Chapter 7
Imprinting Using Smart Polymers

Carmen Alvarez-Lorenzo, Angel Concheiro, Jeffrey Chuang, and Alexander Yu. Grosberg

CONTENTS
7.1 Introduction ................................................................. 211
    7.1.1 Approaches to Molecular Imprinting ..................... 212
    7.1.2 Imprinting in Gels .............................................. 215
7.2 The Tanaka Equation ..................................................... 216
    7.2.1 Theoretical Considerations .................................. 218
    7.2.2 Experimental Assessment of the Tanaka Equation .... 224
7.3 Temperature-Sensitive Imprinted Hydrogels .................... 228
7.4 pH-Sensitive Imprinted Gels ........................................... 237
7.5 Conclusions ............................................................... 241
Acknowledgments ............................................................. 241
References ................................................................. 241

7.1 Introduction

The use of materials as so-called smart polymers is motivated by the simple observation that biological molecules perform incredibly complex functions. While the goal of engineering polymers as effectively as nature remains somewhat in the realm of science fiction, in recent years many researchers have sought to find or design synthetic polymeric materials capable of mimicking one or another "smart" property of biopolymers. Polymer gels are promising systems for such smart functions because of their volume phase transition, predicted theoretically by Dusek and Patterson in 1968 and experimentally demonstrated by Tanaka in 1978. Gel collapse can be driven by any one of the four basic types of intermolecular interactions operational in water solutions and in molecular biological systems, namely, by hydrogen
bonds and by van der Waals, hydrophobic, and Coulomb interactions between ionized (dissociated) groups. According to Flory’s theory, the degree of swelling of a hydrogel is the result of a competition between the entropy due to polymer conformations, which causes rubber elasticity, and the energy associated with internal attractions and repulsions between the monomers in the gel and the solvent. A change in the environmental conditions, such as temperature, pH, or composition, modifies the balance between the free energy of the internal interactions and the elasticity component, inducing a volume phase transition. The variety of external stimuli that can trigger the phase transition as well as the present possibilities of modulating the rate and the intensity of the response to the stimulus guarantee smart gels a wealth of applications.

The interest in stimuli-sensitive hydrogels, especially in the biomedical field, could be remarkably increased if they could mimic the recognition capacity of certain biomacromolecules (e.g., receptors, enzymes, antibodies). The unique details of the protein’s native state, such as its shape and charge distribution, enable it to recognize and interact with specific molecules. Proteins find their desired conformation out of a nearly infinite number of possibilities. In contrast, as known from recent theoretical developments, a polymer with a randomly made sequence will not fold in just one way (see, for example, the review by Pande et al. and references therein and Tanaka and Annaka). Therefore, the ability of a polymer (or polymer hydrogel) to always fold back into the same conformation after being stretched and unfolded, i.e., to thermodynamically memorize a conformation, should be related to properly selected or designed nonrandom sequences. To obtain, under proper conditions, synthetic systems with sequences able to adopt conformations with useful functions, the molecular imprinting technology can be applied. The hydrogels can recognize a substance if they are synthesized in the presence of such a substance (which acts as a template) in a conformation that corresponds to the global minimum energy. The “memorization” of this conformation, after the swelling of the network and the washing of the template, will only be possible if the network is always able to fold into the conformation, upon synthesis, that can carry out its designated function (Figure 7.1). This revolutionary idea is the basis of new approaches to the design of imprinted hydrogels and has been developed at different levels, as explained below.

### 7.1.1 Approaches to Molecular Imprinting

The concept of molecular imprinting was first applied to organic polymers in the 1970s, when covalent imprinting in vinyl polymers was first reported. The noncovalent imprinting was introduced a decade later. Both approaches are aimed at creating tailor-made cavities shaped with a high specificity and affinity for a target molecule inside or at the surface of highly cross-linked polymer networks. To carry out the process, the template is added to the monomers and cross-linker solution before polymerization,
which allows some of the monomers (called “functional” monomers) to be arranged in a configuration complementary to the template. The functional monomers are arranged in position through either covalent bonds or non-covalent interactions, such as hydrogen bond or ionic, hydrophobic, or charge-transfer interactions (Figure 7.2). In the first case, the template is covalently bound to the monomers prior to polymerization; after synthesis of the network, the bonds are reversiblybroken for removal of the template molecules and formation of the imprinted cavities. In the noncovalent or self-assembly approach, the template molecules and functional monomers are arranged prior to polymerization to form stable and soluble complexes of appropriate stoichiometry by noncovalent or metal coordination interactions. In this case, multiple-point interactions between the template molecule and various functional monomers are required to form strong complexes in which both species are bound as strongly as in the case of a covalent bond. The noncovalent imprinting protocol allows more versatile combinations of templates and monomers, and provides faster bond association and dissociation.
kinetics than the covalent imprinting approach.\textsuperscript{15} By any of these approaches, copolymerization of functional monomer–template complexes with high proportions of cross-linking agents, and subsequent removal of the template, provides recognition cavities complementary in shape and functionality. These vacant cavities are then available for rebinding of the template or structurally related analogues. Excellent reviews about the protocols to create rigid imprinted networks can be found in the literature.\textsuperscript{16, 17} Nowadays, molecular imprinting is a well-developed tool in the analytical field, mainly for separating and quantifying a wide range of substances contained in complex matrices.\textsuperscript{18–20} Additionally, there has been a progressive increase in the number of papers and patents devoted to the application of molecularly imprinted polymers (MIPs) in the design of new drug-delivery systems and of devices useful in closely related fields, such as diagnostic sensors or chemical traps to remove undesirable substances from the body.\textsuperscript{21,22}

\textbf{FIGURE 7.2}

Schematic view of the imprinting process: (a) covalent approach, in which the template is covalently bound to polymerizable binding groups that are reversibly broken after polymerization; and (b) noncovalent approach, in which the template interacts with functional monomers through noncovalent interactions (e.g., ionic interaction, ii; hydrophobic interaction, hi; or hydrogen bond, hb) before and during polymerization.
To obtain functional imprinted cavities, several factors must be taken into account. It is essential to ensure that the template

- Does not bear any polymerizable group that could attach itself irreversibly to the polymer network
- Does not interfere in the polymerization process
- Remains stable at moderately elevated temperatures or upon exposure to UV irradiation

Other key issues are related to the nature and proportion of the monomers and to the synthesis conditions (solvent, temperature, etc.). The cavities should have a structure stable enough to maintain their conformation in the absence of the template and, at the same time, be sufficiently flexible to facilitate the attainment of a fast equilibrium between the release and re-uptake of the template in the cavity. The conformation and the stability of the imprinted cavities are related to the mechanical properties of the network and depend to a great extent on the cross-linker proportion. Most imprinted systems require 50 to 90% of cross-linker to prevent the polymer network from changing the conformation adopted during synthesis. Consequently, the chances to modulate the affinity for the template are very limited, and it is not foreseeable that the network will have regulatory or switching capabilities. The lack of response to changes in the physicochemical properties of the medium within the biological range or to the presence of a specific substance notably limits their utility in the biomedical field. A high cross-linker proportion also considerably increases the stiffness of the network, making it difficult to adapt its shape to a specific device or to living tissues.

