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Mimicking Biological Delivery Through Feedback-Controlled Drug Release Systems Based on Molecular Imprinting

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Abstract

Intelligent drug delivery systems (DDS) are able to rapidly detect a biological event and respond appropriately by releasing a therapeutic agent; thus, they are advantageous over their conventional counterparts. Molecular imprinting is a promising area that generates a polymeric network which can selectively recognize a desired analyte. This field has been studied for a variety of applications over a long period of time, but only recently has it been investigated for biomedical and pharmaceutical applications. Recent work in the area of molecularly imprinted polymers in drug delivery highlights the potential of these recognitive networks as environmentally responsive DDS that can ultimately lead to feedback controlled recognitive release systems.

Keywords

molecularly imprinted polymers; drug delivery; feedback controlled release; hydrogels; environmentally responsive

Introduction

In the past few decades, the design of intelligent drug delivery systems (DDS) has been a focal point of scientists in the biomedical and pharmaceutical fields. Specifically, there has been a shift in research from conventional frequent high concentration dosages to the development of DDS that are able to respond directly to an individual patient's requirements.[1,2] These so-called feedback controlled DDS would be able to efficiently deliver a therapeutic agent by rapidly detecting and responding to a biological event, such as an elevated biomarker, even before symptoms are present. Systems of this nature would be an ideal therapeutic vehicle as they would ensure that the drug is released at the correct time (when the biomarker concentration is an elevated level) and at a nontoxic therapeutic concentration. Controlled DDS are becoming even more important because of the

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increasingly complex and powerful drugs and biopharmaceuticals that are being developed, many of which cannot be administered without a controlled dosage system.

One promising platform in this area is the use of molecularly imprinted polymers (MIPs) as they have the ability to selectively recognize a specific analyte, such as a biomarker, in the presence of other similarly structured molecules that may be in biological fluids. Originally used for the separation of small chiral molecules in chromatography columns,[3] MIPs have only recently been investigated for the use in biomedical applications. MIPs have the ability to be incorporated in a completely closed loop process—by not only being implemented as a diagnostic tool to sense the biomarker of interest but also employed as a therapeutic tool. One could envision an implantable device with an array of analyte sensitive MIPs which could be used to diagnose a variety of diseases with known biomarkers in each well in the array and having a swelling response to the biomarker of interest and deliver the desired therapeutic. Once the biomarker level is no longer elevated, the matrix would collapse and release would stop. These devices could even be tailored to each patient depending their risk levels for certain diseases. While the practical application of such systems is a long way off, the potential of MIPs in feedback regulated recognitive release systems is enormous.

Recognitive hydrogels as controlled drug delivery vehicles

Over the past several decades, hydrogels have been extensively studied as vehicles for controlled drug release.[4,5] Hydrogels are insoluble, cross-linked, hydrophilic polymer networks that have the ability to swell significantly in water. Because of this property and corresponding rubbery nature, hydrogels resemble natural tissue, and thus have been used in a variety of biomedical applications, such as in contact lenses, sutures, and in drug delivery and molecular recognition devices. The value of hydrogels in drug delivery can be attributed to the ability to tailor their swelling properties to control the diffusion of drug molecules through their networks. Depending on the functional groups present along the backbone of the polymer chains, a hydrogels' swelling and associated release characteristics are dependent on environmental conditions such as pH, temperature, ionic strength, or even analyte concentration (Figure 1).[5,6]

Natural recognition

Although MIPs are generally composed of synthetic organic polymers, specific recognition of a target molecule is not something that is novel as this occurs frequently in biological systems. For example, noncovalent interactions similar to those found in antigen-antibody and enzyme-substrate associations have been utilized by MIP networks for the specific recognition of a template molecule of interest. In addition, proteins themselves are heteropolymers that contain specific recognition capabilities for other biomolecules.

The unique amino acid residues of the protein direct its final conformation, thus, enabling the protein to form binding pockets specific to certain target molecules.[7] Several groups have successfully developed natural MIP systems by using proteins as the recognition unit.[8,9] Despite these successes and the fact that recognition in nature is far more specific, synthetic biomimetic MIP systems still hold several advantages over their natural counterparts. Namely, synthetic MIPs are straightforward to prepare, easy to customize to a

particular template or integrate into a drug delivery device or sensor, and are relatively stabile in harsh conditions.

Molecularly Imprinted Polymers

Molecular recognition or molecular imprinting is an emerging field of interest in which a polymer network is formed with specific recognition for a desired template molecule. In general, MIPs are prepared in a four step process. First, constituents are chosen based on the specific application. Functional monomers are chosen which exhibit chemical structures designed to interact with the template molecule (drug molecule of interest) via covalent or noncovalent chemistry. Also, the type and amount of cross-linking monomer is selected which provides structural support to the polymer network as well as help define the pore size for diffusion of the template in and out of the matrix. Once selected, these ingredients are dissolved, with the template, in an appropriate solvent.

Second, the prepolymerization complex is formed between the template and functional monomer which will form the basis of the specific binding sites. Third, the monomer mixture is polymerized, typically via a UV or redox initiated free radical polymerization reaction. Fourth, the template is removed which leaves a polymer network with stereo-specific three-dimensional binding cavities based on the template molecule (Figure 2).

Applications of MIPs

MIPs are simple and inexpensive to synthesize as well as are generally robust and stable, which make them suitable for a variety of industrial applications. Several excellent reviews have been written on molecular imprinting.[10–13] Applications of MIPs include analytical chemistry, sensors, and pharmaceutical applications.[12] In addition, several companies have been formed and are now selling products that include MIP-based technology.

Analytical chemistry is the most common application with subsets such as enantiomeric separations,[14,15] solid phase extraction,[16] catalysis,[17,18] artificial enzymes,[19] as well as antibody and receptor mimics.[20,21] For example, MIPs are ideal replacements for antibodies. Conventional lab practice to achieve specific protein entrapment in assays is dependent on antibodies which are expensive and often single use. Chemical sensors are another application of MIPs that have received significant attention.[3,22,23] One such application is the potential to have a single sensor equipped with multiple MIPs that can each specifically recognize an analyte.

A multifunctional unit like this could be helpful for environmental clean-ups or for the production of safe drinking water by sensing and removing contaminants in wastewater treatment.[24] Also, MIPs have been studied for the potential use in pharmaceutical applications, such as drug discovery, drug purification, or drug delivery.[24,25] MIPs can be helpful to pharmaceutical companies attempting to synthesize a novel therapeutic or a medicine similar to one already available by limiting the number of steps required in the screening of combinatorial libraries, which is typically employed to find promising candidates for further testing.

MIPs have been studied as potential platforms for biosensors, specifically via protein/macromolecular recognition. These biomimetic sensors, which incorporate synthetic elements such as imprinted polymers, are viable alternatives to biological entities (i.e. antibodies) because they can be designed to mimic biological recognition pathways while at the same time exhibit abiotic properties that are favorable, such as greater stability in harsh environments.

