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Review

Molecularly imprinted polymers for drug delivery

Carmen Alvarez-Lorenzo*, Angel Concheiro

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

Abstract

Molecular imprinting technology has an enormous potential for creating satisfactory drug dosage forms. Although its application in this field is just at an incipient stage, the use of MIPs in the design of new drug delivery systems (DDS) and devices useful in closely related fields, such as diagnostic sensors, is receiving increasing attention. Examples of MIP-based DDS can be found for the three main approaches developed to control the moment at which delivery should begin and/or the drug release rate, i.e. rate-programmed, activation-modulated, or feedback-regulated drug delivery. The utility of these systems for administering drugs by different routes (e.g. oral, ocular or transdermal) or trapping undesired substances under in vivo conditions is discussed. This review seeks to highlight the more remarkable advantages of the imprinting technique in the development of new efficient DDS as well as pointing out some possibilities to adapt the synthesis procedures to create systems compatible with both the relative instable drug molecules, especially of peptide nature, and the sensitive physiological tissues with which MIP-based DDS would enter into contact when administered. The prospects for future development are also analysed.

Keywords: Drug delivery; Molecularly imprinted polymers

Contents

* Corresponding author. Fax: +34-981-547148.
E-mail address: ffrusdog@usc.es (C. Alvarez-Lorenzo).

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1. Introduction

In the last few years, a number of significant advances have been made in the development of new technologies for optimising drug delivery [1]. To maximise the efficacy and safety of medicines, drug delivery systems (DDS) must be capable of regulating the rate of release (delayed- or extended-release systems) and/or targeting the drug to a specific site. Efficient DDS should provide a desired rate of delivery of the therapeutic dose, at the most appropriate place in the body, in order to prolong the duration of pharmacological action and reduce the adverse effects, minimise the dosing frequency and enhance patient compliance. To control the moment at which delivery should begin and the drug release rate, the three following approaches have been developed [2]—(a) *rate-programmed drug delivery*: drug diffusion from the system has to follow a specific rate profile; (b) *activation-modulated drug delivery*: the release is activated by some physical, chemical or biochemical processes; and (c) *feedback-regulated drug delivery*: the rate of drug release is regulated by the concentration of a triggering agent, such as a biochemical substance, concentration of which is itself dependent on the drug concentration in the body. When the triggering agent is above a certain level, the release is activated. This induces a decrease in the level of the triggering agent and, finally, the drug release is stopped. The sensor embedded in the DDS tries to imitate the recognition role of enzymes, membrane receptors and antibodies in living organisms for regulation of chemical reactions and for maintenance of the homeostatic equilibrium.

Molecular imprinting technology can provide efficient polymer systems with the ability to recognise specific bioactive molecules and a sorption capacity dependent on the properties and template concentration of the surrounding medium; therefore, although imprinted DDS have not reached clinical application yet, this technology has an enormous potential for creating satisfactory dosage forms. A good proof of this is the progressive increase in the number of papers devoted to the application of MIPs in the design of new DDS and also in devices useful in closely related fields, such as diagnostic sensors or chemical traps to remove undesirable substances from the body [3–6]. Enormous interest has also been shown in imprinted materials as they mime biological receptors for the screening of new substances with potential pharmacological activity or to specifically detect drugs in biological fluids in screening assays for drugs of abuse [7]. Such specificity is comparable with monoclonal antibodies used in immunoassay techniques [8].

Molecular imprinting is a well-developed tool in the analytical field, mainly for separating and quantifying very different substances, including drugs and bio-active molecules contained in relatively complex matrices [9–12]. The information generated about polymer synthesis procedures (Table 1) and the properties outlined for optimum performance in separation-based technologies [13] may be a good starting point to create imprinted DDS, although with some subtle differences. The following aspects should be taken into account.

Table 1
Variables of polymerisation of imprinted polymers

Imprinting mechanism
Covalent
Non-covalent
Metal co-ordination
Cross-linker proportion
High
Low
Medium
Organic
Aqueous
No solvent

- Compromise between rigidity and flexibility. The structure of the imprinted cavities should be stable enough to maintain the conformation in the absence of the template, but somehow flexible enough to facilitate the attainment of a fast equilibrium between the release and re-uptake of the template in the cavity. This will be particularly important if the device is used as a diagnostic sensor or as a trap of toxic substances in the gastrointestinal tract. In this sense, non-covalent imprinting usually provides faster equilibrium kinetics than the covalent imprinting approach [3]. The mechanical properties of the polymer and the conformation of the imprinted cavities depend to a great extent on the proportion of the cross-linker. Mostly imprinted systems for analytical applications require around 25–90% of cross-linker agent [13]. These cross-linking levels increase the hydrophobicity of the network and prevent the polymer network from changing the conformation obtained during synthesis. In consequence, the affinity for the template is not dependent on external variables and it is not foreseen that the device will have regulatory or switching capabilities. The lack of response through a change in polymer conformation to the alterations of the physico-chemical properties of the medium or to the presence of a specific substance limits their potential uses as *activation*- or *feedback-modulated* DDS. A high cross-linker proportion also considerably increases the stiffness of the network making it difficult to adapt the shape of the administration site and causing mechanical friction with the surrounding tissues (especially when administered topically, ocularly or as implants).
- High chemical stability. MIPs for drug delivery should be stable enough to resist enzymatic and chemical attack and mechanical stress. The device will enter into contact with biological fluids of complex composition and different pH, in which the enzymatic activity is intense. Ethylene glycol dimethacrylate (EGDMA) and related cross-linkers, which are the most usual ones, have been proved to provide stable networks in a wide range of pHs and temper-

atures under in vitro conditions [14]. However, additional research should be carried out to obtain information about its behaviour in vivo environments, where esterases and extreme pHs seem to be able to catalyse its hydrolysis [15].

Additionally, it has to be taken into account that the adaptability of molecular imprinting technology for drug delivery also requires the consideration of *safety* and *toxicological* concerns. The device is going to enter into contact with sensitive tissues; therefore, it should not be toxic, neither should its components, residual monomers, impurities or possible products of degradation [16,17]. Therefore, to ensure biocompatibility it might be more appropriate to try to adapt the imprinting technique to already tested materials instead of creating a completely new polymeric system. On the other hand, most classical MIPs are created in organic solvents to be used in these media, taking advantage of electrostatic and hydrogen bonding interactions [3]. The presence of residual organic solvents may cause cellular damage and should be the object of a precise control. In consequence, hydrophilic polymer networks that can be synthesised and purified in water are preferable to those that require organic solvents. A hydrophilic surface also enhances biocompatibility and avoids adsorption of proteins and microorganisms [18]. Additionally, many drugs, peptides, oligonucleotides and sugars are also incompatible with organic media.

A wide range of cross-linked hydrogels have been proved to be useful as drug delivery platforms [19,20]. Molecular imprinting in water is still under development and difficulties arise due to the considerable weakness of electrostatic and hydrogen-bonding interactions in this polar medium, which decrease the affinity and selectivity of MIP for the ligand [21]. Nevertheless, hydrophobic and metal co-ordination interactions are proving to be very promising to enhance template and functional monomer association in water [22,23]. It is clear that the polymer composition and solvent are key parameters in the achievement of a good imprinting and that, in consequence, a compromise between functionality and biocompatibility is needed.

Depending on the specific application of the device, an adequate balance between the performance as imprinted systems—that determines the efficiency as drug delivery or biological sensor—and the safety when administered should be reached. Only for applications in which the physiological aspects play a less important role, it may be possible to prepare the networks considering mostly their performance as imprinted devices. This review covers the different approaches proposed to prepare MIPs useful, under in vivo conditions, for delivery of drugs and other biologically active substances, and also as diagnostic sensors or traps of undesirable substances. These approaches range from "classical imprinting", which will refer to imprinted systems prepared in organic solvents with a high cross-linked proportion, to imprinted weakly cross-linked hydrogels and even imprinted stimuli-sensitive gels.

2. MIPs as basis of drug delivery systems

2.1. Rate-programmed drug delivery

2.1.1. Classical imprinted particles as DDS excipients

MIPs prepared in organic solvents with a high cross-linker proportion, as commonly designed for analytical purposes, have been proposed as base excipients for controlled release devices of drugs with a narrow therapeutic index. These drugs present a small difference between the minimum concentration to be active and the concentration at which the side-effects advise against their use. Therefore, they have to be administered in a device able to control their release precisely, as needed, for example for the antiasthmatic drug theophylline [24]. Norell et al. [25] prepared, in chloroform, non-covalent theophylline imprinted particles (65 μm) with a view to oral administration, using the method proposed by Vlatakis et al. [26]. Theophylline-reloaded particles were able to sustain drug release in pH 7.0 phosphate buffer for several hours, especially those loaded with low amounts of theophylline (0.1–2.0 mg/g) (Fig. 1). The increase in release rate observed at greater loadings was attributed to a partial drug adsorption to non-specific binding points to which it was weakly attached. This hypothesis also explains that reference (non-imprinted) systems showed slightly faster release.

To avoid the decrease in interaction intensity between the MIPs and the ligands in water and to enhance their performance as sustaining release excipients of transdermal DDS, Allender et al. [27,28] proposed preventing water from associating with the imprinted binding site by embedding the MIP and the drug within a secondary polymer matrix made of a commercially available non-polar transdermal adhesive. The adhesive material—freely diffusible for drug molecules but relatively hydrophobic—was able to create an environment within which selective binding could occur. The transdermal devices were prepared dispersing propranolol (19.1 mg) and imprinted or non-imprinted poly-

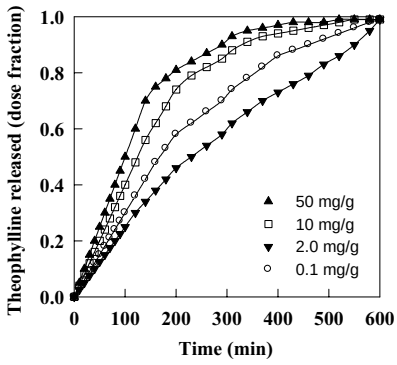

Fig. 1. Theophylline release profiles in phosphate buffer pH 7 from imprinted polymers (theophylline:MAA:EGDMA 5.22 mmol:20.9 mmol: 94.3 mmol in chloroform) loaded with different amounts of drug (reproduced from Norell et al. [25] with permission from John Wiley & Sons Ltd.).

