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## Molecularly Imprinted Polymer Beads-Synthesis, Evaluation and Applications

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# Molecularly Imprinted Polymer Beads

## Synthesis, Evaluation and Applications

Tongchang Zhou



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### DOCTORAL DISSERTATION

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To be defended on Monday, 20<sup>th</sup> June 2016 at 9.15 a.m. in Lecture Hall B at the  
Center for Chemistry and Chemical Engineering, Naturvetarvägen 16, Lund.

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<p><b>Abstract</b></p> <p>Molecularly imprinted polymers (MIPs) are artificial receptors designed for the selective recognition of template molecules. These polymers have been applied in analytical separations, as chemical sensors and in drug delivery system due to their low cost and high stability. In recent years MIP beads, especially those with good selectivity in aqueous solution, have become attractive as they can be potentially used as selective adsorbents for the solid-phase extraction (SPE) and chromatographic separation of various target molecules.</p> <p>The aim of this thesis was to investigate and improve the synthetic methods for the preparation of MIP beads, especially those that can be used in aqueous solution. In the first section, Pickering emulsion is utilized to synthesize water-compatible MIP beads. Scanning electron microscopy (SEM) was used to characterize the morphology and surface structure of the particles. As water-soluble monomers were employed during the imprinting process, the MIP beads had a hydrophilic surface and showed high compatibility with aqueous conditions. Additionally, the MIP beads exhibit high selectivity and specificity to template in aqueous solution. Both organic compounds and macromolecules were used as templates separately and MIP beads with specific binding sites were successfully obtained. The method developed in this thesis has a general applicability and offers a potential approach to synthesize polymer beads for bioseparation and wastewater treatments.</p> <p>Another focus of this thesis was the preparation of multifunctional materials based on MIP beads for the purpose of different applications. Reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization was used to prepare monodisperse beads with RAFT reagents located on their surfaces, which allowed for the straightforward grafting of polymer brushes, or can be converted into new functional groups for further modification. The specific molecular recognition of the beads was not sacrificed during the aminolysis process, and different functionalities were introduced to the MIP beads under mild conditions. The thiol groups introduced onto the MIP surface allowed the MIP beads to be immobilized on a gold-coated substrate, such that these MIP-based sensing surfaces were used for the detection of nicotine by surface enhanced Raman scattering (SERS).</p> <p>The Pickering emulsion systems provide an efficient method with which to prepare water-compatible MIP beads that have high selectivity under aqueous conditions. Further functional materials and new applications can be expected upon the combination of Pickering emulsion systems with more controllable synthetic chemistry. The RAFT polymerization method, in particular, has a general applicability and introduces new possibilities for the development of other functional materials and devices.</p>		
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# Molecularly Imprinted Polymer Beads

Synthesis, Evaluation and Applications

Tongchang Zhou



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*To my family*



# Popular summary

Molecular recognition refers to the specific interaction between two molecules, which plays an important role in biological systems and life processes. An important example of molecular recognition is the interaction of antibodies with antigens: like a key in a lock, antibodies can fit perfectly with their target antigens and are able to recognize and bind them with very high affinity and selectivity. Antibodies have been used to detect and quantify antigen targets and also to treat certain diseases. However, antibodies are not always the best to use because they are unstable out of their native environment. Therefore, they cannot be used on a large scale or under harsh conditions. It has been a long-term scientific goal to chemically synthesize antibody-like materials with a high affinity and selectivity, and at the same time with a high stability and low cost. One simple way of achieving this is through the molecular imprinting technique.

Molecular imprinting is a synthetic technique to create template-shaped cavities in polymer matrices. The cavities are created in such a way that they have a “memory” of the original template molecules. The imprinting is like that you leave your footprints after you walk on a wet beach. The footprints fit with your feet in terms of both their size and shape. During the molecular imprinting process, the template molecule forms a self-assembled complex with specific functional monomers and cross-linkers, which after polymerization and removal of the template leaves well-defined cavities. These imprinted cavities can interact and bind the original template through specific molecular recognition. As molecularly imprinted polymers (MIPs) can recognize and bind specific target molecules, they can be used in many applications to replace biological recognition materials. One of the attractive features of molecular imprinting technique is that it can be used to develop a wide range of affinity materials for different types of target molecules. MIPs are finding important applications in the field of separation science, catalysis, sensors, and immunoassays, and are often called artificial antibodies, synthetic receptors, or enzyme mimics because of their similar functional performances. MIPs can also be used for medical applications, in particular for drug delivery where the high selectivity and affinity of MIPs are utilized to extend and control the residence time of therapeutic compounds.

Various chemical compounds reach the aquatic environment from human activities, and these chemicals have been recognized as emerging pollutants



because of their potential impact on human health and the environment. In recent years, there has been growing concern about the occurrence of chemicals in the environmental water. For complex environmental samples, pre-concentration and clean-up steps are often needed in order to achieve accurate and reliable analytical results. As conventional sorbents lack selectivity, new solid sorbents with pre-defined molecular selectivity have appeared as an attractive alternative. Among them, MIPs appear as excellent candidates due to their high selectivity and affinity. Several polymerization methods have been developed to prepare spherical and uniform MIPs, such as seed polymerization, suspension polymerization and precipitation polymerization. MIPs synthesized by these methods have exhibited molecular recognition properties under non-aqueous conditions. However, the binding selectivity of these MIPs in aqueous solution is generally reduced as water can interfere with the hydrogen bonds between the template and the functional monomers. Since many target molecules of interest are present in aqueous media such as bodily fluids, foods and environmental samples, it is important to develop water-compatible MIPs that can be directly used in aqueous solutions. A general imprinting method to prepare water-compatible MIP beads is therefore highly desired.

In this thesis, a new molecular imprinting method called nanoparticle-stabilized emulsion (Pickering emulsion) polymerization, is employed to prepare spherical MIP beads. The MIP beads are designed to have a hydrophilic surface that enables the MIPs to be used directly under aqueous conditions. This imprinting method has shown great success for the synthesis of selective binding materials for separation and detection of different low molecular weight compounds in aqueous solvents, and also applicable to large biological molecules and living cells. Using a widely applied pharmaceutical compound, diclofenac as template, hydrophilic MIP beads have been prepared using Pickering emulsion polymerization. The MIP beads can be packed into chromatography column to selectively capture diclofenac from environmental water. Moreover, a solid phase extraction process based on the new MIP beads prepared using Pickering emulsion polymerization was performed successfully. The MIP-based treatment could efficiently concentrate target analytes from tap water samples. Another advantage of molecular imprinting in Pickering emulsion is that the analytical targets can be immobilized on the stabilizing nanoparticles, and the immobilized template can be used to carry out molecular imprinting on surface. In this way the surface-immobilized template is used most efficiently to create easily accessible binding sites on the MIP surface, which is important for achieving faster binding kinetics and for shortening response time in chemical sensing.

To improve the functional performance of MIPs, controlled/living radical polymerization (CRP) has been used successfully to synthesize uniform polymer beads with high selectivity for nicotine. With conventional free radical

polymerization (FRP), it is not possible to control the molecular weight of the polymer chains due to the fast propagation and termination reactions. As CRP allows the polymerization process to be controlled, it leads to more homogeneous polymer network and leaves useful chain termini that can be easily converted into new reactive groups. In this thesis the possibility of using living chain termini to construct MIP-based chemical sensors has been demonstrated. The living characteristic of new molecularly imprinted polymers provide many new possibilities to realize multiple functions with a single imprinted material.

# Abstract

Molecularly imprinted polymers (MIPs) are artificial receptors designed for the selective recognition of template molecules. These polymers have been applied in analytical separations, as chemical sensors and in drug delivery system due to their low cost and high stability. In recent years MIP beads, especially those with good selectivity in aqueous solution, have become attractive as they can be potentially used as selective adsorbents for the solid-phase extraction (SPE) and chromatographic separation of various target molecules.

The aim of this thesis was to investigate and improve the synthetic methods for the preparation of MIP beads, especially those that can be used in aqueous solution. In the first section, Pickering emulsion is utilized to synthesize water-compatible MIP beads. Scanning electron microscopy (SEM) was used to characterize the morphology and surface structure of the particles. As water-soluble monomers were employed during the imprinting process, the MIP beads had a hydrophilic surface and showed high compatibility with aqueous conditions. Additionally, the MIP beads exhibit high selectivity and specificity to template in aqueous solution. Both organic compounds and macromolecules were used as templates separately and MIP beads with specific binding sites were successfully obtained. The method developed in this thesis has a general applicability and offers a potential approach to synthesize polymer beads for bioseparation and wastewater treatments.

Another focus of this thesis was the preparation of multifunctional materials based on MIP beads for the purpose of different applications. Reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization was used to prepare monodisperse beads with RAFT reagents located on their surfaces, which allowed for the straightforward grafting of polymer brushes, or can be converted into new functional groups for further modification. The specific molecular recognition of the beads was not sacrificed during the aminolysis process, and different functionalities were introduced to the MIP beads under mild conditions. The thiol groups introduced onto the MIP surface allowed the MIP beads to be immobilized on a gold-coated substrate, such that these MIP-based sensing surfaces were used for the detection of nicotine by surface enhanced Raman scattering (SERS).

The Pickering emulsion systems provide an efficient method with which to prepare water-compatible MIP beads that have high selectivity under aqueous

conditions. Further functional materials and new applications can be expected upon the combination of Pickering emulsion systems with more controllable synthetic chemistry. The RAFT polymerization method, in particular, has a general applicability and introduces new possibilities for the development of other functional materials and devices.

# Abbreviations

AIBN	Azobisisobutyronitrile
Am	Acrylamide
ATR-FTIR	Attenuated total reflectance Fourier transform infrared spectroscopy
BSA	Bovine serum albumin
CDB	Cumyl dithiobenzoate
CRP	Controlled/living radical polymerization
DVB	Divinylbenzene
EGDMA	Ethylene glycol dimethacrylate
Hb	Hemoglobin
HPLC	High-performance liquid chromatography
Lyz	Lysozyme
MAA	Methacrylic acid
Mb	Myoglobin
MIP	Molecularly imprinted polymer
NIP	Non-imprinted polymer
NIPAm	N-isopropylacrylamide
NPM	N-(1-pyrenyl)maleimide
OVA	Ovalbumin
RAFT	Reversible addition-fragmentation chain transfer polymerization
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
SPE	Solid-phase extraction
TRIM	Trimethylolpropane trimethacrylate

# List of papers

Paper I. **Tongchang Zhou**, Xiantao Shen, Shilpi Chaudhary, and Lei Ye, *Molecularly Imprinted Polymer Beads Prepared by Pickering Emulsion Polymerization for Steroid Recognition*. J. Appl. Polym. Sci., 2014, 131, 39606-39612.

Paper II. **Tongchang Zhou**, Ka Zhang, Tripta Kamra, Leif Bülow and Lei Ye. *Preparation of Protein Imprinted Polymer Beads by Pickering Emulsion Polymerization*. J. Mater. Chem. B, 2015, 3, 1254-1260.

Paper III. **Tongchang Zhou**, Tripta Kamra and Lei Ye.  
*Preparation of Diclofenac-Imprinted Polymer Beads for Selective Molecular Separation in Water*. Manuscript.

Paper IV. **Tongchang Zhou**, Lars Jørgensen, Maria Ahlm Matthebjerg, Ioannis S. Chronakis and Lei Ye. *Molecularly Imprinted Polymer Beads for Nicotine Recognition Prepared by RAFT Precipitation Polymerization: A Step Forward Towards Multi-functionalities*. RSC Adv., 2014, 4, 30292-30299.

Paper V. Tripta Kamra, **Tongchang Zhou**, Lars Montelius, Joachim Schnadt, and Lei Ye. *Implementation of Molecularly Imprinted Polymer Beads for Surface Enhanced Raman Detection*. Anal. Chem., 2015, 87, 5056-5061.

Paper not included in this thesis

Paper I. Xiantao Shen, **Tongchang Zhou** and Lei Ye. *Molecular Imprinting of Protein in Pickering Emulsion*. Chem. Commun., 2012, 48, 8198-8200.

Paper II. Lei Ye, **Tongchang Zhou** and Xiantao Shen. *Molecular Imprinting in Particle-stabilized Emulsions: Enlarging Template Size From Small Molecules to Proteins and Cells*. Mol. Impr., 2015, 2, 37-45.

