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Review

Molecular imprinting within hydrogels II: Progress and analysis of the field☆

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ABSTRACT

In the past decade, there has been an exponential increase in the number of papers describing molecular imprinting in hydrogels, a technique which creates memory for template molecules within a flexible macromolecular structure. Macromolecular memory or structural plasticity of polymer chains is a superior description of weakly crosslinked imprinted networks since significant flexibility can occur within the polymer chains. The focus of this article is to review and highlight work in the field describing the imprinting strategy within hydrogels and associated challenges, characterization methods of imprinted gels, current and potential translational applications, and future strategies and directions. This paper also describes ways to improve binding parameter efficacy and presents significant areas of opportunity to further describe, characterize, and understand imprinted gels. An analysis of the literature indicates that imprinting in hydrogels leads to significant improvements in template affinity, capacity, and selectivity over non-templated hydrogels for a number of templates such as ions, small and moderate molecular weight molecules, proteins, viruses, DNA, and cells. However, the influence of imprinting on the transport of template is much more complex, with little attention of most studies to structural analysis or discussion of the gel porosity/tortuosity in the control of template transport. Responsive, intelligent imprinted hydrogels are also highlighted that exhibit reversibly modulated template binding and transport. It is clear that this field has transitioned from infancy and is leading to breakthroughs in a number of areas such as controlled and modulated drug delivery, diagnostic sensors, and separation. For example in drug delivery, imprinting can lead to delayed transport and provides further control of therapeutic transport through the macromolecular structure as well as optimizes the number of therapeutic molecules to polymer chains.

Contents

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1. Introduction

In 2002, a review article entitled "Molecular Imprinting within Hydrogels" (Byrne et al., 2002a) was published, which described the tremendous potential of molecular imprinting techniques in the rational design, synthesis, and applicability of hydrogel structures. Back then, little research work had been conducted using weakly or moderately crosslinked imprinted structures to provide enhanced template binding, responsive recognition, or delayed template transport. The field was in its infancy and 6 years later we have seen significant research progress in this field, demonstrated by the exponential increase in the number of papers published in this period (Fig. 1). There has been a sustained and concerted effort toward the understanding and rational design of such systems, and a considerable increase in proposed applications involving imprinted gel structures.

The purpose of this paper is to examine work in the field since 2002, the year the last comprehensive review was published. In this article, we highlight the recent work in the field with a historical perspective, the benefits and challenges of the imprinting strategy within hydrogels, characterization methods of imprinted gels, responsive imprinted gels, current and potential translational applications, and future strategies and directions. Imprinted structures are leading to solutions of major problems in medicine and pharmacy and will soon provide significant value to address unmet needs in a number of fields.

For example, in drug delivery, the rational design of polymeric structures has led to a number of controlled release products. Molecular imprinting has high potential to provide an additional level of control in the rational engineering of drug delivery matrices. Further control of therapeutic transport through the macromolecular structure as well as optimizing the number of therapeutic molecules to polymer chains are leading to significant enhancements in drug loading and extended transport. This level of control has only been demonstrated recently within a small number of research articles and is expected to grow significantly in the future. This will be particularly important in reduced length scale applications with micro- and nanoscale carriers and devices.

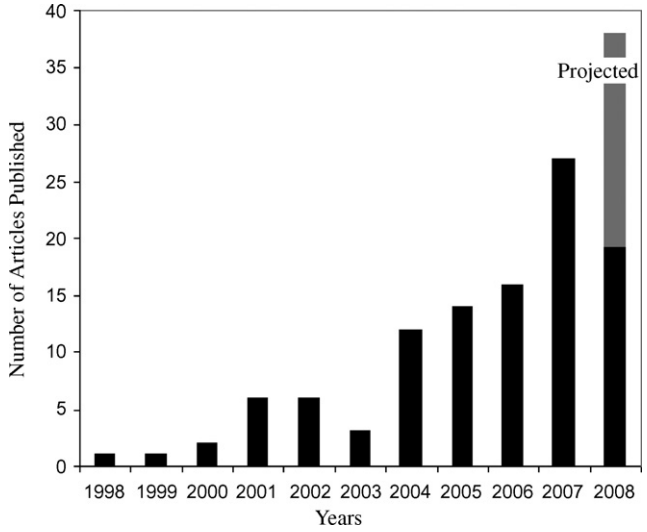

Fig. 1. Exponential increase in imprinted hydrogel research. There has been tremendous progress in the field since 1998. An exhaustive search of imprinted polymer papers was undertaken and only those published papers which describe weakly and moderately crosslinked imprinted gels with binding occurring in aqueous solutions were included in this chart. We have limited inclusion only to gels, where the main constituent is water or other biological fluid. Polymer gels, defined as networks of flexible polymer chains, can be deformed and respond as an elastic body. The projected value for 2008 was calculated by multiplying by a factor assuming the papers in 2008 continue at the same rate.

2. Creating macromolecular memory in hydrogels: molecular imprinting within flexible polymer chains

The concept of molecular imprinting involves the creation of macromolecular memory for a template molecule within a polymer network. There have been many excellent reviews on the topic and we direct the reader to more traditional imprinting strategy reviews (Ye and Mosbach, 2008; Wulff, 2002; Mayes and Whitcombe, 2005; Cormack and Mosbach, 1999; Wei and Mizaikoff, 2007; Whitcombe and Vulfson, 2001; Alexander et al., 2006; Davis et al., 1996), and reviews focusing on imprinting biological molecules, therapeutics, proteins, macromolecules, and cells (Byrne et al., 2002a; Hilt and Byrne, 2004; Ge and Turner, 2008; Hillberg and Tabrizian, 2008; Bergmann and Peppas, 2008; Wei et al., 2006; Zhang et al., 2006; Janiak and Kofinas, 2007; Bossi et al., 2007; Turner et al., 2006). Most important to the non-covalent technique is the inclusion of a template molecule that the polymer must form around that is not covalently incorporated in the polymeric structure. Effective self-assembly of the functional monomer(s)–template complex is crucial toward imprinting efficacy. Macromolecular memory is primarily due to two synergistic effects: (i) shape specific cavities that match the template molecule, which provide stabilization of the chemistry in a crosslinked matrix, and (ii) chemical groups oriented to form multiple non-covalent complexation points with the template (Fig. 2A). Since gel structures can have significant flexibility in the polymer chains as well as collapsible and expansive structures, it is very suitable to use the term macromolecular memory or structural plasticity of polymer chains when describing molecular imprinting in gels. This term is much more appropriate in weakly crosslinked imprinted networks as compared to highly crosslinked networks.

The majority of imprinted polymers produced to date have been highly crosslinked in efforts to limit the flexibility of the associated binding cavities produced between polymer chains. Thus, the idea of the technique translating to polymeric networks with significant flexibility within their polymer chains was highly suspect. It was assumed that flexibility of polymeric chains would lead to fatal deficiencies in the metrics by which imprinted structures are defined, namely template binding affinity, capacity, and selectivity. However, experimental work in the last decade has proven that this is not the case.

An analysis of the recent literature with focus upon imprinted hydrogels indicates that imprinting can occur in hydrogels with significant improvements in binding parameters over non-templated hydrogels (Venkatesh et al., 2007, 2006; Alvarez-Lorenzo et al., 2002, 2006a; Byrne et al., 2008; Hawkins et al., 2005, 2006; Kimhi and Bianco-Peled, 2007; Kublickas et al., 2007; Parmpi and Kofinas,

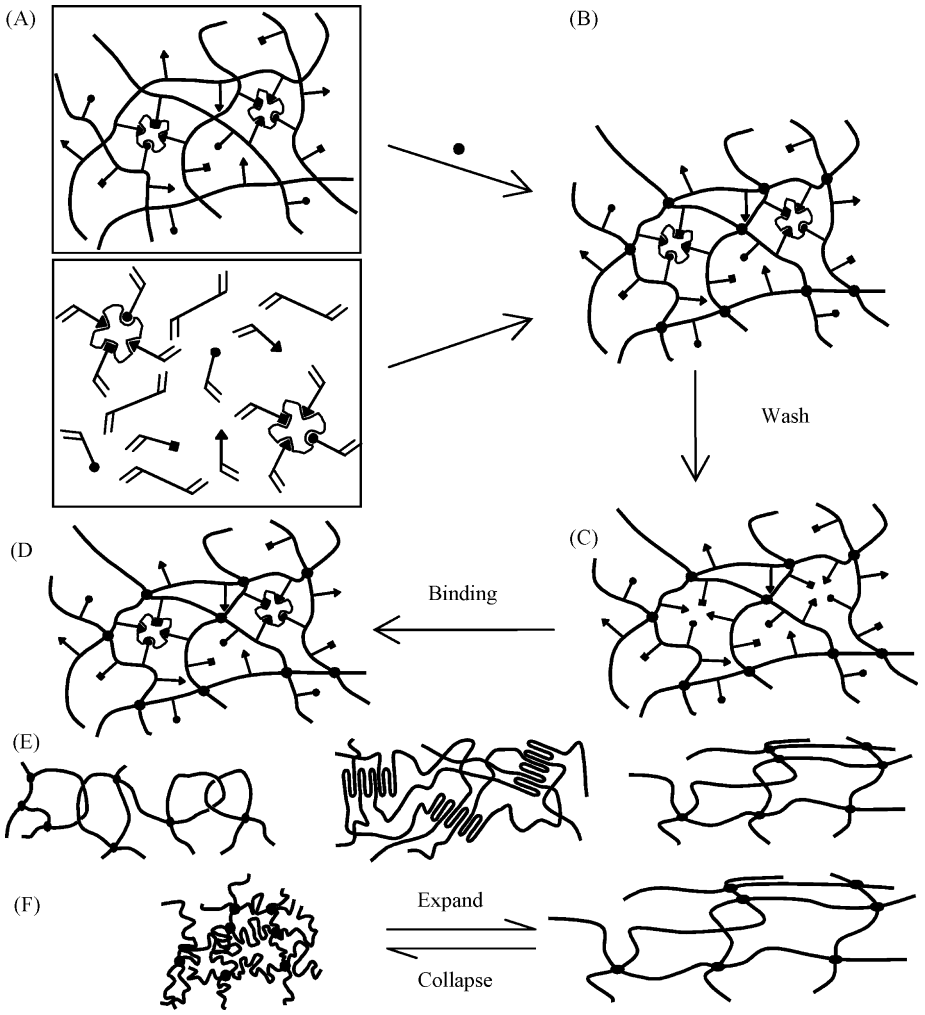

Fig. 2. Macromolecular memory within crosslinked hydrogels. (A) Self-assembly of the functional monomer complexes within the pre-polymerization solution. This can be in the form of monomeric species or oligomers/polymers that have pendant double bonds or are reacted to other chains by another molecule (●). (B) Formation of an idealized network structure (with or without solvent). (C) Wash step where template is removed. (D) Macromolecuar network with recognition sites consisting of functional chemistry on differing polymer chains. (E) Crosslinking can consist of (*left*) permanent physical entanglements, (*center*) microcrystalline regions incorporating various chains, and (*right*) covalent or non-covalent bonds. (F) Expansion and collapse of hydrogel structure as a result of stimulus or solvation of polymer chains.

2004; Xia et al., 2005; Espinosa-Garcia et al., 2007; Wizeman and Kofinas, 2001). From the literature, it is clear that increases in template capacity and affinity within flexible hydrogel structures can be tuned by the imprinting process. While there have been a smaller number of papers that highlight selectivity differences, or the enhanced affinity of the template over closely matched structures, it has also been demonstrated to occur in imprinted hydrogels (Hawkins et al., 2005; Kimhi and Bianco-Peled, 2007; Kublickas et al., 2007; Xia et al., 2005; Wang et al., 2008; Chen et al., 2008; Demirel et al., 2005; Liu et al., 2004a; Oral and Peppas, 2004, 2006; Bolisay et al., 2006, 2007; Guo et al., 2006; Ogiso et al., 2006). Of course, one must recognize that the difficulty of the selective challenge (i.e., how the size and chemical functionality of the competing molecule compares to the original template molecule) is a variable that must be benchmarked in defining selectivity limits. In highly crosslinked polymers, selectivity has been demonstrated between very similar structures, including enantiomers (Maier and Lindner, 2007; Chen et al., 2001a). To date, imprinted hydrogels have not been shown to have this level of selectivity control, and selectivity depends on the level of flexibility within the structure.

A certain degree of flexibility can be seen in nature, where recognition occurs in a polar, protic, aqueous environment due to a diverse group of multiple non-covalent interactions. In most cases, biomolecular recognition, as exhibited by biological macromolecular structures, involves (i) a highly specific recognition event where strong non-covalent bonding is due to the structural orientation of multiple differing chemical functional groups, complementarity, and configuration; and (ii) a recognition event where recognition is usually a constitutive element of a complex functional mechanism that involves the conformational reorganization or flexibility of macromolecular counterparts (Demchenko, 2001; Huber and Bennett, 1983; Blackburne and Hirst, 2001; Lemieux, 1996). Examples of the functional roles of flexibility in protein systems are induced fit mechanisms and regulation of enzyme activity via allosteric mechanisms, in which a molecule binds to a regulation site and subsequent reorganization results in controlled substrate binding at the substrate site. It can also include coupling of protein function by flexible linkage of domains (e.g., immunoglobulins can functionally adapt to the variation of antigenic sites on surfaces) (Huber and Bennett, 1983). Therefore, it is highly probable that the most specific recognition in nature occurs through the ordering of structures that have certain degrees of flexibility.

Much of the theoretical basis of the conformational memory of biological macromolecules and designed heteropolymers

(Demchenko, 2001; Huber and Bennett, 1983; Blackburne and Hirst, 2001; Lemieux, 1996; De Gennes, 1986; Bryngelson and Wolynes, 1987; Pande et al., 1994a,b, 1997, 2000; Jozefowicz and Jozefonvicz, 1997; Peppas and Huang, 2002) can be applied to the concept of molecular imprinting. Macromolecular memory is favored by heteropolymer systems. Typically, one type of functional monomer will not provide optimal interactions between the polymer chains and the template (i.e., depending on the chemistry of the template molecule, each functional monomer will have preferred and more energetically favorable interactions with certain chemical groups on the template). Imprinting can organize the incorporation of monomers within the growing polymer chains in a low energy state conformation that favors multiple point complexation with the template. During network formation, increasing the potential for growing polymer chains with template binding complexes to reach a global energy minimum will lead to increased memorization of the chain conformation and enhance template binding parameters in both highly and weakly crosslinked polymers. Frustrations between the template and polymer chains in forming complexes, as highlighted by Tanaka and co-workers (Enoki et al., 2000; Alvarez-Lorenzo et al., 2000), can be minimized by molecular imprinting.

Demchenko outlines some general principles of recognition between flexible structures, which include: (i) a reaction of complex formation which includes the diffusional formation of an encounter pair and, (ii) a sequential selection isomerization with kinetic proofreading or consecutive elementary steps of stochastic bond-making and breaking events of the encounter pair into a stable complex (Demchenko, 2001). Systems with flexible conformations create configurational complementarity with an ordering process by many trial and error steps until the proper ligand–receptor interaction is reinforced by the ordering of short-range covalent bonds (Huber and Bennett, 1983). This process within the imprinting mechanism is important to produce effective macromolecular memory.

In the next sections, we define hydrogels and outline successful strategies and challenges to create macromolecular memory within imprinted hydrogel networks.

2.1. Hydrogels defined

Hydrogels are insoluble, crosslinked polymer network structures composed of hydrophilic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water and retain their shape without dissolving. Crosslinks (otherwise known as tie-points or junctions) can be covalent bonds, permanent physical entanglements, non-covalent interactions, or microcrystalline regions incorporating various chains and are primarily responsible for preventing the dissolution of the polymer in water (Fig. 2E) (Peppas, 1987a). Since this review focuses on hydrogel networks, we have limited the discussion to gels, where the main constituent of the gel is water. Polymer gels, which are defined as networks of flexible polymer chains, can be deformed and respond as an elastic body. Thus, these solvated polymers are at temperatures above their glass transition, where the amorphous portions of the polymer are in the rubbery state and are flexible. In this regard, we have primarily focused on weakly and moderately crosslinked imprinted networks or networks that are expected to or have been demonstrated to have these properties when solvated. We have also not focused attention on conjugated biomaterials or hybrid structures that contain a complex recognition element such as protein, inclusion complex, etc., unless there were multiple such species on differing polymer chains providing recognition and the structures contained significant flexibility.

When a dry hydrogel is immersed in a thermodynamically compatible solvent, the solvent movement into the hydrogel polymer chains leads to considerable volume expansion and macromolecular rearrangement depending on the nature and extent of crosslinking within the network (Fig. 2F). In addition, the hydrophilicity of the monomers also determines the solvent uptake and thus the observed volume change. The rate at which a polymer expands or swells depends upon two coupled processes, the relative rates of polymer-chain relaxation and solvent penetration into the network. A dry hydrogel transitions in a moving front from an unperturbed, glassy state to a solvated, rubbery state with an increase in macromolecular mobility due to chain extension, and this leads to additional free-volume within the network.

2.2. Imprinting strategies for hydrogel structures

Imprinting within hydrogels is more complex than imprinting within rigid structures. Success depends on structural considerations and the underlying flexibility of polymer chains, limiting the expansion or collapse of polymer chains due to solvation or desolvation forces. It also depends on the number of template molecules in relation to the number of functional monomeric species, the level of diversity of functional monomeric species that interact with the template, the strength of the monomer–template interactions, and the polymerization reaction.

2.2.1. Structural considerations: controlled architecture and limiting the expansion or collapse of polymer chains

One of the most important considerations in effective template recognition is to maintain the binding cavity produced via differing polymer chains close to the state when the original imprint was formed (i.e., close to the relaxed state of the polymer). In other words, the swollen or collapsed polymer volume at equilibrium must not be too different from the relaxed polymer volume fraction. The thermodynamic compatibility of the polymer chains and solvent as well as the number of crosslinking points within the network determines the nature and extent of this transition. At a given crosslinking density in aqueous solvent, an imprinted network that contains more hydrophilic moieties along the polymer backbone will tend to swell or expand more than gels containing hydrophobic groups, which will try to minimize their exposure to aqueous solvent. The expansion of polymer chains increases the free volume available for template transport, but it can decrease the effectiveness of the imprinting site created by multiple polymer chains. Equilibrium is reached when the swelling force is counterbalanced by the retractive force due to crosslinking points in the network structure. In gels where significant collapse of the structure can occur (e.g., the gel is put in a thermodynamically non-compatible solvent), template transport may be significantly reduced and the binding cavity may be significantly altered in a collapsed state.

It is important to note that this does not dictate that gels prepared in the absence of solvent (in these cases, the largest monomer component is the solvent) and subsequently bind template in solvent have been unsuccessful. Typically, these systems have demonstrated higher affinity and capacity (Venkatesh et al., 2007, 2006; Alvarez-Lorenzo et al., 2002, 2006a) compared to non-imprinted gels in aqueous solvents. In these cases, the largest component in the formulation was a hydrophilic monomer.

If the network is prepared in solvent, the growing polymer chains are solvated by the solvent and polymer must form around the solvent molecules. Depending on the amount of solvent in the formulation, the gel may have varying levels of porosity. Fig. 3 highlights the classification of spaces within a hydrogel polymer. Hydrogels can be classified as macroporous, microporous, and nanoporous. As one approaches the nanoscale, the mesh size or

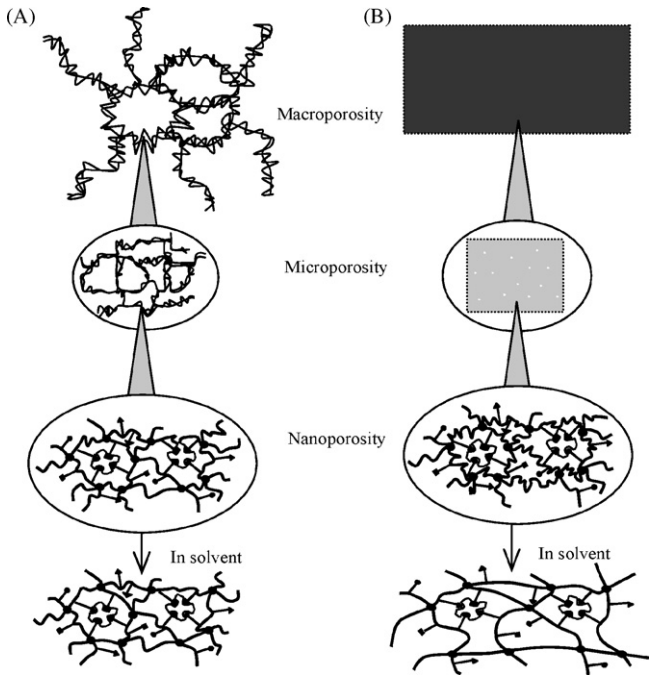

Fig. 3. Porosity within imprinted hydrogels. (A) For templated gels prepared in solvent the polymer will contain significant macro- and microporosity. Template transport will be primarily related to the porosity and tortuosity within the polymer, as in conventional hydrogels. (B) For imprinted gels prepared without solvent, the polymer will not typically exhibit macro and micro-porosity (Peppas, 1987a) and will have small pores that approach the size of the template. Once solvated, the mesh size or the free volume within the polymer chains and the imprinting effect will influence template transport. These type of polymers may exhibit delayed template transport due to the imprinting effect and the binding of the template in the binding cavities between the chains (Venkatesh et al., 2008) and also due to steric hindrance or 'screening effect' due to the crosslinked structure and the mobility of the polymer chains excluding template. Polymers prepared in solvent will typically bind less template due to less polymer volume fraction.

the free-volume available between the polymer chains is reached. If this nanoporous mesh structure is in a collapsed state and the network is not formed in solvent, the polymer can be quite non-porous and significantly limit the transport of solutes. Critical point drying with transmission electron microscopy (Hawkins et al., 2007) and fluorescent and confocal imaging (Hawkins et al., 2006; Byrne et al., 2002b, 2008) have been used to view the imprinted nanocavities or to visualize (Hawkins et al., 2006) and quantify (Byrne et al., 2002b, 2008) the template bound within the gel. Fluorescent spectroscopic methods allow the study of the local environment around the chromophore (Aburto and Le Borgne, 2004). These studies offer evidence as to the size of the imprinted cavities, template binding distribution within sections of the gel, as well as their potential within micro- and nanobiotechnology (Hilt et al., 2006a). Section 3.3 presents these concepts further with discussion of structural and transport considerations of imprinted gels.

