Molecularly imprinted polymers for the detection of caffeine in water

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Molecularly imprinted polymers for the detection of caffeine in water

Abstract
This work investigates the potential of Molecularly Imprinted Polymers (MIPs) for the detection of caffeine in water samples. Different polymerization techniques were explored; namely free-radical bulk, miniemulsion and precipitation polymerization. Building the adsorption isotherms allowed determining the rebinding capacities of the resulting polymers (binding constant and number of sites). Using the nanoparticles prepared by precipitation polymerization in acetonitrile, new analytical techniques were developed. The latex was analyzed directly by High Performance Liquid Chromatography allowing fast template quantification in water samples. In addition, the nanoparticles were coated on a SMHz microbalance quartz crystal. The bi-Langmuir isotherm allowed computing the mass uptakes at the sensor's surface and suggested that as little as 0.1 ng of caffeine could be detected.

Keywords
Chemistry, Polymer
MOLECULARLY IMPRINTED POLYMERS FOR THE DETECTION OF
CAFFEINE IN WATER

BY

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Engineering diploma, ESCPE Lyon (France), 2006

THESIS
Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirement for the Degree of

Master of Science
In
Materials Science

September, 2007
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ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Yvon Durant for his encouragement, support and advice. He taught me how to persevere in my research and how to seek for alternative routes when the initial path fails.

I am also grateful to Dr. Jerome Claverie for his enthusiasm during the first year of this project.

I owe a special thank to Sayantan Roy for countless discussions about imprinted polymers.

I also would like to acknowledge Dr. Don Sundberg; his classes were an outstanding learning experience.

I would like to extend my thanks to all the members of the NPRC for making my experience at UNH a pleasant one.
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MOLECULARLY IMPRINTED POLYMERS FOR THE DETECTION OF CAFFEINE IN WATER

by

Marine Barasc

University of New Hampshire, September 2007

This work investigates the potential of Molecularly Imprinted Polymers (MIPs) for the detection of caffeine in water samples. Different polymerization techniques were explored; namely free-radical bulk, miniemulsion and precipitation polymerization. Building the adsorption isotherms allowed determining the rebinding capacities of the resulting polymers (binding constant and number of sites). Using the nanoparticles prepared by precipitation polymerization in acetonitrile, new analytical techniques were developed. The latex was analyzed directly by High Performance Liquid Chromatography allowing fast template quantification in water samples. In addition, the nanoparticles were coated on a 5MHz microbalance quartz crystal. The bi-Langmuir isotherm allowed computing the mass uptakes at the sensor's surface and suggested that as little as 0.1ng of caffeine could be detected.
INTRODUCTION

A large amount of artificial chemicals is currently being released into the environment, contaminating global resources. Water is typically polluted by pesticides such as triazines and phenylurea, drugs such as theophylline and ibuprofen, endocrine disruptors such as estrogens etc. The potential impact of those chemicals on wildlife and human health requires the development of reliable analytical techniques allowing their monitoring [1, 2].

Several analytical methods that allow the detection of such compounds in water samples already exist. Some of them have been approved by the Association of Official Analytical Chemists (AOAC): pesticides such as diuron, present in river water, are commonly quantified by liquid chromatography; atrazine is also analyzed in water samples thanks to an immunoassay [3].

But there is still a wide ongoing research to either try to enhance the current analytical methods or develop completely new techniques. Molecular imprinting represents an attractive opportunity to improve the current analytical techniques. Imprinted polymers are synthetic materials that exhibit an enhanced affinity toward a specific molecule thanks to their tailor-made recognition sites. Those polymers are inexpensive, readily prepared, reusable and present remarkable stability over a range of temperature, humidity and pH. This is why they have attracted a broad research interest in the last years.
They have already been reported as superior sorbents for solid-phase extraction [4, 5]. Some authors also investigated their use as sensor’s recognition units [6-8]. And several studies examined their potential as packing material for high performance liquid chromatography [9, 10].

The goal of this thesis was to investigate the potential of molecularly imprinted polymers for the detection of analytes in water samples. Caffeine was chosen as the target compound for this work because it is known to occur in river waters and because it has been widely reported in the literature as a template for molecular imprinting.

This thesis has been broken into three chapters. The first one provides some background useful to the understanding of this thesis. The synthesis techniques that have been reported for the preparation of molecularly imprinted polymers (MIPs) are first reviewed. Then the use of MIPs in liquid chromatography methods and sensors development is reported.

The second chapter focuses on the synthesis and characterization of the polymers. The last chapter covers the application of the polymers synthesized as the recognition unit of sensors and as sorbents for liquid chromatography.
CHAPTER 1

BACKGROUND AND LITERATURE REVIEW

1.1. Several imprinting approaches

Molecularly Imprinted Polymers (MIPs) are designed to recognize and bind selectively a specific compound in a complex mixture of chemicals. The recognition process is based upon complimentary interactions that take place between functional groups on the target molecule and on the polymer cavities [11]. Different interactions allowed the development of three types of preparation techniques. Even though different conceptually, those techniques are based on the same three steps approach (figure 1).

Initially the recognition monomer(s) arrange themselves around the target molecule. The former are then copolymerized with a crosslinking agent in presence of a porogen solvent. The high level of crosslinker in the copolymer guarantees the structure rigidity so that after template extraction, pores complementary in size and shape to the target molecule appear within the polymer matrix.
1.1.1. Covalent Imprinting

In covalent imprinting the template is converted into a polymerizable molecule that is then copolymerized with the crosslinking agent. Monomers and target are thus covalently bound which allows precise orientation of the template throughout the polymerization [13]. Removing the target from the matrix involves cleaving the polymer-template linkage. During rebinding, the covalent bonds are reformed [14]. Boronic acid has been used widely in this type of preparation since it is able to react rapidly and reversibly with diols. Figure 2 gives an example of a complex formed between 4-vinylphenylboronic acid and phenyl-α-D-mannopyranoside [15].
1.1.2. Semi-covalent Imprinting

A semi-covalent approach uses covalent interactions during polymerization, but the rebinding takes place thanks to non-covalent interactions [5, 13, 16]. Figure 3 gives a schematic representation of the approach used by Boonpangrak et al. [16]. In this work, the authors relied on a sacrificial carbonate bond: they used cholesteryl (4-vinyl) phenyl carbonate for imprinting which was hydrolyzed afterwards. Cholesterol was then rebound in the cavities through hydrogen bonding in nonpolar organic solvent.

![Schematic representation of the semi-covalent approach used by Boonpangrak et al.](image)

Figure 3: Schematic representation of the semi-covalent approach used by Boonpangrak et al. [16]

1.1.3. Non-covalent Imprinting

Non-covalent imprinting is the most commonly used preparation technique. In this case, imprinting and rebinding rely upon non-covalent interactions between monomers and template. Hydrogen bonding is exploited in many cases [5, 11, 17, 18], but some authors reported imprinting through hydrophobic packing [6, 19], electrostatic interactions [20] and metal coordination [7].
1.1.4. Comparing the imprinting approaches

Covalent imprinting is generally accepted as the method giving the best type of binding sites (homogeneous, well-defined, high binding affinity) since the monomer-template arrangement is precisely oriented and more stable. This technique suffers however a serious lack of flexibility. It requires preparing for each polymer system a template derivative that could be polymerized. Also, the bond maintaining the template in place during polymerization should be formed and cleaved easily, in mild conditions [13, 18].

In contrast, non-covalent imprinting generates some non-specific sites but it can rely on the variety of the commercially available monomers. Besides, target removal consists in a straightforward extraction and rebinding occurs upon simple mixing [5, 17]. For those reasons non-covalent imprinting is the approach that was selected for this thesis work. Only the studies reporting non-covalent techniques will be reviewed further.

1.2. Preparation techniques

Until recently, MIPs were most commonly prepared by bulk polymerization. The term bulk is misleading even though it is widely used in the field of imprinting technology. Generally speaking, a bulk polymerization is defined as the polymerization of monomer alone, i.e., no solvent is present during the reaction. Papers reporting the synthesis of bulk molecularly imprinted polymers refer to the preparation of hard polymers that need to be ground to generate a large imprinted surface area. Those polymers are typically formed by
the free radical polymerization of monomers in the same volume of solvent. Initially the mixture is homogeneous but rapidly polymer chains grow and precipitate out, while the solvent creates pores in the polymer network.

Grinding yields irregularly shaped particles that present a high size polydispersity. They usually need to be sieved which results in loosing almost 80% of the initial polymer: only 20% of the particles being of a useful size [21]. This is why in the last few years many laboratories have been interested in developing polymerization methods that would produce regularly shaped and monodispersed particles. The following sections will review the techniques that allow forming such particles.

1.2.1. Emulsion Polymerization

The most common type of emulsion system describes the polymerization of monomer droplets dispersed in a continuous aqueous phase. The latter consists of water, some kind of surfactant at a concentration greater than its critical micelle concentration and in some cases salts. When the monomer is added to the aqueous phase, a small fraction dissolves in the water and another small portion penetrates inside the micelles (2-10nm). The largest part of the monomer form large hydrophobic droplets (1-100μm) stabilized by surfactant.
The initiator used in such a polymerization is water-soluble so that the reaction takes place almost exclusively in the micelles. As the polymerization proceeds, monomers diffuse from the droplets to the micelles. At this point three types of particles coexist: the monomer droplets that act as reservoir, the active micelles where polymerization takes place and the polymer particles i.e. micelles where polymerization stopped [22]. Using such a technique to prepare imprinted polymers can then result in burying the template inside the particle. This is why a two-step approach is usually preferred for creating imprinting sites. The latter are produced in the second stage of the reaction, in the shell of a core-shell particle so that they are readily accessible for recognition.

Carter et al. used such a technique to imprint caffeine and theophylline [20, 23]. Initially, the authors prepared an aqueous emulsion of inert, highly
crosslinked polystyrene particles. The second stage of the polymerization allowed growing on top of the polystyrene core a thin shell (from 1nm to 5nm) of poly(ethyleneglycol dimethacrylate-co-oleyl phenyl hydrogen phosphate) imprinted with caffeine. To do so the authors relied upon the electrostatic and hydrophobic interactions taking place between caffeine and the recognition monomer (Figure 5). The resulting particles were found to have an average diameter of 40nm.

![Structure of the recognition monomer used by Carter et al. [20, 23]](image)

Figure 5: Structure of the recognition monomer used by Carter et al. [20, 23]

Perez-Moral et al. [24] also chose this approach to imprint the shell of core-shell particles with propranolol. The seed latex was made of methyl methacrylate (MMA) and slightly crosslinked with Ethleneglycol dimethacrylate (EGDMA). In a second-step, methacrylic acid (MAA) and EGDMA were copolymerized around propranolol to form a 10nm thick shell. Toluene was added to the monomer mixture during the imprinting step. Given the relatively small shell thickness, the solvent was not expected to act as a porogen as in bulk polymerization. Its role was rather to swell the forming network and thus ease the diffusion of template molecules in the polymer structure. The authors reported the formation of 60nm imprinted particles (Figure 6).
Creating imprinting sites at the surface of the particles can also be achieved by converting the template into a surface active molecule. For instance, the transformation of cholesterol into a surfactant-like molecule allowed Perez et al. to imprint the surface of poly(styrene-co-divinylbenzene) particles [26] (figure 7).

The new template is able to sit at the water and oil interface since it bears an anionic head that stays in the water while the hydrophobic cholesterol end interacts with the organic phase (figure 8). After template extraction, hydrophobic cavities were thus made available for cholesterol rebinding.
1.2.2. Miniemulsion Polymerization

In a miniemulsion system the oil phase is dispersed into the water phase thanks to high energy shear (sonication) that allows forming monodisperse monomer droplets stabilized by surfactant. Polymerization can start in the oil or in the water phase depending on the type of initiator used but propagation takes place mostly in the organic droplets. A single type of particles is thus present at
all times in the system and by the end of the polymerization polymer particles are the same size as the initial monomer droplets.

Figure 9: Schematic representation of miniemulsion polymerization

Consequently, each droplet is considered as an independent reactor and a one-step reaction yields homogeneous particles. To our knowledge, Vaihinger et al. [21] are the only ones who reported the use of miniemulsion polymerization to produce molecularly imprinted polymers. In a one-stage protocol, the authors imprinted acrylic polymeric particles with L-tert-butyl oxy carbonyl-phenylalanine anilid. The latter is a hydrophobic amino-acid derivative which allows efficient imprinting of the particles in the organic phase. (Figure 10) The authors reported the formation of 200nm particles. (Figure 11)
1.2.3. Suspension Polymerization

Suspension polymerization starts by dispersing monomers into a continuous water phase thanks to mechanical stirring. Polymerization is initiated by an oil-soluble initiator so that the reaction occurs in the monomer droplets. Small amounts of surfactant (stabilizer) ensure that the organic droplets do not coalesce. The relatively low concentration of stabilizer is responsible for the large particles usually obtained via dispersion polymerization (50-500μm) [22]. This technique is similar to the miniemulsion approach in the sense that each droplet is considered as an independent reactor where a miniature bulk reaction occurs. Homogeneous particles are thus generated.

A couple of authors reported the formation of imprinted particles by aqueous suspension polymerization[27-29]. In each case, acrylic polymers were used to complex the templates (triazine, dibutylmelamine, 4-aminopyridine). Reported particle sizes range between 8μm and 63μm. This technique thus
allows generating regularly shaped particles. However, their recognition capacities were limited and this was attributed to the continuous phase used. Water actually tends to destabilize the hydrogen bonds involved in the formation of the template-monomer complex. This is why Mayes et al. explored the use of liquid perfluorocarbons as a new continuous phase for suspension polymerization[30]. Such chemicals make suitable solvents for imprinting polymerization since they are largely immiscible with most organic compounds and thus do not compete with the recognition monomer for complex formation. The authors were able to imprint an acrylic matrix with Boc-DL-phenylalanine. Experimental conditions were optimized to make reproducible and uniform particles. Mechanical stirring, UV irradiation, perfluoro polymers as stabilizers (Figure 12) and chloroform as a porogen allowed forming particles of 2 to 25 um diameter.

Figure 12: Structure of the perfluorocarbons used as surfactants and solvents by Mayes et al [30]
1.2.4. Precipitation Polymerization

Precipitation polymerization is fairly similar to the bulk polymerization. In both cases, the monomers, the template and the porogen are miscible (while emulsion and dispersion systems are completely heterogeneous). However, at some point during the polymerization, the growing chains are no longer soluble in the solvent and consequently phase separate. In bulk polymerization, the crosslinked chains are able to occupy the entire volume of the reaction vessel, which results in a single piece of polymer. On the other hand, precipitation polymerization occurs in such a large excess of solvent that the chains aggregate into nanospheres that precipitate out of the solution. The latter are prevented from coalescing because of the large excess of solvent used.