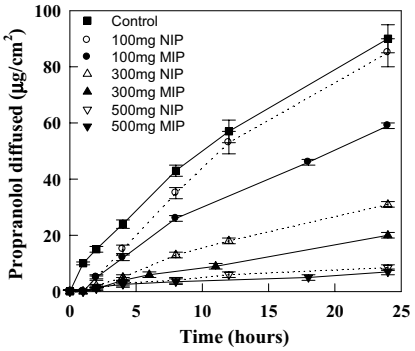

Fig. 2. Influence of polymer content and imprinting effect on propranolol release from 1-cm diameter discs constituted by imprinted (MIP) or non-imprinted (NIP) polymers (MAA:EGDMA 6 mmol:30 mmol in chloroform) embedded in a non-polar transdermal adhesive (reproduced from Allender et al. [28] with permission from Elsevier Science).

mer (100, 300, and 500 mg) in chloroform, and then mixing with the self-curing acrylic co-polymer adhesive. The viscous dispersions were left to cure overnight and then cut in 1 cm diameter discs containing 0.5 mg propranolol. Drug diffusion studies carried out in water:ethanol (50:50) mixture showed that the devices containing MIPs were able to substantially decrease the release rate, compared to the non-imprinted ones (Fig. 2). The lower diffusion rate of propranolol from devices prepared with MIPs indicates that the specific binding characteristic of these systems can provide a useful means of sustaining the delivery profile.

MIPs are also promising as enantioselective release excipients. A growing awareness of the often profoundly different pharmacokinetic—oral bioavailability, clearance, protein binding, elimination half-life—and also pharmacodynamic and toxicological profiles of the enantiomers of chiral substances has motivated recent regulatory requirements governing their use as pharmaceuticals [29]. Large differences in activity and toxicity justify the necessity of administering pure single enantiomers. However, the production of single enantiomers is still difficult and time-consuming, and enantiopure drugs may suffer racemization during pharmaceutical processing, storage, and in vivo dissolution [30]. In consequence, racemates are still being widely used. To obtain a selective release of the most active enantiomer, it has been proposed the inclusion of the racemate in DDS that also contains chiral excipients, which may preferentially interact with the less active enantiomer. Semi-synthetic or natural chiral macromolecules, such as cellulose ethers, alginates or starch, can delay the diffusion of one enantiomer with respect to the other [31,32]. However, the ability of these polymers to provide stereoselective release is quite limited and only observed when their chiral groups and those of the drug are complementary to each other.

The ability of MIPs to recognise, in an organic medium, so subtle structural differences as occur in enantiomers is well-known and valuable for use in the analytical field [33,34], although its efficiency in water seems to be more limited. Suedee et al. [35] have carried out a detailed analysis of the potential of MIPs in the enantioselective-controlled delivery, in aqueous media, of a β-blocker and two non-steroidic antiinflammatory drugs (NSAIDs), included in granules and tablets intended for oral administration. Granulation is a pharmaceutical process intended for agglomerating small powder particles to obtain greater particles with improved morphological, mechanical and drug release properties. Granules are usually intermediate products in the manufacturing of DDS, as hard gelatine capsules and tablets. Suedee et al. [35] used the enantiomers *R*-propranolol, *S*-ibuprofen, and *S*-ketoprofen as templates. Spherical polymer particles were prepared by a multistep swelling of polystyrene particles (aprox. 1 μm in diameter) in monomers microemulsion. The functional monomers were methacrylic acid (MAA) for propranolol, and 4-vinylpyridine (VPy) in the case of the NSAIDs. EGDMA was used as cross-linker. After polymerisation for 24 h at 50 °C under nitrogen atmosphere, the particles were washed and dried under vacuum. Granules, based on either imprinted or non-imprinted polymers, were prepared by mixing of racemic drug and polymer particles with ethanol solution of polyvinylpyrrolidone (PVP, 20%), passing the granules through a 1.18 mm sieve, and open air drying overnight. Drug release profiles were obtained, at 37 °C, in aqueous media of different pHs. The release of the enantiomer used as template was in all cases slower than the release of the non-print one. The granules prepared with non-imprinted polymer showed, for the three drugs, similar release rates for both enantiomers. This indicates that the MIP embedded into the granule is responsible for the enantioselectivity. Fig. 3 shows the effect of drug/polymer ratio on the release rate of *S*-ibuprofen and *R*-ibuprofen from granules prepared with *S*-ibuprofen MIPs. As the polymer proportion increased, drug release rate decreased and the enantioselectivity increased. This means that as more imprinted cavities are available, the imprint molecule finds it more difficult to escape from them and, therefore, the ability of the device to selectively release the non-imprint molecule increases.

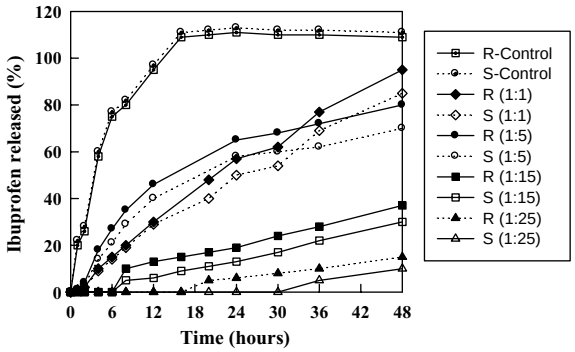

Fig. 3. Influence of drug/polymer ratios on the release profile of the *R* and *S* enantiomers of ibuprofen from granules containing racemic drug and *S*-ibuprofen imprinted polystyrene particles. The dissolution profiles of each pure enantiomer are also shown (reproduced from Suedee et al. [35] with permission from Taylor & Francis).

The enantioselectivity values, estimated as enantiomeric excess release percentage using the equation:

$$\%ee = \left[\frac{S_{isomer} - R_{isomer}}{R_{isomer} + S_{isomer}}\right] \times 100$$

in which S_{isomer} and R_{isomer} represent the amounts of each isomer released at a given time, indicate that the granulation of a mix of *S*-ibuprofen MIP and *S*-ketoprofen MIP particles (combined MIP granules) provide a higher release rate of *R*-ibuprofen and *R*-ketoprofen from the granules, and a lower release rate of *S*-ibuprofen and *S*-ketoprofen. In consequence, the combined MIP granules showed greater enantioselectivity (%ee for ibuprofen = 63.6%; %ee for ketoprofen = 13.5) than that shown by each single MIP granule (%ee for ibuprofen = 43.5; %ee for ketoprofen = 10.0).

In a previous paper, Suedee et al. [36] found that tablets prepared with imprinted polymers obtained by common bulk polymerisation, provided a faster release of the enantiomer (*R*- or *S*-propranolol) used as template. The differences in the procedure followed to obtain the MIPs may explain this apparent contradiction. In this last case, the synthesis was carried out by dissolving *R*- or *S*-propranolol (3 mmol) in chloroform (40 ml), and then adding MAA (12 mmol), EGDMA (310 mmol) and initiator (AIBN, 0.36 mmol). After polymerisation, the bulk polymer was ground to a fine powder, and washed. Tablets were prepared after wet granulation of a mixture of racemic propranolol and the polymer particles with an ethanol solution of PVP and hydrogenated vegetable oil. Those tablets obtained with imprinted particles always released the drug used as the imprint faster than the non-imprint enantiomer; the difference in release rate being dependent on the pH of the medium. The low retention of the enantiomers may be attributed to two causes: a high rate of water uptake by the matrix and an inadequate restoring of the imprinted cavities. When the tablet enters into contact with the dissolution medium, water dissolves the drug at the same time as the polymer is hydrated. If drug dissolution is faster than the time needed for the polymer to be ready to recognise the drug molecules, no delay in the release is observed. On the other hand, these MIP granules swelled considerably in water, which alters the conformation of the imprinted cavities, decreasing their affinity for the imprint molecule.

To compare the behaviour of this high swelling MIP matrix with that of low swelling MIP matrices, tablets were prepared applying a different procedure [37]. Instead of mixing all components, the tablets had a core of racemic propranolol and an outer layer of granules of MIP/PVP covered by a film of hydrogenated vegetable oil. When the shell becomes completely hydrated, the medium penetrates into the core and starts to dissolve the drug. The release of the drug starts only after this lag time when the drug can diffuse outward. The drug has to pass through the hydrated polymer before leaving the matrix, and this process may enhance any possible interaction of the enantiomer with the MIP. Therefore, the tablets initially released the non-print enantiomer, for which the polymer had a lesser affinity, faster. After some days in a phosphate buffer (pH 7.4), the differences between each enantiomer release rate decreased and even reversed (Fig. 4). This last observation is a consequence of that the final swelling to some extent of the tablets causes a distortion of the cavities specific for the template molecule, which increases the release of the imprint enantiomer. The slightly greater degree of enantioselectivity obtained with the *S*-enantiomer-imprinted matrix may be due to low experimental reproducibility.