### 7.1.2 Imprinting in Gels

A further step in the imprinting technology is the development of stimuli-sensitive imprinted hydrogels. The low-cross-linked proportion required to achieve adequate viscoelastic properties can compromise the stability of the imprinted cavities in the hydrogel structure, resulting in some sacrifice of both affinity and selectivity. Strong efforts are being made to adapt the molecular imprinting technology to materials that are more flexible and thus more biocompatible, such as hydrogels for use in the biomedical field. To produce low-cross-linked hydrogel networks capable of undergoing stimuli-sensitive phase transitions, the synthesis is carried out in the presence of template molecules, each able to establish multiple contact points with functional monomers. Multiple contacts are the key for strong adsorption of the template molecules because of the larger energy decrease upon adsorption as well as the higher sensitivity due to the greater information provided for recognition. As in the classical noncovalent approach, the monomers and the template molecules are allowed to move freely and settle themselves into a configuration of thermodynamic equilibrium. The monomers are then
polymerized in this equilibrium conformation at the collapsed state. As the hydrogel is made from the equilibrium system by freezing the chemical bonds forming the sequence of monomers, we might expect such a hydrogel to be able to return to its original conformation, at least to some degree of accuracy, upon swelling–collapse cycles in which the polymerized sequence remains unchanged (Figure 7.3). If the memory of the monomer assembly at the template adsorption sites is maintained, truly imprinted hydrogels will result. The combination of stimuli sensitivity and imprinting can have considerable practical advantages: the imprinting provides a high loading capacity of specific molecules, while the ability to respond to external stimuli modulates the affinity of the network for the target molecules, providing regulatory or switching capability of the loading/release processes.

This chapter focuses on the sorption/release properties of imprinted smart gels compared with those of random (nonimprinted) heteropolymer gels. We first discuss the theoretical ideas behind the adsorption properties of random heteropolymer gels and review the experiments that have been done on adsorption of target molecules by random gels, detailing the dependence of the affinity on structural and environmental factors. We then show the early successes of the imprinting method and highlight the advantages of the imprinted gels compared with random gels.

### 7.2 The Tanaka Equation

Tanaka and coworkers were pioneers in proposing the creation of stimuli-sensitive gels with the ability to recognize and capture target molecules using polymer networks consisting of at least two species of
monomers, each having a different role. One forms a complex with the
template (i.e., the functional or absorbing monomers capable of interacting
ionically with a target molecule), and the other allows the polymers to
swell and shrink reversibly in response to environmental changes (i.e., a
smart component such as \(N\)-isopropylacrylamide, NIPA) (Figure 7.4). The
gel is synthesized in the collapsed state and, after polymerization, is
washed in a swelling medium. The imprinted cavities develop affinity for
the template molecules when the functional monomers come into prox-
imity, but when they are separated, the affinity diminishes. The proximity
is controlled by the reversible phase transition that consequently controls
the adsorption/release of the template (Figure 7.3). A systematic study of
the effects of the functional monomer concentration and the cross-linker
proportion of the hydrogels (for both imprinted and nonimprinted gels)
and of the ionic strength of the medium on the affinity of the hydrogels
for different templates led to the development of an equation, called the
Tanaka equation in memory of the late Professor Toyoichi Tanaka
(1946–2000).39

![Chemical structures of some monomers used to create imprinted smart gels: N-isopropylacrylamide (NIPA, temperature-sensitive), \(N,N\)-methylene-bisacrylamide (BIS, cross-linker), methacrylamidopropyl trimethylammonium chloride (MAPTAC, cationic adsorber), and acrylic acid (AA, anionic adsorber). Structures of some ionically charged derivatives of pyranine used as targets are also shown.](image-url)
7.2.1 Theoretical Considerations

The Tanaka equation\textsuperscript{39} relates the gel affinity for the target molecules with the aforementioned variables as follows:

\[
\text{Affinity} = \frac{[\text{Ad}]^p}{[\text{Re}]^p} \exp(-\beta \varepsilon) \exp\left(-\frac{(p-1)c[Xl][\text{Ad}]^{2/3}}{[\text{Ad}]^{2/3}}\right) \tag{7.1}
\]

where \([\text{Ad}]\) represents the concentration of functional monomers in the gel; \([\text{Re}]\) is the concentration of replacement molecules, i.e., ions that are bound to the target molecule when it is not bound to the functional monomers (in cases where they have ionic or protonized groups); \([Xl]\) is the concentration of cross-linker; \(p\) is the number of bonds that each template can establish with the functional monomers; \(\beta\) is the Boltzmann factor \((1/k_BT)\); \(\varepsilon\) is the difference between the binding energy of an adsorbing monomer to the target molecule and that of a replacement molecule to the target molecule; and \(c\) is a constant that can be estimated from the persistence length and concentration of the main component of the gel chains (e.g., NIPA). The main assumption in Equation 7.1 is that the adsorption of target molecules is dominated by one value of \(p\) at each state of the gel. The value of \(p\) changes from 1 in the swollen state to \(p_{\text{max}}\) in the collapsed state, where \(p_{\text{max}}\) is the number of functional monomers that simultaneously interact with the target molecule.

The affinity of a network for a given target molecule is usually determined through the analysis of sorption isotherms. The suitability and limitations of different binding models, such as those developed by Langmuir, Freundlich, or Scatchard, have been discussed in detail elsewhere.\textsuperscript{40–42} The Langmuir isotherm is derived from the point of view of the binding sites, where each binding site can be filled or unfilled

\[
[T_{\text{ads}}] = S \frac{K[T_{\text{sol}}]}{K[T_{\text{sol}}]+1} \tag{7.2}
\]

where \([T_{\text{ads}}]\) is the concentration of target molecules adsorbed into the gel, \([T_{\text{sol}}]\) is the concentration of target molecules in solution, and \(S\) is the concentration of binding sites. \(K\) is the binding constant, with units of inverse concentration, and indicates the affinity per binding site. The overall affinity of the binding sites in the gel for the target molecule is defined to be the product \(SK\) (also denoted as \(Q\)), which is dimensionless.