Ye et al. were the first ones to report the preparation of molecularly imprinted polymers by precipitation polymerization[31]. Initially, the authors investigated a range of solvents (acetonitrile, dichloromethane, toluene, tetrahydrofuran) and crosslinker (EGDMA, trimethylolpropane trimethacrylate (TRIM), divinylbenzene (DVB)) to determine the optimum conditions for the formation of a stable dispersion. The polymerization of MAA and TRIM in 98% of acetonitrile yielded stable nanoparticles of 300nm diameter. After the first papers by Ye et al., precipitation polymerization was reported many times as an efficient technique to produce imprinted particles. If acetonitrile and methacrylic acid were often the solvent and recognition monomer of choice, a variety of templates were employed. Nicotine[32], ephedrine[33], aconitic acid[34] are some of them. Depending on the system, reported particle diameter ranged from 100nm to 4μm.
1.2.5. Imprinting inorganic particles

Producing monodisperse particles can also be achieved by polymerizing the recognition monomers on top of preformed silica beads. The latter are usually modified to bear on their surface a monomer or an initiator that will fix the imprinted layer on the particles. One of the approaches thus consists in modifying the silica beads with a monomer and further react the double bond with other monomer and crosslinker. To ensure that the monomers actually add to the modified beads and do not polymerize in solution, the reaction is typically carried out in a minimal amount of solvent. The monomers are usually dissolved in the solvent and the mixture is allowed to adsorb on the beads surface. Only when the mixture has penetrated the particles pores is the polymerization initiated. Plunkett and Arnold[35] initially modified 10\(\mu\)m silica beads with 3-(trimethoxysilyl) propylmethacrylate (Figure 13). The grafted acrylic monomer was then copolymerized with EGDMA and copper (4-vinylbenzyl)imino diacetate in methanol. Only 8ml of solvent were used to modify 2g of silica beads. The final polymer coating thickness was found to be around 1\(\mu\)m.
Figure 13: Surface modification of silica beads to introduce a monomer

A similar approach was employed by Hirayama et al. [36] who imprinted relatively large (25-40μm) silica particles with lysozyme. The amino groups present at the surface of the particles were used to introduce vinyl groups. Polymerization of acrylamide, acrylic acid and bisacrylamide crosslinkers was then carried out in a small volume of phosphate buffer.

Sellergen [37] put together a more straightforward approach that consists in modifying the silica beads (10μm diameter) with an azoinitiator (Figure 14). This technique ensures that polymerization takes place only at the particles surface. The reaction can then be carried out in regular conditions. The authors reported the modification of 10μm silica beads with 4,4-azo-bis(4-cyano pentanoic acid). Further grafting of MAA and EGDMA was carried out in toluene, in the presence of L-phenylalanine anilide. The technique allowed coating particles with imprinted films of various thicknesses, from 0.8nm to 7nm.

Figure 14: Surface modification of silica particles to introduce an initiator

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1.2.6. Comparing the preparation technique

Table 2 summarizes some of the essential features of the preparation techniques mentioned in this discussion and compares their drawbacks and advantages. Clearly, none of those techniques appear to be ideal in all cases. However, each of those methods yields various particle sizes, which allows using imprinted polymers in a variety of applications.
<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk polymerization</td>
<td>Well-know process</td>
<td>Yields irregular and polydispersed particles</td>
</tr>
<tr>
<td></td>
<td>Easily applied to a variety of templates</td>
<td>Grinding process results in loosing some of the sites</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous emulsion</td>
<td>50nm particles</td>
<td>Requires a stabilizer</td>
</tr>
<tr>
<td>polymerization</td>
<td>Good control of particle size</td>
<td>Can result in burying the template</td>
</tr>
<tr>
<td></td>
<td>Two-step approach or chemical modification of</td>
<td>Possible partitioning of hydrophilic template</td>
</tr>
<tr>
<td></td>
<td>template allows imprinting the surface</td>
<td></td>
</tr>
<tr>
<td>Aqueous miniemulsion</td>
<td>200nm particles</td>
<td>Requires a stabilizer</td>
</tr>
<tr>
<td>Polymerization</td>
<td>Good control of particle size</td>
<td>Possible partitioning of hydrophilic template</td>
</tr>
<tr>
<td></td>
<td>Efficient for hydrophobic templates</td>
<td></td>
</tr>
<tr>
<td>Suspension</td>
<td>2-60μm particles</td>
<td>Poor control of particle size</td>
</tr>
<tr>
<td>Polymerization</td>
<td>Low concentration of stabilizer</td>
<td>Possible partitioning of hydrophilic template</td>
</tr>
<tr>
<td>Precipitation</td>
<td>100nm-4μm particles</td>
<td>Poor control of the particle size</td>
</tr>
<tr>
<td>Polymerization</td>
<td>Surfactant free surface</td>
<td>Limited to low solid contents</td>
</tr>
<tr>
<td></td>
<td>Efficient for imprinting in organic solvent</td>
<td></td>
</tr>
<tr>
<td>Polymerization on</td>
<td>10-40μm particles</td>
<td>Requires chemical modification of the preformed beads</td>
</tr>
<tr>
<td>preformed silica</td>
<td>Good control of particle size</td>
<td></td>
</tr>
<tr>
<td>particles</td>
<td>Surfactant free particles</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison of the preparation techniques reported for the preparation of molecularly imprinted polymer
1.3. Applications of molecularly imprinted polymers in analytical chemistry

Molecularly imprinted polymers have the ability to recognize a particular compound among a range of chemical which make them good candidates to improve existing analytical techniques or develop new methods. As a matter of fact, they have been reported as antibody mimics in immunoassays, enzyme mimics in catalytic applications, adsorbents for solid-phase extraction etc.[15] In the following sections some of those applications, namely chromatographic and mass sensor applications will be reviewed.

1.3.1. MIPs as recognition elements in mass sensors

Principle

A chemical sensor consists of two units, a recognition element responsible for recognizing and binding a target analyte and a transducer part that converts the binding event into a quantifiable output signal. A simple way of transducing the analyte binding by a MIP consists in measuring the mass accumulation occurring in the polymer with an acoustic device.

The latter rely upon the piezoelectric principle: some material responds to the application of a mechanical stress by the creation of electrical charges. The reciprocal implies that a mechanical stress is produced when the material is submitted to an electrical field (Figure 15).
Quartz material at rest

Quartz material when a stress is applied

Figure 15: Schematic representation of the piezoelectric effect

In the quartz crystal microbalance (QCM), an oscillating electrical field is applied to the gold or silver electrodes, producing a mechanical wave that propagates in the bulk of the quartz (Figure 16, right). Any change in the wave propagation path provokes a variation in the wave resonance parameters (amplitude, velocity and thus frequency). Measuring the crystal resonance frequency thus allows monitoring the changes that occurred at the sensor's surface.

Figure 16: Schematic representations of a quartz crystal microbalance [12]

The quartz crystal microbalance has been reported several times in the literature as a transduction technique for MIPs[6-8, 13, 17, 38, 39]. Conceptually,
the MIP is coupled in some fashion to the quartz crystal and the resulting sensor is placed in presence of a template solution (Figure 16 left). The mass increase occurring when the polymer recognizes and adsorbs the template slows down the crystal oscillations. Monitoring the resonance frequency variations thus allows determining the mass changes. The frequency is usually recorded in a continuous fashion. Figure 17 represents a typical signal, where the frequency is plotted as a function of time.

![Figure 17: Typical frequency signal obtained from a quartz crystal microbalance](image)

Different relationships allow transforming the frequency variations into mass changes. The most well-known is the Sauerbrey's equation that quantifies the pure elastic mass added to the surface (Figure 18). Such calculations yield a new set of data, where the mass variations are plotted as a function of time (Figure 19).
\[ \Delta F = -C_f \Delta m \]
\[ \Delta F = \text{frequency change (Hz)} \]
\[ \Delta m = \text{mass change (g/cm}^2\text{)} \]
\[ C_f = \text{sensitivity factor (Hz ug}^{-1}\text{cm}^2\text{)} \]

Figure 18: Sauerbrey's equation and parameters

Figure 19: Mass variations as a function of time, obtained after treatment of the frequency raw data[8]

Consequently, such sensors are useful to quickly detect analytes in a sample; reported applications include quantification of drugs in biological samples[13] or contaminants in water[6].

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**Sensor Preparation**

Different techniques have been reported for the preparation of the sensor, i.e., coupling of the imprinted polymer to the quartz crystal. The simplest one consists of spin coating a suspension of MIPs particles in a solvent. In order to enhance the adhesion of the film to the electrode, a solvent soluble polymer is often added to the suspension. Several authors used polyvinyl chloride in tetrahydrofuran to couple the crystal to the MIPs particles yielded after bulk polymerization [13, 17, 39].

Another option is to polymerize directly the imprinted matrix on the gold electrode. UV irradiation of a pre-polymerization mixture (monomers, porogen, template, and initiator) cast at the crystal surface is a typical procedure [7, 15, 38]. Again, enhanced adhesion of the polymer film to the crystal is often desired. To do so, the gold surface can be modified with a thiol compound bearing vinyl groups. The latter are further polymerized with the imprinting monomer [7, 38].

**Detection Limit**

Depending upon the quartz thickness, instruments operate at different resonance frequencies [41]. High-frequency sensors are known to be more sensitive. With a 5MHz instrument, Percival et al.[13] reported the detection of $10^{-7}$ mol/L of nandrolone in ethanol while as little as $10^{-5}$ mol/L of glucose in water were detected by the 6MHz QCM of Ersoz et al.[7]. 9MHz instruments allowed several authors to reach lower detection limit: Yao et al.[17] detected as low as $10^{-8}$ mol/L of trimethoprim in ethanol, Liang et al.[39] observed the binding
of $10^{-9}$ mol/L of caffeine in water and Das et al.[6] reported a sensitivity down to $10^{-12}$ mol/L.

1.3.2. MIPs as sorbents for high-performance liquid chromatography (HPLC)

High-Performance liquid chromatography is a separation technique where sample compounds are partitioned differently between a stationary-phase (the column packing material) and a mobile-phase (the solvent stream). Solutes distributed preferentially in the mobile phase will move more rapidly than those distributed in the stationary phase, and will thus elute earlier. The partitioning process relies upon both chemical (polarity, charge or size) and mechanical separation (diffusion along the column). As they elute from the column, the compounds are sensed by a detector which allows quantifying each components (Figure 20)

![Figure 20: Schematic representation of analyte separation and detection in an HPLC system][42]

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Molecularly imprinted polymers were found to be very efficient to enhance the separation of similar compounds or extract analytes from complex samples.

Column Preparation

Both monolith and microspheres were successfully packed in HPLC columns. In the case of monoliths, the polymer is usually ground then sieved and sedimented or centrifuged to reduce polydispersity; the resulting particles present a diameter ranging from 10\(\mu\)m to 25\(\mu\)m. A couple of authors also packed micron-size particles (4-5\(\mu\)m) yielded after suspension or precipitation polymerization[30, 43].

Applications

When used as a packing material, imprinted polymers are able to discriminate structural isomers. For instance Lai et al.[27] reported separation of the 4-aminopyridine (4-AP) from the 2-aminopyridine (2-AP) and the 4-methylpyridine (4-MP) using a column packed with 4-aminopyridine imprinted polymer while a classic C18 column yielded a single peak (Figure 21).
Separation of optical isomers on a MIP column has also been reported many times; analytes include amino acids and their derivatives, peptides and pharmaceuticals [44]. For instance, Sambe et al. packed a short column (50mm×2.0mm I.D) with a S-nicotine imprinted polymer. Upon injecting a mixture of the two enantiomers, the polymer was able to separate the S and the R enantiomer. On the other hand, the column packed with the control polymer (non-imprinted polymer, NIP) was unable to separate both compounds (Figure 22) [32].
Figure 22: Chromatograms of nicotine enantiomers on a column packed with an imprinted polymer (right) and a non-imprinted polymer (left)[32]

In the same token, Ye et al. reported chromatographic separation of the estradiol enantiomers when using an imprinted polymer whereas the control polymer yielded a single peak (Figure 23)

It has also been reported that when the other enantiomer is used as a template during polymerization, the elution order is switched [45].
To quantify the extent of separation, authors typically report
- the capacity factor for each of the analog (Equation 1, where the dead time is to
  the retention time of a compound that is not retained in the column). This is how
Lai et al.[27] confirmed that the 4-aminopyridine imprinted polymer retained
longer the 4-aminopyridine than its isomer or other analogs (Table 3)

$$k = \frac{\text{time of retention}}{\text{dead time}} - 1$$

Equation 1: Equation for the capacity factor
- or the separation factor (equation 2); Sambe et al. for instance separated the S-nicotine from its R-enantiomer with a factor of 2.7

$$\alpha = \frac{k_2}{k_1}$$

Equation 2: Equation for the separation factor, $k_1$ and $k_2$ are the capacity factor for 2 compounds

<table>
<thead>
<tr>
<th>4-aminopyridine vs : $K=4$</th>
<th>2-aminopyridine: $K=1.5$</th>
<th>4-methylpyridine: $K=0.4$</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="4-aminopyridine" /></td>
<td><img src="image" alt="2-aminopyridine" /></td>
<td><img src="image" alt="4-methylpyridine" /></td>
</tr>
</tbody>
</table>

Table 3: Capacity factor of 4-aminopyridine and its analog on a column packed with 4-aminopyridine imprinted polymer

MIPs stationary phases can also allow simplifying sample preparation and reducing method optimization. This is especially true in the case of real samples that usually require several cleaning steps to remove matrix components interfering with the analyte and hampering accurate quantification. Imprinted polymers packed in an HPLC column enable clear separation of the target analyte from the matrix. The polymer specificity allowed Turiel et al. to inject crude fruits extracts in their column and quantify directly thiabendazole[46].

The imprinted polymer can also be packed in a pre-column to be used in a "column switching" approach prior to HPLC analysis. It consists in placing the short column packed with the imprinted polymer before a classic column. A valve...
allows using only the pre-column or both of them (Figure 24). The procedure is as follows:

- A sample is first injected in the pre-column where the imprinted matrix retains the target analyte while the matrix components are eluted
- In a second time, the clean analyte is desorbed from the pre-column and eluted to the analytical column.