# My contribution to the papers

## Paper I.

I performed the majority of the experimental work, and wrote the first draft of the manuscript together with co-authors.

## Paper II.

I planned and performed all of the experimental work, with the exception of the SDS-PAGE measurements and analysis, and wrote the manuscript with the help of co-authors.

## Paper III.

I planned and performed all of the experimental work, and wrote the manuscript with the help of co-authors.

## Paper IV.

I planned and performed all of the experimental work, with the exception of SEM measurements and analysis, and wrote the manuscript with the help of co-authors.

## Paper V.

I designed and prepared the molecularly imprinted polymer beads and took part in the discussion of results. I assisted in writing the first draft of the manuscript.

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# Chapter 1 Introduction

## 1.1 Background

Molecular recognition refers to the specific interactions that occur between two molecules, for example, the specific binding of certain molecular species with biological hosts. These binding events play an important role in biological systems and life processes, and are observed, for example, in receptor-ligand, antigen-antibody, and DNA-protein binding. Biological receptors have been used in many applications, including selective separations and catalytic processes for a long time. Although biological recognition materials possess high molecular selectivity towards their guest molecules, their use on a large scale are limited due to their low stability and high production costs. Compared to biological recognition materials, one type of synthetic materials with predesigned molecular recognition capability called molecularly imprinted polymers (MIPs) has become very attractive due to their higher stability and much lower production cost, which can be used as alternatives to natural receptors [1-3].

In chemistry, molecular imprinting is a technique used to create template-shaped cavities in polymer matrices with a memory of the template molecules, which can be used in molecular recognition to replace biological recognition materials (see Figure 1). Nowadays this approach is considered a straightforward and versatile technique for the generation of synthetic receptors not only for small organic compounds, but also for large biological macromolecules [4-6]. As MIPs are becoming more and more popular because of their easy preparation and chemical stability, a large number of papers on the development and applications of MIPs have been published. MIPs have been considered to be promising candidates for use in many applications, including chromatographic stationary-phase, in solid-phase separation and as enzyme mimics [7-9]. The early examples of MIPs were prepared by a bulk polymerization method. However, the resultant monoliths had to be crushed, ground and sieved in order to produce particles with desirable sizes, which have irregular shapes and wide size distribution. To prepare spherical and monodisperse MIPs, several polymerization methods have emerged, among which, seed polymerization, suspension polymerization and precipitation polymerization can generate MIP beads [10-12]. However, these organic solvent-compatible MIP beads lack the molecular selectivity required in aqueous solution, and therefore,

the applications of MIPs have been limited to organic solvent-based systems. For many practical analyses, similar to those in biotechnology and biomedical systems, they need to be carried out under aqueous conditions. Therefore, a general imprinting method to prepare water-compatible MIP beads is still highly desirable.

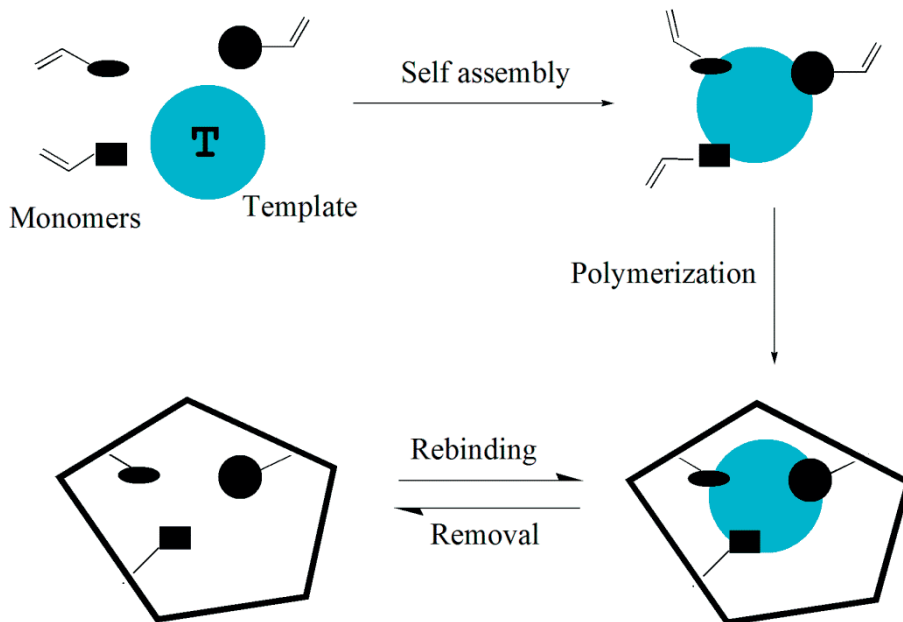


Figure 1. Schematic representation of the molecular imprinting process.

## 1.2 Aim and scope of the thesis

The aim of this thesis was to design and develop new approaches to synthesize MIP beads that would be more suitable for biotechnology and biomedical applications. To reach this goal, synthetic MIPs should enable analysis and treatment of biological samples under aqueous conditions.

In Paper I, II and III, a new imprinting method, Pickering emulsion polymerization, was employed to prepare spherical MIPs with hydrophilic surfaces that enabled the MIPs to be used directly under aqueous conditions. The synthesis of MIP beads towards both small organic molecules and biomacromolecules via Pickering emulsion polymerization was realized. Scanning electron microscopy was used to characterize the morphological features of the MIP beads, and a high binding

affinity for the template molecule in aqueous solutions was confirmed by binding analysis.

In the second section of this thesis (Paper IV and V), reversible addition-fragmentation chain transfer (RAFT) polymerization, one of the modern controlled/living radical polymerization (CRP) methods, was used to prepare monodisperse MIP beads. The RAFT reagents present on the surface of the MIP beads allowed for the straightforward grafting of hydrophilic polymer brushes or layers on the surface of the particle. The RAFT agent could also be used efficiently for post-imprinting modification to generate different complex structures. Use of surface-modified MIP beads for the surface-enhanced Raman scattering (SERS) detection of nicotine was further demonstrated.



# Chapter 2 Molecularly imprinted polymers (MIPs)

Molecular imprinting is a process whereby functional and cross-linking monomers are copolymerized in the presence of a target molecule that acts as a molecular template. It has become one of the most effective techniques in the synthesis of materials with predictable molecular selectivity, especially for low molecular weight compounds. Molecularly imprinted polymers (MIPs) are polymer products obtained using this molecular imprinting technique. MIPs contain cavities in the polymer matrix that have a high affinity for the chosen template molecule. As synthetic receptors, MIPs possess specific molecular recognition, which is the most important feature of biological antibodies and receptors. MIPs are ideal substitutes for biological receptors under harsh conditions due to their advantages of high selectivity, easy preparation and low cost. Recently, MIPs have been widely used in applications such as sensors, drug delivery, chromatography, protein recognition, chiral separation and catalysis.

## 2.1 Approaches for molecular imprinting

The molecular recognition capabilities of MIPs are based on the specific interactions of a template with the functional groups located in the polymer cavity. Depending on the interactions that occur between the template and functional monomers involved in the imprinting and rebinding steps, molecular imprinting can be divided into three different approaches, covalent, non-covalent, and semi-covalent approaches.

The first approach of molecular imprinting in organic network polymers was introduced by Wulff [13], and later by Shea [14], which was based on a covalent attachment strategy, involving a covalent monomer-template or a covalent polymer-template. The approach uses a reversible covalent bond to link the molecular template with the functional monomer during the imprinting reaction, whereby after polymerization, the covalent bond is broken to free the template from the solid polymer to form an imprinted site. The obvious advantage of this

technique is that the binding sites are more homogenous due to the high stability of the template-monomer interaction, as confirmed by Shimizu and coworkers [15,16]. In this method, the monomer-template conjugates are so stable that a wide variety of polymerization conditions (e.g., high temperature, and low or high pH) can be employed. However, there are only a few reversible covalent bonds available for use in this approach, and additionally, it can be difficult to match some templates with suitable monomers for covalent imprinting. Another issue that arises is that the binding kinetics are often slow due to the necessity to reform the covalent bonds during the rebinding process.

In 1993, the group of Klaus Mosbach published a study in *Nature* where, for the first time, non-covalent molecular imprinting was employed in a competitive binding assay for the detection of theophylline and diazepam in human serum [17]. In this approach, non-covalent interactions such as hydrogen bonding, van der Waals forces, ionic interaction and hydrophobic interaction are utilized to stabilize the template-functional monomer complex during polymerization. The template was simply mixed with one or several functional monomers prior to polymerization. In this way, the template could be easily extracted from the polymer and recycled. The rebinding of the template to the imprinted sites was also realized by the same non-covalent interactions. This approach is very attractive due to the simplicity of preparation and the flexibility in selecting functional monomers. Compared with covalent molecular imprinting, the non-covalent approach has become the mainstream of molecular imprinting because many functional monomers are available and the synthetic process is less complicated.

In the non-covalent imprinting approach, monomers are normally used in excess compared to the template to favor the formation of template-monomer assemblies. Consequently, heterogeneous binding sites often exist in MIPs because the pre-polymerization step is a less defined process, which results in the formation of complexes with different ratios of template to monomer, and leads to the formation of different binding sites. Because excess monomers are used in order to form template-monomer complexes, this can also cause higher non-selective binding. To overcome this limitation, a semi-covalent approach (also called hybrid approach) was proposed. This semi-covalent approach was developed by Whitcombe and coworkers, where a covalently attached template was utilized in the imprinting process while the template rebinding step employed a non-covalent interaction [18]. This approach seems to combine the advantages of both the covalent and the non-covalent protocols. However, as occurs in the covalent imprinting approach, application of the semi-covalent approach also requires the complicated synthesis of a template-monomer complex before polymerization, which brings in the same limitations as those that arise in the covalent approach. Since the template-functional group bonds must be readily cleaved for template

removal, the process requires somewhat harsh reaction conditions. Furthermore, the limited choice of functional monomers that can be applied to this methodology restricts the applicability of this technique.

Among the above three approaches, the non-covalent approach has been most commonly used in MIP synthesis nowadays because it allows more straightforward preparation and a large number of functional monomers are commercially available. Imprinted polymers prepared by the non-covalent imprinting approach show much faster rebinding kinetics than those prepared by the covalent approach, which makes them particularly suitable for use as the stationary phase in high-performance liquid chromatography (HPLC) and as the recognition components in sensors. The traditionally heterogeneous binding sites may be overcome by the use of stoichiometric monomer-template complexes based on stronger non-covalent interactions [19].

Since the success of the molecular imprinting process using the non-covalent approach is very much dependent on the monomer-template interaction, the choice of monomer is very important in order to create highly specific cavities designed for the template molecule. Commonly, monomers are chosen according to their complementarity with the chemical groups of the template. A large number of functional monomers and cross-linkers (Figure 2) have been successfully utilized in molecular imprinting systems and they are mostly commercially available. Among them, typical functional monomers are carboxylic acids, such as methacrylic acid (MAA) and heteroaromatic bases, such as 4-vinylpyridine (4-VP). MAA has been the most widely used to create good binding sites for a large variety of template structures containing hydrogen bond or proton-accepting functional groups. 4-VP is frequently used in the imprinting of acidic templates because of its low cost and good solubility in common solvents.



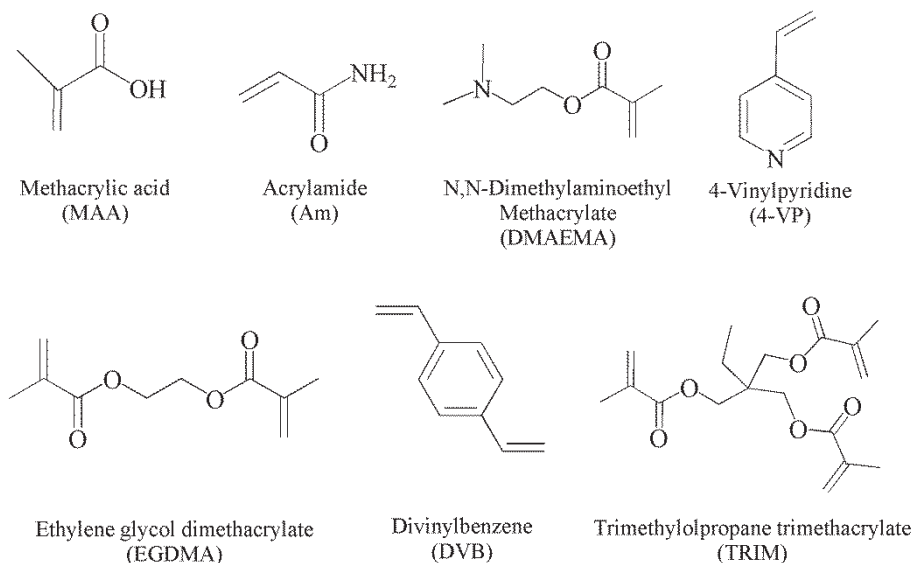


Figure 2. Commonly used functional monomers and cross-linkers.

