Molecularly Imprinted Polymer-New Characterization Methods and Designs

Di Song

University of South Carolina

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MOLECULARLY IMPRINTED POLYMER – NEW CHARACTERIZATION METHODS AND DESIGNS

by

Di Song

Bachelor of Science
Beijing Institute of Technology, 2006

Submitted in Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy in
Chemistry and Biochemistry
College of Arts and Science
University of South Carolina
2013

Accepted by:
Ken D. Shimizu, Major Professor
Linda S. Shimizu, Committee Member
John L. Ferry, Committee Member
Sarah C. Baxter, Committee Member
Lacy Ford, Vice Provost and Dean of Graduate Studies
DEDICATION

This work is dedicated to

My Family

For all your love and support
ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Ken D. Shimizu, for his guidance, advice, knowledge, and patience. None of my accomplishment would be possible without your help and encouragement. I will never forget your efforts to train me how to think in different directions and how to write paper up. My experience in graduate school under your supervision has not only trained me to be a better experimental chemist, but also has made me a good scholar. I feel very grateful to have you as my advisor.

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I would like to thank my family who give me endless love and support. To my parents, no words can express my deep gratitude to you. You always undoubtedly love and support me throughout my life and never push me. Thank you for allowing me to
spend five years to attain my graduate degree thousands of miles away from you. I am grateful for everything you have done for me. I also would like to thank my grandparents who took care of me when I was young. I still can remember these days I lived with you and I miss you so much.

At last, I would like to thank all my friends. We shared many sorrow, happy, bitter, and sweet moments together. Everything would not be possible without you.
ABSTRACT

Chemical sensors are important in a wide range of applications. However, there is no commercially available molecularly imprinted polymers (MIPs) based sensor. Thus, the design and development of sensors utilizing imprinting technique have been an area of active research. In Chapter 1, first a brief introduction to imprinting techniques is given. Then we provide a short review of progresses in design of MIPs sensors using multi-functional monomers. Multi-functional monomers (multi-FMs) are high affinity monomers towards target molecules and are able to introduce other functionally active groups for sensing and catalysis. Two classes of multi-FMs will be reviewed and discussed. Then, new advances which we have achieved and strategies which we have developed will be discussed at the end of this chapter.

A new method of verifying and characterizing the imprinting efficiency of molecularly imprinted polymers (MIPs) was developed and tested. In the new polar solvent titration (PST) method, a series of MIP and non-imprinted polymers (NIPs) are prepared with increasing concentrations of a polar solvent. The templation and monomer aggregation processes can be systematically disrupted by the polar solvent additives. The changes in the binding capacities of the polymers in each series provide a measure of the relative magnitudes of the imprinting effect and monomer aggregation effects. The new method was tested using three different urea functional monomers that had varying degrees of templation and monomer aggregation self-assembly. Diphenyl phosphate
anion was used as template for these polymers. The new MIP characterization method can differentiate differences in binding capacity arising from templation and monomer aggregation. To independently verify the new characterization method, the MIPs were also characterized using binding isotherm analysis. The two methods appeared to give consistent conclusions. However, the results from the PST method provided more information about the presence and relative magnitudes of the templation and processes that influenced the binding properties of the polymers.

In Chapter 3, first we studied the importance of monomer aggregation for molecular imprinting. Monomer aggregation can improve the imprinting effect by suppressing the number of background binding sites. Then, the effects of crosslinking degree were evaluated using MAA and EA9A system. High crosslinking degree was required for imprinting effects. Higher crosslinked polymer exhibits greater imprinting effect. The relative magnitudes of the effect of crosslinking degree are estimated using urea functional monomers and phosphate template system. The effect of decreasing 13% of crosslinking degree was estimated to reduce 24% of the binding capacity. Next, the influence of functional monomer to template ratio on imprinting was studied and the range of this ratio was optimized. Finally, the above results were combined to design new functional monomers and new MIPs with improved imprinting effect. A diacid functional monomer was shown to be a better monomer compared to MAA.

In Chapter 4, a lanthanide-containing polymer sensor was designed and prepared. This polymer showed sensitive and selective response to carboxylates. First a fluorescent europium-containing complex bearing styrene functionalities was synthesized. The complex was co-polymerized with EGDMA in dichloroethane under free radical
polymerization conditions thermally. The sensing properties of the polymer were characterized by monitoring the fluorescence response using fluorimeter after pipetting a series of different anion solutions in varying concentrations. The polymer showed highly selectivity to carboxylate anions over halide and other oxy-anion analytes. Also, MIPs templated with two different carboxylates showed better selectivity to the corresponding carboxylates.
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LIST OF SYMBOLS

$B$ Analyte bound to polymer.

$F$ Free analyte in solution.

$a$ Freundlich isotherm constant.

$m$ Freundlich isotherm constant.

$K_{agg}$ Aggregation constant.

$K_a$ Association constant.

$\lambda_{max}$ The wavelength of maximum absorption.

$J$ Coupling constant.

$\delta$ Chemical shift.
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<tr>
<td>MIP</td>
<td>Molecularly imprinted polymer</td>
</tr>
<tr>
<td>NIP</td>
<td>Non-imprinted polymer</td>
</tr>
<tr>
<td>FM</td>
<td>Functional monomer</td>
</tr>
<tr>
<td>Multi-FM</td>
<td>Multi-functional monomer</td>
</tr>
<tr>
<td>Mono-FM</td>
<td>Mono-functional monomer</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer-Emmett-Teller method</td>
</tr>
<tr>
<td>MAA</td>
<td>Methacrylic acid</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>EGDMA</td>
<td>Ethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>AIBN</td>
<td>2-2'-Azoisobutyronitrile</td>
</tr>
<tr>
<td>TBA-DPP</td>
<td>Tetrabutylammonium diphenylphosphate</td>
</tr>
<tr>
<td>TBA-PhOAc</td>
<td>Tetrabutylammonium phenylacetate</td>
</tr>
<tr>
<td>TBA-OAc</td>
<td>Tetrabutylammonium acetate</td>
</tr>
<tr>
<td>TBA-Bz</td>
<td>Tetrabutylammonium benzoate</td>
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<tr>
<td>TBA-OTs</td>
<td>Tetrabutylammonium tosylate</td>
</tr>
<tr>
<td>TBA-Cl</td>
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</tr>
<tr>
<td>TBA-F</td>
<td>Tetrabutylammonium fluoride</td>
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CHAPTER 1

INTRODUCTION TO MOLECULARLY IMPRINTED POLYMER SENSORS

Abstract

Molecularly imprinted polymers (MIPs) are synthetic polymeric materials that are formed in the presence of a template to form active sites similar to those formed in antibodies and proteins. MIPs are economic and easy to prepare. However, there are currently no MIP-based sensors and catalysts that are commercially available due to the low fidelity of imprinting process. Thus, the design and development of new monomers that have higher imprinting efficiencies have been an area of active research. Multi-functional monomers (multi-FMs) are a new class of monomers to address this problem via high affinity towards target molecules and ability to easily introduce other functionally active groups for sensing and catalysis. The goal of this chapter is to provide an introduction to MIPs with a specific focus on the design of multi-FMs for imprinting. After giving a brief introduction to MIPs, two classes of unique multi-FMs will be reviewed and discussed. Then, new advances which we have achieved and strategies which we have developed will be discussed at the end of this chapter.

1.1 General introduction

Chemical sensors are important in a wide range of applications including environmental hazard assessment, medical monitoring, and pharmaceutical quality control. Sensors are typically constructed from the combination of two basic elements. The first is a signaling platform that provides a measurable change in an optical or...
electrical signal when challenged with a chemical analyte of interest. The second is a recognition platform that can differentiate the analyte of interest from common and structurally similar molecules. Examples of commonly utilized recognition platforms in sensors include materials with innate specificity such as polymer films, silica, and metal oxides and tailored materials such as enzymes, antibodies, and synthetic molecular receptors.\textsuperscript{6-16} The innate recognition materials are typically readily available and inexpensive but possess relatively low affinities and specificities for individual analytes. The tailored materials have much higher affinities and specificities, which result in more sensitive and selective sensors. Their major drawback is that they are generally expensive and require considerable resources and effort to customize to the analyte of interest.

MIPs are recognition materials that have been used in sensors which possess attributes of both of the above classes.\textsuperscript{17-20} MIPs are synthetic polymers that can be inexpensively and readily prepared often from commercially available starting materials. The recognition properties of MIPs can also be tailored using a molecular templation process as shown in Scheme 1.1. The molecular imprinting process involves three steps. First, functional monomers bearing a recognition group are mixed with the template molecule which is either the analyte of interest or a structural analog. Second, the resulting monomer-template complex is polymerized in the presence of a high percentage of a crosslinker to preserve the complementary distance and orientation between the functional monomer recognition groups. Finally, the removal of the template creates complementary cavities within a rigid highly-crosslinked polymer matrix. The combination of synthetic efficiency and versatility of the imprinting process
has facilitated the integration of MIPs into a variety of sensing platforms and sensing applications.\textsuperscript{21-23}

\begin{scheme}
\begin{center}
\includegraphics[width=\textwidth]{molecular_imprinting_process}
\end{center}
\end{scheme}

\textbf{Scheme 1.1} Illustration of the three-step molecular imprinting process. In this example, the urea functional monomer and the diphenylphosphate tetrabutyl ammonium salt template are highlighted in red and blue, respectively.

Due to the highly cross-linked structure, good stability, tolerance in harsh condition such as high temperature, pressure and organic solvents, acid and base, as well as the low cost, MIP is an ideal sensitive material for molecular recognition.

\subsection{1.2 Types of imprinting mechanism}

MIP according to the different types of interaction of template and functional monomer in polymerization process can be divided into covalent, non-covalent, metal ion and non-polar imprinting.\textsuperscript{24} In this section, we will mainly focus on the three most common types, covalent imprinting, metal ion imprinting and non-covalent imprinting.
1.2.1 Covalent imprinting

Covalent imprinting technique is imprinting processes using chemical reactions to form monomer-template complex. The advantages of covalent molecular imprinting are the firm stoichiometry of monomer to template and the homogeneity of imprinted sites. Thus, functional monomers are only associated with templates in recognition sites, forming a majority of templated sites rather than non-selective background sites. The strength of covalent imprinting is quite high since it involves the chemistry of forming and breaking bonds. However, high energy is required for bond formation and cleavage, leading to being time consuming for template to associate with and dissociate from monomers/polymers. Also, covalent imprinting requires synthetic efforts. Thus, the use of molecular imprinting covalent is limited, and commonly used for catalytic applications.

There are two types of covalent imprinting, reversible covalent and semi-covalent. Reversible covalent imprinting is an imprinting technique to both form and dissemble complex covalently through single bond formation and cleavage. These conditions limits the technique to reversible condensation reactions, which are applicable to templates with specific structures. For example, boronate ester is the most successful approach for reversible covalent imprinting.\textsuperscript{25-31} Covalent imprinting also involves weak bond formation and cleavage such as Schiff’s base\textsuperscript{32} and ketal (acetal)\textsuperscript{33, 34} formations, and strong covalent bond formation and cleavage such as esterification.\textsuperscript{35, 36}

The semi-covalent imprinting was covalent imprinting as templation technique during polymerization. Here the rebinding of template is actually a non-covalent process. It covers rebinding of the initial templates and slightly modified structures. For example,
Zimmerman and co-workers published a series of works on dendrimer monomolecular imprinting and recognition of porphyrins, amines and sugars.\textsuperscript{37-44} The origin of this strategy was in 1999 when his first porphyrin-cored dendrimer was synthesized and published (Scheme 1.2).\textsuperscript{45} This monomolecular imprinting idea has both the features of covalent imprinting and non-covalent imprinting, and involves relatively small number of functional monomer that was used. One dendrimer monomer was utilized for the recognition of a template, which was not only efficient, economical, but also environmental friendly. On the other hand, the rebinding study was non-covalent in nature, requiring no synthetic efforts. Porphyrin bearing \textit{m}-dihydroxybenzene functionalities was designed as a covalently-bound template to form complexes before the formation of dendrimer. Large conjugated \( \pi \) systems were introduced to facilitate the monitoring of imprinting effect. Figure 1 below shows an example of the process of monomolecular imprinting. Those cored dendrimer monomers were first synthesized following previous designed procedure with covalently bonded porphyrin core. After making the dendrimer matrix, it was polymerized through ring opening metathesis polymerization (ROMP). Porphyrin was removed chemically with strong base to yield a dendrimer monomer with donut-like hallow inner framework bearing multiple carboxylic acid functionalities. It was carried out with the original template and a series of porphyrin-based analytes bearing different functionalities such as phenol, pyridine and pyrimidine. The association constants between dendrimer and porphyrin-based template lied in the range of \( 10^5 \text{ M}^{-1} \), which is extremely high and efficient compared to ordinary imprinting systems.
Scheme 1.2 Schematic illustrating the preparation of imprinted dendrimer through covalent imprinting process (Figure adapted from reference 45).
1.2.2 Metal ion imprinting

Transition metals have been used to bind to a broad range of both charged and neutral analytes through coordination between heteroatoms of the analyte and the outer unfilled orbitals of the metal. Metal ion imprinting can be classified into three types, metal ion -templated imprinting, metal ion-mediated imprinting, and metal ionic crystal imprinting. Among these three types, metal ion-mediated molecular imprinting has been investigated heavily. This technique normally involves complexation among functional monomer, metal ions, and template. Functional monomers bind to metal ion to form the polymerizable complex which in turn binds to the template through metal ion. More details on this topic will be discussed in Chapter 4.

1.2.3 Non-covalent imprinting

Non-covalent imprinting refers to molecular imprinting strategies in which template and functional monomer form complexes in solution mainly driven by weak forces such as hydrogen bonding, electrostatic interactions, hydrophobic effects and pi-pi interaction. Non-covalent imprinting is the predominant method for imprinting due to the ease in preparation. Typically, this technique requires no or little synthetic chemistry. The imprinting process starts spontaneously when monomer and template are mixed together. The associated monomer/template complex is stable under polymerization conditions such as free radical polymerization. However, non-covalent imprinting has drawbacks. Non-covalent imprinting process is a dynamic equilibration. This leads to the low yield of imprinted sites. It also creates a lot of non-selective binding sites due to the heterogeneity of non-covalent imprinting process, which may largely affect the imprinting efficiency.
Researchers have achieved great success in the area of non-covalent imprinting.\textsuperscript{57-61} Most functional monomers for non-covalent imprinting are commercially available such as acidic monomers including the most widely used methacrylic acid (MAA),\textsuperscript{62} acrylic acid,\textsuperscript{63} and itaconic acid,\textsuperscript{64} neutral monomers including acrylamide,\textsuperscript{65} 2-hydroxyethyl methacrylate,\textsuperscript{66} and basic monomers such as 4-vinylpyridine.\textsuperscript{67} Our group has utilized MAA to prepare non-covalent colorimetric MIP sensor array that can accurately identify seven different aromatic amines in 2005.\textsuperscript{68} Dye displacement method was introduced to visualize the colorimetric response for each analytes (Scheme 1.3). Linear discriminant analysis was utilized to classify the resulting response patterns and proved this array can give 94% accuracy. This array is superior to individual MIPs due to the poor selectivity and cross-reactivity of each MIP toward all seven analytes. This study proved that MIP
sensor array made through non-covalent imprinting technique possess high great accuracy of discrimination even though the functional monomer MAA has no selectivity towards structurally similar templates.

The majority of this dissertation will focus on the use of non-covalent imprinting due to the established techniques and the ease in preparation. Only Chapter 4 investigates metal ion-mediated imprinting to.

1.3 Challenges in MIP and solution

Despite their many attractive qualities, there have not been any commercial examples of MIP-based sensors. A major reason is the relatively limited binding properties of MIPs. MIPs have higher binding affinities than innate recognition materials such as silica and polymer films. However, their average binding affinities and selectivities fall far short of other tailored recognition materials such as antibodies or aptamers.

One reason for the poor binding properties of most MIPs is that they are prepared using commercially available or synthetic-easy functional monomers which contain a single recognition group. This leads to low imprinting efficiencies as mono-functional monomers (mono-FMs) have low binding affinities for the template. Therefore, it is entropically unfavorable to form monomer-template complexes that contain more than one FM. Examples of commonly used mono-FMs include methacrylic acid (MAA), methacrylamide (MA), and 2-vinyl pyridine (2-VP). Mono-FMs are attractive because they are commercially available, inexpensive, and surprisingly versatile. They have been used to successfully imprint a wide range of molecular and macromolecular templates including pharmaceuticals, environmental pollutants, herbicides, pesticides, proteins, and even bacteria.
Mono-FMs impose a number of key limitations on the properties and utility of the resulting MIPs. Specifically, the low binding affinities of mono-FMs for the template and the difficulties in formation of entropically unstable complexes of multiple monomers and one template ultimately lead to poor imprinting efficiencies. To efficiently form monomer-template complexes that contain multiple FMs, a large stoichiometric excess (at least 3- to 5-fold) of monomer is typically used in mono-FMs imprinting protocols. This strategy does lead to the formation of the desired higher ordered monomer-template complexes; however, one side effect is that the excess monomer is also incorporated into the polymer matrix, creating a large population of non-selective background sites that tend to dominate the binding properties of the MIPs.\textsuperscript{90, 91}

One solution to the above problems is multifunctional monomers (multi-FMs). Multi-FMs are monomers containing more than one recognition groups or bearing different functionalities. There are two types of multi-FMs that will be discussed in this chapter, multi-FMs with higher binding affinities and selectivities, and multi-FMs containing a combination of recognition groups and other functional groups capable of signaling (or catalyzing, response to stimuli).

