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INVITED REVIEW PAPER

Engineering molecularly imprinted polymer (MIP) materials: Developments and challenges for sensing and separation technologies

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Abstract–Molecular imprinting, artificial receptors, plastic antibodies are terms associated with synthetic materials capable of chemical and biological sensing. Through the years, these sensors have advanced greatly not only in analytical chemistry; they have high utility for environmental, health, security, military, etc. monitoring and separations applications. New transduction methods and miniaturization have enabled *in-situ* and real-time sensing capabilities. On the other hand, they have high utility as matrices for chemical and biological separations. The challenge of employing molecularly imprinted polymers or MIPs as receptors lie in demonstrating high selectivity and sensitivity. Robustness and cost are also important considerations. Traditional methods of monolith polymerization employing free radical polymerization mechanisms have yielded good performance but lack the ability to demonstrate repeatable selectivity and sensitivity. Thin films have been deemed to be more useful in sensing applications, but may not offer the right throughput for separations applications. Engineering optimized materials require not only adapting to new chemistries but also knowing their structure-property relationships.

Key words: Molecular Imprinting, Sensing, Separation, Extraction, Film

DEFINITION AND BASICS

A Molecularly Imprinted Polymer (MIP) is simply a synthetic polymer that utilizes a template molecule that is removed afterwards, leaving complementary cavities or specific interactions behind (Fig. 1) [1]. The material is not limited to a polymer but perhaps inorganic materials (sol-gel or metal oxides) [2] and may as well be referred to as molecularly imprinted material (MIM). These materials then exhibit a degree of chemical affinity for the original template molecule. Several possible interactions, such as hydrophobic interactions, H-bonding, van der Waals forces and electrostatic interactions determine the spatial arrangement of monomers around the template. Polymerization and/or cross-linking fix these structures into place enabling stability of the arrangement. The method can be used to fabricate sensors, catalysts, and separations solid-support [3]. The components in a molecular imprinting process include: 1) template, 2) functional monomer, 3) crosslinker, 4) initiator, 5) porogenic solvent, and 6) extraction solvent.

1. Biological Mimicry

The inspiration for MIP is the lock-and-key mechanism in enzymes and natural receptors mimicking perhaps the endocrine system response of most living organisms [4]. For example, many basic biological processes, from sensing of odors to signaling between cells, rely on such lock-and-key combinations. In a way, the complement with biologists and endocrinologists is that while they are involved in identifying the chemicals or endocrine disrupting chemicals (EDC)s playing locksmith, and searching for the right key to fit a particular receptor, MIP scientists and materials engineers work on making the locks themselves, artificially that is. Analogously, drug or pharmaceutical research has the goal to find alternative chemicals that

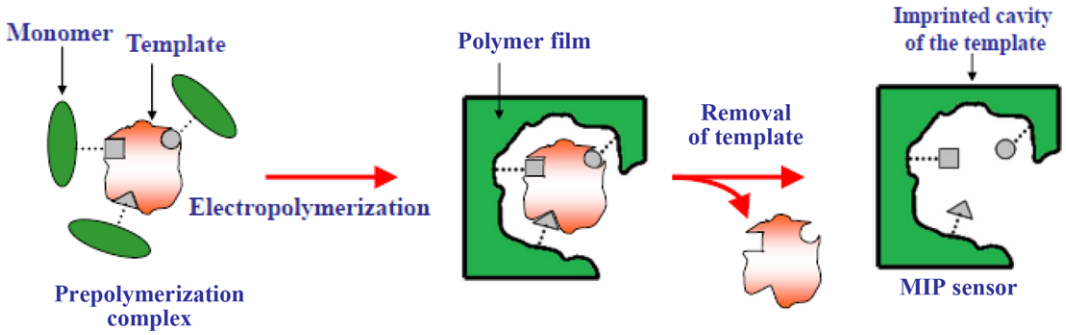

Fig. 1. Schematic diagram of a general molecular imprinting polymerization (MIP) process that can be employed.