The overall affinity \(SK\) can be determined from the partition function that sums over the different possible states of the target molecule: 0 adsorbers bound, 1 adsorber bound, \(\ldots\), \(p_{\text{max}}\) adsorbers bound. The partition function will be of the form:

\[
Z = Z_0 + Z_1 + Z_2 + \cdots + Z_{p_{\text{max}}} \tag{7.3}
\]
where \( Z_p \) indicates the term of the partition function in which a target molecule is bound by \( p \) adsorbing monomers, and \( Z_0 \) corresponds to the case of the target molecule being completely unbound. In the Langmuir equation, the term \( K[T_{\text{sol}}] \) is proportional to the fraction of filled binding sites, and \( SK[T_{\text{sol}}] \) is proportional to the concentration of bound target molecules. Compared with Equation 7.3, we see that

\[
SK[T_{\text{sol}}] \propto Z_0 + Z_1 + Z_2 + \cdots + Z_{p_{\text{max}}}
\]

(7.4)

The partition function component \( Z_0 \) must be proportional to the number of target molecules in solution (i.e., \([T_{\text{sol}}]\)). Therefore

\[
SK \propto \frac{(Z_1 + Z_2 + \cdots + Z_{p_{\text{max}}})}{Z_0}
\]

(7.5)

To calculate each of the terms \( Z_p \), a “fixed-point model” was developed. We can consider the case of a cross-linked gel made of a major component (i.e., a smart one, such as NIPA) and some functional monomers, prepared in the absence of templates (i.e., nonimprinted). The polymerized monomers can move in the gel to some extent, but are also constrained by the connectivity of the chains. When the cross-linking density is high, there are many constraints on the motion of the monomers. Conversely, at low cross-linker densities, monomers can diffuse more freely. The length scale of monomer localization is determined by the concentration of cross-linker in the gel.

If the gel is immersed in the solution of target molecules and these can diffuse in and out of the gel to form an adsorption binding site of \( p \) adsorbing monomers, the monomers have to move in space to properly group together. However, their motions are severely restricted because almost every adsorbing monomer in the gel belongs to a subchain, which means it is connected by the polymer to two cross-links (there might also be a few adsorbing groups on the dangling ends, connected to only one cross-link). Apart from real cross-links, the freedom of subchains is also restricted by the topological constraints, such as entanglements between polymers.

From a qualitative and very simplified approach, one particular adsorbing monomer in the gel can only access some relatively well-defined volume. It is reasonable to assume that in the center of this volume the adsorbing group is free to move, but approaching the periphery of its spherical cage the group feels increasing entropic restrictions. This can be modeled by saying that for each adsorbing monomer in the gel there is a point fixed in space, which is the center of the cage, and then there is a free-energy-potential well (of entropic origin) around this center. Every adsorbing monomer is attached to its corresponding self-consistent center by an effective polymer chain, like a dog on a leash (Figure 7.5). The length of the effective chain must be of the same order as the distance between cross-link points. Thus, it will be a decreasing function of the cross-link density. In a simplified way, we can imagine that each one of the adsorbing monomers is at the end of one of these effective chains. At the other end of the chain is one of these points
fixed in space, the positions of which are distributed randomly in the gel. Each chain is assumed to be made of \( n \) links, where \( n \) is inversely proportional to the cross-linking density of the gel, based on the concept that additional cross-links increase the frustration in the gel. The parameter \( n \) should be proportional to the ratio of main-component monomers to cross-linker monomers.

An advantage of the fixed-point model is that it allows one to determine the entropic properties of the network using the well-known statistics of polymer chains. Adsorption of target molecules in the gel will deform the chain network, and the accompanying entropy loss can be analyzed via the entropy of Gaussian chains. This entropic effect will be affected not only by the cross-linker density, but also by the density of adsorber monomers, which are implicit in the definition of a fixed point. Note that this density can be adjusted via the gel-swelling phase transition. In the swollen state there will be a low density of fixed points, and in the collapsed state there will be a high density.

The dependences shown in Equation 7.1 can be qualitatively explained using this model as follows (Figure 7.5 and Figure 7.6).

1. Power-law dependence of the affinity on \([\text{Ad}]\). For a target molecule to be adsorbed, the \( p \) adsorbing monomers must be clustered together to simultaneously bind it. The probability of such a cluster existing at a given point in a random (nonimprinted) gel is a product of the probabilities for each of the adsorbing monomers. Therefore, the dependence goes as \([\text{Ad}]^p\). Each of these clusters requires \( p \) adsorbing monomers; hence \( SK \) is proportional to \( 1/p \).
2. Power-law dependence of the affinity on [Re]. The replacement molecules (typically salt ions) act as competitors to the adsorbing monomers. In solution, a target molecule can either be adsorbed into the gel or bound by $p$ replacement molecules. Binding to the replacement molecules prevents adsorption by the gel. Similar to a mass action law, the $p$ replacement molecules must cluster around the target. This creates a power-law dependence similar to that for target molecule adsorption by adsorbing monomers, but with an opposite sign exponent ($[\text{Re}]^{-\gamma}$). Note that it was assumed that each replacement molecule binds to one site on the target molecule, as the adsorbing monomers do. If the adsorber monomers and the replacement molecules have different valences, these exponents should be modified.

3. The term $\exp(-\beta \varepsilon)$ represents the enthalpy contribution to the sorption, i.e., the attraction energy of a target molecule to $p$ adsorbing monomers. This is a Boltzmann probability based on a binding energy $\varepsilon$ per adsorbing monomer.

4. The term $\exp[-c(p - 1)[X]/[A]^2/3]$ summarizes the entropy restrictions to the sorption, which are mainly related to the cross-linking density, as mentioned above. The adsorber units in the gel can move rather freely within a certain volume determined by the cross-linking density. Below a certain length scale associated with the cross-linking density, the gel behaves like a liquid, allowing the adsorber groups to diffuse almost freely. Beyond that length scale, however, the gel behaves as an elastic solid body. The adsorber units cannot diffuse.