Multi-FMs with higher binding affinities and selectivities normally contain multiple recognition functionalities towards template within a single molecule. These functionalities are in close proximity and a combination of these functionalities contributes to high association to template molecule compared to a mono-FM. The higher affinity of FMs to template leads to forming reduced/less background sites due to the fewer amounts of FMs that utilized.
Multi-FMs containing a combination of recognition groups and other functional groups consist of some functionality such as a signaling functionality (or catalytic, responsive functionality, and so forth) is a bonus for molecular imprinting since it broadens the applications. Those potentials and advantages will boost the design and development of multi-FMs.

However, there are some limitations in utilization of multi-functional monomers. First, the majority of them are not commercially available. The design of synthetic route and the actual synthesis might be difficult and expensive. The specificity of this kind of monomer could be more limited, but sometimes could be a good feature for imprinting. Thus, rational design and development of efficient and economic friendly multi-FMs is important.

1.4 Examples of multi-FMs with higher binding affinities and selectivities

The most commonly used multi-FM with higher binding affinities and selectivities for MIP is commercially available itaconic acid. It was first reported as a monomer for MIPs by Suedee and co-workers’ in 1999 for the enantioseparation of adrenergic drugs. Phenylenethanolamine adrenergic agonists are widely used nasal congestion medicines. All of them are chiral and only the $R$ absolute configuration of the hydroxyl group-attached carbon is preferred for pharmaceutical and medical applications. Therefore, the resolution of the mixture of stereoisomers is important. Both itaconic acid and MAA, a mono-FM, were utilized to make MIPs as chiral stationary phases (CSPs). The best resolution was achieved using ITA as functional monomer. MIP prepared with itaconic acid is more stereospecific, and it can work in extreme environment such as polar mobile
phase (10% acetic acid) for elution. These results demonstrated that multi-FM itaconic acid is a better monomer than mono-FM MAA in imprinting.

Computational calculation also suggested multi-FM itaconic acid is a better monomer for imprinting compared to mono-acid monomers due to its higher affinity and selectivity. Pavel and Lagowski reported a computational approach for the selection of monomers for imprinting of theophylline and its derivatives in 2005. There were 25 commonly used FMs including itaconic acid and corresponding polymers were screened, 5 out of 25 were acid monomers. Each of the monomer-template complexes was investigated by molecular dynamics simulations to predict interaction energies, contact distances and active binding groups. Multi-FM itaconic acid predicted to form the most stable FM-template complex among all the acid monomers.

Multi-FM tweezers bearing two cholesterol arms has been successfully utilized to recognize and extract cholesterol (Figure 1.1). Compared to analogous ‘one-armed’ tweezers receptor, it was able to discriminate certain structurally related steroids such as stigmasterol and cholesterol acetate. The recognition of cholesterol was of interests due to the current focuses on extraction of the steroid from food sources. The backbone of the tweezers was constructed from 3.5-dibromobenzoic acid, propargyl alcohol, and cholesterol arms through a multi-step synthesis. The optimum stationary phase was determined by chromatographic screening process of multi-component mixture of structurally related steroids. A series of polymers were made thermally, with various co-monomers, various solvents and high density of various cross-linkers. The binding capacities were tested and were found that MAA, EGDMA and THF are the best and most suitable co-monomer, cross-linker and solvent, respectively, for this binding
All the components were proven to contribute to the binding even though the binding affinity was mainly depended on the tweezers. The imprinting efficiency of the resulting polymers made with chosen MAA, EGDMA and tweezers, was characterized by chromatographic study and showed sufficient binding capacity (74%) and selectivity.

**Figure 1.1** The cholesterol-based molecular tweezer system (adapted from reference 94).

Hall *et al.* reported a high affinity multi-functional urea monomer for the enantioselective sensing of oxyanions (Figure 1.2). A series of urea FMs including a bis-urea monomer were synthesized in one step from a polymerizable isocyanate and a nonpolymerizable diamine with a high yield. The association constant of bis-urea multi-FM to template was obtained from titration data to be 1500±200 M⁻¹ in competitive solvent DMSO-d6 with a 1:1 stoichiometry. The association constant of a structurally similar mono-urea was calculated as 30 ± 4 M⁻¹ which was two orders smaller than that of the bis-urea. The MIP made with bis-urea exhibited high affinity for the template over the other enantiomer and other analytes, however, the low affinity of MIP made with mono-urea was demonstrated by its low retention factor which was much lower (k < 1) than that of polymer made with bis-urea (k > 1).
Figure 1.2 Bis-urea and mono-urea monomers (adapted from reference 100).

Our group designed and developed a porphyrin-based tetra-urea multi-FM for the recognition and sensing of carbohydrates based on previous publications.103-105 Both the porphyrin and urea participated in the association with carbohydrates and provide high affinity and differentiate closely related carbohydrates (Figure 1.3). Porphyrins were proved to be an optical-sensitive receptor and could transduce signals when bound to other molecules.106-111 It provides a large contact surface and space for monosaccharide. Even though porphyrin was proved to have binding affinity to carbohydrate, it is hard to design and develop rational sugar receptors due to the complexity of the non-covalent hydrogen bonding. The incorporation of urea functionalities in close proximity provided more hydrogen donors/acceptors for hydrogen bonding thus enlarged the association between monosaccharide and monomer. Both porphyrin and urea functional group together provide the quality and complementary binding sites. They show high association constants with carbohydrates that range between $6.2 \times 10^4$ to $1.2 \times 10^5$ M$^{-1}$ in chloroform. Binding properties of resulting polymers were characterized with structurally similar aromatic-derived carbohydrate instead of alkyl-derived due to their lack of chromophore. MIPs showed higher affinity than corresponding NIPs.
Figure 1.3 Porphyrin-based multi-urea FM and carbohydrate template (adapted from reference 103).

In summary, the above examples demonstrated that multi-FMs normally have greater affinity than structurally similar mono-FMs, thus have better imprinting effect according to the literature.

1.5 Examples of multi-FMs containing a combination of recognition groups and other functional groups

Functional monomers containing sensing, catalytic, or responsive moieties other than recognition sites will be discussed in this section. First, functional monomers are capable of transducing signals may be of interest for making MIPs for sensing. In the past, most of researches showed a way to introduce optical-sensitive properties into molecularly imprinted polymers. That is to use a fluorescent co-monomer to transduce signals. The main drawback is that those optical-sensitive co-monomers randomly distribute on the surface of polymer matrix, causing overestimated or false response because some of the signals are not due to templated-site bindings but background binding. Thus, the design of monomers containing both recognition and signaling groups is crucial.
Wulff and coworkers designed and developed a series of multi-FMs that containing catalytic moiety that could be used in formation of transition state imprinted polymers for carbonate hydrolysis (Figure 1.4).\textsuperscript{112-114} These monomers consisted of two key components, the amidinium functionality and the tri-amine-transition metal functionality. The amidinium functionality was able to bind to phosphate or carboxylate anions, and catalyze. A tri-amine was introduced to threefold coordinate with transition metals (four coordination capacity) to form a complex with a free coordination site for other ligands, such as pyridinyl groups in the template. The incorporation of Zn\textsuperscript{2+} or Cu\textsuperscript{2+} to chelate with tri-amine greatly enhanced the catalysis efficiency (catalyzed to uncatalyzed reaction of 10\textsuperscript{5}-fold). Even higher carbonate hydrolysis efficiency (one order of magnitude bigger) was observed with introduction of two amidinium moieties. These polymers were considered to have higher activity compared to catalytic antibodies and provide a novel strategy to design artificial molecular catalyst.

\textbf{Figure 1.4} Representation of reactive site in MIP matrix. The FM, template, and the coordinated metal were highlighted in green, blue, and red, respectively (adapted from reference 112).
A fluorescent bi-functional monomer that post-modified with FITC dye was designed for the recognition of protein with a post-modification method by Takeuchi and co-workers in 2010 (Figure 1.5).\textsuperscript{115} In the past, biomolecules were needed for the recognition of protein. Considering the high cost and time-consuming, artificial mimics of bio-functional molecules are preferred. Molecular imprinting has been successfully developed in this area.\textsuperscript{116-121} The functional monomer in this paper consists of three components: polymerizable group, NH group which is close to recognition group that can be post-modified to introduce fluorescence molecular after polymerization, and benzoic acid recognition group for protein. After preparation of functional monomers, two series of polymers layers were made on top of glass chips with previously immobilized initiator immersed in 0.1 M Tris–HCl buffer (pH 7.4). After wash then an amino-reactive fluorescent dye FITC was inserted on NH group next to the recognition site. The binding constant between protein and imprinted polymers are at around $10^6$ M$^{-1}$ which proved the high affinity. The selective enhancement of fluorescence by lysozyme enables the post-modification of multi-FM a promising technology for protein imprinting.

\textbf{Figure 1.5} Representation of the three functional groups on monomer (adapted from reference 115).

A multi-FM with introduction of a photo responsive functionality, azobenzene, was designed and the resulting FM and imprinted polymer that showed photo-isomerization property, making it possible to controlled release and uptake (Scheme 1.4).\textsuperscript{122} The
association constants were 2800 and 2200 M\(^{-1}\) for cis and trans isomers obtained in DMSO-d6, respectively. The difference in association constant was 600 M\(^{-1}\) that are not impressive but considering that the competitive solvent DMSO was used, which disrupted the self-aggregation of monomers, the difference should be larger in other solvents when aggregation is present. Pre-polymerization solution and pure monomer in solution were irradiated under UV at 365 nm to get the thermally unstable cis-isomer before polymerization was conducted. Monomer/polymer isomerization was observed when change the irradiation wavelength to 440 nm. Binding efficiency was tested through batch binding assay. Irradiation of polymers at different wavelength allowed the partial uptake/release (40% of load) of N-Z-L-methylesterglutamate anion. This photo-regulated MIP showed great photo responsive properties making it promising to the design of stimuli-responsive MIPs and application in drug delivery systems.

**Scheme 1.4** Schematic demonstration of MIP photo-responsible controlled uptake and release (figure adapted from reference 122).

In summary, multi-FMs that containing moieties other than recognition functionalities have broaden the applications of MIP and make it versatile as imprinting monitor, controlled release agent, or catalysis. It provides a new strategy to design multi-
function polymer materials by introducing multi-functionalities into a single monomer instead of utilizing two or more different monomers that have separate properties.

1.6 Conclusion

The above examples demonstrate the advantages and versatility of both types of multi-FMs. Multi-FMs were proved to have high affinity toward targeted molecules and can greatly reduce the required amount of functional monomers, thus decrease the background binding. Multi-FMs containing a combination of recognition groups and other functionalities can introduce sensing, responsive, and catalytic properties, etc. to MIP. We confidently believe that the future advances in MIP will involve the design of these two types of multi-FMs and development of synthetic routes towards them.

1.7 MIP – new characterizations and designs

This dissertation focuses on improving the imprinting effect in MIPs and investigating better characterization methods. One major challenge in developing new imprinted polymers and in optimizing the imprinting process is finding an accurate method of measuring the imprinting effect. In Chapter 2, a new method of characterizing and verifying the imprinting efficiency of molecularly imprinted polymers (MIPs) was developed and examined. This new PST method not only appeared to give accurate and consistent conclusions with the traditional binding isotherm analysis, it can also differentiate whether differences in binding capacity are due to templation, other self-assembly processes, or a combination of the two in the pre-polymerization solution. Moreover, it can estimate the relative magnitude of imprinting effect to other processes.

Chapter 3 studies the rational design of new functional monomers by examining components/factors that influence imprinting effect, such as monomer aggregation, cross-
linking degree, and monomer to template ratio. Functional monomer aggregation was found to greatly suppress the number of background binding sites. It leads to an improvement in imprinting efficiency by increasing the percentage of actual imprinted sites in total binding sites and thus, the selectivity. Crosslinking degree was shown to be important as it controls the rigidity of the recognition sites within the MIPs. If the crosslinking degree is too low, the MIP will exhibit irreproducible binding properties due to the flexibility of imprinted sites. The amount of templates used in making an MIP is correlated to the resulting imprinting effect. Given these factors, this chapter presents our approaches to design new multi-FMs. Several multi-FMs that aggregate were designed and the synthetic routes were developed. A multi-FM containing two carboxylic acid functionalities within defined proximity was designed and synthesized following this strategy. The resulting MIP showed good imprinting effects towards adenine with reduced background non-selective sites. The suppression of background sites was due to the great monomer intra- and inter-molecular self-assembly.

An interesting study on a carboxylate selective lanthanide polymer sensor is presented in Chapter 4. This preliminary study develops a metal ion-mediated imprinted polymer. The lanthanide containing monomer and resulting polymer were first chosen and synthesized using a slightly modified version of the literature procedure. A polymerizable salen ligand coordinated with Eu$^{3+}$ and became a fluorescent complex monomer bearing two europium functionalities. The complex monomer and resulting polymer were able to recognize carboxylate anions through dative bonding with the europium metal. These interactions could be monitored by fluorimetry due to the sensing property of salen-Eu moieties.
1.7 References


CHAPTER 2

CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMERS USING A NEW POLAR SOLVENT TITRATION (PST) METHOD

Abstract

A new method of characterizing molecularly imprinted polymers (MIPs) was developed, which provides a more accurate means of verifying and measuring the molecular imprinting effect. In the new PST method, a series of imprinted and non-imprinted polymers are prepared in solutions containing increasing concentrations of a polar solvent. The polar solvent additives systematically disrupt the templation and monomer aggregation processes in the pre-polymerization solutions, and the extent of disruption is captured by the polymerization process. The changes in binding capacity within each series of polymers are measured, providing a quantitative assessment of the templation and monomer aggregation processes in the imprinted and non-imprinted polymers. The new method was tested using three different diphenyl phosphate imprinted polymers made using three different urea functional monomers. Each monomer had varying efficiencies of templation and monomer aggregation. The new PST characterization method was found to have several advantages. The method could differentiate differences in binding capacity arising from templation or monomer aggregation. The method was also easy to carry out. To independently verify the new characterization method, the MIPs were also characterized using traditional binding isotherm analyses. The two methods appeared to give consistent conclusions. However,
the results from the PST method were more easily interpreted and provided more information about the presence and relative magnitudes of the templation and processes that influenced the binding properties of the polymers.

2.1 Introduction

The molecular imprinting technique is a synthetically efficient, inexpensive, and rational approach for preparing polymers with tailored molecular recognition properties.\(^1\) Due to these attractive characteristics, molecularly imprinted polymers (MIPs) have been successfully utilized in many applications including chromatographic separations,\(^3,4\) artificial immunoassays,\(^5,6\) solid-phase extraction,\(^7,8\) sensing,\(^9,10\) and even catalysis.\(^11-13\) The three-step imprinting process can typically be carried out in a single vessel using commercially or readily accessible functional monomers (FMs). For example, Scheme 2.1 shows a schematic representation of the imprinting process for the diphenyl phosphate imprinted polymers prepared in this study. First, the urea FM and diphenyl phosphate template form a hydrogen bonded monomer-template complex in the pre-polymerization solution. Polymerization with a cross-linker captures the monomer-template complex within a rigid polymer matrix. Finally, removal of the template molecules generates binding sites with a complementary shape to the template molecule and lined with complementary recognition groups.

A major challenge in developing new imprinted polymers is finding an accurate method of measuring the imprinting effect.\(^14-16\) The enhancements in binding capacity and selectivity imparted by the imprinting process are often very subtle and thus is easily obscured or is mistaken for other processes or factors. For example, one of the most common methods of verifying and characterizing the imprinting effect is via the difference in binding capacity between an imprinted and non-imprinted polymer made in
the presence and absence of the template molecule. The observation of a higher binding capacity for the molecularly imprinted polymer (MIP) versus the non-imprinted polymer (NIP) is interpreted as evidence of an imprinting effect (Scheme 2.2a), and the magnitude of the difference is used as a measure of the imprinting effect. However, the NIP is not always a good control polymer for identifying the imprinting effect. Thus, this simple analysis can lead to the incorrect assignment of polymers as imprinted polymers.

Scheme 2.1 Illustration of the three-step molecular imprinting process. In this example, the urea functional monomer and the diphenylphosphate tetrabutyl ammonium salt template are highlighted in red and blue, respectively. The higher binding capacity of an MIP versus an NIP can arise from three sources. These are: 1) the imprinting effect (Scheme 2.2a), 2) differences in polymer surface area and morphology, or 3) functional monomer aggregation (Scheme 2.2b) that leads to a suppression of the number of binding sites in the NIP. In most cases, the differences
are due to some combination of the above sources. Differences arising from variations in morphology are easily identified by material characterization methods via microscopy (SEM) or surface area measurements (BET). One the other hand, identifying differences arising from FM aggregation presents a much more difficult challenge. Monomer aggregation in the NIP polymerization solution reduces the number of recognition groups that are available to form binding sites making the NIP a poor control polymer (Scheme 2.2b). This can lead to large differences in binding capacities between MIPs and NIPs even in cases where there was no imprinting effect (Scheme 2.2b). Even in the case of strongly imprinted polymers, FM aggregation in the NIP can augment the differences between MIP and NIP, leading to an overestimation of the imprinting effect. Furthermore, our recent studies found that the influence of monomer aggregation is extremely prevalent.\textsuperscript{19, 20} For example, the most common used molecular imprinting monomer, methacrylic acid (MAA), shows very strong FM aggregation effects. Other common FMs containing self-associating amide and urea recognition groups also show strong monomer aggregation effects.