The ability to selectively recognize a specific protein in a complex solution (such as blood) would have many applications, including serving as a biosensor/biodiagnostic tool. Because of this, protein imprinting has gained a great deal of attention from the scientific community as several excellent reviews have been written on this area recently.[26–30] However, efforts to do so have achieved limited success because of the inherent properties of proteins and macromolecules. These include size, complexity, conformation, and solubility.[26]

Traditional imprinted polymers tend to be relatively dense networks (small pore sizes for diffusion of the template into and out of the matrix) to retain the binding sites created during polymerization. This creates a problem for large templates like proteins as they can become entrapped in the network after polymerization and make it difficult for the protein to diffuse back into the network subsequently, both of which result in inadequate recognition properties.

Unlike smaller templates, proteins are complex biopolymers composed of linear sequences of amino acids that present a large number of potential recognition sites. Different portions of a protein exhibit distinct chemical functionality. It is accepted that the greatest selectivity for a specific molecule occurs when the number of points of interactions is small but each interaction is strong. Because of the large number of potential heterogeneous binding sites, proteins lend themselves to having multiple weak interactions, which favors nonspecific binding.

The polymerization conditions employed during traditional imprinting procedures are often nonphysiological. This may denature proteins or force them into conformations different than those found in their natural environment, causing the binding sites to be specific to this alternate conformation. Therefore, when rebinding is attempted later under physiological conditions, specific recognition for the template is not observed.

Since solvents are used to dissolve all components before polymerization, one must be chosen such that it does not interfere with the template-monomer interactions. As a result, the majority of imprinting takes place in nonpolar organic solvents in an attempt to maximize electrostatic interactions, such as hydrogen bonding, upon which many systems rely for recognition. However, proteins are often insoluble and unstable in organic solvents. While most proteins are completely miscible in water, it is far from an ideal solvent for imprinting as it will compete for and potentially disrupt any hydrogen bonds that are formed between the monomer and template. In addition, using an aqueous solvent also limits the choices of monomers available because many of the common monomers used for imprinting are insoluble in water.

Recently, MIPs have been investigated as possible controlled drug delivery vehicles. MIPs would be advantageous for drug delivery for many reasons including the ability to sustain the release of a therapeutic agent, enhance the loading capacity, tailor the cross-linking type and amount, intelligently release the therapeutic by responding to the environment, and enantioselectively loading or releasing the eutomer (isomer of interest).[31] MIPs can help achieve sustained release because of the affinity of the template to the functional monomer thereby increasing the residence time of the drug within the body. If the drug has a narrow therapeutic window, MIPs as DDS can keep the plasma concentration below toxic levels while also above the minimum effective level (Figure 3). Traditional drug delivery, for these cases, requires pulsatile type delivery, in which frequent high concentration doses are required, thus, resulting in the possibility for toxic side effects. By optimizing the size and amount of cross-linker, the MIP could potentially act as a reservoir to slow the release of the therapeutic agent because of diffusion limitations.

In general, drug release profiles from MIPs have been relatively uninteresting as similar profiles could have been achieved with slight modifications to the control polymers. However, the potential exists for MIPs to incorporate intelligent drug delivery characteristics. In such a system, the MIP could act as a feedback controlled device that would sense an event in the environment, such as an overexpressed biomarker, and be able to deliver the drug while continuing to monitor its surroundings. When this biomarker is no longer elevated, it would respond by terminating the template release.

Types of monomer/template interactions

To create the specific recognition properties of MIPs, exploiting the interactions between the template and functional monomer(s) is of paramount importance. The monomer/template complex must be stable under reaction conditions but at the same time the bond must be easily broken for template cleavage and with no disruption to the network so that subsequent selective uptake is observed. There are three main types of interactions—covalent, noncovalent, and metal-ion.

The covalent approach, pioneered by Wulff and collaborators,[32–34] utilizes covalent bonds to link the template and monomer(s). This approach is advantageous as it should produce more homogeneous binding sites and display a very strong affinity between the monomer and template. However, the template removal is difficult because of the strong attraction, subsequent rebinding is slower because of the necessary formation of covalent linkages, and only a limited number of compounds can be imprinted with this approach (diols, aldehydes, ketones, amines, and carboxylic acids).[35] Common monomers used with this approach are boronic acid esters, ketals, disulfide bonds, acetals, and Schiff bases.[29] Wulff et al. synthesized highly cross-linked imprinted polymers for the racemic separation of several free sugars using 4-(vinylpheny1)boronic acid as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker.[32] Enantiomeric selectivity up to 2.4 was displayed by the imprinted polymers.

Molecular imprinting has received a significant increase in attention from the scientific community because of the widespread adoption of noncovalent imprinting which is a direct result of the introduction of organic polymers as an alternative to silica matrices.[12] The

noncovalent approach, first championed by Mosbach and coworkers,[36,37] is much more common than its counterparts. This is because of the ease of fabrication and template removal, rapid template rebinding since covalent bond formation is not required, and because most biological interactions are noncovalent. Unfortunately, this approach also has significant drawbacks. Namely, since noncovalent bonds are, on a per bond basis, 1–3 orders of magnitude weaker than covalent,[38] this approach generates a large number of heterogeneous and low affinity binding sites. The interactions commonly utilized are H-bonding, van der Waals, electrostatic, and hydrophobic. Acidic (i.e. methacrylic acid (MAA),[39] acrylic acid (AA),[40] and 4-vinylbenzoic acid[41]), basic (i.e. 2-(dimethylamino)ethyl methacrylate (DMAEMA),[42] 2-(diethylamino)ethyl methacrylate (DEAEMA),[43] 4-vinylpyridine[44]) and neutral (i.e. Acrylamide (Aam),[45] *N*-vinyl pyrrolidone,[46] and 2-hydroxyethyl methacrylate (HEMA)[47]) functional monomers are commercially available and have all been utilized to generate successful imprints of a variety of template molecules.[35]

Methacrylic functional monomers, however, are employed most often in MIPs for biomedical applications because their anionic acid pendant groups allow the template to be removed without harsh conditions. Frequently, two or even three of these monomers are included in a single system to improve upon the recognition observed with just a single monomer.[40,42,45] Often times, though, an excess of functional monomer is used in an attempt to increase the complexation with the template, which has been shown to lead to more nonspecific adsorption.[48] One important consideration with noncovalent imprinting is the solvent. As discussed above, solvents, especially those that are aqueous based, can interfere with the monomer-template complex.

Attempts have been made, recently by Whitcombe and coworkers, to combine the covalent and noncovalent approaches, in which the initial imprinting establishes covalent linkages between the monomer and template, and subsequent rebinding utilizes noncovalent interactions.[49,50] Whitcombe et al. attached cholesterol, the template, to 4-vinylphenyl using a carbonyl spacer.[49] After the polymerization, cholesterol was removed via base hydrolysis and the carbonyl group was lost, which left a phenolic hydroxyl group capable of noncovalent H-bonding with the template.