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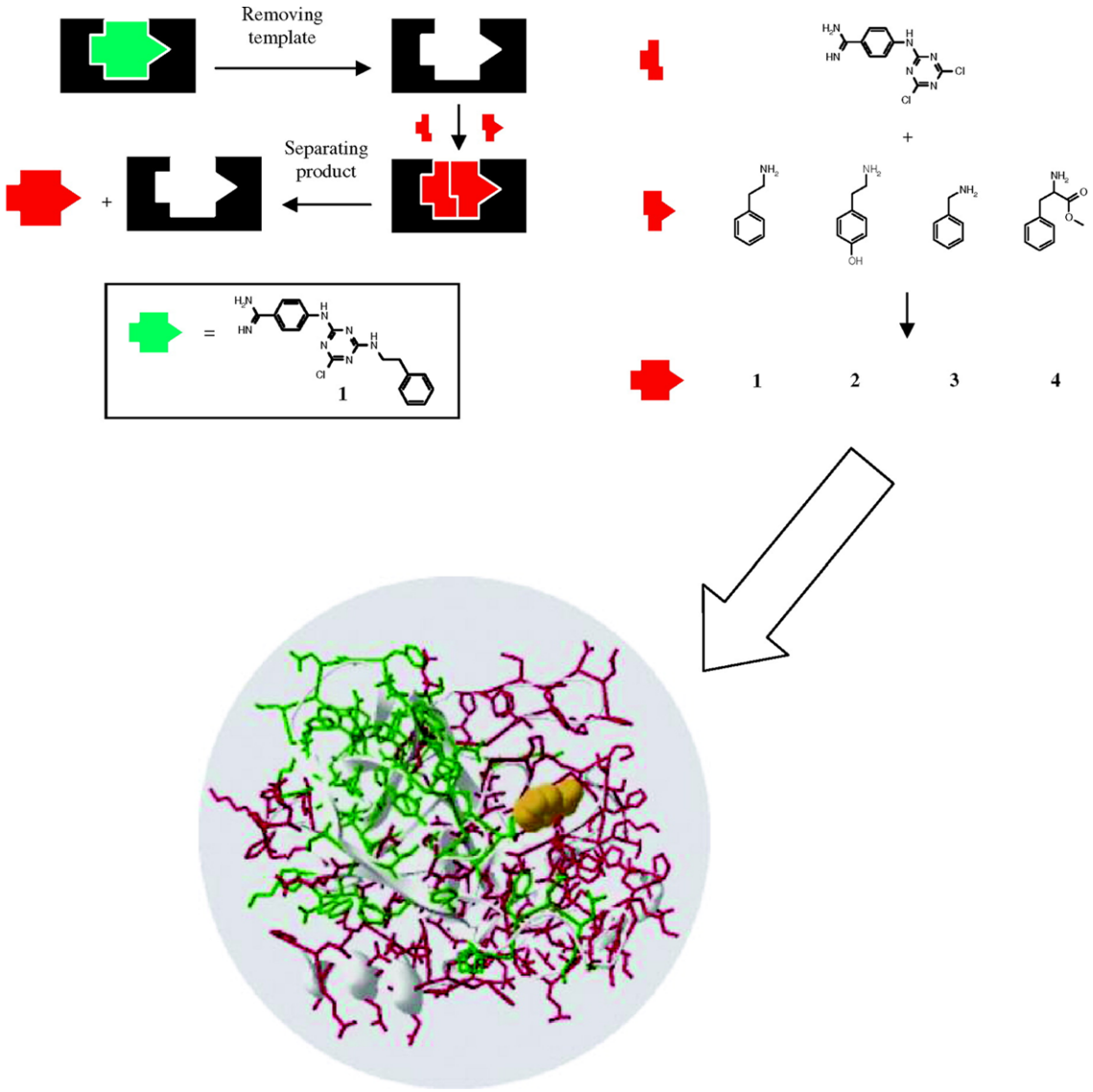

Fig. 2. Shows the generation of new enzyme inhibitors using imprinted binding sites which shows removal of the template (in green) from the MIP (in black) where the binding cavity was used to direct the assembly of reactants (in red). A new inhibitor (3) showed Kallikrein inhibition activity similar to that of the original template (1). Where the enzyme's active site is blocked by an inhibitor (in yellow) (REF. 7).

can elicit a biological response on natural receptors towards the treatment of diseases, MIP scientists can artificially mimic the action of a drug by building a synthetic chemical receptor for it [5]. One can see that a great advantage of artificial receptors over naturally occurring receptors is the ability to design new molecules or use new synthetic methodologies on demand. Also the ability to adapt these two solid support matrices enable better analysis. Compared to natural receptors which require DNA-based information or proteins, a variety of chemical components (e.g., organic or inorganics) can be used. This gives MIP the stability, flexibility, and other properties that can be selectively modulated by materials design. Even functional groups that are not found in nature can be employed to create synthetic guest-host systems. Lastly, it is possible to apply external fields (irradiation, temperature, pH change, electric or magnetic field, and others) to extend their utility or stimuli-responsiveness [6]. It is indeed possible to develop a totally new artificial system not even found in nature. In one example, the generation of new enzyme inhibitors using imprinted binding sites has been demonstrated based on the direct assembly of reactants where a new inhibitor showed Kallikrein inhibition activity similar to that of the original template (Fig. 2) [7]. In a "direct molding" to mimic an active site of an enzyme - Kallikrein was studied and a stereospecific inhibitor obtained allowing the constituents to interconnect in the active site. This approach is appealing for finding new drugs [8], in particular if not much is known about the biomolecule (enzyme) in question.

RECENT REVIEWS

The interest in the technique of MIP has increased rapidly in academia and in industry. The history of the MIP method can be traced to the 1930s experiment by Polyakov [9]. Key developments by

Table 1. Summary of some of the recent reviews on MIP

General review on molecular imprinting strategies	Ye and Mosbach [16]; Mayes and Whitcombe [17]; Wei and Mizaikoff [18]; Alexander et al. [19]; Holthof et al. [20]
Reviews focusing on imprinting biological molecules, drugs, therapeutics, proteins, macromolecules, and cells	Picci [6]; Conchiero [8]; Hillberg and Tabrizian [21]; Verheyen et al. [22]; Bergmann and Peppas [23]; Janiak and Konas [24], Piletsky et al. [25]; Turner et al. [26]; Reddy [38]
Different chemistries and analytical methods	Haupt et al. [27]; Walcarius et al. [28]; Byrne et al. [39]; Miyata [37]
Applications in separation and purification methods	Wulff [12]; Turiel et al. [29]; Augusto et al. [30]; Kist et al. [31]; Row et al. [34]

Mosbach [10], Katz [11], Wulff [12], Andersson [13], Peppas [14], Shea [15], etc. have been reported through the years. Many methods and attempts have been developed. Much progress has been made in utilizing new materials, polymerization methods, substrates (transduction) format to produce MIP materials with very good binding properties in monoliths, beads, films or nanoparticles. This review is not meant to be comprehensive compared to recent reviews but is designed to introduce and highlight the potential and recent challenges of the field in engineering new materials. There have been many excellent and recent reviews that are accessible to the reader. These reviews are summarized in Table 1.