To try to address some of the above problems, recent studies have recommended the characterization of the binding properties of MIPs over a range of concentrations using binding isotherms.\textsuperscript{21-24} These multipoint characterization methods specifically address the highly concentration-dependent-binding properties of MIPs\textsuperscript{25} that make single-point comparisons subject to a high degree of imprecision. Although absorption isotherms provide a more accurate and comprehensive measure of the binding properties, they still do not provide data on the precise origins of differences in binding properties between imprinted and non-imprinted polymers.
Therefore, a new characterization method was developed that could differentiate the effects arising from the templation process and from other sources such as monomer aggregation. The method was named “polar solvent titration” because of its resemblance to titration-based strategies that measure association constants via systematically disruption of the binding equilibrium via changes in concentration, temperature, pH or solvents. Similarly, in the PST method, the formation of the monomer-template complex in the imprinting process is systematically disrupted by the addition of a polar
solvent. This is performed by preparation of a series of MIPs in solutions that contain increasing concentrations of the polar solvent. The binding capacities of these MIPs are then characterized. The expected results of a PST of an MIP are shown in Figure 2.1a (solid line). The measured binding capacities of the MIPs asymptotically decrease as more polar solvent is added to the prepolymerization solution. The final polymers contain only background binding sites as the imprinting process has been completely disrupted. The strength of the imprinting effect is qualitatively assessed by the difference in binding capacity between the MIPs formed in the absence and presence of the highest concentration of the polar solvent additive. MIPs with imprinting effects will show large drops in binding capacity (Figure 2.1a, b, and e). MIPs that are not imprinted will show little or no difference (Figure 2.1c and d).

A similar series of NIPs is prepared to characterize the functional monomer aggregation process (Figure 2.1, broken lines). These NIPs are prepared in solutions containing increasing concentrations of the same polar solvent additive. The polar solvent systematically disrupts functional monomer aggregation in the prepolymerization solution allowing the formation of more background sites. Thus, NIPs with functional monomers aggregation will display asymptotic increases in binding capacities with increasing polar solvent (Figure 2.1c). The strength of the functional monomer aggregation is characterized by the difference in binding capacity between the NIP formed in the absence and in the presence of the highest concentration of polar solvent additive. NIPs with FMs that have strong aggregation will display large increases in binding capacities (Figure 2.1a, c, and e), and NIPs with weak or no FM aggregation will show no increase in binding capacity (Figure 2.1b and d).
Figure 2.1. Examples of the results of the PST methods of five possible pairs of MIPs and NIPs. Each plot represents the binding capacities of a series of MIPs (solid line) and NIPs (broken line) formed in solutions with increasing concentrations of a polar solvent additive.

The key advantage of the PST method is that it can accurately identify and characterize imprinted polymers. The reason is that the method measures the relative magnitudes of the imprinting and FM aggregation processes in MIPs and NIPs (Figure 2.1a-d). Thus, the method can differentiate MIPs and NIPs containing: (a) a combination of imprinting and FM aggregation, (b) imprinting and no FM aggregation, (c) no imprinting and FM aggregation, and (d) no imprinting and no FM aggregation. The PST
method also has the ability to determine whether an NIP is a good control polymer. If the NIP is a poor control polymer, the method can also identify better control polymers.

In this study, the new PST MIP characterization method was tested and evaluated using three different MIPs (Figure 2.2). The three MIPs were all imprinted using diphenyl phosphate (DPP) as a template but were made using different urea monomers (1–3). The tetrabutyl ammonium salt of diphenyl phosphate (TBA-DPP) was chosen as the template due to its complementarity and affinity to urea groups via hydrogen bonding.\textsuperscript{27-29} Specifically, we have previously established that the three urea FMs 1, 2, and 3 can efficiently imprint TBA-DPP within a crosslinked ethylene glycol dimethacrylate (EGDMA) polymer matrix.\textsuperscript{19,29}

There were two primary reasons for the selection of the three urea monomers used in this study. First, FMs 1, 2, and 3 were expected to have greatly different imprinting efficiencies (1 \(\gg\) 2 \(\gg\) 3). FM 1 was predicted to have the strongest imprinting efficiency because it can form stronger multipoint monomer-template complexes with its three urea groups. In contrast, FMs 2 and 3 contain only one urea recognition group and should form much weaker monomer-template complexes. However, FM 2 should have enhanced templation efficiencies versus FM 3 because it contains two polymerizable groups versus FM 3 which has only one polymerizable group. Thus, the urea recognition groups in FM 2 polymers will be held more rigidly within the polymer matrix and more efficiently preserve the shape and functional group complementary of the template.

The second reason for selecting FMs 1, 2, and 3 is that they should have different degrees of FM aggregation. The expected order of aggregation strength is again 1 \(\gg\) 2 \(\gg\) 3 for similar reasons. The urea recognition groups have a strong propensity for self-
association.\textsuperscript{31} FM 3 with three urea recognition groups should show the strongest aggregation effects.\textsuperscript{32} FM 2 with two polymerizable groups should better preserve the FM aggregation.

![Chemical structures of FM 1, FM 2, FM 3, and TBA-DPP](image)

**Figure 2.2** Three urea FMs and tetrabutyl ammonium diphenyl phosphate template (TBA-DPP).

2.2 Experimental Section

2.2.1 General

\textsuperscript{1}H NMR spectra were recorded on a Varian 300 MHz NMR at ambient temperature. Chemical shifts (ppm) were referenced to tetramethylsilane or residual protonated solvent. UV measurements were made using a Jasco V-530 spectrometer. Solvents were purchased from Sigma-Aldrich, Fisher and VWR. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. All other reagents were purchased from Sigma-Aldrich and were used as received.
2.2.2 Synthesis of FM 1

To an ice-cooled solution of tris (2-aminoethyl)amine, (0.37 mL, 2.47 mmol) in dry 
CH₂Cl₂ (50 mL) under nitrogen atmosphere, 2-isocyanatoethyl methacrylate (1.05 mL, 
7.42 mmol) was slowly added and cooled in ice bath for 15 min. The reaction mixture 
was stirred at room temperature for 4 h. The mixture was concentrated to give 1.51 g 
(100% yield) of FM 1 as a white solid. ¹H NMR (300 MHz, CDCl₃) δ: 6.11 (t, 3 H, J = 
1.2 Hz), 5.85 (m, 3 H), 5.66 (m, 3 H), 5.56 (m, 3 H), 4.18 (t, 6 H, J = 5.8 Hz), 3.44 (dd, 6 
H, J = 5.7 Hz), 3.15 (m, 6 H), 2.49 (m, 6 H), 1.92 (s, 9 H).

2.2.3 Synthesis of FM 2

To a solution of 2-aminoethyl methacrylate hydrochloride (1.22 g, 6.95 mmol) in dry 
CH₂Cl₂ (80 mL) and triethylamine (1.15 mL, 8.36 mmol) under nitrogen atmosphere, 2-
isocyanatoethyl methacrylate (1.00 mL, 6.95 mmol) was slowly added while stirring in an 
ice bath for 15 min. The reaction mixture was stirred at room temperature overnight. 
Then the reaction mixture was washed with 4 M HCl 4x100 mL, then water 4x100 mL. 
After dried with anhydrous sodium sulfatethe organic layer was concentrated to give 
1.580 g (80% yield) of FM 2 as a white solid. ¹H NMR (300 MHz, CDCl₃) δ: 6.09 (m, 2 
H), 5.60 (m, 2 H), 4.78 (t, 2 H, J = 5.7 Hz), 4.22 (m, 2 H), 3.50 (m, 2 H), 1.93 (m, 3 H).

2.2.4 Synthesis of FM 3

To a solution of benzylamine (0.35 mL, 3.19 mmol) in dry CH₂Cl₂ (60 mL) under 
nitrogen atmosphere 2-isocyanatoethyl methacrylate (0.46 mL, 3.19 mmol) was slowly 
added and stay in ice bath for 15 min. The reaction mixture was stirred at room 
temperature for 4 h. The mixture was concentrated to give 0.850 g (100% yield) of FM 3 
as a white solid. ¹H NMR (300 MHz, CDCl₃) δ: 7.22 (m, 5H), 6.06 (t, 1 H, J = 0.9 Hz), 
5.54 (m, 1 H), 5.12 (t, 1 H, J = 5.3 Hz), 5.00 (t, 1 H, J = 5.3 Hz), 4.30 (d, 2 H, J = 5.7
Hz), 4.16 (t, 2 H, J = 5.4 Hz), 3.33 (dd, 2 H, J = 5.5 Hz), 1.92 (dd, 3 H, J = 1.5 Hz, J = 0.9 Hz). $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$: 167.41, 158.63, 139.27, 135.98, 128.39, 127.16, 125.92, 64.10, 44.16, 39.24, 18.24. HRMS (EI) calculated for C$_{14}$H$_{18}$N$_2$O$_3$: 262.1317; obs: 262.1324.

**2.2.5 Preparation of TBA-DPP**

To a stirred solution of diphenyl phosphate (2.00 g, 8.00 mmol) in dry methanol (150 mL) under nitrogen was added a 1.0 M solution of tetrabutylammonium hydroxide in methanol (8 mL, 8.0 mmol) in one portion. The resulting mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, and the resulting solid was dried for 12 h under vacuum to give 3.83 g (98% yield) of TBA-DPP as a clear solid. The resulting tetrabutylammonium salt was stored under anhydrous conditions. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.28 (d, J = 7.8 Hz, 4 H), 7.18 (t, J = 7.8 Hz, 4 H), 6.93 (t, J = 7.2 Hz, 2 H), 3.25 (t, J = 8.4 Hz, 8 H), 1.58 (m, 8 H), 1.39 (m, 8 H), 0.93 (t, J = 7.5 Hz, 12 H).

**2.2.6 Polymerization-general**

Three series of MIPs (MIPs 1-3) and NIPs (NIPs 1-3) were made using FM 1, FM 2 and FM 3 with varying concentrations of DMSO. These were all made using similar conditions that followed previously reported molecularly imprinting procedures for TBA-DPP (Wu et al. 2008; Zhang et al. 2011). A typical procedure is described below.

These polymers were all prepared using crosslinker (EGDMA), free radical initiator (AIBN) in chloroform in screw-capped vials with (MIPs) and without (NIPs) template (TBA-DPP). Dissolved oxygen in the polymerization solutions was removed by ultrasonication under nitrogen for 10 min. The vials were sealed and then heated in a
water bath at 65 °C for 6 h. The resulting polymer monoliths were crushed and ground to a fine powder in a mortar and pestle, and the template and the unreacted species were removed by Soxhlet extraction with methanol for 24 h and then with a methanol/acetonitrile mixture (1:4 v/v) for 24 h. The polymer particles were dried overnight under vacuum.

To provide an accurate comparison of binding properties of the polymers made with FM 1, 2, and 3, the number of urea groups in each polymer was kept constant. FM 1 in MIP 1 has three ureas per monomer unit. Thus, MIP 1 has 1/3 the number of monomer units than MIP 2 and 3 which were made with FM 2 and FM 3, which has only one urea group. NIPs 1, 2, and 3 were made under same conditions as the corresponding MIP but without the template.

2.2.7 Preparation of MIP 1-3 and NIP 1-3

FM 1 (0.1224 g, 0.2 mmol), 0.0984 g (0.2 mmol) TBA-DPP, 0.754 mL (4 mmol) EGDMA, 0.0164 g (0.1 mmol) AIBN were dissolved in 2.5 mL of solvent. The solution was degassed and then polymerized at 65 °C to yield MIP 1.

The preparation of MIP 2 is to keep everything the same as above except the amount of monomer was increased. Three equivalent of FM 2 (0.1707 g, 0.6 mmol) was used.

The preparation of MIP 3 is to keep everything the same as above except the amount of monomer was increased. Three equivalent of FM 3 (0.1574 g, 0.6 mmol) was used.

Corresponding NIPs were made under the same condition without the TBA-DPP template.
2.2.8 Batch binding study

The binding capacities of these polymers were measured by shaking a fixed weight of polymer in 3.5 mL of a 0.5 mM solution of TBA-DPP in CHCl$_3$. The suspension was filtered, and the concentration of unbound TBA-DPP remaining in the supernatant was measured by UV-vis analysis (266 nm). The amount bound was calculated simply by subtracting unbound concentration from the 0.5 mM TBA-DPP solution. The binding capacity (μmol/g) is amount bound per weigh unit of polymers.

2.2.9 Gas Adsorption Porosimetry

Polymers were degassed for 12 h and tested by nitrogen adsorption porosimetry using a Quantachrome Autosorb automated gas sorption system. Surface areas were obtained by the Brunauer-Emmett-Teller (BET) method at 77.35 K.

2.2.10 Surface morphology image

Polymers were ground and sieved, and then dispersed in acetonitrile. Several drops of the suspension were transferred onto conductive carbon adhesive tabs and the acetonitrile was allowed to evaporate. Images were taken using Tescan Vega3 SBU variable pressure scanning election microscopy (SEM).

2.3 Results and Discussion

The goal of this study was to test the ability of the PST MIP characterization method to accurately characterize the imprinting and FM aggregation effects. MIPs (1-3) and NIPs (1-3) were prepared using urea FMs 1-3 and TBA-DPP as the template. The binding properties of the polymers for the template, TBA-DPP were measured by three different methods for comparison. First, the MIPs and NIPs were characterized using conventional single-point batch binding studies of the MIP. Second, the polymers were
characterized using the new PST method. Finally, the polymers were characterized using binding isotherm analyses.

**Single-point batch binding study.** First, the three MIP and NIP pairs were compared by single-point batch binding studies. The binding capacities of each polymer for TBA-DPP in chloroform were measured (Figure 2.3). This simple analysis suggested that all three MIPs were strongly imprinted. All the MIPs bound at least twice as much as their corresponding NIPs. Also, MIP 2 appeared to be the most strongly imprinted, displaying the largest difference in binding capacity between MIP 2 and NIP 2. However, as will be shown by the next two analyses, these conclusions are not entirely accurate. Only MIP 1 and 2 are imprinted, and MIP 1 is much more strongly imprinted than MIP 2 as expected.

![Figure 2.3](image)

**Figure 2.3** Binding capacities to 3.5 mL 0.5 mM TBA-DPP of 105 g MIPs and NIPs polymerized with FM 1, FM 2, and FM 3.

**PST analysis.** Next, MIPs 1-3 were characterized using the new PST method. Thus, for each MIP and NIP, a series of polymers were made in CHCl₃ containing varying
concentration of a polar solvent additive (0% to 45% DMSO). DMSO was chosen because it is a highly polar solvent that can disrupt the hydrogen bonding interactions of the urea FMs. Four polymers (0, 10, 25, 45% DMSO v/v) were prepared for each MIP and NIP, which was sufficient to unveil any trends. Then the binding capacities for each series of polymers were measured via batch binding studies using TBA-DPP (0.5 mM) as the analyte and a fixed weight of polymer in CHCl₃ (3.5 mL).

Originally, the batch binding studies were all carried out with the same weights of polymer (105 mg). However, due to the large differences in binding capacity of the polymers made with the different functional monomers, the conditions for the batch binding experiments for the PST had to be optimized for each set of MIPs and NIPs. When the polymers had binding capacities above 80% bound or below 20% bound the polymers differences in binding capacity could not be accurately measured. An example is shown below in Figure 2.4 for MIP 1. When the binding studies were carried out below the 80% limit (40 mg polymer), the differences in the binding capacities of the polymers made with varying percentages DMSO were clear. However, when the same polymers were measured above the 80% limit (105 mg polymer), the differences in binding capacity of the polymers were not apparent.

The amount of polymer used in the binding studies was optimized so that all the polymers for an MIP-NIP series bound between 20% to 80% of TBA-DPP from a 0.5 mM solution (Figure 2.5). The optimal weights of the polymer for polymers made with FMs 1, 2, and 3 were 40 mg, 60 mg, and 105 mg, respectively. These different amounts were consistent with the expected binding efficiencies of the respective FMs. FM 1 with three urea groups had the highest binding affinity and thus required the lowest weight of
polymer. Alternatively, FM 3 with a single urea group required 105 mg of polymer to bind sufficient TBA-DPP for the analysis.

Figure 2.4 Percent bound for 0.5 mM TBA-DPP CHCl₃ solution of 105 mg (triangle), and 40 mg (circle) mg MIP 1 (solid) prepared in solutions of increasing polarity from 0% to 45% v/v DMSO/CHCl₃.

The PST analyses of MIPs 1-3 and NIPs 1-3 (Figure 2.5) displayed similar trends to those in the hypothetical examples (Figure 2.1). In general, the binding capacities of the MIPs decreased and the NIPs increased. The magnitudes of these two effects varied for each polymer, providing a means to evaluate whether the MIPs were imprinted and whether the NIPs displayed strong FM aggregation effects.

The imprinting effect in each MIP was assessed by the difference in binding capacity between the first (0% v/v DMSO) and last (45% v/v DMSO) polymer in the PST. The PST analyses of MIPs 1-3 showed that MIPs 1 and 2 were strongly imprinted but MIP 3 was not imprinted. MIPs 1 and 2 showed a pronounced difference between the first and last polymer, which was indicative of a strong imprinting effect. MIP 3, however,
displayed no difference in binding capacity and thus was not imprinted. These imprinting
trends are consistent with the expected imprinting efficiencies of the FMs 1-3.