Lastly, metal coordination has shown promise as another viable imprinting platform.[51–53] There are potentially a wide range of functional groups that can be exploited through the donation of electrons with the strength of interaction varying considerably. In general, this approach consists of having a polymerizable ligand complexing with a metal ion, typically a transition metal, which in turn complexes to the template. In a recent article, Striegler et al., copper (II) was employed as the coordination metal for the preparation of carbohydrate recognitive networks.[52] However, research is limited in comparison to the other two interaction types.

Responsive release systems

Molecularly imprinted intelligent analyte sensitive hydrogel polymer networks have the potential to be used in feedback controlled DDS.[10,54] These include swelling induced delivery, loss of effective cross-link delivery, and delivery via artificial systems. While the

following examples are not MIP systems, they demonstrate the robust abilities of hydrogels in environmentally responsive drug delivery. In analyte induced swelling, systems have been designed in which a cationic hydrogel with an attached enzyme swells and releases a loaded drug as a result of a local pH change because of an enzymatic reaction caused by a free analyte binding to the enzyme. These systems have been applied for the feedback controlled release of insulin, in which the analyte is glucose, covalently attached enzyme is glucose oxidase (GOD), and the cationic hydrogel is DEAEMA.[55–57] When the concentration of glucose is high, a reaction with GOD occurs to locally reduce the pH below the pKa value of DEAEM, thus causing it to swell and release insulin. As the amount of glucose decreases, the enzymatic reaction subsides which reverses the pH change and the gel shrinks back to its original size preventing further amounts of insulin from releasing.

Secondly, in loss of effective cross-links, systems have been developed in which both pendent and free analyte compete for binding with a pseudo cross-linker protein. As the amount of free analyte increases, the effective cross-linked protein binds to it instead of the pendent analyte causing the network mesh size to increase and release of the drug occurs. As the amount of free analyte decreases, the protein will bind, once again, to the pendant analyte groups and effectively shrink the matrix structure. Once again, these systems have been employed as glucose sensitive hydrogels for the feedback controlled release of insulin.[58–61] In this work, HEMA based neutral hydrogels were prepared with concavalin A (Con-A) as the effective protein cross-linker and glucose as the analyte.[61] This study demonstrated another way to regulate the release of insulin based on the concentration of glucose in the bulk phase.

Along the same lines, Miyata et al. developed a reversible antigen/antibody responsive competitive binding system.[62] Instead of a protein, the system consisted of a rabbit antibody IgG as an effective cross-linker grafted into the polymer network. When free goat anti-rabbit antigen is introduced into the solution, it specifically binds to the antibody causing the network to lose this portion of its cross-linking, thus allowing the hydrogel to swell and release a therapeutic. This swelling response was also shown to be reversible by removing the free antigen from the solution.

The competitive binding approaches described above can be extended to developing systems that contain nonim-printed molecules (i.e. therapeutic drug) noncovalently attached to the polymer network. In such a system, when the imprinted molecule is introduced into the surrounding media it competes for these binding sites and the nonim-printed molecule is released as the network has a stronger affinity for the template. Once the concentration of the template decreases, the release will stop. Several studies have been conducted which employ this phenomenon.[63–65] In one such report, Sreenivasan developed a HEMA based MIP in which the rate of release of testosterone was dependent on the bulk concentration of the imprinted hydrocortisone with minimal release reported when hydrocortisone was not present.[63]

Thirdly, in the bound analyte induced swelling artificial system, the complexation of an analyte to specific functional groups along a polymer backbone can change the ionic character and thus the swelling of a hydrogel. Kataoka et al. have demonstrated this

phenomenon for insulin regulated release in response to glucose concentrations.[66] Their system has a swelling response to both glucose and temperature because of the glucose sensing moiety phenylboronic acid pendent chains attached to the temperature sensitive monomer backbone, poly(*N*-isopropyl acrylamide) (PNIPAAm). Phenylboronic acid compounds, which are known to form covalent complexes with polyols such as glucose, are in equilibrium between an uncharged and a charged form while in aqueous solutions and this equilibrium can be shifted to the charged (and more hydrophilic) form through such a complex. Therefore, an increase in the bulk concentration of glucose increases the fraction of total borate ions and causing an increasing the swelling. This investigation demonstrated not only a sharp transition in swelling degree based on temperature and glucose concentration, but also rapid on/off regulation of insulin release based on changes in glucose concentration.

While these novel intelligent release systems have been successful in regulating the release of a therapeutic agent, they could be further improved through the molecular imprinting process. Combining these concepts would not be trivial, however, doing so would be advantageous. MIPs could be employed as enzyme or antibody mimics or effective cross-linkers and replace their natural counterparts. In addition, the incorporation of MIPs in these systems would enhance the amount of drug that could be loaded into the hydrogel. This would allow the feedback regulated release elements to have a longer therapeutic lifespan.

Comparison of imprinting methods

Approaches to molecular imprinting can be classified into four categories—bulk, particle, surface, or epitope. Bulk imprinting, which consists of the synthesis of a macroporous monolithic film, is the most common and straightforward approach, especially in small template molecular imprinting. The general bulk synthesis method is detailed in Figure 2. Its major advantages are that the entire molecule is imprinted, thus, when it is removed after polymerization a three-dimensional cavity remains with which rebinding can occur in addition to the volumes of literature investigating this approach.

However, most bulk imprinting relies on a high degree of cross-linking which can create diffusional limitations, especially with large molecule templates. Sacrificing density of the polymer to facilitate transport will create flexibility in the network, and consequently cause the binding pockets to lose their three-dimensional orientation. Also, most bulk imprinting procedures involve crushing the film after polymerization and before the template removal steps to create more rapid recognition systems. This produces irregularly shaped and polydisperse particles[67] in addition to being time intensive, and having the potential to destroy binding sites.[68]

As a result, various studies have investigated the use of emulsion or suspension polymerizations to directly obtain micro-/nanoparticles.[69–72] This approach enjoys many of the same advantages as bulk (i.e. imprint entire molecule, relatively straightforward, and has some well-characterized procedures), while eliminating the need for crushing after polymerization.

The synthesis procedures for this approach are very similar to bulk imprinting, with the main differences being that the monomer/template mixture is at a much lower concentration in the solvent and the addition of surfactants or stabilizers. The more solvent added to the prepolymerization solution, the more porous the resultant polymer will become. If enough solvent is added, particles will form instead of a film as the polymer chains will terminate during the polymerization when they cannot find another chain with a radical upon which to grow.

Particle size, size distribution, and particle morphology can be optimized with the appropriate monomer concentration and surfactant/stabilizer.[31] However, in some cases residual amounts of the surfactants/stabilizers have been found in the polymer particles. In a recent study, Pang et al. synthesized polyacrylamide particles using an inverse suspension polymerization using bovine serum albumin, a common model protein, as the template.[71] Ethyl cellulose, the stabilizer, was dissolved in toluene to form the continuous phase into which the monomer solution (aqueous buffer solvent) was added. Binding studies on the resultant microparticles showed affinities of ~4 (amount loaded into MIPs vs. NIPs) and adequate selectivities over similar protein molecules.