Variations in network structure itself have been demonstrated to influence template binding and control the size of the imprinted cavities (Byrne et al., 2008; Espinosa-Garcia et al., 2007; Noss et al., 2008; Spizzirri and Peppas, 2005; Djourelov et al., 2007; Yamashita et al., 2003). Not surprisingly, crosslinking strategies have primarily included covalent crosslinks with very little work exploiting other methods. Imprinted networks linked by non-covalent interactions or permanent physical entanglements have not been explored while interpenetrating (Xia et al., 2005; Wang et al., 2008; Yamashita et al., 2003) and semi-crystalline and crystalline imprinted networks (Binet et al., 2007; Marty et al., 1999; Palaprat et al., 2006) have received little attention. Typically, the low mechanical strength of gels and increased flexibility of the network can be overcome by increasing and controlling the junction points. If the junction points are reversible, binding, release, or other functions of the gel could be modulated. For example, Binet et al. (2007) recently demonstrated liquid crystalline imprinted networks with enhanced affinity and very large loading capacities for template. They also demonstrated catalytic activity after disorganization of the network by temperature increases or swelling, signifying memory within the network.

For covalent crosslinks, the crosslinker length (i.e., linear size), concentration (i.e., the percent of crosslinking monomer reacted in network or degree of crosslinking), and crosslinker double bond functionality (i.e., two or more double bonds) influence imprinting effectiveness (Fig. 4A–C). Covalent crosslinking can be classified by two distinct approaches, which depend on the monomer or polymer building blocks.

With additive polymerization, such as the case with free-radical polymerizations, the double bond of a vinyl monomer will open and begin to build linear chains. Thus, the first approach involves a bifunctional or divinyl crosslinker being added to the growing polymer chain and serving as a bridge to link two distinctive chains (Fig. 4A). The crosslink bridge is usually much smaller in molecular weight than the chains between two consecutive crosslinks and is sometimes represented in a similar manner as Flory, as a volumeless point in respect to the rest of the polymer chains. In reality, the crosslinking structure is far from ideal and can have a number of defects, which may or may not participate in template recognition depending on the accessibility of the binding cavity and flexibility of the chains that comprise the binding cavity. The formation of primary loops, secondary loops, entanglements, and dangling ends can also occur within a crosslinked network (Fig. 4D).

Gels of well-defined mesh size can be prepared if the length of the chains between the crosslink is controlled. Recently, our group was the first to demonstrate that living polymerization strategies can significantly enhance binding parameters of highly crosslinked imprinted networks, hypothesized to be due to a more appropriately sized template binding cavities between the chains and a more homogeneous mesh size (Fig. 4E) (Vaughan et al., 2007). We have also demonstrated this effect in weakly crosslinked gels (Vaughan, unpublished data). Living polymerization with a reversible termination reaction also increased the potential for the growing polymer network and template binding complexes to reach a global energy minimum and led to further memorization of the chain conformation.

The second covalent crosslinking approach is to start with oligomers or polymer chains with double bonds or suitable chemistry along the polymer chain, either along the polymer chain or branched pendant groups, that can be covalently reacted to different chains (Fig. 2A). A few groups have used this approach in imprinting, namely the use of oligomers with functional groups that complex with the template and then are covalently bonded into a growing network structure (Parmpi and Kofinas, 2004; Espinosa-Garcia et al., 2007; Wizeman and Kofinas, 2001; Bolisay et al., 2006; Guo et al., 2006; Kanekiyo et al., 2002, 2003; Zhao et al., 2006; Moritani and Alvarez-Lorenzo, 2001; Jacob et al., 2008; Silvestri et al., 2005a). Recently, this has been demonstrated using a polyvinyl alcohol backbone oligomer functionalized with double bonds and pyridyl groups, which were randomly distributed with 3–5 per chain (Guo et al., 2006). The complex was then immobilized onto the rigid surface of macroporous spheres with modified groups containing double bonds. Recognition and specificity was demonstrated with bovine serum albumin as the template. Using different oligomers, this technique has also been demonstrated with cloned porcine cyclophilin 18 protein (Zhao et al., 2006). It has also been used to produce membranes with a high separation factor for

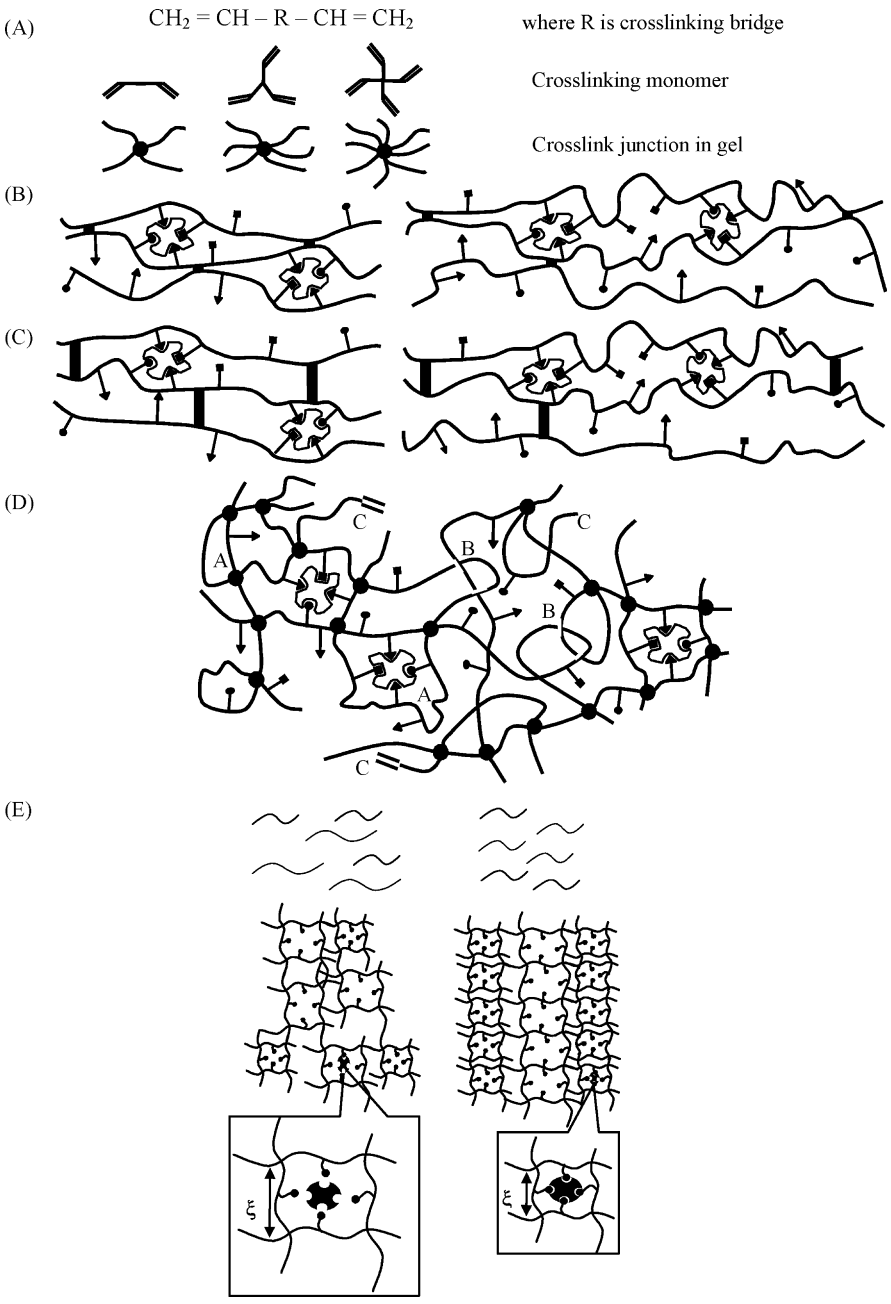

Fig. 4. Structural considerations and flexibility of imprinted hydrogels. (A) Bi-functional crosslink where *R* is crosslinking bridge. As the length of *R* increases this will lead to additional flexibility in the hydrogel structure. bi-functional crosslinking monomer exhibits a tetra-functional crosslink in the gel, a tri-functional crosslinker exhibits a hexa-functional crosslink, and a tetra-functional crosslinker exhibits an octa-functional crosslink with the polymer gel. (B) Small bi-functional crosslinking monomer at moderate concentration (*left*) and low concentration (*right*). (C) Longer bi-functional crosslinking monomer (increased *R*) at moderate concentration (*left*) and low concentration (*right*). Structures have increasing flexibility moving top to bottom with the most flexible structure on the bottom right. If the functional monomer is grafted to the polymer chain (i.e., branched, dangling functional chain), it can also have segment flexibility. (D) A non-ideal network where A represents primary and secondary loops, B represents entanglements, and C represents dangling ends. Primary and secondary loops may participate in binding template depending on the chain flexibility and accessibility of such structures to the template. All crosslinks are tetra-functional and are represented by (●), unreacted, non-incorporated polymer chains are not shown. (E) Living/controlled polymerization of imprinted gels and the effect on the network structure (Vaughan et al., 2007). (*Top*) In mono-vinyl polymerization, the use of iniferter yields a lower polydispersity of kinetic chains and decreased average chain length. (*Bottom*) Within crosslinked networks, addition of iniferter leads to a more uniform and higher population of appropriately sized imprinted macromolecular cavities for the template. An optimal mesh size, ξ, gives the binding site a better functional configuration which leads to enhanced binding properties.

uracil using supercritical carbon dioxide as solvent (Kobayashi et al., 2008).

This is an exciting transition in the field, but more experimental work is needed with controlled studies that vary degrees of substitution, chain length, chain flexibility, and the diversity of functional chemistry in the chain that will interact with the template. Thus, it is expected that in the near future there will be more work creating oligomers with varying distributions of two types of chain functionality, one that promotes covalent linking into a network or onto a surface and one that non-covalently interacts with the template. This will lead to a better understanding of the imprinting process and the building of chains from monomeric units as in conventional methods, but it may also lead to more control of the imprinted network structure and lead to enhanced template bind-

ing parameters. However, this depends on efficient integration of the oligomer chain into the network structure with the binding site able to reach an energy minimum.

2.2.2. Compositional considerations: the number and diversity of functional monomeric species and the strength of monomer–template interaction

The strength of the monomer–template non-covalent interaction is paramount to a successful imprinting strategy. As the polymer is forming, any cross interaction between the solvent and the intended functional monomer–template non-covalent bonding will lead to decreased macromolecular memory. This depends on the strength of the intended non-covalent interactions (i.e., ionic, hydrogen bonding, hydrophobic interactions, pi–pi orbital interactions, and Van der Waals forces). For example, ionic non-covalent bonds are the strongest non-covalent bonds, with bond strengths of 5–35 kcal/mol, approaching a third of covalent bond strength (Meot-Ner, 2005; Lodish et al., 2000). Hydrogen bonding decreases and hydrophobic forces increase as the temperature increases. Thus, a strong interaction between functional monomer and the template is needed. Highly crosslinked imprinted polymers typically employ solvents as porogens, in the absence of which template diffusion out of the rigid structure of the highly crosslinked gel is severely limited. However, very high template removal can be achieved in hydrogels which have low crosslinking densities.

The relative amount of functional monomers and template is also very important to imprinting efficacy. The functional monomer to template (M/T) ratio is a major variable in an effective design. There is considerable evidence of an optimal M/T ratio within highly crosslinked imprinted structures. There is also an optimum in weakly crosslinked structures, and the work of Alvarez-Lorenzo and co-workers has been at the forefront (Hiratani et al., 2005; Alvarez-Lorenzo et al., 2006a). In non-covalent systems, usually an excess of functional monomer is needed to push the reversible template-functional monomer interaction to the complexed state. Therefore, M/T ratios are usually much higher than unity and are not commonly in stoichiometric amounts based on the functionality of the template. This optimum can be distinctively seen by looking at two extreme cases. At a very large M/T ratio, the memorized configuration of monomer within the polymer chains is very small compared to the randomly incorporated monomer, and there is no difference between the non-imprinted and imprinted gels. At very small M/T ratios, there are not effective multiple monomer interactions with the template, resulting in no recognition.

A diversity of functional monomers has also been demonstrated to increase binding parameters of imprinted hydrogels (Venkatesh et al., 2007, 2008). Our group was the first to demonstrate the potential of this technique in gels, and this work shows substantially larger template binding than other systems (Venkatesh et al., 2007, 2008). Single non-covalent bond energies are much less than single covalent bond energies, and they are slightly higher than the average kinetic energy of molecules at room temperature. Thus, many molecules almost possess sufficient kinetic energy to break their non-covalent bonds. However, when multiple non-covalent bonds exist, they produce very stable binding complexes, such as those found in proteins and receptor ligand binding pockets (Meot-Ner, 2005; Lodish et al., 2000). Our group has demonstrated that at a fixed M/T ratio partitioning of drug into networks synthesized from multiple functional monomers (four different monomers) was 8 times greater than networks synthesized from single monomers (Venkatesh et al., 2007). To date, there is not much diversity in the choice of functional monomers and most synthetic gels use one or at the most two different monomeric units (not counting the crosslinking monomer) to interact with the template. Depending on the reaction and the interactions between monomers, integration of multiple monomeric species into the polymer gel is sometimes difficult to achieve. A discussion of reactivity ratios and monomer incorporation is highlighted in Section 2.2.4.

Cross interaction and reactivity between the functional monomers must also be avoided (Lubke et al., 2000). With a cocktail of functional monomers, if they prefer to associate with each other or other monomers or are energetically more favored to have self- or other monomer-association rather than with the template, less effective recognition will occur. In many respects, this lack of diversity and difficulties associated with the use of multiple monomers has not furthered the field.

2.2.3. Template size, chemical functionality, and mobility

The imprinting field has only recently moved toward more biologically relevant applications and aqueous environments. Most work on weakly crosslinked gels has been in aqueous solution with most of the chain building monomer being hydrophilic. Just as a more diverse group of functional monomers increases imprinting effectiveness, templates with more chemical functionality and diverse chemical functionality are, in theory, easier to imprint.

The majority of imprinted gels have been with relatively small sized, hydrophilic templates such as ions (Alvarez-Lorenzo et al., 2000, 2001a,b; Hiratani et al., 2001; Ito et al., 2003; Basavaraja et al., 2007) and low molecular weight molecules (Venkatesh et al., 2007, 2006; Alvarez-Lorenzo et al., 2002, 2006a, 2001a; Byrne et al., 2008; Parmpi and Kofinas, 2004; Espinosa-Garcia et al., 2007; Wizeman and Kofinas, 2001; Wang et al., 2008; Liu et al., 2004a; Oral and Peppas, 2004, 2006; Enoki et al., 2000; Kanekiyo et al., 2002, 2003; Hiratani et al., 2005, 2001; Gong et al., 2008, 2006; Suedee et al., 2006; Lavine et al., 2007; Watanabe et al., 1998, 2001; Oya et al., 1999; Hu et al., 2008; Bodugoz et al., 2007). Protein recognition (Hawkins et al., 2005; Kimhi and Bianco-Peled, 2007; Kublickas et al., 2007; Xia et al., 2005; Hawkins et al., 2006; Chen et al., 2008; Demirel et al., 2005; Guo et al., 2006; Zhao et al., 2006; Brown and Puleo, 2008; Takatsy et al., 2006a) has increased dramatically in the last few years, but it has been demonstrated to a lesser extent. DNA fragments (564 (Ogiso et al., 2006) and 34 base pair (Slinchenko et al., 2004), double-stranded fragments), viruses (Bolisay et al., 2006, 2007; Takatsy et al., 2006b), and even cells (Bacskay et al., 2006) have been imprinted within hydrogels. Of course as the size of the template increases, a determination of the hydrodynamic radius is needed to ascertain the template's ability to diffuse within the spaces between the polymer chains. Thus, the size of the template may impose structural considerations that must be reflected in the choice of crosslinking monomer size and concentration, which will affect the mesh size of the network. For example, a monomeric human growth hormone imprinted gel, which bound approximately seventy times more template than the non-imprinted gel, has been shown to bind approximately four and twenty-three times more template than the dimeric and trimeric forms, respectively (Kublickas et al., 2007).

The generality of the technique has been explored. Recently, moiety molecular imprinting techniques (Byrne et al., 2008) have been developed for the preparation of polymer networks that can recognize a general moiety, D-glucose, and the novel evaluation of loading and release of a larger molecule with glucose as an integral part of its structure (i.e., fluorescently tagged glucose). Poly(acrylamide-co-poly(ethylene glycol)dimethacrylate) networks with varying crosslinking monomer percentages (80, 67, and 30%) and crosslinker lengths (average number of ethylene glycol units of 1, 4, and 14) were prepared and characterized using fluorescent microscopy, which allowed for microscale observation of the dynamic binding and release within the polymer film. Experimental results indicate that tighter mesh-sized networks had increased affinity and capacity toward the glucose function-

alized molecule as well as increased diffusional transport times, indicating the strong potential to load significantly higher amounts of therapeutic within intelligent carriers as well as control and extend the rate of release via macromolecular structure.

The imprinting of hydrophobic templates in gels has not received much attention due to problems such as template/monomer mutual solubility and, if solvent is used, finding a suitable solvent to dissolve the water-soluble monomers and the hydrophobic template. These networks require the presence of hydrophilic backbone monomers since recognition will occur in aqueous solutions. Spizzirri and Peppas (2005) describe a network where cholesterol, a largely hydrophobic molecule, is used as template, and mutual solubility during the polymerization is achieved by the use of two solvents of significantly differing polarity. This provides a dual benefit of countering the hydrophobicity of cholesterol as well as providing a means to make the hydrogel more porous to improve the transport of the large cholesterol molecule.

2.2.4. Polymerization reaction, monomer reactivity and incorporation

Since template binding properties are strongly dependent on the network structure, it is important to study the details of the reaction. For example, network structure of free-radical polymerizations of multifunctional monomers depends upon monomer/macromer size, flexibility, functionality, the amount of solvent and concentration of monomers and initiators, initiation methods and initiation rate, as well as diffusional reaction constraints of propagating polymer chains. For example, monomer double bonds may possess different reactivities that are influenced by conversion (i.e., pendant double bonds typically have reduced reactivity) and significantly affect structural characteristics of the polymer network. It is this reason why most synthetic polymers do not typically consist of a large diversity of monomers. Questions arise on the equal or equivalent incorporation of the monomers within the polymer chains. These are very real concerns and difficulties especially when imprinting to achieve well-functionalized, diverse binding cavities. However, these issues can be overcome by proper understanding and study of monomer reactivities and reactivity ratios, the interaction between various monomers in the formulation, and the imprinting polymerization reaction itself.

The study of the imprinting polymerization reaction has been virtually unexplored and little effort has been expended in trying to understand the imprinting process on mechanistic or a basic molecular level. There have been a number of computational studies, but most groups have primarily focused on studying the pre-polymerization template–functional monomer-complex in the pre-polymerization solution (Wei and Mizaikoff, 2007; Chianella et al., 2006). Only recently have researchers begun to analyze larger polymer chains and the polymerization process (Pavel and Lagowski, 2005; Henthorn and Peppas, 2007; Monti et al., 2006). For example, a method simulating the formation of densely crosslinked networks was recently developed incorporating intra- and intermolecular interactions. An all-atom kinetic gelation simulation technique utilizing an off-lattice approach tracked the position and interaction of all atoms during imprinted polymer formation (Henthorn and Peppas, 2007). This type of work will lead to much insight into the mechanism of imprinted polymer formation.