The magnitudes of the FM aggregation effects in NIPs 1-3 were compared via the
differences in binding capacities of the first and last polymers in the PST analysis (Figure
2.5). Large increases in binding capacities were observed for all three polymers, which is
indicative of a strong FM aggregation effect. This was consistent with the DMSO
additive disrupting the aggregation of the urea FMs 1-3, resulting in more background
sites.

The analysis revealed that NIPs 1-3 were generally poor control polymers for the
imprinting efficiencies of MIPs 1-3. The majority of the difference between the MIPs
and NIPs observed in the single-point batch binding studies were due to FM aggregation
and not from the imprinting effect. In the case of MIP 3 and NIP 3, the binding capacity
difference was due entirely to FM aggregation. In the cases of MIPs 1 and 2, the single-
point analysis greatly overestimated the imprinting effect due to the contributions of the
strong FM aggregation effects. The PST analysis identified the NIP formed in the
presence of 45% DMSO as a better control polymer, as this high concentration of DMSO
was sufficient to suppress the influence of FM aggregation.
Figure 2.5 Binding capacities for TBA-DPP of series of MIPs (solid lines) and NIPs (broken lines) prepared in solutions of increasing polarity from 0% to 45% v/v DMSO/CHCl₃: a) 40 mg MIP 1 and NIP 1; b) 60 mg MIP 2 and NIP 2; c) 105 mg MIP 3 and NIP 3.
Finally, very strong templation and FM aggregation effects could be identified by PST curves that did not reach their asymptotic limits. For example, the curves for MIPs 1 and 2 (Figures 2.5a and 2.5b) are still falling at the end of the PST. Thus, the templation effects in these MIPs were particularly strong, as they still retained measurable imprinting effects at 45% v/v DMSO. This was corroborated by the significant difference in binding capacity between the MIPs and NIPs at 45% v/v DMSO. If the templation and FM aggregation effects had been completely disrupted by the solvent additive, then the MIP and NIP at the end of the PST should have had the same intermediate binding capacity, as shown in Figure 2.1a. The strong templation effects even in such polar solvent environments were surprising but did have literature precedence. For example, the formation of charge-enhanced hydrogen bonding interactions between urea-based receptors and anionic guests, similar to the template in this study, have been characterized in DMSO.33

By comparison, the PST analysis found that the FM aggregation effects were much weaker, as the curves for all three NIPs 1-3 had reached their asymptotic maximums (Figure 2.5). Thus, the FM aggregation effects were more easily disrupted by the DMSO additive. This is consistent with the much weaker self-association hydrogen bonding interactions of the neutral urea FMs.

In summary, the PST analysis appears to provide a more accurate assessment of the imprinting effect. For example, the PST analysis found that MIPs 1 and 2 were imprinted and MIP 3 was not imprinted. This is in contrast to the single-point MIP versus NIP comparison that found that all three MIPs were imprinted. The PST analysis also identified the origins of the incorrect assignment of MIP 3 as an imprinted polymer by
the single point analysis. All three urea FMs were found to display strong FM aggregation effects, which made the corresponding NIPs poor control polymers. In the cases of MIPs 1 and 2, this leads to an overestimation of the imprinting effect by the single point comparison. In the case of MIP 3, the FM aggregation in NIP was the sole source of the difference in binding capacity of the MIP and NIP. The PST analysis also found that NIPs polymerized with 45% v/v DMSO were good control polymers, as all of the FM aggregation effects had been suppressed. For example, if the 45% v/v DMSO NIP were used in the single-point analysis, then the imprinting effects in MIPs 1 and 2 would be more accurately quantified and the lack of an imprinting effect in MIP 3 would be apparent.

**Surface area and Morphology analysis.** One concern with the PST analysis was that the observed trends might be due to effects other than the templation and FM aggregation effects. Specifically, the analysis compares polymers formed in different solvent environments that might have significant differences in polymer surface area and morphology. Therefore, the surface areas of representative polymers were measured by BET analysis (Table 2.1) and the surface morphologies studied by environmental SEM (Figure 2.6).  

**Table 2.1** BET surface areas for polymers.

<table>
<thead>
<tr>
<th>polymers</th>
<th>functional monomer</th>
<th>polymerization solvent</th>
<th>surface area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 1</td>
<td>FM 1</td>
<td>CHCl₃</td>
<td>17</td>
</tr>
<tr>
<td>NIP 1</td>
<td>FM 1</td>
<td>CHCl₃</td>
<td>260</td>
</tr>
<tr>
<td>MIP 2</td>
<td>FM 2</td>
<td>CHCl₃</td>
<td>150&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NIP 2</td>
<td>FM 2</td>
<td>CHCl₃</td>
<td>330&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MIP 3</td>
<td>FM 3</td>
<td>CHCl₃</td>
<td>300</td>
</tr>
</tbody>
</table>
While there was some variation in the surface areas of the polymers (Table 2.1), there was no clear correlation between the surface areas and measured binding capacities. Therefore, the differences and changes in binding capacities observed in the PST studies could not be attributed to differences in surface area. Most of the MIPs and NIPs had very similar surface areas of around 260 to 330 m$^2$/g (Table 2.1), which is characteristic of the high surface area rigid monoliths that are formed using high mol percentages of the crosslinker EGDMA. Thus, polymers with very different binding capacities, such as NIP 2 formed in pure in CHCl$_3$ and NIP 2 formed in 45% v/v DMSO/CHCl$_3$, had similar surface areas. Even polymers that fell outside this range of surface areas did not show any correlation with their binding capacities. For example, MIP 1 and 2 had significantly lower surface areas ($\leq$150 m$^2$/g) than their corresponding NIPs ($\geq$260 m$^2$/g) despite having much higher binding capacities.

More importantly from the point of view of the PST analyses, the addition of the polar solvent additives did not lead to significant differences in surface area and polymer morphology that might bias the analyses. For example, NIPs 1, 2, and 3 (Table 2.1) made in the highest percent of polar solvent additive (45% v/v DMSO) had surface areas within the typical range 260 to 330 m$^2$/g for these crosslinked polymers. SEM images of the polymer surfaces showed that NIP 2 made in the absence (0%) and presence (45%) of DMSO had very similar surface morphologies (Figure 2.6a and b).

<table>
<thead>
<tr>
<th>NIP</th>
<th>FM</th>
<th>Solvent</th>
<th>Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>45% DMSO/CHCl$_3$</td>
<td>290</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>45% DMSO/CHCl$_3$</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>45% DMSO/CHCl$_3$</td>
<td>290</td>
</tr>
</tbody>
</table>

$^a$ these data come from reference 19

NIP 3 FM 3 CHCl$_3$ 140
NIP 1 FM 1 45% DMSO/CHCl$_3$ 290
NIP 2 FM 2 45% DMSO/CHCl$_3$ 320
NIP 3 FM 3 45% DMSO/CHCl$_3$ 290

48
Figure 2.6 SEM images of a) NIP 2 made in chloroform; and b) NIP 2 made in 45% DMSO/CHCl₃.
**Binding Isotherm Analysis.** Finally, a binding isotherm study was performed to check the consistency of our PST method as a MIP characterization method. The binding isotherm analysis is a proven and well-established method of characterizing the binding properties of MIPs and validating the imprinting effect. Binding isotherms were measured for MIPs 1-3 made in pure chloroform and NIPs 1-3 made in 45% DMSO/CHCl₃. The NIPs formed in the most polar solvents were chosen based on the results of the PST analyses which showed that they were better control polymers for evaluating the imprinting effect than the NIPs made in pure chloroform.

The binding isotherm analysis was performed using a constant concentration of guest (0.5 mM TBA-DPP) and vary weights of polymers. The binding isotherms for the six polymers were fitted to a Freundlich isotherm (Figure 2.7).

The Freundlich isotherm (Eq. (1)) is a power function relationship between $B$ (analyte bound to polymer) and $F$ (free analyte in solution), where the fitting variables $a$ and $m$ which varies from 0 and 1. Previously, we have shown that lower values for the heterogeneity index $m$ correspond to more strongly imprinted MIPs than contain a higher percentage of templated sites. The fitting variable $a$ provides a measure of the polymer’s binding capacity. To aid in visualization of the Freundlich isotherm, a linear form of the equation (Eq. (2)) was used to enable simple linear regression curve fitting methods. Thus, the slope ($m$) and $y$-intercept ($\log a$) provide the two key binding parameters that will be used in comparing the polymers.

$$B = a F^m \quad (1)$$

$$\log B = m \log F + \log a \quad (2)$$
Figure 2.7 Binding isotherms of MIPs (solid) and NIPs (empty) prepared with FM 1 (circle), FM 2 (square), and FM 3 (triangle) measured for their binding to 0.5 mM TBA-DPP in CHCl₃. The resulting data were fitted to Freundlich isotherm model to give straight lines.
The log $B$ vs log $F$ plots for all six polymers were linear and were well-fit by the Freundlich isotherm (Figure 2.7). The $R^2$ values and the key binding parameters $m$ and $a$ for each isotherm are shown in Table 2.2.

**Table 2.2** Polymerization conditions, correlation factors, and calculated constants for the isotherms.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>monomer</th>
<th>$R^2$</th>
<th>$m$</th>
<th>$a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 1 (CHCl$_3$)</td>
<td>FM 1</td>
<td>0.89</td>
<td>0.31</td>
<td>79</td>
</tr>
<tr>
<td>NIP 1 (45% DMSO/CHCl$_3$)</td>
<td>FM 1</td>
<td>0.90</td>
<td>0.66</td>
<td>69</td>
</tr>
<tr>
<td>MIP 2 (CHCl$_3$)</td>
<td>FM 2</td>
<td>0.96</td>
<td>0.32</td>
<td>43</td>
</tr>
<tr>
<td>NIP 2 (45% DMSO/CHCl$_3$)</td>
<td>FM 2</td>
<td>0.94</td>
<td>0.51</td>
<td>27</td>
</tr>
<tr>
<td>MIP 3 (CHCl$_3$)</td>
<td>FM 3</td>
<td>0.90</td>
<td>0.52</td>
<td>20</td>
</tr>
<tr>
<td>NIP 3 (45% DMSO/CHCl$_3$)</td>
<td>FM 3</td>
<td>0.94</td>
<td>0.50</td>
<td>18</td>
</tr>
</tbody>
</table>

Comparison of the heterogeneity indexes ($m$) of an MIP and its NIP provides a measure of the strength of the imprinting effect. This analysis showed that MIPs 1 and 2 were imprinted but MIP 3 was not imprinted. The $m$-values for MIPs 1 and 2 (0.31 and 0.32) were significantly lower than for NIPs 1 and 2 (0.66 and 0.51), respectively. In contrast, the $m$-value for MIP 3 (0.52) and NIP 3 (0.50) were similar.

Comparison of the binding capacity measurements from the binding isotherm analyses provided additional support that only MIPs 1 and 2 were imprinted. Due to the differences in slope of the Freundlich isotherms for the polymers, the binding capacities were assessed by the binding capacities of the fitted isotherms at an intermediate value of 50% bound ($F = 0.25$ mM). These binding capacity measurements are shown in Figure
2.8. Large differences were observed in the binding capacities of the MIP and NIP for MIPs 1 and 2, which is indicative of a strong imprinting effect. In contrast, the binding capacities of MIP 3 and NIP 3 were very similar. These analyses also showed the large difference in binding capacity between the imprinted and non-imprinted polymers formed with FMs 1-3.

![Figure 2.8](image-url) **Figure 2.8** Calculated binding capacities of MIPs (solid) and NIPs (empty) from binding isotherm analysis when $F = 0.25$ mM.

In summary, the binding isotherm analyses give the same conclusions as our new PST method, confirming that the new method provides an accurate assessment of the imprinting effect.

2.4 Conclusions

From the studies of the three different MIPs formed using three different urea monomers, we conclude that the common method of characterizing the imprinting effect by single point comparison of the binding capacities of MIPs and NIPs is often
inaccurate. FM aggregation in the prepolymerization solution of the NIPs makes them poor control polymers, which can lead to a misassignment or overestimation of the imprinting effect. This problem is endemic most of the common MIP formulations that use hydrogen bonding monomers such as methacrylic acid, methacrylamide, and urea-based monomers. The PST analysis addresses this problem by differentiating the contributions of the imprinting and FM aggregation processes to the binding capacities of MIPs and NIPs. Furthermore, the new method can also identify polymerization conditions for NIPs that yield better control polymers for the imprinting process. This method requires multiple binding capacity measurements like the binding isotherm analysis and the preparation of a series of imprinted and non-imprinted polymers. However, due to the synthetic efficiency of the imprinting process, the synthesis of additional polymers is not a significant burden.

2.5 References


CHAPTER 3

A STUDY OF THE VARIABLES IN THE MOLECULAR IMPRINTING PROCESS AND THE NEW DESIGN OF MOLECULARLY IMPRINTED POLYMER

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Abstract

In this chapter, we studied the importance of monomer aggregation for molecular imprinting. Then the effect of crosslinking degree was evaluated, and the relative magnitude of this effect was estimated. Next, the influence of functional monomer to template ratio on imprinting was studied, and the range of this ratio was optimized. Finally, the above results were combined and evaluated to design new MIPs with improved binding properties. A series of multi-functional monomer (multi-FM) were designed and discussed. Also, a diacid functional monomer was shown to be a better monomer compared to MAA.

3.1 Introduction

The first section 3.3.1 in this chapter focuses on the influence of monomer aggregation on the imprinting effect. Due to the low fidelity of imprinting process, a large excess of functional monomer (FM) is used to drive the formation of the functional monomer-template complexes in the prepolymerization solution. Thus, the majority of the FMs are not associated with template; instead, they generate a large percentage of background binding sites, which led to poor selectivity. As discussed in Chapter 1, one solution to this problem is to design functional monomers with high affinity. Also, the
development of MIPs with high imprinting efficiencies involves optimizing imprinting conditions. Thus, variables including crosslinking degree, monomer to template ratio, temperature, solvents, template aggregation, and concentration in the imprinting process, have been extensively studied.

This led us to set out to further explore the relationship and importance of monomer aggregation with imprinting effect in section 3.3.1. There have been few discussions in literature of the influence of monomer aggregation in the imprinting process. Our recent studies with a triurea-based MIP showed surprisingly low numbers of background binding sites. We hypothesized that this might be due to FM aggregation.

![Figure 3.1](image_url)  
**Figure 3.1** Structure of FMs, templates and cross-linkers utilized in the study of effect of monomer aggregation.

Monomer aggregation has two important consequences: first, it greatly enhances the selectivity of the resulting MIP by reducing the number of background sites in MIP. Second, it can lead to large differences in the binding properties between the imprinted
and non-imprinted polymers by reducing the background sites in NIP. This can lead to over-estimations of molecular imprinting efficiencies and to the incorrect assignment of the imprinting effect.

We studied the effect of FM aggregation using two different functional monomers. The first is methacrylic acid (MAA) that dimerizes and the second is urea-based functional monomers that aggregate. MAA and urea functional monomers were chosen due to their strong dimerization/aggregation ability. EA9A and TBA-DPP were used as the template due to their high affinity towards MAA and urea monomer, respectively. The structure of these functional monomers and templates are shown in Figure 3.1. In each case, the functional monomer aggregation was verified by NMR dilution studies. Then, we applied the polar solvent titration (PST) method to measure the influence of aggregation to the imprinting effect and the relative magnitude of aggregation effect.

In section 3.3.2, the goal is first to verify that molecular imprinting requires high crosslinking degree and to test the minimum crosslinking degree for imprinting. The structure of the functional monomer and template can be seen in Figure 3.2. MAA and EA9A system was chosen because they are commercially available and this system has been proved to be able to form imprinted polymers. Methyl methacrylate (MMA) was chosen as the co-monomer because it is not a crosslinker and has no carboxylic acid functionality. The crosslinking degree was varied by replacing a certain percentage of EGDMA with a non-hydrogen bonding co-monomer methyl methacrylate (MMA).
Then, the magnitude of effect of the crosslinking degree was confirmed using a second MIP system (Figure 3.3). Two mono-urea functional monomers were chosen. FM 1 is a crosslinking FM and FM 2 is non-crosslinking. These two FMs were used to study our PST method in Chapter 2 and interestingly we found that MIPs made with FM 1 were successfully imprinted, however MIPs made with FM 2 were not imprinted at all. These observations encouraged us to study whether the lack of imprinting effect in MIPs made with FM 2 is due to the lower crosslinking degree compared to the MIPs made with FM 1. The magnitude of the effect of crosslinking can be estimated using these two FMs. TBA-DPP was used as the template, and EGDMA was used as crosslinker. MMA that bears no urea functionality was used as a co-monomer to reduce crosslinking degree. The crosslinking degree was varied by replacing a certain percentage of EGDMA with a non-hydrogen bonding MMA.

The role of the crosslinking agent is to make a highly crosslinked, rigid polymer matrix, which holds the functional groups in the monomer recognition group in specific locations around the template molecule. The amount of crosslinking agent varies according to the application for the molecularly imprinted polymer. For chromatographic analysis, molecularly imprinted polymers need to contain a large amount of cross-linking
agent to achieve sufficient mechanical stability and good selectivity. For sensing applications, lower percentages of cross-linking agent help solvent accessibility of molecularly imprinted polymer which shortens their recognition and response times.