THE PROTOCOL FOR MIP

The MIP process is essentially a phenomenon of creating macromolecular memory. In general, this rely on two synergistic effects: (i) cavity formation - shape specic cavities that images the template molecular shape stabilized in a crosslinked (or gel) matrix, and (ii) interactions- chemical groups oriented to elicit several non-covalent complexation points with the template. Non-covalent forces of interaction may also be observed in non-crosslinked structures but they tend to be weak and destroyed by heating, e.g. DNA de-association of H-bonds upon reaching melting temperature. Crosslinking is a form of polymerization forming networks or even interpenetrating network (IPN) structures. MIPs rely on a controlled degree of cross-linking which can predetermine selectivity for a single molecule or a group of structurally related molecules (selectivity is important). In a covalent approach, the monomer and template are covalently bound. In non-covalent imprinting, selectivity is introduced by designing the complexing monomer to surround or include the template analyte during cross-linking.

What then constitutes an ideal molecular imprinting?

1) *Complexation-Dissolution:* Dissolution of a template molecule in a solvent, together with one or more of the functional and complexing monomers. This complexation depends on the degree of complementarity of the chemical functionalities in the template and the monomer. The solvent properties e.g., dielectric constant and miscibility for both is important. Simulation or the application of quantum mechanics may be important to predict optimum monomer-template combination.

2) *Polymerization-deposition:* the template-monomer complex in the presence of an initiator and crosslinker or simply a polymerization protocol, results in the incorporation of the template monomer to form a matrix that is deposited or precipitated as a monolith, particle, or a film. These different polymerization techniques can include bulk, precipitation, emulsion, suspension, dispersion, gelation, and multi-step swelling polymerization. One can add electropolymerization, surface-initiated polymerization, and supramolecular assembly methods.

3) *Removal of Template:* A very important and if not un-optimized procedure in many cases is the removal of the template. Often a variety of factors have to be taken into account including the miscibility of the matrix to the solvent, surface energy, hierarchical porosity, interpenetration of structures, and surface area. Problems with incomplete removal of the template can lead to "bleeding" or generation of false-positive results in sensing or poor separation methods.

4) *Establishment of Analytical Figures of Merit.* An important aspect for establishing the validity of method includes kinetics of binding (or capture and release), limit of detection, dynamic range, and throughput (combinatorial). It has been largely demonstrated that MIPs offer the highest selectivity when samples are dissolved in the solvent used for the MIPs preparation. As most MIPs are synthesized in organic solvent, MIPs seem to be well adapted to the clean-up of complex matrices on organic solvents. A perennial problem though is the lack of affinity for sensing and separations in aqueous systems.

5) *Other factors.* This includes: a) robustness of the materials against a variety of temperature, pressure, and solvents, b) adaptability to surfaces and devices is a problem for sensing, c) reproducibility of data based on un-optimized protocols and d) cyclic stability and reusability.

MONOMER AND TEMPLATE COMPLEX PAIRING

It is important that the tandem monomer-template complex or concept be understood and optimized prior to MIP protocol preparation. While MIP can be divided into a covalent and a non-covalent approach, the non-covalent approach is advantageous in that it does not require the use of post-MIP reactions to remove the template but can simply involve solvent washing.

What are the important parameters for template design and monomer selection?

1) *Analyte.* Identify components of an analyte that results in effective interaction with the monomer in the solvent or media to which the polymerization occurs. Thus not all analytes may have the necessary non-covalent interactions and may be chemically derivatized covalently to allow MIP.

2) *Monomer and polymerizability.* Choose monomers that are likely to form strong interactions in a chosen solvent or exhibit cage effects. This will increase the capacity and influence homogeneity of the binding cavities. It is also important that the monomer be tailored towards a particular polymerization process (solution, bulk, or even

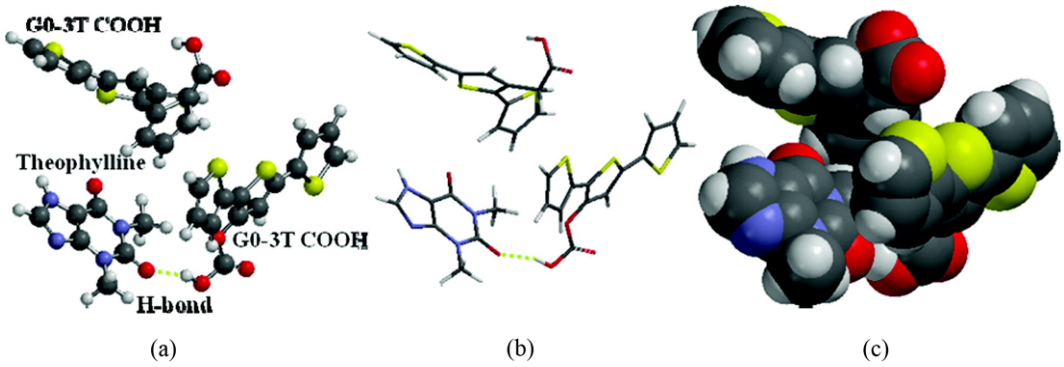

Fig. 3. Importance of complexation is demonstrated in 2D optimized structures (ball and spoke (a), tube (b), and space filling (c) models) of the prepolymerization complex between a polymerizable monomer and theophylline template (Spartan, Wavefunction Inc., semi-empirical AM1 quantum calculations). Note color representation of elements: carbon (gray), hydrogen (white), nitrogen (blue), oxygen (red), and sulfur (yellow).

electropolymerization). Modeling can be used to optimize the ratio and composition of the monomer-template complex even with simple semi-empirical quantum chemical methods (Fig. 3).

