}$P NMR interaction studies. M2 (4’-vinyl-biphenyl-4-ol) showed the most favourable interaction, at a 1:2 T:M ratio, with both PT and PPA forming multiple hydrogen bonds.

As the solubility of PT is limited in most organic solvents, only PPA MIPs were prepared by thermal and microwave polymerisation utilising M2. As with the theophylline MIPs, higher PPA sorption was obtained for microwave PPA MIPs compared to their thermal counterparts, but both polymers exhibited binding discrimination between their MIPs and NIPs confirming the success of the imprinting process. Further, Freundlich analysis of the thermal polymers confirmed the imprinting effect with the MIP exhibiting a lower $K_D$ and higher binding capacity ($N_T$) than the NIP.

The effect of the mode of polymerisation is evident in the physical characteristics of the polymers. The imprinted and non-imprinted thermal polymers exhibit higher surface area and surface charges but a lower microporosity than the microwave polymers, consistent with the results observed for the theophylline MW-PPA polymers.
5.4 Experimental

5.4.1 Chemicals and Reagents

All biphenyl monomers used were synthesised as previously described in Chapter 2. HPLC grade (Fluka) tetrahydrofuran (THF) and methanol were used as received. Ethylene glycol dimethacrylate (EGDMA), from Sigma Aldrich, was purified using alumina prior to use. Azobisbutyronitrile (AIBN, Dupont) was recrystallised from acetone prior to use. Phenylphosphonic acid and phosphotyrosine were purchased from Sigma-Aldrich and used as received.

5.4.2 Template-Monomer Interaction Studies

**Molecular modelling.** Template-monomer molecular interactions were modelled using Spartan ’04 software implementing the semi-empirical Austin Model 1 (AM1) using equilibrium geometry calculations. This molecular orbital computational method predicts the stable configuration of the template (T), monomer (M), M-M clusters and T-M clusters, and calculates their standard heats of formation ($\Delta H^\circ$). The molecules were randomly positioned and the T-M clusters were modelled with respect to increasing monomer-template molecular ratio from 1 to 5. To account for the monomer-to-monomer interaction, M-M clusters of up to four molecules were also surveyed. The energy of interaction of the T-M clusters, $\Delta E^\circ_{(\text{cluster})}$, at different molecular ratios, were then calculated using Equation 4.1 (as shown in Section 4.2.1). 19

**$^{31}$P NMR titration.** Typically, from a stock solution of 20.0 mM in DMSO, incremental amounts of 50.0 µL of the biphenyl monomer was added to a 0.50 mL of 2.00 mM solution of the template (phosphotyrosine or phenylphosphonic acid) in deuterated DMSO. Phosphoric acid was used as reference at 0.00 ppm. The phosphorous signal from the phosphate group of the template was monitored as incremental amounts of monomers were added. The titration curve was then constructed from the plot of change in chemical shift, $\delta$ (ppm), of the phosphorous signal against increasing amount of monomers added.
Job’s plots. Appropriate volumes of the template phosphotyrosine or phenylphosphonic acid (2.00 mM) and monomer (2.00 mM) stock solutions prepared in deuterated DMSO were combined in an NMR tube to make up a 0.5 mL solution with resulting phosphotyrosine or phenylphosphonic acid:monomer ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. The phosphorous signal from the phosphate group of the template was followed and recorded. A Job’s plot was then constructed by plotting the complex-induced shift (ppm) of the phosphorous against the mole fraction of the template.

5.4.3 Polymer Synthesis

Thermal synthesis. Predetermined quantities of phenylphosphonic acid (0.50 mmol, 0.79 g), M2 (1.00 mmol, 0.196 g), EGDMA (10.00 mmol, 1982.0 g) and AIBN (1 mol%, 34.5 mg) were dissolved in 4 mL THF in a 10-mL vial, purged with nitrogen gas for 5 min, sealed and polymerised at 60 ± 1.0°C in a Thermoline oven. The polymers were then ground and sieved to a particle size <38 µm. The template was extracted via Soxhlet extraction in methanol:acetic acid (90:10 v/v) for 24 h and further washed in methanol for another 24 h. The polymers were then dried at 40°C in a vacuum oven. A similar procedure was applied to the non-imprinted polymers.

Microwave synthesis. A similar procedure was used as for the thermal synthesis, except that polymers were polymerised in a 10 mL pressure vessel via microwave heating using a CEM Discoverer Benchmate at 60°C using 100W for 15 min.

Table 5.6 Preparation of T-MIP 2 and T-NIP 2, MW-MIP 2 and MW-NIP 2 and P-EGDMA for phosphate specific imprinted polymer.