Conventional free-radical polymerization is highly non-ideal and differences in theory and experimental data indicate heterogeneity within the network structure (Monti et al., 2006). Research work on non-imprinted structures has involved the examination of major variables that control crosslinking polymerization rate, conversions, and final cross-link density. While a lower concentration of crosslinking monomer and lower final conversion will produce less densely crosslinked networks, other factors, such as the pendant double bond reactivity which is based upon monomer size, monomer stiffness, comonomer ratio, and solvent concentration (Elliott and Bowman, 2001), significantly affect the structure and heterogeneity of the resulting polymeric network. Three types of cycles can form in a polymer network when a pendant double bond reacts with a free radical. Typically, a pendant double bond is formed when one end of a crosslinking agent reacts in a polymer chain leaving the other reactive end dangling from the polymer chain and free to react. Primary cyclization or loops (where a radical reacts with the radical on its own propagating chain) will reduce the crosslinking density. Primary and secondary cycles or loops (where a radical reacts with the radical on secondary chain) do not contribute as crosslinks to the overall structure of the polymer (Fig. 4D). These lead to more heterogeneously crosslinked imprinted networks, which may negatively impact binding effectiveness, decrease the overall material strength, and alter template transport.

Reaction conditions such as the type of free-radical initiation mechanism and the polymerization temperature have been explored, but more analysis is needed. Reactions that have controlled temperature have been demonstrated to lead to better imprinted structures and free-radical, UV-initiated imprinted polymerizations have been demonstrated to lead to better binding parameters compared to thermally initiated polymerizations due to lower temperatures of polymerization (Spivak et al., 1997; Lin et al., 1997; Rampey et al., 2004; Piletsky et al., 2002; Spivak and Shea, 2001). However, the benefits of reduced temperatures gained in the stability of the monomer-template complex (O'Shannessy et al., 1989) can negatively influence the structure of the network since reduced temperatures will decrease reaction rates and monomer conversion.

Imprinted polymer reaction analysis and living/controlled imprinted polymerization are other areas that have not received much attention. Recently, our group was the first to demonstrate that reaction analysis of a typical highly crosslinked poly(methacrylic acid-co-ethylene glycol dimethacrylate) molecularly imprinted network revealed low double bond conversion ($35 \pm 2.3\%$ at $0\,^{\circ}C$ to $54 \pm 1.9\%$ at $50\,^{\circ}C$) which was due to severely constrained network formation (Vaughan et al., 2007). This work highlighted that the final composition of imprinted polymers does not represent the initial formulation when using significant amounts of short bifunctional crosslinking monomer. Also, living/controlled polymerization with a reversible termination reaction can provide much more control over the network structure. It increased the potential for the growing polymer network and template binding complexes to reach a global energy minimum and led to further memorization of the chain conformation. Compared to conventional techniques, controlled/living polymerization resulted in a 63% increase in the number of binding sites at approximately equivalent average binding affinity while retaining selectivity for the template. This was hypothesized to be attributed to a decrease in kinetic chain length and/or a more narrow dispersity of kinetic chains which leads to increased structural homogeneity with more stability and integrity of more appropriately sized binding sites.

3. Characterization of imprinted hydrogel structures

Analysis of template binding and structural parameters of imprinted hydrogels is crucial to properly characterize and understand macromolecular recognition. In order to assess structural and recognition/transport property relationships, additional study is needed. The sections below provide a basis for characterizing imprinted hydrogel structures.

3.1. Determination of imprinted gel binding parameters

Imprinting effectiveness can be determined by assessment of the binding parameters of template binding affinity (i.e., the equilibrium association or dissociation constant between the ligand molecule and the network), capacity of loading (i.e., the maximum ligand bound per mass or volume of polymer), and selectivity (i.e., the ability to differentiate between the ligand and other molecules). Compared to highly crosslinked imprinted networks, weakly crosslinked gels can remove most of the template before rebinding experiments. Proper template washing procedures and verification of template removal is very important in the analysis of binding parameters. Binding characterization can be incorrect if post-polymerization washing of template is not verified.

Binding affinity is a measure of how well the template molecule is attracted to the macromolecular binding site or how well a ligand is held to the receptor site formed between the macromolecule chains. The equilibrium dissociation constant, K_d, or equilibrium association constant, K_a, provide a quantitative measure of this level of attraction. Ligands with low K_d or high K_a values bind tightly to the receptor and have high affinity. Conversely, high K_d and low K_a values are indicative of weak template binding.

Template affinity and loading capacity can be estimated from equilibrium binding isotherms analyzing template bound versus the equilibrium template concentration. At a particular binding concentration, the amount of template bound or template partition/distribution coefficients have been used. It is common knowledge that imprinting leads to a distribution of binding sites of varying affinity. The distribution has been demonstrated in a number of systems to be bi-modal, but affinity distribution models have been successfully applied (Rampey et al., 2004; Umpleby et al., 2000) and better approximate the heterogeneity of binding cavities within imprinted networks. Analysis has been conducted in a number of ways using theoretical or empirical based binding isotherms. Care must be taken when applying the best isotherm based on the fit of the data but also on the inherent assumptions in the underlying equations, especially if a theoretical equation is used. Imprinted networks have been analyzed via limiting slope Scatchard analysis (Lubke et al., 2000), Langmuir isotherms (Kimhi and Bianco-Peled, 2007; Xia et al., 2005; Espinosa-Garcia et al., 2007; Hiratani and Alvarez-Lorenzo, 2004), Freundlich isotherms (Xia et al., 2005; Espinosa-Garcia et al., 2007; Umpleby et al., 2001), bi- (Chen et al., 2001b; Li and Husson, 2006) and tri- (Kim et al., 2005) Langmuir isotherms, and Langmuir–Freundlich isotherms (Li and Husson, 2006). Recently, isothermal titration calorimetry has thermodynamically verified differences in the binding enthalpy of template within imprinted and non-imprinted gels (Kimhi and Bianco-Peled, 2007). Using this microcalorimetric method, the binding mechanism can be elucidated with quantification of the enthalpic and entropic contributions during the binding event (Chen et al., 2001c). This technique has also been used to determine the optimum monomer template ratio (Alvarez-Lorenzo et al., 2006a). We direct the reader to the following review which classifies typical dissociation constants of molecularly imprinted polymers and those of common classes of receptor–ligand interactions (Hilt and Byrne, 2004).

Selectivity, α, can be determined by a ratio of the equilibrium association or dissociation constants between two molecules (one which differs from the template in chemical functionality, orientation of chemical functionality, or physical size).

$$\alpha = \frac{K_{\text{a, template molecule}}}{K_{\text{a, other molecule}}} \quad (1)$$

In the last 6 years, there has been a substantial increase in the number imprinted hydrogels displaying template selectivity. Table 1 highlights a number of selective hydrogels imprinted for a large variety of templates of varying sizes (i.e., drug molecules, proteins, viruses, DNA) and associated composition and selectivity values. In most hydrogel imprinted networks, selectivity has been most commonly evaluated as the ratio of the loading capacities or partition/distribution coefficients of the template to the template analog in the gel. Also, most papers in the field, whether highly or weakly crosslinked imprinted structures, do not provide a competitive challenge of various molecules including the template.

Demirel et al. (2005) describe a hydrogel with *N*-*tert*-butylacrylamide and maleic acid as functional monomers interacting with bovine serum albumin as template. Acrylamide was used to form the backbone while *N*,*N*′-methylene-bis-acrylamide served as the crosslinking monomer. The selectivity of the gels increased as the template percentage in the formulation increase. A maximum selectivity of 4.2 was reached at 8.63 weight% of template whereas the non-imprinted gel was unselective (with a value of 0.9).

Similarly selectivities were demonstrated by Hawkins et al. (2005), Liu et al. (2004a) and others (Wang et al., 2008; Gong et al., 2006). Liu and co-workers determined selectivity on the distribution coefficients of the template (L-pyroglutamic acid) and analog molecules in separate binding experiments. Three template analogues were used (2-pyrrolidone, L-proline, pyrrolidone), and imprinted gels exhibited selectivities of 2.5, 3.0, and 5.1, respectively. The poly(AM-co-MBA) prepared by Hawkins and co-workers bound more than 90% of the template (Bovine Hemoglobin) while rejecting nearly 80% of the template analogs (cytochrome *c* and myoglobin).

Larger molecules such as viruses (Bolisay et al., 2006; Bolisay et al., 2007) and DNA (Ogiso et al., 2006) have been imprinted and these networks have demonstrated selectivity. For example, fragments of DNA were imprinted and electrophoresis experiments with a cocktail of fragments revealed decreased migration of template within the imprinted gel. Also, most analogues, that only differed by a few base pair substitutions, did not demonstrate decreased migration.

Notably, Fazal and Hansen (2007) have shown that the differing binding capacities of poly(allylamine hydrochloride) imprinted networks crosslinked with epichlorohydrin are correlated with the octanol/water partition coefficients. Their gels were prepared in a manner similar to the original papers that highlighted selective recognition (Parmpi and Kofinas, 2004; Wizeman and Kofinas, 2001), but multiple templates were used (D-glucose-6-phosphate monobarium salt, D-glucose, L-glucose, barium hydrogen phosphate and D-gluconamide) to prepare five different imprinted gels. All gels showed preferential binding for glucose and the selectivities were reasonably similar irrespective of the template used.

These results illustrate that better analysis must be conducted when performing selectivity studies. It is recommended that "reverse selectivity" experiments be carried out where the selectivity of analog imprinted polymers toward the template is measured. This emphasizes selective imprinting and eliminates the effect of preferential binding of the template to the networks via non-specific interactions. Some other important steps that may affect the results include the selection of the template analog and the verifying of template removal post-polymerization. Insufficient washing results in the reported binding values being lower than the actual values due to residual template present before the rebinding step. Also, it is also recommended that template octanol/water partition coefficients be calculated, which are not presented for the majority of systems in the literature.

However, some groups do carry out additional experiments in the form of competitive challenge (Kimhi and Bianco-Peled, 2007; Xia et al., 2005; Wang et al., 2008; Ogiso et al., 2006) and reverse selectivity (Kublickas et al., 2007; Chen et al., 2008; Fazal

Table 1
Imprinted gels displaying selectivity.

Functional Monomers/Oligomers (Crosslinker)	Crosslinking Amount (%), Template, Solvent	Selectivity Assessment (Template Analogues)	Reference(s)
MAA/DEAEM/AM (MBA)	Low (0.9%), Lysozyme, Tris–HCl buffer(pH 7)	Competitive binding at conc. ratios of CyC/template of less than one, IP bound 3.5× more than NIP, for conc. ratios >1 IP bound ~12× more template. Analogue imprinted gel demonstrated more analogue binding than lysozyme. (cytochrome *c*)	Kimhi and Bianco-Peled (2007)
MAA/AM (MBA)	Low (1.4%), Lysozyme, Tris–HCl buffer (pH 7)	Competitive binding with Lys-IP preferentially bound Lys over CyC (~5×), myoglobin (~2.5*x*). The other proteins, unlike the template in size/structure, show very little binding to Lys-IP and NIP. (CyC, myoglobin, BSA, horseradish peroxidase, trypsin inhibitor, Hb)	Chen et al. (2008)
TBA/AM/Maleic Acid (MBA)	Low (<1%), Bovine Serum Albumin, Methanol/Water (1:1)	Template/analogue bound ratio increases with template percentage in monomer mix (0.9 for NIP to 4.2 for IP made with 8.63 wt% template) (casein)	Demirel et al. (2005)
Semi-IPN Chitosan/AM (MBA)	Low (2.4%), Bovine Hemoglobin, Buffered water	Competitive binding distribution coefficient (DC) of IP for template was 83 and too little to be detected for analogue. NIP DC for template was 1.9 and 2.9 for analogue. (bovine serum albumin)	Xia et al. (2005)
VDAT/AM (MBA)	Low (2.3%), dsDNA (564 base pair fragment) and verotoxin DNA, Buffered water	Electrophoresis detection experiments with mixed DNA samples, template had decreased migration distance due to hindrance binding in gel. 2 of 3 base pair substitutions with Verotoxin DNA IP did not show decreased migration	Ogiso et al. (2006)
DEAEM/MAA/AM (MBA)	Low (4.3%), Human growth hormone (22 kDa) and dimer (44 kDa), PBS buffer (pH 6.2)	IP for dimer binds monomeric hGH ~2× less than dimeric template. IP for dimer binds trimer ~3× less as dimeric template. Other proteins demonstrate very low binding (0.4–1.5%) (Human growth hormone dimers (44 kDa), trimers (66 kDa), BSA(66 kDa), HSA (66 kDa), lysozyme)	Kublickas et al. (2007)
PAA-HCl (MW 15,000) (epichlorohydrin)	Low, Tobacco Mosaic Virus, Water	IP bound 8.8 mg TMV/g IP, NIP bound 4.2 mg TMV/g NIP. Ratio of binding capacity of TMV/TNV is ~2 for IP and identical for NIP. TMV is rod shape (300 nm length, 18 nm diameter) and TNV is icosahedral shape (24 nm diameter). (TNV)	Bolisay et al. (2006)
PAA-HCl (MW 15,000) (ethylene glycol diglycidyl ether)	Low, Tobacco Mosaic Virus, Water	IP bound 2.7 mg TMV/g IP, NIP bound 1.18 mg TMV/g NIP. Ratio of binding capacity of TMV/TNV is ~7.8 for IP and ~1.8 for NIP (TNV)	Bolisay et al. (2007)
AM (MBA)	Low (10%), Bovine Hemoglobin, Water	For IP, 90% of template in solution bound as opposed to 20% of analogues (CyC, myoglobin)	Hawkins et al. (2005)
Star PEG polymers/MAA (PEG600DMA)	Low (11% v/v), D-Glucose; Water	Selectivity of ~3.1 is shown for glucose over fructose by the 31 arm polymer (5970 g/mol per arm; 450,000 M_n (g/mol)) while the 75 arm polymer (20,000 g/mol per arm; 624,000 M_n (g/mol)) showed no selectivity. (D-fructose)	Oral and Peppas (2004)
MAA (EGDMA)	Low (12.5%), L-Pyroglutamic Acid Methanol	IP distribution coefficient (DC) for template ~5, 3, 2.5× larger than that for corresponding analogues. DCs in NIP similar for all analytes and ~4× less than IP for template. (pyrrolidine, L-proline, 2-pyrrolidine)	Liu et al. (2004a)
Semi-IPN-PVA (MW 70,000–80,000)/AA (trihydroxymethyl propane glycidol ether)	Moderate (33%), MMTCA, DMSO/Water (~1:1)	Competitive binding capacity for MMTCA/riboflavin was ~2.6 and MMTCA/aspirin was ~10. *Note*: aspirin is smaller and riboflavin has a different molecular geometry. (aspirin, riboflavin)	Wang et al. (2008)

Table 1 (*Continued*)

Functional Monomers/Oligomers (Crosslinker)	Crosslinking Amount (%), Template, Solvent	Selectivity Assessment (Template Analogues)	Reference(s)
MAPASA (Various MBA sizes)	High (83%), Paracetamol, DMF/Water	As crosslinker increased in length, template binding affinity decreased and capacity increased. Analogues show ~10% or less binding compared to 45% for the template. (phenacetin, antifebrin)	Gong et al. (2008)

Note: Binding takes place in aqueous solution unless specified. When appropriate, crosslinking % was calculated and is equivalent to (100% × (mole crosslinking monomer/(mole crosslinking monomer and all other monomers))). *Abbreviations*: AA: Acrylic Acid; AM: Acrylamide; BSA: Bovine serum albumin; CyC: Cytochrome *c*; DBT: Dibenzothiophene; DBTS: Dibenzothiophene Sulfone; DEAEM: 2-(dimethylamino)ethyl methacrylate; DMF: Dimethylformamide; EGDMA: Ethylene Glycol Dimethacrylate; Hb: Hemoglobin; HSA: Human serum albumin; IP: Imprinted Polymer; IPN: Interpenetrating Network; Lys: Lysozyme; MAA: Methacrylic acid; MAPASA: 4-[(4-methacrylolyoxy)phenylazo] benzenesulfonic acid; MBA: *N*,*N*–methylene-bis-acrylamide; MMTCA: 1-(4-methoxyphenyl)-5-methyl-1,2,3-triazol-4-carboxylic acid; NIP: Non-Imprinted Polymer; NIPA: *N*-isopropylacrylamide; PAA-HCl: Poly(allylamine hydrochloride); PEG: Polyethylene glycol; PEG600DMA: Poly(ethylene glycol)600 dimethacrylate; PVA: Poly(vinyl alcohol); TBA: *N*-*tert*-butylacrylamide; TFMAA: 2-(trifluoromethyl)acrylic acid; TRIM: Trimethylolpropane Trimethacrylate; DMDBT: 4,6-dimethyl dibenzothiophene; TNV: Tobacco Necrosis Virus; TMV: Tobacco Mosaic Virus; VDAT: 2-vinyl-4,6-diamino-1,3,5-triazine; VPD: 4-vinylpyridine.

and Hansen, 2007). For example, Chen et al. (2008) have shown excellent results for lysozyme recognition in hydrogels. Not only are seven different template analogs used to perform competitive selectivity studies, but lysozyme and cytochrome *c* are used to create two separate imprinted gels in addition to the non-imprinted hydrogels. Both imprinted gels selectively bound the template with lysozyme imprinted gels exhibiting selectivities of 2.5 and 5. Xia et al. (2005) have also demonstrated competitive selectivity experiments of hemoglobin imprinted semi-interpenetrating hydrogel networks. Kimhi and Bianco-Peled (2007) also carried out reverse selectivity and competitive binding experiments between the lysozyme and cytochrome *c*, demonstrating selectivity of lysozyme imprinted gels.

3.2. Imprinted gel structural characterization

There have been many excellent reviews characterizing hydrogel structures. We direct the reader to the following references (Peppas, 1987a; Peppas et al., 2000). Structural assessment of imprinted hydrogels can be achieved by analysis of the following related parameters: the polymer volume fraction in the swollen state (i.e., the amount of water absorbed by the gel), the average molecular weight between two adjacent crosslinking or junction points, and the average correlation distance between two adjacent crosslinking or junction points (i.e., the average mesh size or free space between the macromolecular chains available for transport).

Factors such as the extent or degree of crosslinking in the network, the size and flexibility of the crosslinking monomer, and the type of chemical groups that comprise the polymer chains, both functional that non-covalently interact with the template and those that do not, primarily affect the swelling and mesh structure of imprinted hydrogel structures. Typically, higher crosslinked imprinted gels have a tighter mesh structure and swell to a lesser extent than weakly crosslinked gels. Imprinted gels containing more hydrophilic moieties tend to swell more than gels containing hydrophobic groups, which will minimize their chain exposure to aqueous solvent.

Equilibrium swelling and rubber elasticity theories have been used to characterize the structural parameters of polymer gels. Flory–Rehner theory states that a crosslinked polymer gel in equilibrium is subject to two opposing forces, the thermodynamic force of mixing and the elastic, retractive force of the polymer chains (Flory and Rehner, 1943a; Flory, 1953). At equilibrium, the total Gibbs free energy is zero and these two forces are equal as Eq. (2) denotes.

$$\Delta G_{\text{total}} = \Delta G_{\text{elastic}} + \Delta G_{\text{mixing}} \tag{2}$$

Differentiating Eq. (2) with respect to the number of solvent molecules, while keeping the temperature and pressure constant, will yield an equivalent equation in terms of chemical potential. At equilibrium, the chemical potential of the solvent outside and inside the gel must be equal. The change in chemical potential due to elastic retractive forces of the polymer chains can be determined from the theory of rubber elasticity (Flory, 1953), and the change in chemical potential due to mixing can be expressed using the heat and entropy of mixing. Essentially, this measures the interaction and compatibility of the polymer chains with the solvent molecules. By equating these two contributions, an expression can be written for the determination of the molecular weight between two adjacent crosslinks of a neutral, non-ionized, imprinted hydrogel prepared without solvent,

$$\frac{1}{\overline{M_c}} = \frac{2}{\overline{M_n}} - \frac{(\bar{\upsilon}/V_1)[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1 \upsilon_{2,s}^2]}{[(\upsilon_{2,s})^{1/3} - (\upsilon_{2,s}/2)]} \tag{3}$$

where M_c is the average molecular weight between crosslinks, M_n is the average molecular weight of the polymer chains prepared under identical conditions in the absence of crosslinking agent, V_1 is the molar volume of solvent (e.g., V_1 for water is 18.1 cm^3/mol), υ is the specific volume of the polymer (i.e., the reciprocal of density), χ_1 is the Flory polymer–solvent interaction parameter, and $\upsilon_{2,s}$ is the polymer volume fraction in the swollen state. The polymer volume fraction in the swollen state is related to the volume swelling ratio, Q, which can calculated from equilibrium swelling experiments as follows:

$$Q = \frac{1}{\upsilon_{2,s}} = \frac{V_{2,s}}{V_{2,d}} \tag{4}$$

where $V_{2,s}$ is the swollen gel volume at equilibrium, $V_{2,d}$ is the volume of the dry polymer, and $\upsilon_{2,s}$ is the polymer volume fraction in the swollen state. The relaxed polymer volume fraction is the volume of the dry polymer divided by the relaxed polymer volume. The volume of the gel in the swollen, relaxed, or dry state can be obtained by using Archimedes buoyancy principle (Peppas, 1987a).