FIGURE 7.6
Binding site formation and gel microstructure. (a) Candidate adsorber pairs can be located near a cross-link (1), on a single chain (2), and on distant chains without (3) and with (4) intervening entanglements. (b) With increasing cross-linking density [XL], adsorbers are frustrated in non-imprinted gels. (c) In imprinted gels, flexibility at low [XL] leads to competition for targets, with possible topological consequences (mispairings) that diminish at high [XL]. (Reproduced from Stancil, K.A. et al., J. Phys. Chem. B, 109, 6636, 2005. With permission from the American Chemical Society.)
further than that length scale. As shown earlier in Figure 7.5, we assume that each adsorber is at one end of a fictitious Gaussian chain with a length half the average polymer length between the nearest cross-links, which can be estimated as follows:

\[ l = nb = ([NIPA]/2[Xl])a \]  

(7.6)

where \( n \) is the number of monomer segments of persistent length \( b \) contained in the chain. In the case of a bifunctional cross-linker, i.e., with two polymerizable groups, there are \((NIPA)/2[Xl])\) monomers between the cross-link point and an adsorbing monomer group. Then, \( n = ([NIPA]/2m[Xl]) \) and \( b = ma \), where \( m \) is the number of monomers involved in the persistent length, and \( a \) is the length of each monomer. At a concentration \([Ad]\) of adsorbing monomers, the average spatial distance between adsorbing monomers is \( R = [Ad]^{-1/3} \). For a molar concentration \( C_{ad} \), this corresponds to \( R = 1 \text{ cm}/(C_{ad}N_A)^{1/3} \), where \( N_A \) is the Avogadro number. This fictitious Gaussian chain represents the restricted ability of the adsorber groups to diffuse within a certain volume in the gel. We expect that the probability for two adsorber monomers to meet should be proportional to the Boltzmann factor of the entropy loss associated with the formation of one pair of adsorbers

\[ P = P_0 \exp(-c[Xl]/[Ad]^{2/3}) \]  

(7.7)

where the quantity \( c \) is determined by the persistence length, the number of monomers in a persistence length, and the concentration of the main component of the chains through the relation

\[ c = 2m/([NIPA]b^2) \]  

(7.8)

Since the adsorption of a divalent target by two adsorbers brings together each end from two fictitious Gaussian polymers, the affinity should be proportional to this probability. If more than two contact points are expected, the equation can be generalized as

\[ SK \propto \exp[-c(p-1)[XI]/[Ad]^{2/3}] \]  

(7.9)

where \( p \) is the number of contact points. If the target molecule is adsorbed only by a single contact \((p = 1)\), the affinity should be independent of the cross-linker concentration. In contrast, if the target binding site requires several adsorber monomers \((p > 1)\), the cross-links may frustrate the formation of the binding site, and the frustration will increase with \( p \). This will be particularly significant at low concentrations of [Ad] (since only a small fraction of the adsorber monomers will be close enough together to participate in multiple binding sites) and high cross-linking concentration. It should also be taken into account that the adsorption process itself can increase the cross-linking density, since the complexes will act as tie junctions.
Imprinting Using Smart Polymers

of polymeric chains. This could modify the value of \( n \) as the sorption is going on. Nevertheless, usually this last contribution to the cross-linking is low enough to be negligible.

It can be shown by consideration of the relative weights of the terms \( Z_p \) that for a given set of experimental parameters, the affinity \( SK \) will almost always be dominated by a single value of \( p \), either \( p = 1 \) or \( p = p_{\text{max}} \). This can be understood by considering whether the attraction of the target molecule to adsorber monomers (due to energetic and concentration effects) is stronger than the repulsion due to the entropy loss required to deform the gel. If attraction is sufficiently favored, then the target will be bound by as many adsorber monomers as possible and, therefore, \( p = p_{\text{max}} \). However, if the entropy loss to deform the gel is stronger, then the adsorption will only be possible by single adsorber monomers, i.e., \( p = 1 \), which would not require deformation of the network (no chain entropy penalty).

The basic concept of gels as smart materials is that they will have high affinity for the target in the collapsed state, but low affinity in the swollen state. By controlling the phase transition of the gel, one will be able to create a switchlike behavior in the affinity. The Tanaka equation allows one to predict the composition of gels that will drastically change affinity during the gel phase transition. If the gel is to have a low affinity in the swollen state, the adsorber monomers should have only a weak attraction to the target molecules, i.e., any adsorption should be single-handed (\( p = 1 \)). To have a high gel affinity, adsorption in the collapsed phase should involve as many adsorber monomers as possible (\( p = p_{\text{max}} \)). The \( p \) value transition should occur where the entropic and energetic contributions to the affinity are equal, i.e., the crossover should occur when

\[
\ln(\frac{\text{[Ad]}}{\text{[Re]}}) = (\frac{\text{[Ad]}^{2/3} \ n b^2}{\text{[Re]}})^{-1} + \beta \varepsilon \tag{7.10}
\]

A more detailed description of the Tanaka theory has been published by Ito et al.\textsuperscript{39,40}

The experiments discussed below use gels in which \( p \) changes across the phase transition. However, the design of such gels still requires a significant amount of research, since it is difficult to know \textit{a priori} the exact value for the binding energy \( \varepsilon \).

Since the Tanaka equation is based on the idea that there are many sets of adsorber monomers that work together locally to form binding sites for the target molecules and that the probability for chain stretching drops off exponentially with distance, it can also explain the greater affinity of imprinted gels compared with the nonimprinted ones. The synthesis in the presence of the target molecules leads to the distribution of the adsorbing monomers in groups of the \( p \) members required for the binding of each target molecule. In the imprinted gels, the \( p \) members are closely fixed during polymerization in the collapsed state due to the template. The template is removed from the gel in the swollen state because of the deformation of the binding sites. Once again, in the collapsed state, the binding sites can be reconstructed, and since
each binding site possesses the needed \( p \) adsorbing monomers close together, the entropic restrictions to the sorption should be minimized (Figure 7.6). Representative examples of the enhancement in affinity observed for imprinted smart gels are given in the following subsections.

### 7.2.2 Experimental Assessment of the Tanaka Equation

Among the studies carried out to assess the Tanaka equation in nonimprinted heteropolymer gels,\(^{36–38,43}\) those carried out with pyrene derivatives as target molecules are particularly representative.\(^{36–39}\) The gels were prepared by free-radical polymerization using 6\(M\) \( N \)-isopropylacrylamide (NIPA) as the stimuli-sensitive component, 0 to 120\(mM\) methacrylamido propylammonium chloride (MAPTAC) as functional monomer, and 5 to 200\(mM\) \( N,N' \)-methylenbis(acrylamide) (BIS) as cross-linker. The monomers were dissolved in dimethylsulfoxide and polymerized inside micropipettes (i.d. 0.5 mm). Once washed with water, all gels were collapsed at 60\(^{\circ}\)C and showed the same degree of swelling as during polymerization. As adsorbates or target molecules, several different types of pyrene sulfonate derivatives were used: 1-pyrene sulfonic acid sodium salt (Py-1·Na), 6,8-dihydroxy-pyrene-1,3-disulfonic acid disodium salt (Py-2·2Na), 8-methoxy pyrene-1,3,6-trisulfonic acid trisodium salt (Py-3·3Na), and 1,3,6,8-pyrene tetrasulfonic acid tetrasodium salt (Py-4·4Na), portrayed in Figure 7.4. These chemicals present 1 (Py-1), 2 (Py-2), 3 (Py-3), or 4 (Py-4) anionic charges, which can interact electrostatically with a cationic charged site such as on MAPTAC. Pieces of cylindrical gel (5 to 20 mg dry weight) were placed in 2- or 4-ml target aqueous solution, the concentration of which ranged from 2 to 0.5\(mM\). The solutions also contained \( NaCl \) of a prescribed concentration (27 to 200\(mM\)) to provide chloride ions to replace the target molecules. The samples were kept swollen (20\(^{\circ}\)C) or shrunken (60\(^{\circ}\)C) for 48 h, and the adsorption isotherms were analyzed in terms of the Langmuir equation (Equation 7.2) to calculate the affinity \( SK \).