3) *Solubility and stability of the complex.* Choose templates and monomers that will be soluble in the porogenic solvent to be used in the polymerization process. This cannot be taken for granted since dissociation of the complex due to poor solubility can result in a poor MIP protocol with non-homogeneous distribution of cavities. One should ensure that the template-monomer mixture is stable and does not undergo side reactions under the polymerization conditions.

4) *Porosity and removability.* Ensure the porosity of the matrix from which the analyte will eventually be extracted by selecting the proper crosslinking monomer—a range of di- or tri-unsaturated crosslinking monomers with varying chemistries are available to create the porous organic network material. The analytes has to be able to go in and out of the cavities. For separations, this is important as throughput is a primary consideration. The template should be removable to a high degree, i.e. up to ppb levels if the MIP is to be used to clean up trace-level compounds. This should enable it to have more quantitative properties and demonstrate reusability. The use of a redox potential on a conducting polymer MIP matrix to improve template removal was recently highlighted by the Advincula group in a recent publication [32].

5) *Molecular cross-reactivity.* Sometimes, it is necessary to use an analog of the desired template molecule especially if the original template is unstable, non-soluble, or is not efficiently removed. The problem with incomplete removal of the template is that of "bleeding" or generation of false-positive results. One can use this concept to prepare an MIP with one template molecule that is not only selective for that molecule but also for other target molecules with a similar three-dimensional orientation of interacting functional groups. This is analogous to the "pharmacophore" concept in drug design, and for separations, a "selectophore". This means that the device is useful for a host of other molecules yet to be synthesized and has valuable applications in high throughput combinatorial synthesis methods and drug development.

POLYMERIZATION PROTOCOLS

It is important to focus on applying the right polymerization protocols. The monolith approach is by far the most common way to prepare MIPs and is also the simplest. This is often based on "bulk" or solution polymerization due to its versatility and the commercial availability of common monomers. It is mainly used exclusively with organic solvents and consists basically of homogeneously mixing all the components (template, monomer, solvent and initiator) and subsequently polymerizing them. The resultant polymeric block is then pulverized, freed from the template, crushed and sieved to obtain particles of irregular shape and size between 20 and 50 μm which contain the templated cavities. In principle, depending on the target (template) type and the final application of the MIP, MIPs can be prepared in different formats such as nano/micro spherical particles, nanowires and thin film or membranes. Thin films in particular are very important for sensing. For vinyl or acrylate polymerization, the most common materials for MIP synthesis are mainly based on the use of methacrylic acid monomer (MAA) or vinyl pyridine monomer (VP). Crosslinkers include divinylbenzene or diacrylate derivatives. Other polymers have been developed for better optimization of MIP selectivity. It is possible to do a fishing expedition for the best combination of components. High-throughput synthesis and evaluation of large groups of MIPs has recently been demonstrated by Sellergen et al. [10]. In this case, a library of polymers can be prepared in a very short time and a complete evaluation of binding properties assessed on each combination. This technique can lead to rapid optimization of MIP synthesis for the extraction and analysis of analytes within a matrix but may not be as useful for sensors. To make the process more targeted, computational method for a custom synthesis of MIP can be used to optimize the process. This can be based on a molecular modeling software that enables energy minimization of a particular complex. This then leads to a library of functional monomers and crosslinkers that can be designed and screened against the template to identify the best monomers and composition ratio for an MIP process.

APPLICATIONS

Due to a number of possible applications, it is worthwhile summarizing first into two types prior to listing the challenges in this field:

1) *Sensing and monitoring*. By far, a more relevant mimick to

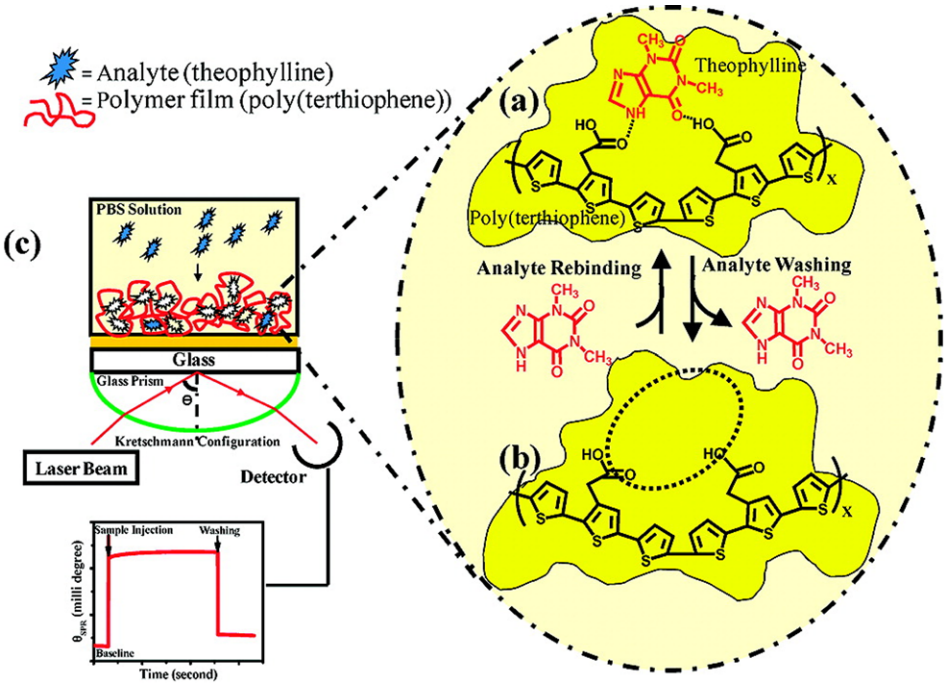

Fig. 4. Sensing of theophylline demonstrated via surface plasmon resonance spectroscopy (SPR): (a) Molecular imprinting of the template. (b) Formation of cavity after washing the template. (c) SPR setup for sensing of the template (REF. 30).