If the imprinted network is prepared in the presence of solvent, the Peppas and Merrill (1977) equation must be used (Eq. (5)). It is a modification of the Flory–Rehner theory to include the presence of solvent, which modifies the chemical potential due to elastic forces. The molecular weight between adjacent crosslinks in a neutral, imprinted gel prepared in solvent is determined by Eq. (5),

$$\frac{1}{\overline{M_c}} = \frac{2}{\overline{M_n}} - \frac{(\bar{\upsilon}/V_1)[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1 \upsilon_{2,s}^2]}{\upsilon_{2,r}[(\upsilon_{2,s}/\upsilon_{2,r})^{1/3} - (\upsilon_{2,s}/2\upsilon_{2,r})]} \tag{5}$$

where $\upsilon_{2,r}$ represents the polymer volume fraction in the relaxed state. It is defined as a physical property of the polymer immediately after crosslinking before swelling or collapse of the network.

If the imprinted network contains a number of ionic moieties, an ionic contribution is added to the expression of chemical potential,

$$\mu_1 - \mu_{1,0} = \Delta\mu_{\text{elastic}} + \Delta\mu_{\text{mixing}} + \Delta\mu_{\text{ionic}} \tag{6}$$

where μ_1 is the chemical potential of the solvent in the polymer gel, $\mu_{1,0}$ is the chemical potential of pure solvent, and μ_{ionic} is the ionic contribution term. Equations have been derived for both anionic and cationic gels prepared in the presence of solvent (Ricka and Tanaka, 1984; Brannon and Peppas, 1991). For anionic, imprinted gels, Eq. (7) can be used,

$$\frac{V_1}{4I}\left(\frac{\upsilon_{2,s}^2}{\bar{\upsilon}}\right)\left(\frac{K_a}{10^{-\text{pH}} - K_a}\right)^2 = [\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1\upsilon_{2,s}^2] + \left(\frac{V_1}{\bar{\upsilon}\bar{M}_c}\right)\left(1 - \frac{2\bar{M}_c}{\bar{M}_n}\right)\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \left(\frac{\upsilon_{2,s}}{2\upsilon_{2,r}}\right)\right] \tag{7}$$

where ionic strength is I and K_a is the equilibrium constant for the acid. For basic, imprinted gels, the term containing the acid equilibrium constant K_a is replaced by K_b in the numerator and $10^{\text{pH-14}}$-K_a in the denumerator. In networks containing weakly acidic or basic pendent groups, water uptake can result in ionization of pendent groups depending on the solution pH and ionic composition. The gels then act as semi-permeable membranes to the counterions influencing the osmotic balance between the hydrogel and the external solution. For ionic gels containing weakly acidic pendent groups, the equilibrium degree of swelling increases as the pH of the external solution increases. For gels containing weakly basic pendent groups, the equilibrium degree of swelling increases as the pH decreases. In an ampholyte gel, containing both acidic and basic groups, the isoelectric pH determines the transitional pH of swelling of the gel. An increase in the ionic content of the gel increases the hydrophilicity leading to faster swelling and a higher equilibrium degree of swelling. Numerous physicochemical parameters contribute to the swelling of ionic hydrogels, including the ionic content, ionization equilibrium considerations, nature of counterions, and nature of the polymer (Peppas and Khare, 1993; English et al., 1996, 1998).

In cases where the swelling of the gel can be influenced by the complexation of multiple chains via multiple non-covalent interactions with the template, we believe that another free energy term must be added to reflect the complexation contribution of multiple polymer chains due to imprinting. While the numerical significance and weight of this contribution has yet to be realized, in theory, multiple, strong, non-covalent interactions, such as those with highly functionalized templates interacting with multiple chains, can lead to less expansion of imprinted networks compared to non-imprinted networks. For imprinted hydrogels that demonstrate template associated swelling differences, the free energy associated with template-chain complexation may need to be included as a term in Eq. (2), presented below as Eq. (8). Thus, there is now opportunity and need to develop this theoretical framework.

$$\Delta G_{\text{total}} = \Delta G_{\text{elastic}} + \Delta G_{\text{mixing}} + \Delta G_{\text{Imprinting complexation}} \tag{8}$$

The average molecular weight between crosslinks, $\bar{M}_c$ of imprinted hydrogels can be determined from the theory of rubber elasticity (Flory and Rehner, 1943a,b; Flory, 1953). For an isotropic, swollen, imprinted hydrogel synthesized without solvent, with a constant deformation volume, Eq. (9) is valid for short elongation ratios of up to 2:

$$\tau = \left(\frac{RT\upsilon_{2,s}^{1/3}}{\bar{\nu}\bar{M}_c}\right)\left(1 - \frac{2\bar{M}_c}{\bar{M}_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \tag{9}$$

where τ is the stress, α is the elongation ratio in any direction (final length/initial length), R is the universal gas constant, and T is the absolute temperature.

If, $\bar{M}_c \ll \bar{M}_n$, $\left(1 - \frac{2\bar{M}_c}{\bar{M}_n}\right) \to 1$, and Eq. (9) becomes,

$$\tau = \left(\frac{RT\upsilon_{2,s}^{1/3}}{\bar{\nu}\bar{M}_c}\right)\left(\alpha - \frac{1}{\alpha^2}\right) \tag{10}$$

Stress–strain data are typically obtained by performing tensile studies on a dynamic mechanical analyzer. Polymer gels can be cut into thin sheets, clamped between the two parallel arms of the dynamic mechanical analyzer and subjected to a linear load until breaking point. Strain values are converted into the elongation function $(\alpha - 1/\alpha^2)$ and the slope can be used to calculate $\bar{M}_c$.

If the imprinted gel is prepared in the presence of solvent, Eq. (11) is used.

$$\tau = \left(\frac{RT}{\bar{\nu}\bar{M}_c}\right)\left(1 - \frac{2\bar{M}_c}{\bar{M}_n}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} \tag{11}$$

The space available for diffusion, occurring through the space available between macromolecular chains is regarded as the pore or mesh size. A structural parameter that is often used in describing the size of pores is the correlation length, ξ, which is defined as the linear distance between two adjacent crosslinks (Peppas, 1987a; Canal and Peppas, 1989). It can be calculated by the following equation,

$$\xi = \alpha\left(\bar{r}_o^2\right)^{1/2} = \upsilon_{2,s}^{-1/3}\left(\bar{r}_o^2\right)^{1/2} \tag{12}$$

where α is the elongation ratio, which for gels that swell isotropically can be related to the swollen polymer volume fraction, $\upsilon_{2,s}$ as shown.

The unperturbed end-to-end distance of the polymer chain between two adjacent crosslinks can be calculated using the following,

$$\left(\bar{r}_o^2\right)^{1/2} = l(C_n N)^{1/2} \tag{13}$$

where C_n is the Flory characteristic ratio or rigidity factor of the polymer, l is the length of the bond along the polymer backbone (e.g., for vinyl polymers equals 1.54 Å), and N is the number of links per chain between crosslinks that can be calculated from Eq. (14).

$$N = \frac{2\bar{M}_c}{M_r} \tag{14}$$

where M_r is the molecular weight of repeating units from which the polymer chain is composed. For heteropolymers, this can be a weighted average of the monomers that make up the polymer chains assuming equal reactivity. If reactivity ratios differ greatly, the polymer chains will not reflect the feed composition. In light of recent results demonstrating enhanced network binding properties of multiple, different functional monomers interacting non-covalently with the template (Venkatesh et al., 2007, 2008), there is a need to explore differences in reactivity of monomers due to complexation with the template pre-polymerization, which has not been adequately studied to date. Combining equations (Peppas, 1987a; Canal and Peppas, 1989), yields:

$$\xi = \upsilon_{2,s}^{-1/3}\left(\frac{2C_n\bar{M}_c}{M_r}\right)^{1/2} l \tag{15}$$

The crosslinking density, ρ_x, can be calculated from:

$$\rho_x = \frac{1}{\bar{\upsilon}\bar{M}_c} \quad (16)$$

These calculations along with one-dimensional transport studies of template and template analogues can fully characterize the structural architecture of imprinted gels. Nitrogen porosimetry experiments or positron annihilation lifetime spectroscopy (Bogershausen et al., 2006) can complement gravimetric analysis of gel swelling and can lead to pore size analysis. In template transport applications, such as membrane separation, drug delivery carriers, etc., a proper analysis of structural parameters and their relation to binding parameters and template transport is crucial to the design and understanding of such systems.

3.3. Characterization of template transport within imprinted gels

Mass transfer of the template through the imprinted polymer will depend on a number of structural and physiochemical considerations. For imprinted gels prepared in solvent (i.e., macro or microporous gels), the template diffusion coefficient will be primarily related to the porosity and tortuosity within the polymer, as in conventional hydrogels (Peppas, 1987a; Peppas et al., 2000). For polymers with relatively small pores that approach the size of the template and nanoporous polymers (i.e., polymers exhibiting free volume mostly within the polymer chains or with the mesh), the template can experience delayed transport due to steric hindrance of the polymer chains or the 'screening effect' due to the crosslinked structure and the mobility of the polymer chains excluding template. The chains can also increase the frictional drag on the template (Amsden, 1998a). Delayed transport can also occur due to the imprinting effect and binding of the template in the binding cavities between the chains (Venkatesh et al., 2008).

Table 2 highlights work in the field that has analyzed template transport within or from imprinted hydrogel structures. From this table, it is clear that imprinting enhances loading capacity/affinity but the influence of imprinting on the transport of template is much more complex, with little attention of most studies to structural analysis or discussion of how much porosity/tortuosity are controlling transport.

Recently, our group has exploited molecular imprinting methods to delay the transport of drug via interaction of the drug with numerous functional groups organized within the network (Venkatesh et al., 2008). The drug's heightened interaction with the memory pockets slowed its transport within the hydrogel despite comparable free volume within the polymer chains for drug transport. One-dimensional permeation studies showed that the gel with maximum incorporated chemical functionality had the lowest diffusion coefficient, which was at least an order of magnitude lower than all other gels studied. All imprinted networks had significantly lower diffusion coefficients than non-imprinted networks, in spite of comparable mesh sizes and equilibrium polymer volume fractions in the swollen state. It is important to note that no solvent was used in the formulation. Since permeation studies showed different template permeation rates after lag/breakthrough times, we proposed the "tumbling hypothesis", wherein a molecule tumbling through an imprinted network with multiple, organized functionalities and an appropriate mesh size, experiences heightened, transient interactions with memory sites and shows delayed transport kinetics. This could also be partially due to bound template temporarily obstructing free template transport. Thus, the structural plasticity of polymer chains, i.e., the organization of functional groups into memory sites, may be responsible for enhanced loading and extended release. Similar delayed template transport with dependence on the M/T ratio have been demonstrated in hydrogels produced without solvent with timolol as template and methacrylic acid as functional monomer; however a structural analysis of the mesh size was not conducted (Hiratani et al., 2005). Table 2 highlights the papers that display the highest binding (Venkatesh et al., 2007, 2008) and most delayed release (Hiratani et al., 2005; Venkatesh et al., 2008, 2007) of imprinted gels studied to date.

Template transport depends on the size and chemistry of the template, the macromolecular structure and organization of the network chains (i.e., mesh size or mesh free-volume), the macro/micro porosity (which is reflected in the polymer volume fraction of the gel), and tortuosity of the polymer. Without careful consideration of structure and porosity, transport analysis and the effect of imprinting on template transport cannot be correctly ascertained. Recent work has demonstrated that network mesh structure as well as the micro- and macro-porosity of the polymer can significantly impact the ability to extend drug release via enhanced affinity networks prepared by molecular imprinting (Byrne et al., 2008). Essentially differences in polymer porosity, which can even exist between imprinted and their corresponding non-imprinted control structures due to the inclusion of template or aggregation of template, can overshadow the contributions of enhanced affinity for the template. It is clear that more study is needed on the design, formation, and understanding of structure–property relationships of such systems.

Since recognition and loading take place between the polymer chains, a smaller polymer volume fraction in the swollen state will decrease template loading capacity. Thus, imprinted hydrogels prepared with solvent will have reduced template binding capacities and demonstrate less 'imprinted' control over the release profile compared to a similar imprinted polymer prepared without solvent. This can be distinctly seen in Table 2 comparing binding parameters and template transport of reference (Singh and Chauhan, 2008 includes solvent in the formulation and references (Hiratani et al., 2005; Venkatesh et al., 2007, 2008; Alvarez-Lorenzo et al., 2006a), which do not include solvent.

We direct the reader to an excellent review of mechanisms and models of solute diffusion within hydrogels (Amsden, 1998a) and a number of references that discuss free-volume (Peppas, 1987a; Lustig and Peppas, 1988), hydrodynamic (Peppas, 1987a; Cukier, 1984), and obstruction (Amsden, 1998b) theories and models as well as solute–polymer interactions (Amsden, 1998b; Valente et al., 2003; Cheng and Sun, 2005). These models can be applied to imprinted hydrogels; however, depending on the porosity, the binding of the template within the polymer chains should be considered and will decrease transport. Thus, considering most theories do not include solute–polymer interactions, there is considerable opportunity to provide a theoretical framework for template diffusion phenomena with imprinted hydrogels.

4. Responsive intelligent imprinted hydrogels

Stimuli-sensitive hydrogels that respond to changes in the external environment have been the subject of much research and have been demonstrated in a wide variety of hydrogel systems (Peppas et al., 2006; Ulijn et al., 2007). To date, molecularly imprinted responsive networks have been demonstrated with triggers such as pH (Wang et al., 2008; Demirel et al., 2005; Kanekiyo et al., 2002, 2003), temperature (Chen et al., 2008; Demirel et al., 2005; Liu et al., 2004a,b; Aburto and Le Borgne, 2004), light (Gong et al., 2008, 2006-highly crosslinked), and salt concentration (Chen et al., 2008) for templates such as ions (Alvarez-Lorenzo et al., 2000, 2001a,b; Hiratani et al., 2001), low to moderate molecular weight molecules (Enoki et al., 2000; Watanabe et al., 1998, 2001), and proteins (Chen et al., 2008) (Table 3). Of course, flexible polymer chains with con-

Table 2
Imprinted hydrogel template release/transport.

Functional Monomers (Crosslinker)	Crosslinking Amount (%), Template, Solvent	Characterization of Binding Parameters	Characterization of Template Release/Transport	IP Structural/Swelling Analysis	Reference
HEMA/MAA (EGDMA)	Low (0.128%), Timolol, No solvent	HEMA/MAA IPs bind 12 mg/g at pH 5.5, NIPs bind 4 mg/g	90–100% release in 9 h	Swelling	Alvarez-Lorenzo et al. (2002)
HEMA/MMA (EGDMA)		HEMA/MMA IPs demonstrate poor binding			
MAA (EGDMA)	Low, Timolol, No solvent	IP demonstrated higher affinity than NIP	Template diffusion coefficients were calculated from template release studies. The diffusion coefficient from IP at certain M/T ratios (16/1, 32/1) 2 orders of magnitude lower than NIP. Lowest IP diffusion coefficient approaching 10^{-10} cm^2/s)	Swelling	Hiratani et al. (2005)
HEMA/AA/AM/NVP (PEG200DMA)	Low (5%), Ketotifen fumarate, No solvent	Most biomimetic IP (AA/AM/NVP/HEMA functional monomers) bound 21.3 mg/g, with NIP binding 3.4 mg/g (~8× greater than single functional monomer IP)	IP demonstrated delayed release compared to NIP. Most biomimetic, or more functionalized gels exhibited more delayed release compared to less functionalized gels. Most diverse network released 65% of template in 3.5 days and 100% in 5 days	Swelling	Venkatesh et al. (2007)
HEMA/AA/AM/NVP (PEG200DMA)	Low (5%), Ketotifen fumarate, No solvent	Partitioning of template into IP gels composed of multiple functional monomer was ~8× greater than less diverse IPs. Each IP bound more template than corresponding NIP	One-dimensional transport studies show template diffusion coefficient 2 orders of magnitude lower (7×10^{-10} cm^2/s) in most diverse IP with comparable gel mesh sizes and equilibrium polymer volume fractions. All IP gels had lower template diffusion coefficients than corresponding NIP with comparable mesh sizes	Swelling and Structural Analysis	Venkatesh et al. (2008)
AA or VPD (EGDMA)	Low (1%), Norfloxacin, No solvent	IP loaded more than NIP highest loading at M/T of 3:1 and 4:1	IP polymer release extended compared to NIP. IP at M/T 4/1 released 40% template in 24 h	Swelling	Alvarez-Lorenzo et al. (2006a)
AM; (EGDMA; PEGnDMA)	Variable (80, 67, 30%), Glucose, DMSO	Binding of glucose analogue in water, tighter mesh-sized IP networks demonstrate increased affinity and capacity	No differences between IP and controls, different porosity and mesh structure affected release in water	Swelling	Byrne et al. (2008)
HEMA/AA (MBA)	Low (~2.5%) 5-fluorouracil, water	IP bound 1.3 mg/g gel and NIP bound 0.7 mg/g gel	Gels were dried and release evaluated under swelling when placed in water. Template diffusion coefficients calculated from release data indicate 2.3×10^{-7} cm^2/s with no evidence of imprinting delaying release	Swelling	Singh and Chauhan (2008)
Self-assembled silica and hybrid organo-silica sol-gel	Persantin, Ethanol/water	Drug included in formulation, but no binding information presented	Hydrophobic interactions dominated drug dissolution and release. Polar end of drug interacts with hydrophilic SiOH groups	Pore Size	Paul et al. (2007), Bogershausen et al. (2006)
MAA (EGDMA)	Moderate (57%), Sulfasalzine, Acetonitrile/Toluene (77/23 vol%)	None reported	IP particle release slower than NIPs. NIPs release 100% and IPs release 80% in 5 h. Average IP and NIP particle sizes are different	No	Puoci et al. (2004)
MAA (EGDMA)	Moderate and High (44% and 77%), cholesterol, THF; THF/DMSO; THF/water; THF/water/salt	Higher crosslinked structures had higher binding compared to NIP (maximum at 77% was ~13 times)	Transport of template into gel for up to 1500 min. IP had lag time in binding	Swelling and Structural Analysis	Spizzirri and Peppas (2005)

Table 2 (*Continued*)

Functional Monomers (Crosslinker)	Crosslinking Amount (%), Template, Solvent	Characterization of Binding Parameters	Characterization of Template Release/Transport	IP Structural/Swelling Analysis	Reference
MAA (EGDMA)	Moderate and High (50–90%), Tetracycline	Range with highest partition coefficient (80% crosslinked) of 107.4 and 3.84 mg/g; IP partition coefficients 3–5 times higher than controls. 50% crosslinked gels bound 2.19 mg/g template	IP shows slower release kinetics. However, only 20% released at 8 hrs with no further release	No	Cai and Gupta (2004)

Note: Adapted from reference Venkatesh et al. (2008). We direct the reader to reference Venkatesh et al. (2008) for a list that contains additional entries of transport or release of template from highly crosslinked, rigid networks. When appropriate, crosslinking % was calculated and is equivalent to (100% × (mole crosslinking monomer/(mole crosslinking monomer and all other monomers))). *Abbreviations*: AA: acrylic acid; AM: acrylamide; DMF: dimethylformamide; DMSO: dimethyl sulfoxide; EGDMA: ethylene glycol dimethacrylate; HEMA: 2-hydroxyethylmethacrylate; IP: imprinted polymer; MAA: methacrylic acid; MBA: *N*,*N*-methylene-bis-acrylamide; MMA: methyl methacrylate; M/T: Monomer template ratio; NAA: *N*-acryloyl-alanine polymer; NIP: non-imprinted polymer; NVP: *N*-vinyl 2-pyrrolidinone; PEG200DMA: poly(ethylene glycol)200 dimethacrylate; THF: Tetrahydrofuran; VPD: 4-vinylpyridine.

siderable mobility such as those found in hydrogel networks can expand and collapse depending on the solvent and the interaction between the solvent and the polymer chains. Therefore, imprinted hydrogels can also alter binding differences in response to changes in the solvent (i.e., introducing a better or worse solvent which affect the solvation of polymer chains).

It is no surprise that molecular imprinting strategies have been used in the development of intelligent, recognitive, heteropolymer networks that contain stimuli sensitive moieties within the polymer chains. The first paper from Watanabe et al. (1998) in the field was classical molecular imprinting, but this type of work primarily began by using variations of the molecular imprinting technique (i.e., using templating of charged molecules (e.g., anionic small molecules and ions) with post-crosslinking (Enoki et al., 2000; Alvarez-Lorenzo et al., 2000, 2001b) and acrylated multiple-functionality inclusion complexes (Kanekiyo et al., 2003). Work has now progressed to include a much wider variety of molecules of increasing functionality and molecular size exploiting more non-covalent interactions with comparable synthesis techniques that match traditional molecular imprinting strategies.