![Figure 3.3](image)

Figure 3.3 Urea monomers and template TBA-DPP for the study of effect of crosslinking degree.

The next section 3.3.3 studies the influence of the FM to template ratio on the imprinting effect. The goal of this section is to find the best range of monomer to template ratio for imprinting efficiency of MAA polymers templated with EA9A. A series of polymers containing varying amount of EA9A template were prepared using MAA as functional monomer, EGDMA as cross-linker, acetonitrile as solvent and the binding capacities were characterized using PST analysis.

The formation of functional monomer-template complexes creates the recognition sites in the final MIP. The ratio of functional monomers to template molecule greatly influences the quantity and quality of recognition sites. If the ratio is too low, an insufficient number of monomer recognition sites are formed. A large monomer to template ratio is more commonly used to increase the number of templated recognition sites, as well as when template molecule is expensive, difficult to dissolve, or hard to prepare in synthesis process. However, if the ratio is too high, there are a large number of
unbound monomers, resulting in the formation of large quantities of unselective background sites, which greatly reduce the selectivity of MIP.

Finally, in section 3.3.4, the results from the above studies will be combined to design new MIPs. Specifically a new multi-arm FM 3 was designed which has high self-aggregation and association towards EA9A (Figure 3.4). This new multi-FM should have: 1) high affinity towards template molecules to form templated sites when template is present; 2) strong molecular aggregation or intra-molecular binding to reduce number of untemplated background sites; 3) present multiple polymerizable groups lining the recognition sites. Also, crosslinking degree and monomer to template ratio is also considered. The imprinting effect of MIP templated with EA9A was characterized using PST method and the comparison of MAA polymers was performed.

![Figure 3.4 New multifunctional diacid FM 3 and template EA9A.](image)

### 3.2 Experimental Section

#### 3.2.1 Polar solvent titration (PST) analysis

Polar solvent titration (PST) analysis was applied for each polymer to characterize the binding capacities. First, a series of polymers were prepared in solutions containing increasing concentrations of a polar solvent (polar solvent additive). Then the batch binding studies were performed for each series. The resulting plots for MIP and NIP pair
were compared and analyzed with the five PST scenarios. Detailed introductions and explanations of PST analysis can be found in Chapter 2.

Scheme 3.1 The 5 PST analysis outcomes showing varying combinations of imprinting effects in the MIP and FM aggregation in the NIP.

### 3.2.2 Polymer preparation

**General procedure**

Prepolymerization mixtures containing functional monomer, template, crosslinker, initiator, and solvent were degassed in an ultrasonic bath for 5 min under nitrogen. The tightly capped vials were then immersed in a water bath at 65 °C for 6 h. The resulting monoliths were crushed and ground with a mortar and pestle. The templates and unreacted species were removed by Soxhlet extraction with methanol for 24 h and then, with a mixture of methanol/acetonitrile (1:4 v/v) for another 24 h. The MIP particles were finally dried overnight under vacuum. The corresponding NIPs were synthesized following the same protocol but without template.
Preparation of MIP 0 and NIP 0 (FM = MAA)

EA9A (0.025 g, 0.11 mmol), MAA (0.094 g, 1.1 mmol), EGDMA (1.89 g, 9.54 mmol), and AIBN (0.033 g, 0.20 mmol) were dissolved in 2 mL of solvent of varying polarities in a screw capped vial. For MIP 0, methanol was used as the polar solvent additive. MIP 0(AcOH) was prepared using acetic acid as the polar solvent additive. NIPs were synthesized following the same protocol but without EA9A. The polymerization compositions are shown in Table 3.1.

**Table 3.1** Polymerization components for preparation of MIP 0.

<table>
<thead>
<tr>
<th>polymer</th>
<th>MAA (mmol)</th>
<th>EA9A (mmol)</th>
<th>EGDMA (mmol)</th>
<th>AIBN (mmol)</th>
<th>Solvent (2 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 0</td>
<td>1.10</td>
<td>0.11</td>
<td>9.54</td>
<td>0.20</td>
<td>CH₃COOH/CH₃CN (0 to 25% v/v)</td>
</tr>
<tr>
<td>MIP 0</td>
<td>1.10</td>
<td>0.11</td>
<td>9.54</td>
<td>0.20</td>
<td>CH₃OH/CH₃CN (0 to 45% v/v)</td>
</tr>
</tbody>
</table>

Preparation of MIP 0 and NIP 0 with varying degree of crosslinking

MIP 0(90%), MIP 0(80%), MIP 0(60%), MIP 0(50%), and MIP 0(20%) were made using the same amount of MAA, EA9A, AIBN and solvent as MIP 0. The amounts of EGDMA were 8.95 mmol, 7.63 mmol, 3.82 mmol, 4.77 mmol, and 1.91 mmol, respectively, corresponding to 90%, 80%, 60%, 50%, and 20% of the crosslinking percentage of MIP 0. The concentration of the combination of EGDMA and MMA was kept constant (9.54 mmol) to keep the monomer concentration constant for each polymer. Then 0.95 mmol, 1.91 mmol, 3.85 mmol, 4.77mmol, and 7.63 mmol MAA were added to the prepolymerization solutions of MIP 0(90%), MIP 0(80%), MIP 0(60%), MIP 0(50%), and MIP 0(20%), respectively. The polymerization compositions and the calculated crosslinking degree are in Table 3.2.
Table 3.2 Polymerization compositions for MIP 0 and NIP 0 with varying degree of crosslinking.

<table>
<thead>
<tr>
<th>polymer</th>
<th>monomer (mmol)</th>
<th>MMA (mmol)</th>
<th>EA9A (mmol)</th>
<th>EGDMA (mmol)</th>
<th>MMA (mmol)</th>
<th>Crosslinking degree (mol %)</th>
<th>AIBN (mmol)</th>
<th>Solvent (2 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 0 (90%)</td>
<td>1.10</td>
<td>0.11</td>
<td>8.59</td>
<td>0.95</td>
<td>80.7</td>
<td>0.20</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
<tr>
<td>MIP 0 (80%)</td>
<td>1.10</td>
<td>0.11</td>
<td>7.63</td>
<td>1.91</td>
<td>71.7</td>
<td>0.20</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
<tr>
<td>MIP 0 (60%)</td>
<td>1.10</td>
<td>0.11</td>
<td>5.72</td>
<td>3.82</td>
<td>53.8</td>
<td>0.20</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
<tr>
<td>MIP 0 (50%)</td>
<td>1.10</td>
<td>0.11</td>
<td>4.77</td>
<td>4.77</td>
<td>44.8</td>
<td>0.20</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
<tr>
<td>MIP 0 (20%)</td>
<td>1.10</td>
<td>0.11</td>
<td>1.91</td>
<td>7.63</td>
<td>18.0</td>
<td>0.20</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
</tbody>
</table>

Preparation of MIP 1, NIP 1, MIP 2, and NIP 2

The preparation of these polymers was presented in Chapter 2. FM 1 (0.1707 g, 0.6 mmol), 0.0984 g (0.2 mmol) TBA-DPP, 0.754 mL (4 mmol) EGDMA, 0.0164 g (0.1 mmol) AIBN were dissolved in 2.5 mL of solvent of increasing polarities (Table 3.3). MIP 3 were made under the same condition using FM 2 instead of FM 1. Corresponding NIPs were synthesized following the same protocol but without EA9A.

Table 3.3 Polymerization conditions for MIP 1, MIP 2 and MIP 1a.

<table>
<thead>
<tr>
<th>polymer</th>
<th>monomer (mmol)</th>
<th>MMA (mmol)</th>
<th>TBA-DPP (mmol)</th>
<th>EGDMA (mmol)</th>
<th>AIBN (mmol)</th>
<th>Solvent (2.5 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 1</td>
<td>FM 1 (0.60)</td>
<td>0</td>
<td>0.20</td>
<td>4.00</td>
<td>0.10</td>
<td>DMSO/chloroform (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 2</td>
<td>FM 2 (0.60)</td>
<td>0</td>
<td>0.20</td>
<td>4.00</td>
<td>0.10</td>
<td>DMSO/chloroform (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 1a</td>
<td>FM 1</td>
<td>0.70</td>
<td>0.20</td>
<td>4.00</td>
<td>0.10</td>
<td>DMSO/chloroform</td>
</tr>
</tbody>
</table>
Preparation of MIP 1a, NIP 1a, and MMA control polymer

FM 1 (0.7 mmol), TBA-DPP (0.2 mmol), EGDMA (4 mmol), MMA (0.7 mmol), and AIBN (0.1 mmol) were dissolved in 2.5 mL of solvent of increasing polarities. NIP 1a were made using same compositions but without TBA-DPP. MMA control polymers were made under the same condition but without FM 1 and TBA-DPP.

Preparation of MIP 0 (a – e) and corresponding NIPs

MIP 0 (a - e) were made using the same amount of MAA, EGDMA, AIBN and solvent as MIP 0 (Table 3.4). However, the amounts of EA9A were 0.143 mmol, 0.077 mmol, 0.044 mmol, 0.022 mmol, and 0.011 mmol, respectively, which were 130%, 70%, 40%, 20%, and 10% of the amount of EA9A in MIP 0.

Table 3.4 Polymerization compositions for MIP 0(a - e).

<table>
<thead>
<tr>
<th>polymer</th>
<th>MAA (mmol)</th>
<th>EA9A (mmol)</th>
<th>EGDMA (mmol)</th>
<th>AIBN (mmol)</th>
<th>M/T</th>
<th>Solvent (2mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 0</td>
<td>1.10</td>
<td>9.54</td>
<td>0.20</td>
<td>10</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
<td></td>
</tr>
<tr>
<td>MIP 0(a)</td>
<td>1.10</td>
<td>0.143</td>
<td>9.54</td>
<td>0.20</td>
<td>7.7</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 0(b)</td>
<td>1.10</td>
<td>0.077</td>
<td>9.54</td>
<td>0.20</td>
<td>14</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 0(c)</td>
<td>1.10</td>
<td>0.044</td>
<td>9.54</td>
<td>0.20</td>
<td>25</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 0(d)</td>
<td>1.10</td>
<td>0.022</td>
<td>9.54</td>
<td>0.20</td>
<td>50</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
</tr>
<tr>
<td>MIP 0(e)</td>
<td>1.10</td>
<td>0.011</td>
<td>9.54</td>
<td>0.20</td>
<td>100</td>
<td>Methanol/acetonitrile (0 to 45% v/v)</td>
</tr>
</tbody>
</table>
Preparation of MIP 3 and NIP 3

FM 3 (0.55 mmol), EA9A (0.11 mmol), AIBN (0.2 mmol), and EGDMA (9.54 mmol) were mixed and polymerized in 2 mL of methanol/acetonitrile solvent (0 to 45% v/v) for 4 hours. NIP 3 were synthesized following the same protocol but without EA9A.

3.2.3 Batch rebinding study

MIP 1, NIP 1, MIP 1a, NIP 1a, MMA control polymer, MIP 3, and NIP 3

The binding capacities of these polymers were measured by shaking 60 mg of each polymer in 3.5 mL of a 0.5 mM solution of TBA-DPP in CHCl₃. The suspension was filtered, and the concentration of unbound TBA-DPP remaining in the supernatant was measured by UV-vis analysis (266 nm). The amount bound was calculated simply by subtracting unbound concentration from the 0.5 mM TBA-DPP solution. The binding capacity (µmol/g) is amount bound per weigh unit of polymers.

MIP 2 and NIP 2

MIP 2 and NIP 2 (105 mg) were tested under the same conditions as MIP 1.

MIP 0 and NIP 0 series

For the batch binding study, 2.5 mL of a 0.1 mM solution of EA9A in acetonitrile was shaken for 2 hours with 60 mg of polymer. The solution was filtered to remove all particles and the absorbance (257 nm) of the supernatant was measured. The percent of EA9A bound by the polymer was determined by the change in absorbance value of the measured supernatant compared to a stock solution 0.1 mM solution of EA9A in acetonitrile. The binding capacities were calculated based on the percent bound.
3.2.4 Synthesis of FM 3

The 2,2-diethylpentane-1,3-diol (528.8 mg, 4 mmol) and itaconic anhydride (986.3 mg, 8.8 mmol) were dissolved in toluene and heated for 96 h at 100 °C to give FM 3 as yellow viscous oil with 80% yield. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 11.51 (s, 2 H), 6.42 (s, 2 H), 5.80 (s, 2H), 3.90 (d, $J = 7.1$, 4 H), 3.33 (s, 4 H), 1.30 (m, $J = 7.8$, 4 H), 0.86 (m, $J = 7.8$, 6 H). $^{13}$C NMR (75 MHz, Acetone-d$_6$): 171.32, 137.75, 128.99, 125.27, 66.02, 39.53, 37.49, 22.91, 6.90. HRMS (EI) $m/z$ Calc. For M$^+$ (C$_{17}$H$_{24}$O$_8$) observed = 355.14, calculated = 355.13.

3.2.5 Synthesis of FM 4

Isopropyl alcohol (0.415 mL, 5.4 mmol) and itaconic anhydride (465 mg, 4.15 mmol) was dissolved in toluene and the mixture was heated for 96 h at 100 °C. The residue was collected with a 70% yield after removal of solvent. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 11.18 (s, 2 H), 6.20 (s, 1 H), 5.62 (s, 1 H), 4.80 (d, $J = 7.4$, 2 H), 3.43 (m, $J = 7.1$, 1 H), 1.01 (d, $J = 7.8$, 6 H).

3.2.6 Aggregation constant

The aggregation constant $K_{agg}$ of MAA, FM 1, FM 3 and FM 4 were determined by following the chemical shift of the carboxylic acid proton (MAA, FM 3, and FM 4) or urea proton (FM 1) through a $^1$H NMR dilution in the concentration range of 0.02 to 8.0 M (MAA, FM 3, and FM 4) or 0 to 80.0 M (FM 1). The aggregation constant was calculated by using a numerical curve fitting procedure described by William et al. $^{20}$

3.2.7 Association constant

The association constant $K_a$ of FM 3 and FM 4 to template EA9A was determined by following the chemical shift of a specific proton that participated in template-monomer
association through a $^1$H NMR titration. The association constant was calculated using a 1:1 binding model. $^{19}$

3.2.8 Surface area study

The surface area was measured by using Quantachrome Autosorb Automated Gas Sorption System (under $N_2$). The values were calculated using Branauer-Emmett-Teller (BET) model.

3.3 Results and Discussion

3.3.1 Functional monomer aggregation

The goal in this section is to study the effect of monomer aggregation on the imprinting. Until recently, researchers have believed that the higher binding capacities of MIPs versus NIPs could be entirely attributed to the imprinting effect. Our recent studies$^{16}$ led us to postulate that FM aggregation in the prepolymerization solution can lead to dramatic overestimations or even misassignment of the imprinting effect by reducing the number of non-selective background binding sites in the NIPs making them poor control polymers.

The common use of a large excess of FM to drive the formation of the monomer-template complexes in prepolymerization solution leads to the formation of large numbers of background sites in addition to the desired templated sites (Scheme 3.2, left). On the other hand, MIPs made with FMs that dimerize or aggregate contain templated sites, background sites, and aggregation sites. FM aggregation also can largely reduce the number if background sites in NIP (Scheme 3.2, right).
Scheme 3.2 Schematic illustration of the influence of functional monomers dimerization upon MIPs and NIPs.

In this section, first MIPs and NIPs were prepared using MAA (or urea FM) as functional monomer, EA9A (or TBA-DPP) as template, EGDMA as crosslinker, AIBN as initiator. The PST method introduced in Chapter 2 provided us the ability to differentiate differences in binding capacity arising from the imprinting effect and FM aggregations. It can also provide a measure of the magnitudes of each effect. Thus, this new characterization method was applied to each set of polymers by making polymer using varying degree of polar solvent additives. Then the aggregation constant of MAA was measured using NMR dilution study. Finally, batch binding studies of resulting polymers were performed and the magnitude of the changes can provide a measure of the effect of monomer aggregation and imprinting effect in MIPs.

The study in this section is a collaborative project with Dr. Yagang Zhang. My role was making polymers and performing some of the batch rebinding studies.

First, in NMR dilution study, the chemical shifts of the carboxylic acid hydrogen in MAA was followed and fitted to an isodesmic aggregation EK model to yield a $K_a = 2.5$ in acetonitrile.

Then, a series of polymers were prepared and our PST analysis was applied.
The batch binding study results of the resulting polymers MIP 0 and NIP 0 are plotted in Figure 3.5. MIP 0 and NIP 0 are two series of MAA polymers prepared in the presence and absence of EA9A in increasing concentration of polar solvent additive methanol. MIP 0 (AcOH) and NIP 0 (AcOH) are polymers prepared under the same conditions except the polar solvent additive was switched to acetic acid.

The value of increases in binding capacities of NIPs provided a measure of effect of monomer aggregation according to PST analysis (Scheme 3.1). In Figure 3.5, the binding capacities of the last NIPs (made in the most polar solvent) were a lot higher than the binding capacities of the first NIPs (made in the least polar solvent), which indicated that the addition of either polar solvent (methanol or acetic acid) to the pre-polymerization mixture dramatically increased the binding capacities of the NIPs (four fold increases). The large increase in binding capacity showed that there was significant FM dimerization which reduced the background binding sites in the first NIPs. The high concentration of polar solvent in the last NIPs disrupted FM dimerization which led to an increase in binding capacities. The magnitudes of the increases were calculated by subtracting the binding capacity of first NIP 0 from the binding capacities of last NIP 0.