## 2.2 Different forms of MIPs

Particular attention has been paid to the physical forms of MIPs as their potential for incorporation into a number of technological applications continues to increase. In order to be employed in different practical applications, the MIPs have been prepared in different forms using different synthetic methods, such as membranes, monoliths, films, micro- and nano-structured beads. Many efforts have been made towards the preparation of uniform spherical MIP beads in the micrometer range. This is the consequence of the need for this kind of materials as fillers for HPLC columns, cartridges for solid-phase extractions (SPE) and in other applications requiring uniform and molecularly selective adsorbents.

### 2.2.1 MIP monoliths

Traditionally, MIPs have been prepared as porous monoliths by bulk polymerization of mixtures containing monomers, template molecule and a certain amount of solvent to act as a porogen [20]. The resulting polymer monolith had to be ground and sieved in order to produce particles in the desired size ranges required by the specific application. Although the process of bulk polymerization is simple, the rest of the preparation steps are tedious and time-consuming.

Particles that are irregular in size and shape are often produced, which are often unsuitable for more advanced applications. In addition, some imprinted sites are destroyed during grinding, which reduces the MIPs loading capacity. Therefore this method suffers from high consumption of template molecules and other starting materials, since only a portion of the original polymer can be used.

Monolithic MIPs have also been prepared by a simple, one-step, in situ free-radical polymerization process directly within a chromatographic column without the need of grinding, sieving and column packing [21,22]. The advantages of monolithic MIP stationary phases are that they are quite simple to prepare and less expensive. However, some drawbacks are obvious such as the poor morphology control of the monoliths and lower column efficiencies.

Although this method is the most popular and has been the only method for MIP synthesis for a very long time, it is clearly a rough method, which is not suitable for the large-scale production of MIPs. In order to overcome these problems, alternative methods to prepare novel MIP forms have been developed in recent years.

### **2.2.2 Nano-sized MIPs**

Nanotechnology is used in many research fields and various nano-structural materials have been produced for uses in affinity separation and sensor construction. By combining nanotechnology and molecular imprinting, MIP nanoparticles have also been prepared and applied in several areas. The benefits of nano-sized MIP materials are not only their size, but also their fast equilibration with the analyte. Compared to bulk MIPs, MIP nanoparticles have higher surface area-to-volume ratios and the imprinted cavities are more easily accessible by the templates, which leads to a fast analyte equilibration to bring in many performance improvements, for example, in sensing and affinity separation [23,24]. Also, an enhanced catalytic activity can be expected, which has been previously observed only for metal nanoparticle catalysts. Due to their superior physical and chemical properties, nano MIP particles have already been used as enzyme substitutes, in drug delivery systems and as sensors [25-29]. A large number of publications have been reported on the development of MIP nanoparticles. The most general preparation methods are precipitation polymerization, mini- and micro-emulsion polymerization, synthesis of core-shell particles and nanogels [30-34,26,35].

The precipitation polymerization approach to prepare MIP nanoparticles was first described in 1999 [36] and it is still the most popular method nowadays because it has several advantages. This technique is easy, less time-consuming than other methods, and provides good yields. For emulsion polymerization, surfactants or stabilizers are often required, which may affect the assessment of binding to the

template. Moreover, surfactant or stabilizers are not easy to remove from the system after polymerization. These drawbacks affect the efficiency and binding capacity of the MIP nanoparticles. The synthesis of core-shell particles involves the deposition of a MIP layer on preformed core nanoparticles, such as silica, polymers and magnetite. The main advantages of the core-shell approach are that the MIP layer can be made very thin and uniform. In this way it is also possible to use cores with specific properties to prepare functional materials. Functional materials based on MIP nanoparticles have attracted much interest as additional useful functions such as magnetism or fluorescence can be integrated into the fabricated MIP materials to get magnetic nanoparticles or fluorescent nanoparticles. These multifunctional MIP materials maintain their high selectivity and can be more easily handled in assays. Nanogels can be synthesized by solution polymerization, which provides an efficient way to imprint biomolecules. Each method described here has its advantages, but also its limitations. Generally, different methods can be chosen according to the requirement of the intended applications.

### **2.2.3 MIP membranes**

A biological membrane is a selective barrier that allows certain molecules to pass through it, while preventing others. Lipid-based membranes are difficult to use on a large scale due to their high cost and low stability, while commercial membranes are more stable but lack molecular selectivity. MIP membranes are of interest for their highly porous morphology and high selectivity. The MIP membranes could overcome the problems associated with the limited accessibility of the imprinted sites of the bulk polymers as well as the lack of selectivity of the commercial membranes. The transport properties across a MIP membrane are controlled by not only a sieving effect due to the membrane pore structure, but also a selective absorption effect due to the imprinted cavities [37,38]. As a consequence, their separation performance depends not only on the efficiency of molecular recognition, but also on the membrane morphology, in particular the barrier pore size and the thickness of the membrane.

Two types of MIP membranes have been proposed, one is self-supported MIP membranes and another one is MIP composite membranes. Self-supported MIP membranes are synthesized traditionally by in situ cross-linking polymerization of a monomer mixture containing the template [39]. However, their applicability is limited significantly due to their low permeability and stability. Composite membranes have attracted much attention because of the combination of mechanical strength of the starting membrane and the selectivity of the MIPs. Normally, MIP composite membranes are composed of an appropriate porous membrane support that is functionalized with either a thin layer of MIP or with

MIP particles. This strategy can improve the mechanical stability of the imprinted polymer phase, similar to the preparation of MIP composite beads.

Two approaches, in situ MIP synthesis on a supporting membrane and incorporation of pre-synthesized MIP particles into membranes, have been reported to prepare composite membrane [40-43]. In the former approach, the support membrane is soaked in a pre-polymerization mixture consisting of functional monomers, cross-linkers and template. A MIP layer is formed on or in the porous support by in situ photo- or thermal- polymerization. MIP composite membranes can also be obtained by incorporation of the MIP particles into the membrane polymer matrix by mixing the particles in an appropriate solvent with the membrane-forming polymer, which is then solidified by a phase inversion process. The mechanical stability and morphology of the resulting membranes depends on the polymeric matrix and the presence of additives, as well as on the MIP particle size and concentration.

#### **2.2.4 MIP beads**

The shape and size of the MIPs are of critical importance for their performances when used in affinity separation applications. When MIPs are used as affinity-based chromatographic media, HPLC stationary phases or SPE media, it is desirable to prepare the beads with regular sizes and shapes. Up until now, most efforts have been made and important breakthroughs have been obtained towards the preparation of MIP beads in the micrometer size range. As new selective sorbents for SPE procedures, MIPs allow not only the pre-concentration and cleaning of samples but also the selective extraction of target analytes from complex matrices. To prepare spherical and monodisperse MIPs, several polymerization methods have emerged, including bulk polymerization, seed polymerization, suspension polymerization and precipitation polymerization, which will be discussed below.

### **2.3 Methods to prepare MIP beads**

As outlined in Section 2.2.1, MIPs are typically prepared by a bulk polymerization method, where the resultant monoliths had to be crushed, ground and sieved in order to produce particles with desired sizes to be packed into HPLC or SPE columns as stationary phase. However, these MIP particles are not well suited to achieve a high separation performance due to their irregular shapes and sizes. It is therefore necessary to prepare uniform spherical MIP beads.

### **2.3.1 Seed polymerization**

Seed polymerization is a multi-step swelling and polymerization process. This method involves a combination of stepwise swelling of preformed seed particles followed by polymerization. Initially, the seed particles are swollen by micro-emulsion droplets containing an activating solvent, and are then further swollen by a pre-polymerization mixture. After photo- or thermal- polymerization, monodisperse MIP beads are obtained. This strategy involves the generation of a thin MIP layer on the surface of the seed and was first applied in the preparation of MIP-based stationary phases for HPLC column packing for the separation of diaminonaphthalene [10]. MAA and EDMA were used as the functional monomer and cross-linker, respectively. After polymerization, the linear polystyrene seed was removed by extensive washing, leaving MIP beads with a highly porous structure.

The significant advantages of this method are that it is easy to prepare MIP beads with a narrow size distribution and to perform in situ modification, although the synthetic process is somewhat more complicated due to the multistage swelling process required. For example, hydrophilic monomers can be incorporated into these beads for hydrophilic surface modification. Unlike directly synthesized beads, these core-shell particles from the multistep swelling polymerization method keep the binding sites presumably located near or at the particle surface, which can be helpful in achieving fast binding kinetics.

### **2.3.2 Suspension polymerization**

Over the years, many efforts to address the limitations of conventional molecular imprinting techniques (bulk polymerization) have resulted in the development of new imprinting methodologies. Methods such as suspension polymerization have been developed and applied to prepare MIP beads successfully. In conventional suspension polymerization, water is used as a continuous phase to suspend droplets of pre-polymerization mixtures (template molecule, functional monomer, cross-linker and initiator) in the presence of a stabilizer or surfactant [44]. The biggest advantage of this method is its excellent heat dispersion, which makes it suitable for industrial scale-up without heat transfer limitations. However, the MIP beads prepared by suspension polymerization have a broad size distribution as the size of the droplet formed is always polydisperse. Additionally, water hampers the non-covalent interactions such as hydrogen bonding and electrostatic interactions between the template molecule and functional monomer required for non-covalent molecular imprinting. Therefore, suspension polymerization using water as a continuous phase is typically only compatible with the covalent molecular imprinting technique.

Attempts to prepare MIP beads by suspension polymerization in water have led to only very poor molecular recognition. To overcome the limitations posed by the presence of water in this polymerization process, one possible way is to substitute the continuous water phase with an alternative liquid that can still act as a dispersion medium for the monomer mixture, but that better preserves the non-covalent interactions in the template-monomer assembly. Two new suspension polymerization techniques based on droplets of pre-polymerization mixtures formed in liquid perfluorocarbons [45] and mineral oil [46,47] have been developed. The use of liquid perfluorocarbons as the continuous phase might be preferred to avoid the effects of water on the non-covalent complex. MIP beads have been prepared using perfluorocarbon as the dispersion media, and excellent chromatographic performance was obtained. The limitation of this method is that the cost of the synthesized MIP beads may increase as it requires expensive perfluorinated solvents, which limits the applicability and practicality of this method. Kempe introduced the use of mineral oil as continuous phase in suspension polymerization towards the synthesis of propranolol imprinted polymer beads. The disadvantage of this approach is the wide range of bead sizes prepared, which requires extensive sieving in order to obtain the narrow size distribution required.

As described above, MIP beads have been commonly prepared via suspension polymerization, and templates suitable for this system include metal ions, drugs and proteins. In the meantime, it was found that this approach was limited to certain compositions, that the MIP beads prepared may have a wide size distribution. Furthermore, the stabilizer or surfactant, which is required for the formation and stabilization of droplets, may interfere with the interactions between the template molecule and functional monomer.

### **2.3.3 Precipitation polymerization**

Another common method of preparing spherical MIP beads is by precipitation polymerization, a relatively simple one-step polymerization method. A crucial difference between bulk polymerization and precipitation polymerization is the volume of the polymerization medium used, where precipitation polymerization requires a larger volume of medium. In this approach, the key point is the use of a solvent in which the monomers are soluble but the resulting polymer is not. Starting with a very dilute homogeneous monomer solution, the growing polymers precipitate out from the solution due to their low solubility in the solvent while continuously capturing and consuming monomers, eventually to form cross-linked polymer beads in the micro- or even sub-micrometer size range under the appropriate conditions. Because no interfering reagents, i.e., surfactant or stabilizer, are used during the polymer synthesis, precipitation polymerization has

been shown to be applicable to a broad range of template structures and is the most suitable method for preparing high-quality MIP beads by non-covalent molecular imprinting. Moreover, the most commonly employed solvents are aprotic, which can preserve the necessary hydrogen bonds between monomer and template during the polymerization process.