![Figure 24: Schematic of the column switching method][5]

This technique also referred to as on-line solid-phase extraction yields clean and enriched extracts. Figure 25 and 26 (reproduced from Koeber et al.[47]) nicely illustrate this feature. The top chromatogram on Figure 25 represents the analysis of a water sample when only an HPLC column was used. The UV signal indicates the presence of matrix interferents. On the other hand, when both an HPLC column and a MIP column were used, the chromatogram displayed a single peak due to the analyte of interest (Figure 26).
This column coupling technique has been used successfully for the quantification of phenolic compounds[5], triazine in river water[47], and drugs in biological samples[48, 49]

Figure 25: Chromatogram obtained after injection of a water sample in an HPLC column[47]

Figure 26: Chromatograms resulting from the injection of a water sample in a MIP column coupled to an HPLC column[47]
This review focused upon High Performance Liquid Chromatography and Quartz Crystal Microbalance applications of imprinted polymers since those techniques are discussed in the third chapter of this dissertation. One should know however that those are only some of the applications of the imprinted polymers. MIPs have also been reported as antibody mimics in immunoassay [15] and transducing unit in sensor based for instance on surface plasmon resonance [50].
All of the syntheses were carried out by free radical polymerization since it is a chain-growth mechanism where long chains that surround the polymer complex are quickly formed. Furthermore, all the MIPs were prepared by copolymerization of ethylene glycol dimethacrylate (EGDMA) and methacrylic acid (MAA). The latter associates with caffeine through hydrogen bonds and thus forms non-covalent complexes. A range of preparation techniques and porogen solvents were tested. The polymers abilities to bind again caffeine were evaluated by adsorption experiments. The results are compared and discussed in this chapter.

Figure 27: Schematic of the imprinting matrix formation
2.1. Bulk Polymerization

2.1.1. Synthesis

A bulk polymer was first prepared in acetonitrile according to the recipe displayed in table 4. Prior to polymerization, the monomers were passed through a column packed with alumina in order to remove any inhibitors. Afterward, the monomers solvent and caffeine were heated to 60°C and purged with nitrogen for ten minutes to remove oxygen, a polymerization inhibitor. The reaction was then initiated by adding a solution of 2-2'-azo-bis-isobutylnitrile (AIBN) in acetonitrile.

<table>
<thead>
<tr>
<th></th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>75.2</td>
</tr>
<tr>
<td>Methacrylic acid</td>
<td>3</td>
</tr>
<tr>
<td>EGDMA</td>
<td>18</td>
</tr>
<tr>
<td>Caffeine</td>
<td>3.3</td>
</tr>
<tr>
<td>AIBN</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 4: Formulation of mjb-1, prepared by bulk polymerization

The reaction was allowed to run for twenty four hours and yielded a hard polymer that was further ground and ball milled. Extraction of the caffeine from the porous structure was performed by soxhlet extraction first with methanol for 24 hours then with water for 24 hours as well. The resulting powder was analyzed by laser light diffraction on a Microtrac S3500. As expected, the particle size distribution was found to be fairly broad (Table 5).

<table>
<thead>
<tr>
<th>Mean Diameter of the volume distribution - MV</th>
<th>Mean diameter of the number distribution - MN</th>
<th>Mean diameter of the area distribution - MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>153.1 µm</td>
<td>4.40 µm</td>
<td>34.11 µm</td>
</tr>
</tbody>
</table>

Table 5: Particle size distribution observed for mjb-1
2.1.2. Rebinding experiments

The polymer rebinding abilities were evaluated with an adsorption isotherm. The experiment consists in equilibrating samples of polymer (mjb-1) with caffeine solutions of increasing concentrations, for five minutes. The equilibration time was chosen according to Sayantan Roy’s kinetics studies[51]. The total reaction volume was maintained constant from one experiment to another so that the solid content was constant and equal to 2% by weight. The solid polymer was then separated by centrifugation and the amount of free caffeine remaining in the solution was quantified by UV detection on a High Performance Liquid Chromatography using a Hewlett Packard 1100 Series. The concentration of caffeine bound to the solid phase (gram of caffeine per gram of polymer) was further deduced and plotted as a function of the concentration of free caffeine (gram of caffeine per liter of solution). The resulting isotherm is plotted in Figure 28.
2.1.3. Modeling the polymer rebinding capacities

**Binding models**

**The bi-Langmuir model**

A binding model is a mathematical relationship relating the amount of analyte bound to the amount of analyte free. One of the simplest binding models is the homogeneous Langmuir isotherm which assumes that there are a finite number of adsorption sites that are all equivalent and independent (Equation 3).

---

**Figure 28: Experimental binding isotherm for mjb-1**

---

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[Analyte bound] = \frac{K \cdot S \cdot [Analyte free]}{1 + K \cdot [Analyte free]}

with \ S \ total \ number \ of \ adsorption \ sites
\ K \ binding \ constant

Equation 3: Langmuir binding model

In the case of imprinted polymer, this simple system is somewhat inaccurate since it does not take into account the matrix heterogeneity. A modified version of this isotherm, the bi-Langmuir model gives a more realistic description of the imprinted polymer matrix. This model consists in considering two types of binding sites:
- High-affinity binding sites that result from complexes where a large number of interactions existed between the monomer and the template.
- Low-affinity binding sites.

Typically high-affinity binding sites dominate the binding properties at low concentration of template and low-affinity binding sites dominate at high concentration of template. Equation (4) is the mathematical expression of the bi-Langmuir isotherm relating the concentration of caffeine bound by the polymer phase (in gram of caffeine per gram of polymer) to the concentration of caffeine free in solution (in gram of caffeine per liter of solution).

\[
[Caffeine \ bound] = \frac{G_0}{1 + K_{\text{specific}}[Caffeine \ free]} + \frac{B_0}{1 + K_{\text{nonspecific}}[Caffeine \ free]}
\]

Equation 4: Mathematical expression of the Bi-Langmuir isotherm

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In this equation, $G_o$ and $B_o$ are the number of specific and nonspecific sites, expressed in gram per gram of polymer. $K_{\text{specific}}$ and $K_{\text{nonspecific}}$ are the corresponding binding constants, expressed in liter per gram. The derivation of the mathematical expression can be found in Appendix A.

The Freundlich isotherm

The Freundlich isotherm was initially reported as an empirical binding model. Since then, some mathematical derivations allowed justifying theoretically the isotherm. One of those computations imagines a continuous distribution of independent sites of varying affinities [52]. Such a model thus gives a more realistic description of the polymer matrix since it allows taking into account the wide variety of the sites sizes, shapes and rigidities.

The Freundlich isotherm (equation 5) relates the concentration of caffeine bound by the polymer (in gram of caffeine per gram of polymer) to the concentration of caffeine in solution (in gram of caffeine per liter of solution) with two constants, $a$ and $m$. The constant $m$ is an unitless number greater than 0 and less than 1, and has been referred to as the heterogeneity index[53]. As a matter of fact, when $m$ is equal to 1, equation (5) describes a linear adsorption isotherm, corresponding to a homogeneous surface. Since $m$ induces a curvature it is thought to give an idea of the surface heterogeneity. The constant has units of $(L/g)^m$

$$[\text{Caffeine bound}] = a \times [\text{Caffeine free}]^m$$

Equation 5: Mathematical expression of the Freundlich isotherm

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Data fitting

The Excel solver tool allowed fitting mjb-1 experimental data to the Freundlich and the bi-Langmuir isotherms (Figure 29). The software minimized the residual sum of squares by adjusting some target parameters, namely the binding constants and number of sites for the bi-Langmuir model and the heterogeneity parameter and constant a in the case of the Freundlich isotherm (Table 6). Errors on m are accessible fairly easily with a linear regression on the logarithm form of the Freundlich isotherm. In contrast, the statistics for the bi-Langmuir parameters obtained from a non-linear regression yield complex calculations that were not performed for this dissertation.

<table>
<thead>
<tr>
<th>Bi-Langmuir isotherm</th>
<th>Specific sites</th>
<th>Non-specific sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding constant (L/g)</td>
<td>300</td>
<td>1.40</td>
</tr>
<tr>
<td>Number of sites (g/g)</td>
<td>0.00028</td>
<td>0.024</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Freundlich isotherm</th>
<th>Specific sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (L/g)^m</td>
<td>0.017</td>
</tr>
<tr>
<td>m</td>
<td>0.73±0.03</td>
</tr>
</tbody>
</table>

Table 6: Bi-Langmuir and Freundlich fitting parameters for mjb-1
Figure 29: Experimental data and binding model for mjb-1

**Comparing the Freundlich and bi-Langmuir model**

Evaluating the goodness of fit was achieved with equation (6) that calculates the difference between the theoretical and calculated points. The results of those computations are presented in Table 7.

\[
\text{Goodness of fit} = \frac{1}{n} \sum (\text{Caffeine bound theory} - \text{Caffeine bound experimental})^2
\]

Equation 6: Mathematical expression of the goodness of fit
Table 7: Evaluation of goodness of fit for Freundlich and bi-Langmuir models

Table 7 points out that the goodness of fit is slightly superior in the case of
the bi-Langmuir model than that of the Freundlich. This is a result of the greater
number of parameters used to fit the bi-Langmuir model compared to that used
for the Freundlich isotherm. The latter model thus describes more accurately the
experimental data, given the number of fitting parameters used.

If, overall both models properly describe the MIP adsorption behavior,
each model is thought to be more or less useful depending upon the discussion.

Theoretical derivations of the Freundlich isotherm rely upon an exponentially
decaying distribution of binding constants. Based upon the first derivations of the
isotherm, Umpleby et al. [54] reported a simplified equation for the affinity
distribution of binding sites in MIPs. Equation 7 relates the number of binding
sites having a particular binding constant $N(K)$ to the binding constant. It is a
function of the heterogeneity index, $m$ deduced from the Freundlich isotherm.
(The reader is referred to the mentioned references for more details concerning
the derivations.)

$$N(K) = 0.02 \exp(-2.3 \times m \times \log K)$$

Equation 7: Affinity distributions calculated from the Freundlich isotherm
To give the reader an idea of the kind of curve observed, Mjb-1 site distribution was calculated according to equation (3) and plotted in figure 30. The chart also displays the polymer binding parameters obtained from the bi-Langmuir fitting. Both models describe the same trend i.e. the MIP possesses only few specific sites (high binding constant) but many low-affinity binding sites. This feature is typical of imprinted polymers. Sayantan Roy calculated several binding parameters with the bi-Langmuir model for his caffeine imprinted polymers[55]; they are also plotted on figure 30. One can clearly see how all of the reported parameters describe the same area, namely, the polymer present only few specific sites.

![Figure 30: Site distribution for mjb-1 compared with some of the binding parameters reported in the literature](image-url)
Clearly when comparing two imprinted polymers, the Freundlich model becomes very interesting since it allows comparing the whole site distribution rather than a set of four data. On the other hand, if one was to consider a single polymer, the set of binding parameters extracted from the bi-Langmuir model would be enough to give an idea of the MIP properties (Table 3). Therefore, depending on the discussion, either the Freundlich or the bi-Langmuir isotherm will be used to fit the experimental data.

The initial work on bulk polymerization allowed understanding the basics of imprinting technology and synthesizing a decent polymer compared to what other reported (figure 30). However, as already stated in chapter 1 of this dissertation, polymers prepared by bulk polymerization feature some major drawbacks such as broad size distributions, large particle sizes etc. This is why alternative synthesis routes yielding monodisperse nanoparticles were explored.
2.2. Miniemulsion polymerization

2.2.1. Principle

Formation of nanoparticles was intended by free radical miniemulsion polymerization. The synthesis was designed to imprint the shell of a core-shell particle, making imprinted sites more accessible for rebinding.

The first step consisted in forming a crosslinked poly(methyl methacrylate) (PMMA) core while the second stage aimed at forming the imprinted poly-(MAA-co-EGDMA) (10:90) shell. Crosslinking the seed particles and polymerizing a polar monomer in the second stage should guarantee polymerization of the imprinted phase on the outside of the particles. To verify this hypothesis, simulations of the experiment were performed using the UNHLATEX KMORPH (3.0) and UNHLATEX EQMORPH (6.0) software.

The latter calculates the morphology that is thermodynamically favored under the reaction conditions. Those morphologies are referred to as equilibrium morphologies and result from the necessity to minimize the interfacial energies.

Figure 31: Schematic representation of the surface imprinted nanoparticles
In order to run the software, a couple of simplifications concerning the nature of the polymers and surfactants were made. Namely:

- the nature of the crosslinking agent used in the seed polymer could not be specified
- the second stage monomer was modeled as methyl methacrylate containing 10% of acid monomer
- the amount of crosslinking agent in the second stage was not specified
- the surfactants, Sodium Dodecyl Sulfate (SDS) and Tergitol Nonyl Phenol (NP-50) were modeled as SDS

<table>
<thead>
<tr>
<th>Software inputs</th>
<th>Reaction conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed polymer</td>
<td>90% Crosslinked PMMA (Mc/M=50)</td>
</tr>
<tr>
<td>Second stage monomer</td>
<td>PMMA acidified at 10%</td>
</tr>
<tr>
<td>Surfactant</td>
<td>SDS - 5.6pphm</td>
</tr>
<tr>
<td>Stage Ratio</td>
<td>0.40</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 8: Comparison of the reaction conditions and the parameters set to run the UNHLATEX EQMORPH software

Figure 32a represents the energy map that plots the free energy (vertical axis) for each of the possible morphology (horizontal axis). The morphology map is reproduced on Figure 32b where the blue areas correspond to the second stage polymer and the dark areas symbolize the seed polymer. From those
charts, it clearly appears that the core-shell structure presents the minimum free energy and is thus the thermodynamically preferred morphology (Figure 32c).

UNHLATEX EQMORPH software outputs; a: energy surface map, b: base topology, c: preferred morphology

UNHLATEX KMORPH on the other hand calculates the kinetics of polymerization and predicts the morphology development when the
polymerization occurs under kinetics conditions. Again, the reactions conditions were modeled in order to run the software (table 8).