Figure 7.7 shows the dependence of affinity for Py-3 and Py-4 on the MAPTAC concentration. Above a certain MAPTAC concentration (20\(mM\)) and in the collapsed state, both the log–log plots show a straight line, with slope 3 for Py-3 and with slope 4 for Py-4. These power-law relationships are due to three and four adsorption points, respectively. Adsorption sites are formed when three (or four) equivalent adsorbing molecules (MAPTAC) gather to capture one Py-3 (or Py-4) molecule. The obtained power laws are in agreement with the Tanaka equation. At MAPTAC concentrations below 10\(mM\), the major component of the gel (NIPA) contributes more to the adsorption of pyranine (due to a hydrophobic interaction) than do the MAPTAC groups, and the power-law exponent becomes zero. In the swollen state, the log–log slope becomes 1, indicating that MAPTAC adsorbs the target molecule with a single contact. Single-point adsorption is favored because the MAPTAC monomers are well separated one from another, and it becomes entropically unfavorable for the multipoint adsorption complex to assemble.
The slope returns to 3 or 4 upon shrinking, indicating recovery of the multipoint binding sites. The reversible adsorption ability is controlled by the volume phase transition.

The effect of external salt concentration on the binding of target molecules to the gel is shown in Figure 7.8. As mentioned above, when these low-cross-linked gels are in the collapsed state, a number of MAPTAC groups equal to the number of charges of the target ($p_{\text{max}}$) can gather to form a binding site. Since the attraction is electrostatic, coexistent ions in the solution can make the target molecule adsorption difficult. Ions with the same charge sign as the target molecules should compete with the target molecule for binding with the adsorber monomers. In the cases Py-1, -2, and -3, the log–log plot showed slopes of $-1$, $-2$, and $-3$, respectively, i.e., $-p_{\text{max}}$, where $p_{\text{max}}$ is the number of charged groups on the target molecule (affinity $\propto [\text{Re}]^{-p_{\text{max}}}$). Py-4 followed a similar behavior, though there was a small discrepancy below 40 mM of salt concentration, probably because of the increasing effect of the Donnan potential at lower salt concentrations. In the swollen state ($p = 1$), the log–log plots showed slopes of $-1$. Therefore, the Tanaka equation fits the results quite well in a wide range of salt concentrations.

As explained above, the multipoint adsorption should lead to an entropy loss in the polymer chains that are bound together by the target molecules. The effect of this entropy loss, which is a function of the concentration of cross-linker and adsorber, is to reduce the affinity of the gel for the target. Several experimental studies have shown that the affinity of a heteropolymer gel involved in multiple contact adsorption decreases with its cross-linking degree.
Hsein and Rorrer\textsuperscript{44} showed an exponential decrease in calcium adsorption by chitosan as the extent of cross-linking increases. Eichenbaum et al.\textsuperscript{45} found that, for alkali earth metal binding in methacrylic acid-co-acrylic acid microgels, the cross-links prevent the carboxylic groups from achieving the same proximity as in a linear polymer, which affects the binding properties of the metals. In the case of a thermosensitive gel, the influence of the degree of cross-linking has also been shown.\textsuperscript{24,31}

The volume phase transition of a NIPA gel induced by a stimulus is responsible for separating the adsorber monomers (e.g., MAPTAC) in the swollen state, which decreases their probability of coming close to each other to adsorb a multicharged target. Consequently, in the swollen state, the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.8.png}
\caption{Dependence of the affinity for pyranines on the replacement ion concentration. The solid line displays a slope with the value shown in each plot. In this experiment, the concentrations of the adsorber (MAPTAC) and the cross-linker (BIS) were fixed at 40 and 10mM, respectively. (Reproduced from Watanabe, T. et al., \textit{J. Chem. Phys.}, 115, 1596, 2001. With permission from the American Institute of Physics.)}
\end{figure}
affinity for the target increases slightly with the degree of cross-linking, since the degree of swelling is reduced. In contrast, in the collapsed state an exponential decrease of the affinity with the cross-linking, which was especially significant for the cases of contact numbers above 2, was observed (Figure 7.9). The affinity values predicted by the Tanaka equation agree well with those experimentally obtained. This unfavorability for the affinity has been understood to be a “frustration” to the mobility of the adsorbing sites.\textsuperscript{31,46,47} The frustration can also be viewed in terms of the “flexibility” of the polymer chains, which is critical for allowing the adsorber groups to come into proximity for a multipoint adsorption. A detailed analysis of the data shown in Figure 7.9 revealed that a plot of the exponential decay rate vs. the number of contact points gives a linear relationship in which the slope is 0.32. This slope associates with the parameter $c$ in Equation 7.1, and

![Figure 7.9](image-url)

**FIGURE 7.9**
Dependence of the affinity of gels at 60°C for pyranines on the concentration of cross-linker BIS (upper $x$-scale) or on the ratio of cross-linker concentration to that of adsorber monomer to the two-thirds power (lower $x$-scale). (Reproduced from Ito, K. et al., *Prog. Polym. Sci.*, 28, 1489, 2003. With permission from Elsevier.)
can be used to calculate the persistence length $b = ma$ for the polymer chains, applying Equation 7.8. For $c = 0.32$, the value of $b$ equals 2.9 nm. The persistent length is theoretically predicted to be around 2 nm, e.g., $10$ monomers ($m = 10$) of 2 Å ($a = 2 \times 10^{-10}$ m). Thus, the obtained result is somewhat reasonable. Therefore, the theory predicts and explains well the exponential decay with concentration of the cross-linker. The cross-links and polymer connections create frustrations so that the adsorber groups (MAP-TAC) cannot lower the energy of the polymer by forming pairs, triplets, or groups of $p$ members for capturing target molecules. As will be shown below, such frustrations can be overcome using molecular imprinting.