Application of this technique to synthesize MIP beads was first proposed by the group of Ye and Mosbach [36]. MIP beads in the sub-micrometer size range were prepared using acetonitrile as the polymerization medium, and MAA as the functional monomer. The method was also shown to work satisfactorily with different template molecules such as theophylline, caffeine, 17 $\beta$ -estradiol, (S)-propranolol and 2,4-dichlorophenoxyacetic acid [30,48]. MIP beads prepared by precipitation polymerization have been used in applications including ligand binding assays, liquid chromatography, capillary electrochromatography, solid-phase extraction and chemical sensing [49-53]. Cormack and co-workers also found that copolymerization of DVB in a mixture of acetonitrile and toluene gave monodisperse MIP beads [54]. This approach was used to prepare monodisperse theophylline imprinted polymer beads of about 5  $\mu$ m in diameter using MAA as a functional monomer. The prepared MIP beads displayed good separation performance when they were packed into an HPLC column for the separation of theophylline.

The effects of the cross-linking monomers on the final sizes of MIP beads were examined by Yoshimatsu et al [55]. The sizes of MIP beads could be controlled by varying the ratio of DVB and TRIM, and ranged in size from 130 nm to 2.4  $\mu$ m. This result illustrates that by changing the ratio between the two cross-linkers employed in precipitation polymerization, the MIP sizes can be tuned from the nanometer to the micrometer scale.

The simplicity of this method, the omission of stabilizers or other additives and its compatibility with high cross-linking degrees have made precipitation polymerization very popular among MIP researchers. The disadvantage of this method, however, is that a large amount of template molecule is required for the preparation process because of the high dilution factor. Additionally, such high dilution conditions have a negative effect on the interactions between the monomers and the template, so a careful choice of sufficiently strong binding interactions between template and monomers is also necessary.

### **2.3.4 Additional methods**

Other than the direct preparation of MIP beads from a monomer solution, the preparation of MIP beads from preformed supporting spheres, such as spherical silica or organic polymers, has also been reported [56,57]. The beaded silica



approach has drawn more attention, as silica with a small bead size and narrow size distribution is commercial available, and additionally, the silica scaffold can be removed by HF without destroying the structure of the final MIP beads. Furthermore, the imprinted sites are typically located at the surface of the macropores within the MIP beads, if the template molecule is linked to the pore surface of the silica scaffold prior to polymerization.

Although these methods have been successfully demonstrated to give well-defined imprinted polymer beads, up until now, such methodologies have not become general for the synthesis of MIP beads. The reason is possibly due to the fact that they involve multi-step reactions and are somewhat time-consuming.

## 2.4 Highlighted applications of MIP beads

In recent years, the number of reports of MIPs that have been used in sensors, as artificial antibodies and as catalysts has increased dramatically. Moreover, MIPs are most frequently utilized as affinity adsorbents for sample pre-concentration and separation in HPLC, SPE, and in capillary electrochromatography (CEC) systems based on their selective recognition towards the template or its analogs.

MIP packed HPLC columns have been used to carry out analytically relevant separations, such as the separation of templates from other substances in a complex sample. Compared with classical SPE that lacks selectivity, MIP-SPE is very efficient for extraction and cleanup due to its selective binding of the target analyte, which is crucial, particularly when the sample is complex and impurities can interfere with quantification. Therefore, MIPs are believed to be a good choice to replace the traditional stationary phases in HPLC or SPE, especially when spherical and monodisperse MIPs on the micrometer scale can be prepared. In recent years there have been many applications of MIPs for real samples. Various compounds have been extracted from environmental samples (e.g., tap water, river water, lake, surface, waste and pond water samples), biological samples (e.g., urine, plasma, serum, and blood) and food matrices (e.g., pork, milk, tomato and egg).

MIP beads as sorbent phases also have some limitations, especially those prepared using non-covalent interactions between the template and the monomers. The bonds formed with the target analyte, typically hydrogen bonds, are relatively weak and are sensitive to interferences from the external conditions, which means that when MIP-SPE is performed for extraction of aqueous solutions, water molecules will compete with the target analytes leading to the loss of binding selectivity and capacity. To overcome this problem, a certain amount of organic



solvent can be added to the mobile phase during the extraction, or a liquid-liquid extraction can be applied prior to the MIP-SPE process. However, these additional procedures are complicated and time-consuming. The easiest way to overcome this problem is to prepare MIP beads that can be used directly in an aqueous media. Therefore, the next strategy is focused on the development of water-compatible MIP beads.

# Chapter 3. Synthesis, characterization and applications of MIP beads

## 3.1 Methodologies to obtain water-compatible MIPs

One challenging task in molecular imprinting is the generation of MIPs that could eventually replace biological receptors, such as enzymes and antibodies in practical applications, in particular generating MIPs that show outstanding molecular recognition abilities in aqueous media. Additionally, other target molecules of interest that are present in aqueous media such as body fluids, foods, and environmental samples, MIPs with molecular recognition in aqueous media are greatly needed. However, most previously developed MIPs using non-covalent imprinting are only compatible with organic solvents, and they often fail to show specific binding in aqueous solutions. As mentioned earlier, the traditional MIPs are synthesized in organic solvent and they offer the highest binding selectivity in the organic solvent used in the polymerization process. When these MIPs are used in aqueous media, water molecules can disrupt the hydrogen bonding interactions required for selective recognition, and can cause significant non-specific binding due to the hydrophobic effect. In recent years, many efforts have been made to develop water-compatible MIPs that are applicable in aqueous solution for potential uses in environmental protection, food safety, and clinical diagnostics. Several efficient approaches for the preparation of water-compatible MIPs have been reported in the literature.

### 3.1.1 Optimization of binding conditions

Although the strongest and most selective rebinding is often observed in a non-polar organic solvent, some MIPs prepared via the conventional molecular imprinting strategy can still be used in an aqueous buffer containing some organic solvents, for example, in the specific molecular recognition of low molecular weight analytes. As pure aqueous conditions can lead to significant hydrophobic interactions, non-specific adsorption is typically observed. One strategy employed to improve the specific binding of MIPs in water was shown through the addition

of a small amount of organic solvent to the system, in order to minimize the extent of hydrophobic interactions that occur. This strategy was first reported by Andersson, who demonstrated that the non-specific binding of hydrophobic MIPs in buffer solutions could be minimized by the addition of organic modifiers (e.g. surfactants or organic solvents). A certain amount of organic solvent can minimize non-specific interactions, however the conditions must be carefully optimized.

In Paper IV, we investigated the effects of water on the binding of nicotine by MIP and NIP beads. In pure water, the uptake of nicotine by the MIP was only slightly higher than that of the NIP, and both MIP and NIP displayed high nicotine binding due to non-specific interactions. The best specific binding of nicotine (40.5%) was obtained in pure acetonitrile which was used as porogen to prepare the MIP beads. The non-specific binding can be significantly reduced by optimizing the acetonitrile content of the solutions, which lead to some specific binding as shown in Figure 3.

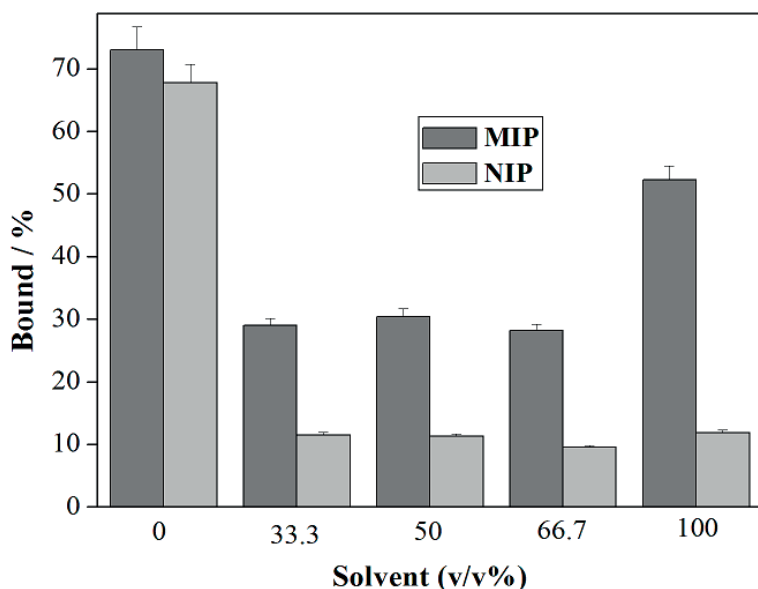


Figure 3. Uptake of nicotine by polymer beads (5 mg) in water containing different amount of acetonitrile.

The total binding to a MIP can be divided into two parts, specific binding to the imprinted cavities of the MIP and non-specific binding to the MIP surfaces. Some pre-treatment is frequently used to remove the non-specific binding and redistribute non-specifically bound analytes to the imprinted sites when organic solvent-compatible MIPs are used as the stationary phases of HPLC or SPE to

treat aqueous samples. A washing solvent, the same or close to the porogen used during the imprinting reaction containing a small amount of polar modifier, can be used in order to limit the non-specific interactions between the MIP and the target molecule [58]. The modifier can remove non-specific binding effectively, while still retaining the selective binding for the target analyte, which significantly enhances the MIP binding selectivity.

To use organic solvent-compatible MIPs in aqueous solution directly, optimization of the binding conditions, such as the buffer used, the amounts of organic solvent added and the solvents pH values, must be carried out carefully. These procedures are complicated and time-consuming, which largely limits its broad applications. Therefore, efficient approaches that can be used to prepare water-compatible MIPs directly are highly desirable.

### **3.1.2 Surface modification after molecular imprinting**

It is well known that recognition of the template by traditional MIPs is mainly mediated by hydrogen bonds between the template and the functional groups on the MIPs, and non-specific adsorption is mainly a result of the hydrophobic interaction of the materials. Grafting hydrophilic polymer layers onto the organic solvent-compatible MIPs to improve their surface hydrophilicity has been utilized to improve the binding selectivity of MIPs in aqueous conditions. As this strategy only requires the modification on the preformed MIP particles to improve the hydrophilicity of the MIPs, it offers better application potential.

Haginaka and co-workers managed to reduce the non-specific adsorption of MIPs in water by the addition of a hydrophilic external layer using surface modification [59]. The experimental results revealed that the chiral recognition sites of (S)-naproxen in the MIP beads remained unchanged with hydrophilic surface modification.

Core-shell structured MIPs with a hydrophilic shell are another method that allows specific binding in aqueous media to be maintained. Ye and co-workers reported a one-pot synthesis of hydrophilic core-shell MIP nanoparticles [60]. Propranolol-imprinted nanoparticles were initially prepared via a simple precipitation polymerization method, and hydrophilic polymer layers were later grafted onto the surface via conventional free radical polymerization of acrylamide (Am) and N,N-methylene bis acrylamide (MBA). The resulting hydrophilic MIP nanoparticles showed good specific template binding in a mixture of buffer and acetonitrile.

Similarly, introduction of hydrophilic groups onto MIPs surface by post-imprinting surface modification has also been shown to improve water compatibility. Puoci et al. successfully prepared hydrophilic MIP beads with

hydroxyl groups on their surface [61]. P-acetaminophenol imprinted polymer beads with surface-bound epoxy groups via precipitation polymerization were synthesized firstly using MAA as the functional monomer, divinylbenzene (DVB) as the cross-linker, and glycidyl methacrylate (GMA) as the co-monomer. After polymerization, the epoxide ring from GMA was opened to introduce hydrophilic groups by treatment with perchloric acid (Figure 4).

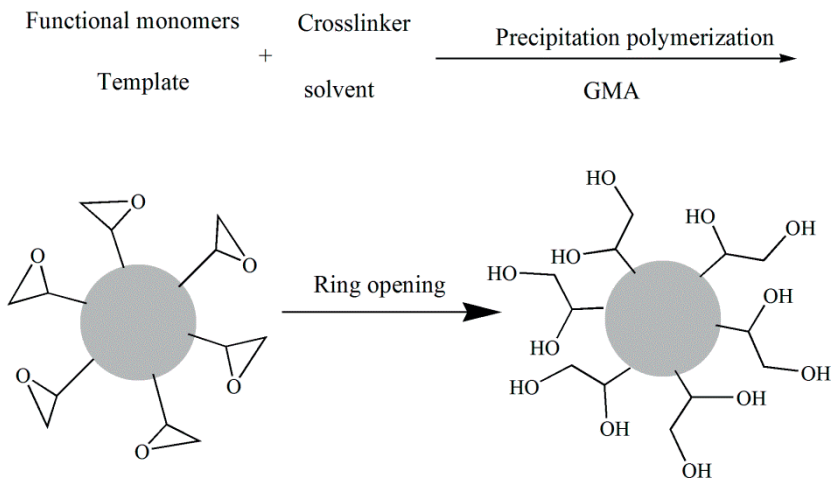


Figure 4. Preparation of hydrophilic MIP beads with surface-bound hydroxyl groups from precipitation polymerization and epoxide ring opening.