The magnitudes of the decreases in binding capacities of MIPs provided a measure of the imprinting effect (scheme 3.1). The binding capacities of the last MIPs (made in the most polar solvent) were a lot lower than the binding capacities of the first MIPs (made in the least polar solvent), which indicated that the addition of either polar solvent (methanol or acetic acid) to the pre-polymerization mixture dramatically decreased the binding capacities of the MIPs. The magnitudes of the decreases were calculated by subtracting the binding capacity of last MIP 0 from the binding capacities of first MIP 0.
The large decrease (40% decreases) in binding capacity in Figure 3.5 demonstrated that the imprinting effect in MIP 0 was disrupted by the addition of polar solvent additive (methanol or acetic acid). The binding affinity of MIP is mainly based on the number and quality of the imprinted sites. Thus, the significant decreases caused by disruption of imprinting led to a 40% decrease in binding capacities.

The largest difference in binding capacity between the first MIP 0 and the first NIP 0 is due to a combination of imprinting effect and monomer aggregation. As a result of the disruption of imprinting and monomer aggregation processes, MIP and NIP eventually became identical as the amount of polar solvent increased. Thus, the simple MIP versus NIP batch binding study may lead to an overestimation of the imprinting effect because the differences in binding capacities of MIP and NIP may come from two sources, imprinting effect and monomer aggregation.

In comparison of two MIP series in Figure 3.5, it took more methanol than acetic acid to disrupt the dimerization and imprinting processes. This is probably because not only is acetic acid more polar but also acetic acid is more structurally similar to MAA and can more efficiently compete with MAA in the dimerization and templation processes.

Next, NMR dilution study was performed to measure the aggregation ability of FM 1. The chemical shifts of urea -NHs in FM 1 was followed and fitted to an isodesmic aggregation EK model to yield a $K_a = 3.5$ in chloroform.
Figure 3.5 Binding capacities for EA9A of a series MIPs and NIPs polymerized in acetonitrile solutions containing varying percentages of methanol or acetic acid.\textsuperscript{18}

In batch binding study, similar trends were observed for MIP 1 and NIP 1 made with FM 1 and TBA-DPP template, which can be seen in Figure 3.6. The addition of DMSO dramatically increased the binding capacities of the NIPs by disrupting the urea monomer aggregation. The increase in binding capacities of NIPs provided a measure of the effect of monomer aggregation. The DMSO additive also dramatically decreased the binding capacities of the MIPs by disrupting the imprinting effect. The magnitude of this decrease provided a measure of the imprinting effect. The largest difference between MIP and NIP was observed when both of the polymers were prepared in the least polar solvent due to a combination of imprinting effect and monomer aggregation. The deactivation of the background sites by monomer aggregation in NIP 1 can lead to an overestimation of imprinting effect of MIP 1.
Figure 3.6 Binding capacities for TBA-DPP of MIPs (solid) and NIPs (empty) prepared in solutions of increasing polarity from pure CHCl₃ to 45% v/v DMSO–CHCl₃, as measured by uptake studies using 60 mg polymer in 3.5 mL of 0.5 mM TBA-DPP in CHCl₃.

In summary, monomer aggregation or dimerization can increase the differences in binding capacity of the MIP and NIP by suppressing the background binding sites in NIP. The relative magnitude of the effect of monomer aggregation can be estimated by subtracting the binding capacity of the first NIP (made in the least polar solvent) from the binding capacity of the last NIP (made in the most polar solvent) using PST analysis. Also, the monomer aggregation reduced the number of background sites in MIP leading to an improvement of the selectivity of the imprinting effect. Thus, monomer aggregation can be utilized to design MIP with improved selectivity by designing functional monomers with strong monomer aggregation.
3.3.2 Crosslinking degree

The goal of this section is to study the effect of crosslinking degree in imprinting. The quality of specific recognition sites in molecularly imprinted polymers is related to the mole ratio of crosslinking agent in molecularly imprinted polymer structure. Increasing the amount of cross-linking agent can improve imprinting efficiency by increasing the rigidity of the polymer which helps hold/maintain imprinted sites in polymer matrices. Reduction of the amount of cross-linking agent can improve the solvent accessibility by increasing the flexibility of polymer chains that creates pores and channels for molecule access.

In this section, we first tested if high crosslinking degree is required for imprinting. We use a series of MAA polymers templated with EA9A prepared with different amount of crosslinker. Then the minimum amount of crosslinking degree needed for maintaining imprinting effect was obtained for this system. Another two urea FMs were introduced to estimate the magnitude of the effect of crosslinking degree to imprinting effect.

MIP 0 represents the standard polymer made under our predesigned conditions which was discussed in previous section in this chapter. MIP 0(90%), MIP 0(80%), MIP 0(60%), MIP 0(50%), and MIP 0(20%) are polymers series made under the same conditions of MIP 0 except 10%, 20%, 40%, 50%, and 80% of the amount of EGDMA as in MIP 0 were replaced by the same amount of a non-crosslinking monomer MMA. Then the crosslinking degree in MIP 0(90%), MIP 0(80%), MIP 0(60%), MIP 0(50%), and MIP 0(20%) are 90%, 80%, 60%, 50%, and 20% of the crosslinking degree of MIP 0, respectively. NIP 0(90%), NIP 0(80%), NIP 0(60%), NIP 0(50%), and NIP 0(20%) are corresponding NIPs made without the template.
Figure 3.7a shows that the binding capacities of MIP 0, MIP 0(90%), and MIP 0(80%) decreased gradually with increasing polarity of solvents. It suggested that highly crosslinked polymers including MIP 0, MIP 0(90%), and MIP 0(80%) were successfully imprinted according to PST analysis. However, the curve of the binding capacities of MIP 0(60%) series started to become slightly undulate. It suggested MIP 0(60%) with 54% crosslinking density (this number was calculated and is shown in Table 3.2) started to lose some of the imprinting effects due to the lower crosslinking degree.

The binding capacities of NIP 0, NIP 0(90%), and NIP 0(80%) increased gradually with increasing polarity of solvents; whereas the binding capacities of NIP 0(60%) series is slightly undulate which is similar to MIP 0(60%). These observations suggested that NIP started to lose some of the monomer aggregation at 54% crosslinking density.

In Figure 3.7b, the binding capacities of MIP 0(50%) and MIP 0(20%) do not decrease while the concentration of polar solvent increases, which suggested that polymers showed no imprinting effect when the crosslinking degree was below 45 w/w % (Table 3.2) according to PST analysis. It is likely that at such a low crosslinking density, the spaces and pores within the polymer allow ready access for solvents and templates. Such a fluid, porous environment makes it harder to form imprinted sites and monomer aggregation sites. Thus, polymers totally lost their ability to hold the pre-formed recognition sites during imprinting process.

In Figure 3.7a, higher crosslinking polymers exhibited greater imprinting effect. For example, MIP 0(80%) had overall smaller binding capacities than MIP 0(90%) which in turn were smaller than MIP 0. This indicated that higher rigidity of the polymer matrix can better preserve the imprinting effect.
Figure 3.7 The binding capacities of MIPs (solid) and NIPs (empty) that made with varying crosslinking degree.

MIP 1 and MIP 2 were prepared with FM 1 and FM 2, respectively. FM 1 and FM 2 are urea monomers containing only one urea recognition functionality. The only difference between these two monomers is that FM 1 is a crosslinking urea monomer but FM 2 is non-crosslinking. MIP 1 and MIP 2 represent TBA-DPP templated polymers.
made with FM 1 and FM 2, respectively. NIP 1 and NIP 2 are corresponding NIPs made under the same condition but without TBA-DPP.

Figure 3.8 Binding capacities towards TBA-DPP of MIPs (solid) and NIPs (empty) made with a) FM 1 (0.6 mM), b) FM 2 (0.6 mM), c) FM 1 (0.7 mM) and a co-monomer MMA (0.6 mM), and d) MMA (0.6 mM).

The binding capacities of polymers made with FM 1 and FM 2 showed large differences in binding capacities towards TBA-DPP (Figure 3.8a and 3.8b). The binding capacities of MIP 1 decreases with the increasing concentration of polar solvent.
However, the binding capacities of MIP 2 did not decrease with the increasing amount of polar solvent additive DMSO. According to our PST analysis, MIP 1 were imprinted but MIP 2 were not imprinted. There are two reasons for this observation: 1) MIP 2 and NIP 2 have lower crosslinking degree, which is about 13% that was calculated from the polymerization compositions showed in Table 3.3, because FM 1 is a crosslinking monomer, but FM 2 is not; 2) the urea group in FM 2 is too flexible to hold imprinted sites after polymerization.

In order to test whether the lack of imprinting effect observed in MIP 2 was due to the lower crosslinking degree, we synthesized a series of polymers (MIP 1a and NIP 1a) with FM 1 and an additional monomer MMA (0.7 mM) to reduce the crosslinking degree to be the same as in MIP 2 and NIP 2. MMA was chosen as the co-monomer because it has no hydrogen bonding ability to TBA-DPP. The amount of FM 1 was also increased to 0.7 mM in order to keep the monomer concentration of MIP 1, MIP 2, and MIP 1a the same. MMA control polymers were made with 0.7 mM of MMA co-polymerized with EGDMA but without FM 1 and TBA-DPP. These polymers were prepared to check whether the introduction of MMA influence the imprinting effect or not.

According to Figure 3.8d, binding capacities of the control MMA polymers made in the same solvent were extremely low (in average 1.5 μmol/g). Compared to the binding capacities of MIP 1a (11.1 – 17.9 μmol/g) which are shown in Figure 3.8c, the binding capacity of control MMA polymer can be neglected. It proved that the introduction of MMA did not influence the affinity of urea polymer to TBA-DPP.

MIP 1a, which contain the same crosslinking density as MIP 2, were shown to have decreased binding capacities while the concentration of polar solvent increases, which
showed that MIP 1a have imprinting effect according to PST method (Figure 3.8c). The binding capacities of MIP 1a made in increasing amount of DMSO dropped dramatically and steadily. This observation suggested that the lack of imprinting effect in MIP 2 was not due to the lower crosslinking degree compared to MIP 1 because the imprinted MIP 1a has the exactly same crosslinking degree as MIP 2. Thus, it was mainly due to the flexibility of recognition sites. The urea functionality in the templated sites has a high degree of mobility leading to lower affinities and selectivities.

The overall binding capacities of MIP 1a series were a lot lower than that of MIP 1 (Figure 3.8b and 3.8c). The differences between them were entirely caused by the lower crosslinking degree in MIP 1a. Considering MIP 2 and MIP 1a have the same crosslinking degree, thus, the lower binding capacities of MIP 2 were not only due to the flexibility of recognition sites in MIP 2, but also partially due to the lower crosslinking degree, however, which is not the main reason for the loss of imprinting effect.

To estimate the relative magnitude of changing 13% of crosslinking degree from 100% in MIP 1 to 87% in MIP 1a, we subtracted the binding capacity of MIP 1a made in pure chloroform (17.9 μmol/g) from the binding capacity of MIP 1 made in the same solvent (23.6 μmol/g) to give a value of 5.7 μmol/g. This value was calculated to be 24% of the binding capacity of MIP 1 made in chloroform. Thus, the effect of reducing 13% of the crosslinking degree is to decrease 24% (about double of the decrease in crosslinking degree) of the binding capacity of MIP 1.

In summary, high crosslinking degree was required for imprinting effects. Higher crosslinked polymer exhibits greater imprinting effect. Thus, to make an MIP with high binding capacity, the crosslinking degree should be kept high. Also, the polymerizable
groups near recognition groups are required to form rigid recognition sites, otherwise, loss of imprinting effect will be observed. Finally, we observed that little variations might leads to huge differences in binding capacities. The effect of decreasing 13% of crosslinking degree was estimated to reduce 24% of the imprinting effect in urea polymers templated with TBA-DPP.

3.3.3 Functional monomer to template ratio

The goals of this section is first to test the importance of functional monomer/template ratio, and then find the best range of this ratio for imprinting. To test this, a series of polymers were prepared that contain varying amount of EA9A template using MAA as functional monomer, EGDMA as cross-linker, acetonitrile as solvent. Next, we evaluated the binding capacities of each of the polymer by using PST analysis. The PST method was applied to this study.

MIP 0, MIP 0(a), MIP 0(b), MIP 0(c), MIP 0(d), and MIP 0(e) are MAA polymers templated with varying amount of EA9A. The concentrations of the monomer MAA for each polymer were kept the same. In order to test polymers with different monomer/template ratio, the amount of template was varied. The monomer/template ratios for MIP 0, MIP 0(a), MIP 0(b), MIP 0(c), MIP 0(d), and MIP 0(e) are 10/1, 7.7/1, 14/1, 25/1, 50/1, and 100/1, respectively (Table 3.4).

Figure 3.9 suggested that the imprinting effect is related to the amount of template used in making polymer. For example, MIP 0(a) are the polymers made with the smallest monomer/template (M/T) ratio as 7.7:1. Yet, this series of polymers had the largest binding capacities (imprinting). To totally disrupt the imprinting, at least 45% methanol/acetonitrile is needed. MIP 0(c) series have a bigger monomer/template ratio of
25:1, and their binding capacities are a lot lower than MIP 0(a) due to smaller number of imprinted sites. Also, it showed to have weak imprinting effect (worse quality of the imprinted sites) because 5% methanol/acetonitrile is strong enough to mostly disrupt the imprinting. MIP 0(e) series were tested to have the lowest binding capacities and their monomer/template ratio is the largest (100:1). MIP 0 and MIP 0(b) have similar monomer/template ratio (10:1 and 14:1) and showed similar imprinting effect (binding capacities).

![Graph](image)

**Figure 3.9** The binding capacities of polymers that made with varying monomer/template ratio.

Interestingly in Figure 3.9, MIP 0(d) and MIP 0(e) showed increasing binding capacities while the polarity of solvents is increasing until the percentage of polar solvent reached 5%. This observation suggested that both of MIP 0(d) and MIP 0(e) were not imprinted in the presence of too little template. This means that the amount of template is too little to form quality imprinted sites with functional monomers. Templates were only able to compete with monomer aggregation to disrupt some of the aggregation sits.
The presence of little amount of template disrupted some of the monomer aggregation, thus, the binding capacities of these two series of polymers were higher than NIP 0. The binding capacities did not change after the percentage of polar solvent reached 5%, which suggested that only a small amount of monomer aggregation sites were formed in the presence of little amount of template.

In summary, to form sufficient and quality templated sites, the monomer/template ratio should be equal to or smaller than 25:1 for MAA and EA9A system. On the other hand, the ratio can go down to 7.7:1 or even less. But remember, as discussed in the introduction, this ratio cannot be too low because the low fidelity of the imprinting process requires the large excess of functional monomer to drive the imprinting equilibrium to form monomer-template complexes. Also, a large monomer/template ratio is desirable when template molecule is expensive, difficult to dissolve, or hard to prepare in synthesis process.

3.3.4 New design of molecularly imprinted polymers

As introduced in Chapter 1, multi-functional monomers are a good choice for imprinting due to their higher affinity to template compared to mono-functional monomer. According to our studies in this chapter, we have shown that functional monomer aggregation helps the imprinting effect by suppressing the background sites to improve the selectivity of the resulting polymer. Also, more polymerizable groups near recognition functionalities in monomer should be a plus for imprinting by maintaining the rigidity of recognition sites within polymer matrices. We drew upon these ideas for our designs for new functional monomers.
Specifically, we targeted multi-functional monomers that could form strong intra- or inter-molecular aggregation. In the absence of template, functional monomer interacts with itself through intramolecular hydrogen bond or aggregated with each other; in the presence of template, the template break into the interaction center and form stable complexes with monomer. Our design positions these functionalities in close proximity to interact with templates. Our framework should be rigid enough to hold functionalities at specific distances and positions, but should have sufficient flexibility to form a cavity for templates. Finally, polymerizable groups must be placed adjacent to each recognition
group to rigidly anchor these functionalities in the desired conformation within the polymer matrix. We designed a series of multi-functional monomer and predicted them to be better monomers for imprinting (Figure 3.10).

However, due to the poor solubility, most of the above multi-FMs were ruled out. For example, FM (b, c, h) were bisamide functional monomers that can be synthesized from diamine and itaconic anhydride. They were successfully synthesized but proved to have poor solubility and only dissolve in strong polar solvent such as DMSO and methanol. These observations suggested that amide functional monomers typically have poor solubility and we should avoid this structure in design of new monomers. Triurea FM d was successfully synthesized from triamine and isocyanide but this monomer only dissolves in organic solvents containing a high concentration of DMSO. FM g was synthesized using bisphenol and methyl 2-(bromomethyl)acrylate then deprotected with lithium hydroxide. It only dissolves in methanol. The poor solubility of these above monomers limited the utilization in molecular imprinting.

Also, after many trials, due to the difficulty in synthetic approaches, we were not able to synthesize FM (a, e, f).