On-demand or triggered release, where a stimulus invokes a reversible alteration of the template binding memory site leading to a decrease in template affinity and subsequent template release, has been demonstrated via two mechanisms (Fig. 5). Primarily, it has been demonstrated to occur via changes in the chemistry along the main polymer chains which leads to the movement of the backbone chain and thereby, in a secondary way, disrupting the orientation of the chemistry that makes up the binding cavity. To a much lesser extent, template release has also involved only the disruption of the chemistry that makes up the binding site. This has been accomplished via alteration of the functional monomer(s) charge within the binding site or charges on the template, such as a pH change (Demirel et al., 2005) or changing the conformation of the functional monomer, such as using photoswitchable azobenzene chromophores (Gong et al., 2006), which undergo *cis–trans* isomerization. Of course, decrease in template affinity has also been demonstrated in a variety of highly crosslinked imprinted polymers by knocking out hydrogen bonding between the template and binding site chemistry with increased temperatures or using a solvent that competes or interferes with the binding site non-covalent interactions.

Modulatory mechanisms, or turning binding and release on and off through various cycles has been demonstrated in a number of systems with the successful reorientation of the binding site after the removal of the stimulus. However, in most systems to date the number of cycles has been relatively low, approaching approximately 3–4 cycles, with the sustainability of the binding mechanism decreasing slightly.

Comparing to conventional, intelligent hydrogels without imprinting mechanisms, Gong et al. (2008) are correct when they say "molecularly imprinted responsive materials are able to provide additional degrees of control over the transfer of targeted substrates". The rational design and engineering of intelligent hydrogels using imprinting mechanisms will lead to greater control of the diffusional transport of template in these systems, and may solve some of the limitations of conventional systems, such as drug release or leaking in the collapsed state.

Before 1998, there were no papers discussing the incorporation of stimuli responsive monomers in the design of molecularly imprinted gels. The first paper in the field from Watanabe et al. (1998) demonstrated recognition of template during the shrunken state of a temperature-sensitive polymer with a volume change in response to the template. Subsequent work began by elucidating the memory of conformations in heteropolymer systems exploiting previous work using statistical mechanics to understand

Table 3
Responsive, intelligent imprinted hydrogels.

Stimuli Sensitivity/Trigger	Functional Monomers/ Oligomers (Crosslinker)	Stimuli Sensitive Monomer(s)	Crosslinking Amount (%), Template, Solvent	Characterization of Binding Parameters	Cycles	Reference
pH	Acryloylamylose inclusion complex (MBA)	AA	Low (<1%), Bisphenol-A, Water	At higher degrees of substitution of acryloyl-amylose, enhanced binding was observed. As pH increased template binding decreased as network swelled. IP bound 2.5× NIP	Yes-3 cycles. Reversibility gradually decreased due to hydrolysis of ester bonds of inclusion complex	Kanekiyo et al. (2003)
	Semi-IPN-PVA (MW 70,000-80,000)/AA (trihydroxymethyl propane glycidol ether)	AA	Moderate (33%); MMTCA; DMSO/water (1:1)	At acidic conditions (pH 2 and 4), hydrogen bonding predominate to promote absorption while at pH 7 and 9 stereo shape effect becomes important	No	Wang et al. (2008)
Temperature	MAA (EGDMA)	NIPA	Low (8.7%); 4-aminopyridine; DMF	IP bound ~2.2× NIP in shrunken state	Yes-3 cycles, Nearly equal amount bound after each cycle with 85% release	Liu et al. (2004c)
	MAA (EGDMA)	NIPA	Low (12.5%), L-pyroglutamic acid, Methanol	IP bound ~2.5× NIP in shrunken state	No	Liu et al. (2004a)
	MAA/AM (MBA; EGDMA)	MBA	High, Dopamine HCl, Methanol/Water (4:1)	Imprinting factor increased as temperature increased and gel shrinks. It peaked at 35 °C after which it decreased due to increased non-specific binding	No	Suedee et al. (2006)
pH/Temperature	TBA, AM, Maleic Acid (MBA)	TBA	Low (<1%); BSA; Methanol/Water (1:1)	Max binding at pH 5. Binding increased with template increase in formulation (at 8.63 weight % binding nearly 5× that of NIP) As temperature increases, gel shrinks and binds less template (however still more than corresponding NIP)	No	Demirel et al. (2005)
Light	MAPASA (Various MBA sizes)	MAPASA	High (83%); Paracetamol; DMF/Water	Long crosslinker means high photoisomerization and low binding strength (2C~5 times 8C) *N,N′*-hexylene-bis-acrylamide (6C) is optimal crosslinker. NIP not photoregulated	Yes-3 Cycles Decrease in release and uptake (29.1% decrease by third cycle) explained by deformation of receptors	Gong et al. (2008)
Template, Temperature, Salt Concentration	MAA/AM (MBA)	NIPA	Low (1.4%), Lysozyme, Tris–HCl Buffer (pH 7.0)	IP shrinks with Lys concentration. Binding in IP increases with temperature and salt concentration which decreases gel swelling (max at 20 mM NaCl after which ionic interactions dominate to reduce binding; little binding at 100 mM)	No	Chen et al. (2008)
Template	AM/MAA/DMAEM (MBA)	–	Low, Lysozyme, Tris–HCl Buffer (pH 7.0)	IP for native lysozyme promotes folding of denatured lysozyme. NIP does not promote folding	No	Haruki et al. (2007)
	Poly(AM-g-Con A)/AM (MBA)	–	Low, α-fetoprotein; Phosphate buffer (pH 7.4)	α-Fetoprotein (AFP) provides a recognition link between Con-A and antibody on differing polymer chains. When AFP is in solution, Con-A and anti-AFP are bound together by AFP which leads to shrinking of the gel	No	Miyata et al. (2006)

Table 3 (*Continued*)

Stimuli Sensitivity/Trigger	Functional Monomers/ Oligomers (Crosslinker)	Stimuli Sensitive Monomer(s)	Crosslinking Amount (%), Template, Solvent	Characterization of Binding Parameters	Cycles	Reference
	MAA/NNPA (MBA)	–	Low (5.5%), Theophylline, acetonitrile	IP swells with theophylline concentration but is unaffected by caffeine. NIP does notrespond to either molecule	No	Lavine et al. (2007)
	MAA (EGDMA)	–	Moderate (~20%), Bovine serum albumin, anhydrous ethanol	Albumin causes hydrogel swelling in IP and not NIP. Excellent selectivity between template and lysozyme were demonstrated in competitive binding experiments	No	Hu et al. (2007)
	AA (MBA)	NIPA	Low (4.7%), Dopamine, DMSO	Swelling on analyte binding that is demonstrated to be reversible	Yes. Increasing concentrations of template are studied	Matsui et al. (2005)

Note: For brevity, notable responsive imprinted gels references are missing for ions (Alvarez-Lorenzo et al., 2000, 2001a,b; Hiratani et al., 2001; Basavaraja et al., 2007) and small molecules (Enoki et al., 2000; Watanabe et al., 1998, 2001; Oya et al., 1999). When appropriate, crosslinking % was calculated and is equivalent to (100% × (mole crosslinking monomer/(mole crosslinking monomer and all other monomers))).*Abbreviations*: AA: Acrylic Acid; AFP: α-fetoprotein; AM: Acrylamide; BSA: Bovine Serum Albumin; DMAEM: 2-(dimethylene amino)ethyl methacrylate; DMF: Dimethylformamide; DMSO: dimethyl sulfoxide; EGDMA: Ethylene Glycol Dimethacrylate; IP: Imprinted Polymer; IPN: interpenetrating network; Lys: Lysozyme; MAA: Methacrylic Acid; MAPASA: 4-[(4-methacryloyloxy)phenylazo]benzenesulphonic acid; MBA: *N*,*N*–methylene-bis-acrylamide; MMTCA: 1-(4-methoxyphenyl)-5-methyl-1,2,3-triazol-4-carboxylic acid; NIP: Non-Imprinted Polymer; NIPA: *N*-isopropylacrylamide; NNPA: *N*-(*N*-propyl)acrylamide; PVA: poly(vinyl alcohol); VA: Vinyl Acetate; TBA: *N-tert*-Butylacrylamide.

macromolecular recognition. Tanaka and co-workers (Oya et al., 1999) demonstrated that a target molecule could be captured by multiple-point electrostatic adsorption of flexible polymer chains and have differing affinity for the target. By varying polymer conformation and concentration, polymer gels demonstrated reversible thermosensitive affinity for target molecules by two or three orders of magnitude. Ionic recognition of Pyranine with 3 or 4 negative charges was proven in high salt concentration thereby limiting a mechanism based on Donnan potential. It is important to note that these gels were not polymerized in the presence of the template molecule as is the case with molecular imprinting. Nonetheless, this work outlined the strong possibilities of controlling and modulating target molecule affinity by the concentration and conformation of chemical groups along the polymer chain. The proximity of these groups and subsequent recognition was controlled by the reversible phase transition of groups along the polymer chains. This involved one monomer with non-covalent interaction with the target and another monomer that would undergo a transition.

Shortly afterward, three papers by Tanaka and co-workers were published that utilized molecular imprinting concepts in the creation of weakly crosslinked gels with reversible thermosensitive affinity (Enoki et al., 2000; Alvarez-Lorenzo et al., 2000, 2001b). The first published paper utilized the same target, Pyranine tri- and tetrasulfonic sodium salt with 3 or 4 negative charges, and involved a post-crosslinking thiol reaction in the presence of the target as template. This was the earliest attempt in these systems to fix the conformation of polymer chains in a global energy minimum to impart memory within the flexible polymer network chains. However, templating was accomplished after the network was polymerized and was limited by not allowing a preferred, templated sequence of monomers. The other two papers included the molecular imprinting concept using a template (lead and calcium ions) in the polymerization reaction, which is more consistent with conventional molecular imprinting techniques (Alvarez-Lorenzo et al., 2000, 2001b). This is the first evidence of the use of molecular imprinting beginning with functional monomeric units demonstrating recognition responsiveness to capture template ions.

Later work by some of the authors included concurrent multiple point adsorption of two oppositely charged ions and a reversible crosslinker to further ascertain an enhancement of the imprinting technique (Alvarez-Lorenzo et al., 2001a; Hiratani et al., 2001). A physical theory has been suggested for adsorption of templates to random heteropolymer gels, which were modeled as a set of adsorbing monomers connected by Gaussian chains to fixed crosslinking points (Ito et al., 2003). Recently, Basavaraja et al. (2007) presented a self-oscillating imprinted hydrogel depending on the oxidation state of a metal catalyst group contained in the polymer chains. The oscillation is induced by redox change of the covalently bound catalyst, which alters the hydrophilicity of the polymer chains leading to swelling and less effective recognition of ions.

An article published in 2003 by Tao and co-workers, was one of the first to demonstrate a pH-responsive molecularly imprinted polymer for a relatively small molecule, bisphenol-A (Kanekiyo et al., 2003). It involved a helical inclusion-complex between amylose, a polymer of glucose which adopts a helical structure, and bisphenol-A. The paper keenly controlled the stability of the inclusion complex by controlling the degree of substitution of the amylose, which was substituted with acryloyl groups. The backbone polymer was acrylic acid and the crosslinking monomer was *N*,*N*′-methylene-bisacrylamide. As the pH of solution increased, acrylic acid groups along the main polymer chains ionized leading to network swelling and a disruption in the inclusion complex structure which decreased bisphenol-A binding. As the degree of substitution increased, higher template binding was demonstrated at every

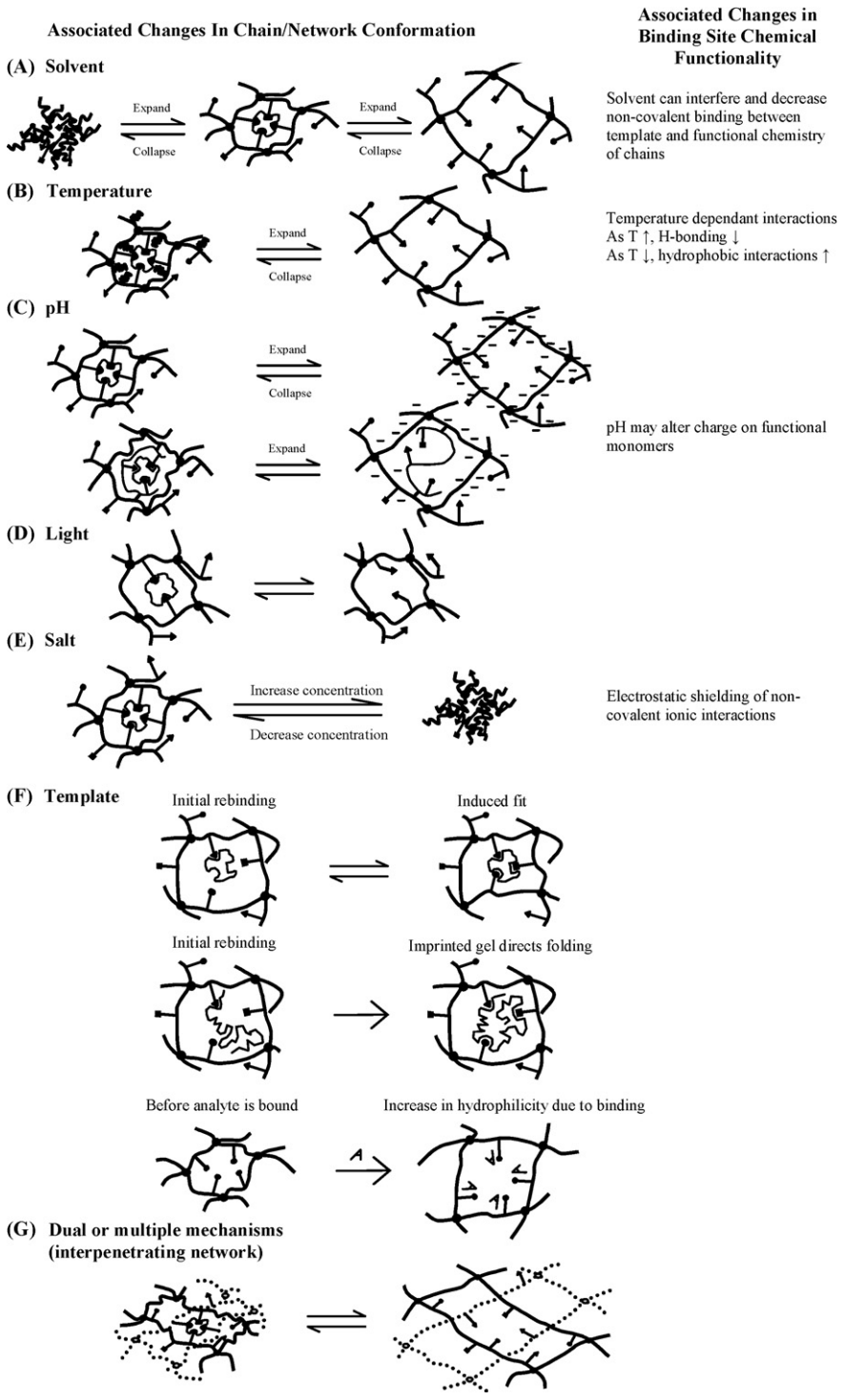

Fig. 5. Modulatory mechanisms within responsive imprinted hydrogel networks. Changes can occur in the chain/network conformation (*left*) and also in the binding site functional groups (*right*) leading to template recognition or release. (A) Solvent-sensitive imprinted network. The gel can collapse or expand depending on the polymer–solvent interaction. In a good solvent, the polymer chains will be solvated and the network will expand. In a poor solvent, the chains will prefer to be in close proximity to each other and the network will collapse. (B) Temperature-sensitive imprinted network. Temperature sensitive groups along the polymer chains aggregate as they become more hydrophobic with an increase in temperature, leading to collapse of the polymer network (Liu et al., 2004a,c; Suedee et al., 2006). (C) pH-sensitive imprinted network. As groups along the polymer ionize (acrylic acid groups ionize at pH above ~4.5), the chains become more hydrophilic and also exhibit charge repulsion, leading to swelling and disrupting of the binding cavity. This has been demonstrated using a covalently attached amylose inclusion complex that is altered in conformation due to the movement of the network chains (Wang et al., 2008). If the chain monomers are cationic, the gel expands as pH is decreased. (D) Light-sensitive imprinted network. Light affects the conformation of functional monomers disrupting complexation (Gong et al., 2008) (E) Salt-sensitive imprinted network. As salt concentration is increased (Chen et al., 2008), the NIPA-based network collapses due to a dehydration mechanism via to destabilization of water molecules clustered around the isopropyl group. (F) Template-sensitive imprinted network. As template binds, the imprinted network shrinks to accommodate the template (Chen et al., 2008) and also has been demonstrated to depend on concentration of template (Watanabe et al., 1998). Other methods have included imprinted gels that swell with binding of template (Lavine et al., 2007) and biomolecular methods using lectin and antibody chains that complex as glycoprotein enters the network (Miyata et al., 2006). Imprinted networks have also assisted in the folding of protein (Haruki et al., 2007). (G) Dual mechanisms or interpenetrating imprinted networks. Using networks with differing stimuli-sensitivities that are physically entagled, template binding and recognition can be further controlled (Wang et al., 2008).

pH value. This work was also first to highlight a number of cycles indicating macromolecular memory could be switched on and off multiple times. The sustainability of the cycles depends on a number of factors, and in this case it decreased slightly due to hydrolysis of the ester bonds connecting the inclusion complex to the polymer network.

Stimuli-responsive lysozyme imprinted polymers were demonstrated to respond to temperature and salt concentration, but also to the template proteins producing significant volume shrinking (Chen et al., 2008). The polymer gel decreased in volume and the volume fraction in the swollen state increased as template protein increased. At concentration of lysozyme greater than 1 mg/mL, the polymer reached a limit of 85% of original volume. A small decrease was apparent using the template as well as bovine serum albumin with the non-imprinted system.

Another interesting notion is the movement of flexible polymer chains in the assisted assembly of template guided by multiple complexation points. Recently, exciting work has demonstrated that an imprinted polymer for native lysozyme promoted the folding of chemically denatured lysozyme (Haruki et al., 2007). At protein concentrations of 0.125 mg/mL with refolding yield measure by enzymatic activity, imprinted structures demonstrated substantially higher refolding yields as compared to non-imprinted polymers, which did not demonstrate template refolding. The authors also compared the refolding of lysozyme in a cytochrome *c* imprinted polymer (i.e., a similarly sized protein), and found the correct pore size alone did not lead to folding. The mechanism of molecularly imprinted assisted folding and the potential of an "induced fit mechanism" is convincing and plausible, but more details of the mechanism are needed. It is important to note that the poly(acrylamide-co-2-(dimethylamino)ethyl methacrylate-co-methacrylic acid-co-*N*,*N*′-methylenebisacrylamide) hydrogel network had multiple complexation points with the template.

Temperature-sensitive imprinted polymers for larger molecules have also been demonstrated using *N*-isopropylacrylamide as temperature sensitive monomer along with functional monomers that interact with the template. This has been shown for L-pyrogluamic acid (Liu et al., 2004a) and 4-aminopyridine templates (Liu et al., 2004b) using methacrylic acid as functional monomer with ethylene glycol dimethacrylate as crosslinking monomer. It is notable that reference (Liu et al., 2004a) demonstrates the reusability of these gels showing four loading/release cycles with similar template loading and release amounts.

Light responsiveness has also been demonstrated. The first paper in the field demonstrating photoresponsive imprinting had caffeine as template (Gong et al., 2006). Recently, a molecularly imprinted polymer has been synthesized with photoregulated affinity for paracetamol. Irradiation at 440 nm resulted in binding and irradiation at 353 nm resulted in release. The polymer contained 4-[(4-methacryloyloxy)phenylazo] benzenesulfonic acid (MAPASA) that undergoes *trans–cis* photoisomerization within a polyacrylamide hydrogel. Non-imprinted gels did not demonstrate photoregulated release and two structural analogs had significantly less photoregulated uptake and release. Three uptake and release cycles were demonstrated with a reduction in the template bound and released, hypothesized to be due to a gradual deformation of the imprinted receptor sites (Gong et al., 2008).

Recently, interpenetrating polymers of poly(acrylic acid) and poly(vinyl alcohol) were imprinted for 1-(4-methoxyphenyl)-5-methyl-1,2,3-triazol-4-carboxylic acid (MMTCA) (Wang et al., 2008). The imprinted interpenetrating network (IPN) exhibited higher binding capacity than the non-imprinted IPN and networks of either polymer alone. Work has also included the imprinting of metal ions (Yamashita et al., 2003) and hemoglobin (Xia et al., 2005) via interpenetrating networks.