Reversible addition-fragmentation chain transfer (RAFT) polymerization is a highly efficient approach for the preparation of MIP beads with well-defined surface-grafted hydrophilic polymer layers or brushes, which can exhibit excellent molecular recognition ability in aqueous solutions. Normally, this two-step approach involves the synthesis of “living” MIP beads with surface-bound chain transfer groups, and subsequent surface-grafting of hydrophilic polymer layers or brushes via surface-initiated polymerization of hydrophilic monomers. Zhang developed a “one-step approach” to prepare narrowly dispersed MIP microspheres with surface-grafted hydrophilic polymer brushes by using a series of hydrophilic macromolecular chain transfer agents, such as poly(N-isopropylacrylamide) (polyNIPAm) and polyethylene glycol (PEG) with a dithioester end-group and different molecular weights [62,63]. The addition of hydrophilic macromolecular chain transfer agents to the molecular imprinting systems proved to have a negligible effect on the formation of specific binding sites.

### 3.1.3 Using hydrophilic monomers

To obtain water-compatible MIPs, some hydrophilic functional monomers have been used in molecular imprinting systems to prepare hydrophilic MIPs. The principal of this strategy is the reduction of the non-specific binding of the MIPs towards the template molecules in aqueous solutions by increasing the surface hydrophilicity of the MIPs, while avoiding complicated post-surface modifications. Acrylamide (Am) [64,65], methacrylamide [19] and 2-hydroxyethyl methacrylate (HEMA) [66] are commonly used hydrophilic monomer or co-monomers, while N,N-methylene bis acrylamide (MBA) [67] and N,N-ethylene bis acrylamide [68] are often used as hydrophilic cross-linkers.

Sellergren and co-workers found that the water compatibility of MIPs was affected by the amount of hydrophilic co-monomer and/or cross-linkers added to the reaction mixture [69]. The hydrophilic MIPs prepared under optimal conditions showed high imprinting factors in aqueous solution, however, the amounts of hydrophilic co-monomers added had to be thoroughly optimized for each imprinting system, as they had a significant influence on the imprinting effects of the resulting MIPs.

Water-compatible MIPs have been successfully prepared by the addition of certain amounts of hydrophilic monomers or cross-linkers in the molecular imprinting processes, and their improved surface hydrophilicity proved to be responsible for their water-compatibility. Although some MIPs with specific molecular recognition abilities in aqueous solutions could be obtained by utilizing hydrophilic functional co-monomers or cross-linkers in the molecular imprinting systems, the use of these monomers or cross-linkers could only be applied to particular systems. Furthermore, a large amount of hydrophilic co-monomers is normally required to obtain noticeable improvements in the surface hydrophilicities of the MIPs, which also results in a negative effect on the molecular recognition ability of the MIPs. Therefore, new and more efficient approaches for the preparation of water-compatible MIPs are highly desirable.

## 3.2 Pickering emulsion polymerization

Many efforts have been devoted to the development of MIPs that are directly useable in aqueous solutions (water-compatible MIPs) because of their great potential for use in environmental protection and food safety applications. Successful results have been reported in the literature, however, most approaches cannot become general method due to different kinds of limitation. Recently, a new approach based on Pickering emulsion was applied to obtain water-

compatible MIPs, which showed distinct advantages over the previously mentioned methods [70,71].

Solid particle stabilized emulsions, often called Pickering emulsions, are known to display long-term stability against coalescence, in contrast to systems stabilized by small molecular weight surfactants. The high stability against coalescence makes Pickering emulsion an interesting system for the synthesis of spherical polymer beads using free radical polymerization. The most common Pickering emulsions are oil-in-water (o/w) or water-in-oil (w/o) droplets stabilized by different partial wetting solid particles. The type of emulsion generated is largely determined by the hydrophobicity of the particles. Generally, the phase that preferentially wets the particle will be the continuous phase in the emulsion system (Figure 5). The adsorption of colloidal particles at the oil/water interface is a crucial factor in preparing stable Pickering emulsions. In most cases, adjustment of the surface hydrophobicity (also expressed by the three-phase contact angle  $\theta$ ) of colloidal particles is used to control the adsorption or assembly of the colloidal particles at oil/water interfaces. Similar to traditional suspension polymerization based on surfactant-stabilized emulsions, Pickering emulsion polymerization can be used to prepare polymer beads.

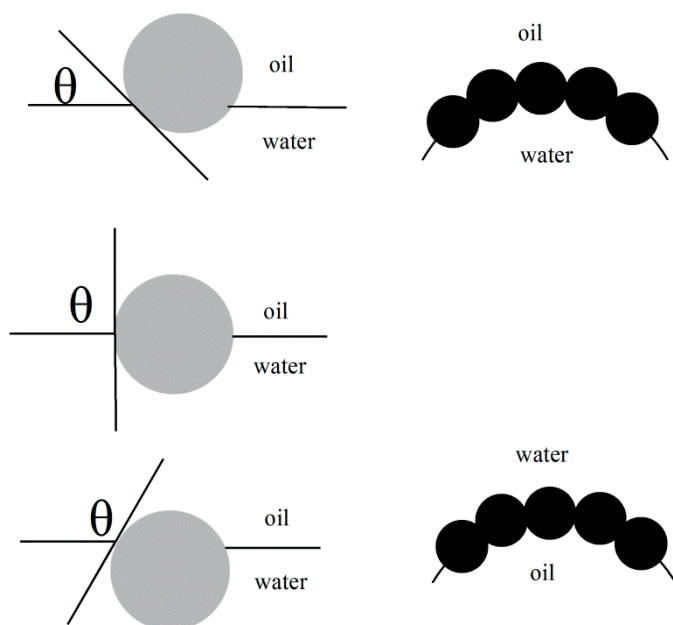


Figure 5. Schematic of particle stabilization for oil-in-water (o/w) and water-in-oil (w/o) Pickering emulsions.

Our group recently developed a new method of molecular imprinting based on Pickering emulsion, a new synthetic platform that can offer hydrophilic and water-compatible MIPs possessing selective molecular recognition in aqueous media, not only for small organic molecules [72], but also for proteins [73] and cells [74]. The water-compatibility of such MIPs is due to the presence of a high density of hydrophilic functional groups on the MIP surfaces from the partitioning of monomers between the water and oil phases during the Pickering emulsion polymerization. Although molecular imprinting in Pickering emulsion is a relatively new development, it has shown great promise for the preparation of MIP beads for selective recognition of an increasing number of pre-defined molecular targets.

### **3.2.1 Influence of colloidal particles on Pickering emulsion**

As mentioned in section 3.2, colloidal particles adsorbed at the water/oil interface are a crucial factor in preparing stable Pickering emulsions. A variety of colloidal particles, such as silica, clays, polymeric latexes, and poly(N-isopropylacrylamide) (polyNIPAm) microgels [75-78], have been used as Pickering emulsion stabilizers. The properties of particles can affect emulsion stabilization. For example, the particle size is in close relationship with the hindering droplet coalescence, and the particle concentration determines the emulsion coverage, which may influence the emulsion formation and stabilization.

Silica nanoparticles are the most commonly used stabilizer as they can be easily removed by hydrofluoric acid (HF) after the polymers are obtained. Furthermore, the synthesis of monodisperse silica particles is relatively simple to perform and well documented in the literature. Besides, it is straightforward to transfer the initially hydrophilic silica into hydrophobic particles by chemical modification. In Paper I, two types of silica nanoparticles with different polarities were selected to prepare Pickering emulsions. The surface properties of the silica nanoparticles were first studied by attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis as shown in Figure 6. For clearer comparison, the IR spectra were normalized to give the same intensity for the asymmetric Si-O-Si stretching vibration peaks present at around  $1100\text{ cm}^{-1}$ . As can be seen, the density of OH groups on SiO<sub>2</sub>-I was much higher than that on SiO<sub>2</sub>-II, which suggested that SiO<sub>2</sub>-I was more hydrophilic than SiO<sub>2</sub>-II. The different surface polarities between SiO<sub>2</sub>-I and SiO<sub>2</sub>-II were also confirmed by the measurement of their colloidal stability in water. When the two SiO<sub>2</sub> nanoparticles (10 mg) were added to water (3 mL), SiO<sub>2</sub>-I produced a more stable dispersion than SiO<sub>2</sub>-II, which aggregated and settled quickly.



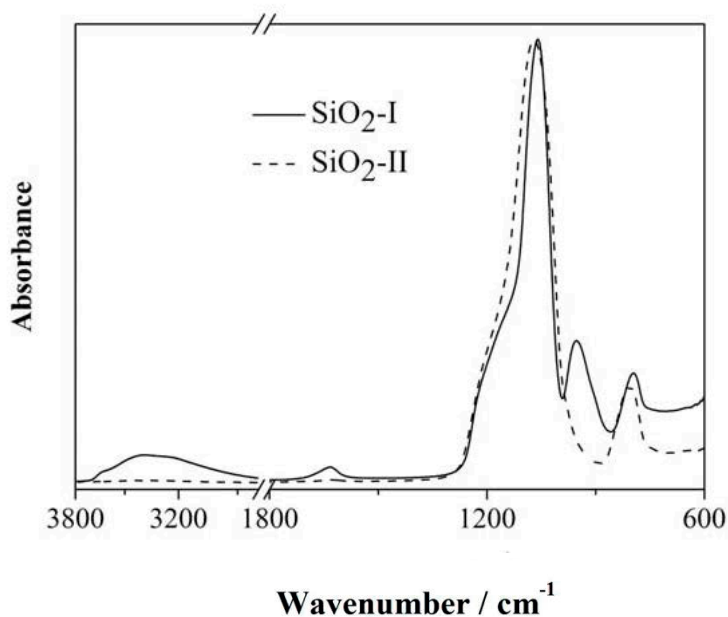


Figure 6. FT-IR spectra of SiO<sub>2</sub>-I and SiO<sub>2</sub>-II.

As the surface characteristics of silica particles are one important factor that affects the morphology and droplet size of the obtained Pickering emulsions, optical microscopic images of the Pickering emulsions stabilized by SiO<sub>2</sub>-I and SiO<sub>2</sub>-II were shown to approve that. The emulsions stabilized by SiO<sub>2</sub>-I had a wide size distribution from 20 to 200  $\mu\text{m}$ . When SiO<sub>2</sub>-II nanoparticles were used as the stabilizer to prepare the Pickering emulsions, the dispersed droplets became much smaller (Figure 7).

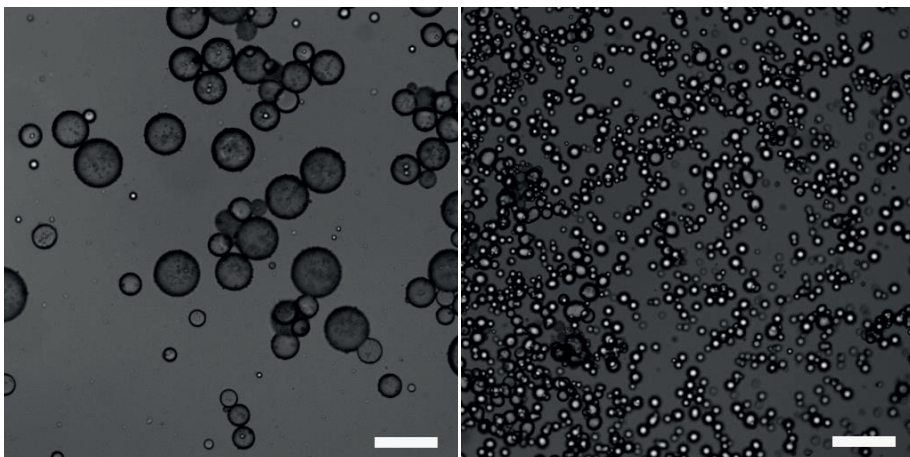


Figure 7. Optical images of the Pickering emulsions from the SiO<sub>2</sub>-I (left) and SiO<sub>2</sub>-II (right) nanoparticles.