<table>
<thead>
<tr>
<th>Software inputs</th>
<th>Reaction conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed polymer</td>
<td>PMMA</td>
</tr>
<tr>
<td>Second stage monomer</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>Surfactant</td>
<td>SDS</td>
</tr>
<tr>
<td>Stage Ratio</td>
<td>0.4</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>70</td>
</tr>
<tr>
<td>Monomer Feed Rate (g/hr)</td>
<td>2.2</td>
</tr>
<tr>
<td>Solid Content</td>
<td>11%</td>
</tr>
</tbody>
</table>

Table 9: Comparison of the reaction conditions and the parameters set to run the UNHLATEX KMORPH software

Figure 33 shows the calculated radial distribution and Figure 34 provides the predicted final morphology where the dark areas represent the second stage polymer.
Both figures point out that full penetration of the second stage polymer was not possible. Kinetics thus predicts the formation of a core-shell particle, where the core consists of the seed polymer and the shell is the second-stage polymer.
In summary, both thermodynamics and kinetics predict a core-shell morphology where the core consists of the seed, inert particles and the shell is the imprinted second-stage. One should note however that those simulations could not take into account the presence of caffeine during the polymerization. Since the latter is a surface active molecule, it could, depending on its concentration, modify the expected morphology.

2.2.2. Synthesis

In the first stage, poly (methyl methacrylate) crosslinked particles were synthesized. Details of the formulation are given in table 10. Apart from the initiator, all the reactants were magnetically stirred for 15 minutes and then sonicated (Branson sonifier probe, power=9 and level=90%) over ice, for 2 minutes. Afterward, the mixture was degassed with N₂ and heated. When the temperature reached 70°C, a solution of Potassium Persulfate (KPS) in water was added, and the reaction was allowed to run for 2 hours.

<table>
<thead>
<tr>
<th></th>
<th>m (g)</th>
<th>% of organic phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA</td>
<td>25.9382</td>
<td>85</td>
</tr>
<tr>
<td>Egdma</td>
<td>2.8907</td>
<td>10</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>1.5235</td>
<td>5</td>
</tr>
<tr>
<td>Water</td>
<td>102.14</td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>0.6064</td>
<td>3.47 pphm</td>
</tr>
<tr>
<td>NP-50</td>
<td>0.4101</td>
<td></td>
</tr>
<tr>
<td>KPS</td>
<td>0.029</td>
<td>0.1 pphm</td>
</tr>
<tr>
<td>Theoretical Solid content</td>
<td></td>
<td>23%</td>
</tr>
</tbody>
</table>

Table 10: Formulation of the seed latex, mjb-20 (pphm stands for parts per hundreds monomer)
Table 11 provides the recipe used to grow a 10nm shell on top of the seed particles. The reaction was conducted at pH=3 so that methacrylic acid was largely under its protonated form (pKa=4.66) and thus likely to polymerize in the organic phase.

<table>
<thead>
<tr>
<th></th>
<th>m (g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>24.9031</td>
<td>33.74</td>
</tr>
<tr>
<td>MAA</td>
<td>0.2286</td>
<td>0.31</td>
</tr>
<tr>
<td>EGDMA</td>
<td>1.9835</td>
<td>2.69</td>
</tr>
<tr>
<td>Buffer solution</td>
<td>42.64</td>
<td>57.77</td>
</tr>
<tr>
<td>Caffeine</td>
<td>4.0138</td>
<td>5.44</td>
</tr>
<tr>
<td>KPS</td>
<td>0.0385</td>
<td>0.05</td>
</tr>
<tr>
<td>Stage ratio</td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>Theoretical Solid Content</td>
<td></td>
<td>11%</td>
</tr>
</tbody>
</table>

**Table 11: Formulation of the second stage latex, mjb-22**

The buffer solution and first stage latex were mixed and heated. When the temperature reached 70°C, the caffeine was added and the mixture was degassed during 15 minutes. A concentrated solution of KPS was then injected.

The monomers, previously mixed together, were fed to the reactor with a syringe pump over one hour. The reaction was stopped 30 minutes after the end of the addition. The limited solubility of caffeine in water at room temperature compared to that at 80°C could cause the particles to coagulate (Table 12). Thus, the latex was diluted in a warm water solution before allowing the system to cool down.

<table>
<thead>
<tr>
<th></th>
<th>T = 25°C</th>
<th>T = 80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility of caffeine (g/L)</td>
<td>22</td>
<td>180</td>
</tr>
</tbody>
</table>

**Table 12: Solubility values for caffeine in water [56]**

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2.2.3. Characterization

The solid content of the latices produced was evaluated by gravimetry i.e. by weighing samples before and after evaporating off the volatile components in a drying oven; conversion was further deduced (Table 13).

<table>
<thead>
<tr>
<th>Solid Content (%)</th>
<th>Mjb-20</th>
<th>Mjb-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion (%)</td>
<td>98</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 13: Solid Content and conversion values for miniemulsion lattices

The limited conversion of the second stage experiment was observed at several instances and was attributed to caffeine acting as an inhibitor of the polymerization. The caffeine ring structure is thought to adsorb a radical and stabilize it. This could be counterbalanced by initiating the reaction with increasing amounts of KPS.

Particle sizes of the resulting latices were examined by capillary hydrodynamic fractionation on a CHDF 2000 from Matec Applied Science (Table 14). Results showed a clear increase of the particle size after the second stage experiment and thus indicate that a shell was actually grown on top of the seed particles. Scanning electron microscopy (Figure 35) confirmed that the final latex presented a size distribution centered on 100nm.

<table>
<thead>
<tr>
<th>CHDF</th>
<th>Mjb-20</th>
<th>Mjb-22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mv</td>
<td>88.7</td>
<td>98.6</td>
</tr>
<tr>
<td>Mn</td>
<td>82.4</td>
<td>94.6</td>
</tr>
</tbody>
</table>

Table 14: Particle Size characterization for miniemulsion lattices
2.2.4. Rebinding experiments

In order to perform the rebinding experiments, caffeine was extracted from the latex by ultrafiltration on a 30,000 Daltons regenerated cellulose membrane. To maintain colloidal stability during the process, the latex was cleaned with a 0.1g/L SDS solution. The extraction efficiency was monitored by injecting samples of the filtrate in HPLC. Finally, the clean latex was concentrated to 4% solid content. The rebinding experiments were performed by Sayantan Roy, according to the method described in section 1.2 of this dissertation. Fitting the experimental data to the bi-Langmuir isotherm (figure 36 and table 15) revealed the total lack of specific sites in the polymer matrix.

Figure 35: Scanning electron micrographs for mjb-22 imprinted by aqueous miniemulsion
<table>
<thead>
<tr>
<th></th>
<th>Specific sites</th>
<th>Non-specific sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding constant (L/g)</td>
<td>-</td>
<td>0.0026</td>
</tr>
<tr>
<td>Number of sites (g/g)</td>
<td>-</td>
<td>4.2</td>
</tr>
<tr>
<td>Goodness of fit</td>
<td></td>
<td>1.8*10^-9</td>
</tr>
</tbody>
</table>

**Table 15: Binding parameters for mjb-22**

This result was attributed to the unfavorable polymerization medium. Most likely in the polymerization mixture, water competed for the formation of hydrogen bond with caffeine, thereby reducing the interactions between caffeine and methacrylic acid. This feature is even more prominent since water is present in large excess compared to the recognition monomer.

![Figure 36: Experimental and theoretical binding isotherms for mjb-22](image-url)
2.3. Precipitation polymerization

2.3.1 Choosing the polymerization solvent

The poor recognition capacities of the latex prepared by aqueous miniemulsion resulted in seeking an alternative route for the preparation of nanoparticles. Precipitation polymerization was investigated since it allows preparing particles in organic medium. Several solvents were tested, namely acetonitrile, dimethyl sulfoxide (DMSO), polyethylene glycol dimethyl ether (PEGDME, MW=500g/mol), and diethylene glycol dimethyl ether (DIGLYME). All the reactions were carried out in the same conditions; details of the recipe are given in Appendix B). The monomers and caffeine were dissolved in a large excess of solvent (96%) and the resulting homogeneous mixture was degassed and heated to 70°C. When the reaction temperature was reached, polymerization was initiated by the addition of a concentrated solution of AIBN and was allowed to run for two hours. Only acetonitrile allowed forming a suspension of particles (Figure 37); the other solvents produced a gel structure. Running the experiment while sonicating the medium allowed breaking the macro-gel structure that was formed in some cases. However, scanning electron microscopy clearly showed that a micro-gel structure still existed and that no independent particles were formed (Figures 38).
Table 16: List of solvents tested for precipitation polymerization

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Polymer form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>Particles</td>
</tr>
<tr>
<td>PEGDME-500</td>
<td>Macro-gel</td>
</tr>
<tr>
<td>PEGDME-500</td>
<td>Micro-gel</td>
</tr>
<tr>
<td>DIGLYME</td>
<td>Gel</td>
</tr>
<tr>
<td>DMSO</td>
<td>Macro-gel</td>
</tr>
<tr>
<td>DMSO – Sonication</td>
<td>Micro-gel</td>
</tr>
<tr>
<td>DMSO/ACN 66/34</td>
<td>Gel</td>
</tr>
</tbody>
</table>

Figure 37: SEM micrograph of the particles formed by precipitation polymerization in acetonitrile

Figure 38: SEM micrograph of the structure formed by precipitation polymerization in PEGDME
The mechanism of particle formation during precipitation polymerization can help explaining the different morphologies observed. The chemical structure of poly (EGDMA-co-MAA) is fairly similar to that of PMMA. For the purpose of the discussion, PMMA will thus be considered as the polymer of interest. Also, ethylene glycol dimethyl ether will model DIGLYME.

Complete miscibility of a polymer in a solvent is achieved when the Flory-Huggins parameter, $X$, is less than 0.5. $X$ was computed according to equation 8 for the combinations of PMMA and solvents used in the polymerization (Table 17).
\[ \chi = \frac{V_1}{RT} \cdot (\delta_1 - \delta_2)^2 + 0.34 \]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_1)</td>
<td>solvent molar volume (m³/mol)</td>
</tr>
<tr>
<td>(\delta)</td>
<td>solubility parameter (MPa¹/²)</td>
</tr>
<tr>
<td>(R)</td>
<td>gas constant (JK⁻¹mol⁻¹)</td>
</tr>
<tr>
<td>(T)</td>
<td>temperature (K)</td>
</tr>
</tbody>
</table>

**Equation 8: Equation for the polymer-solvent interaction [57]**

The results of those computations indicate that acetonitrile is a good solvent for the polymer, unlike dimethyl sulfoxide and ethylene glycol dimethyl ether. Consequently acetonitrile should dissolve most of the nuclei particles so that only few of them are formed. As the polymerization proceeds, oligomers precipitate out of solution on those few nuclei, yielding some large polymeric particles that are prevented from aggregation due to the high level of dilution (94% of the reaction mixture is solvent). On the other hand, the solubility parameters of dimethyl sulfoxide and ethylene glycol dimethyl ether are somewhat different from that of PMMA. Thus, those solvents should induce the formation of lots of nuclei particles. Later on, the oligomers precipitate on those nuclei but the particles are so numerous that they tend to be bridged together by the crosslinker. In such solvents, a network of particles then develops.

Those hypotheses are in agreement with the particle size observed on the micrographs: Figure 37 clearly displays independent particles of less than 500nm in diameter while Figure 38 indicates the presence of 10nm particles trapped in a gel structure. However, this explanation is somewhat incomplete since it only...
considers the particle nucleation and does not take into account the particle
growth mechanism.

<table>
<thead>
<tr>
<th></th>
<th>PMMA</th>
<th>Acetonitrile</th>
<th>Dimethyl sulfoxide</th>
<th>Ethylene glycol dimethyl ether</th>
<th>Mixture 70/30 DMSO/ACN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility parameter (MPa$^{1/2}$)</td>
<td>22.69</td>
<td>24.3</td>
<td>29.7</td>
<td>17.6</td>
<td>27.8</td>
</tr>
<tr>
<td>$X$</td>
<td>N/A</td>
<td>0.39</td>
<td>1.77</td>
<td>1.44</td>
<td>1.05</td>
</tr>
</tbody>
</table>

Table 17: Solubility parameters for PMMA and solvents used in the polymerization [57] and corresponding Flory-Huggins parameter

Carrying out the reactions in a larger volume of DMSO or DIGLYME might have allowed forming independent spheres. However this option was not explored since yielding interesting quantities of polymer would have required preparing very large batches. Acetonitrile was thus used in all of the polymerizations. Details of the final formulation are given in table 18.

<table>
<thead>
<tr>
<th></th>
<th>m (g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>286.7</td>
<td>94.11</td>
</tr>
<tr>
<td>Methacrylic acid</td>
<td>1.2504</td>
<td>0.41</td>
</tr>
<tr>
<td>EGDMA</td>
<td>6.4401</td>
<td>2.11</td>
</tr>
<tr>
<td>Caffeine</td>
<td>10.0863</td>
<td>3.31</td>
</tr>
<tr>
<td>AIBN</td>
<td>0.1686</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 18: Formulation of mjb-77, the precipitation polymerization in acetonitrile

2.3.2. Evaluating the extraction efficiency

The extraction of caffeine after polymerization was first performed by ultrafiltration on a 30,000 Daltons regenerated cellulose membrane. Water was the cleaning solvent so that at the end of the filtration all the particles were...
suspended in water. The resulting latex was characterized for its rebinding capacities. To do so, increasing concentrations of caffeine were equilibrated with a constant mass of latex. Afterward, the whole system was injected directly in a short stainless steel column (50* 4.6mm id) packed with silica and connected to the HPLC instrument (details of this analytical technique will be provided in chapter three of this dissertation). The amount of free caffeine was quantified by UV detection. Fitting the experimental data with the bi-Langmuir isotherm indicated that the polymer did not contain any specific sites for caffeine but a large number of low-affinity binding sites (Table 19)

<table>
<thead>
<tr>
<th></th>
<th>Specific sites</th>
<th>Non-specific sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding constant (L/g)</td>
<td>-</td>
<td>$8 \times 10^{-5}$</td>
</tr>
<tr>
<td>Number of sites (g/g)</td>
<td>-</td>
<td>26.2</td>
</tr>
</tbody>
</table>

Table 19: Binding parameters obtained for mjb-77 after ultrafiltration in water

This result can be the consequence of using an inadequate porogen solvent during the polymerization as it was the case for the miniemulsion system. However, mjb-1 was prepared by bulk polymerization in acetonitrile and showed interesting rebinding capacities (section 1.2. of the dissertation); this hypothesis was thus discarded. But the bulk and the precipitation polymer present a significant difference: they were not extracted by the same technique. While mjb-1 was cleaned by soxhlet extraction at high temperature, caffeine in mjb-77 was removed by ultrafiltration at room temperature.