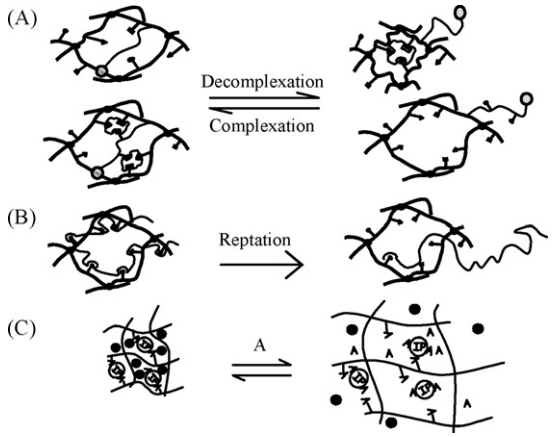

Fig. 6. Future modulatory template release of imprinted hydrogels. (A) A grafted chain that reversibly complexes with group on other chains can create imprinted gels that turn recognition on and off. This grafted chain can also have functionality that can non-covalently bind with the template. Disruption of the complex may lead to disruption of the binding site. (B) The transport of long molecular weight molecules can be also be controlled by imprinting mechanisms. While the size of the macromolecule and its conformation as well as the polymer mesh size will influence release, imprinting will lead to an additional level of control to delay release or turn release on and off (Ali and Byrne, 2008). (C) An imprinted polymer particle, covalently attached or bigger than network mesh, can be used as effective crosslinks in a polymer gel that has pendent attached template to the polymer chains. When free template enters the gel, the gel will expand (Byrne et al., 2002a).

Exciting progress has been made in small molecule and protein-sensitive gels, where the template either alters the water solvation of polymer chains or results in effective crosslinks in the gel. These papers are discussed in Section 5.2.

In the last few years, there has been a considerable increase in the number of imprinted gels with responsive mechanisms. As with conventional intelligent hydrogels, the number of responsive mechanisms will increase (e.g., there are no magnetic or electric field responsive imprinted gels to date) and the type of networks will expand to include more multiple-responsive mechanisms. Considerable effort will also be put forward to increase the dynamics associated with the recognition/release cycle. This will be obtained by carefully controlling network structure and the size of the gel. Maintaining cycles without reduction in binding/affinity is not a significant problem to overcome, but significant response lifetimes have not been demonstrated. We highlight some of our envisioned intelligent imprinted systems of the future in Fig. 6.

5. Emerging and future translational applications of imprinted hydogels

Imprinted gels are currently being used to create functional materials in controlled and modulated drug delivery, sensors and diagnostics, membrane separation, and solid phase extraction. In these fields, they are leading to significant solutions and the development of new materials. Future applications of imprinted gels include tissue engineering, fluidic valves and actuators, as well as coatings for a number of drug delivery carriers and medical devices.

5.1. Controlled and modulated therapeutic delivery and controlled recognition/transport processes

Controlled drug release from hydrogels has been extensively studied for the past three decades. Molecular design and control of the network architecture are driving new developments in the field. Only recently has molecular imprinting been applied to drug delivery, which is highlighted in the following reviews (Byrne et

al., 2002a; Hilt and Byrne, 2004; Alvarez-Lorenzo and Concheiro, 2004; Sellergren and Allender, 2005; Allender et al., 2000; Cunliffe et al., 2005).

In order to emphasize the significance imprinting may have on drug delivery, it is best to highlight the past. Langer and co-workers (Bawa et al., 1985) studied the interconnected pore network structures formed when proteins were included within the creation of normally non-porous polymers. The study of the voids left behind or the amount of protein trapped within the structure led to the rational development of formulations to extend or delay the transport of the therapeutic protein from the polymer structure. These early studies indicated how the nature of embedded molecule and the concentration and size of these molecules influenced the underlying porosity of the resulting polymer, and subsequent release.

Other than highlighting non-specific interactions between the drug and polymer such as hydrophobicity affecting drug transport, the field has done very little to understand the influence of the molecule on the organization of the chemical functionality and orientation of polymer chains when it is included in the polymerization process. To be clear, this is exactly where molecular imprinting is gaining a new role in drug delivery. Imprinted network formation, with a proper optimization of drug affinity relating to number and strength of functional monomer interactions, crosslinking structure, and mobility of polymer chains, has a strong potential to influence a number of hydrogel carriers and add to the variables one can alter to tune the release profile. Imprinting can lead to delayed transport of therapeutic.

Two schemes are typically used to load therapeutics into hydrogels – produce the gel in the presence of drug or synthesize the gel and then load drug into the gel via equilibrium partitioning. It is common knowledge that preparation conditions of hydrogels can lead to significant changes in the network structure and resulting properties. Surprisingly, no work has addressed the potential impact of the inclusion of drug and its affect on the organization of polymer chains. Only recently has work addressed the potential to extend or optimize controlled release by tailored drug–polymer chain interactions, such as those produced by molecular imprinting. Table 2 presents imprinted hydrogels with analysis of template release or transport. It is clear that there have not been many drug release studies conducted on weakly crosslinked imprinted structures or hydrogels. Recently, we have proven that imprinting leads to delayed template transport and it is not due to differing mesh structures or porosity (Venkatesh et al., 2008).

To further clarify the role of imprinting in controlled release, one must first look at mechanisms of drug release from hydrogel structures. We direct the reader to the following references (Peppas et al., 2000; Brazel and Peppas, 2000; Peppas, 1987b; Lin and Metters, 2006). Molecular imprinting can provide control of the drug release profile in swollen networks, dynamically swelling or swelling-controlled networks (i.e., drug-loaded networks going from a dry to a swollen state), and responsive-swollen networks (i.e., a swollen gel that undergoes a reversible volume transition based on a stimulus such as pH, temperature, ionic strength, etc.). Responsive hydrogels can be engineered to change network structure in response to a stimulus due to the presence of specific chemical/biological species along their backbone polymer chains.

For swelling-controlled release from hydrogels, if there is a constant rate of solvent front penetration which is much smaller than the drug diffusion rate in the swollen gel, a constant drug release rate, or zero-order release, arises (Brazel and Peppas, 2000). Imprinting may aid this process in swelling-controlled gels, decreasing the drug diffusion rate. If the polymer relaxation rate is high and the drug diffusion is rate limiting, this results in the drug release rate being proportional to the concentration gradient between the drug source and the surroundings (i.e., a Fickian drug release profile). In this situation, a number of strategies have been attempted to achieve an extended zero-order release such as bioerodible and biodegradable systems with solvent penetration fronts moving with similar velocities as the outer eroding front (Tahara et al., 1995), hydrogels with rate controlling-barriers such as higher crosslinked outer edges (Lee et al., 1980), and non-uniform drug distribution (Lee, 1984).

Molecular imprinting has led to the development of extended drug releasing contact lenses, which cannot use conventional strategies to delay drug transport from the equilibrium swollen polymer network (Venkatesh et al., 2007, 2006; Alvarez-Lorenzo et al., 2002, 2006a; Hiratani et al., 2005; Hiratani and Alvarez-Lorenzo, 2004). Recently, dynamic, *in vitro* drug release studies from imprinted hydrogel contact lenses within a novel microfluidic device that simulated the volumetric flow rate, tear volume, and composition of the eye resulted in a constant, zero order release (Ali et al., 2007). Imprinting delays transport from the polymer chains, and a tumbling hypothesis was recently proposed analyzing one-dimensional template transport (Venkatesh et al., 2008). We direct the reader to the following articles discussing the impact of such systems in ocular drug delivery (Ali and Byrne, 2008; Alvarez-Lorenzo et al., 2006b).

Recently, multi-nuclear heteroneuclear correlation solid state NMR spectroscopy has been used to provide evidence of monomer–drug interactions leading to sustained release (Paul et al., 2007), but it is important to note that hydrophobic effects dominated the drug release profile of the imprinted porous gels prepared in ethanol/water (Bogershausen et al., 2006). Hydrophobic interaction with the drug retarded the drugs dissolution or release kinetics and the hydrophobicity of the gel reduced water diffusion in the pores. However, studies such as these will lead to validation of imprinting mechanisms delaying transport.

Further control of transport at decreasing thicknesses will be paramount for the success of micro- and nanoscale drug delivery carriers. Imprinted drug delivery networks will be especially important is situations where the carrier or film must be limited in volume or extended release is needed from thin layers.

Work has also included transdermal drug delivery (Allender et al., 2000; Bodhibukkana et al., 2006), solid-phase extraction (Suedee et al., 2006; Aburto et al., 2004), and membrane separation (Silvestri et al., 2005a,b; Aburto et al., 2004); however, most of these networks are not flexible gels. Recently, selective enrichment has been demonstrated (Zhao et al., 2006), and dopamine-imprinted, temperature-sensitive polymer gels have been used for selective separation (Suedee et al., 2006). Imprinted, temperature-sensitive networks have also been grafted on non-woven polypropylene films (Tokuyama et al., 2008), where separation of heavy metal ions by temperature swing adsorption has been demonstrated with ion adsorption/desorption kinetics (Tokuyama et al., 2008, 2005). Also, enantioselective-controlled delivery was investigated by Suedee et al. (2000) (Bodhibukkana et al., 2006), where they applied imprinted networks as enantioselective excipients and transdermal systems. Composite membranes for transdermal delivery of *S*-propranolol have been developed using pore functionalization via imprinting with selective transport of the enantiomer (Bodhibukkana et al., 2006). Also, an enantioselective membrane for L-phenyalanine (template) over D-phenylalanine was prepared by sol–gel process (Jiang et al., 2006). Hybrid membranes of chitosan and γ-glycidoxypropyltrimethoxysilane with varying degrees of crosslinking demonstrated increase in enantiomeric selectivity factor as membrane swelling decreased or the crosslinking content increased. The imprinted gels had higher template binding and decreased template permeation.

Combining imprinted poly(acrylamide-co-*N*,*N*′-methylene-bis-acrylamide) gels with electrophoresis has created an exciting,

powerful analytical tool to selectively separate protein (Takatsy et al., 2006a), virus (Semliki Forest Virus, diameter 70 nm) (Takatsy et al., 2006b), and *E. coli* bacteria (rod shape, 1-2 μm in length, 0.1–0.5 μm in diameter) (Bacskay et al., 2006). The artificial gels can sense the difference between the template virus and a mutant virus which only differs by three amino acids in one of the three proteins on the surface of the virus particle (Takatsy et al., 2006b). Neutral imprinted gel particles migrated in the electric field when complexed with charged virus, protein (Takatsy et al., 2006a), or cells (Bacskay et al., 2006).

Imprinted hydrogels have also performed catalysis with pH sensitivity decreasing the activity, as demonstrated by Karmalkar et al. (1997). This work highlights the proximity of active functional groups by imprinting and demonstrates on-off release of catalyzed molecule. Coating silica beads with imprinted hydrogels have been used to bind and detect lysozyme (Hirayama et al., 2001), and imprinted calcium alginate gel microspheres have been prepared with recognition for albumin (Zhao et al., 2008).

5.2. Sensor substrates, diagnostics, and biomarker detection

Molecularly imprinted hydrogels are gaining popularity as recognition elements due to their ability to translate analyte binding event into a mechanical or chemical signal. Imprinted polymers are more robust than biological sensing elements and have economic advantage in terms of raw material price as well as manufacturability. Recently, Lotierzo et al. (2004) showed that imprinted polymers outperformed monoclonal antibody natural receptors with a wide detection range and long stability. These studies are prompting the transition of imprinted networks toward point-of-care diagnostics, sensors that must work in areas outside the controlled environment of the laboratory. The main problem associated with large molecules is decreased transport through gels, which increases response time. However, with the use of extremely thin films satisfactory results can be achieved (Noss et al., 2008; Hilt et al., 2006b). We direct the reader to the following reviews of imprinted sensors (Haupt and Mosbach, 2000; Henry et al., 2005). A selection of papers that utilize imprinted hydrogels is highlighted in the following paragraphs.

Lavine et al. (2007) describe molecularly imprinted, temperature-sensitive nanogel particles which selectively bind theophylline template. The binding event increases the phase transition temperature of the gel, and the increased hydrophilicity results in volume swelling of the gel. The volume transition decreases the refractive index which is used to quantify the amount of theophylline bound using surface plasmon resonance spectroscopy. Theophylline concentration values as low as 10^{-6} M were detected using the sensor. In addition, caffeine, which is similar in structure to theophylline, does not cause a volume transition in the particles even at values as high as 10^{-2} M.

Another system where sensing is based on swelling of the imprinted gel utilizes immobilized gold nanoparticles within a temperature-sensitive, imprinted gel coated on a gold substrate (Matsui et al., 2005). As the template, dopamine, is bound, the gel swells resulting in greater distance between the gold nanoparticles and the substrate. This change in the distance is reflected as a change in the contact angle in a surface plasmon resonance curve.

Results have also been very encouraging with large molecules such as proteins, viruses, and DNA. Miyata et al. (2006) used imprinted hydrogels to recognize tumor marker glycoproteins by lectin and antibody ligands. Lectin (Con-A) and antibodies (polyclonal anti-AFP) were first functionalized with vinyl groups using *N*-succinimidylacrylate and then a copolymer of Con A and acrylamide was prepared. Then poly(acrylamide-g-Con-A) was copolymerized with acrylamide with *N*,*N*′-methylenebisacrylamide as crosslinker in the presence of the template, α-fetoprotein (AFP). AFP is a glycoprotein widely used for the serum diagnosis of primary hepatoma. AFP provides a recognition link between the Con-A and the antibody, which are on different polymer chains. Thus, when free AFP is present, Con-A and anti-AFP are bound together by AFP which leads to shrinking of the gel. The work also demonstrates selectivity for AFP over ovalbumin, another glycoprotein with a saccharide chain similar to AFP but with a different peptide chain. This work promises an intensive application for cancer detection.

Slinchenko et al. (2004) have successfully imprinted dsDNA molecules in a gel using an epitope approach. The functional monomer, 2-vinyl-4,6-diamino-1,3,5-triazine, interacts with the adenine–thymine-base pair. This method is simple and inexpensive to detect or separate specific dsDNA in gel electophoresis using the imprinted gel (Ogiso et al., 2006).

Recently, a three-dimensional highly ordered macroporous structure was produced using silica colloidal crystal templating (Hu et al., 2007). In this highly innovative work, an albumin imprinted hydrogel was polymerized within the void spaces of a silica colloidal crystal array. When the silica and protein was removed, a surface imprinted macroporous gel was produced. Selective protein binding and subsequent hydrogel swelling was determined optically via color change of the imprinted film without the use of a transducer. This system was fast (i.e., on order of minutes) and very sensitive with a 1 ng/mL concentration of albumin detected. Recently, this same technique was used to produce selective theophylline and ephedrine photonic-imprinted hydrogels (Hu et al., 2008). Biosensing via optical detection of molecule-sensitive hydrogels is very promising, demonstrated by using bioconjugated hydrogels as microlenses (Ehrick et al., 2007). Upon recognition of molecules and loss of effective crosslinks within the gel, swelling occurs and allows a pattern on the substrate to be visualized. It is important to note that this work (Ehrick et al., 2007) did not exploit imprinting strategies; however, it is evident that molecular imprinting will be very beneficial in the transition of these devices to market and the generalization of these systems.

Sol gel procedures exploiting molecular imprinting are increasing. Imprinted sol–gels using silanes have demonstrated template recognition for nafcillin (Guardia et al., 2008) and have been used to functionalize tin oxide sensor surfaces for the template dopamine (Gao et al., 2007). They have also been used to functionalize the surface of chitosan microspheres. Sol–gel processes for chitosan spheres have included using siloxanes to imprint albumin (Li et al., 2008), grafting polyacrylamide to imprint hemoglobin (Guo et al., 2005; Fu et al., 2008) or albumin (Guoqi et al., 2007), or grafting poly (methacrylic acid-co diethylene glycol diacrylate) gel imprinted for quercetin (Xia et al., 2006).

Recently, Brown and Puleo (2008) produced porous silica scaffolds using a sol–gel process imprinting protein using the epitope approach. Epitope imprinted gels demonstrated recognition for the template protein over controls, but it did not demonstrate selectivity under competitive binding experiments. Also, the epitope imprinted gels demonstrated less differential binding compared to full protein imprinted gels. This was attributed to the short size of the epitope peptide (approximately 12% of the protein).

5.3. Future applications

Tissue engineering utilizes hydrogel scaffolds for bioactive molecule delivery which allows for very specific localized delivery (Biondi et al., 2008; Malafaya et al., 2002; Saltzman and Olbricht, 2002). Hydrogel systems have been studied (Drury and Mooney, 2003) as a vehicle to deliver proteins such as the angiogenesis promoting growth factor (VEGF), basic fibroblast growth factor (bFGF) and osteogenesis promoting bone morphogenic pro-

tein (BMP). Kiick and co-workers (Nie et al., 2007) describe the synthesis of heparin-functionalized hydrogels for the controlled delivery of bFGF. The system consists of high molecular weight heparin functionalized with maleimide crosslinked into PEG-based hydrogels. These hydrogels bind to the growth factors on the basis of noncovalent protein–glycosaminoglycan interactions. Molecular imprinting offers tissue engineers the ability to control binding and release of growth factors as well as a scaffold of controlled porosity and pore-size uniformity to enhance cell-adhesion and tissue in-growth. Molecular imprinting can also provide a means for increasing the interaction between the cells and the hydrogel scaffold by imprinting specific protein molecules or cells as well as providing modulatory events that aid in cellular organization and protection. These interactions may promote faster cell differentiation and growth. For example, Shin et al. (2002) used hydrogels modified with RGD peptide sequences to promote specific binding of marrow stromal cells. Recently, a biodegradable nanotemplated hydrogel network was produced using a self-assembling lyotropic crystal crystalline mesophase as template. This led to a highly organized lamellar matrix geometry that enhanced mesenchymal cell attachment (Clapper et al., 2008).

In 2004, Eddington and Beebe (2004a) published an insightful review describing the potential for stimuli-responsive hydrogels in controlling the flow of fluids through micro-channels. Stimuli responsive hydrogels are naturally suited to being used as flow control valves or actuators due to their ability to collapse or swell 'on demand' (Liu et al., 2002; Eddington and Beebe, 2004b; Richter et al., 2003; Lee et al., 2003). Combined with sharp responses at micro length scales and independence from external control, hydrogel valves also offer the additional advantage of in-situ formation, greatly reducing complexity of fabrication. Imprinted responsive gels, which have not been demonstrated within fluidic systems yet, show tremendous potential as robust, template-sensitive valves. Binding of template can reduce or slow the transport of a detector molecule or lead to a volume change in the polymer valve, which would then allow transport of template or detector molecule. Of course responsive hydrogels, as discussed in Section 4, can also be used in micro-fluidic applications to capture or release templates for use in micro-scale reactors, separators, etc.

In conclusion, it is easy to imagine imprinted hydrogel films/coatings on medical devices, polymer carriers, and drug particles. Imprinted films would provide an additional level of control in these decreased length scale applications where delayed release of therapeutic is imperative and other mechanisms cannot be used.

6. Conclusions, challenges, and future considerations

Recent progress in the field of imprinted hydrogels is leading to exciting developments. The field of hydrogel imprinting did not exist 10 years ago and has seen significant growth with the realization of large molecule imprinting within flexible structures. The field has transitioned to protein and larger particle imprinting and has begun to confirm selectivity of imprinted gels. Also, responsive gels have exploded in both the number of modulatory mechanisms to bind or release template and in the control of such mechanisms. The future is indeed bright, and the next few years will see unprecedented progress in the control and fabrication of such systems and the translational application of these intelligent structures at all length scales within pharmacy, medicine, tissue engineering, sensors and diagnostics, micro and nanodevices, and separation processes.

Challenges exist and substantial work will be necessary to characterize and direct template diffusional phenomena within imprinted gels, to relate the complexation contribution of multiple polymer chains and template to thermodynamic theories of polymer network dynamics, to control and engineer the network structure, and to understand the templating polymerization reaction and diverse functional monomer incorporation. This work will invariably lead to further understanding of designed heteropolymer recognition and potentially provide valuable insight into biohybrid conjugated structures and protein folding theories.

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References

Aburto, J., Le Borgne, S., 2004. Selective adsorption of dibenzothiophene sulfone by an imprinted and stimuli-responsive chitosan hydrogel. Macromolecules 37, 2938–2943.

Aburto, J., Mendez-Orozco, A., LeBorgne, S., 2004. Hydrogels as adsorbents of organosulphur compounds currently found in diesel. Chem. Eng. Process. 43, 1587–1595.

Alexander, C., Andersson, H.S., Andersson, L.I., Ansell, R.J., Kirsch, N., Nicholls, I.A., O'Mahony, J., Whitcombe, M.J., 2006. Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. J. Mol. Recognit. 19, 106–180.

Ali, M., Byrne, M.E., 2008. Challenges and solutions in topical ocular drug-delivery systems. Expert Rev. Clin. Pharmacol. 1, 145–161.