<table>
<thead>
<tr>
<th>Polymer(^a)</th>
<th>M2 (mmol)</th>
<th>PPA (mmol)</th>
<th>EGDMA (mmol)</th>
<th>THF (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-MIP 2</td>
<td>1.000</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>MW-MIP 2</td>
<td>1.000</td>
<td>0.500</td>
<td>10.00</td>
<td>4.00</td>
</tr>
<tr>
<td>P-EGDMA</td>
<td>-</td>
<td>-</td>
<td>10.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

\(^a\)Polymerisation was conducted at 60°C for 18 h. The same formulation was used for the preparation of T-NIP 2 and MW-NIP 2 except in the addition of PPA.
5.4.4 Sorption Study

A sorption study was performed using batch binding experiments where various amounts of polymers ranging from 10.0 to 50.0 mg were placed into a 5 mL vial to which was added 1.00 mL of 0.0800 mM phenylphosphonic acid in acetonitrile. The mixture was shaken for 18 h, filtered and the filtrate analysed directly by HPLC. The amount of free phenylphosphonic acid was subtracted from the initial binding solution concentration to obtain the amount of phenylphosphonic acid bound to the polymer. All binding experiments for this study were done in triplicate to assess reproducibility. A sorption isotherm was generated from the plot of phenylphosphonic acid bound (mM) versus amount of polymer (mg).

5.4.5 Time-Binding Study

Thirty milligrams, the optimal weight obtained from the adsorption study, was used for determining the optimum time of template binding. To a triplicate set of 30.0 mg of polymer, 1.00 mL of 0.0800 mM phenylphosphonic acid was added and the mixture shaken for a designated period of time. The reaction times investigated were 0.5, 2.0, 6.0, 18.0 and 24 h. After binding, the mixtures were filtered and the filtrates analysed by HPLC. The amount of bound phenylphosphonic acid was then obtained by subtracting the amount of phenylphosphonic acid left in solution from the initial concentration. A plot of the amount of phenylphosphonic acid bound versus time of contact was generated to determine the optimum time of contact for binding phenylphosphonic acid.

5.4.6 Saturation Binding

The optimum weight and time of contact obtained from the sorption and time-binding studies were used for the saturation binding experiment. A series of 30.0 mg of each polymer was incubated with different concentrations of phenylphosphonic acid for 2 h, after which, the mixtures were filtered and the filtrates analysed directly by HPLC. A plot of bound template against free phenylphosphonic acid concentration was then generated to visualise the saturation binding isotherm of the polymers.
5.4.7 HPLC analysis

Phenylphosphonic acid rebinding was measured using a Shimadzu Prominence HPLC equipped with a SPD-20A/M20A lamp and LC-20AD pump. Analyses were performed on an Econosphere C18 5µ column (150 x 4.6 mm) using isocratic elution at 45°C and an acetonitrile:buffer (10.0 mM HEPES, pH 7) at 1:4 v/v and 1.5 mL/min. The injection volume used was 30 µL and the chromatograms were recorded at 212 nm. The peak at 0.85 min attributed to phenylphosphonic acid was measured.

A five-point calibration curve from five standard concentrations of PPA with linear regression values ($R^2$) of 0.997 was used to determine the concentrations of PPA after binding measurements.

5.4.8 Scanning Electron Microscopy

Morphology of the polymers was examined using a Phillips XL30 scanning electron microscope. Each polymer was deposited on a sticky carbon tab and coated with gold using a SPI gold spotter coating unit. SEM micrographs of the polymers were obtained at 20000x magnification at 15.0 kV.

5.4.9 Zeta Potential

Zeta potential measurements were performed using a Malvern Nanosizer S fitted with a maintenance-free folded capillary cell (DTS 1060). Very dilute suspensions of polymers were prepared using ~ 0.75 mL deoxygenated distilled deionized water (non-equilibrated in air, 18.2 MΩ cm$^{-1}$). Measurements were performed at 25.0 °C, pH 7.0 in 5 replicates.

5.4.10 Specific Surface Area (Brunauer-Emmett-Teller)

Gas adsorption analysis was carried out using a Micromeritics ASAP 2020 Accelerated Surface Area and Porosity instrument (Norcross, GA, USA). The analysis was carried out using 100 mg of sample and degassed at 110°C under vacuum for 12 h to remove any adsorbed solvent and water. The adsorption isotherm of this degassed sample was then measured using carbon dioxide as the adsorbate at a temperature of 773 K, covering the partial pressure ($P/P_0$) range 1x10$^{-6}$ to 0.03. The specific surface area of each sample was
determined from the adsorption data using the linearised BET equation\textsuperscript{20}, whilst the pore size distribution was calculated using a BJH model.