A new extraction technique was thus devised. The bulk of the caffeine was first eliminated by ultrafiltration in water. To extract any residual template, the latex was then transferred into a flask equipped with a condenser and heated to
80°C for 48 hours. The cleaning procedure was completed by another ultrafiltration step to replace the medium by fresh water.

Samples of the filtrate were analyzed by HPLC. Analysis of a sample taken before the heating cycle indicated that all the caffeine was removed from the latex. However, the same analysis performed right after the heating cycle revealed the presence of caffeine (figure 40). This result confirmed that ultrafiltration at room temperature was not efficient enough to extract the template from some of the imprinted sites. Most likely, higher temperature helped extracting the template from the sites by raising its solubility in water (table 20). Analysis of the filtrate after the final ultrafiltration did not indicate the presence of any caffeine (figure 40).

<table>
<thead>
<tr>
<th>Solubility of caffeine (g/L)</th>
<th>T = 25°C</th>
<th>T = 80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22</td>
<td>180</td>
</tr>
</tbody>
</table>

Table 20: Solubility values for caffeine in water [56]
Figure 40: HPLC chromatograms of the various filtrates

Particle size was also monitored before and after the heating cycle by dynamic light scattering. The extraction procedure did not seem to change the overall size distribution (Figure 41).

Figure 41: Particle size before and after the heating cycle and ultrafiltration

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The rebinding experiments performed on the resulting polymer indicated this time the presence of two types of sites, some specific sites having a high binding constant and some low-affinity binding sites presenting a poor binding constant (Table 20). This result clearly suggests that the initial extraction technique was responsible for the poor rebinding capacities observed at first. Adding a heating cycle in the cleaning procedure helped extracting caffeine from the polymer matrix; thereby making sites available for rebinding.

<table>
<thead>
<tr>
<th>Specific sites</th>
<th>Non-specific sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding constant (L/g)</td>
<td>100</td>
</tr>
<tr>
<td>Number of sites (g/g)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Proportion of high affinity binding sites (number of high specific sites divided by total number of sites)</td>
<td>0.03%</td>
</tr>
</tbody>
</table>

Table 21: Binding parameters obtained for mjb-77 cleaned at high temperature

2.3.3. Influence of the amount of caffeine

Optimization of the polymer performances by adjusting the template concentration in the formulation was also investigated. To do so, two polymers were prepared and characterized for their binding capacities according to the method described in section 3.2. MJB-58 was imprinted with a small quantity of caffeine, namely 1% of the total mixture. Three times more caffeine were employed in the preparation of MJB-64 (Figure 42).
Figure 42: Comparison of the formulation of mjb-58 and mjb-64

Figure 43 shows the experimental adsorption isotherm for both polymers as well as the Freundlich model that was successfully fit to the set of data (table 22). From the theoretical adsorption isotherm, the heterogeneity index, $m$ was deduced. The latter was found to be greater in the case of mjb-58 than for mjb-64 which indicates that increased proportion of caffeine in the formulation yields a more heterogeneous structure. This result is in agreement with what was reported in the literature by Rampey et al. [53]. The latter found a heterogeneity index constantly decreasing upon going from the non-imprinted polymer (no template in the formulation) to high concentration of template in the MIP preparation. To compare the two polymers, their site distributions were calculated and plotted on Figure 44, on a logarithm scale to give a better idea of the distributions differences.
Table 22: Freundlich fit parameters

<table>
<thead>
<tr>
<th></th>
<th>Mjb-58</th>
<th>Mjb-64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goodness of fit</td>
<td>4.367*10^-7</td>
<td>4.37*10^-7</td>
</tr>
<tr>
<td>m</td>
<td>0.75±0.01</td>
<td>0.62±0.02</td>
</tr>
</tbody>
</table>

Figure 43: Experimental and theoretical adsorption isotherms for mjb-58 and mjb-64
Figure 44: Site distribution in the logarithm format for mjb-64 and mjb-58

Those charts indicate that overall, both mjb-58 and mjb-64 present similar capacities. The plots also point out that mjb-64 has a better ratio of high- to low-affinity sites than mjb-58. This result suggests that increasing the amount of caffeine in the formulation induced the formation of additional specific sites. This is in line with other reports concerning the influence of the template concentration on the imprinting effect [43, 53]. To explain this result, one can refer to equation (9) that states the formation of a prepolymerization complex having a particular binding constant. Le Chatelier's principle predicts that an increase in one of the reactant concentration will drive the system toward the formation of more of the prepolymerization complex. Thus raising the amount of caffeine in the formulation induced an increase formation of prepolymerization complex and
consequently the creation of additional specific binding sites. This clearly occurred at the expense of the low-affinity binding sites.

\[
K = \frac{[\text{Caffeine. MAA}]}{[\text{Caffeine}]*[\text{MAA}]}
\]

Equation 9: Equation describing the formation of a prepolymerization complex

Therefore, in the final recipe (Table 18) for the preparation of imprinted nanoparticles, large concentrations of caffeine were employed.

2.3.4. Comparing the bulk and the latex polymer

Mjb-1 and mjb-77 were prepared in similar conditions, namely same proportion of crosslinker compared to recognition monomer. More caffeine was used for the preparation of mjb-77 according to the conclusions drawn from section 3.3. And obviously the proportion of solvent was also different. Mjb-77 was prepared in 94% acetonitrile while mjb-1 required only 75% of solvent.

The rebinding capacities of both polymers were tested in a similar concentration range to allow comparison and the experimental data were fitted to the Freundlich model (figure 44).
Figure 45: Experimental and theoretical adsorption isotherms for mjb-1 and mjb-77

<table>
<thead>
<tr>
<th></th>
<th>Mjb-1</th>
<th>Mjb-77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goodness of fit</td>
<td>$1.14 \times 10^{-7}$</td>
<td>$1.22 \times 10^{-7}$</td>
</tr>
<tr>
<td>$M$</td>
<td>$0.73 \pm 0.03$</td>
<td>$0.8 \pm 0.04$</td>
</tr>
</tbody>
</table>

Table 23: fitting parameters for mjb-1 and mjb-77

This chart points out the overall increased capacity of mjb-1 compared to that of mjb-77. The different levels of solvent used in the each polymerization probably accounts for this difference. The latter preparation technique yields a single piece of porous polymer containing all of the acetonitrile and caffeine. On the other hand, precipitation polymerization produces particles dispersed in an excess of solvent. In this case, there is thus a partitioning of the template and solvent between the solution and the solid phase. Consequently only a fraction of the initial acetonitrile (and caffeine) finds itself in the polymer matrix. The
particles are thus expected to present less sites than the bulk polymer. This hypothesis is in line with Sayantan Roy's BET analysis [55]. The technique allows measuring the surface area of porous structures. It is related to the extent of porosity of the material. Roy characterized several polymers prepared by bulk polymerization in increasing volumes of porogen, all other things kept constant. His results are presented in table 24.

<table>
<thead>
<tr>
<th>Proportion of acetonitrile (%)</th>
<th>Surface area (m²/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk 1</td>
<td>50</td>
</tr>
<tr>
<td>Bulk 1C</td>
<td>75</td>
</tr>
<tr>
<td>Bulk 1D</td>
<td>85</td>
</tr>
</tbody>
</table>

*Table 24: Influence of the polymerization solvent volume on the polymer surface area [55]*

Clearly, increasing the volume of acetonitrile during the polymerization resulted in a decrease of the surface area. It was thus concluded that the amount of solvent in the polymerization was responsible for the polymer surface area and number of sites. This proposed mechanism explains reasonably well why preparing spheres by precipitation polymerization produced fewer sites than in the equivalent monolith.

Another interesting feature arises from the site distributions displayed on figure 46. The plot shows that mjb-1 presents a more favorable ratio of high- to low-affinity binding sites compared to that of mjb-77.
This suggests that mjb-1 was prepared with a more favorable proportion of monomer and template. As mentioned earlier, the bulk preparation ensures that all the caffeine is locked in the polymer matrix. Figure 47 shows that mjb-1 was synthesized with about one recognition monomer for one template molecule. On the other hand, the precipitation procedure does not allow determining how much caffeine is actually fixed in the nanoparticles. The previous section revealed that the proportion of caffeine had to be maximized to create a maximum of high-affinity sites. What Figure 45 suggests is that there is still not enough caffeine in the precipitation system to allow forming as much specific sites as in the bulk polymer.
Precipitation polymerization yields a polymer with a somewhat smaller imprinting effect than that of the bulk polymer, due mostly to the high level of dilution required to form independent particles. However, when developing analytical methods, the latex form offers several advantages over the monolith. Those will be discussed in the following chapter.
3.1. Applications to High Performance Liquid Chromatography

3.1.1. Imprinted monolith

Since molecularly imprinted polymers are designed to recognize and bind a specific compound over a range of chemicals, they are good candidates as stationary phases in High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE). Both techniques rely upon the different partitioning of compounds between a sorbent phase and a solvent phase, but SPE columns (cartridges) present usually a lower plate number than HPLC columns (50 vs. 10,000). This is why SPE is typically used as an extraction technique for sample preparation while HPLC allows separation and quantification of a mixture of compounds. The SPE procedure is normally made up of five steps. After conditioning the sorbent, the sample is applied in the cartridge. The latter retains the compound of interest while the matrix components are eluted. A next step consists in washing off any interferents slightly retained on the cartridge. Finally, a good solvent of the target analyte allows eluting the latter. MIPs have been widely used as sorbents for off-line SPE allowing extraction of pesticides or drugs from complex samples [58-60]. A couple of authors also reported on-line techniques that simplify the experimental procedure [5, 49].
In this work, the potential of mjb-1 as sorbent for on-line SPE type of chromatographic analysis was evaluated. Stainless steel guard columns (50*4.6mm id) were manually packed using the dry material, and then connected to the HPLC system. Three columns containing silica and increasing amounts of imprinted polymer were actually prepared. The quantity of imprinted matrix in the packing material was increased to 15% and 50% by weight. Adding more than 50% of bulk polymer was found to be impossible. The resulting high back-pressure was most likely a consequence of the polymer polydispersity.

<table>
<thead>
<tr>
<th></th>
<th>Column A</th>
<th>Column B</th>
<th>Column C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica</td>
<td>100%</td>
<td>85%</td>
<td>50%</td>
</tr>
<tr>
<td>MIP</td>
<td>0%</td>
<td>15%</td>
<td>50%</td>
</tr>
</tbody>
</table>

Table 25: List of the HPLC stationary phases tested

In a first time, the chromatographic performances of the various stationary phases were compared by injecting a solution of caffeine at 0.01g/L. Based on the adsorption isotherms (presented in chapter 2), choosing such a low concentration allows relying upon the polymer specific sites for separation. Details of the analytical conditions are given in table 26 below.

<table>
<thead>
<tr>
<th>Time</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;t&lt;2 minutes</td>
<td>100% Water</td>
</tr>
<tr>
<td>2&lt;t&lt;4 minutes</td>
<td>50% Water, 50% Acetonitrile</td>
</tr>
<tr>
<td>t&gt; 4 minutes</td>
<td>50% Water, 50% Acetonitrile</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 254nm</td>
</tr>
</tbody>
</table>

Table 26: Summary of the chromatographic conditions used for the evaluation of MIP as a stationary phase
Figure 48 compares the chromatographic profiles obtained after injection of caffeine in each of the columns.

![Figure 48: Chromatographic profiles of a caffeine solution injected in various HPLC columns](image)

Caffeine was eluted very rapidly, around one minute, when run on the reference stationary phase. On the other hand, when the content of MIP in the column was increased to 50%, caffeine got delayed by more than three minutes, clearly indicating the stronger interactions taking place between the analyte and the stationary phase. On the intermediate column, caffeine came out as a shouldered peak. The elution profile can be explained by the relatively low amount of imprinted polymer in the stationary phase that could interact with only limited quantity of caffeine. Those results are very encouraging but they do not allow concluding on the efficiency of the imprinted polymer as a stationary phase. To do so, a 0.01g/L solution of theobromine, whose chemical structure is very similar to caffeine (Figure 49), was injected in the same columns using identical conditions. The chromatograms obtained are compared on Figure 50.

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Theobromine peak broadens considerably as the concentration of MIP in the column increases. This suggests that some interactions are actually taking place between the polymer and the theobromine. However, comparison of the analytes elution profiles obtained after injection in the 50% MIP column (Figure 51) proves that the polymer interacts more strongly with caffeine than with theobromine. The former yields a sharp peak delayed at about four minutes while the elution of theobromine starts around one minute.

Figure 50: Chromatographic profiles of a theobromine solution injected in various columns
Theobromine at 0.01 g/L  
Caffeine at 0.01 g/L  
Mixture theobromine caffeine, each at 0.005g/L

Figure 51: Comparison of the chromatographic profiles obtained for a caffeine and a theobromine solution when injected in a column containing 50% of imprinted polymer

Injecting a mixture of the two analytes confirmed the previous observations: a peak corresponding to that of caffeine was observed around four minutes. It was clearly preceded by a broad shoulder corresponding to the elution of theobromine. From those experiments, it was concluded that mjb-1 can differentiate and separate two closely related analytes. It could thus be used as an HPLC stationary phase for the separation and quantification of caffeine and its analogs. However achieving full separation of the two analytes would require careful optimization of the chromatographic conditions.

The advantage of such an imprinting technique seems obvious: in theory one can prepare a variety of tailor made packing materials to use for a range of analyses. However, packing the polymer in the column is a tedious process. And preparing columns containing only the imprinted material would require sieving the powder which is also a very cumbersome procedure. A method using
imprinted nanoparticles was found to resolve the mentioned issues while taking advantage of the general imprinting technique.

3.1.2. Imprinted nanoparticles

The particles prepared by precipitation polymerization present a size distribution centered on 400nm. Since such a diameter does not allow packing the particles in an HPLC column, a new analysis method was devised to use the imprinted particles along with the HPLC. The principle of the technique is the following:

- a sample containing caffeine is mixed with a caffeine imprinted latex
- the system is left equilibrating for several minutes and is then injected in a short HPLC column packed with silica
- diffusion effects in the column allow separating the large latex particles from the free caffeine molecules; UV detection thus yields two traces (Figure 52)
- the peak corresponding to the caffeine free in solution is then integrated and a calibration curve allows quantifying the amount of caffeine detected

Figure 52: Typical chromatogram yielded after injection of a mixture of latex and caffeine (caffeine peak at 2mins)
The technique was initially tested with caffeine solutions of known concentrations. And equilibrating the latex with increasing amount of caffeine allowed plotting the whole adsorption isotherm for the polymer. The latter was presented in chapter 2 of this thesis. More interestingly, a chart relating the amount of free caffeine to the amount of caffeine initially present was plotted (Figure 53). Such a modified isotherm becomes especially useful when analyzing samples of unknown caffeine concentration. One can imagine repeating the procedure described above with the latex and the sample of interest. After quantification of the free caffeine by HPLC, one would refer to the modified latex isotherm to determine the initial concentration of template.