Ali, M., Horikawa, S., Venkatesh, S., Saha, J., Hong, J.W., Byrne, M.E., 2007. Zero-order therapeutic release from imprinted hydrogel contact lenses within in vitro physiological ocular tear flow. J. Control. Release 124, 154–162.

Allender, C.J., Richardson, C., Woodhouse, B., Heard, C.M., Brain, K.R., 2000. Pharmaceutical applications for molecularly imprinted polymers. Int. J. Pharm. 195, 39–43.

Alvarez-Lorenzo, C., Concheiro, A., 2004. Molecularly imprinted polymers for drug delivery. J. Chromatogr. B 804, 231–245.

Alvarez-Lorenzo, C., Guney, C., Oya, O., Sakai, T., Kobayashi, Y., Enoki, M., Takeoka, T., Ishibashi, Y., Kuroda, T., Tanaka, K., Wang, K., Grosberg, G., Masamune, A.Y., Tanaka, S.T., 2000. Polymer gels that memorize elements of molecular conformation. Macromolecules 33, 8693–8697.

Alvarez-Lorenzo, C., Hiratani, H., Tanaka, K., Stancil, K., Grosberg, A.Y., Tanaka, T., 2001a. Simultaneous multiple-point adsorption of aluminum ions and charged molecules a polyampholyte thermosensitive gel: controlling frustrations in a heteropolymer gel. Langmuir 17, 3616–3622.

Alvarez-Lorenzo, C., Guney, O., Oya, T., Sakai, Y., Kobayashi, M., Enoki, T., Takeoka, Y., Ishibashi, T., Kuroda, K., Tanaka, K., Wang, G.Q., Grosberg, A.Y., Masamune, S., Tanaka, T., 2001b. Reversible adsorption of calcium ions by imprinted temperature sensitive gels. J. Chem. Phys. 114, 2812–2816.

Alvarez-Lorenzo, C., Hiratani, H., Gomez-Amoza, J.L., Martinez-Pacheco, R., Souto, C., Concheiro, A., 2002. Soft contact lenses capable of sustained delivery of timolol. J. Pharm. Sci. 91, 2182–2192.

Alvarez-Lorenzo, C., Yanez, F., Barreiro-Iglesias, R., Concheiro, A., 2006a. Imprinted soft contact lenses as norfloxacin delivery systems. J. Control. Release 113, 236–244.

Alvarez-Lorenzo, C., Hiratani, H., Concheiro, A., 2006b. Contact lenses for drug delivery. Am. J. Drug Deliv. 4, 131–151.

Amsden, B., 1998a. Solute diffusion within hydrogels. Mechanisms and models. Macromolecules 31, 8382–8395.

Amsden, B., 1998b. Solute diffusion in hydrogels. An examination of the retardation effect. Polym. Gels Networks 6, 13–43.

Bacskay, I., Takatsy, A., Vegvari, A., Elfwing, A., Ballagi-Pordany, A., Kilar, F., Hjerten, S., 2006. Universal method for synthesis of artificial gel antibodies by the imprinting approach combined with a unique electrophoresis technique for detection of minute structural differences of proteins, viruses, and cells (bacteria). III. Gel antibodies against cells (bacteria). Electrophoresis 27, 4682–4687.

Basavaraja, C., Park, D.Y., Choe, Y.M., Park, H.T., Zhao, Y.S., Yamaguchi, T., Huh, D.S., 2007. Dependence of molecular recognition for a specific cation on the change of the oxidation state of the metal catalyst component in the hydrogel network. Bull. Kor. Chem. Soc. 28, 805–810.

Bawa, R., Siegal, R.A., Marasca, B., Karel, M., Langer, R., 1985. An explanation for the controlled release of macromolecules from polymers. J. Control. Release 1, 259–267.

Bergmann, N.M., Peppas, N.A., 2008. Molecularly imprinted polymers with specific recognition for macromolecules and proteins. Prog. Polym. Sci. 33, 271–288.

Binet, C., Ferrere, S., Lattes, A., Laurent, E., Marty, J.D., Mauzac, M., Mingotaud, A.F., Palaprat, G., Weyland, M., 2007. Benefit of liquid crystal moieties in the MIP technique. Anal. Chim. Acta 591, 1–6.
Biondi, M., Ungaro, F., Quaglia, F., Antonio Netti, A., 2008. Controlled drug delivery in tissue engineering. Adv. Drug Deliv. Rev. 60, 229–242.
Blackburne, B.P., Hirst, J.D., 2001. Evolution of functional model proteins. J. Chem. Phys. 115, 1935–1942.
Bodhibukkana, C., Srichana, T., Kaewnopparat, S., Tangthong, N., Bouking, P., Martin, G.P., Suedee, R., 2006. Composite membrane of bacterially-derived cellulose and molecularly imprinted polymer for use as a transdermal enantioselective controlled-release system of racemic propranolol. J. Control. Release 113, 43–56.
Bodugoz, H., Guven, O., Peppas, N.A., 2007. Glucose recognition capabilities of hydroxyethyl methacrylate-based hydrogels containing poly(ethylene glycol) chains. J. Appl. Polym. Sci. 103, 432–441.
Bogershausen, A., Pas, S.J., Hill, A.J., Koller, H., 2006. Drug release from self-assembled inorganic–organic hybrid gels and gated porosity detected by positron annihilation lifetime spectroscopy. Chem. Mater. 18, 664–672.
Bolisay, L.D., Culver, J.N., Kofinas, P., 2006. Molecularly imprinted polymers for tobacco mosaic virus recognition. Biomaterials 27, 4165–4168.
Bolisay, L.D., Culver, J.N., Kofinas, P., 2007. Optimization of virus imprinting methods to improve selectivity and reduce nonspecific binding. Biomacromolecules 8, 3893–3899.
Bossi, A., Bonini, F., Turner, A.P.F., Piletsky, S.A., 2007. Molecularly imprinted polymers for the recognition of proteins: the state of the art. Biosens. Bioelectron. 22, 1131–1137.
Brannon, L., Peppas, N.A., 1991. Equilibrium swelling behavior of pH-sensitive hydrogels. Chem. Eng. Sci. 46, 715–722.
Brazel, C.S., Peppas, N.A., 2000. Modeling of drug release from swellable polymers. Eur. J. Pharm. Biopharm. 49, 47–58.
Brown, M.E., Puleo, D.A., 2008. Protein binding to peptide-imprinted porous silica scaffolds. Chem. Eng. J. 137, 97–101.
Bryngelson, J.D., Wolynes, P.G., 1987. Spin glasses and the statistical mechanics of protein folding. Proc. Natl. Acad. Sci. U.S.A. 84, 7524–7528.
Byrne, M.E., Park, K., Peppas, N.A., 2002a. Molecular imprinting within hydrogels. Adv. Drug Deliv. Rev. 54, 149–161.
Byrne, M.E., Oral, E., Hilt, J.Z., Peppas, N.A., 2002b. Networks for the recognition of biomolecules: molecular imprinting and micropatterning poly(ethylene glycol)-containing films. Polym. Adv. Technol. 13, 798–816.
Byrne, M.E., Hilt, J.Z., Peppas, N.A., 2008. Recognitive biomimetic networks with moiety imprinting for intelligent drug delivery. J. Biomed. Mater. Res. Part A 84A, 137–147.
Cai, W., Gupta, R.B., 2004. Molecular-imprinted polymers selective for tetracycline binding. Sep. Purif. Methods 35, 215–221.
Canal, T., Peppas, N.A., 1989. Correlation between mesh size and equilibrium degree of swelling of polymeric network. J. Biomed. Mater. Res. 23, 1183–1193.
Chen, Y., Kele, M., Quinones, I., Sellergren, B., Guiochon, G., 2001a. Influence of the pH on the behavior of an imprinted polymeric stationary phase-supporting evidence for a binding site model. J. Chromatogr. A 927, 1–17.
Chen, Y., Kele, M., Quinones, I., Sellergren, B., Guiochon, B., 2001b. Influence of the pH on the behavior of an imprinted polymeric stationary phase—supporting evidence for a binding site model. J. Chromatogr. A 927, 1–17.
Chen, W., Chen, C., Lin, F., 2001c. Molecular recognition in imprinted polymers: thermodynamic investigation of analyte binding using microcalorimetry. J. Chromatogr. A 923, 1–6.
Chen, Z.Y., Hua, Z.D., Xu, L., Huang, Y., Zhao, M.P., Li, Y.Z., 2008. Protein-responsive imprinted polymers with specific shrinking and rebinding. J. Mol. Recognit. 21, 71–77.
Cheng, M., Sun, Y., 2005. Observation of the solute transport in the permeation through hydrogel membranes by using FTIR-microscopy. J. Membr. Sci. 253, 191–198.
Chianella, I., Karim, K., Piletska, E.V., Preston, C., Piletsky, S.A., 2006. Computational design and synthesis of molecularly imprinted polymers with high binding capacity for pharmaceutical applications-model case: adsorbent for abacavir. Anal. Chim. Acta 559, 73–78.
Clapper, J.D., Pearce, M.E., Guymon, C.A., Salem, A.K., 2008. Biotinylated biodegradable nanotemplated hydrogel networks for cell interactive applications. Biomacromolecules 9, 1188–1194.
Cormack, P.A.G., Mosbach, K., 1999. Molecular imprinting: recent developments and the road ahead. React. Funct. Polym. 41, 115–124.
Cukier, R.I., 1984. Diffusion of Brownian spheres in semidilute polymer-solutions. Macromolecules 17, 252–255.
Cunliffe, D., Kirby, A., Alexander, C., 2005. Molecularly imprinted drug delivery systems. Adv. Drug Deliv. Rev. 57, 1836–1853.
Davis, M.E., Katz, A., Ahmad, W.R., 1996. Rational catalyst design via imprinted nanostructured materials. Chem. Mater. 8, 1820–1839.
De Gennes, P.G., 1986. Pattern recognition by flexible coils. In: Grinstein, G., Mazenko, G. (Eds.), Directions in Condensed Matter Physics. World Scientific, Singapore, pp. 83–99.
Demchenko, A.P., 2001. Recognition between flexible protein molecules: induced and assisted folding. J. Mol. Recognit. 14, 42–61.
Demirel, G., Ozcetin, G., Turan, E., Caykara, T., 2005. pH/temperature-sensitive imprinted ionic poly (*N-tert*-butylacrylamide-co-acrylamide/maleic acid) hydrogels for bovine serum albumin. Macromol. Biosci. 5, 1032–1037.
Djourelov, N., Ates, Z., Guven, O., Misheva, M., Suzuki, T., 2007. Positron annihilation lifetime spectroscopy of molecularly imprinted hydroxyelthyl methacrylate based polymers. Polymer 48, 2692–2699.
Drury, J.L., Mooney, D.J., 2003. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24, 4337–4351.
Eddington, D.T., Beebe, D.J., 2004a. Flow control with hydrogels. Adv. Drug Deliv. Rev. 56, 199–210.
Eddington, D.T., Beebe, D.J., 2004b. A valved responsive hydrogel microdispensing device with integrated pressure source. JMEMS 13, 586–593.
Ehrick, J.D., Stokes, S., Bachas-Daunert, S., Moschou, E.A., Deo, S.K., Bachas, L.G., Daunert, S., 2007. Chemically tunable lensing of stimuli-responsive hydrogel microdomes. Adv. Mater. 19, 4024–4027.
Elliott, J.E., Bowman, C.N., 2001. Monomer functionality and polymer network formation. Macromolecules 34, 4642–4649.
English, A.E., Tanaka, T., Edelman, E.R., 1996. Polyelectrolyte hydrogel instabilities in ionic solutions. J. Chem. Phys. 105, 10606–10613.
English, A.E., Tanaka, T., Edelman, E.R., 1998. Polymer and solution ion shielding in polyampholytic hydrogels. Polymer 39, 5893–5897.
Enoki, T., Tanaka, K., Watanabe, T., Oya, T., Sakiyama, T., Takeoka, Y., Ito, K., Wang, G., Annaka, M., Hara, H., Du, R., Chuang, J., Wasserman, K., Grosberg, A.Y., Masamune, S., Tanaka, T., 2000. Frustrations in polymer conformation in gels and their minimization through molecular imprinting. Phys. Rev. Lett. 85, 5000–5003.
Espinosa-Garcia, B.M., Argelles-Monal, W.M., Hernandez, J., Felix-Valenzuela, L., Acosta, N., Goycoolea, F.M., 2007. Molecularly imprinted chitosan-genipin hydrogels with recognition capacity toward *o*-xylene. Biomacromolecules 8, 3355–3364.
Fazal, F.M., Hansen, D.E., 2007. Glucose-specific poly(allylamine) hydrogels—a reassessment. Bioorg. Med. Chem. Lett. 17, 235–238.
Flory, P.J., 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, NY.
Flory, P.J., Rehner, J., 1943a. Statistical mechanics of cross-linked polymer networks. II Swelling. J. Chem. Phys. 11, 521–526.
Flory, P.J., Rehner Jr., J., 1943b. Statistical mechanics of cross-linked polymer networks. I. Rubberlike elasticity. J. Chem. Phys. 11, 512–520.
Fu, G., Yu, H., Zhu, J., 2008. Imprinting effect of protein-imprinted polymers composed of chitosan and polyacrylamide: a re-examination. Biomaterials 29, 2138–2142.
Gao, N., Xu, Z., Wang, F., Dong, S.J., 2007. Sensitive biomimetic sensor based on molecular imprinting at functionalized indium tin oxide electrodes. Electroanalysis 19, 1655–1660.
Ge, Y., Turner, A.P., 2008. Too large to fit? Recent developments in macromolecular imprinting. Trends Biotechnol. 26, 218–224.
Gong, C.B., Lam, M.H.W., Yu, H.X., 2006. The fabrication of a photoresponsive molecularly imprinted polymer for the photoregulated uptake and release of caffeine. Adv. Funct. Mater. 16, 1759–1767.
Gong, C.B., Wong, K.L., Lam, M.H.W., 2008. Photoresponsive molecularly imprinted hydrogels for the photoregulated release and uptake of pharmaceuticals in the aqueous media. Chem. Mater. 20, 1353–1358.
Guardia, L., Badia-Laino, R., Diaz-Garcia, M.E., Ania, C.O., Parra, J.B., 2008. Role of surface adsorption and porosity features in the molecular recognition ability of imprinted sol–gels. Biosens. Bioelectron. 23, 1101–1108.
Guo, T., Xia, Y., Hao, G., Zhang, B., Fu, G., Yuan, Z., He, B., Kennedy, J.F., 2005. Chemically modified chitosan beads as matrices for adsorptive separation of proteins by molecularly imprinted polymer. Carbohydr. Polym. 62, 214–221.
Guo, M.J., Zhao, Z., Fan, Y.G., Wang, C.H., Shi, L.Q., Xia, J.J., Long, Y., Mi, H.F., 2006. Protein-imprinted polymer with immobilized assistant recognition polymer chains. Biomaterials 27, 4381–4387.
Guoqi, F., Zhao, J., Yu, H., Liu, L., He, B., 2007. Bovine serum albumin-imprinted polymer gels prepared by graft polymerization of acrylamide on chitosan. React. Funct. Polym. 67, 442–450.
Haruki, M., Konnai, Y., Shimada, A., Takeuchi, H., 2007. Molecularly imprinted polymer-assisted refolding of lysozyme. Biotechnol. Prog. 23, 1254–1257.
Haupt, K., Mosbach, K., 2000. Molecularly imprinted polymers and their use in biomimetic sensors. Chem. Rev. 100, 2495–2504.
Hawkins, D.M., Stevenson, D., Reddy, S.M., 2005. Investigation of protein imprinting in hydrogel-based molecularly imprinted polymers (HydroMIPs). Anal. Chim. Acta 542, 61–65.
Hawkins, D.M., Trache, A., Ellis, E.A., Stevenson, D., Holzenburg, A., Meininger, G.A., Reddy, S.M., 2006. Quantification and confocal imaging of protein specific molecularly imprinted polymers. Biomacromolecules 7, 2560–2564.
Hawkins, D.M., Ellis, E.A., Stevenson, D., Holzenburg, A., Reddy, S.M., 2007. Novel critical point drying (CPD) based preparation and transmission electron microscopy (TEM) imaging of protein specific molecularly imprinted polymers. J. Mater. Sci. 42, 9465–9468.
Henry, O., Cullen, D.C., Piletsky, S.A., 2005. Optical interrogation of molecularly imprinted polymers and development of MIP sesnors: a review. Bioanal. Chem. 382, 947–956.
Henthorn, D.B., Peppas, N.A., 2007. Molecular simulations of recognitive behavior of molecularly imprinted intelligent polymeric networks. Ind. Eng. Chem. Res. 46, 6084–6091.
Hillberg, A.L., Tabrizian, M., 2008. Biomolecule imprinting: developments in mimicking dynamic natural recognition systems. ITBM-RBM 29, 89–104.
Hilt, J.Z., Byrne, M.E., 2004. Configurational biomimesis in drug delivery: molecular imprinting of biologically significant molecules. Adv. Drug Deliv. Rev. 56, 1599–1620.