the lock and key analogy in enzymes. This can require a simple yes or no answer to determine the presence of a particular analyte. Quantification may not be the main goal but can be obtained as a matter of calibration. More importantly, it is the ability to distinguish from interfering non-analytes that optimizes the application, i.e. sensitivity combined with selectivity. For example, the use of surface plasmon resonance (SPR) sensor is an important transducer for achieving high sensitivity and selectivity in sensing (Fig. 4) [32,33]. Another is the development of artificial receptors or antigen-antibody interactions. The monitoring of banned substances and quantitation of pharmaceutically active compounds and their metabolites in blood, serum, or urine is a challenge. It can be *in-situ* or *ex-situ*. Likewise, the monitoring hormones and growth promoters in biological and environmental matrices can be problematic with the presence of non-specific interference. It is important that for these types of compounds, the extremely low detection limits set by various organizations and monitoring agencies match the length of time needed to utilize current spectroscopic and chromatographic analytical detection methods. This is an interesting growth area and necessitates alternative and on-site techniques that can deliver information in real time.

2) *Extraction or Separations.* An important application of MIPs is separations and isolation methods (either for sample isolation, improved detection, or concentration enhancement). This can involve liquid-liquid extraction and solid-phase extraction methods. Some limitations include extraction of polar pharmaceuticals from aqueous solutions and the presence of a complex matrix or media containing many different chemical classes. These factors often compromise isolation of the desired molecules and results in poor performance. The optimization of an MIP for extraction also allows for rapid, simple, and near-quantitative analysis of analyte. Compared to liquid-phase extraction in a solid-phase extraction (SPE), one needs to estimate the capacity value corresponding to the maximum amount of a compound retained on the MIP for a particular procedure (Fig. 5) [34]. The determination of a saturation curve is often necessary which is obtained by loading constant volumes of a sample spiked with increasing amounts of target analyte. The amount of extracted analyte can be reported as a function of the amount of analyte present in the percolated sample. A plateau corresponding to the saturation of binding sites is eventually observed.

A fast and cost-effective MIP technique has many potential ap-

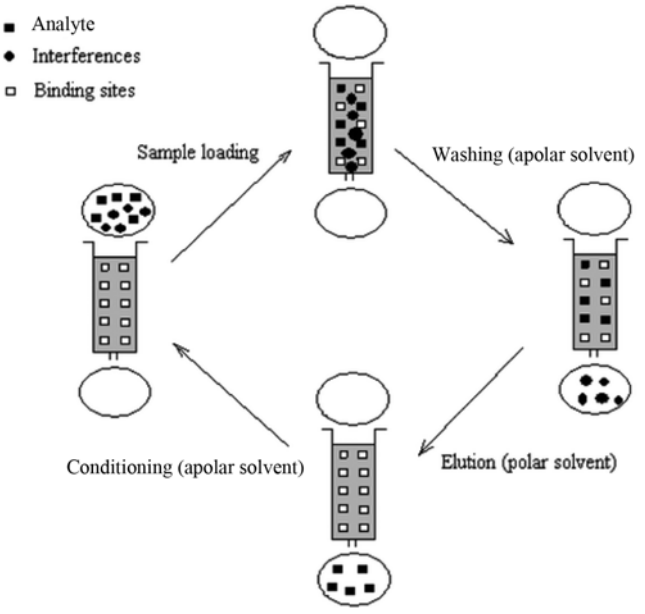

Fig. 5. Schematic procedure of molecularly imprinted solid phase extraction (REF. 34).

plications in the fields of chemistry, biology and engineering, particularly as: affinity material for sensors, detection of banned chemicals, separations in manufacturing processes, adsorbents for solid phase extraction, chromatographic stationary phase, catalysis, binding assays, artificial antibodies, and drug development and screening. It should be noted that despite all the successes in demonstrating the robustness of the MIP for specific applications in literature and in patents, the successful commercial demonstration of portable sensors, integrated monitoring, process scale separations, and analytical chromatography are still wanting. Other specialized applications of MIPs in the future include microfluidic devices, circulating medicinal or plastic antibodies, strip sensors, and field-effect transistor devices.