![modified isotherm for mjb-77, using the bi-Langmuir parameters presented in table 21](image)

**Figure 53:** Modified isotherm for mjb-77, using the bi-Langmuir parameters presented in table 21
However, real samples rarely contain a single analyte. Consequently, understanding how the imprinted latex would behave in presence of several components is a question that needs to be addressed. This can be achieved with a co-adsorption experiment, where the imprinted polymer is equilibrated with caffeine, its template, and a caffeine analog, that acts as a competitor. Benzotriazol (BTA) was the compound of choice for this experiment since it is similar in size and shape to caffeine (Table 27). To understand how good a competitor benzotriazol is, a first binding experiment was performed. It consists in comparing the binding isotherm obtained when the polymer is in the sole presence of benzotriazol to that of the polymer when it is equilibrated only with caffeine. The latex was thus equilibrated with increasing concentrations of BTA and the resulting system was injected in HPLC. It is important to note here that the initial concentrations of BTA were identical to that used for the caffeine rebinding experiments. Quantification of the BTA in solution was achieved by UV detection at 280nm and the amount of BTA bound by the polymer was deduced. The resulting isotherm was plotted and compared to that of the caffeine on figure 54.

Table 27: Structure of BTO and Caffeine

<table>
<thead>
<tr>
<th>Caffeine</th>
<th>Benzotriazol</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Caffeine Structure" /></td>
<td><img src="image2.png" alt="Benzotriazol Structure" /></td>
</tr>
</tbody>
</table>

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Figure 54 points out two interesting features. First of all, benzotriazol is an adequate caffeine competitor since using the same initial concentrations yields similar adsorption levels.

On the other hand, one can clearly see that the polymer binds more caffeine than BTA. This was actually confirmed by fitting both sets of experimental data with the bi-Langmuir model and comparing the binding parameters (Table 28). Overall, it appears that fitting the model to the BTA adsorption yields sites of lower affinity compared to that obtained from the caffeine adsorption. This result indicates that the imprinted polymer is more selective to caffeine.
Table 28: Comparison of the binding parameters for the same polymer, mjb-77, equilibrated with caffeine and benzotriazol (separately)

<table>
<thead>
<tr>
<th></th>
<th>Caffeine</th>
<th>Benzotriazol</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-affinity sites</td>
<td>Binding constant (L/g)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Number of sites (g/L)</td>
<td>0.01</td>
</tr>
<tr>
<td>Low-affinity sites</td>
<td>Binding constant (L/g)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Number of sites (g/L)</td>
<td>30.4</td>
</tr>
</tbody>
</table>

Those results allowed concluding that benzotriazol was a competitor of choice for a co-adsorption experiment. To model the results of such an experiment, a binding model was then developed. The nomenclature used in the following derivations is summarized in table 29.

Table 29: Nomenclature used for the derivation of the co-adsorption model

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>Total concentration of caffeine</td>
</tr>
<tr>
<td>To</td>
<td>Total concentration of benzotriazol</td>
</tr>
<tr>
<td>Go</td>
<td>Total number of high-affinity site</td>
</tr>
<tr>
<td>Bo</td>
<td>Total number of low-affinity site</td>
</tr>
<tr>
<td>C</td>
<td>Caffeine free</td>
</tr>
<tr>
<td>T</td>
<td>Benzotriazol free</td>
</tr>
<tr>
<td>G</td>
<td>High-affinity site</td>
</tr>
<tr>
<td>B</td>
<td>Low-affinity site</td>
</tr>
<tr>
<td>CG</td>
<td>Caffeine bound in a high-affinity site</td>
</tr>
<tr>
<td>CB</td>
<td>Caffeine bound in a low-affinity site</td>
</tr>
<tr>
<td>TG</td>
<td>Benzotriazol bound in a high-affinity site</td>
</tr>
<tr>
<td>TB</td>
<td>Benzotriazol bound in a low-affinity site</td>
</tr>
</tbody>
</table>

The model is inspired by the bi-Langmuir equations, namely, it assumes that the polymer is made up of two types of sites, high and low-affinity binding sites. It is also assumed that both caffeine and benzotriazol are able to adsorb on each type of site so that four binding events are distinguished:
- Adsorption of caffeine in a high-affinity site; the binding constant associated to this binding event is noted $K_1$
\[ C + G \rightleftharpoons^{K_1}_{\text{CG}} \]

- Adsorption of benzotriazol in a high-affinity site. Since the sites are not specific to BTA, the binding constant associated to this event is different than that of caffeine and is noted $K_2$
\[ T + G \rightleftharpoons^{K_2}_{\text{TG}} \]

- Adsorption of caffeine or BTA in a low-affinity site. Since those sites are not specific to either compound, the same binding constant $K_2$ was associated to each of those events. It is to note here that this assumption constitutes a major simplification of the model.
\[ C + B \rightleftharpoons^{K_2}_{\text{CB}} \]
\[ T + B \rightleftharpoons^{K_2}_{\text{TB}} \]

One can now write the binding constants as

\[ K_1 = \frac{CG}{C \cdot G} \]

\[ K_2 = \frac{TG}{T \cdot G} = \frac{CB}{C \cdot B} = \frac{TB}{T \cdot B} \]

and the total number of binding sites as

\[ Go = CG + TG + G \]
\[ Bo = CB + TB + B \]

Also the total number of analyte at any instant is

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\[ Co = CG + CB + C \]
\[ To = TB + TG + T \]

From the binding constant equations, one can write the total amount of caffeine bound as

\[ CG + CB = K1 \cdot C \cdot G + K2 \cdot C \cdot B \]

Using the equation of the number of sites to express G and B yields

\[ G = \frac{Go}{K1 \cdot C + K2 \cdot T + 1} \]
\[ B = \frac{Bo}{K2 \cdot C + K2 \cdot T + 1} \]

Consequently, the amount of caffeine bound is

\[ CG + CB = C \left( \frac{K1 \cdot Go}{K1 \cdot C + K2 \cdot T + 1} + \frac{K2 \cdot Bo}{K1 \cdot C + K2 \cdot T + 1} \right) \]

As expected, the total concentration of caffeine bound is related to the amount of caffeine and benzotriazol free.

The amount of benzotriazol bound was also written as:

\[ CG + CB = K2 \cdot T \left( \frac{Go}{K1 \cdot C + K2 \cdot T + 1} + \frac{Bo}{K2 \cdot C + K2 \cdot T + 1} \right) \]

To gain an idea of how the polymer could behave, a couple of co-adsorption experiments were simulated. For the purpose of those simulations, the binding constants and number of sites were determined as follows. The caffeine and benzotriazol adsorption set of data were fitted simultaneously using
- A bi-Langmuir model with two binding constants and number of sites (\(K_1, K_2, G_0, B_0\)) for the caffeine adsorption experiment

- A Langmuir with a single binding constant and site number for the benzotriazol adsorption (\(K_2\) and \(B_0\), equal to that of the caffeine binding parameters)

The resulting parameters are summarized in table 30.

<table>
<thead>
<tr>
<th>High-affinity sites</th>
<th>Binding constant (L/g)</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of sites (g/L)</td>
<td>0.03</td>
</tr>
<tr>
<td>Low-affinity sites</td>
<td>Binding constant (L/g)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Number of sites (g/L)</td>
<td>1.015</td>
</tr>
</tbody>
</table>

Table 30: Parameters used to simulate the co-adsorption experiments

With those numbers, the co-adsorption experiment was simulated in two cases. The first simulation was performed assuming that the concentration of caffeine was equal to that of benzotriazol. The isotherms yielded in this case are presented in Figure 55. The latter clearly points out that the polymer would bind over the whole concentration range more for caffeine than for benzotriazol. This was in line with what was suggested in the comparison of the two adsorption isotherms (Figure 54).
Figure 55: Simulated co-adsorption isotherm when the concentration of caffeine is equal to that of benzotriazol

In an attempt to model a more realistic situation, another experiment was simulated. That is to say, the concentration of benzotriazol was assumed to be five times greater than that of caffeine. The resulting isotherms are displayed in Figure 56. The chart suggests that the specific sites allow the polymer to bind more caffeine than BTA in the low concentration range. However, the parameters set for the simulation indicate that there are 20 times more non-specific sites than specific sites. This is why in the medium to high concentration range, the polymer adsorbs more benzotriazol than caffeine.
To understand how the polymer would actually behave and to verify the accuracy of the model, the co-adsorption experiment was performed. As the above equations point out, quantifying the amount of caffeine bound requires knowing how much of caffeine and benzotriazol are free in the solution at any instant. The analysis technique was devised to quantify each compound without severe optimization of the HPLC method. The co-adsorption experiment was designed as follows:

* Known amounts of latex were equilibrated with equal amounts of caffeine and BTA

* After equilibration, the whole system was injected twice in the HPLC on a short column packed with silica
The first injection allowed detecting the mixture of benzotriazol and caffeine at 240nm. The analytes were then detected at 280nm in the second run.

The UV detector was calibrated at both wavelengths for both compounds so that four calibration curves were obtained. The Beer Lamber law then allows writing

\[ \text{Area} = a \times [\text{Analyte}] \]

where \( a \) depends upon the compound and the wavelength (Table 31).

<table>
<thead>
<tr>
<th></th>
<th>240 nm</th>
<th>280 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>( a_1 )</td>
<td>( a_3 )</td>
</tr>
<tr>
<td>Benzotriazol</td>
<td>( a_2 )</td>
<td>( a_4 )</td>
</tr>
</tbody>
</table>

Table 31: Calibration curves slopes

Integrating the HPLC traces at 240nm and 280nm yields a set of two equations

\[ \begin{align*}
\int \text{HPLC}_{240nm} \, dt &= A_1 \\
\int \text{HPLC}_{280nm} \, dt &= A_2
\end{align*} \]
\[
\begin{align*}
A(240\text{nm}) &= a_1 \cdot C + a_2 \cdot T \\
A(280\text{nm}) &= a_3 \cdot C + a_4 \cdot T
\end{align*}
\]

Which can be solved for \( C \) and \( T \)

\[
\begin{align*}
C &= \frac{A(240\text{nm}) - a_2 \cdot T}{a_1} \\
T &= \frac{\text{Area}(280\text{nm}) - \frac{a_3}{a_1} \cdot \text{Area}(240\text{nm})}{a_4 - \frac{a_3 \cdot a_2}{a_1}}
\end{align*}
\]

This set of two equations was used to compute the amount of caffeine and benzotriazol free in solution as well as the amount of caffeine and benzotriazol bound by the polymer. The resulting isotherm showed dramatic scattering of the experimental data (Figure 58) and did not allow concluding about the co-adsorption experiment.

This was attributed to an accumulation of errors throughout the experimental process. To confirm this hypothesis, a reproducibility experiment was performed on a latex equilibrated with caffeine and benzotriazol. The system was injected five times at each wavelength, and the error on the area was evaluated. The statistics presented in table 32 (the mathematical expression can be found in appendix C) already points out one of the drawbacks of the technique. Namely, the coefficient of variation indicates that the data points at 240nm are more scattered than that at 280nm. This could be the consequence of the limited sensitivity of the compound at that wavelength. It could also result from the lower detector sensitivity.
The impact of those errors on the final concentration calculations was then evaluated by computing the relative standard error on the final concentrations. The results are displayed in table 33. The latter clearly points out the drastic scattering of the final data. It was thus concluded that the way the experiment was designed did not allow accurate quantification of the analyte concentration.
<table>
<thead>
<tr>
<th></th>
<th>[T]free (g/L)</th>
<th>[C]free (g/L)</th>
<th>[T]bound</th>
<th>[C]bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative standard error</td>
<td>5.4%</td>
<td>120%</td>
<td>12%</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 33: Reproducibility statistics on the final concentrations

It is likely that relying upon two injections in order to compute a single value produced inaccuracy in the concentration calculations. This could be avoided in future experiments by using a diode array detector since the latter allows monitoring two wavelengths at the same time.

Another possible source of error is the choice of the caffeine competitor. In this case, both benzotriazol and caffeine adsorb in the same wavelengths range (Figure 57). A competitor presenting a UV spectrum very different from that of caffeine could be used. This could allow detecting each compound at its maximum of absorption. One can imagine for instance replacing some of the hydrogen in the caffeine molecule by fluorine. Such a molecule should present a UV spectrum rather different than caffeine without affecting much of the structure.

3.2. Applications to chemical sensing

As mentioned in the first chapter of this review, coupling an imprinted polymer to a quartz crystal microbalance (QCM) allows recording in real time the mass uptake occurring in the polymer. The instrument records the variations of oscillation frequency as a function of time. Using different mathematical expressions, the latter are transformed into mass variations occurring at the
QCM electrode surface as a function of time. Such a sensor thus enables direct quantification of an analyte in a sample. This section focuses on the development of a sensor that allows quantifying caffeine concentrations in water samples. To do so, imprinted nanoparticles prepared by precipitation polymerization in acetonitrile and a 5MHz quartz crystal microbalance from Stanford research systems were used (Figure 59).

![Figure 59: QCM 200 5MHZ Quartz Crystal Microbalance [61]](image)

3.2.1. Preparation of the crystals

The first step toward chemical sensing consists in coupling in some fashion the imprinted particles to the quartz crystal. The crystals used were 5MHz quartz crystals with chrome and gold electrodes on each side. This process should ensure proper adhesion of the polymer to the crystal and guarantee signal stability. Several coupling strategies were investigated and compared. Because the adhesion of the polymer film is also dependent upon the surface cleanliness, all of the crystals were immersed for five minutes in a piranha solution. The latter consists of a mixture of three volumes of sulfuric acid and one volume of hydrogen peroxide at 30%; it allows removing organic coatings.

Using an inert polymer
The initial strategy consisted in coating a layer of some inert polymer, followed by a layer of nanoparticles. Some important parameters for this process were identified. They include the nature of the polymer, its glass transition temperature, its concentration and the coating technique. Evaluating the influence of those factors was achieved by recording the resonance frequency before and after coating, and looking at the signal stability upon immersion in water.