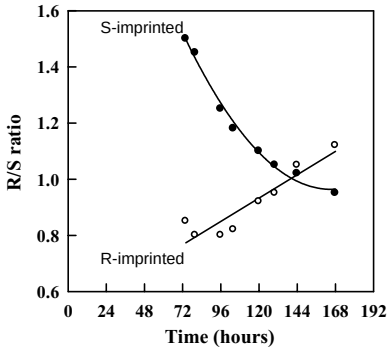

Fig. 4. Time-course of the relative percentage of *R*-propranolol and *S*-propranolol enantiomers released from tablets made of a racemic propranolol core and a low-swelling imprinted polymer shell (template:MAA:EGDMA 3 mmol:12 mmol:310 mmol in chloroform) (reproduced from Suedee et al. [37] with permission from Marcel Dekker).

Although much more work should be done to achieve an optimum enantioselectivity, the data already obtained are quite encouraging and suggest that the transition of the original polymer from the dry to the swollen state and the possible loss of conformation at the active sites, are key factors influencing the rate of drug release and selectivity of this type of MIP matrices.

2.1.2. Imprinted polymers in water as DDS

2.1.2.1. Imprinting of peptides and proteins. Relatively low molecular weight compounds are generally used as templates in molecular imprinting. The synthesis of MIPs selective to macromolecules such as proteins is mainly hindered by steric and thermodynamic reasons. Bulky protein cannot easily move in and out through the mesh of a polymer network [38], although this may be overcome by synthesising macroporous MIPs [39] or creating surface imprinting using metal(Cu^{2+})–ligand monomers [40]. On the other hand, the use of large non-rigid templates, such as polypeptides and proteins, yields less well-defined recognition sites [41]. These considerations led Rachkov and Minoura [42] to create protein-imprinted polymers using a short peptide (epitope) that represents only a part of the large protein as a template. The MIPs are intended to recognise such a portion of amino acids in any protein, as the antibodies recognise specific sequences in macro-

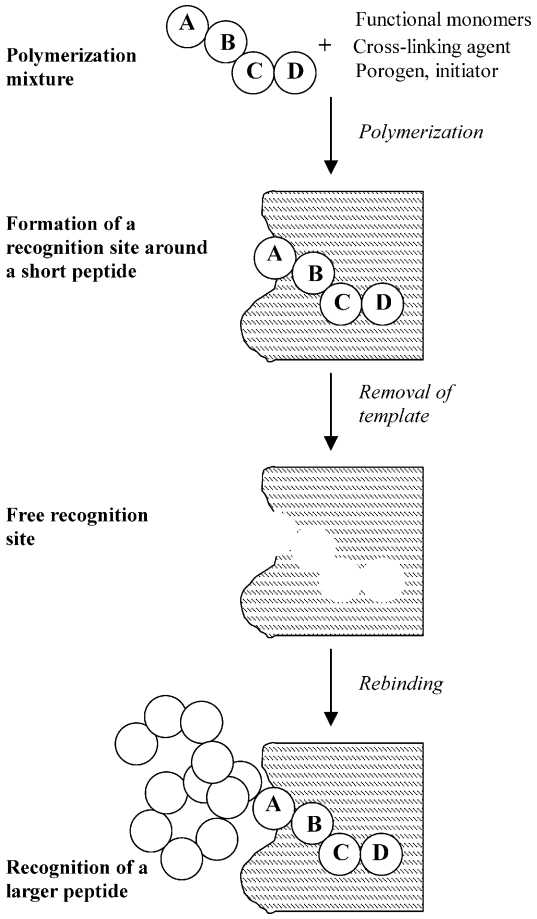

Fig. 5. Schematic draw of the imprinting process of a peptide using the epitope approach (reproduced from Rachov and Minoura [42] with permission from Elsevier Science).

molecular antigens. For example, a sequence of four amino acids (Tyr-Pro-Leu-Gly) can be chosen as the template of oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2) using methacrylic acid as the functional monomer, EGDMA as the cross-linker (monomer to cross-linker ratios ranged from 1:30 to 1:7.5), and water–acetonitrile mixture as the polymerisation medium (Fig. 5). The imprinted macroporous polymer efficiently recognised both the template and the whole protein, even in pH 6.5 aqueous medium. The high selectivity and affinity of these MIPs for peptides and proteins make them potentially useful for the development of DDS with a high loading capacity and able to control the release of these macromolecules in an adequate physiological environment.

2.1.2.2. Imprinting with cyclodextrins. Asanuma et al. [43] have developed a new molecular imprinting technique based on the ability of cyclodextrins to form inclusion complexes in water with relatively hydrophobic drugs. Cyclodextrins are non-toxic cyclic oligomers of 6–9 glucopyranose units, which present a hydrophilic outer surface and a non-polar cavity, of 5–8 Å i.d. and 7 Å depth [44]. Complex formation depends on the size of the hydrophobic region of the drug and the dimensions of the cavity, and on the intensity of the interactions between the chemical groups of both. The strategy developed by this research group consists of assembling several host cyclodextrins in a polymer network, with a spatial distribution that allows each of them to fit a designated portion of the target molecule and, at the same time, the assembly as a whole to recognise the guest—sized in a range from angstroms to nanometers—very exclusively (Fig. 6). Applying this procedure, receptors for various peptides having two or more hydrophobic residues have been successfully obtained. High affinity polymers for vancomycin and cefazolin were also created [45]. The large size and the complex structure of these antibiotics made it possible the simultaneous interaction with several cyclodextrins and, in consequence, the imprinted polymers showed a considerably greater binding constant than the non-imprinted ones ($630\,M^{-1}$ versus $240\,M^{-1}$ for vancomycin; $320\,M^{-1}$ versus $140\,M^{-1}$ for cefazolin). When a smaller antibiotic was used as template, the polymers were not able to bind the large antibiotics, which emphasizes its high specificity. The release rate of the drug from the cyclodextrin–MIPs is expected to be mainly regulated by the dissociation constant of the host-guest complex [44]. The stronger the association, the slower the release rate. These results make it foreseeable that imprinted systems with cyclodextrins are particularly useful for the development of DDS of peptides and hydrophobic drugs. The combination of cyclodextrins and ionic monomers has been proposed by Piletsky et al. [22] to enhance the affinity to amphiphilic molecules containing hydrophobic and ionic groups.

2.1.3. Weakly cross-linked MIPs prepared without solvents as drug delivery soft contact lenses

Some drugs may be directly dissolved in the monomers mixture, without using additional solvents. Sreenivasan [46] demonstrated the feasibility of using imprinted polyhydroxyethylmethacrylate (HEMA, 3 ml) cross-linked with EGDMA (3.5 ml) as hydrocortisone (250 mg) sustained-release materials. The quantity of hydrocortisone reloaded by the MIP (9.20 mg/g) was almost 40 times greater than the amount loaded by the non-imprinted polymer (0.23 mg/g). Additionally, this last system released, in methanol/water solutions, all the loaded drug in less than 1 day, while the release from the MIP was considerably slower. The affinity for the drug was too high to be easily broken and after one month there was still nearly 96% of hydrocortisone inside the MIP. This over-enhanced affinity might cause the release of an insufficient fraction of dose of the drug included in the system, limiting its therapeutic utility. Nevertheless, imprinted HEMA networks without using solvents open interesting possibilities for the development of DDS since HEMA is a widely used substance for preparing biocompatible hydrogels, such as those intended for implants and contact lenses [47].

Recently, our group has developed an imprinted technique to create soft contact lenses able to load and re-

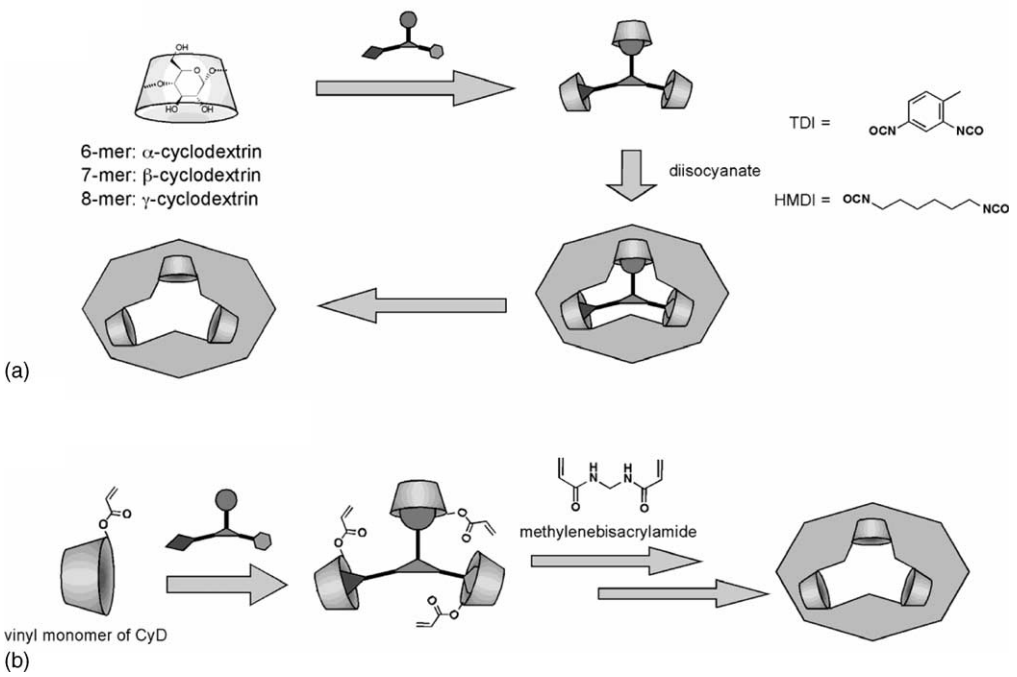

Fig. 6. Molecular imprinting of cyclodextrins as receptors of nanometer-scaled templates. (a) The cyclodextrin is cross-linked by diisocyanate, in dimethylsulfoxide, in the presence of the template. (b) The vinyl monomer of cyclodextrin is copolymerised with methylenebisacrylamide, in water, in the presence of the template (reproduced from Asanuma et al. [43] with permission from Wiley–VCH Verlag).