7.3 Temperature-Sensitive Imprinted Hydrogels

Like proteins, a heteropolymer gel can exist in four thermodynamic phases:

1. Swollen and fluctuating
2. Shrunken and fluctuating
3. Shrunken and frozen in a degenerate conformation
4. Shrunken and frozen in the global minimum energy conformation

The order parameter that describes the phase transition between the first and the second phases is the polymer density or, equivalently, the swelling ratio of the gel. The third and fourth phases are distinguished by another order parameter: the overlap between the frozen conformation and the minimum energy conformation. In the third phase, the frozen conformation is random. In the fourth phase, the frozen conformation is equivalent to that of the global energy minimum. Proteins in this fourth phase take on a specific conformation, which may be capable of performing catalysis, molecular recognition, or many other activities. Tanaka and colleagues strove to recreate such a fourth phase in gels by designing a low-energy conformation and then testing whether the gel could be made to reversibly collapse into this “memorized” conformation. According to developments in the statistical mechanics of polymers, to achieve the memory of conformation by flexible polymer chains, several requisites must be satisfied.

1. The polymer must be a heteropolymer, i.e., there should be more than one monomer species, so that some conformations are energetically more favorable than others.
2. There must be frustrations that hinder a typical polymer sequence from being able to freeze to its lowest energy conformation (as considered in absence of the frustration). Such frustrations may be due to the interplay of chain connectivity and excluded volume, or
they may be created by cross-links. For example, a cross-linked polymer chain will not freeze into the same conformation as the non-cross-linked polymer chain, at least for most polymer sequences.

3. The sequence of monomers must be selected so as to minimize these frustrations, i.e., a particular polymer sequence should be designed such that the frustrating constraints do not hinder the polymer from reaching its lowest energy conformation.

These three conditions allow the polymer to have a global free-energy minimum at one designed conformation.

The nonimprinted gels described in previous sections satisfy the first two conditions, and can be engineered to satisfy the third. The adsorber and the main-component monomers provide heterogeneity in interaction energies, since adsorber interactions are favorably mediated by the target molecules. Frustrations to the achievement of the global energy minimum exist due to the cross-links in the gel as well as chain connectivity. To meet the third condition, the minimization of the frustration, the molecular imprinting technique can be particularly useful. In this section, we discuss the early experimental successes of the imprinting method and the application of the Tanaka equation to imprinted gels.

Two approaches were investigated to achieve elements of conformational memory in gels:

1. Cross-linking of a polymer dispersion or preformed gel in the presence of the target molecule (two-step imprinting or post-imprinted)
2. Simultaneous polymerization and cross-linking of the monomer solution (one-step imprinting)

The first approach was studied in heteropolymer gels consisting of NIPA as the major component sensitive to stimuli and MAPTAC as the charged monomer able to capture pyranine target molecules. The polymer networks were randomly copolymerized, in the absence of pyranines, with a small quantity of permanent cross-links and thiol groups (–SH). The gels were then further cross-linked by connecting thiol group pairs into disulfide bonds (–S–S–). The process was carried out directly (nonimprinted gels) or after the gels were immersed in a solution of pyranines and had all charged groups forming complexes with the target molecules (postimprinting). These post-cross-links were still in very low concentrations, in the range 0.1 to 3 mol%, and therefore the gels could freely swell and shrink to undergo the volume phase transition. The postimprinted gels showed higher affinity for the target than those that were randomly post-cross-linked (Figure 7.10). However, this post-cross-linking approach has a fundamental drawback. Before the post-cross-linking, the sequence of the components has already been determined and randomly quenched. The minimization of the frustration is allowed only in the freedom of finding best partners among –SH groups. For this reason, the imprinting using a post-cross-linking technique can give only a partial success.
Ideally, the entire sequence of all monomers should be chosen so that the system will be in its global energy minimum. The complete minimization of the frustration can be achieved by polymerizing monomers while they self-organize in space at a low-energy spatial arrangement.\textsuperscript{31,32,35} This second approach controls the sequence formation, allowing the monomers to equilibrate in the presence of a target molecule. These monomers are then polymerized with a cross-linker. It is hoped that, upon removal of the template species, binding sites with the spatial features and binding preferences for the template are formed in the polymer matrix. The choice of functional monomers and the achievement of an adequate spatial arrangement of functional groups are two of the main factors responsible for specificity and reversibility of molecular recognition.

The first experiments carried out by Alvarez-Lorenzo et al.\textsuperscript{31,32} used gels prepared by polymerization of NIPA, small amounts of methacrylic acid (MAA), and BIS in the absence (nonimprinted) or the presence of divalent cations. MAA was used as the functional monomer able to form complexes...
in the ratio 2:1 with divalent ions. The effect of temperature on the adsorption capacity of the imprinted copolymers prepared with different template ions and in different organic solvents was compared with that of the nonimprinted ones. Successful imprinting was obtained using calcium or lead ions as template. After removing the template and swelling in water at room temperature, the affinity for divalent ions notably decreased. When the gels were shrunken by an increase in temperature, the affinity was recovered (Figure 7.11). The measurements of the affinity suggested that multipoint adsorption occurs for both imprinted and nonimprinted gels in the collapsed state (saturation $S = [\text{Ad}]/2$), but that in the imprinted gel, the multipoint

![Figure 7.11](image-url)

**FIGURE 7.11**
The overall affinity $SK$ of the nonimprinted and imprinted gels for calcium ions is plotted as a function of methacrylic acid monomer concentration for (a) the swollen state and (b) the shrunken state. The values of the slope for nonimprinted gels duplicate the slope of the imprinted ones prepared with $Ca^{2+}$ as template or with the alternative template $Pb^{2+}$. (Reproduced from Alvarez-Lorenzo, C. et al., *J. Chem. Phys.*, 114, 2812, 2001. With permission from the American Institute of Physics.)
adsorption is due to memorized binding sites. After recollapsing, two-point adsorption is recovered in the random gels with a power law of $SK \propto [Ad]^2$, while the imprinted gels showed a stronger affinity with a power law of $SK \propto [Ad]^1$. The difference in the power dependence of the affinity is due to a different $[Ad]$ dependence for $K$. The affinity per adsorption site is actually independent of $[Ad]$ for imprinted gels. This is because the adsorbers are ordered such that they already form sites with their unique partners. Hence, the adsorbers are ordered in groups of two to give the highest possible $K$ at all values of $[Ad]$. Furthermore, the affinity of the imprinted gels does not change at all with an increase of the cross-linking density, while the affinity decreases for random gels (Figure 7.12). The nondependence of the affinity on $[XI]$ proves the minimization of the frustration with regard to the cross-links and chain connectivity. Thus, the requirements 1, 2, and 3 necessary for obtaining conformational memory have been achieved.