The other two approaches (surface and epitope) have been utilized mainly for the imprinting of proteins or macromolecules. In surface imprinting, the template is partially imprinted on the surface of a bulk polymer which is placed on a support, thereby enabling easy access of the binding sites for the template. Therefore, it is not necessary for the template to diffuse through the network to be targeted and, additionally, it can be easily applied as a sensor. However, nonspecific binding increases while both selectivity and capacity decreases because only a portion of the template is imprinted for and thus, later bound. Several papers have recently investigated this approach.[73–76] Lin et al. spincoated albumin imprinted films onto quartz crystal microbalance (QCM) gold electrodes with different functional groups.[76] The binding studies showed excellent affinities and selectivities, despite the fact that the competing proteins were much smaller than the template. These results are among several others that show promise for surface imprinting especially for biosensor applications.

Lastly, epitope imprinting imprints for only a general moiety of the larger template molecule. This approach attempts to mimic the specific interactions between an antigen and antibody in which antibodies are ‘Y’ structures that recognize a specific arrangement of amino acids at the terminus, known as the epitope. This methodology is advantageous because specific interactions with a small fragment can minimize nonspecific binding, organic solvents can be employed in the polymerization (which is critical for protein templates as whole proteins are typically not soluble in organic mediums), and is inexpensive. In bulk imprinting, the entire molecule is used in the polymerization. Proteins and pharmaceutical compounds can be extremely expensive, so the ability to use short peptides or a protein or a general moiety of a pharmaceutical would be far more economical. In addition, this process could lead to a very broad range of therapeutic drugs or biologicals that could be loaded and release from a given polymer.

On the other hand, several drawbacks are present with this method. Namely, if not commercially available, synthesizing or purifying functionalized polypeptides may be

difficult, selectivity over structurally similar compounds will likely decrease, and successful epitope imprinting may not translate to its parent protein molecule. Several studies have been conducted to test this approach.[45,77–79] Nishino et al. imprinted thin films using polypeptides of three proteins.[79] Excellent affinities and capacities, ~7 for the cytochrome c imprinted films, were demonstrated by the MIPs over the control films. Also, highly specific binding was shown in the albumin imprinted films and when single amino acid substitution was tested under the same conditions, as almost no adsorption displayed by the albumin MIP. However, each nonapeptide was synthesized through a relatively complex process.[79]

MIP considerations

There are several important things to consider when designing an imprinted polymer. Obviously, one of the essential parameters is the capacity for specific interactions between the functional monomer(s) and template. As discussed earlier, covalent, noncovalent, and metal ion imprinting interactions are possibilities. One must be mindful of the strengths and weaknesses of each type and choose appropriately based on the desired template and/or potential application. Not only does one need to choose the appropriate monomer type, the monomer to template ratio must also be optimized. A ratio too small will not create enough binding sites while a ratio too high may create nonspecific adsorption.[48]

Another important consideration is the type and amount of cross-linking monomer in comparison to the functional monomer (Figure 4). The most commonly used cross-linkers are poly (ethylene glycol) (PEG) derivatives, especially those in biological applications, as they have been approved by the FDA for several medical applications.[80] Other cross-linkers commonly used in MIPs include *N*,*N*′-methylene bisacrylamide (MBA)[42,75] in polyacrylamide gels, Trimethylolpropane trimethacrylate (TRIM),[65,81] and pentaerythritol tetraacrylate[52,82] in metal-ion imprinting. The majority of MIPs rely upon a high degree of cross-linking, typically between 10 and 80 mol % (moles cross-linker/total moles of monomer and cross-linker),[29] to retain the three-dimensional orientation of the binding sites. Also, the length of cross-linker plays a key role in the pore sizes of the networks, thus the recognition ability and template diffusion.[42] In general, an increase in the amount of or decrease in the length of cross-linker leads to a decrease of the average molecular weight between cross-links, thus the mesh size. However, for use in drug delivery or protein imprinting, it is advantageous to prepare MIPs that are not densely cross-linked. In drug delivery, lightly cross-linked environmentally sensitive hydrogels can be employed which adsorb the template in its contracted state and release the template in its swollen state.[83]

Yet another paramount consideration is the solvent type, especially in noncovalent imprinting. As discussed previously, solvent interaction with the monomers, such is the case in using aqueous based solvents, will compete for H-bonds and ultimately yield a less effective recognition. Therefore, nonpolar organic solvents are favored in molecular imprinting. Commonly used solvents are chloroform,[63] dimethyl sulfoxide (DMSO),[45] dimethylformamide (DMF),[81,84] and tetrahydrofuran (THF).[85] Typically, the solvent used in the synthesis of the MIP gives the best results for the subsequent rebinding studies. Therefore, while aqueous solvents are far from ideal in terms of recognition, they may need

to be used in biomedical applications because the uptake and release studies should be conducted under conditions that simulate physiological fluids.

Lastly, the template removal strategy must be fully evaluated before a successful MIP system can be realized. If the template is not completely removed from the network, the number of possible binding sites in the loading studies is decreased as those sites are already occupied. The ease with which a template can be removed is directly related to the network mesh size in relation to the size of the template (Figure 4D) and the strength of the template-monomer complex, in addition to the type of solvent used in the removal process.

Recent studies verify template removal via HPLC or UV-vis spectrophotometry; however, on average, a few percent (<5%) of the template remains in the network after washing[12] and even up to 27% has been reported to be left in the network for protein templates.[42] It was originally thought that this few percent of the template was permanently trapped in the matrix, but reports from several years ago clearly show otherwise.[86,87] Even after exhaustive MIP washing strategies, these studies show that the template slowly leaches out of the network over a long period of time which can seriously effect the subsequent rebinding and release measurements. Nevertheless, it is important to consider the template removal strategy as the inability to remove the template before rebinding experiments will render the MIP to be less effective, regardless of how well the other considerations are addressed.

Important metrics

Depending on the particular application, there are several important metrics with which the effectiveness of MIPs is measured. In general, affinity (recognition of the template via the MIP over the control), selectivity (recognition of the template against other structurally similar ligands), as well as capacity (how much template is adsorbed per mass of polymer) are the most common.

As an intelligent drug delivery system, the release time of the template in the therapeutically relevant range-both how quickly the supernatant concentration reaches this level and how long this level is sustained-are of the utmost importance. For sensor or separation applications, the MIP must be able to quickly recognize the molecule of interest and also have a high selectivity even against enantiomers of the template.

MIPs as DDS

The first article reporting MIPs as a potential drug delivery platform was a study that investigated the template release characteristics of theophylline from a matrix consisting of MAA and EGDMA.[88] Theophylline, a methyl xanthine commonly employed for the treatment of asthma, has a narrow therapeutic window (30–100 μM) with toxic effects likely at concentrations larger than 110 μM. Theophylline is normally administered via an oral sustained release platform; however, more efficient systems would be desirable. The resultant bulk monoliths were crushed and packed into columns for the recognition and release studies. A selectivity of 18 was demonstrated for the template over the structurally similar caffeine. Release studies were conducted at various pH values[6–8] and template-loading concentrations (0.1–50 mg template/g dry polymer) with release profiles differing

between the imprinted and control, but an overall clear pattern was not shown. However, the slowest release characteristics were displayed at a pH = 7 and the lowest theophylline loading concentrations.