The amount of silica particles used to stabilize Pickering emulsion also has an important effect on the binding performances of the resulting polymer beads. As more silica was used, the binding of the MIP beads decreased, while the binding of NIP remained almost unchanged (Figure 8). The reason is that more silica can block the interaction between template and functional monomers. In the work reported in Paper III, the template was dissolved in an oil phase while the functional monomer was in water and oil phase. The silica particles self-assembled at the oil-water interface. With increased silica particles, more functional monomers were attracted to the interface, leaving fewer functional monomer in the oil phase to create effective imprinted sites. This results led to a reduced imprinting effect. In the case of the NIP system, the binding was not affected because it was purely non-specific adsorption.

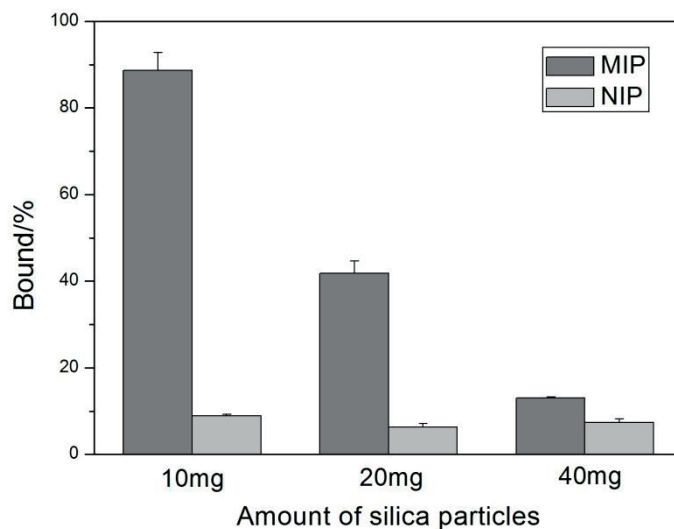


Figure 8. Diclofenac acid (DFC) binding by MIP and NIP beads prepared with different amounts of silica particles.

Under optimized conditions, MIP beads in the micrometer size range with hydrophilic surfaces were obtained, and were successfully used as SPE absorbents for the effective enrichment of low concentration organic pollutants ( $\beta$ -blockers) from tap water. When the water-compatible MIP beads from Pickering emulsion were packed into HPLC columns, chromatographic evaluation was easily conducted using water as the mobile phase as shown in Paper III. The results of the chromatographic separation showed that the MIPs maintained specific molecular recognition selectivity for the template.

Although the first molecular imprinting from Pickering emulsions was reported only a few years ago, the number of publications in this area has increased very rapidly. For recognition of large biological molecules and living cells, Pickering emulsion is particularly interesting because various templates and epitope structures can be immobilized on the stabilizing particles to create surface imprinted binding sites.

### 3.2.2 Protein imprinted polymer beads

As a versatile technique for preparing synthetic polymers with specific recognition property for molecular targets, molecular imprinting has been primarily successful for small molecules. To date, the imprinting of larger molecules and biological macromolecules has been hindered by a number of complicating factors. The size, complexity, conformational flexibility and environmental sensitivity of such

molecules, have made imprinting against biomacromolecules particularly challenging.

A variety of approaches have been developed for the preparation of macromolecule-imprinted polymers over the past decades, and significant progress has been reported especially in the molecular imprinting of proteins. Protein imprinting strategies can be divided into several categories, which typically include bulk polymerization [79], epitope imprinting [80], protein imprinted hydrogel [73] and surface imprinting [81,82]. Bulk imprinting is the most straightforward approach to macromolecular imprinting, in which three-dimensional binding sites are formed for the entire protein within the bulk of the matrix. However, it is difficult for the transfer of protein molecules within the highly cross-linked polymer networks. In addition, bulk imprinting requires grinding and sieving that produces irregularly shaped MIP particles, which can be time consuming and results in a loss of polymers. Epitope imprinting uses a small structural element of the protein as the template that substitutes the targeted larger polypeptide or protein. The use of a short peptide sequence as template can avoid most of the problems of observed in protein imprinting, such as restricted mobility and sensitivity to environments. However, the challenge that arises is that the synthesis and purification of peptides can be very difficult. In recent years, a class of novel and swellable polymers known as hydrogels with selectivity has been synthesized, especially for protein imprinting [83]. Protein-imprinted hydrogels are soft materials due to their low cross-linker density and have a relatively low mechanical strength. In order to use protein imprinted polymers for protein separation, it is necessary to develop non-compressible MIP beads that can ideally be used in a chromatography column, while at the same time ensure that the protein binding sites are easily accessible. Guo and co-workers proposed a possible answer. In order to improve the mechanical strength of polyacrylamide (PAm) gel, macroporous chitosan beads were used as a matrix and the selective soft PAm gel was entrapped in the beads [84]. The obtained polymer beads possessed good mechanical strength, specific binding ability and chemical stability.

Surface grafting of MIP layers onto preformed beads has been recently proposed as an attractive technique to obtain MIP beads with selectivity for proteins. The most common type of material used as support beads is silica mainly due to its stability, favorable physical properties and ease of derivatization. Other support materials include magnetic nanoparticles [85], polystyrene, chitosan [86] or other types of polymers, depending on the requirements of the application. Surface imprinting can generate easily accessible binding sites on the seed particle for large molecules, which make it a promising approach for the preparation of chromatography-grade imprinted materials. The imprinted sites are located at or very near to the surface of the polymer layer, which addresses the issues of

restricted mass transfer and facilitates removal of the protein. Among the techniques used for protein imprinting, surface imprinting is currently the most popular and generalized method. Typically protein molecules are covalently immobilized on the surface of solid substrates. Commonly, the process of protein immobilization involves three steps, as shown in Figure 9. First, amino-groups are introduced onto the support surface followed by introduction of aldehyde groups via the reaction of amines and glutaraldehyde. The protein is immobilized on the support surface by the formation of a covalent bond after mixing a protein solution with the aldehyde-modified support. After polymerization and removal of the template, specific binding sites for the protein are generated at the surface of the imprinted materials. The disadvantage of this kind of protein imprinting method is that it requires a multistep treatment of the support matrix to immobilize the protein. Additionally, only a limited number of imprinted sites are produced, resulting in a low loading capacity for protein adsorption.

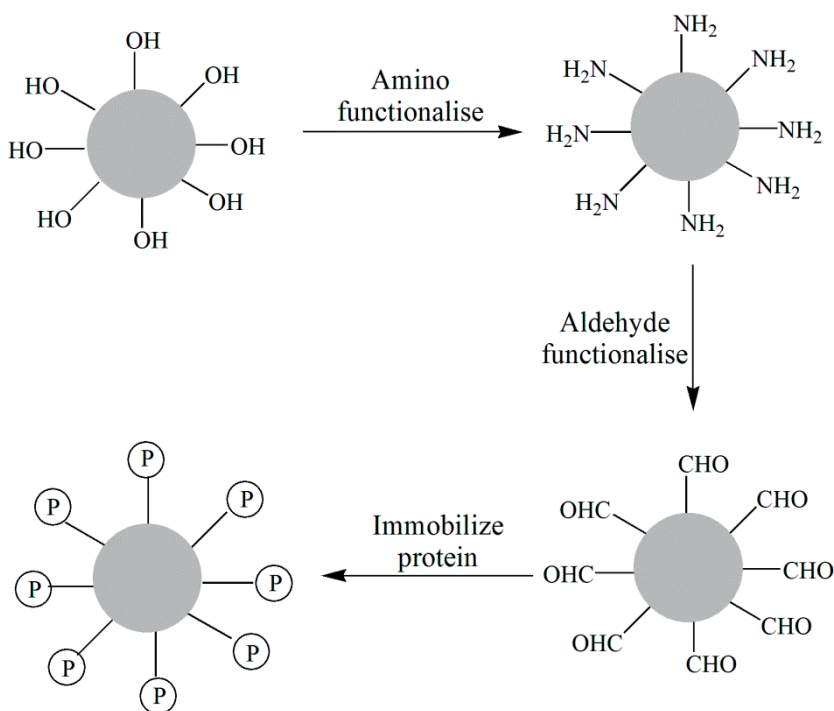


Figure 9. Covalently immobilization of protein on silica surface.

In our recent studies, we have demonstrated that Pickering emulsions offer a versatile system for preparing MIP beads. In addition to MIP beads that are

selective for small organic molecules, we have shown that hydrophilic MIP beads containing protein-imprinted sites can be synthesized by Pickering emulsion polymerization. In the previous protein-imprinting work, we used silica nanoparticles to stabilize the water-in-oil emulsion, where the water phase contained the functional monomer and protein template. Although the obtained hydrophilic MIPs showed high protein selectivity, they had low mechanical strength due to the low cross-linking density in the interior of the polymer particles. In Paper II, we showed that by presenting the protein template on the surface of the stabilizing nanoparticles, it was feasible to synthesize protein imprinted sites on MIP surfaces. This new method involved the use of protein-coated silica as the stabilizing particles to establish an oil-in-water Pickering emulsion. The oil phase contained the cross-linking monomer and initiator, while the functional monomer that interacted with the protein template was enriched at the oil-water interface due to the protein template (Figure 10). This interfacial protein imprinting led to the formation of protein recognition sites on the surface of highly cross-linked polymer beads. The advantage of this synthetic method resides in its general applicability and being able to be scaled up for preparation of large quantities of protein selective MIPs.

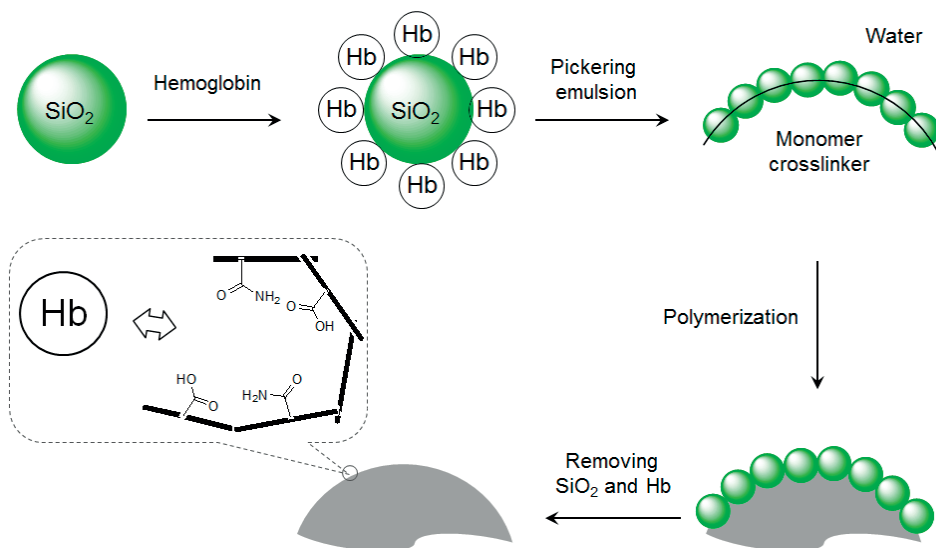


Figure 10. Schematic illustration of the interfacial protein imprinting process.

As shown in Figure 11, when MAA and Am were used separately as the functional monomer, the obtained MIP and the corresponding NIP beads showed very little

Hb binding. It was only when both MAA and Am were used as the functional monomers that the obtained MIP beads displayed significantly higher Hb binding than the NIP beads (Paper II). This result was in agreement with previous findings that multiple functional monomers often lead to successful protein imprinted polymers. The reason is that imprinting of biological macromolecules is mainly carried out in aqueous solvent, where combined hydrogen bonds, ionic forces, and hydrophobic effects have been suggested to contribute to a positive imprinting effect.

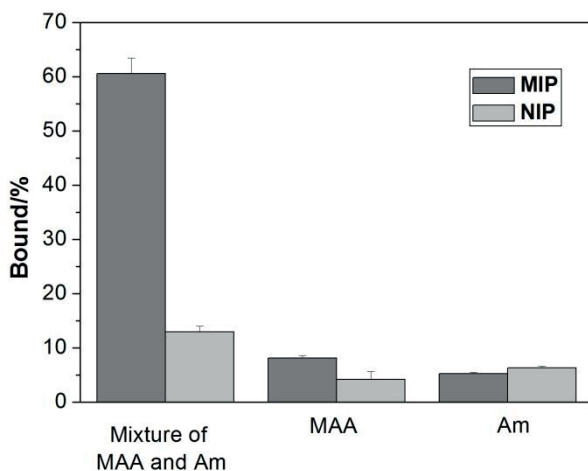


Figure 11. Hb binding to polymer beads prepared using different functional monomers.