Hilt, J.Z., Byrne, M.E., Peppas, N.A., 2006a. Microfabrication of intelligent biomimetic networks for recognition of D-glucose. Chem. Mater. 18, 5869–5875.
Hilt, J.Z., Byrne, M.E., Peppas, N.A., 2006b. Microfabrication of intelligent biomimetic networks for recognition of glucose. Chem. Mater. 18, 5869–5875.
Hiratani, H., Alvarez-Lorenzo, C., 2004. The nature of backbone monomers determines the performance of imprinted soft contact lenses as timolol drug delivery systems. Biomaterials 25, 1105–1113.
Hiratani, H., Alvarez-Lorenzo, C., Chuang, J., Guney, O., Grosberg, A.Y., Tanaka, T., 2001. Effect of reversible cross-linker, *N*,*N*′-bis(acryloyl)cystamine, on calcium ion adsorption by imprinted gels. Langmuir 17, 4431–4436.
Hiratani, H., Mizutani, Y., Alvarez-Lorenzo, C., 2005. Controlling drug release from imprinted hydrogels by modifying the characteristics of the imprinted cavities. Macromol. Biosci. 5, 728–733.
Hirayama, K., Sakai, Y., Kameoka, K., 2001. Synthesis of polymer particles with specific lysozyme recognition sites by a molecular imprinting technique. J. Appl. Polym. Sci. 81, 3378–3387.
Hu, X., Li, G., Huang, J., Zhang, D., Qiu, Y., 2007. Construction of self-reporting specific chemical sensors with high sensitivity. Adv. Mater. 19, 4327–4332.
Hu, X.B., Li, G.T., Li, M.H., Huang, J., Li, Y., Gao, Y.B., Zhang, Y.H., 2008. Ultrasensitive specific stimulant assay based on molecularly imprinted photonic hydrogels. Adv. Funct. Mater. 18, 575–583.
Huber, R., Bennett, W.S., 1983. Functional significance of flexibility in proteins. Biopolymers 22, 261–279.
Ito, K., Chuang, J., Alvarez-Lorenzo, C., Watanabe, T., Ando, N., Grosberg, A.Y., 2003. Multiple point adsorption in a heteropolymer gel and the Tanaka approach to imprinting: experiment and theory. Prog. Polym. Sci. 28, 1489–1515.
Jacob, R., Tate, M., Banti, Y., Rix, C., Mainwaring, D.E., 2008. Synthesis, characterization, and ab initio theoretical study of a molecularly imprinted polymer selective for biosensor materials. J. Phys. Chem. A 112, 322–331.
Janiak, D.S., Kofinas, P., 2007. Molecular imprinting of peptides and proteins in aqueous media. Anal. Bioanal. Chem. 389, 399–404.
Jiang, Z., Yu, Y., Wu, H., 2006. Preparation of CS/GPTMS hybrid molecularly imprinted membrane for efficient chiral resolution of phenylalanine isomers. J. Membr. Sci. 280, 876–882.
Jozefowicz, M., Jozefonvicz, J., 1997. Randomness and biospecificity: random copolymers are capable of biospecific molecular recognition in living systems. Biomaterials 18, 1633–1644.
Kanekiyo, Y., Naganawa, R., Tao, H., 2002. Molecular imprinting of bisphenol A and alkylphenols using amylose as a host matrix. Chem. Commun., 2698–2699.
Kanekiyo, Y., Naganawa, R., Tao, H., 2003. pH-responsive molecularly imprinted polymers. Angew. Chem. Int. Ed. 42, 3014–3016.
Karmalkar, R.N., Kulkarni, M.G., Mashelkar, R.A., 1997. Pendent chain linked delivery systems: II. Facile hydrolysis through molecular imprinting effects. J. Control. Release 43, 235–243.
Kim, H., Kaczmarski, K., Guiochon, G., 2005. Mass transfer kinetics on the heterogeneous binding sites of molecularly imprinted polymers. Chem. Eng. Sci. 60, 5425–5444.
Kimhi, O., Bianco-Peled, H., 2007. Study of the interactions between protein-imprinted hydrogels and their templates. Langmuir 23, 6329–6335.
Kobayashi, T., Leong, S.S., Zhang, Q.Q., 2008. Using polystyrene-co-maleic acid for molecularly imprinted membranes prepared in supercritical carbon dioxide. J. Appl. Polym. Sci. 108, 757–768.
Kublickas, R., Werner, C., Jariene, G., Voit, B., Lasas, L., 2007. Polyacrylamide gels containing ionized functional groups for the molecular imprinting of human growth hormone. Polym. Bull. 58, 611–617.
Lavine, B.K., Westover, D.J., Kaval, N., Mirjankar, N., Oxenford, L., Mwangi, G.K., 2007. Swellable molecularly imprinted poly*N*-(*N*-propyl)acrylamide particles for detection of emerging organic contaminants using surface plasmon resonance spectroscopy. Talanta 72, 1042–1048.
Lee, P.I., 1984. Novel approach to zero-order drug delivery via immobilized non-uniform drug distribution in glassy hydrogels. J. Pharm. Sci. 73, 1344–1347.
Lee, E.S., Kim, S.W., Cardinal, J.R., Jacobs, H., 1980. Drug release from hydrogel devices with rate controlling barriers. J. Membr. Sci. 7, 293–303.
Lee, S., Eddington, D.T., Kim, Y., Kim, W., Beebe, D.J., 2003. Control mechanism of an organic self-regulating microfluidic system. JMEMS 12, 848–854.
Lemieux, R.U., 1996. How water provides the impetus for molecular recognition in aqueous solution. Acc. Chem. Res. 29, 373–380.
Li, X., Husson, S.M., 2006. Adsorption of dansylated amino acids on molecularly imprinted surfaces: a surface plasmon resonance study. Biosens. Bioelectron. 22, 336–348.
Li, F., Li, J., Zhang, S., 2008. Molecularly imprinted polymer grafted on polysaccharide microsphere surface by the sol–gel process for protein recognition. Talanta 74, 1247–1255.
Lin, C.C., Metters, A.T., 2006. Hydrogels in controlled release formulations: network design and mathematical modeling. Adv. Drug Deliv. Rev. 58, 1379–1408.
Lin, J., Nakagama, T., Uchiyama, K., Hobo, T., 1997. Temperature effects on chiral recognition of some amino acids with molecularly imprinted polymer filled capillary electrochromatography. Biomed. Chromatogr. 11, 298–302.
Liu, R.H., Yu, Q., Beebe, D.J., 2002. Fabrication and characterization of hydrogel-based microvalves. JMEMS 11, 45–53.
Liu, X.Y., Guan, Y., Ding, X.B., Peng, Y.X., Long, X.P., Wang, X.C., Chang, K., 2004a. Design of temperature sensitive imprinted polymer hydrogels based on multiple-point hydrogen bonding. Macromol. Biosci. 4, 680–684.
Liu, X.Y., Ding, X.B., Guan, Y., Peng, Y.X., Long, X.P., Wang, X.C., Chang, K., Zhang, Y., 2004b. Fabrication of temperature-sensitive imprinted polymer hydrogel. Macromol. Biosci. 4, 412–415.
Liu, X., Ding, X., Guan, Y., Peng, Y., Long, X., Wang, X., Chang, K., Zhang, Y., 2004c. Fabrication of temperature-sensitive imprinted polymer hydrogel. Macromol. Biosci. 4, 412–415.
Lodish, H., Berk, A., Zipursky, L.S., Matsudaira, P., Baltimore, D., Darnell, J., 2000. Molecular Cell Biology. Section 2.2 Noncovalent Bonds. W.H. Freeman and Co., New York, NY.
Lotierzo, M., Henry, O.Y.F., Piletsky, S., Tothill, I., Cullen, D., Kania, M., Hock, B., Turner, A.P.F., 2004. Surface plasmon resonance sensor for domoic acid based on grafted imprinted polymer. Biosens. Bioelectron. 20, 145–152.
Lubke, C., Lubke, M., Whitcombe, M.J., Vulfson, E.N., 2000. Imprinted polymers prepared with stoichiometric template–monomer complexes: efficient binding of ampicillin from aqueous solutions. Macromolecules 33, 5098–5105.
Lustig, S.R., Peppas, N.A., 1988. Solute diffusion in swollen membranes 9. Scaling laws for solute diffusion in gels. J. Appl. Polym. Sci. 36, 735–747.
Maier, N.M., Lindner, W., 2007. Chiral recognition applications of molecularly imprinted polymers: a critical review. Anal. Bioanal. Chem. 389, 377–397.
Malafaya, P.B., Silva, G.A., Baran, E.T., Reis, R.L., 2002. Drug delivery therapies II. Strategies for delivering bone regenerating factors. Curr. Opin. Solid State Mater. Sci. 6, 297–312.
Marty, J.D., Tizra, M., Mauzac, M., Rico-Lattes, I., Lattes, A., 1999. New molecular imprinting materials: liquid crystalline networks. Macromolecules 32, 8674–8677.
Matsui, J., Akamatsu, K., Hara, N., Miyoshi, D., Nawafune, H., Tamaki, K., Sugimoto, N., 2005. SPR sensor chip for detection of small molecules using molecularly imprinted polymer with embedded gold nanoparticles. Anal. Chem. 77, 4282–4285.
Mayes, A.G., Whitcombe, M.J., 2005. Synthetic strategies for the generation of molecularly imprinted organic polymers. Adv. Drug Deliv. Rev. 57, 1742–1778.
Meot-Ner, M., 2005. The ionic hydrogen bond. Chem. Rev. 105, 213–284.
Miyata, T., Jige, M., Nakaminami, T., Uragami, T., 2006. Tumor marker-responsive behavior of gels prepared by biomolecular imprinting. Proc. Natl. Acad. Sci. U.S.A. 103, 1190–1193.
Monti, S., Cappelli, C., Bronco, S., Giusti, P., Ciardelli, G., 2006. Towards the design of highly selective recognition sites into molecular imprinting polymers: a computational approach. Biosens. Bioelectron. 22, 153–163.
Moritani, T., Alvarez-Lorenzo, C., 2001. Conformational imprinting effect on stimuli-sensitive gels made with an "Imprinter" monomer. Macromolecules 34, 7796–7803.
Nie, T., Baldwin, A., Yamaguchi, N., Kiick, K., 2007. Production of heparin-functionalized hydrogels for the development of responsive and controlled growth factor delivery systems. J. Control. Release 122, 287–296.
Noss, K.R., Vaughan, A.D., Byrne, M.E., 2008. Tailored binding and transport parameters of molecularly imprinted films via macromolecular structure: the rational design of recognitive polymers. J. Appl. Polym. Sci. 107, 3435–3441.
O'Shannessy, D.J., Ekberg, B., Mosbach, K., 1989. Molecular imprinting of amino-acid derivatives at low temperature using photolytic homolysis of azobisnitriles. Anal. Biochem. 177, 144–149.
Ogiso, M., Minoura, N., Shinbo, T., Shimizu, T., 2006. Detection of a specific DNA sequence by electrophoresis through a molecularly imprinted polymer. Biomaterials 27, 4177–4182.
Oral, E., Peppas, N.A., 2004. Responsive and recognitive hydrogels using star polymers. J. Biomed. Mater. Res. Part A 68A, 439–447.
Oral, E., Peppas, N.A., 2006. Hydrophilic molecularly imprinted poly(hydroxyethylmethacrylate) polymers. J. Biomed. Mater. Res. Part A 78A, 205–210.
Oya, T., Enoki, T., Grosberg, A.Y., Masamune, S., Sakiyama, T., Takeoka, Y., Tanaka, K., Wang, G., Yilmaz, Y., Feld, M.S., Dasari, R., Tanaka, T., 1999. Reversible molecular adsorption based on multiple-point interaction by shrinkable gels. Science 286, 1543–1545.
Palaprat, G., Weyland, M., Phou, T., Binet, C., Marty, J.D., Mingotaud, A.F., Mauzac, M., 2006. Introduction of unusual properties into polymers by the use of liquid-crystalline moieties. Polym. Int. 55, 1191–1198.
Pande, V.S., Grosberg, A.Y., Tanaka, T., 1994a. Folding thermodynamics and kinetics of imprinted renaturable heteropolymers. J. Chem. Phys. 101, 8246–8257.
Pande, V.S., Grosberg, A.Y., Tanaka, T.A., 1994b. Thermodynamic procedure to synthesize heteropolymers that can renature to recognize a given target molecule. Proc. Natl. Acad. Sci. U.S.A. 91, 12976–12979.
Pande, V.S., Grosberg, A.Y., Tanaka, T.A., 1997. Statistical mechanics of simple models of protein folding and design. Biophys. J. 73, 3192–3210.
Pande, V.S., Grosberg, A.Y., Tanaka, T.A., 2000. Heteropolymer freezing and design: towards physical models of protein folding. Rev. Mod. Phys. 72, 259–314.
Parmpi, P., Kofinas, P., 2004. Biomimetic glucose recognition using molecularly imprinted polymer hydrogels. Biomaterials 25, 1969–1973.
Paul, G., Steuernagal, S., Koller, H., 2007. Non-covalent interactions of a drug molecule encapsulated in a hybrid silica gel. Chem. Commun., 5194–5196.
Pavel, D., Lagowski, J., 2005. Computationally designed monomers and polymers for molecular imprinting of theophylline. Part II. Polymer 46, 7543–7556.
Peppas, N.A. (Ed.), 1987a. Hydrogels in Medicine and Pharmacy. Volume I: Fundamentals. CRC Press, Boca Raton, FL.
Peppas, N.A., 1987b. Hydrogels in Medicine and Pharmacy, Vol. II&III. CRC Press, Boca Raton, FL.
Peppas, N.A., Huang, Y., 2002. Polymers and gels as molecular recognition agents. Pharm. Res. 19, 578–587.

Peppas, N.A., Khare, A., 1993. Preparation, structure and diffusional behavior of hydrogels in controlled release. Adv. Drug Deliv. Rev. 11, 1–35.
Peppas, N.A., Merrill, E.W., 1977. Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks. J. Appl. Polym. Sci. 21, 1763–1770.
Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharamaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.
Peppas, N.A., Hilt, J.Z., Khademhosseini, A., Langer, R., 2006. Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv. Mater. 18, 1345–1360.
Piletsky, S.A., Piletska, E.V., Karim, K., Freebairn, K.W., Legge, C.H., Turner, A.P.F., 2002. Polymer cookery: influence of polymerization conditions on the performance of molecularly imprinted polymers. Macromolecules 35, 7499–7504.
Puoci, F., Iemma, F., Muzzalupo, R., Spizzirri, U.G., Trombino, S., Cassano, R., Picci, N., 2004. Spherical molecularly imprinted polymers (SMIPs) via a novel precipitation polymerization in the controlled delivery of sulfasalazine. Macromol. Biosci. 4, 22–26.
Rampey, A.M., Umpleby, R.J., Rushton, G.T., Iseman, J.C., Shah, R.N., Shimizu, K.D., 2004. Characterization of the imprint effect and the influence of imprinting conditions on affinity, capacity, and heterogeneity in molecularly imprinted polymers using the freundlich isotherm-affinity distribution analysis. Anal. Chem. 76, 1123–1133.
Richter, A.K., Howitz, D., Arndt, S.T.G., 2003. Electronically controllable microvalves based on smart hydrogels: magnitudes and potential applications. JMEMS 12, 748–753.
Ricka, J., Tanaka, T., 1984. Swelling of ionic gels: quantitiative performance of the Donnan theory. Macromolecules 17, 2916–2921.
Saltzman, W.M., Olbricht, W.L., 2002. Building drug delivery into tissue engineering. Nat. Rev. Drug Discov. 1, 177–186.
Sellergren, B., Allender, C.J., 2005. Molecularly imprinted polymers: a bridge to advanced drug delivery. Adv. Drug Deliv. Rev. 57, 1733–1741.
Shin, H., Jo, S., Mikos, A.G., 2002. Modulation of marrow stromal osteoblast adhesion on biomimetic oligo[poly(ethylene glycol) fumarate] hydrogels modified with Arg-Gly-Asp peptides and a poly(ethyleneglycol) spacer. J. Biomed. Mater. Res. 61, 169–179.
Silvestri, D., Cristallini, C., Ciardelli, G., Giusti, P., Barbani, N., 2005a. Molecularly imprinted bioartifical membranes for the selective recognition of biological molecules. Part 2: Release of components and thermal analysis. J. Biomater. Sci. Polym. Edn. 16, 397–410.
Silvestri, D., Cristallini, C., Ciardelli, G., Giusti, P., Barbani, N., 2005b. Molecularly imprinted bioartificial membranes for the selective recognition of biological molecules. Part 2: Release of components and thermal analysis. J. Biomater. Sci. Polym. 16, 397–410.
Singh, B., Chauhan, N., 2008. Preliminary evaluation of molecular imprinting of 5-fluorouracil within hydrogels for use as drug delivery systems. Acta Biomater. 4 (5), 1244–1254.
Slinchenko, O., Rachkov, Miyachi, H., Ogiso, M., Minoura, N., 2004. Imprinted polymer layer for recognizing double-stranded DNA. Biosens. Bioelectron. 20, 1091–1097.
Spivak, D.A., Shea, K.J., 2001. Investigation into the scope and limitations of molecular imprinting with DNA molecules. Anal. Chim. Acta 435, 65–74.
Spivak, D., Gilmore, M.A., Shea, K.J., 1997. Evaluation of binding and origins of specificity of 9-ethyladenine imprinted polymers. J. Am. Chem. Soc. 119, 4388–4393.
Spizzirri, U.G., Peppas, N.A., 2005. Structural analysis and diffusional behavior of molecularly imprinted polymer networks for cholesterol recognition. Chem. Mater. 17, 6719–6727.
Suedee, R., Srichana, T., Martin, G.P., 2000. Evaluation of matrices containing molecularly imprinted polymers in the enantioselective-controlled delivery of β-blockers. J. Control. Release 66, 135–147.
Suedee, R., Seechamnanturakit, V., Canyuk, B., Ovatiarnporn, C., Martin, G.P., 2006. Temperature sensitive dopamine-imprinted (*N*,*N*-methylene-bis-acrylamide cross-linked) polymer and its potential application to the selective extraction of adrenergic drugs from urine. J. Chromatogr. A 1114, 239–249.
Tahara, K., Yamamto, K., Nishihata, T., 1995. Overall mechanism behind matrix sustained release tablets prepared with hydroxypropyl methylcellulose 2910. J. Control. Release 35, 59–66.
Takatsy, A., Sedzik, J., Kilar, F., Hjerten, S., 2006a. Universal method for synthesis of artificial gel antibodies by the imprinting approach combined with a unique electrophoresis technique for detection of minute structural differences of proteins, viruses, and cells (bacteria): Ia. Gel antibodies against proteins (transferrins). J. Sep. Sci. 29, 2802–2809.
Takatsy, A., Sedzik, J., Kilar, F., Hjerten, S., 2006b. Universal method for synthesis of artificial gel antibodies by the imprinting approach combined with a unique electrophoresis technique for detection of minute structural differences of proteins, viruses, and cells (bacteria): II. Gel antibodies against virus (Semliki Forest Virus). J. Sep. Sci. 29, 2810–2815.
Tokuyama, H., Kanazawa, R., Sakohara, S., 2005. Equilibrium and kinetics for temperature swing adsorption of a target metal on molecular imprinted thermosensitive gel adsorbents. Sep. Purif. Technol. 44, 152–159.
Tokuyama, H., Naohara, S., Fujioka, M., Sakohara, S., 2008. Preparation of molecular imprinted thermosensitive gels grafted onto polypropylene by plasma-initiated graft polymerization. React. Funct. Polym. 68, 182–188.
Turner, N.W., Jeans, C.W., Brain, K.R., Allender, C.J., Hlady, V., Britt, D.W., 2006. From 3D to 2D: a review of the molecular imprinting of proteins. Biotechnol. Prog. 22, 1474–1489.
Ulijn, R.V., Bibi, N., Jayawarna, V., Thornton, P.D., Todd, S.J., Mart, R.J., Smith, A.M., Gough, J.E., 2007. Bioresponsive hydrogels. Mater. Today 10, 40–48.
Umpleby II, R.J., Bode, M., Shimizu, K.D., 2000. Measurement of the continuous distribution of binding sites in molecularly imprinted polymers. Analyst 125, 1261–1265.
Umpleby, R.J., Baxter, S.C., Bode, M., Berch, J.K., Shah, R.N., Shimizu, K.D., 2001. Application of the Freundlich isotherm in the characterization of molecularly imprinted polymers. Anal. Chim. Acta 435, 35–42.
Valente, A.J.M., Polishchuk, A.Y., Burrows, H.D., Miguel, M.G., Lobo, V.M.M., 2003. Sorption/diffusion behavior of anionic surfactants in polyacrylamide hydrogels: from experiment to modeling. Eur. Polym. J. 39, 1855–1865.
Vaughan, A.D., Zhang, J., Byrne, M.E. unpublished data.
Vaughan, A.D., Sizemore, S.P., Byrne, M.E., 2007. Enhancing molecularly imprinted polymer binding properties via controlled/living radical polymerization and reaction analysis. Polymer 48, 74–81.
Venkatesh, S., Sizemore, S.P., Zhang, J.B., Byrne, M.E., 2006. Therapeutic contact lenses: a biomimetic approach towards tailored ophthalmic extended delivery. Abstr. Pap. Am. Chem. Soc., 231.
Venkatesh, S., Sizemore, S.P., Byrne, M.E., 2007. Biomimetic hydrogels for enhanced loading and extended release of ocular therapeutics. Biomaterials 28, 717–724.
Venkatesh, V., Saha, J., Pass, S., Byrne, M.E., 2008. Transport and structural analysis of molecularly imprinted hydrogels for controlled drug delivery. Eur. J. Pharm. Biopharm. 69, 852–860.
Wang, B., Liu, M.Z., Liang, R., Ding, S.L., Chen, Z.B., Chen, S.L., Jin, S.P., 2008. MMTCA recognition by molecular imprinting in interpenetrating polymer network hydrogels based on poly(acrylic acid) and poly(vinyl alcohol). Macromol. Biosci. 8, 417–425.
Watanabe, M., Akahoshi, T., Tabata, Y., Nakayama, D., 1998. Molecular specific swelling change of hydrogels in accordance with the concentration of guest molecules. J. Am. Chem. Soc. 120, 5577–5578.
Watanabe, T., Ito, K., Alvarez-Lorenzo, C., Grosberg, A.Y., Tanaka, T., 2001. Salt effects on multiple-point adsorption of target molecules by heteropolymer gel. J. Chem. Phys. 115, 1596–1600.
Wei, S., Mizaikoff, B., 2007. Recent advances on noncovalent molecular imprints for affinity separations. J. Sep. Sci. 30, 1794–1805.
Wei, S.T., Jakusch, M., Mizaikoff, B., 2006. Capturing molecules with templated materials—analysis and rational design of molecularly imprinted polymers. Anal. Chim. Acta 578, 50–58.
Whitcombe, M.J., Vulfson, E.N., 2001. Imprinted polymers. Adv. Mater. 13, 467–478.
Wizeman, W.J., Kofinas, P., 2001. Molecularly imprinted polymer hydrogels displaying isomerically resolved glucose binding. Biomaterials 22, 1485–1491.
Wulff, G., 2002. Enzyme-like catalysis by molecularly imprinted polymers. Chem. Rev. 102, 1–27.
Xia, Y.Q., Guo, T.Y., Song, M.D., Zhang, B.H., Zhang, B.L., 2005. Hemoglobin recognition by imprinting in semi-interpenetrating polymer network hydrogel based on polyacrylamide and chitosan. Biomacromolecules 6, 2601–2606.
Xia, Y., Guo, T., Song, M., Zhang, B., Zhang, B., 2006. Selective separation of quercetin by molecular imprinting using chitosan beads as functional matrix. React. Funct. Polym. 66, 1734–1740.
Yamashita, K., Nishimura, T., Ohashi, K., Ohkouchi, H., Nango, M., 2003. Two-step imprinting procedure of inter-penetrating polymer network-type stimuli-responsive hydrogel adsorbents. Polym. J. 35, 545–550.
Ye, L., Mosbach, K., 2008. Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors. Chem. Mater. 20, 859–868.
Zhang, H.Q., Ye, L., Mosbach, K., 2006. Non-covalent molecular imprinting with emphasis on its application in separation and drug development. J. Mol. Recognit. 19, 248–259.
Zhao, Z., Wang, C.H., Guo, M.J., Shi, L.Q., Fan, Y.G., Long, Y., Mi, H.F., 2006. Molecular imprinted polymer with cloned bacterial protein template enriches authentic target in cell extract. FEBS Lett. 580, 2750–2754.
Zhao, K., Cheng, G., Huang, J., Ying, X., 2008. Rebinding and recognition properties of protein-macromolecularly imprinted calcium phosphate/alginate hybrid polymer microspheres. React. Funct. Polym. 68, 732–741.