The nature of the inert polymer was first investigated. Three commercial polymers were tested, namely Poly-(methyl acrylate), Polystyrene and Polyisobutylene. Each of the polymers was dissolved in tetrahydrofuran at 1% by weight. 10μL of the solution were then dropped on the center of the sensor and spread over the whole surface by spin-coating at 7000rpm for 20 seconds. The possibility of polymerizing in situ a layer of monomers was also examined. A prepolymerization mixture consisting of EGDMA, HEMA (1:4) and AIBN was prepared. A 10 μL aliquot was further dropped on the sensor's surface and spin-coated at 3000rpm for 20 seconds to form a uniform film. The latter was then polymerized under UV irradiation for 2 hours.

Upon immersion of the resulting crystals in water, all of the commercial polymers showed, at first sight, decent adhesion to the substrate. In contrast, the coating that was polymerized in situ quickly peeled off the surface. This phenomenon was reported before in the literature and attributed to the polymer film shrinking during the UV polymerization [62].
Table 34: Structure and Glass transition temperature of the polymer used to coat the quartz crystal

<table>
<thead>
<tr>
<th>Polystyrene</th>
<th>Poly (methyl acrylate)</th>
<th>Polyisobutylene</th>
<th>Poly-(hydroxyethyl methacrylate)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Polystyrene structure" /></td>
<td><img src="image2" alt="Poly (methyl acrylate) structure" /></td>
<td><img src="image3" alt="Polyisobutylene structure" /></td>
<td><img src="image4" alt="Poly-(hydroxyethyl methacrylate) structure" /></td>
</tr>
<tr>
<td>$T_g = 100^\circ C$</td>
<td>$T_g = 9^\circ C$</td>
<td>$T_g = -76^\circ C$</td>
<td>$T_g = 85^\circ C$</td>
</tr>
</tbody>
</table>

In addition, crystals coated with poly-(methyl acrylate) consistently presented a signal decreasing over several hours and a large range of frequencies. Figure 60 displays a typical signal where a 2000Hz decrease was observed within 2 hours. This could be due to the polymer ability to form hydrogen bonds with water. A network of water and polymer molecules is thought to develop, thus constantly increasing the mass at the surface of the crystal. On the other hand, both polyisobutylene and polystyrene showed stable signals, i.e. no large frequency drifts were observed in the time frame of the experiment. Polyisobutylene forms a soft film at room temperature that tends to deform upon oscillation and thus can end up not fully coupled to the crystal. Consequently, polystyrene which is glassy at room temperature was the polymer of choice for the following experiments.
To gain an idea of the effective maximum film thickness, several concentrations and coating techniques were experimented. A solution of polystyrene at 10% by weight in tetrahydrofuran was found to form a film too thick and heavy for the crystal. Both spin-coating and painting the solution resulted in loosing the resonance frequency.

Films formed with a solution at 5% in THF were thus examined. Two crystals were painted with the polystyrene solution and allowed to dry. Then a layer of nanoparticles prepared by precipitation polymerization in acetonitrile was applied. 10μL of the latex were spin-coated on the crystal while on the second crystal, the particles (10μL as well) were simply deposited and allowed to dry. The crystal's resonance frequencies recorded before and after coating allowed estimating the coating thickness (Table 35). The calculations were performed according to Sauerbrey's equation (Figure 18). As expected, spin-coating the particles
resulted in forming a thinner film than by simple deposition. At first sight, this coating technique would thus be the method of choice since thinner films typically guarantee better coupling between the crystal and the coating outer layer.

<table>
<thead>
<tr>
<th></th>
<th>ΔF (Hz)</th>
<th>Δm (μg)</th>
<th>Coating thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal spin-coated</td>
<td>-5055</td>
<td>36</td>
<td>0.9</td>
</tr>
<tr>
<td>Crystal deposited</td>
<td>-14090</td>
<td>100</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 35: Comparison of the coating thickness obtained from two coating techniques

However, when both crystals were immersed simultaneously in water and the frequency variations were recorded, significant differences in the signal stabilities were observed. Figure 61 points out that the sensor with deposited particles generated a signal more stable than the crystal that was spin-coated. It is not really clear why such a trend was observed. To understand what kind of coating the deposition process yielded, two crystals were prepared and examined using scanning electron microscopy. The sensors were coated in a similar fashion. After that, one of them was left in the air while the other one was immersed in water for 72 hours. Both sensors were then fractured; SEM allowed imaging of the crystal cross-sections (figure 62 and 63) and measuring the layer thicknesses (Table 36).
Figure 61: Comparison of the signal stability of a crystal where particles are deposited and a crystal where particles are spin-coated

Figure 62: SEM micrographs of a sensor left in air
Figure 63: SEM micrographs of a sensor immersed in water for 72 hours

<table>
<thead>
<tr>
<th></th>
<th>Crystal left in air</th>
<th>Crystal emerged in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured from SEM micrographs</td>
<td>PS layer</td>
<td>473 nm</td>
</tr>
<tr>
<td></td>
<td>Particles layer</td>
<td>4.8 μm</td>
</tr>
<tr>
<td>Calculated from Sauerbrey's equation</td>
<td>Total film thickness</td>
<td>2.5 μm</td>
</tr>
</tbody>
</table>

Table 36: Comparison of measured and calculated thickness

From table 36, several features can be underlined. First of all, it is interesting to note the difference between the particles layer thicknesses. The crystal that was left in the air presented a layer about twice as thick as that of the crystal immersed in water. The immersion process most likely resulted in loosing some of the upper layers of the coating. To verify this hypothesis, two sensors were painted with the solution of polystyrene and one of them was further coated with the nanoparticles. Both crystals were then immersed in water and their frequency signal was recorded. Figure 64 shows how the frequency of the crystal
coated with polystyrene decreases upon immersion in water and then equilibrates. On the contrary, the frequency of the other sensor decreases first but quickly increases to finally level off. This frequency increase was clearly the fate of particles leaving the crystal surface, confirming what the SEM suggested. This coating process thus requires equilibrating in water the crystals till a stable baseline is reached, before starting an experiment.

![Graph showing frequency signals](image)

**Figure 64: Comparison of the frequency signals of a sensor painted with polystyrene and a crystal coated with polystyrene and nanoparticles**

Another interesting feature arises from table 36, namely the difference between the calculated and measured thicknesses for the crystal left in air. Two hypotheses were formed to explain such a variation. Either the coating process was not reproducible enough or Sauerbrey’s equation does not fully explain the mass increase that occurred at the crystal surface. To check the reproducibility of the coating procedure, four crystals were prepared in parallel. Depositing the
particles on the sensor surface was assumed to generate only little variations, as long as the same latex is employed. In contrast, the painting step could be responsible for non-reproducibility. Thus, the four crystals were all painted with the polystyrene solution at 5% by weight in THF. Their resonance frequencies before and after coating were recorded and their thicknesses were calculated. The relative standard deviation was found to be equal to 20%. It was concluded that the painting process was reasonably reproducible. This then suggests that Sauerbrey's equation does not describe well the mass increase situation. In fact, this equation assumes that a rigid, uniform and thin film is deposited on the sensor so that virtually no energy is lost when the crystal oscillates. In a situation where a soft or thick film is applied (as it might be the case here) the dampening can become important and Sauerbrey's equation is no longer applicable. Overall, painting the crystal surface with a polystyrene solution and then depositing the nanoparticles allowed coating the crystal with the imprinted polymer. The resulting frequency signal was found reasonably stable so that this technique was used in the first experiments. However, in an attempt to better control the film thickness, another preparation technique was investigated.

**Building a layer-by-layer assembly**

The layer-by-layer assembly method relies upon electrostatic interaction to build an assembly of polyelectrolyte by consecutive adsorption steps. Figure 65 illustrates the principle of such a coating technique.
Details of the experiment are given below. In summary, the substrate, that is to say, the crystal gold surface was modified to bear negative charges. Then, a commercial polycation was self-assembled on the surface. In a third time, the imprinted particles that present some negative charges were adsorbed. Such a technique allows controlling the film thickness by adjusting the number of adsorption steps. Also, it should enable forming a structure less dense in nanoparticles than that resulting from the deposition technique. This could make a tremendous difference during the adsorption experiments by easing the access of the template to the imprinted particles.

The experimental procedure detailed in this section is adapted from a paper by Baba et al. [64]. In a first time, the crystal gold surface was modified with an anionic thiol compound, namely 3-Mercapto-1-Propanesulfonic acid Sodium salt (MPS). The sulfur atoms that strongly coordinate on the gold surface
ensure adhesion of the assembly (to-be-built) to the sensor. This step was performed by immersing the crystal in a MPS solution at 0.1mol/L in ethanol for 30 minutes.

![Figure 66: Structure of 3-Mercapto-1-Propanesulfonic acid Sodium salt (MPS)](image)

After that, the crystal was rinsed and immersed in an aqueous solution of Poly(diallyldimethylammonium chloride) (PDADMAC) at 5g/L for 20 minutes. The electrostatic interactions taking place between this polycation and the negatively charged thiol should ensure adsorption of the PDADMAC on the crystal.

![Figure 67: Structure of Poly-(diallyldimethylammonium chloride) PDADMAC](image)

In the next step, a layer of imprinted particles was added to the assembly. A latex prepared by precipitation polymerization in acetonitrile possess about 10% of methacrylic acid so that the particles once transferred in water and at pH above 4.5, bear negative charges. The latex was thus ultrafiltered in water and the pH of the final dispersion was set at 5. The crystal was immersed in this final solution for 20 minutes.
The second and third steps constitute an adsorption cycle that was repeated several times to build a 10-layers thick assembly. To gain an idea of how stable the signal was, the resulting crystal was immersed in water. Figure 68 shows a typical frequency signal. The vertical axis scale clearly points out that the signals are very stable. However, upon reproducing the coating technique on four crystals, it appeared that the process was non-reproducible. The relative standard deviation on the coating thickness was indeed found to be around 90%. Although this technique seemed promising, it showed for some unknown reasons severe non-reproducibility. Consequently, the first adsorption experiments were carried out using sensors prepared by painting a polystyrene solution and depositing the imprinted particles.

![Typical signal recorded upon immersion of crystals prepared by layer-by-layer assembly](image)

Figure 68: Typical signal recorded upon immersion of crystals prepared by layer-by-layer assembly
3.2.2. Adsorption – Desorption experiment

Experimental set-up

Because the quartz crystal microbalance is virtually sensitive to any kind of variations (mechanical stress at the sensor's surface, temperature, pressure, viscosity of the medium) understanding how to set-up the experiment was a challenge. Overall two modes of operation were experimented:

- A flow mode where caffeine solutions are flowed over the crystal
- A batch mode that consisted in immersing the crystal in solutions of various caffeine concentrations.

The initial set-up consisted in bringing the crystal in contact with the liquid using a flow cell (Figure 69).

![Figure 69: Picture and schematic of a flow cell mounted on the crystal holder (from SRS website)](image)

To run the solution to the cell, a peristaltic pump was first used. The flow rate was set-up at 4ml/min to avoid excessive stress on the crystal. However, very large noises namely 50Hz shifts, were constantly observed on the resulting frequency signal. This was attributed to the mechanical vibrations produced by the pump.
In an attempt to reduce the noise, the peristaltic pump was thus replaced by a syringe pump. Using such an instrument allows reducing the flow rate to 1ml/min. and limiting the noise to a couple of hertz. Although this was encouraging, other problems arose upon putting together the adsorption-desorption experiment. The principle of such an experiment is the following:

- The crystal is first equilibrated in water
- When a stable baseline is reached, the sensor is placed in contact with a template solution (here a caffeine solution). If the polymer binds the template, the frequency signal decreases.
- As soon as the signal is stabilized, caffeine is desorbed from the polymer binding sites by flowing a cleaning solution over the sensor.

Such an experiment thus requires being able to easily change the liquid in contact with the crystal. Consequently, the following set-up was put together: two syringe pumps were connected to a valve placed before the flow cell (Figure 70). This allowed switching the flowing liquid fairly easily. However, air bubbles circulating or located at the sensor's surface were a frequently encountered issue when using such a set-up. This induced variations on the frequency signal. To limit this problem as much as possible the liquids were degassed before each experiment.
A simpler, batch mode of operation was also experimented. It consisted in immersing the sensor in a beaker filled with degassed solutions. Switching liquids was achieved by taking the crystal out and reemerging it in a new solution. This operation typically yielded large frequency shifts that were attributed to the change in the medium viscosity and density (Table 38, Figure 71). As Figure 72 points out, the signal was quickly stabilized (in about 30 seconds) but a signal difference of about 10Hz was observed before and after immersion. This issue was not addressed at first since only high concentrations of caffeine provoking large frequency variations were tested.

<table>
<thead>
<tr>
<th>Property</th>
<th>Air</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity at 20°C (Pa.sec)</td>
<td>$1.8 \times 10^{-5}$</td>
<td>$1.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Density (kg/m$^3$)</td>
<td>1.168 at 25°C</td>
<td>0.9982 at 20°C</td>
</tr>
</tbody>
</table>

**Table 37: Comparison of the viscosity of air and water**
\[ \Delta f = -f^{3/2} \cdot \left( \frac{\rho_L \eta}{\pi \mu \rho_q} \right)^{1/2} \]

\( \Delta f \) = measured frequency shift

\( \rho_L \) = density of the fluid

\( \eta \) = viscosity of the fluid

\( \rho_q \) = density of the quartz

\( \mu_q \) = shear modulus of the quartz

\( f \) = resonance frequency of the crystal

Figure 71: Equation relating the frequency variation to the density and viscosity of the medium [61]

Figure 72: Typical signal recorded upon taking a crystal out of solution and reemerging it in the same solution

With the goal of lowering the instrument detection limit, the method was modified; the crystals were equilibrated in water and an aliquot of caffeine solution was injected in the beaker. This successfully smoothed the frequency signal (Figure 73). Consequently small frequency changes occurring upon
adsorption of limited quantities of caffeine in the polymer were monitored. Obviously such a procedure was limited and did not allow running a desorption step.

![Graph showing frequency vs. time](image)

**Figure 73:** Typical signal recorded upon injecting an aliquot of solution to a beaker filled with water

**Array detection**

**Polystyrene – Molecularly Imprinted Polymer**

In an effort to distinguish the frequency shifts due to environmental changes (temperature, pressure, viscosity etc.) from the signal variations occurring upon adsorption of caffeine, two crystals were run in parallel. This array detection set-up is illustrated on figure 74. It is important to note here that to limit...
the frequency variations due to temperature changes (8Hz/°C according to the manufacturer), this set-up was modified. Namely, the crystals were insulated in a polystyrene box.