NEW CHALLENGES AND DIRECTIONS FOR MIP

1. Solid Phase Extraction Technologies

The most readily demonstrable commercial application of MIP perhaps is in selective solid-phase extraction (SPE) methodologies. This is a very useful method for the clean-up and the direct selective extraction of trace level of compounds from various complex matrices. This includes: 1) environmental pollutants and EDCs, 2) trace molecules from pathogens, 3) pharmaceutical drugs and combinatorial synthesis, etc. There are a number of academic researchers and start-up companies that synthesize MIPs for new selective extraction methods of various molecules. MIP Technologies AB of Lund, Sweden, has made commercially available custom made SPEs based on MIP. The MIPs are synthesized with vinyl or acrylic monomers and generate selective interactions based on H-bonding or electrostatic attraction. There are important challenges though. A primary limitation is the nature of selective interactions or phases that take place during the extraction in which it is important to match the organic or aqueous solvent, i.e. dielectric constant or polarty. This requires a comprehensive investigation into the retention mechanism to optimize the extraction procedure. The method can be documented for very common compounds or family of compounds. These MIPs should find utility in high throughput analysis methods as well. *The use of combinatorial screening is certainly an efficient tool to develop more selective MIPs with new monomers.* Also MIPs can have a high potential in miniaturized system like microfluidics. This includes the coating of solid-phase microextraction fibers and in electrochromatography.

2. Challenges for Protein and Large Biomolecule MIPs

There is much interest recently towards imprinting larger biomolecules or biotemplates. This is relevant for the monitoring of diseases or pathogenic infections based large on proteomics and genomics. The difficulty is that MIP as a technique has been primarily developed to create articial receptors around small template molecules. However, adapting the methodology toward MIPs for selective recognition of proteins, DNA, viruses and bacteria is very challenging. Some of the important considerations include: proper monomer selection, optimized washing method/template removal, and the quantication of the rebinding and reproducibility. The biggest problem is perhaps porosity or transport of analyte for removal and rebinding studies. Monoliths are not optimized for this consideration since many sites will be inaccessible if deeply located within the matrix. The use of detergents commonly used for template removal, can lead to experimental artifacts, and must be avoided. Although, the use of charged monomers can lead to strong electrostatic interactions between monomers and the biotemplate, this can also lead to undesirable high specic binding or even denaturation. Thus with most template rebinding studies of large biomolecules, they are unreliably quantied and the results may lack statistical validity. Strong electrostatic interactions between monomers and template can lead

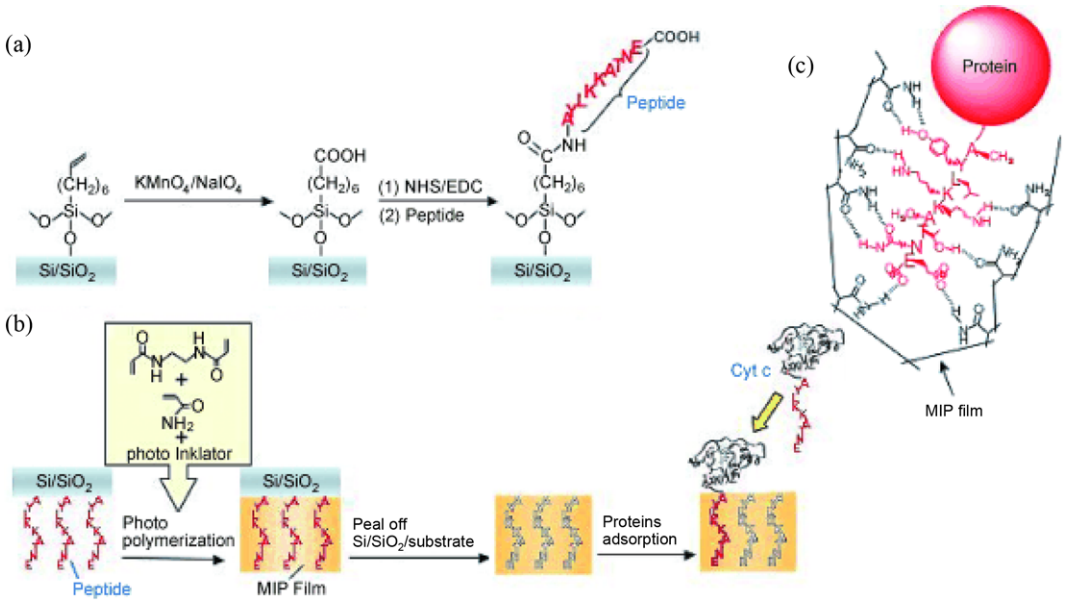

Fig. 6. Protocol for template imprinting with protein epitopes. (a) Method for glass modification and peptide attachment. (b) Illustration outlining MIP film fabrication and evaluation and (c) Proposed mechanism for the target protein recognition of C-terminus peptide-sequence-imprinted surfaces. A nano-cavity-bound target protein is caused by many cooperative weak interactions, involving hydrogen bonds and hydrophobic interactions (REF. 35).

to very high non-specic binding in which case, the use of charged monomers should therefore be considered carefully. Lack of consideration of factors such as pH and ionic strength can also complicate matters. In natural systems, for example high afnity between antibody and antigen or ligand and receptor, H-bonding is not the only primary consideration. Other non-covalent interactions such as electrostatic and hydrophobic interactions also play a major role. However, for imprinting of biomolecules in aqueous media, hydrophobic and electrostatic interactions may substantially contribute to an imprinting effect. For validation of a method, elemental or compositional analysis of the MIP could be used to rule out minute amounts remaining in the polymer. Kinetic measurements should always be employed to determine the time needed to reach binding equilibrium. The use of control non-imprinted polymers (NIP)s should ensure that changes in concentration correlate to binding on the MIP and not due to e.g. protein aggregation/adsorption. Reproducibility should also be conrmed with different batches. Perhaps for proteins the best approach should be in terms of the *epitope approach* in which a segment or peptide fragment of the protein which is prominently exposed naturally and can represent the binding of the whole protein synergistic with other surface or interactions "around" the protein (Fig. 6) [35].