In these hydrogels the relative proportion of functional monomers, compared with the other monomers, is quite low (1 mol%), and it should be difficult for the gel to take on a strong-binding conformation other than the one imprinted during synthesis. For random gels, it was difficult to pair randomly distributed MAA, and their affinity for divalent ions decreased exponentially as a function of cross-linker concentration. In contrast, in the imprinted gels, the local concentration of MAA in the binding site is very high, i.e., the members of each pair are closely fixed by the template during polymerization. If the gel did not memorize such pairs after washing the template out, swelling, and reshrinking, an MAA would have to look for a new partner nearby, and such a probability would be the same as that in a nonimprinted gel. Therefore, it can be concluded that the greater adsorption

FIGURE 7.12
Influence of the cross-linker (BIS) concentration on the overall affinity for calcium ions of the imprinted (full symbols) and nonimprinted (open symbols) NIPA (6M) gels in the shrunken state in water. The concentration of functional monomers (MAA) was fixed at 32mM. (Reproduced from Alvarez-Lorenzo, C. et al., *Macromolecules*, 33, 8693, 2000. With permission from the American Chemical Society.)
capacity of the imprinted gels comes from the successfully memorized MAA pairs. Güney et al. followed a similar procedure to prepare temperature-sensitive gels that specifically recognize and sorb heavy metal ions from aqueous media in an effort to develop chemosensors. Kanazawa et al. used another functional monomer N-(4-vinyl)benzyl ethylenediamine (Vb-EDA) that contains two nitrogen groups that can specifically form coordination bonds with one copper ion; each copper ion requires two functional monomers to complete its bonding capacity. This occurs when the gel is formed at the shrunken state. At the swollen state, the bonds are broken and the affinity for the ions disappears. These gels showed a high specificity for Cu$^{2+}$ compared with Ni$^{2+}$, Zn$^{2+}$, or Mn$^{2+}$. The different coordination structure—square planar for Cu$^{2+}$ and Ni$^{2+}$, tetrahedral for Zn$^{2+}$, and octahedral for Mn$^{2+}$—together with the differences in ion radii explains this specificity, which was not observed in the nonimprinted gels. The amphiphilic character of the functional monomer Vb-EDA made it possible to develop an emulsion polymerization procedure to prepare imprinted microgels. The main advantage of these microgels is that they can adsorb and release the copper ions faster because of their quick volume phase transitions.

Detailed calorimetric studies of the thermal volume transition of polyNIPA (PNIPA) hydrogels and the influence of ligand binding on the relative stability of subchain conformations have been carried out by Grinberg and coworkers. The dependence of the critical temperature of PNIPA hydrogels on the proportion of ionic co-monomers is an obstacle to obtaining devices with a high loading capability while still maintaining the PNIPA temperature-sensitive range. This can be overcome by synthesizing interpenetrated polymer networks (IPN) of PNIPA with ionizable hydrophilic polymers. Although only a few papers have been devoted to this topic, the results obtained in those studies have shown the great potential of IPNs. A two-step approach to imprint interpenetrated gels with metal ions was identified by Yamashita et al. It basically consists in (a) polymerization of AA monomers to have a loosely cross-linked (1 mol%) polyAA network; (b) immersion of polyAA in copper solution to enable the ions to act as junction points between different chains; and (c) transfer of polyAA-copper ion complexes to a NIPA solution containing cross-linker (9.1 or 16.7 mol%) and synthesis of the NIPA network in the collapsed state (Figure 7.13). The nonimprinted IPNs (i.e., prepared in the absence of copper ions) showed a similar affinity for Cu$^{2+}$ and for Zn$^{2+}$. In contrast, the imprinted IPNs in the collapsed state could discriminate between the square planar structure of Cu$^{2+}$ and the tetrahedral structure of Zn$^{2+}$.

NIPA-based imprinted hydrogels have also been prepared using organic molecules as templates. Watanabe et al. observed that NIPA (16 mmol) - acrylic acid (4 mmol) cross-linked (1 mmol) polymers synthesized in dioxane and in the presence of norephedrine (2 mmol) or adrenaline (2 mmol) showed, after template removal, an increase in the swelling ratio in the collapsed state as the target molecule concentration in water increases.
Since the molar concentration of the adsorbing monomers was much higher than the cross-linking density, the cross-links should not have created frustration. Thus the imprinting effect may only be due to the phase separation caused by the template during polymerization, which is macroscopically evidenced as a change in the conformation when the network collapses. Liu et al.\textsuperscript{64,65} obtained temperature-sensitive imprinted gels for 4-aminopyridine and L-pyroglutamic, which showed the same ability to sorb and release the drug after several shrinking–swelling cycles. These gels had significantly higher saturation and affinity constants than the nonimprinted ones and were also highly selective. These results indicate that temperature-sensitive imprinted gels have a potential application in drug delivery.

Natural polymers, such as chitosan, have also been evaluated as a basis for temperature-sensitive hydrogels instead of synthetic monomers.\textsuperscript{66} Chitosan is an aminopolysaccharide (obtained from chitin) that can be chemically cross-linked through the Schiff base reaction between its amine groups and the aldehyde ends of some molecules, such as glutaraldehyde.\textsuperscript{67} A recent study has shown that if the reaction is carried out in the presence of target molecules, such as dibenzothiophenes (DBT), imprinted networks with a remarkably greater adsorption capability than nonimprinted ones can be obtained. This effect was particularly important when the gel was collapsed in the same solvent (acetonitrile) and at the same temperature (50\degree C) as were used during the cross-linking.\textsuperscript{66} Additionally, the DBT-imprinted gels showed a high selectivity for the target molecules compared with other
structurally related compounds (Figure 7.15). These gels have been proposed as traps for organosulfur pollutants. In general, stimuli-sensitive imprinted gels are very weakly cross-linked (less than 2 mol%) and, therefore, the success of the imprinting strongly depends on the stability of the complexes of template/functional monomers during polymerization and after the swelling of the gels. If the molar ratio in the complex is not appropriate or if the complex dissociates to some extent during polymerization, the functional monomers will be far apart from both the template and each other, and the imprinting will be thwarted. However if the interaction is too strong, it may be difficult to remove the templates.
completely, which leads to a reduction of the number of free binding sites and template bleeding during the assays.