lease drugs in a controlled way for the treatment of ocular pathologies [48,49]. Conventional ophthalmic formulations generally show low ocular bioavailability because of protective mechanisms, such as tear drainage and blinking. Although the efficacy of drops and ointments can be improved by viscosity enhancers, *in situ* gelling systems and mucoadhesive polymers, the time of permanence of drug on the eye is still quite limited [50]. Medicated contact lenses may be particularly useful for increasing drug bioavailability while, at the same time, if the patient requires it, they can correct impaired vision [51,52]. The feasibility of using drug-loaded soft contact lenses depends on whether drug and contact lens material can be matched so that the lens uptakes a sufficient quantity of drug and releases it in a controlled fashion. In general, drug loading capacity of conventional soft contact lenses is insufficient and, therefore, they have rarely been employed for ophthalmic drug delivery [52,53]. To overcome this drawback, the application of the molecular imprinting technology in the design of soft contact lenses has been shown to be particularly useful. Timolol maleate, a drug used in glaucoma therapy, is a suitable molecule to provide imprinted systems since it offers multiple sites for interaction with the functional monomer. To prepare timolol-imprinted soft-contact lenses, a small proportion of methacrylic acid (MAA) was added as a functional monomer able to interact through ionic and hydrogen bonds with timolol maleate. Imprinted HEMA-based and *N*,*N*-diethylacrylamide (DEAA)-based lenses, synthesised with different proportions of MAA (1.28–5.12 mol%) and cross-linker EGDMA (0.32–8.34 mol%), took up more timolol than the corresponding non-imprinted systems (Fig. 7). The lenses prepared with the lowest MAA proportion and the highest cross-linking degree evaluated were able to load therapeutically useful amounts of timolol, to sustain drug release in lachrymal fluid for more than 12 h (Fig. 8), and to reload timolol overnight, being ready to use the next day [48,49,54].

Due to the requirements of optical clarity, flexibility and oxygen permeability, the main composition of soft contact lenses is restricted to some approved monomers that differ in water affinity and hydrogen-bonding capacity [47].

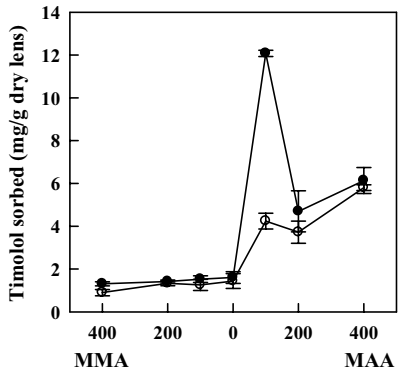

Fig. 7. Timolol uptake from water by imprinted (solid symbols) and non-imprinted (open symbols) cross-linked hydrogels made of HEMA copolymerised with different amounts (in mM) of methylmethacrylate (MMA) and methacrylic acid (MAA) as functional monomers (reproduced from Alvarez-Lorenzo et al. [48] with permission from John Wiley & Sons Ltd.).

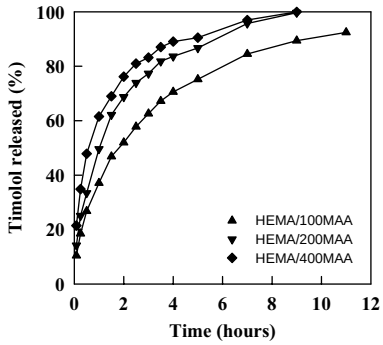

Fig. 8. Timolol release profiles, in artificial lachrymal fluid at 37 °C, from re-loaded imprinted hydrogels made of HEMA copolymerised with different amounts (in mM) of methacrylic acid (MAA) (reproduced from Alvarez-Lorenzo et al. [48] with permission from John Wiley & Sons Ltd.).

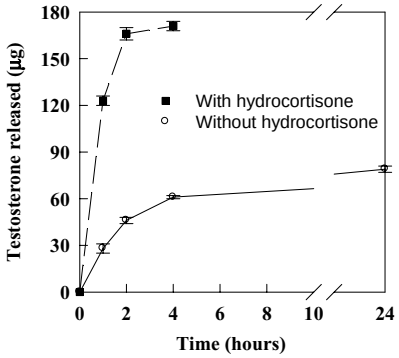

Fig. 9. Effect of the presence of hydrocortisone in the aqueous medium (50 mg/l) on testosterone release rate. The initial load of testosterone was 175 μg in 100 mg of hydrocortisone-imprinted polymers (HEMA:EGDMA 1 g:4 g) (data taken from Sreenivasan [56]).

Furthermore, the proportions of functional monomer and cross-linking agent have to be relatively low and, in consequence, the physical stability of the binding site is a main concern. Therefore, to try to generalise the applicability of the molecular imprinting technology to manufacture therapeutic contact lenses, the influence of the backbone monomers on the achievement of the imprinting effect, keeping the proportions of the functional monomer and cross-linker constant, is currently analysed in our laboratory [55].

2.2. Activation-modulated drug delivery

In this section, we comment on a series of systems potentially useful to develop DDS in which the release occurs as a response to a change in the conditions of the environment, which affects the binding of the drug directly (competitive binding or hydrolysis of the bounds) or through a change in the swelling state of the polymer (volume phase transition induced by a external stimulus).

2.2.1. Competitive binding

An activation-modulated delivery may be achieved with an imprinted gel that releases the drug because of the competitive binding to the polymer of another substance in the solution. The network includes a non-imprint drug and, when the imprint molecule appears in the surrounding medium, the network binds it and releases the drug. If the concentration of the imprint substance decreases, the release stops. A system of these characteristics able to release testosterone at a rate depending on the concentration of hydrocortisone in the medium was described by Sreenivasan [56]. HEMA (1 g) cross-linked with EGDMA (4 g) and imprinted for hydrocortisone (100 mg) in chloroform (6–8 ml) absorbed, after removing the imprint, a considerable amount of testosterone (175 μg/100 mg MIP versus 36 μg/100 mg control polymer). The release of the loaded drug to the aqueous medium was considerably enhanced in the presence of the template molecule (hydrocortisone) (Fig. 9).

Other competitive binding experiments, in aqueous environment -between bupivacaine and other local anaesthetic drugs [57], and between theophylline and other methylxantines (caffeine, theobromine) and 17-β-estradiol and very close structurally related sterols (17-α-estradiol, 17-α-ethynylestradiol) [58]—have shown the ease with which a non-imprint specie bound to imprinted polymer particles may be replaced by the template molecule.

2.2.2. Hydrolytically-induced drug release

A particularly useful approach to modulate drug delivery consists of creating erodible systems from which the drug cannot be released unless the polymer degrades or polymer–drug bonds are broken. The external conditions that can induce these processes are, usually, extreme physiological pH or the catalytic activity of certain enzymes. For example, drugs that are unstable under the gastric conditions may be selectively released in the colon by preparing DDS with polymers that serve as substrates of the enzymes of this intestinal region or that degrade at slightly alkaline pH environments. In other cases, the rate of hydrolysis of the drug linkage to the polymer network controls the release rate. Ester bonds usually need alkaline pHs much stronger (pH 10–11) than those found in the physiological environment to be broken. Additionally, the presence of electron donating groups in the drug molecule (*p*-metoxy or *p*-amino) considerably suppresses the rate of hydrolysis [59].

To enhance the hydrolysis of polymer–drug ester or amide bonds under mild pH conditions, Karmalkar et al. [60] proposed incorporating imidazole groups (nucleophilic catalyst) near the drug linkage using a molecular imprinting technique. The hydrogels, designed for the release of *p*-amino benzoic acid, were prepared dissolving HEMA, *N*-vinylimidazole (NVIm) and 2-methacryloylethyl *p*-aminobenzoate (PAP) in methanol, in the proportions indicated in Table 2. Imprinted hydrogels were only obtained when PAP and NVIm were previously mixed together with Co^{2+} ions. The metallic ions bring together both monomers forming a co-ordination complex, as shown in Fig. 10.

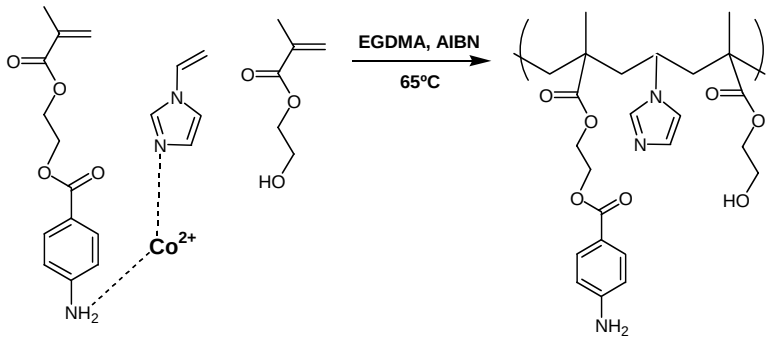

Fig. 10. Synthetic route proposed by Karmalkar et al. [60] for creating imprinted cavities in which the imidazole catalytic group is positioned close to the drug-polymer bound. The procedure consisted on the pre-organisation of the monomeric drug with a metal-complexing monomer and subsequent polymerisation.

Table 2
Amounts (g) of monomers used to synthesise hydrogels capable of selective release *p*-amino benzoic acid, in slightly alkaline pH medium, by a hydrolytically induced mechanism

Polymer	HEMA	PAP	NVIm	$CoCl_2 \cdot 6H_2O$
PAP-1	4.22	0.5	0.17	0.106
PAP-2	4.30	0.5	0.17	0
PAP-3	4.50	0.5	0	0

To enhance the release rate, imprinted cavities containing imidazole groups and the monomeric drug were created using Co^{2+} co-ordination interactions [60].