3. Imprinting with Hydrogels

Although most of the demonstrated MIPs rely on highly crosslinked systems, macromolecular memory or structural plasticity of polymer chains can also be observed with weakly crosslinked imprinted networks. Taking advantage of stimuli response, molecular imprinting in hydrogels, creates memory for templated molecules within a exible macromolecular structure that responds to pH, ionic strength, and solvent changes [36]. Not only is it possible to improve binding parameters in hydrogels, it should be interesting to demonstrate field-effects or stimuli response [37]. It has been shown that imprinting in hydrogels leads to signicant improvements in template afnity, capacity, and selectivity over non-templated hydrogels for templates such as ions, small and moderate molecular weight molecules, proteins, viruses, DNA, and cells [38]. An important advantage of hydrogels is different levels of porosity (Fig. 7) [39]. Responsive imprinted hydrogels can also exhibit reversibly modulated template binding and transport. Compared to more rigid crosslinked systems, hydrogels has a number of advantages for controlled and modulated drug delivery, diagnostic sensors, and separation. In drug delivery for example, imprinting can lead to modulated transport and can provide further control of therapeutic transport with a multiple number of molecules. The basis of improved response compared to rigid crosslinked systems is not unwarranted. A certain degree of exibility has been observed in nature, where recognition occurs in a polar, protic, aqueous environment due to a diverse group of multiple non-covalent interactions. Biomolecular recognition, as exhibited by biological macromolecular structures such as enzymes, involves a highly specic recognition event where strong non-covalent bonding is due to the structural orientation of multiple differing chemical functional groups, complementarity, and conguration. This is usually a complex functional mechanism that involves the conformational reorganization or exibility of macromolecular counterparts. The *induced t mechanisms* and regulation of enzyme activity via *allosteric mechanisms* are examples of this flexibility requirement or trigger effects in which a molecule binds to a regulation site and

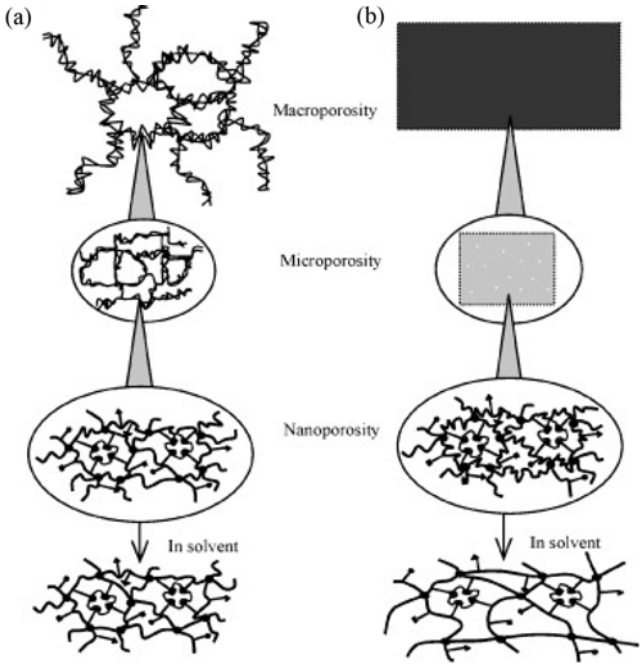

Fig. 7. Hierarchical porosity within imprinted hydrogels. (a) For templated gels prepared in solvent the polymer will contain significant macro- and microporosity. Template transport will be primarily related to the porosity and tortuosity within the polymer, as in conventional hydrogels. (b) For imprinted gels prepared without solvent, the polymer will not typically exhibit macro and micro-porosity and will have small pores that approach the size of the template (REF. 39).

subsequent reorganization results in controlled substrate binding at the substrate site. It can also include coupling of protein function by exible linkage of domains, e.g., immunoglobulins can functionally adapt to the variation of antigenic sites on surfaces. It is highly probable that the most specic recognition in nature occurs closer to that of hydrogels through the ordering of structures that have certain degrees of exibility. Recent progress in the eld of imprinted hydrogels should lead to exciting developments in the future and may be combined with nanomaterials. Responsive gels are not only for recognition but can exhibit "catch and release" function on template or drugs in a controlled mechanism. Thus, the application of these systems can find its way in pharmacy, medicine, tissue engineering, sensors and diagnostics, micro and nanodevices, and separation processes.

4. Imprinting with Electropolymerization

MIP is a highly viable method of preparing the recognition elements for chemical and biological sensors because of its high selectivity and sensitivity toward a wide array of analytes. Most of these sensors rely on metal-electrode transduction either by electrochemical, optical, or frequency (acoustic) methods. Robustness would allow for its use in various demanding applications with higher pH and temperature. *Direct electropolymerization* on surfaces is regarded as a most promising technique to interface the imprinted polymer layer on the surface of the transducer/electrode. Polymers which exhibit p-conjugation in its structure, commonly called conducting polymers, can be formed by the electropolymerization process due to the presence of electrochemically active sites for radical cation