### 3.3 Controlled/living radical polymerization (CRP)

Among all of the polymerization methods used to prepare MIPs, conventional free radical polymerization (FRP) is the most flexible in terms of reagent purity, experimental conditions, and choice of monomers, etc. This makes it the most widely used polymerization approach that is employed in preparing MIPs. However, a major drawback is that it does not allow one to control the molecular weight of the polymer synthesized, due to the high reactivity of the radicals produced constantly during the polymerization process and the uncontrolled and premature chain termination that occurs. As a result, polymers with a broad molecular weight distribution are generally produced, which are not suitable for applications where good control over the polymeric structures is desirable. It is

therefore of great interest to be able to control the growing and/or the termination steps of the reaction by using controlled/living radical polymerization (CRP).

CRP has drawn much attention as it has the potential to overcome the limitations of FRP. The polymerization takes place in a living way and early chain termination becomes negligible during the polymerization process. In CRP, the degree of polymerization increases linearly with monomer conversion, and can be controlled by adjusting the ratio of monomer to initiator. It has been well established that one of the main advantages of CRP is that living polymers with reactive end groups are readily obtained, which can be further extended to yield polymers with other more complicated architectures. Therefore, many kinds of advanced functional polymers can be obtained by direct grafting of polymer layer or brushes based on the end-capped living groups of the resulting polymers. The presence of extra surface-immobilized functional groups on the polymers therefore make them very useful in many further applications.

Significant progress has been made in the development of effective CRP approaches in recent years. Currently, the most popular CRP techniques are reversible addition-fragmentation chain transfer polymerization (RAFT) [87], atom transfer radical polymerization (ATRP) and nitroxide mediated polymerization (NMP) [88], which represent key strategies for the preparation of polymers with narrow molecular weight distributions. It is important to emphasize that every CRP approach has its own advantages and limitations and an understanding of these factors is of great importance in the choice of CRP used in designing advanced functional polymers.

So far, CRP has proven very powerful in preparing well-defined polymers with predetermined molecular weights, narrow molecular weight distributions, and desired molecular structures (e.g., block, graft, and star polymers). These methods should have a great potential for improving the morphology of MIPs and thus imprinting efficiency. However, CRP is still not used commonly in molecular imprinting.

### **3.3.1 Nitroxide mediated polymerization (NMP)**

NMP involves the cleaving of a C-ON bond using heat to generate a stable aminoxyl or nitroxyl radical and a reactive carbon radical, which brings control to the reaction. So far, there have been few reports on the applications of NMP in molecular imprinting due to the high activation temperature required. Although NMP has good functional group tolerance, the polymerization requires a high reaction temperature ( $>100\text{ }^{\circ}\text{C}$ ) in order to activate the dormant radicals, which makes NMP a slow process and only applicable for some special template systems as high temperatures are not compatible for MIPs synthesized with non-covalent



approach. Ye and coworkers employed NMP to synthesize a MIP specific for cholesterol through the covalent approach, since covalent bonds are more stable even at higher temperatures [89]. Compared to the MIPs prepared under the same conditions using traditional free radical polymerization, the template cleavage from the MIPs prepared by NMP was much more efficient and template binding was much higher.

### **3.3.2 Atom transfer radical polymerization (ATRP)**

Atom transfer radical polymerization (ATRP) has rapidly attracted considerable interest because of its easy availability for many kinds of initiators and catalysts. It is also known as metal-catalyzed radical polymerization, as the active species are produced by a reversible redox reaction, catalyzed by a transition metal/ligand complex. So far, many transition metals such as Cu, Ru, Ni, and Fe, together with various ligands have been successfully utilized to mediate ATRP, where Cu/ligand complexes are the most widely used.

ATRP can be applied to various vinyl monomers including methacrylates, acrylates, and styrenics, and it has been applied in molecular imprinting for the controlled preparation of MIPs with tailor-made structures and improved properties. The first successful application of ATRP in molecular imprinting was demonstrated by Husson and coworkers [90], where smooth MIP films were readily grafted onto the surfaces of gold-coated silica wafers in one step at room temperature. The living nature of ATRP allowed for the growth of MIP films with adjustable thickness and no solution-phase polymerization was observed. ATRP leaves easily convertible functional groups on the MIP surface and therefore makes further surface modification straightforward.

As a new approach in the preparation of uniform MIP beads, the ATRP technique is believed to represent a general and promising methodology. The possibility to perform polymerization at ambient temperature makes it particularly suitable for non-covalent molecular imprinting and for heat-sensitive systems. Compared with the traditional thermally initiated radical polymerizations, ATRP can be carried out under mild reaction conditions at room temperature in aqueous solutions. Hence, it is suitable for the imprinting of proteins on the surface of nanomaterial supports. A lysozyme-imprinted polymer placed at the surface of superparamagnetic particles was prepared using the ATRP protocol and reported by Gai et al. [91]. The authors claimed that the use of ATRP for surface grafting produced favorable results in achieving homogeneous binding sites, and improved the binding affinity and selectivity of MIPs.

A major limitation for this technique in MIP synthesis is that the deactivation of the metal catalysts in the presence of large amounts of acidic functional monomers

is possible. Therefore, typical monomers used for molecular imprinting such as methacrylic acid are incompatible, as they inhibit the metal-ligand complex involved in ATRP. With other monomers, like methacrylamide and vinylpyridine, it is difficult to achieve high monomer conversion as template molecules that carry functional groups may also inhibit the catalyst activity. The requirement for the complete removal of the metal catalysts from the final product in some specific applications such as drug delivery is also quite complicated. These limitations suggest that ATRP may not be the best choice of polymerization techniques for molecular imprinting.

### 3.3.3 Reversible addition-fragmentation chain transfer polymerization (RAFT)

Reversible addition-fragmentation chain transfer polymerization (RAFT) is the most recently developed polymerization technique that does not use metal catalysts, and is applicable to the polymerization of a wide variety of vinyl monomers, such as styrenic, (meth)acrylic, (meth)acrylamido and vinylic monomers under mild reaction conditions. RAFT polymerization is typically initiated by a classical FRP initiator. This method involves reversible addition-fragmentation sequences in which the transfer of a dithioester moiety between active and dormant chains serves to maintain the living character of the polymerization. RAFT precipitation polymerization technique combines the advantages of the traditional precipitation polymerization and CRP, and can thus be performed in a controlled manner without using any surfactant or stabilizer, leading to the precise control over the sizes, surface functionalities and living groups of the resulting polymer beads. In addition, the resultant polymer obtained by RAFT polymerization is end-capped by the moieties derived from the RAFT agent. As a result, the functional groups can be easily introduced onto the chain ends of the polymer by adjusting the structure of the RAFT agent used in the RAFT process. The moieties from the RAFT agent can also be used efficiently for subsequent post-polymerization modification to generate more complex structures.

The RAFT mechanism begins with the formation of an initiator derived radical ( $I^\cdot$ ) that reacts with the monomer ( $M$ ) to give a polymeric radical ( $P_n^\cdot$ ) as shown in Figure 12. The  $P_n^\cdot$  reacts efficiently with the RAFT agent 1 to form an intermediate 2, which quantitatively gives the macro-RAFT agent 3 and the expelled RAFT agent-derived radical ( $R^\cdot$ ), which re-initiates the polymerization.  $R^\cdot$  can generate its own active center by reacting with monomer molecules, providing a new polymeric radical  $P_m^\cdot$ . Finally, an equilibrium is established between the actively growing polymeric radicals ( $P_m^\cdot$  and  $P_n^\cdot$ ) and the macro-RAFT agent 3, which provides an equal probability for all of the chains to grow,


$$\text{Termination} \quad \text{Pm}^\bullet + \text{Pn}^\bullet \longrightarrow \text{Dead polymer}$$

ated the successful grafting of MIP films onto

As shown in Figure 12, MIP beads with surface bound dithiolester groups could

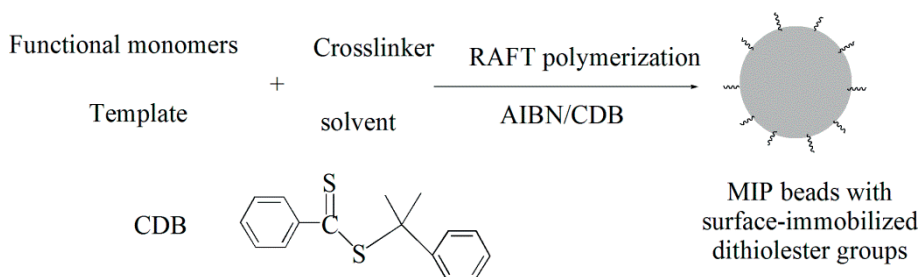


Figure 13. Schematic illustration for the controlled synthesis of MIP beads with surface-immobilized dithiolester groups by RAFT polymerization.

Zhang and co-workers have shown that monodisperse MIP microspheres with well retained molecular recognition properties could be prepared using a CRP technique [93]. The “living” end groups on the surface of the MIP beads were utilized to graft a new layer of polymer for straightforward surface functionalization. The introduction of hydrophilic polymer layers onto the MIP beads significantly improved their surface hydrophilicity and suppressed non-specific interactions between the MIPs and template molecules, thus leading to MIPs with pure water-compatible template binding properties. In addition, they claimed that the MIP beads prepared via RAFT have improved binding capacity, larger binding constant and significantly higher high-affinity binding site density in comparison with those prepared via FRP, which was believed to be due to the controlled polymerization mechanism of RAFT [94], thus leading to increased structural homogeneity and improved stability and integrity of the binding sites.

In Paper IV, nicotine imprinted polymer beads were synthesized by RAFT precipitation polymerization. As seen from the images by scanning electron microscopy (SEM), the size of the MIP and the NIP beads were  $1.55 \pm 0.04 \mu\text{m}$  (Figure 14a) and  $4.33 \pm 0.07 \mu\text{m}$  (Figure 14b), respectively.

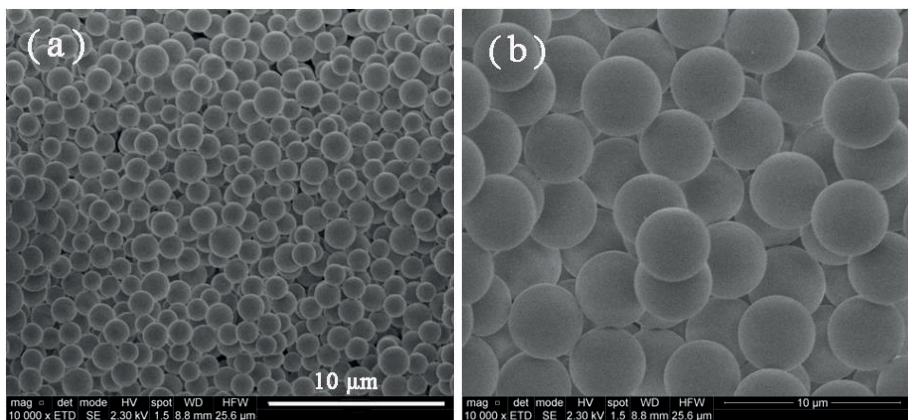


Figure 14. SEM images of (a) MIP and (b) NIP beads prepared by RAFT polymerization.

For many practical applications, it would be very beneficial if MIPs could have multiple functions rather than just target recognition alone. Based on the living groups of MIP beads from RAFT polymerization, it is possible to prepare multifunctional MIPs (Figure 15) using further post-imprinting modification. Several additional functions can be introduced into the MIPs without negatively affecting their selective molecular recognition.