![Figure 74](image)

**Figure 74**: Picture of a batch mode set-up for the adsorption-desorption experiments

At first, one of the crystals was painted with polystyrene and then coated with imprinted nanoparticles while the other one was simply painted with polystyrene. The second sensor was thus used as a control polymer. After equilibration in a buffer solution, the crystals were immersed in a concentrated caffeine solution. The frequency signal, reproduced on figure 75 clearly points out the difference between the two signals. After switching solutions, the crystal coated with polystyrene quickly stabilizes around its initial baseline. In contrast, the crystal coated with MIP particles presents a frequency decrease of about 200Hz over a couple of hours. This indicates that the polymer actually binds some of the caffeine present in the solution. It is interesting to notice the time frame of the adsorption process. It is considerably larger than what Roy reported in his kinetics study of the nanoparticles [55]. This was attributed to the different physical form of the nanoparticles during those two experiments. Roy performed
his studies on nanoparticles free in solution whereas in the case of the QCM experiments, the particles are assembled in a dense structure. The latter could result in slowing down the adsorption process.

![Figure 75: Frequency signal obtained from an array detection experiment – Comparison of a crystal coated with polystyrene and a crystal coated with imprinted nanoparticles](image)

Molecularly Imprinted Polymer – Non Imprinted Polymer

To understand how much of the caffeine adsorption is due to specific binding, the array detection was modified to monitor in parallel an imprinted polymer and a non-imprinted polymer (NIP). The latter is typically made in the same conditions as a MIP but no template is added in the formulation. NIPs are known to adsorb some template during the rebinding experiments. Clearly, the
polymer binds in this case with non-specific sites. Such a set-up should thus allow differentiating the different binding events.

Figure 76 compares the frequency signal of a crystal coated with MIP particles to that of a crystal coated with NIP particles during equilibration in an acidic solution (pH=2). Such a solution was employed to extract caffeine from the imprinted sites since the latex was not ultrafiltered before coating. Both signals indicate that particles are leaving the sensor’s surface upon immersion in water. This was in line with what was previously reported. Comparing both signals also points out an increased mass loss on the MIP sensor compared to that of the NIP. This was due to caffeine leaving the polymer matrix.

![Figure 76: Frequency signal obtained from an array detection experiment – Comparison of a crystal coated with MIP particles and a crystal coated with NIP particles upon immersion in an acidic solution](image)
When a stable baseline was obtained, both sensors were immersed in a concentrated caffeine solution (10g/L). The resulting signals are displayed on figure 77. The latter indicates a steeper frequency decrease in the case of the MIP crystal. This in turn suggests that the imprinted polymer binds more quickly than the non-imprinted one. This is in agreement with what Roy reported previously [55]. The author noticed that a polymer with good recognition capacities tended to respond faster than a polymer with only poor recognition abilities.

![Figure 77: Frequency signal obtained from an array detection experiment – Comparison of a crystal coated with MIP particles and a crystal coated with NIP particles upon immersion in a concentrated caffeine solution](image)

Computing the mass changes from the frequency variations obtained in a liquid measurement is a challenge. Some authors reported computations based upon Sauerbrey’s equation [36] while others calibrated the QCM so that the
concentration of solution was linearly related to the observed frequency changes [39]. Here, the mass variations were computed using the bi-Langmuir isotherm.

First, the coating parameters were computed. The SEM micrographs of the crystals equilibrated in water (Figure 63) indicate that about 7 layers of particles were deposited on the sensor’s surface. Consequently, the active surface of the sensor was coated with about $14 \times 10^8$ particles. The number of particles can be translated into the total polymer mass on the active surface i.e. 92ng. Those computations are summarized in table 38.

<table>
<thead>
<tr>
<th>Number of layers</th>
<th>7 layers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active sensor surface</td>
<td>0.4 cm$^2$</td>
</tr>
<tr>
<td>Number of particles on the active surface</td>
<td>$14 \times 10^8$ particles</td>
</tr>
<tr>
<td>Total volume of the imprinted coating</td>
<td>$9.1 \times 10^{-5}$ cm$^3$</td>
</tr>
<tr>
<td>Total mass of the imprinted coating</td>
<td>92 ng</td>
</tr>
</tbody>
</table>

**Table 38: Characteristics of the prepared sensor**

The bi-Langmuir isotherm presented on figure 46 allows calculating the theoretical mass of caffeine bound per gram of polymer for the imprinted polymer. Neglecting the specific term in the bi-Langmuir model allowed calculating the theoretical amount of caffeine bound by the non-imprinted polymer. Given the mass of imprinted coating one can then deduce the mass of caffeine theoretically bound at the sensor’s surface (table 39).
Theoretical mass bound (g/g) | Theoretical mass bound (ng) | ΔF
--- | --- | ---
MIP | 0.045 g/g | 4.14 | -350 Hz
NIP | 0.044 g/g | 4.05 | -310 Hz
Specific adsorption | 0.001 g/g | 0.09 | 40 Hz

Table 39: Computations of the mass of caffeine adsorbed on a MIP and a NIP sensor

Overall, those results indicate that the imprinted polymer binds faster and more caffeine than its non-imprinted equivalent.

Also, from table 39, one can predict the detection limit of such a sensor. Assuming that 5Hz frequency changes can be detected, mass uptakes as low as 0.06ng could be quantified. [22]

Detection Limit

To verify the proposed detection limit, a crystal coated with MIP particles was equilibrated in an acidic solution to extract caffeine and was then immersed in water. When a stable baseline was reached, 10ml of a solution at 10g/L of caffeine was injected in the beaker, so that the final concentration of caffeine was 0.1g/L. The frequency signal recorded is displayed on figure 78. One should note on this signal that as previously mentioned, injecting an aliquot of solution considerably improved the signal stability and consequently eased the frequency reading.
It is interesting to compare this signal with the one recorded when the crystal was immersed in the 10g/L solution. In the first case, the crystal was equilibrated again almost two hours after switching solutions. In contrast, about 30 minutes were enough for the sensor to equilibrate in this experiment. Also, one should note the difference in frequency shifts compared to that of the previous experiment. This experiment yielded a frequency shift of about 6 Hz which after calculations should account for a mass uptake of about 0.12 ng (table 40). This result is slightly above the previously estimated detection limit.

<table>
<thead>
<tr>
<th></th>
<th>Δt</th>
<th>C (g/L)</th>
<th>ΔF (Hz)</th>
<th>Mass bound (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>2 hours</td>
<td>10</td>
<td>350</td>
<td>4.14</td>
</tr>
<tr>
<td>Solution B</td>
<td>30 minutes</td>
<td>0.1</td>
<td>5.55</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 40: Computations of the mass of caffeine adsorbed on two MIPs sensors immersed in caffeine solution of different concentrations
It is interesting to compare at this point the previous mass computations with what would yield the Sauerbrey's equation. As mentioned at the beginning of the discussion, care must be taken in manipulating this equation. Table 41 points out a considerable difference between the computed mass values and suggests that the Sauerbrey's equation is not valid in the conditions of the experiment (liquid phase). Using the sensor as a fast analytical technique would thus require calibrating the QCM so that recorded frequency variations allow determining the analyte concentration.

<table>
<thead>
<tr>
<th></th>
<th>Theoretical mass bound calculated from bi-Langmuir</th>
<th>Theoretical mass bound calculated from Sauerbrey's equation</th>
<th>ΔF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution A</td>
<td>4.14 ng</td>
<td>2.48 μg</td>
<td>-350Hz</td>
</tr>
<tr>
<td>10g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solution B</td>
<td>0.12 ng</td>
<td>40 ng</td>
<td>-5.55 Hz</td>
</tr>
<tr>
<td>0.1g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 41: Comparison of the frequency and mass shifts calculated from the bi-Langmuir model and the Sauerbrey's equation
CONCLUSIONS

This dissertation focused on the development of nanoparticles for the detection of caffeine in water. In a first time, several routes for the synthesis of imprinted nanoparticles were explored. Aqueous miniemulsion polymerization was found to be an inappropriate synthesis method to imprint polymers with polar template. The dispersion medium tends to compete with the recognition monomer and prevents formation of specific binding sites. In contrast, precipitation polymerization in acetonitrile allowed imprinting nanoparticles with caffeine. The synthesis and extraction methods were devised to optimize the polymer rebinding capacities. Namely, removal of caffeine from the binding sites was found to be more efficient when operated at high temperatures. Furthermore, increasing the concentration of template in the formulation allowed creating more high-affinity binding sites. However, comparison of the site distributions of the nanoparticles and a bulk polymer suggested that the high level of dilution used during the precipitation polymerization limited the formation of specific sites. Consequently, the imprinted nanoparticles were found to have slightly inferior binding capacities to that of the bulk polymer.

The second part of this dissertation was centered on the applications of imprinted polymers. The polymer prepared by bulk polymerization was packed in a short HPLC column and was found to be able to separate caffeine from one its...
analog. It was thus considered as a good candidate for chromatographic applications.

In addition, two new analytical techniques based upon imprinted nanoparticles were devised. An HPLC technique allowed injecting a latex equilibrated with a caffeine solution and separating the nanoparticles from the caffeine free. Based upon the polymer adsorption isotherm, the initial concentration of caffeine can be determined for any sample without drastic sample preparation. The method was successfully applied to caffeine and benzotriazol samples. Simulations of a co-adsorption experiment forecasted that only in the low-concentration range would the polymer be able to bind more caffeine than the competitor if the latter is the major component of the sample. Unfortunately, a propagation of errors in the experimental procedure and the computations did not allow concluding about the validity of the co-adsorption model.

The possibility to develop a sensor consisting of an imprinted polymer as a recognition unit and a quartz crystal microbalance as a transducer was also examined. The experimental procedure was optimized to limit the frequency changes due to mechanical oscillations, temperature changes or viscosity variations. The final procedure allowed observing a stable frequency signal for several hours. Low to high concentrations of caffeine were tested on a sensor. The resulting signal pointed out a slow response time, most likely due to the dense nanoparticles structure formed on the sensor. Computations of the theoretical mass uptake suggested that the well-known Sauerbrey's equation
was not valid in the conditions of the experiment. The analytical technique would thus require calibrating the instrument.
RECOMMENDATIONS

Future work could focus on optimizing the precipitation polymerization synthesis to prepare a polymer with binding capacities similar to that of the bulk polymer. Increasing the concentration of template might allow enhancing the polymer binding capacities. The effect of the recognition monomer proportion in the formulation should also be explored.

Concerning the applications of the imprinted polymer, one should work on improving the HPLC application. The experimental procedure devised should allow fast quantification of an analyte without severe method optimization and sample preparation. To do so, one should focus on evaluating the current model using a competitor different than benzotriazol. A compound with a UV spectrum radically different than that of caffeine should be sought. Expounding on that, a diode array detector could be used as a replacement of the simple UV lamp. Both modifications should allow improving the quality of the experimental data and comparing the developed model to the actual adsorption data.
LIST OF REFERENCES


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APPENDICES
APPENDIX A: DERIVATION OF THE HETEROGENEOUS BINDING MODEL

Caffeine free = C
Caffeine bound = C_{bound}
Caffeine bound in specific - site = CG
Caffeine bound in a non - specific site = CB
Specific site = Go
Non - specific site = Bo
Specific sites unoccupied = G
Non - specific sites unoccupied = B

\[
C + G \overset{K_{\text{specific}}}{\underset{C \cdot G \cdot K_{\text{specific}}}{\rightleftharpoons}} CG
\]
\[
C + B \overset{K_{\text{nonspecific}}}{\underset{C \cdot B \cdot K_{\text{nonspecific}}}{\rightleftharpoons}} CB
\]

\[
K_{\text{specific}} = \frac{CG}{C \cdot G}
\]

\[
K_{\text{nonspecific}} = \frac{CB}{C \cdot B}
\]

\[
Go = G + CG
\]
\[
Go = \frac{CG}{K_{\text{specific}} \cdot C} + CG
\]
\[
Go = CG \cdot (1 + \frac{1}{K_{\text{specific}} \cdot C})
\]

\[
Bo = B + CB
\]
\[
Bo = \frac{CB}{K_{\text{nonspecific}} \cdot C} + CB
\]
\[
Bo = CB \cdot (1 + \frac{1}{K_{\text{nonspecific}} \cdot C})
\]

\[
C_{\text{bound}} = CG + CB
\]
\[
C_{\text{bound}} = \frac{Go}{1 + \frac{1}{K_{\text{specific}} \cdot C}} + \frac{Bo}{1 + \frac{1}{K_{\text{nonspecific}} \cdot C}}
\]
APPENDIX B: RECIPES USED FOR THE PRECIPITATION POLYMERIZATIONS

Mjb-45

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<table>
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<tr>
<td>DIGLYME (mL)</td>
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</tr>
<tr>
<td>Methacrylic acid (μL)</td>
<td>100</td>
</tr>
<tr>
<td>EGDMA (μL)</td>
<td>500</td>
</tr>
<tr>
<td>Caffeine (g)</td>
<td>0.2348</td>
</tr>
<tr>
<td>AIBN (g)</td>
<td>0.0330</td>
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Mjb-51

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<tr>
<td>EGDMA (μL)</td>
<td>1167</td>
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<tr>
<td>Caffeine (g)</td>
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<tr>
<td>AIBN (g)</td>
<td>0.0261</td>
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Mjb-52

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<tbody>
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<td>Methacrylic acid (μL)</td>
<td>235</td>
</tr>
<tr>
<td>EGDMA (μL)</td>
<td>1167</td>
</tr>
<tr>
<td>Caffeine (g)</td>
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</tr>
<tr>
<td>AIBN (g)</td>
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</tr>
<tr>
<td>Component</td>
<td>Quantity</td>
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<td>---------------------------</td>
<td>----------</td>
</tr>
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<tr>
<td>ACN (g)</td>
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<tr>
<td>EGDMA (µL)</td>
<td>1167</td>
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<tr>
<td>Caffeine (g)</td>
<td>0.5699</td>
</tr>
<tr>
<td>AIBN (g)</td>
<td>0.0298</td>
</tr>
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</table>
APPENDIX C: MATHEMATICAL DEFINITION OF THE STATISTICS

EMPLOYED

Mean $\equiv \mu = \frac{\sum_{i=1}^{n} x_i}{n}$

Variance $\equiv \sigma^2 = \frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n}$

Standard deviation $\equiv \sigma = \sqrt{\sigma^2}$

Relative standard deviation $\equiv RSD = \frac{\sigma}{\mu} \times 100$