Polymerisation of such a complex and subsequent removal of the metal ion would lead to polymers having the labile bond and imidazole located in contiguous positions on the same chain. In ethanol–phosphate buffer pH 8 medium, the release of *p*-amino benzoic acid from the imprinted system (PAP-1) was considerably easier (first order rate constant 39.8×10^{-3} per day) than from the other two gels (PAP-2: 8.1×10^{-3} per day; PAP-3: 6.1×10^{-3} per day) and even faster than from PAP-3 gels in 0.01 N NaOH (pH 11). The cross-linking was essential for the catalytic activity of the hydrogel; the release rates from linear imprinted and non-imprinted polymers being similar.

To bring into close proximity imidazol and drugs that cannot form a complex with the metal ion, another technique was developed [60,61]. The polymerisation method, based on that previously proposed by Leonhardt and Mosbach [62], consisted of using template molecules of similar size and structure to the drug, but that can form complexes with the metal ions. After polymerisation and removal of the template, the drug was loaded into the hydrogel and immobilised by polymerisation. Since the cavity contained an imidazol group, the release rate was considerably enhanced compared to control hydrogels. These polymers also had the ability to switch on and off the release in response to a change in pH from 3 to 6.8 [61]. This is because the catalytic activity is lost at pH below 3.5 and, in consequence, the release does not occur while the system is kept in that environment. The switch on and off effect might also be achieved by a change in the distance between the imidazole group and the substrate molecule, caused by a temperature-induced polymer swelling, as reported by Wang et al. [63] for related systems.

With a view to their use as bases of DDS, the polymers described above have a considerable interest for targeting drugs, when orally administered, to specific regions of the gastrointestinal tract.

2.2.3. Stimuli sensitive imprinted hydrogels

Polymer gels that modify their structure and, in consequence, their properties in response to changes in the physicochemical characteristics of the physiological medium are very promising candidates to achieve an optimum control of the moment and rate of drug release [64,65]. In consequence, stimuli-sensitive polymer hydrogels that experiment reversible volume phase transitions have attracted special attention. An enormous volume change can be triggered by small changes in temperature, pH, solvent composition, ionic strength, electric field, light or the presence of specific molecules [66]. Poly(*N*-isopropylacrylamide) (NIPA) hydrogel is one of the most widely studied systems that undergo a temperature-controlled volume phase transition. The gel is swollen at temperatures lower than 33 °C and collapses when the temperature is raised [66,67].

The combination of stimuli-sensitivity and imprinting may have considerable practical advantages: the imprinting provides a high loading capacity of specific molecules, while the ability to respond to external stimuli contributes to modulate the affinity of the network for the target molecules, providing regulatory or switching capability of the loading/release processes. From a theoretical point of view, it is also interesting to study the ability of the polymer network to memorise a specific conformation after a dramatic change in swelling degree. In this sense, Watanabe et al. [68] observed that NIPA (16 mmol)–acrylic acid (4 mmol) cross-linked (1 mmol) polymers synthesised in the presence of norephedrine hydrochloride or adrenaline hydrochloride showed, after template removal and in the collapsed state, an increase in swelling ratio with increasing imprint molecule concentration in water. Curiously, this template-induced swelling was observed only for the

polymers prepared in dioxane but not for those prepared in water.

Tanaka and co-workers [69–72] proposed the creation of stimuli sensitive gels able to recognise 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, and the other allows the polymers to swell and shrink reversibly in response to environmental changes. The gel is synthesised in the collapsed state and, after polymerisation, washed in a swelling medium. The imprinted cavities develop affinity for the template molecules when the functional monomers come into proximity, but when they are separated, the affinity diminishes. The proximity is controlled by the reversible phase transition of the gel that consequently controls the adsorption/release of the template (Fig. 11).

In order to obtain a system with the capacity to recognise calcium ions, imprinted and non-imprinted copolymers of *N*-isopropylacrylamide (NIPA) and different methacrylic monomers with carboxyl groups, which form complexes with divalent ions in the relation 2:1, were prepared. The effect of temperature on the adsorption capacity of the imprinted copolymers prepared with different templates and in different organic solvents was compared with that of the non-imprinted ones. Successful imprinting was obtained with NIPA-lead dimethacrylate monomers in dioxane. After washing lead out and swelling in water at room temperature, the affinity for divalent ions disappeared. When the gels were shrunken by an increase in temperature, the affinity was recovered and the original relative position of the carboxylic groups was recalled. Control gels made using randomly distributed MAA experienced difficulty in forming pairs ("frustration") and their affinity for divalent ions decreased exponentially as a function of cross-linker concentration (Fig. 12). In contrast, the topological constraints were completely absent in the imprinted gels, showing that memorisation had been achieved. The success of the imprinting can be attributed to the fact that the degree of dissociation

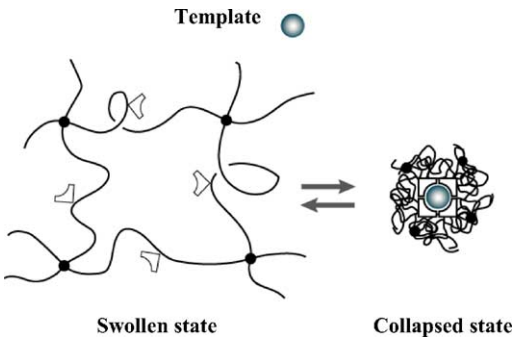

Fig. 11. Diagram of the recognition process of a template by a stimuli sensitive imprinted hydrogel as proposed by Tanaka et al. [69]. The volume phase transition of the hydrogel -induced by an external stimuli such as a change in pH, temperature or electrical field—modify the relative distance of the functional groups inside the imprinted cavities. This alters their affinity for the template.

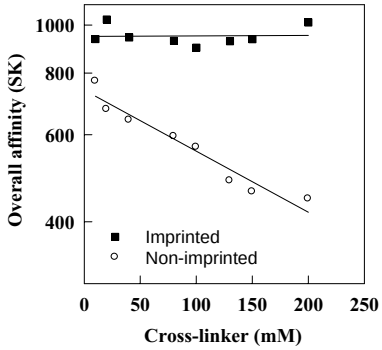

Fig. 12. Influence of the cross-linker (methylenebis-(acrylamide)) proportion on the overall affinity for calcium ions of the imprinted and non-imprinted NIPA (6 M) gels in the shrunken state in water. The amount of functional monomers (MAA) was fixed at 32 mM (reproduced from Alvarez-Lorenzo et al. [70] with permission from American Chemical Society).

between lead and two methacrylate molecules during polymerisation is negligible, and therefore lead is responsible for fixing the juxtaposition of pairs of methacrylates [70,71].

These stimuli-sensitive imprinted gels are very weakly cross-linked (>2 mol%) systems and, therefore, the success of the imprinting strongly depends on the stability of the complexes template/functional monomers during polymerisation and in the aqueous medium. However if the interaction in water is too strong, it may be difficult to remove the template completely after polymerisation to obtain the pure gel. To circumvent some of these drawbacks, functional monomers directly bonded to each other prior to polymerisation, which we call "imprinters", were used [73,74]. An imprinter is a molecule that has three functional parts, two or more polymerisable double bonds, two or more functional groups and a link connecting the functional groups that is easily cleaved afterwards, such as a disulphide bond or a 1,2-glycol structure. The functional groups can be separated after polymerisation to obtain pairs of ionic groups with the same charge. Since the members of each pair are close in the space, they can capture target molecules through multiple-point ionic interactions (Fig. 13). An imprinted hydrogel for cationic divalent ions was obtained, without template, using a monomer with a disulphide bond that, after polymerisation, was oxidised and transformed in two sulfonic groups in contact each other. Thus, the hydrogel was suitable for the binding/release of calcium ions more efficiently than the gels prepared with randomly distributed sulfonic groups [73].

Additionally, NIPA gels were prepared with a new monomer that carries two quaternary ammonium groups linked by a 1,2-glycol bound, which was broken after polymerisation [74,75]. Since a well-defined distance separates the members of each pair, they can capture specific molecules containing two carboxylic groups. The presence of two acidic groups is frequent in drug substances such as antibiotics, glucocorticoids, or colinergic drugs. The adsorption of disodium 5-nitroisophthalate (DPA) and dipotassium

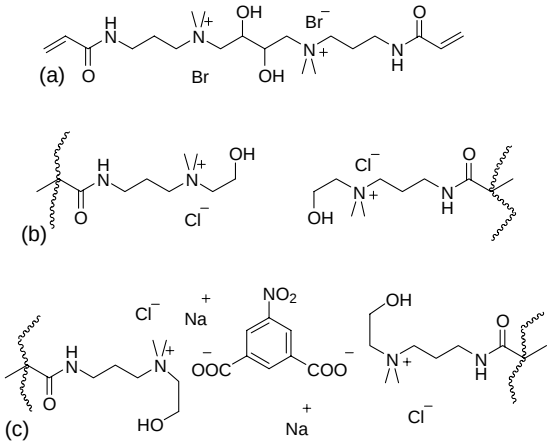

Fig. 13. Structure of Imprinter-Q monomer (a) and of the binding sites of the gel made of Imprinted-Q after breakage of the 1,2-glycol bond (b), and schematic representation of the capture of a target molecule (c) (reproduced from Moritani and Alvarez-Lorenzo [74] with permission from American Chemical Society).

2,6-naphthalenedicarboxylate was considerably greater than that of sodium benzoate, which only carries an anionic group. When the dry DPA-loaded gels were immersed in water at temperature below the phase transition of the gel (20 °C), a fast hydration and swelling took place during which the gels quickly released DPA until equilibrium was reached [74]. Since the adsorption behaviour of the gels follows the Langmuir model and the amount of DPA loaded in each gel depends on the proportion in cationic groups, the percentage of DPA released decreases as the proportion of cationic groups in the hydrogel increases. It is interesting to notice that when the temperature increased above a certain value, the gels were able to re-adsorb a significantly high amount of the DPA previously released (Fig. 14).