One monomer containing two methacrylic acid functionalities between moderately rigid alkyl frameworks stood out. Methacrylic acid (MAA) was known to be a good functional monomer for bases such as adenine, thus in this study, this monomer 3 was studied for the recognition of ethyl adenine-9-acetate (EA9A) template (Figure 3.11). It contains two carboxylic acid functionalities which should have great binding affinity to adenine templates comparable to MAA. Also, the self-aggregation was expected to be stronger than MAA for the same reason. Also, the alkyl framework in between contains a
bulky quaternary carbon center which might free rotate to push the two carboxylic acid groups towards the same direction and form not only inter-molecular aggregation but also strong intra-molecular hydrogen bonds. The imprinting effects of the resulting MIPs and NIPs were characterized using PST method.

![Figure 3.11](image_url)

**Figure 3.11** The intra-molecular hydrogen bonding and monomer aggregation in FM 3.

This is a collaborative project with Diana Rishmawi and Narmina Tyger. My role was synthesizing and characterizing monomers shown in Figure 3.10 including FM 3, preparing polymers, and performing some of the batch rebinding studies.

The association constant $K_a$ of FM 3 to template EA9A was determined by following the chemical shift of a specific proton that participated in template-monomer association through a $^1$H NMR titration. Titration was initially performed in acetonitrile and was calculated using a 1:1 binding model to give an unreasonably low $K_a$ (approximate to zero). Possible explanations for the low $K_a$ in acetonitrile are: 1) low overall binding affinity of FM 3 to EA9A; 2) a stronger competitive monomer aggregation, an intra-molecular monomer hydrogen bonding, or a combination of both of them are present within the monomer which binding constants calculations do not account for. Thus, the NMR titration was performed in less polar/competing solvent. The calculated $K_a$ (using the same 1:1 binding model) towards EA9A in chloroform is 230 M$^{-1}$, which is in the
same magnitude of MAA (140 M\(^{-1}\)). It suggested that FM 3-EA9A association is present and comparable to MAA-EA9A association.

The aggregation property of FM 3 was proved through NMR dilution study according to its concentration dependent behavior. It was done in chloroform in order to directly compare with the calculated \(K_a\). The aggregation constant was calculated by using a numerical curve fitting procedure described by William et al. \(^{20}\) The fitted and calculated \(K_{agg}\) is 1130 M\(^{-1}\) in chloroform indicating FM 3 aggregates more strongly than mono-functional monomer MAA as reported to be 330 M\(^{-1}\).\(^{14}\) It is even stronger than monomer-template association which provides an evidence for the unreasonable low \(K_a\) in acetonitrile. However, the large \(K_{agg}\) cannot prove the presence of intra-molecular hydrogen bonding that we predicted.

![Figure 3.12](image)

**Figure 3.12** Concentration dependent behavior of FM 3(solid) and FM 4 (empty).

Thus, a structurally similar FM 4 was designed and synthesized following the same synthetic approach. The calculated \(K_{agg}\) for FM 4 is 600 M\(^{-1}\) in chloroform which is one order magnitude smaller than FM 3 which suggests multi-FM 3 aggregates stronger than
mono-FM 4. FM 3 should be less concentration dependent compared to FM 4 when intra-molecular hydrogen bonds exist in FM 3. In other words, the changes in chemical shift of FM 3 carboxylic acid proton are smaller than that of FM 4 when diluted. More visually proving is the plotted chemical shift of carboxylic proton versus the log of calculated FM concentrations due to the difficulty to visualize and compare high order exponential plot of chemical shift versus concentration (Figure 3.12). At high concentration, especially within the normal polymerization FM concentration range, the chemical shift of FM 4 drops faster than the chemical shift of FM 3 as the concentration is lowering. This behavior suggests that the intra-molecular hydrogen bonding is probably present in FM 3 in polymerization conditions.

As discussed above, FM 3 has good affinity to EA9A. Therefore, we went ahead to test it as a functional monomer for imprinting. And it has both monomer aggregation and intra-molecular hydrogen bonding which was expected to dramatically reduce the background binding capacity thus the total binding capacities of resulting MIP. Therefore, FM 3 was polymerized using PST method under heat-initiated free radical polymerization. MIP 0 and NIP 0 (MAA as monomer) from section 3.2.2, page 64 were used as controls. A non-crosslinking monomer methyl methacrylate (MMA) was added to the FM 3 pre-polymerization solution to keep the crosslinking degree (mole %) the same as the control MAA polymers with no introduction of additional carboxylic acid functionalities. Since each FM 3 bears two carboxylic acid functionalities, the amount of FM 3 was reduced to half of the amount of MAA. As discussed in previous section, higher crosslinking degree works better for imprinting. Thus, a 90% crosslinking degree was chosen for this study. The monomer to template ratio for MAA/EA9A system should
be smaller than 25:1, then the ratio of 10:1 for MAA/EA9A and 5:1 for FM 3/EA9A was chosen. Considering MAA and EA9A were considered a 1:1 binding model, the 5:1 ratio in MIP 3 actually accounts for 10:1. The polymerization compositions are shown in Table 3.5. MIP 3 were prepared with 0.55 mmol of FM 3, 1.05 mmol of MMA, 9 mmol of EGDMA, 0.11 mmol of EA9A, and 0.2 mmol of AIBN in varying degree of methanol/acetonitrile mixture. MIP 0 were prepared under the same condition except functional monomer was switched to MAA (0.99 mmol) and no MMA was used. The concentration of functional monomer and crosslinking degree for these two series of polymers were kept the same.

**Table 3.5 Composition of the pre-polymerization mixture of MIP 3 and MIP 0.**

<table>
<thead>
<tr>
<th>polymer</th>
<th>FM (mmol)</th>
<th>MMA (mmol)</th>
<th>EGDMA (mmol)</th>
<th>EA9A (mmol)</th>
<th>AIBN (mmol)</th>
<th>Solvent (2 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP 3</td>
<td>FM 3 (0.55)</td>
<td>1.05</td>
<td>9</td>
<td>0.11</td>
<td>0.20</td>
<td>MeOH/ CH$_3$CN (0% to 50% v/v)</td>
</tr>
<tr>
<td>MIP 0</td>
<td>MAA (0.99)</td>
<td>----</td>
<td>9</td>
<td>0.11</td>
<td>0.20</td>
<td>MeOH/ CH$_3$CN (0% to 50% v/v)</td>
</tr>
</tbody>
</table>

Batch binding studies were carried out to examine the binding capacities of all the polymers as shown in Figure 3.13. The decrease in binding capacities of MIP 3 when made in increasing polarity provided a proof of successful imprinting. MIP bound more than twice of NIPs made in acetonitrile indicating an overall good imprinting effect. The difference in binding capacities of first MIP 3 (made in the least polar solvent) and last MIP 3 (made in the most polar solvent) provided a measure of imprinting effect. The value was calculated to be 0.78 μmol/g by subtracting the binding capacity of the last MIP 3 from the binding capacity of the first MIP 3.
Figure 3.13 Binding capacities of MIPs (solid) and NIPs (empty) made with FM 3 (triangle) and MAA (diamond).

As mentioned above, polymers made with FM 3 contain the same number of binding sites as MAA polymers. But it was expected to have lower binding capacities compared to MAA polymers due to the suppression of background binding sites by stronger monomer aggregation than MAA and the relatively smaller $K_a$ compared with $K_{agg}$ (weaker monomer-template association compared to monomer aggregation).

The batch binding results suggested that there are much lower binding capacities of MIP 3 compared to MIP 0 (Figure 3.13). First it indicates stronger intra- and inter-molecular FM interactions were present in FM 3 and the background binding sites were reduced which can be visualized directly from the difference between NIPs that made in pure acetonitrile.

One the other hand, considering the relatively small FM 3 $K_a/K_{agg}$ (approximately 0.2, one order smaller), monomer-template association is not strong enough to compete with the strong monomer-monomer interactions. This results in the very small amount of
templated sites (low binding capacities) observed in MIP 3 compared to MIP 0 (1< \( K_a/K_{agg} < 10 \), in the same order). For example, the difference in binding capacities of MIP 3 to MIP 0 that were made in least polar solvent is larger than that of NIP 3 to NIP 0, proving the reduced overall binding capacities in both MIP 3 were only partially due to the suppression of background sites caused by a combination of monomer aggregation and intra-molecular hydrogen bonds (Figure 3.13). It is also partially due to the low imprinting effects caused by the small \( K_a \).

In Figure 3.13, the tremendous strength of monomer aggregation is even greater than the polar solvent additives. For example, NIP 3 that made in the most polar solvent has almost identical binding capacity to (only 0.15 μmol/g larger than) NIP 3 made in pure acetonitrile, which again proves the great strength of monomer aggregation and intra-molecular interaction that was stable even in polar protic environment.

The relatively weaker FM 3-template association can also be observed from the decrease in binding capacity with increasing polarity (Figure 3.13). We observe that the association is not as strong as polar solvent methanol. The addition of methanol disrupts all the templated sites and then immediately these disrupted sites self-assemble into the stronger monomer aggregation and intra-molecular bonding sites making the binding capacities go even lower. Therefore, the combination of monomer inter- and intra-molecular interactions and relatively low \( K_a \) contributes to the overall low binding capacities observed in MIP 3.

In summary, the new FM 3 is able to immobilize to form MIP with good recognition efficiency. It can suppress the number of background sites when polymerized due to strong inter- and intra-molecular hydrogen bonding. The relatively weaker monomer-
template association also lowers the number of templated sites compared to MAA. A combination of above two factors results in the overall lower binding capacities in resulting MIP. However, the reduced background sites might contribute to the selectivity of MIP thus improves the overall quality of the monomer for imprinting.

3.4 Conclusions

In this chapter, we optimized monomer aggregation and high crosslinking degree to improve the imprinting efficiency. The amount of template was shown to be related to the imprinting effect. A new MIP which contains FM with strong monomer aggregation, high crosslinking degree, and good monomer to template ratio, was successfully developed. It was proved to have better imprinting efficiency compared to MAA polymer due to the efficient suppression of background sites by monomer aggregation.

3.5 References


CHAPTER 4

CARBOXYLATE SELECTIVE LANTHANIDE POLYMER “TURN-ON” SENSOR

Abstract

The work presents in this chapter involves the design and characterization of lanthanide-containing NIPs and MIPs that sensitively and selectively respond to carboxylates. First a fluorescent europium-containing complex bearing polymerizable vinyl groups was synthesized. The complex was immobilized in a polymer matrix with EGDMA as crosslinker in dichloroethane under free radical polymerization conditions. The sensing properties of the polymer were characterized by monitoring the titration of the polymers with different anion solutions via changes in the fluorescence spectra. The polymer was proved to be highly selective for carboxylate anions over halide and other oxy-anion analytes. Also, MIPs made with two different carboxylates showed better selectivity to the corresponding carboxylates.

4.1 Introduction

Luminescent materials for anion detection have been an area of active research.\textsuperscript{1-3} Lanthanide complexes have been previously investigated by researchers to make chemosensors for anions due to their high luminescent efficiencies and strong coordination properties.\textsuperscript{4-9} Sensors for carboxylates are of interest because of the importance of carboxylates in organic processes and biological systems. However, due to the small size of most anions and the high sensitivity of lanthanide sensor to all different kinds of anions,\textsuperscript{6, 10} no specific lanthanide sensor targeting carboxylates was successfully
designed and developed. In comparison to a small molecule sensor, the immobilization of lanthanide chemosensors into a polymer matrix can greatly reduce lanthanide self-quenching, improve stability and durability, and help regulate lanthanide-template interactions.\textsuperscript{11,12} Thus, we have been specifically interested in developing a carboxylate selective lanthanide polymer for use as colorimetric and fluorometric sensors (Scheme 4.1).

\textbf{Scheme 4.1.} Representation of a lanthanide polymer sensor for carboxylates.

There are several requirements for incorporating lanthanide complexes into a polymer matrix. 1) Ligands that coordinate lanthanides with polymerizable groups must be synthesized. 2) The lanthanide complex must be stable during polymerization. 3) The lanthanide complex must be soluble in the polymerization solvent. 4) The lanthanide complex must show a colorimetric or fluorometric response and selectivity upon binding anion analytes. A polymerizable salen lanthanide complex was chosen for this study due to its ease of synthesis and demonstrated stability to free radical polymerization conditions.\textsuperscript{13}

After making the lanthanide-complexes polymer, there are several requirements being a polymer sensor. 1) It must show a similar response and selectivity to the complex upon binding anions analytes. 2) It must possess long-lived excited-state life times. 3)
The response must be reproducible. 4) It must be reused for several cycles. In this chapter, the lanthanide containing polymer was prepared and the ability to be a carboxylate sensor was characterized.

4.2 Experimental section

4.2.1 General

$^1$H NMR spectra were recorded on a Varian 300 MHz NMR at ambient temperature. UV measurements were made using a Jasco V-530 spectrometer. Solvents were purchased from Sigma-Aldrich, Fisher and VWR. Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. All other reagents were purchased from Sigma-Aldrich and were used as received.

4.2.2 2-hydroxy-4-(4-vinylbenzyloxy)benzaldehyde

The aldehyde, 2,4-dihydroxylbenzaldehyde (75 mmol), was dissolved in 30 mL methanol, then 75 mmol of potassium hydroxide was added and stay for 30 minutes. After evaporation of solvent, the brownish-red residue was collected. The residue was suspended in 50 mL of acetonitrile to which an acetonitrile solution of 60 mmol of 4-vinylbenzyl chloride was added. Potassium iodide (25 mmol) was added and the reaction mixture heated at 50 °C for 10 hours. The reaction solution was filtered and solution was collected. After evaporation of the solvent, 50 mL of water and 100 mL of ethyl acetate was added. The organic layer was collected and was washed with 3% potassium carbonate, water, 5% citric acid each for three times. The solvent was removed by rotary evaporator and the residue was recrystallized in ethyl acetate. The pure product is white rod-like crystal (6.8 g, 45% yield). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 9.72 (s, 1 H), 7.40 (m,
5 H), 6.73 (dd, \( J = 17.7 \) Hz, \( J = 10.8 \) Hz, 1 H), 6.61 (dd, \( J = 8.7 \) Hz, \( J = 1.8 \) Hz, 1 H), 6.51 (m, 1 H), 5.78 (d, \( J = 17.7 \) Hz, 1H), 5.28 (d, \( J = 10.8 \) Hz, 1H), 5.10 (s, 2 H).

**4.2.3 Bis[2-hydroxy-4-(4-vinylbenzyloxy)benzaldehyde]ethylenediimine (salen)**

The 2-hydroxy-4-(4-vinylbenzyloxy)benzaldehyde (11 mmol) was suspended in 50 mL of dry methanol and sonicated. The solution was put under nitrogen and ethylenediamine (5 mmol) was slowly added. The reaction mixture was stirred for 16 hours. The precipitates were filtered and washed with ether. The greenish-yellow solid (2.26 g, 85% yield) was collected and dried. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) : 8.19 (s, 2H), 7.39 (m, 8 H), 7.08 (m, \( J = 8.4 \) Hz, 2 H), 6.71 (dd, \( J = 17.7 \) Hz, \( J = 10.8 \) Hz, 2 H), 6.45 (m, 4 H), 5.75 (d, \( J = 17.7 \) Hz, 2H), 5.25 (d, \( J = 10.8 \) Hz, 2H), 5.03 (s, 4 H), 3.85 (s, 4 H).

**4.2.4 Eu\(_2\) (salen)\(_3\)(H\(_2\)O)\(_2\) (salen-europium complex)**

Salen (6 mmol) was suspended in 50 methanol and 9 mmol of KOH was added. A methanolic solution of europium nitrate (4 mmol) was added and stir for 12 hours at 40 °C. The precipitate was filtered and washed with methanol to yield 3.6 g (93% yield) yellow solid. No further purification was needed. UV-vis UV/Vis (DMF, \( \lambda_{\text{max}} \), nm): 340 nm. FT-IR (Nujol, cm\(^{-1}\)): \( \nu \) (C=N) 1617 (s), 1594 (s). Fluorescent emission (DMF, \( \lambda_{\text{max}} \), nm): 614.

**4.2.5 Tetrabutylammonium phenylacetate**

To a 50 mL methanol solution of 4 mmol phenylacetic acid, 4 mL of 1 M tetrabutylammonium hydroxide solution was added. The solvent was removed and the residue was dried under vacuum to yield 1.5 g clear oil with a 98% yield. The resulting salt was stored under anhydrous conditions. \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) : 7.37 (d, \( J = \)
7.4, 2 H), 7.21 (m, 2 H), 7.08 (t, $J = 7.1$ Hz, 1 H), 3.51 (m, 2 H), 3.18 (m, 8 H), 1.52 (m, 8 H), 1.33 (m, 8 H), 0.93 (m, 12 H).

4.2.6 Tetrabutylammonium tosylate

To a 50 mL methanol solution of 4 mmol $p$-toluene sulfonic acid, 4 mL of 1 M tetrabutylammonium hydroxide solution was added. The solvent was removed and the residue was dried under vacuum to yield 1.72 g clear oil with a 95% yield. The resulting salt was stored under anhydrous conditions. $^1$H NMR (300 MHz, CDCl$_3$) δ: 7.76 (d, $J = 7.5$ Hz, 2 H), 7.09 (d, $J = 7.2$ Hz, 2 H), 3.23 (m, 8 H), 3.08 (m, 3 H), 1.52 (m, 8 H), 1.33 (m, 8 H), 0.93 (m, 12 H).