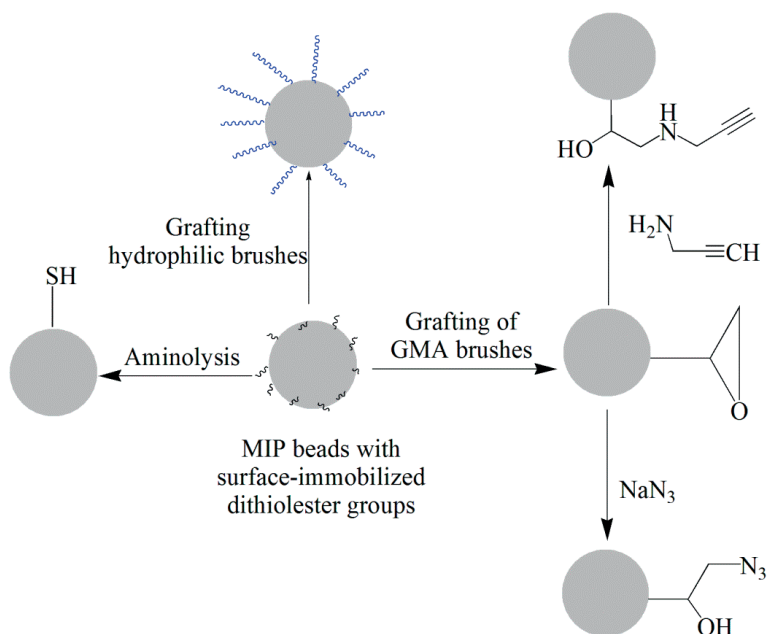


Figure 15. Preparation of multifunctional MIP beads.

Surface modification of organic polymer particles can improve their compatibility with environmental application and therefore has attracted increasing interest. The living character of RAFT polymerization products makes it possible to graft new layers of polymers by simply carrying out a second RAFT polymerization with a new monomer feed. To demonstrate the living property of imprinted polymer microspheres, we grafted poly(N-isopropylacrylamide) (polyNIPAm) brushes onto the surface of the MIP. After the graft polymerization, we used ATR-FTIR to characterize the modified MIP particles (MIP-polyNIPAm). As shown in Figure 16, the IR bands at  $1654\text{ cm}^{-1}$  and  $1541\text{ cm}^{-1}$  can be assigned to the fundamental stretching vibrations of Amide I and Amide II, respectively, which arise from the polyNIPAm brushes. These characteristic IR bands show the presence of the RAFT reagent in the MIP beads, and allowed for the continuous grafting of new polymer chains and effective surface modification of the polymer.

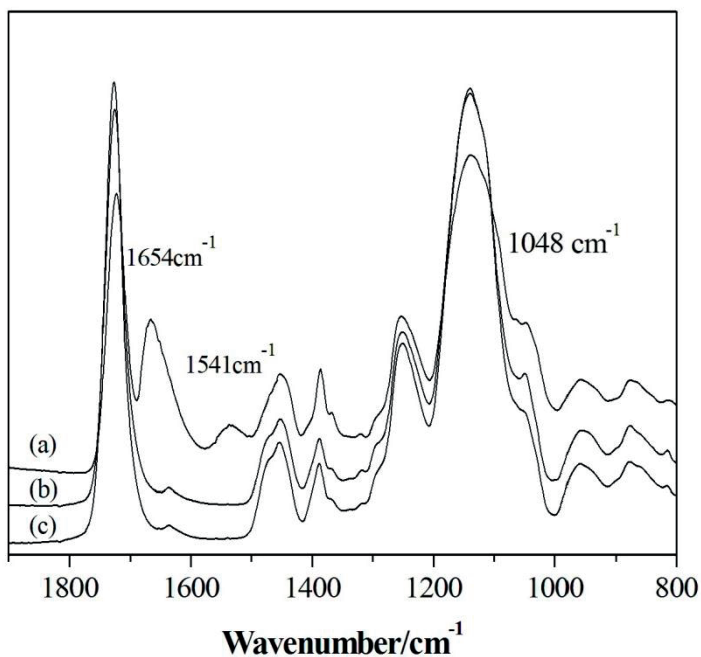


Figure 16. ATR-FTIR spectra of (a) MIP-polyNIPAm, (b) MIP, and (c) MIP-SH.

The covalently bound RAFT agent (CDB) in the MIP and NIP particles was converted into thiol groups by treating the particles with organic amines. After aminolysis reaction, the color of the particles changed from pink to white. In the ATR-FTIR analysis, the intensity of the IR band at  $1048\text{ cm}^{-1}$  (corresponding to the C=S stretch) decreased (Figure 16), which suggests that part of the CDB moieties has been altered. To show that the CDB moieties had been converted into

terminal thiols, we labelled the MIP-SH particles with NPM, a thiol-selective and fluorogenic pyrene derivative. After mixing the MIP-SH particles with NPM, the particle suspension became strongly fluorescent. As only the product of the Michael addition reaction between MIP-SH and NPM could be fluorescent, it is clear that the aminolysis reaction has converted the RAFT agent into thiol groups.

As a very versatile functional group, terminal thiols can be utilized to conjugate different organic molecules through S-S bridges, or via simple Michael addition or thiol-ene click reaction. The possibility of using terminal thiols to immobilize MIP particles on gold-coated transducers to develop different chemical sensors and introduce additional functional properties (e.g. stimuli responsiveness) to MIPs is also appealing for the practical applications of molecularly imprinted polymers.

In conclusion, RAFT polymerization has rapidly drawn increasing interest in recent years because of its simplicity, good controllability, and excellent compatibility with almost all monomers suitable for conventional free radical polymerization. The well-controlled nature of RAFT polymerization and its applicability to a large number of different hydrophilic functional monomers makes this approach a general and promising way to develop advanced water-compatible MIP materials. In the future, it is likely to become the most popular CRP technique and will be applied to many different fields.

### 3.4 Application of MIP beads in chemical sensing

A sensor is a device that can respond to a physical or chemical stimulus to produce a measurable signal. Two types of sensors are often studied that are referred to as chemical sensors or biosensors. In biosensors, the recognition elements are obtained from different biological systems, for example, antibodies and aptamers, which are particularly useful for the detection of biomacromolecules. However, antibodies and aptamers have limited stability and are not particularly successful in recognizing low molecular weight analytes. Molecular imprinting is a promising way to overcome many of the limitations imposed by biosensors. MIP-based chemical sensors for low molecular weight compounds have been reported in numerous publications [95-97]. Combining the molecular selectivity of MIPs with a spectroscopic transducer has proven very powerful in the detection of organic molecules in complex samples. For example, MIPs have been combined with IR evanescent wave spectroscopy [98] and a surface enhanced Raman scattering (SERS) [99] transducer to detect small organic analytes.



In Paper V, we investigated three approaches for implementing MIP beads to realize label-free detection of nicotine using SERS. The MIP beads were synthesized by RAFT precipitation polymerization and were decorated with terminal thiol groups (Paper IV), which allowed the MIP beads to be easily immobilized on a gold substrate. A common feature of these approaches employed was the use of surface thiol groups to immobilize MIP beads on a gold-coated surface to provide a selective molecular recognition layer. In the first approach, the immobilized MIP layer was sputter-coated with gold nanoparticles, and the activated MIP-SH surface was subsequently exposed to a nicotine solution and then analyzed by Raman spectroscopy. When the MIP-SH surface sputter-coated with gold was exposed to a nicotine solution, it generated strong Raman signal at  $1034\text{ cm}^{-1}$  (Figure 17). For the activated surface alone no nicotine signal could be detected. Under the same condition, the NIP-SH surface activated by Au sputter coating did not generate any nicotine signal, which is in line with the lack of binding sites in the NIP-SH particles. Au sputter coating on MIP-SH transformed the nicotine selective surface into a Raman active substrate, making it possible to detect the selective nicotine binding via the Raman “hot spots”. This method of gold sputtering to activate MIP for Raman measurement was very easy to carry out and was much cheaper than using commercial SERS substrates.



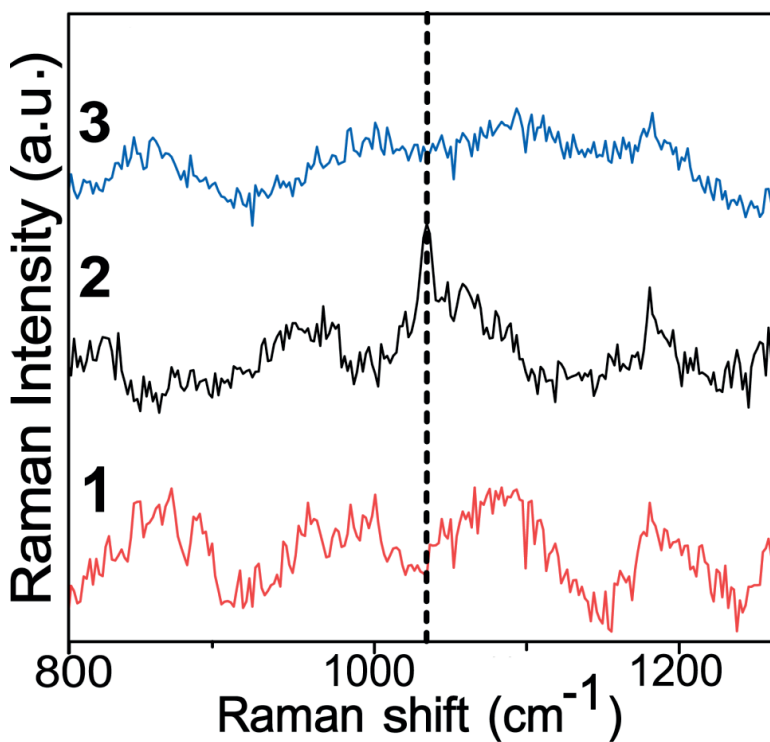


Figure 17. Raman spectra collected from (1) a MIP surface after gold sputtering, (2) a MIP surface after gold sputtering and exposure to  $6 \times 10^{-5}$  M nicotine, (3) a NIP surface after gold sputtering and exposure to  $6 \times 10^{-5}$  M nicotine.

## 4. Summary and Future outlooks

Recently, functional MIP materials have drawn much attention due to their potential applications in practical environment. The advantages of MIP materials are that they possess high recognition capacities for template and also have multifunctional properties. In this thesis, different approaches have been developed to prepare water-compatible MIP beads and multifunctional MIP materials.

Pickering emulsion is a new approach to directly synthesize hydrophilic and water-compatible MIP beads directly. The emulsion is stabilized by solid particles instead of surfactant. In Paper I,  $17\beta$ -estradiol was used as a model template because this steroid molecule has a very different polarity and functional groups in comparison with the previously studied propranolol template. Additionally, we succeeded in expanding the scope of the Pickering emulsion polymerization method and were able to synthesize different types of MIP beads. Silica nanoparticles with different polarity were applied in this study to reduce the bead size of the MIPs. We discovered that the nanoparticle stabilizer plays an important role in controlling the droplet size in Pickering emulsion.

For recognition of protein (In Paper II), Pickering emulsion is particularly interesting because templates can be immobilized on the stabilizing particles to create surface imprinted binding sites. This surface imprinting method is also applicable to other large biological molecules and living cells. Adult human hemoglobin (Hb) was adsorbed on silica nanoparticles, then the protein-coated silica particles were used to stabilize an oil-in-water emulsion (Pickering emulsion) composed of cross-linking monomer in the oil phase. The high selectivity of the protein imprinted beads and their high stability were attractive for a number of applications involving bioseparation processes, for example, in protein purification and selective depletion of abundance proteins in proteomics research. In future developments, it will be interesting to use specific epitopes selected from a target protein to modify solid particles to prepare the Pickering emulsion. In this way it will be possible to avoid using large proteins as templates for imprinting.

New monodisperse MIP beads were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization (In Paper IV). Equilibrium binding results indicate that the amount of nicotine bound to the MIP beads was

significantly higher than that bound to the non-imprinted polymer in both acetonitrile as well as in a mixture of acetonitrile and water. The RAFT reagent present on the surface of the polymer beads allowed for the straightforward grafting of hydrophilic polymer brushes on the particle surface. In addition, the dithioester end groups on the surface of the polymer beads could be converted into new thiol groups, through which a fluorescent dye was conveniently conjugated to the MIP beads via Michael addition reaction. Many applications can be conceived for the MIP beads, for example as basic building blocks to construct chemical sensors as well as to build polymer-enzyme conjugates for analytical applications. The thiol groups on the MIP surface allowed the MIP beads to be immobilized on a gold-coated substrate (In Paper V), whereby these MIP-based sensing surfaces were used for the detection of nicotine by surface enhanced Raman scattering (SERS).

As an efficient approach, the unique property of Pickering emulsion systems provides many possibilities to design the location of molecularly imprinted sites in the final products, and the complexity and functions of the final products. I believe that, more functional materials and new applications can be expected by combining Pickering emulsion systems with more controllable synthetic chemistry, such as click chemistry and controlled radical polymerization techniques.

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