Since this study, many others have been published that attempt to use MIPs as DDS for sustained release. Several excellent reviews have been published in this area[10,31,54,89–92] but none since 2005. Because of this, this review focuses on the literature published since then.

Ocular drug delivery with MIPs

The ocular bioavailability of therapeutic agents administered on the surface of the cornea is only 1–7% of the applied dose because of lacrimation, tear turnover, and drainage.[93] Thus, to be effective, current dropwise administration necessitates frequent high concentration doses, which results in adverse side effects such as burning and itching and ultimately leads to decreased patient compliance. And, while drug soaked traditional contact lenses may be more comfortable than drops or ointments, without controlled release of the therapeutic the bioavailability remains low. Effective ocular drug delivery relies on enhancing the bioavailability of the drug by increasing the retention time on the cornea thereby limiting loss and minimizing the need for repeated administration. MIPs have the potential to fill this need by controlled and constant release of the therapeutic agent for extended periods of time. Several papers have been published for this purpose over the last few years.[40,47,94–99]

Recently, timolol imprinted hydrogels were tested, in vivo, for their effectiveness in sustaining physiological drug levels in rabbit tear fluids.[97] The contact lenses were prepared as previously reported.[94] Briefly, 50 mM MAA, 140 mM EGDMA, and 12.5 mM timolol (1:4 template:monomer ratio) were dissolved in the backbone monomer *N*,*N*-Diethylacrylamide (DEAA). Two control experiments were also conducted with the in vivo studies—eyedrops with same amount of template as the commercially available drops and with the same amount as loaded into the imprinted contact lenses were directly instilled into the rabbit eye. Loading studies revealed an affinity (ratio of timolol sorbed for imprinted to control) of ~1.6. The template was detected in the tear fluid for three times longer (180 min) in the rabbit with a MIP and two times longer in rabbit with a nonim-printed polymer (NIP) than either of the control eyedrop cases. Also, ocular bioavailability in tear film, as defined by AUC (area under concentration-time curves), was considerably higher for the imprinted gels as compared to the others, whereas no difference was observed between the NIP gels and the control eyedrops. These results not only suggest the possibility of using MIP as extended release vehicles, it also confirms that an increase in the dose of eye drops does not lead to sustained levels of drug in the tear fluid, in contrast, it may lead to more adverse effects.

In another study from the same group, Hiratani et al. investigated how different ratios of template to functional monomer (1:4 to 1:32) affect in vitro recognition and release of the template timolol from hydrogels containing *N*,*N* dimethylacrylamide and tris(trimethylsiloxy)silylpropyl methacrylate as the backbone monomers, MAA as the functional monomer, EGDMA as the cross-linker, and no solvent.[98] Additionally, different concentrations of functional monomer and cross-linker were studied (100–400 mmol for MAA and 150–600 mmol for EGDMA) keeping the ratio of monomer to cross-linker the

same for all cases. All of the MIPs showed affinities greater than unity, with the largest ~2.1 existing for the lower template/monomer ratios (1:16 and 1:32). The systems prepared with 400 mmol MAA and 600 mmol EGDMA and template/MAA molar ratios of 1:16 and 1:32 exhibited diffusion coefficients that were two orders of magnitude lower than the corresponding control gels with the complete release of timolol taking 72 hours. It is important to note that while the lower template/monomer ratio systems showed better affinity and extended release, the total amount of template released was about one-third that of the other systems, including the control. While the 200 mmol MAA/300 mmol EGDMA with 1:16–1:32 template/monomer ratio did not have as large of a discrepancy with the diffusion coefficients (they were ~0.5–1 order of magnitude lower than the control), the affinities were similar (~2.0) and the total amount of timolol released was the same as the control and was released over a period of 24 hours compared to 6 hours for the control.

These results are a significant improvement over those reported previously by the same group in which the types of backbone monomers were varied while keeping the amounts of functional monomer and cross-linker constant.[96] Affinities were between 1 and 2 and drug release was seen only over 8 hours. This is not surprising as the monomer-template interaction and cross-linker percentage are widely understood to be most important parameters that lead to the imprinting effect and subsequent sustained release.

In the latest paper from this group on this subject, a new model template, norfloxacin, was investigated as it shows relatively good intracorneal and intracameral penetration.[47] In this study, the imprinted polymers were prepared with HEMA as the backbone monomer, AA as the functional monomer, EGDMA as the cross-linker, and norfloxacin as the template with various ratios of template to monomer (1:2–1:16). The loading studies were conducted with different ratios of template to monomer (1:3–1:6), disc thicknesses (0.4, 0.9 mm), and loading solution concentrations of the template (0.025–0.1 mM). Affinities ranged from 0.85 to 2.46 with the higher values corresponding to lower loading concentrations, smaller thickness discs, and smaller template to monomer ratio (1:6) systems although these MIPs exhibited the smallest overall loading capacity. These systems also showed selectivities against timolol from 3 to 8 with the most optimal value at 1:2 ratio of template to monomer.

Release studies were conducted in simulated lachrymal fluid (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L $CaCl_2 \cdot 2H_2O$, pH = 8) at a temperature of 37°C. Significant differences in release profiles were demonstrated depending on the template to monomer ratio, although all of the hydrogels were able to sustain drug release over 1 day. The imprinted hydrogels prepared with higher ratios of template to monomer (1:3 and 1:4) showed the greatest ability to control the release as it was sustained for 2–5 days. As was the case with the recognition studies, the most dilute solutions of norfloxacin showed the best enhancement of release rate for the imprinted over the control with release rate constants up to 3.5 times larger for the nonimprinted. Interestingly, the release rate was shown to be independent of the disc thickness which is an indication that release is controlled by the affinity of the cavities rather than diffusion of the drug through the network.

In yet another ocular drug delivery study, Venkatesh et al. successfully showed enhanced affinity and extended release of the antihistamine Ketotifen fumarate via a hydrogel with

multiple functional monomers.[40] The best formulation was a polymer consisting of AA, Aam, *N*-vinyl 2-pyrrolidinone, and HEMA as the functional monomers with PEG(200)DMA as the cross-linker. This recognitive network demonstrated an affinity of six (recognition over control) and a three times enhanced loading over the systems that contained two or three functional monomers. Also, this network displayed a therapeutically relevant concentration of drug release of over 5 days which was much longer than the control.

In another study from the same group, Ali et al. designed a novel microfluidic device that simulates the ocular conditions to study to release characteristics of the same systems.[99] Under physiological flow rates, volume, and composition, the results showed that the drug is released independent of concentration or time (i.e. zero order release—see Figure 2) for approximately three and a half days at a therapeutically relevant concentration. This linear release is in contrast to the release seen from the infinite sink conditions reported in which a Fickian diffusion or concentration dependent profile was exhibited. However, it is important to note that these results should be taken as estimates since these studies were conducted under conditions with several differences from physiological conditions. Namely, the studies were operated at room temperature (25°C) instead of physiological temperature and the hydrogels were 3–4 times thicker than commercial contact lenses. Despite these discrepancies, the results are extremely promising for providing therapeutic medications at a constant rate for an extended time.