4.2.7 Tetrabutylammonium benzoate

To a 50 mL methanol solution of 4 mmol benzoic acid, 4 mL of 1 M tetrabutylammonium hydroxide solution was added. The solvent was removed and the residue was dried under vacuum to yield 1.45 g clear oil with a 97% yield. The resulting salt was stored under anhydrous conditions. $^1$H NMR (300 MHz, CDCl$_3$) δ: 8.06 (m, 2 H), 7.24 (m, 3 H), 3.21 (m, 8 H), 1.52 (m, 8 H), 1.33 (m, 8 H), 0.93 (m, 12 H).

4.2.8 Tetrabutylammonium diphenylphosphate

To a stirred solution of diphenyl phosphate (2.00 g, 8.00 mmol) in dry methanol (150 mL) under nitrogen was added a 1.0 M solution of tetrabutylammonium hydroxide in methanol (8 mL, 8.0 mmol). The resulting mixture was stirred for 2 h at room temperature. The solvent was evaporated and the resulting solid was dried for 12 h under vacuum to give 3.83 g (98% yield) of TBA-DPP as a colorless solid. The resulting tetrabutylammonium salt was stored under anhydrous conditions. $^1$H NMR (300 MHz,
CDCl$_3$ $\delta$: 7.28 (d, $J = 7.8$ Hz, 4 H), 7.18 (t, $J = 7.8$ Hz, 4 H), 6.93 (t, $J = 7.2$ Hz, 2 H), 3.25 (t, $J = 8.4$ Hz, 8 H), 1.58 (m, 8 H), 1.39 (m, 8 H), 0.93 (t, $J = 7.5$ Hz, 12 H).

4.2.9 Polymerization

Eu$_2$(salen)$_3$(H$_2$O)$_2$ (0.054 mmol) was dissolved in 7 mL dichloroethane then heated up to 80 °C to dissolve. Then crosslinker EDGMA (10.7 mmol) and initiator AIBN (0.1 mmol) were added and the mixture was put back to oil bath at 80 °C. The resulting polymer monoliths were ground and washed first with methanol for 12 hours in a Soxhlet extractor, and then with a methanol/acetonitrile mixture for another 12 hours. Finally the polymer was dried under vacuum to yield a light yellow polymer NIP 1. The MIP 1 and MIP 2 were prepared under the same conditions with an addition of 0.054 mmol of tetrabutylammonium phenylacetate (TBA-PhOAc) and tetrabutylammonium acetate (TBA-OAc), respectively, to the polymerization solutions.

4.2.10 Fluorescence

The polymers were ground with mortar and pestle and sieved in a 100 micrometer size sieve. The powders were transferred into chloroform, and acetonitrile was slowly added until a homogenous suspension was formed. The suspension was allowed to stay overnight to check whether polymer powders precipitate. The best suspension was obtained when the ratio of acetonitrile to chloroform is 6:1. Certain amount of suspension (contain 9.5 mg or 9.9 mg polymer) was transferred to each well of a 96 wells microtiter plate and dried. A series of analyte solutions (200 μL) with varying concentrations (0 to 3 mM) were pipetted into each well and the fluorescent properties (excited at 350 nm) were characterized through bottom reading using MDS SpectraMax M2 microplate reader.
4.3 Results and Discussion

Scheme 4.2 Schematic illustration of synthetic approach of lanthanide-containing polymer.

The synthesis of the europium-salen complex was prepared using a slightly modified version of the literature procedure (Scheme 4.2). First, a stronger base, potassium hydroxide instead of potassium carbonate, was introduced to improve the yield of 2-hydroxy-4-(4-vinylbenzyloxy)benzaldehyde. The temperature of this step was reduced to 50 °C to prevent polymerization. Then in the complexation step, nitrate salt of Eu$^{3+}$ was used instead of europium (III) trifluoromethanesulfonate to prepare salen-europium
complexes. Also, a weaker base, potassium hydroxide instead of potassium hydride, was used due to its sufficient ability to deprotonate phenols. For the polymerization, the concentration of salen-europium complex was greatly reduced to 1/10 of the literature concentration because of its high luminescent efficiency and poor solubility in the prepolymerization solution.

**Figure 4.1** UV-vis spectra of salen solution (53 mM, solid line) and salen-europium complex suspension (broken line) in DMF.

First, UV-vis experiments were performed to verify the incorporation of europium (Figure 4.1). Due to the low solubility of salen-europium complex in a broad range of organic solvents, the UV-vis spectra were taken using a DMF suspension of the complex. The $\lambda_{\text{max}}$ and shape of the absorption spectra was repeatable, but the absorbance values were not repeatable due to the inaccuracy brought by suspension. The absorbance also varied when different suspension solvents were used due to the different solubilities of the salen-europium complex in each solvent. However, although the UV-vis
measurements cannot provide a quantitatively interpretation, the spectra still can reveal the changes. In the spectrum of salen-europium complex, the peak of salen at 307 nm went down and a new peak appeared at 339 nm. These observations were consistent with the literature we followed, which proved that salen-europium complex was successfully formed.  

The DMF solution of salen and DMF suspension of salen-europium complex were then examined with a fluorimeter. The luminescent properties were shown in Figure 4.2a. The peak at 615 nm in plot of salen-europium complex is the characteristic emission peak for europium indicating that europium was successfully incorporated into the salen ligand. However, this experiment cannot be used for the next step characterization of responses to anions due to the inaccuracy introduced by suspension.

FT-IR was performed and showed that the C=N stretch (at 1615-1700 cm\(^{-1}\)) and C-O (phenol) stretch (at 1500 cm\(^{-1}\)) shifted suggesting Eu\(^{3+}\) was successfully incorporated into salen ligand (Figure 4.3).  

Due to the poor solubility of the resulting europium-salen complex in most of commonly used organic solvents, characterization was conducted using a 96 wells microtiter plate and a microplate reader. Thus, lanthanide-salen complex showed stable luminescent properties in several organic solvents and was tested with a series of anions. It shows responsive to fluoride and acetate anions. But poor quantitative correlations were observed due to the different influences of the anions on the solubility of the europium-salen complex. The addition of some anions improved the solubility of the europium-salen complex, whereas other anions made the solubility worse, leading to
inaccuracy in screening and comparison of anions (some in solid state and some is liquid state).

**Figure 4.2** Fluorescent emission spectra (excited at 350 nm) a) before polymerization: salen solution in DMF (solid) and salen-europium complex suspension in DMF (dotted); b) salen-europium polymer in solid state.
In order to make a more accurate and reproducible europium anion sensor, the complex was immobilized into a polymer matrix. The formation of lanthanide-containing EGDMA (ethylene glycol dimethacrylate)-crosslinked polymer followed the literature procedure. However, the concentration of salen-europium complex was greatly reduced due to its high luminescent efficiency and poor solubility in the prepolymerization solution. The resulting polymer then was crushed and ground into fine powder and extracted with methanol for 1 day and sequenced with methanol/acetonitrile (1:4 v/v) for another 24 hours. The polymer was then dried under vacuum.

**Figure 4.3** FT-IR spectra of a) salen, and b) salen-europium complex.
solubility of polymer never changed when titrated with high concentration of different anions.

Next, the dried lanthanide containing polymer was ground into a finer powder and sieved. In order to make a good polymer suspension in organic solvent, several mixtures of acetonitrile/CHCl₃ solution with varying ratio (density) were made and polymer powder was added and the mixture was shaken and allowed to settle down overnight. The best ratio of acetonitrile/CHCl₃ was found (1:6) which kept the polymer powder suspended after 12 hours. The reason for making a polymer suspension is to accurately transfer the same small weight of polymer into each well of a microtiter plate without introducing errors during weighing. After distribution of the polymer solutions to the wells of the microtiter plate, the solvent was allowed to evaporate in air and then the plate was put in oven.

The fluorescent properties of the resulting polymer were also characterized. The presence of a strong 615 nm emission in Figure 4.2b, indicated that salen-europium complex was successfully incorporated into the polymer matrix.

Then the luminescent properties of the polymer (9.5 mg in each well) in the presence of a series of different neutral molecules and anions were investigated to characterize the binding efficiencies and selectivities of europium-containing polymer (Figure 4.4). Neutral analytes that were tested include triphenylphosphine oxide, triphenylphosphate, DMF and DMSO. Tetrabutyl ammonium (TBA) cation was chosen as the counter ion for the anion salts which include phenylacetate (TBA-PhOAc), acetate (TBA-OAc), benzoate (TBA-Bz), tosylate (TBA-Ts), diphenylphosphate (TBA-DPP), fluoride (TBA-F) and chloride (TBA-Cl). Solutions of these anions and neutral analytes were prepared
in the following concentrations 0, 0.15, 0.30, 0.60, 0.90, 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.00 mM in acetonitrile and were added to the solid polymer in the microtiter plate. Then the response was visualized under long wavelength UV light and then quantified with fluorimeter (excitation wavelength 350 nm).

![Chemical Structures](image)

**Figure 4.4** The analyte list used in this study.

Interestingly, the polymer selectively responded to the carboxylate anions. It also responded to fluoride anion over other anions and neutral analytes. The response was readily visualized under long wavelength UV irradiator (Figure 4.5). The 1, 3, 5 and 8 rows were polymer titrated with increasing concentrations (from left to right) of benzoate anion, phenylacetate anion, acetate anion, and fluoride anion, respectively. Those polymers emit strongly under UV light and the emissions increased with the increase of analyte concentration, suggested they are “turn on” sensor which is unusual and much more desirable. Specifically all polymers titrated with carboxylates were emitting bright pink light and polymers titrated with fluoride were emitting bright red light. All other polymers titrated with other analytes emit weakly.
The polymer responded to carboxylate anions no matter they are aromatic or non-aromatic (Figure 4.5). For example, the analytes in 1 and 3 rows were benzoate anion, and phenylacetate anion, which were aromatic carboxylates. The polymer also showed strong response to non-aromatic acetate anion (Figure 4.5, row 5). These observations suggested that the recognition of carboxylates by this polymer does not require any additional chromophores, thus this polymer can be used to sense a broad spectrum of carboxylates.

The fluorimeter was used to quantify the emission response. The changes ($I/I_0$) in fluorescent property for each anion were shown in Figure 4.6. In order to quantitatively compare each analyte, $I/I_0$, which represents the ratio of fluorescent emission intensity of the polymer with analytes to that of neat solid, were plotted versus concentrations of each analytes. The response to DMF, DMSO and triphenylphosphate showed no response and are not shown in Figure 4.6. The strongest responses were acetate anion, phenylacetate anion and benzoate anion, respectively, indicating this polymer showed high affinity and sensitivity targeting to acetate anions. The rank of responses can be explained that polymers preferred the smallest carboxylate, acetate anion, which can easily access the europium recognition sites. On the other hand, benzoate anion is the most bulky one among the three carboxylates since the carboxylate group was directly connect to the huge benzene ring which prevented the anion from entering recognition cavities. Also, the electro-rich benzene is able to quench fluorescence. This polymer showed responses to fluoride anion which is the smallest halide anion (Figure 4.6). It is because anion response is not only related to size, but also correlated to the basicity of the anion and fluoride anion is the most basic anion in organic solvent.\textsuperscript{18, 19}
Figure 4.5 Titration results of europium-containing polymers (9.9 mg) to 200 μL solution of benzoate anion, tosylate anion, phenylacetate anion, diphenyl phosphate anion, acetate anion, triphenyl phosphate, chloride anion, fluoride anion, DMF, DMSO, and triphenyl phosphine oxide, from top to bottom row, in a series of concentrations including 0, 0.15, 0.30, 0.60, 0.90, 1.20, 1.50, 1.80, 2.10, 2.40, 2.70 and 3.00 mM, from left to right, in acetonitrile.
Figure 4.6 Changes in fluorescent intensity (excitation at 350 nm) of the polymer (9.5 mg) in response to 200 μL of increasing concentrations of different anions and neutral analytes in concentration ranges (0 to 3 mM) in acetonitrile. Benzoate anion (solid triangle), tosylate anion (empty circle), phenylacetate anion (solid diamond), diphenyl phosphate anion (empty diamond), acetate anion (solid square), triphenyl phosphoshpine oxide (check), chloride anion (empty triangle), fluoride anion (solid circle).

Surprisingly, in Figure 4.6, the responses of the polymer for carboxylates were higher than the response for fluoride which is unusual, suggesting that the selective responses were not entirely due to the size or the Brønsted–Lowry basicity of the analytes. The selective response to carboxylates probably was due to the Lewis basicity which is the ability to donate electron pair for coordination with europium ions.\textsuperscript{20}

Another advantage of this polymer sensor is that it is a “turn-on” sensor which has broader detection limits and can be more easily monitored than “turn-off” sensors. The addition of analytes strongly enhanced the fluorescent signal and increasing signal was observed with increasing concentration of analytes.
Figure 4.7 Changes in fluorescent intensity (excitation at 350 nm) of polymer (9.5 mg) when tested with 200 μL of 3 mM acetonitrile solution of fluoride anion (cube), acetate anion (diamond), phenylacetate anion (cross), benzoate anion (round), and chloride anion (triangle) after 3 cycles.

The ability to reuse of the polymer sensor was also characterized (Figure 4.7). After first characterization cycle (tested with different anions), polymer powders were combined and washed together using the same washing procedure as in polymer preparation. The fluorescent response of the polymer dropped about 50%; however it stabilized after the second testing. It means that europium polymer immediately reached its saturation after the first usage; afterwards it keeps its sensing properties no matter how many times it is used, which is economy and environmental friendly.

MIP 1 was made with TBA-PhOAc (monomer: template 1:1) and the fluorescent responses to all 11 analytes were measured. The results of 8 analytes were shown in Figure 4.8. The overall $I/I_0$ of MIP 1 to all the carboxylates decreased. This is partially because the imprinting process made europium ion in MIP 1 saturated with TBA-PhOAc.
This is consistent with the observations in recycle experiment that the capacities of NIP decreased after first cycle. However, the magnitudes of decreases for the three carboxylates were different. In other word, the response of MIP 1 to acetate anion decreased dramatically which is twice of the decrease of the response to TBA-PhOAc and four times of that of TBA-Bz. Especially, MIP 1 showed similar responses to TBA-PhOAc and TBA-OAc which proved that MIP 1 is successfully imprinted with TBA-PhOAc. The response to fluoride anion was similar to NIP again proved our explanation that the response is size-related. MIP 1 again showed tiny or no response to other anions and neutral analytes.

Figure 4.8 Change in fluorescent intensity (excitation at 350 nm) of 9.5 mg of MIP (TBA-PhOAc) in response to 200 μL of increasing concentrations of different anions and neutral analytes in concentration ranges (0 to 3 mM) in acetonitrile. Benzoate anion (solid triangle), tosylate anion (empty circle), phenylacetate anion (solid diamond), diphenyl phosphate anion (empty diamond), acetate anion (solid square), triphenyl phosphophine oxide (check), chloride anion (empty triangle), fluoride anion (solid circle).
MIP 2 was made with TBA-OAc and the responses of 9.9 mg polymer in each well were measured and shown in Figure 4.9b. In order to compare with MIP 2, 9.9 mg (each well) of NIP was also tested (Figure 4.9a). In this experiment, NIP showed similar response to previously made NIP. MIP 2 showed relatively low response to carboxylates compared to NIP due to the same reason that cause the low response of MIP 1. The imprinting is not obvious in this study due to same patterns of response were shown in both MIP 2 and NIP. However, MIP 2 did differentiate TBA-PhOAc and TBA-OAc, indicating MIP 2 is quite different from MIP 1. Also, 9.9 mg of MIP 2 showed lower response to TBA-PhOAc and TBA-Bz; whereas it exhibited higher response to TBA-OAc than 9.5 mg of MIP 1. These observations implied that MIP 2 was successfully imprinted. Further studies and solid evidences are needed.

4.4 Future work

In future, first, a series of MIP 1 will be prepared using various concentration of TBA-PhOAc template to find the minimum amount of carboxylate that is required to entirely saturate the europium ions. Next, a series of MIPs imprinted with various carboxylate anions at the same concentration will be prepared. The resulting polymers (including both MIPs and NIP) will be quenched into corresponding anion solutions before washing. Finally, all the polymers will be characterized and more accurate results will be obtained.

Moreover, due to the poor solubility of the europium-salen complex, we were unable to prepare polymer sensors with a higher concentration of complex. Thus, to prepare a structurally similar complex with better solubility is needed. The first thing we could do
is to prepare the complex using another europium reactant, Europium (III) triflate which has better solubility, to replace our old reactant Eu (III) nitrate.

**Figure 4.9** Change in fluorescent intensity (excitation at 350 nm) of 9.9 mg of a) NIP; b) MIP (TBA-OAc) in response to 200 μL of increasing concentrations of different anions and neutral analytes in concentration ranges (0 to 3 mM) in acetonitrile. Benzoate anion (solid triangle), tosylate anion (empty circle), phenylacetate anion (solid diamond), diphenyl phosphate anion (empty diamond), acetate anion (solid square), triphenyl phosphosphine oxide (check), chloride anion (empty triangle), fluoride anion (solid circle).
4.5 Conclusions

This work demonstrated that the europium containing polymer selectively respond to both aromatic and non-aromatic carboxylate anions. And the response is size-related. Imprinted polymers showed selectively response to the corresponding template, making it possible to prepare a sensor array for detection of different carboxylates. Although it also responded to fluoride, but considering the lack of abundance of fluoride in nature, this polymer sensor could be an efficient and economy friendly sensor for carboxylates.

4.6 References


REFERENCES


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