Recent efforts showed the possibilities of using adsorbing monomers directly bonded to each other prior to polymerization, which avoids the use of the template polymerization technique. Each adsorbing monomer can be broken after polymerization to obtain pairs of ionic groups with the same charge. Since the members of each pair are close together, they can capture target molecules through multipoint ionic interactions (Figure 7.16). The adsorption process was found to be independent of the cross-linking density, and the entropic frustrations were completely resolved. Divalent ions or molecules with two ionic groups in their structures can be loaded in a greater amount and with higher affinity by these “imprinted” hydrogels. Furthermore, the hydrogels prepared with PNIPA and imprints were gifted with a new ability not observed in common stimuli-sensitive gels: they can re-adsorb, at the shrunken state, a significantly high amount of the templates previously released at the swollen state. Common (nonimprinted) temperature-sensitive PNIPA hydrogels have a pulsate release behavior that allows a substance entrapped in the polymer network to diffuse out of the hydrogel in the swollen state, but then stops the release when the temperature increases and the network collapses. In contrast, a change from the swollen to the shrunken state of the imprinted PNIPA hydrogels not only stops the release, but also promotes a re-adsorption process. This process occurs...
quickly and in a way that can be reproduced after several temperature cycles (Figure 7.17). At 37°C, the high affinity for the template provokes a stop in the release when an equilibrium concentration between the surrounding solution and the hydrogel is reached. This type of hydrogel has a great potential for development of drug-delivery devices capable of maintaining stationary drug levels in their environment. The gel would stop the release while the released drug has not yet been absorbed or distributed but remains near the hydrogel.

7.4 pH-Sensitive Imprinted Gels

The term “pH-sensitive imprinted gel” can comprise two different behaviors:

1. Imprinted gels with adsorber monomers that show an affinity for the target molecule that can be tuned by changes in pH, but that do not undergo phase transitions
2. Imprinted gels that contain protonizable groups capable of causing pH-induced phase transitions and adsorber groups responsible for the interactions with the target molecule
In the first group we could insert almost all temperature-sensitive gels described in the previous section, as well as many other examples of non-smart imprinted networks, since the recognition relies upon strong ionic interactions between the functional monomers and the template. If a change in pH modifies the degree of protonization of their chemical groups (although no change in degree of swelling takes place), a strong change in binding energy and, therefore, in affinity will occur.

In this section, we will mainly focus on gels that undergo pH-sensitive phase transitions, these changes in volume being mainly responsible for the control of the sorption process. Nevertheless, in some cases, the pH can alter both the swelling and the binding energy.

Kanekiyo et al. have developed pH-sensitive imprinted gel particles using hydrophobic interactions to sorb the target molecules. The method consists of using a polymerizable derivative of amylose capable of wrapping around a hydrophobic template, such as bisphenol-A. The helical inclusion complex formed is then copolymerized with a cross-linker and a monomer having ionizable groups (e.g., acrylic acid, AA) (Figure 7.18). Similarly, other imprinted polymers were prepared with acrylamide instead of acrylic acid. The rebinding ability of MIPs prepared with AA showed a strong dependence....
on pH: the greater the pH, the lower the binding of bisphenol-A. These results indicate that the binding cavity created through the imprinting process is disrupted by a conformational change in the amylose chain arising from the electrostatic repulsion between the anionic groups. A decrease in pH restores the cavities and the binding affinity.

The group of Peppas has developed an imprinting procedure using star-shaped polyethylene glycols (PEGs) copolymerized with methacrylic acid (MAA) for the recognition of sugars in aqueous solution. Star polymers, also called hyperbranched polymers, have a large number of arms emanating from a central core and, therefore, they can contain a large number of functional groups in a small volume. The star-shaped PEGs provide the multiple hydrogen bonds required to interact with sufficient strength in water with sugars or proteins (Figure 7.19). Two different star-PEGs were evaluated. Grade 423 with 75 arms (MW = 6,970) and Grade 432 with 31 arms (MW = 20,000). Imprinted networks were obtained by carrying out the polymerization in the presence of glucose. The imprinted 31-arm star-PEG gel showed...
a 213% increase in glucose sorption over the nonimprinted polymer (253 vs. 118 mg/g), while for the 75-arm star-PEG gels no improvement in sorption ability by the imprinting was observed (198 vs. 199 mg/g). This last finding could be attributed to the ratio of adsorber groups to the cross-linking density being too high for the cross-linker to create frustrations and therefore resulting in no observable difference between imprinted and nonimprinted systems. The incorporation of MAA enabled the system to become pH-responsive. At pH below the pK\(_a\) of MAA (4.5), the gel collapses and the template is strongly held in the network cavities. As the pH rises, the MAA dissociates and the gel swells. This also decreases the hydrogen bonding between the PEGs and the target molecules, allowing them to diffuse out of the gel.

The synthesis of MIPs selective to natural macromolecules, such as peptides and proteins, is not common. It is difficult because bulky protein cannot easily move in and out through the mesh of a polymer network. The attempts to overcome these limitations have been focused on synthesizing macroporous MIPs\(^{75,76}\) or creating imprinted cavities at the surface of the network.\(^{77,78}\) Stimuli-sensitive networks can be particularly useful for overcoming steric impediments, as recently shown by Demirel et al.\(^{79}\) using pH- and temperature-sensitive gels imprinted for serum albumin (BSA). The ionic poly(N-tertbutylacrylamide-co-acrylamide/maleic acid) hydrogels synthesized in the presence of BSA showed a remarkably greater affinity for the protein compared with the nonimprinted ones, the adsorption being dependent on both pH and temperature. The hydrogels were synthesized at 22.8°C, at the swollen state. At this temperature, the adsorption is maximal. In contrast, when the gel collapses it is difficult for the protein to diffuse into the gel, the imprinted cavities are distorted, and the nature of the interactions can also be altered. At low temperature, the interactions between BSA and the hydrogel are based on hydrogen bonds. As the temperature rises, hydrogen bonds become weaker, while hydrophobic interactions get stronger. These results clearly highlight the relevance of the memorization of the conformation achieved during polymerization, which provides the gel with the ability to recognize a given template.
7.5 Conclusions

The synthesis of stimuli-sensitive hydrogels in the presence of target molecules enables one to design the sequence of adsorbing or functional monomers in the polymer network. Through such design, cavities with size and chemical properties complementary to the target can be imprinted in the hydrogel. The appropriate polymer sequence is fixed during polymerization, which allows the hydrogel to memorize the desired conformation. After undergoing cycles of swelling and collapse, the gel maintains the ability to recognize and host the target molecules. Adsorption occurs through multiple contacts in the collapsed state, but through single contacts in the swollen state. This multipoint adsorption makes the collapsed-state affinity significantly larger than that in the swollen state. The abrupt change in affinity during the gel volume phase transition allows one to turn gel adsorption on and off. The Tanaka equation can theoretically explain and predict the effects on a gel’s affinity for the target molecules of the adsorbing monomer and cross-linker proportions in the network and of the salt or replacement molecules concentration in the sorption medium. The Tanaka equation is also a useful tool for better understanding the advantages of imprinted stimuli-sensitive hydrogels, compared with nonimprinted ones, and for optimizing their properties for application in different fields.

Acknowledgments

This work was financed by the Ministerio de Ciencia y Tecnología, FEDER (RYC2001-8; SAF2005-01930), and Xunta de Galicia (PGIDIT03PXIC20303PN), Spain.

References


Imprinting Using Smart Polymers