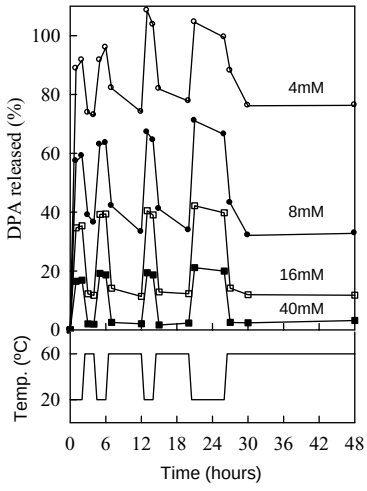

Fig. 14. Influence of temperature on the release and re-adsorption of disodium 5-nitroisophthalate (DPA) in water by imprinted NIPA (6 M) gels prepared with different concentrations of Imprinter-Q. Cross-linker concentration was 40 mM. Degrees of swelling at 20 and 60 °C were 6.0–6.5 and 0.9, respectively (reproduced from Moritani and Alvarez-Lorenzo [74] with permission from American Chemical Society).

This effect is not observed in common stimuli-sensitive gels. Temperature responsiveness of NIPA gels has been proposed by several authors to obtain an oscillating release behaviour, consisting of allowing a substance entrapped in the polymer network to diffuse out of the gel in the swollen state, but stopping the release when the temperature increases and the network collapses [64,65]. In our gels, thanks to the affinity of the receptor groups for DPA, a change from the swollen to the shrunken state induces, not only the cessation of the release, but also the promotion of a re-adsorption process. This process occurs quickly and in a way that can be reproduced after several temperature cycles. The non-imprinted gels presented swelling/collapse behaviour similar to the Imprinter-Q gels, however since their affinity for DPA is smaller, shrunken non-imprinted gels could not re-absorb as much as the Imprinter-Q gels. In physiological conditions, in which temperature slightly oscillates around 37 °C, the release process mainly depends on the pH and ionic strength of the medium, which alter the strength of the ionic interactions [76,77].

The stimuli-sensitive imprinted systems have a great potential in drug delivery. The reversibility of drug release and re-uptake as a function of temperature may be useful for the treatment of some pathological events that are accompanied by local changes in temperature, or to stop delivery of drug, by externally applied local hyperthermia, when the dose released from hydrogels accumulated in an organ is enough.

2.3. Feedback-regulated DDS

Frequently, the levels of some physiological substances are used as direct indicators of the degree of dysfunction of a certain organ. The availability of systems capable of selective recognition of these substances is an essential step to create feedback-regulated DDS able to modulate drug release as a function of the level of these substances in the body [78].

Most work carried out until now has been focused on glucose-regulated insulin delivery systems, using non-imprinted systems. However, since recently, the obtaining of glucose-imprinting has been tackling the issue from quite different approaches. The prevalence of diabetes in developed countries is quite considerable [79]. Therefore, from a practical point of view, simple methods of quantifying blood glucose concentration are required and, consequently, some MIPs have been designed as sensors in this field. Arnold et al. [80] developed a glucose sensing device based on α-methyl-D-glucoside imprinted polymer, applying metal co-ordination, that exhibits a change in pH proportional to the glucose concentration of its environment. This procedure was later improved by Striegler [23,81,82] for enhancing glucose binding at $5.5 < \text{pH} < 7.5$. New functional monomers such as [4-(*N*-vinylbenzyl)diethylenetriamine) copper(II)] diformate or [(diethylenetriamine) copper(II)] dinitrate, which can form 1:1 complexes with carbohydrates in this range of pH, were synthesised (Fig. 15). Although

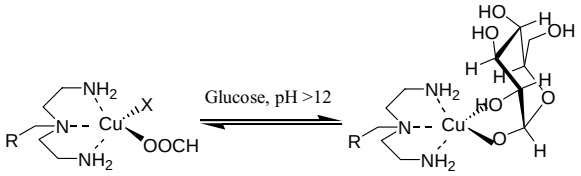

Fig. 15. Pre-organisation of [(4-(*N*-vinylbenzyl)diethylenetriamine) copper(II)] diformate and a carbohydrate template prior to polymerisation at alkaline pH to obtain glucose sensors (reprinted from Striegler [81] with permission from Elsevier).

the ligand–Cu^{2+}–glucose apparent binding constant is lower at physiological conditions (pH 7.4) than at the pH used for polymerisation (12.4), the imprinted systems were still much more efficient than the control ones and showed a high selectivity over other 1,2-*cis*-diols, namely mannose and galactose (Fig. 16) [81]. Adequate imprinted networks even allowed selective discrimination of α- and β-glycosidic linkages of cellobiose and maltose [82]. Finally, to raise the overall loading amount of carbohydrates, cross-linkers and monomers that may establish hydrogen-bonding interactions were also included. As the matrix polarity increased, the polymer preference for the large and polar disaccharide lactose also rose, in detriment of the less polar monosaccharide glucose [83]. Thus, depending on the specific carbohydrate to be recognised, it maybe practical to use polar or non-polar cross-linking monomers. In this way, Mayes et al. [83] and Oral and Peppas [84] demonstrated the feasibility of hydrogen bonding interactions between carbohydrates and methacrylic monomers, to produce high affinity binding polymer networks. These sensors may serve as a basis for the obtaining of glucose-responsive DDS.

On the other hand, Kataoka et al. [85] observed that it is possible to regulate insulin release from a glucose-responsive gel incorporating 3-acrylamidophenylboronic acid as functional monomer. The apparent pK_a of phenylboronic acid is lowered from 8.9 to 7.9 when interact with hydroxyl groups of glucose (Fig. 17). In consequence, at pH 9 and 28 °C, the solubility of the polymer is abruptly

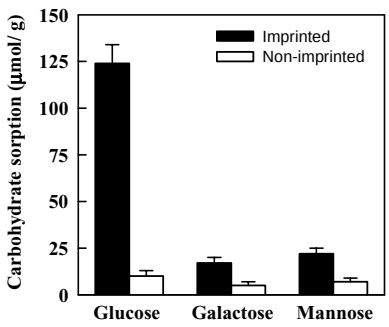

Fig. 16. Rebinding capability of glucose-imprinted and non-imprinted polymers for glucose, galactose and mannose in water. The polymers consisted of [(4-(*N*-vinylbenzyl)diethylenetriamine) copper(II)] diformate (5 mol%) cross-linked with pentaerythritol tetraacrylate (95 mol%) (reprinted from Striegler [81] with permission from Elsevier).

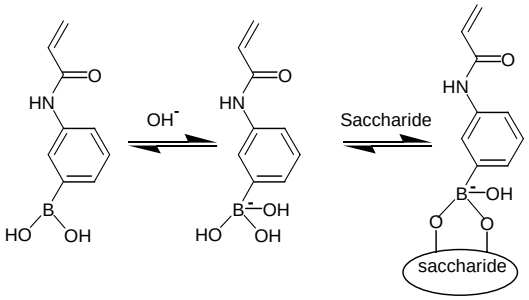

Fig. 17. Reversible covalent binding of glucose to phenylboronic acid in alkaline medium (reproduced from Kataoka et al. [85] with permission from American Chemical Society).

increased when glucose concentration in the medium rises. This provokes a rapid release of insulin that is effectively shut off by decreasing glucose concentration below a critical value. Although this initial polymer system was no imprinted, several authors have shown that sugar imprinting of boronic groups in polymer networks is achievable. Imprinted systems prepared using the reversible formation of boronate esters between pairs of hydroxyl groups of the sugar molecule and *p*-vinylphenylboronic acid, may even distinguish racemates of glycosides and free carbohydrates [86]. Neighbouring amino functionalities in the boronic acids greatly enhance the formation of boronic acid–sugar complexes, making it possible to create carbohydrate sensors at neutral pHs [87]. Therefore, the application of the molecular imprinting technology to the development of this type of systems could contribute to make them more selective, improving their performance significantly.

3. MIPs as trap systems

There is a very long list of substances included in the food that may be related to the apparition/worsening of certain illness (e.g., sugars, hormones, cholesterol, some amino acids). Cholesterol is receiving increasing attention as a template to create sequestrant devices, owing to the influence of high blood cholesterol levels on the onset of atherosclerosis and myocardial infarction. The systems able to bind such substances in the gastrointestinal tract, hindering their absorption by the body, may be used in stead of or as a complement of the pharmacological therapy, for the efficient treatment of these illnesses. The information obtained in the development of these systems might also serve as a base for the design of *feedback*-regulated DDS. This section includes some examples of applications of MIPs as traps.

3.1. Glucose traps

Starting from poly(allylamine hydrochloride), a commercially available polymer (MW 15,000 Da), and glucose phosphate monosodium salt, Wizeman and Kofinas [88] prepared a non-covalent imprinted network by ionic association, with

Table 3
Sugar binding by cross-linked imprinted with glucose phosphate monosodium salt (1%) and non-imprinted poly(allylamine hydrochloride) hydrogels [88]

Cross-linker	Imprinting	Glucose binding (g/g)	Fructose binding (g/g)
Epichlorohydrin	Yes	0.54 (0.03)	0
	No	0.20 (0.01)	0
Ethyleneglycol diglycidylether	Yes	0.53 (0.03)	0.21 (0.03)
	No	0.18 (0.01)	0.11 (0.02)
Glycerol diglycidylether	Yes	0.34 (0.06)	0.18 (0.03)
	No	0.13 (0.01)	0.05 (0.03)

Mean values (S.D.).

the aim of binding glucose in the stomach and small intestine and, then, passing unabsorbed through the body. The networks were prepared with three different cross-linkers using, for both the synthesis and testing, aqueous solutions under air. Dried polymers added to a 50 mg/ml aqueous solution of either glucose or fructose, showed very different binding capability depending on the cross-linker used. The higher degree of specificity for glucose was achieved with the smallest molecular size cross-linker, epichlorohydrin, after 4 h of immersion in the sugar solutions (Table 3). The MIP system would simply be ingested along with foods that are high in glucose with the aim of reducing the sharp rise in blood sugar associated with the ingestion of significant amounts of monosaccharides. This MIP system may also have applications in the preparation of a MIP glucose sensor.