5.4.11 TGA/DSC

Approximately 2.5 mg of polymer were sealed in a crimped aluminium pan. A matching mass of aluminium silicate was measured and sealed in a crimped pan. Both were placed on the balance within the furnace of a DSC-60 scanning calorimeter (Shimadzu, Japan). Samples were then heated from 30°C to 200°C at 10°C/min, held at 200°C for 3 min, and then cooled to 30°C at the same rate.
5.5 References


Chapter 6

Summary and Recommendations
6.1 Summary

This work was spearheaded with the synthesis of biphenyl monomers using Suzuki cross-coupling reactions. Initial investigations on the preparation of 4'-vinyl-biphenyl-4-ol (M2) using thermal acceleration of the Suzuki reaction was found to be slow (5 days) with unsatisfactory (13% yield) for the preparation of large-scale amounts. However, application of microwave heating to the preparation of M2 by the Suzuki reaction significantly improved the reaction yield (98%) in a short reaction time (30.0 min) with the successful suppression of the homocoupling and Heck-coupling side products upon the introduction of a nitrogen atmosphere and modification of the solvent system. The optimised microwave conditions for preparation of the biphenyl monomers were successfully and continually used throughout this research period.

Foreseeing the potential use of these novel vinyl biphenyl compounds as functional monomers for phosphate-containing templates in MIP preparations, i.e., amino acid mimics, the acid dissociation constants of the monomers were determined by potentiometric titrations. However, the nonpolar and water insoluble nature of the biphenyl monomers, limits the potentiometric titration from aqueous solutions. As an alternative, a method developed in this research, employing semi-aqueous titrations, was applied to the biphenyl monomers to derive their aqueous pK_a values from their semi-aqueous pK_a values. The first aqueous pK_a values of these novel biphenyl monomers, ranging from pH 4-9, are reported herein.

The biphenyl monomers were first used as functional monomers in the preparation of molecularly imprinted polymers for theophylline. Prior to MIP synthesis, the biphenyl monomers were subjected to interaction studies via semi-empirical molecular modelling and 1H NMR titrations to obtain the most favourable T:M ratio. Evaluation of the prepared MIPs was performed via sorption experiments. T-MIP2, prepared from M2 (4'-vinyl-biphenyl-4-ol), emerged as the best MIP among the polymer systems investigated. Further studies involved the preparation of the same polymer mixture by microwave polymerisation. The microwave prepared polymers exhibited higher affinity and selectivity towards theophylline over the competing analyte (caffeine) compared to the thermal MIP.
After successful evaluation of biphenyl monomers for theophylline imprinting, the biphenyl monomers were then screened for imprinting phosphate-containing templates. M2 was chosen as the functional monomer after a series of interaction studies via semi-empirical molecular modelling and $^{31}$P NMR titrations with phosphotyrosine. Because of the limited solubility of phosphotyrosine in organic solvents, the more soluble phenylphosphonic acid (PPA) was used as a template for a M2-based MIP. As with theophylline, PPA MIPs were prepared by thermal and microwave polymerisation. The PPA sorption of MW MIPs was consistent with the observed high sorption capacity of theophylline MW MIPs, which were higher than their thermal counterparts.

The effect of the mode of polymerisation was found to affect the physical characteristics of the microwave theophylline and PPA polymers. The microwave polymers exhibited higher surface areas, low surface charges and a higher microporosity compared to thermal polymers.
6.2 Future Work

The results obtained in this study have provided insights into the effect of microwave polymerisation on the physical characteristics of microwave polymers. Having compared microwave polymerisation and thermal polymerisation, further work will focus on the investigation of the effects of microwave polymerisation time, porogen and microwave power on the binding performance of the MIP. The use of microwave radiation proved to be helpful in reducing reaction time, therefore realisation of its full potential towards MIP synthesis provides an exciting opportunity for the continued development of highly specific MIPs.

Initial investigation of the utilisation of biphenyl monomers for phosphate MIPs has demonstrated the interaction capability of the monomers towards phosphate-containing templates. Therefore, future work would involve investigation into the potential of the biphenyl monomers for the preparation of MIPs as enzyme mimics. Preparation of GTPase MIPs using GTP as a template, the long term aim of this project, should utilise a mixture of these biphenyl monomers to maximise interactions between monomers and template. This should be possible because these biphenyl monomers possess various functional groups representing a wide range of $pK_a$ values.