Transdermal drug delivery with MIPs

The ability to differentiate between enantiomers and facilitate the transport of the isomer of interest while blocking the transport of the unwanted isomer would be extremely beneficial as a drug delivery system. Molecularly imprinted polymers have been studied as a possible means by which to accomplish enantioselective intelligent delivery over the past several years by Suedee et al. using MAA and propanolol as the monomer and template, respectively.[100–105] *S*-propanolol is 100–130 times more pharmacologically active as a beta blocker for the treatment of hypertension than the other enantiomer, *R*-propanolol.[104]

Recently, MAA and EGDMA based MIPs and bacterially-derived cellulose membranes were integrated to form composites for the potential application as a transdermal delivery of *S*-propanolol.[104] Cellulose and its derivatives have been shown to have stereoselectivity to propanolol in chromatographic applications.[106,107] In this study, in vitro studies showed enantioselectivity of the S-isomer imprinted MIP as the binding of *S*-propanolol was reversible and fast enough to have a far greater diffusion (ca. 45 times more *S* than *R*) of the eutomer (isomer of interest) across the rat skin independent of the feed proportion of the two isomers. This is interesting because in most MIP systems, the template molecule is released slower because of the complexation between the template and polymer matrix.

In another study, transdermal patches were developed that included the same MIP composite system as discussed above for in vivo studies in Wistar rats.[105] A racemic mixture of propanolol was mixed with one of two gel reservoirs, chitosan and poloxamer, and situated next to a MIP membrane along with a backing support layer and release liner to prepare the patches. The chitosan gel patch allowed enantioselective delivery of approximately a 2:1 and 4:1 ratio for the release rate (flux) and cumulative amount permeated of *S* to *R*-propanolol,

respectively. These results are encouraging for the possibility of enantioselective drug delivery devices for chiral mixtures of pharmaceutical compounds.

Metal ion MIPs for drug delivery

As discussed earlier, metal-coordination is a potential interaction that can be utilized to create recognitive systems. One recent example using this approach successfully imprints for the metal-based nonsteroidal anti-inflammatory drug, copper salicylate, using 4-vinyl pyridine and HEMA as functional monomers and EGDMA as the cross-linker.[107] The MCEP, metal chelating embedded polymer, displayed a higher (factor of 1.3–2.1 depending on the monomer:template ratio) and more sustained release of copper salicylate against the control polymer for up to 5 days. Despite that these results are, in themselves, not groundbreaking, the fact that this paper imprints for a metal based drug makes this paper noteworthy because of the limited literature in this area of MIPs and the nature of metal based drugs. Namely, metal-based drugs frequently have specific requirements for delivery because these therapeutic agents can undergo substitution or redox processes before they perform their desired function and they are often more chemically reactive than organic therapeutics. Both of which makes the potential for adverse side effects even higher.

Moiety imprinting for drug delivery

In one recent study, Byrne et al. investigated the efficacy of intelligent drug delivery via biomimetic moiety imprinting.[45] In contrast to traditional molecular imprinting where the entire drug molecules are imprinted, the researchers were able to imprint D-glucose for the subsequent recognition of a larger molecule which contains a glucose moiety, 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-glucose (2-NBDG) using Aam as the functional monomer and PEGD-MAs of various lengths as the cross-linker. In this study, the effect of cross-linker length and cross-linker amount was investigated to see the impact on recognition ability and drug release times. Not surprisingly, tighter networks (i.e. those with higher percentage and/or shorter cross-linkers) had increased affinity, capacity, and release times. One interesting result was that for each set of imprinted and control hydrogels, the controls exhibited a longer release time of 2-NBDG as compared to the imprinted. This is in contrast to what is seen for the majority of other MIP studies—which show extended release with the imprinted polymers. This can possibly be attributed to the fact that the loaded and released drug does not have as high of an affinity since only a portion of it was imprinted.

This approach would have significant potential if molecular imprinting was to ever become industrially applicable because many pharmaceutical compounds are extremely expensive. Traditional molecular imprinting requires that entire drug molecule be used in the polymerization and then be completely removed to produce the specific binding sites. This entails losing the drug entirely or trying to separate it out of the wash solutions, both of which are not economical. In theory, this process can lead to a very broad range of therapeutic drugs that could be loaded and release from a given gel as long as each of the drugs has a common moiety.

Photoresponsive MIPs for drug delivery

In another potential platform for MIPs as drug delivery devices, Gong et al. investigated the efficacy of photoresponsive imprinted polymers using azobenzene based functional monomers.[81,108] The trans-cis photoisomerization of azobenzene causes significant changes to its geometry, which, in theory, could be used as an on/off switch for specific recognition. Minoura et al. have previously reported the use of photoresponsive imprinted polymers, although not for controlled drug delivery applications.[109,110] In the first study, 4-[(4-methacryloyloxy)phenylazo]benzoic acid (MPABA—functional monomer) and Trimethylolpropane trimethacrylate (TRIM—cross-linker) are mixed in DMF/Acetonitrile in a 6:1 cross-linker/monomer ratio with caffeine as the template.[81] Upon irradiation at 365 nm for 120 min, 58% of the previously bound template is release from the MIP and subsequent exposure to 440 nm light for the same amount of time loads over 96% of the template just released. This process is repeatable; however, the template uptake and release decreases gradually for each cycle and longer irradiation times are needed (~150 min.). The selectivity of the process against the structurally similar theophylline molecule was ~2.3 with no reversibility displayed for this competitor. Also, the control polymer did not have any response to the cycles of irradiation, with only a small amount of template nonspecifically bound in the initial phases of the study.

In the more recent study, different materials were selected.[108] Namely, 4-[(4-methacryloyloxy)phenylazo] benzenesulfonic acid (MAPASA—functional monomer) and *N*,*N*′-hexylenebismethacrylamide (cross-linker) were dissolved in DMF/DI water with Paracetamol, a common analgesic, as the template.[108] Upon irradiation at 353 nm for 120 min, almost 84% of the previously bound template was released from the MIP and subsequent exposure to 440 nm light for 90 min. caused over 94% of the released paracetamol to be readsorbed. Once again, this process was repeatable with a decrease in the uptake and release decreasing for each cycle. In addition to the quantitative advantage of this system over that of the previous, the uptake/release studies in this investigation were conducted in the aqueous HEPES buffer and not DMSO, as was the case in the previous studies. The selectivity against two competitor analogs was ~4 with very little photoresponse and the control polymer, once again, had no response to light outside of the initial nonspecific adsorption. These studies demonstrate specific recognition of two template molecules using photoresponsive polymers, which have the potential for intelligent drug delivery.