3.2. Cholesterol traps

A comprehensive review about MIPs for steroids and, in particular, for cholesterol has been carried out by Davidson and Hayes [89]. Polymer networks containing imprinted cyclodextrins were found particularly useful to enhance their capability to form inclusion complexes with cholesterol [90]. Free cyclodextrins are commonly used to precipitate cholesterol from concentrated solutions. However, the yield of the reaction is limited by the fact that cholesterol is too large to be accommodated within the cavity of a single β-cyclodextrin. To be effectively bound, two or more β-cyclodextrins must interact simultaneously with one cholesterol molecule. Komiyama et al. [43] prepared polymer networks that hold groups of two cyclodextrins at the adequate distance to complex cholesterol, by cross-linking vinyl monomers of β-cyclodextrins with hexamethylene diisocyanate or toluene-2,4-diisocyanate in dimethylsulfoxide in the presence of cholesterol (see Fig. 6). The cholesterol–MIP systems showed strong and selective rebinding of the substrate in the aqueous medium; the hydroxyl group at the 3-position and the alkyl chain at the 17-position playing an important role in the recognition.

Also taking advantage of van der Waals interactions, Sellergren et al. [91] obtained cholesterol–MIPs using amphiphilic monomers under conditions favouring apolar association between the monomers and the template. These amphiphilic monomers were polymerisable derivatives of cholesterol or bile acids (3 mmol), which were mixed with EGDMA (30 mmol) and MAA (6 mmol) and dissolved in ethanol. To obtain an imprinting effect, cholesterol (1.5 mmol) was added before polymerisation. In intestine-mimicking medium, cholesterol-imprinted polymers exhibited a considerably greater adsorption capacity (ca. 45 μmol/g) than the non-imprinted ones (ca. 33 μmol/g). Zhong et al. [92] observed that combining the use of polymerisable cholesterol and cyclodextrins, the MIPs take up cholesterol more efficiently. Non-covalent imprinting using conventional monomers, such as methacrylic acid or 4-vinylpyridine, usually results in lower binding affinity [93].

3.3. Other molecular traps

Another way to decrease the dietary uptake of cholesterol consists of using imprinted polymers for bile acid salts, which participate in its emulsification in the gastrointestinal tract, prior to its absorption. A strong decrease in bile acid salts concentration was achieved with deoxycholic acid-imprinted polymers using *N*,*N*′-diethyl(4-vinylphenyl)amide as functional monomer [94] or cholate-imprinted poly(allylammonium chloride) systems [95].

Although with a lower interest in the therapeutic field, MIPs useful as caffeine traps have been also developed using cross-linker (divinylbenzene) and functional monomers, to which caffeine is adsorbed through hydrophobic π–π interactions [96].

4. A view to the future

Despite the already developed interesting applications of MIPs, commented in the previous sections of this paper, the incorporation of the molecular imprinting approach for the development of DDS is just at its incipient stage. Nevertheless, it can be foreseen that in the next few years significant progress will occur in this field, taking advantage of the improvements of this technology in other areas. Among the evolution lines that should contribute more to enhance the applicability of imprinting for drug delivery, the application of predictive tools for a rational design of imprinted systems and the development of molecular imprinting in water may be highlighted.

The optimisation, using predictive tools, of the nature and amount of functional monomers should overcome the time and material consuming method of “trial and error” and improve the specificity and the affinity of the template [97]. This may be achieved with the use of rapid experimental screening processes [98], combinatorial libraries [99] and with the application of isothermal titration calorimetry anal-

ysis, which has been proven to be a suitable method to investigate the thermodynamics of molecular recognition and to assess the efficiency of the molecular imprinting process [100]. A biomolecular binding database, to include binding affinities for proteins, nucleic acids, carbohydrates, drugs, and synthetic host and guest molecules obtained from literature, has been developed by Gilson et al. [101] and it is still in a growing process. Additionally, new ways of tackling the challenge of preparing MIPs that work efficiently in aqueous solutions are required. Although MIPs made in organic solvents can often recognise the template in water, much more work should be devoted towards making good imprints in aqueous media. This may open the possibility of obtaining imprinted systems for very labile molecules such as some peptide drugs and oligonucleotides, making it possible even to use them in gene therapy [102]. For example, MIPs able to recognise specific compounds of cell surface, especially oligosaccharides or lectins, may be suitable for targeting the DDS to specific tissues or cells, increasing the residence time at the absorption site and providing an intimate contact to the absorptive tissue. Lectin-imprinted polymers have recently been prepared in aqueous solutions using the ability of this glycoprotein to reversibly bind certain saccharidic functional monomers [103]. The affinity of each lectin for specific mucosal surfaces of the gastrointestinal, respiratory, and urogenital tract may serve as a basis for the development of lectin-mediated drug delivery systems of poorly absorbable drugs by opening endocytic pathways [104]. The advances in the preparation of MIPs as spherical particles [58] and films [105] should also enhance the possibilities of the application of these polymers in drug delivery.

Acknowledgements

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References

[1] M.J. Rathbone, J. Hadgraft, M.S. Roberts (Eds.), Modified-Release Drug Delivery Technology, Marcel Dekker, New York, 2003.
[2] Y.W. Chien, S. Lin, Clin. Pharmacokinet. 41 (2002) 1267.
[3] C.J. Allender, K.R. Brain, C.M. Heard, in: F.D. King, A.W. Oxford (Eds.), Progress in Medicinal Chemistry, vol. 36, Elsevier, Amsterdam, 1999, p. 235.
[4] L.I. Andersson, R. Müller, G. Vlatakis, K. Mosbach, Proc. Nat. Acad. Sci. U.S.A. 92 (1995) 4788.
[5] N.A. Peppas, Y. Huang, Pharm. Res. 19 (2002) 578.
[6] S. Striegler, Current Organic Chem. 7 (2003) 81.
[7] O. Ramström, R. Ansell, Chirality 10 (1998) 195.
[8] K. Mosbach, O. Ramström, Biotechnol. 14 (1996) 163.
[9] P.K. Owens, L. Karlsson, E.S.M. Lutz, L.I. Andersson, Trends Anal. Chem. 18 (1999) 146.
[10] L.I. Andersson, J. Chromatogr. B 739 (2000) 163.
[11] P.D. Martin, G.R. Jones, F. Stringer, I.D. Wilson, Analyst 128 (2003) 345.
[12] B.B. Prasad, S. Banerjee, React. Funct. Polymers 55 (2003) 159.
[13] G. Wulff, Angew. Chem., Int. Ed. Engl. 34 (1995) 1812.
[14] J. Svenson, I.A. Nicholls, Anal. Chim. Acta 435 (2001) 19.
[15] D.M. Yourtee, R.E. Smith, K.A. Russo, S. Burmaster, J.M. Cannon, J.D. Eick, E.L. Kostoryz, J. Biomed. Mat. Res. 57 (2001) 522.
[16] O. Aydin, G. Attila, A. Dogan, M.V. Aydin, N. Canacankatan, A. Kanik, Toxicol. Pathol. 30 (2002) 350.
[17] M.Z. Fiume, Int. J. Toxicol. 21 (Suppl. 3) (2002) 1.
[18] J.M. Anderson, Eur. J. Pharm. Biopharm. 40 (1994) 1.
[19] K.A. Davis, K.S. Anseth, Crit. Rev. Ther. Drug Carrier Syst. 19 (2002) 385.
[20] W.E. Hennink, C.F. van Nostrum, Adv. Drug Del. Rev. 54 (2002) 13.
[21] M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanuma, Molecular Imprinting, Wiley–VCH, Weinheim, 2003, p. 119.
[22] S.A. Piletsky, H.S. Andersson, I.A. Nicholls, Macromolecules 32 (1999) 633.
[23] S. Striegler, Macromolecules 36 (2003) 1310.
[24] S. Al-Habet, E.D. Bashaw, L. Lesko, R. Williams, J. Balian, Clin. Pharmacol. Therap. 67 (2000) PIII23.
[25] M.C. Norell, H.S. Andersson, I.A. Nicholls, J. Mol. Recog. 11 (1998) 98.
[26] G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, Nature 361 (1993) 645.
[27] C.J. Allender, K.R. Brain, A.C. Watkinson, C.M. Heard, in: K.R. Brain, V.J. James, K.A. Walters (Eds.), Perspectives in Percutaneous Penetration, vol. 5b, STS Publishing, Cardiff, 1997, p. 183.
[28] C.J. Allender, C. Richardson, B. Woodhouse, C.M. Heard, K.R. Brain, Int. J. Pharm. 195 (2000) 39.
[29] N.R. Srinivas, R.H. Barbhaiya, K.K. Midha, J. Pharm. Sci. 90 (2001) 1205.
[30] M. Reist, B. Testa, P.A. Carrupt, Enantiomer 2 (1997) 147.
[31] C. Alvarez, J.J. Torrado, R. Cadórniga, Chirality 11 (1999) 611.
[32] M.A. Solinís, Y. De la Cruz, R.M. Hernández, A.R. Gascón, B. Calvo, J.L. Pedraz, Int. J. Pharm. 239 (2002) 61.
[33] B. Sellergren, K.J. Shea, J. Chromatogr. 635 (1993) 31.
[34] C.J. Allender, K.R. Brain, C.M. Heard, M.A. Pellett, in: K.R. Brain, V.J. James, K.A. Walters (Eds.), Perspectives in Percutaneous Penetration, vol. 5a, STS Publishing, Cardiff, 1997, p. 88.
[35] R. Suedee, T. Srichana, T. Rattananont, Drug Delivery 9 (2002) 19.
[36] R. Suedee, T. Srichana, G.P. Martin, J. Control. Release 66 (2000) 135.
[37] R. Suedee, T. Srichana, R. Chotivatesin, G.P. Martin, Drug Dev. Ind. Pharm. 28 (2002) 545.
[38] M.F. Refojo, F.L. Leong, J. Appl. Polym. Sci. 66 (1979) 227.
[39] K.J. Shea, Trends Polym. Sci. 2 (1994) 166.
[40] D.S. Shnek, D.W. Pack, D.Y. Sasaki, F.H. Arnold, Langmuir 10 (1994) 2382.
[41] I.A. Nicholls, Chem. Lett. (1995) 1035.
[42] A. Rachkov, N. Minoura, Biochim. Biophys. Acta 1544 (2001) 255.
[43] H. Asanuma, T. Hishiya, M. Komiyama, Adv. Mater. 12 (2000) 1019.
[44] D.C. Bibby, N.M. Davies, I.G. Tucker, Int. J. Pharm. 197 (2000) 1.
[45] H. Asanuma, T. Akiyama, K. Kajiya, T. Hishiya, M. Komiyama, Anal. Chim. Acta 435 (2001) 25.
[46] K. Sreenivasan, Angewandte Makromol. Chem. 246 (1997) 65.
[47] P.C. Nicolson, J. Vogt, Biomaterials 22 (2001) 3273.
[48] C. Alvarez-Lorenzo, H. Hiratani, J.L. Gómez-Amoza, R. Martínez-Pacheco, C. Souto, A. Concheiro, J. Pharm. Sci. 91 (2002) 2182 (see also Modern Drug Discovery, October (2002) 13).
[49] H. Hiratani, C. Alvarez-Lorenzo, J. Control. Release 83 (2002) 223.
[50] S. Ding, Pharm. Sci. Technol. To. 1 (1998) 328.
[51] M.F. Refojo, F.L. Leong, I.M. Chan, F.I. Tolentino, Retina 3 (1983) 45.
[52] C.C.S. Karlgard, N.S. Wong, L.W. Jones, C. Moresoli, Int. J. Pharm. 257 (2003) 141.