coupling of its monomers. In contrast to the traditional methods of bulk/solution polymerization employing free radical polymerization mechanisms which yield monoliths, thin, electropolymerized imprinted polymer films can be obtained via this process. Thin films are more useful in sensing applications as film thickness is one of the factors that can affect efficient detection. Transduction techniques that can be viably coupled to these electropolymerized films can be more commercially attractive. Recently, picomolar sensing of a nerve agent analog was demonstrated on an electropolymerized precursor dendrimer by SPR [40]. Electropolymerized MIPs or E-MIPs require the use of specialized monomer and an optimized recipe. Thus, there can be a significant demand for the synthesis of the new and novel conjugated monomers for this type of application. Moreover, the application of such new monomers or cross-linkers for sensor applications must be thoroughly studied and modeled (quantum calculations and simulations). For one, issues pertaining to homogeneity of the surface must be given attention to ensure reproducibility and to minimize nonspecific interactions with the polymeric receptor. Reproducibility is important only to demonstrate that the system is more robust and analogous to enzymes and other natural biological receptors. Other issues for electropolymerization and optimization include: template-monomer matching of the electrochemical window, concentration template-monomer ratio, solvent quality, potential washing protocols for the removal of template and sensor recycling. The key issues are based on developing a sensing element that is optimized with the transducer. Other than optimizing the electrochemical conditions, a thorough investigation must be performed aiming to characterize the polymer-electrode/transducer interface. For the development of E-MIPs, one must also address the suitability of these for mass-production and for low-cost portable transducers. Though several studies have already shown the feasibility of introducing these directly onto the surface of mass sensitive transducers or conducting substrates, focus must be given to other modes of transduction which can adopt real-world sample measurement such as in field monitoring. Stability and robustness must also be considered in the design of integrated E-MIP/transducer sensor systems. The optical properties of new and novel conjugated polymers can also be exploited to develop fluorescent- or UV-based chemical/biological sensing. Fluorescent based biological sensors are considered one of the most sensitive methods of detection. In this case, the electrochemical method is simply used for materials preparation, e.g. on a fiber optic probe. Other types of transducers including QCM, SPR, waveguides, interferometers, etc. coupled with an electrochemical apparatus, can facilitate the in-situ deposition of the E-MIP film directly on the transducer surface. Hyphenated methods facilitate in-situ characterization of the film deposition as well. Equally attractive is the development of E-MIP based sensors that are tailor-made for the detection of metal ions for environmental monitoring or even for extraction purposes. Studies that will put more emphasis on demonstrating complete analytical protocols and portable devices based on E-MIPs can be a lot more interesting. The Advincula group recently emphasized the use of the E-MIP method in demonstrating high sensitivity and selectivity in SPR and QCM (Fig. 8) sensors [41].

CONCLUSIONS

The challenge of employing MIPs as artificial receptors lie in demonstrating high selectivity and sensitivity. Robustness and cost are also important considerations, but more importantly as sensors, they have to be reliable. Traditional methods of monolith polymerization employing free radical polymerization mechanisms have yielded good performance but lack the ability to demonstrate repeatable selectivity and sensitivity. They are more sufficient for separa-

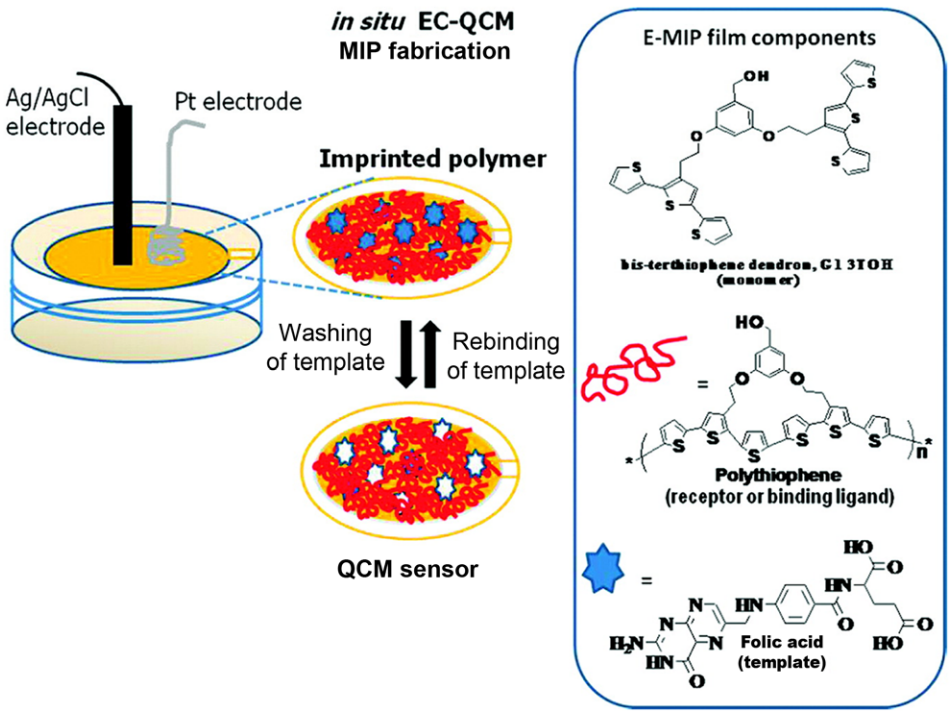

Fig. 8. Schematic illustration of the fabrication of a polythiophene-based sensor for folic acid using quartz crystal microbalance (REF. 41).

tion methods. Thin films have been deemed to be more useful in sensing applications, but may not offer the right throughput for separations applications. Engineering optimized materials require not only adapting to new chemistries but also knowing their structure-property relationships. The sensing and separation of proteins, DNA, cells, bacteria, and viruses will continue to be a challenge. However, advances are still being made. This has been demonstrated with new inorganic materials, stimuli-responsive hydrogel materials, direct electropolymerized thin film matrices, and in the future many such combinations incorporating nanomaterials (not highlighted in this review) should come out in literature and patents.

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