Temperature sensitive MIPs for drug delivery

Liu et al. have published two articles recently that investigate temperature sensitive imprinted polymers as another possible platform for intelligent drug delivery.[111,112] First, 4-aminopyridine (Apy—1:4 template to monomer ratio), a therapeutic used in the treatment of multiple sclerosis, was utilized as the template in MIPs with *N*-isopropyl Acrylamide (NIPAAm—87 mol %) as the temperature sensitive monomer, MAA as the functional monomer (4 mol %), and EGDMA as the cross-linker (9 mol %).[109] The majority of the network is made up of NIPAAm to obtain the desired temperature responsive result. NIPAAm has been well characterized as a component in temperature sensitive hydrogels for biomedical applications as it exhibits a large swelling transition near physiological

temperatures (called a lower critical solution temperature or LCST).[113–115] The loading studies were conducted at a temperature above the LCST where the gel was in its collapsed state to retain the binding sites while the release studies were held at a temperature below the LCST (swollen state) both at pH = 9.6 in an aqueous solution. Depending on the free template concentration in solution, affinities of 2–3 were reported with the larger values at higher concentrations of Apy; however, the capacities of these gels were much lower than traditional MIPs because such a large proportion of the network was not a functional monomer. Approximately 84% of the drug loaded was released in the MIPs (compared to 60% in the control) and this process was repeatable as the same amount was released for three subsequent loading/release cycles. Time of release was reported for neither the MIPs nor the NIPs.

Second, L-pyroglutamic acid (Pga) was imprinted for under nearly the same conditions with the only difference being slightly more cross-linker in the prepolymerization solution and a different solvent (Methanol instead of DMF).[112] The loading and release were conducted similarly, with the exception that the pH = 3.5. The affinity was slightly higher (~3 to 4) and similar results were obtained for the loading studies-88% of Pga loaded was released in the MIPs consistently over five cycles compared to 62% in the NIPs. Selectivity against a variety of structurally similar molecules was shown for the MIPs (~2.6 to 5.1), while none was exhibited with the NIPs (~0.9 to 1.3). Once again, time of release was not shown for either gel. While these systems show promise as drug delivery vehicles, more studies must be performed to validate these temperature responsive hydrogels as controlled release platforms.

MIPs in oral drug delivery

Puoci et al. have published several papers over the past few years for the potential use of MIPs as oral drug delivery devices.[69,84,116] To mimic the path an oral therapeutic might encounter when taken by a patient, the release studies were conducted at two conditions—simulated gastric fluid (pH = 1.0) for the first 2 hours after which sodium phosphate was added to simulate intestinal fluid (pH = 6.8). First, Spherical Molecularly Imprinted Polymers (SMIPs) were prepared using EGDMA cross-linked poly(MAA) with sulfasalazine, a common prodrug used in diseases of the colon.[69] The resultant imprinted microparticles displayed an extended release time (~18 hours, 80% release in 8 hours) compared to the control (~4 hours) to completely release the template.

Secondly, Puoci et al. imprinted for 5-fluorouracil (5-FU), a common anticancer agent, using the same network components as previously reported.[84] The ability to control the release of 5-FU is highly desirable since it is quickly metabolized in the body and causes severe adverse effects above the toxic level, thus currently requiring continuous administration and monitoring. In this study, three systems were synthesized which varied the crosslinker concentration (56–83 mol %) and template to monomer ratio (1:4 to 1:8) and evaluated in simulated physiological fluid (pH = 7.4) and as gastro-intestinal fluid (pH = 1.0 for t = 0–2 hours, pH = 6.8 for t = 2–30 hours). The optimal formulation was the system containing the lowest cross-linker (56 mol %) and template to monomer ratio (1:8). For this system, the affinity values ranged from 3 to 6, selectivities against uracil from 1.9 to 3.2, and

only 60% release of 5-FU from the imprinted gel in 30 hours whereas 100% of the drug was released in 8 hours from the control gel.

The most recent paper from the group once again used poly(MAA-g-EGDMA) polymers using α-tocopherol, one of several forms of vitamin E, as the template.[116] The recognition characteristics of the MIPs, 60 mol % cross-linker and 1:16 molar ratio of template to monomer, were tested in both organic (acetonitrile) and aqueous (ethanol/water 6/4 v/v mixture) solvents, while the release properties were studied, once again, in simulated gastro-intestinal fluid. The affinity of the networks ranged from 1.5 to 27 and the selectivities ranged from 2.25 to 3, with the higher values both taking place in acetonitrile. Once again, the MIPs displayed extended release of the template, 50% release in 4 hours and 100% release in 40 hours, compared to the control polymers (100% release in 4 hours). While these results are promising, in vivo experiments need to be conducted to verify not only the extended release of the therapeutic at therapeutically relevant plasma concentrations but also the efficacy of the systems in gastro-intestinal aqueous mediums.

Other MIP systems for drug delivery

In another study, TRIM cross-linked (30 mol %) poly(methylmethacrylate-co-MAA) nanospheres were imprinted with theophylline.[67] Commonly, MIPs are prepared as bulk monoliths which are subsequently crushed and sieved to a desired particle size range. Not only is this time consuming, it also generates irregularly shaped particles that are often times not monodisperse. This paper attempts to use one of the many polymerization methods that have been applied to preparing hydrogel micro-/nanoparticles, namely, precipitation polymerization where the monomers and template are highly diluted. The resultant imprinted nanospheres exhibited affinities ranging from 1.1 to 2.5 and a selectivity of ~5.1 against the structurally similar caffeine. However, the selectivity of the control samples were almost as high (~4.1) which most likely can be attributed to nonspecific binding. In addition, while the template was released over 7 days, 50% of the release occurred within 3 hours and ~95% of the drug was released in the first 2 days.

Supercritical fluids, especially supercritical CO_2 ($scCO_2$), have recently been identified as potential candidates in the development of novel clean processes for pharmaceutical applications.[117] One major drawback to the use of MIPs for drug delivery applications is that they are often synthesized using organic solvents. Any trace amounts of these solvents or the monomers, for that matter, can potentially be toxic to the patient. However, as mentioned above, competition for H-bonding in aqueous solvents limits the effectiveness of the systems. $ScCO_2$ based procedures have the potential to be used in pharmaceutical applications as they provide many advantages over traditional preparation of controlled release systems. These include that CO_2 is readily available, environmentally acceptable, nonflammable, it has relatively low critical constants (T_c = 31°C, P_c = 74 bar), and does not leave a toxic residue.[117,118]

In this study, Poly(diethylene glycol dimethacrylate) was prepared in $scCO_2$ in the presence of two templates, salicylic acid and acetylsalicylic acid, both of which are commonly used antipyretics.[118] The monomer, stabilizer, template, and liquid CO_2 were loaded into a cell and the completely homogeneous mixture was polymerized at high pressure, 19 MPa, for

three hours. After template removal, the polymeric matrix was impregnated by passing a saturated steam of template in CO_2 at 20 MPa. The affinities of the MIPs ranged from 0.7 to 3.2 depending on the template and ratio of template to monomer in the initial polymerization. Subsequently, release studies were conducted which showed that 90% of the template was released within 8 hours with no extended release shown in the imprinted polymers. In this initial attempt to synthesize MIPs using supercritical fluid technology, two weaknesses are present—the template must be soluble in $scCO_2$ in order for impregnation to occur and equipment suitable for working with supercritical fluids is required. Despite these drawbacks, the potential is high for the preparation of clean and environmentally friendly pharmaceutical processes including MIPs for controlled drug delivery.