[53] T.P. Heyman, M.L. McDermott, J.L. Ubels, H.F. Edelhauser, J. Cat. Ref. Surg. 15 (1989) 169.
[54] H. Hiratani, C. Alvarez-Lorenzo, Imprinted Soft Contact Lenses for Drug Delivery, PTC/JP02/08720, April 26, 2002.
[55] H. Hiratani, C. Alvarez-Lorenzo, Biomaterials 25 (2004) 1105.
[56] K. Sreenivasan, J. Appl. Polym. Sci. 71 (1999) 1819.
[57] J.G. Karlsson, L.I. Andersson, I.A. Nicholls, Anal. Chim. Acta 435 (2001) 57.
[58] L. Ye, P.A.G. Cormack, K. Mosbach, Anal. Chim. Acta 435 (2001) 187.
[59] S.S. Shah, M.G. Kulkarni, R.A. Mashelkar, J. Membr. Sci. 51 (1990) 83.
[60] R.N. Karmalkar, M.G. Kulkarni, R.A. Mashelkar, J. Control. Release 43 (1997) 235.
[61] R.A. Mashelkar, M.G. Kulkarni, R.N. Karmalkar, Polymer composition for controlled release of active ingredients in response to pH, and a process of preparing the same, US Patent 5,851,546 (December 22, 1998).
[62] A. Leonhardt, K. Mosbach, React. Polym. 6 (1987) 285.
[63] G. Wang, K. Kuroda, T. Enoki, A. Grosberg, S. Masamune, T. Oya, Y. Takeoka, T. Tanaka, Proc. Nat. Acad. Sci. 97 (2000) 9861.
[64] S.H. Yuk, Y.H. Bae, Crit. Rev. Ther. Drug Carrier Syst. 16 (1999) 385.
[65] T. Okano, Y.H. Bae, H. Jacobs, S.W. Kim, J. Control. Release 11 (1990) 255.
[66] Y. Hirokawa, T. Tanaka, J. Chem. Phys. 81 (1984) 6379.
[67] H.G. Schild, Prog. Polym. Sci. 17 (1992) 163.
[68] M. Watanabe, T. Akahoshi, Y. Tabata, D. Nakayama, J. Am. Chem. Soc. 120 (1998) 5577.
[69] T. Tanaka, C. Wang, V. Pande, A.Yu. Grosberg, A. English, S. Masamune, H. Gold, R. Levy, K. King, Faraday Discuss 102 (1996) 201.
[70] C. Alvarez-Lorenzo, O. Guney, T. Oya, Y. Sakai, M. Kobayashi, T. Enoki, Y. Takeoka, T. Ishibashi, K. Kuroda, K. Tanaka, G. Wang, A.Yu. Grosberg, S. Masamune, T. Tanaka, Macromolecules 33 (2000) 8693.
[71] C. Alvarez-Lorenzo, O. Guney, T. Oya, Y. Sakai, M. Kobayashi, T. Enoki, Y. Takeoka, T. Ishibashi, K. Kuroda, K. Tanaka, G. Wang, A.Yu. Grosberg, S. Masamune, T. Tanaka, J. Chem. Phys. 114 (2001) 2812.
[72] K. Ito, J. Chuang, C. Alvarez-Lorenzo, T. Watanabe, N. Ando, A.Yu. Grosberg, Prog. Polym. Sci. 28 (2003) 1489.
[73] R. D'Oleo, C. Alvarez-Lorenzo, G. Sun, Macromolecules 34 (2001) 4965.
[74] T. Moritani, C. Alvarez-Lorenzo, Macromolecules 34 (2001) 7796.
[75] T. Moritani, C. Alvarez-Lorenzo, A novel functional cross-linker and gels made thereof. US Patent 20,020,115,738 (February 2001).
[76] C. Alvarez-Lorenzo, A. Concheiro, J. Control. Release 80 (2002) 247.
[77] T. Watanabe, K. Ito, C. Alvarez-Lorenzo, A.Yu. Grosberg, T. Tanaka, J. Chem. Phys. 115 (2001) 1596.
[78] T. Miyata, T. Uragami, K. Nakamae, Adv. Drug Del. Rev. 54 (2002) 79.
[79] http://www.diabetes.org, accessed on June 2003.
[80] G. Chen, Z. Guan, C.T. Chen, L. Fu, V. Sundaresan, F.H. Arnold, Nat. Biotechnol. 15 (1997) 354.
[81] S. Striegler, Tetrahedron 57 (2001) 2349.
[82] S. Striegler, Bioseparation 10 (2002) 307.
[83] A.G. Mayes, L.I. Andersson, K. Mosbach, Anal. Biochem. 222 (1994) 483.
[84] E. Oral, N.A. Peppas, Polymer Preprints 42 (2001) 111.
[85] K. Kataoka, H. Miyazaki, M. Bunya, T. Okano, Y. Sakurai, Polymer Preprints 40 (1999) 301.
[86] G. Wulff, S. Schauhoff, J. Org. Chem. 56 (1991) 395.
[87] J.C. Norrild, I. Sotofte, J. Chem. Soc. Perkin Trans. 2 (2001) 727.
[88] W.J. Wizeman, P. Kofinas, Biomaterials 22 (2001) 1485.
[89] L. Davidson, W. Hayes, Curr. Organic Chem. 6 (2002) 265.
[90] T. Hishiya, M. Shibata, M. Kakazu, H. Asanuma, M. Komiyama, Macromolecules 32 (1999) 2265.
[91] B. Sellergren, J. Wieschemeyer, K.S. Boos, D. Seidel, Chem. Mater. 10 (1998) 4037.
[92] N. Zhong, H.-S. Byun, R. Bittman, Tetrahedron Lett. 42 (2001) 1839.
[93] C.C. Hwang, W.C. Lee, J. Chromatogr. A 962 (2002) 69.
[94] B.S. Green, M. Priwler, Molecularly imprinted polymers for the treatment and diagnosis of medical conditions, US Patent 2002/0015690 A1 (February 7, 2001).
[95] C.C. Huval, M.J. Bailey, W.H. Braunlin, S.R. Holmes-Farley, W.H. Mandeville, J.S. Petersen, S.V. Polomoscanik, R.J. Sacchiro, X. Chen, P.K. Dhal, Macromolecules 34 (2001) 1548.
[96] F.A. Villamena, A.A. De La Cruz, J. Appl. Polym. Sci. 82 (2001) 195.
[97] I.A. Nicholls, K. Adbo, H.S. Andersson, et al., Anal. Chim. Acta 435 (2001) 9.
[98] F. Lanza, B. Sellergren, Anal. Chem. 71 (1999) 2092.
[99] T. Takeuchi, D. Fukuma, J. Matsui, Anal. Chem. 71 (1999) 285.
[100] A. Weber, M. Dettling, H. Brunner, G.E.M. Tovar, Macromol. Rapid. Commun. 23 (2002) 824.
[101] X. Chen, Y. Lin, M. Liu, M.K. Gilson, Bioinformatics 18 (2002) 130.
[102] D.A. Spivak, K.J. Shea, Macromolecules 31 (1998) 2160.
[103] N. Nagahori, S.-I. Nishimura, Biomacromolecules 2 (2001) 22.
[104] F. Gabor, M. Wirth, STP Pharma. Sci. 13 (2003) 3.
[105] D.J. Duffy, K. Das, S.L. Hsu, J. Penelle, V.M. Rotello, H.D. Stidham, J. Am. Chem. Soc. 124 (2002) 8290.