In another recent study, Wei and Mizaikoff looked at heterogeneity of binding sites using the Freundlich binding isotherm methodology via three MIP formats for the same template molecule, 17β-estradiol: irregular shaped particles crushed/sieved from bulk polymerization, microspheres, and submicrospheres with the latter two synthesized via precipitation polymerization.[70] The most common strategy for estimating the binding parameters is a Scatchard plot, which assumes a bimodal distribution of binding sites. However, monomer/template interactions are commonly characterized by binding site heterogeneity and low average binding affinities. This study, on the other hand attempts to provide a more robust model using a more generic approach based on the Freundlich isotherm which describes the relationship between the concentration of bound and free molecules using an association constant and heterogeneity index from which the affinity distribution can be calculated and subsequently the number of high and low affinity binding sites.[119]

From the loading experiments, all of the MIPs displayed more homogeneous binding sites, with microspheres the most homogenous, than their corresponding controls, as displayed with the heterogeneity indices. Also, the MIPs exhibited several orders of magnitude higher association constants than their controls, with the microspheres, once again, being the optimal format. Subsequently, 70–80% of template release was seen within 2 hours with little difference in the release kinetics between the three formats. Calculation of binding site distribution showed that the number of low affinity sites is 20 times higher than the number of high affinity sites for the particles from bulk polymerization. And, despite the differences in the constants, the other two formats displayed very similar binding site distributions. The binding site distribution characterization used in this study has the potential to give a better understanding of the affinity complexes between a monomer and template to produce networks better able to recognize and intelligently deliver a therapeutic agent.

Conclusions

While MIPs have enormous potential as intelligent DDS, most of the literature to date has focused on extended release through the interactions between the functional monomer(s) and template which slow the diffusion out of the matrix. As it was highlighted in this paper, we believe that the potential exists for the field of MIPs to progress into feedback controlled and targeted drug delivery devices as well as biosensors. Not only could MIPs be a main component in intelligent drug delivery such as the case in induced swelling, loss of effective cross-links, and artificial systems, but MIPs could also act as a sensing/actuating element in

a closed loop drug delivery device. In each case, a high bulk phase concentration of the target analyte causes a reversible response to the network, usually swelling, whereby the therapeutic agent is delivered. Once the analyte is no longer in excess, the original response is reversed to prevent over-medication of the therapeutic.

In essence, systems of this nature are able to respond directly to an individual patient's needs and resolve it even before symptoms become present, thereby improving the patient's quality of life.

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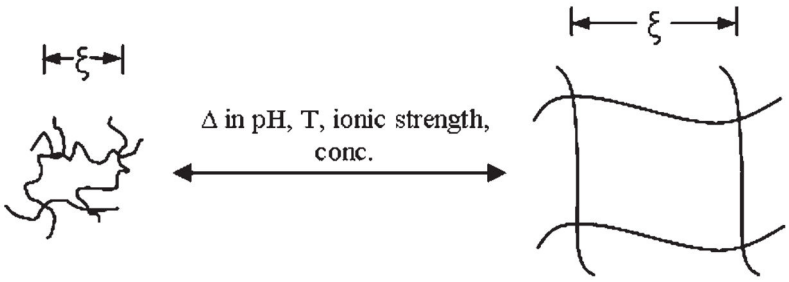

Figure 1.

Hydrogels can reversibly swell or contract because of changes in the environment, such as pH, temperature, ionic strength, or concentration.

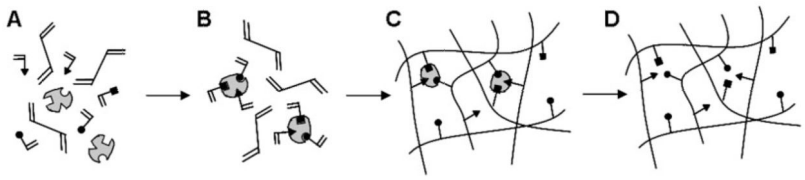

Figure 2.

(A) Solution mixture of template, cross-linking monomer, and functional monomers (triangles, squares, circles), (B) Complex formation between functional monomers and template via covalent or noncovalent chemistry, (C) The formation of the polymer network typically via free radical polymerization, and (D) Template removal step which leaves binding sites specific to the original template.

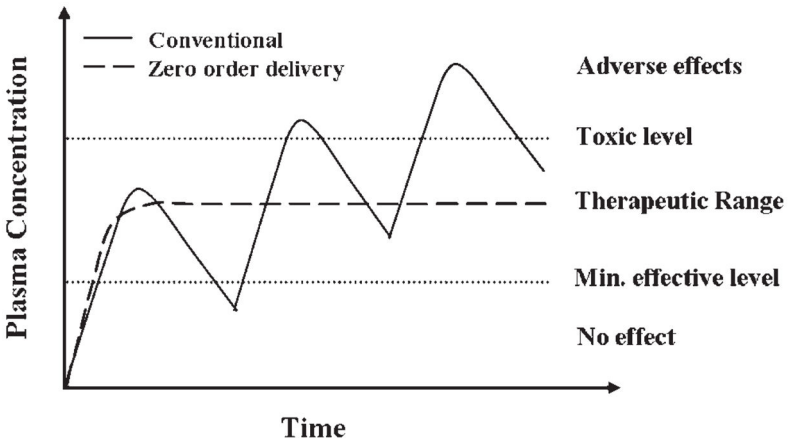

Figure 3. Conventional drug delivery (solid line) involves taking frequent high concentration doses of a therapeutic which often results toxic levels in the body

Zero-order controlled release (dashed line), on the other hand, delivers the drug, independent of concentration, in the relevant therapeutic range for extended periods of time without adverse side effects.

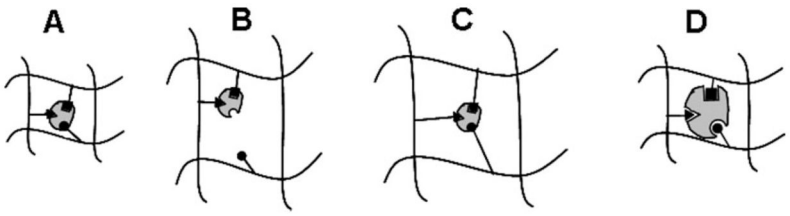

Figure 4.

(A) Appropriate cross-linker and functional monomer size and concentration, (B) Increase in cross-linker length without a change in functional monomer size, (C) Corresponding increase in functional monomer size to the increase in cross-linker length, and (D) Template molecule too large for polymer mesh size, which will create diffusion limitations both into and out of the network.