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Molecularly imprinted polymers in drug delivery: state of art and future perspectives

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Introduction: Molecularly imprinted polymers (MIPs) are synthetic receptors, characterized by a high selectivity for the selected template. Among the different applications of MIPs, their use as controlled/sustained drug delivery devices has been extensively explored, even though the optimization of such devices needs to be performed before they are applied in clinical practice.

Areas covered: Within drug delivery, one of the most promising fields is the possibility to modulate the drug release profile in response to a specific external stimulus; MIPs represent potentially suitable vehicles, because of the possibility to insert a stimuli-responsive co-monomer in their structure. This review discusses recent advances in the use of external stimuli to modulate drug release, as well as the synthetic strategies devoted to increase the water compatibility of these systems, which is a base requirement for their application in biomedicine.

Expert opinion: Although it is easy to imagine imprinted polymers for biomedical applications, several aspects have to be further investigated, such as the *in vivo* studies, efficiency and biocompatibility. However, we think that in the next few years it will possible to see unprecedented progress in the preparation of such systems and the translational application of these intelligent structures in medicine.

Keywords: drug delivery, drug targeting, molecularly imprinted polymers, stimuli-responsive materials, water compatibility

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1. Introduction: molecular imprinting technology

Molecularly imprinted polymers (MIPs) are macromolecular networks characterized by specific recognition sites for a desired target molecule, named template [1]. Two main synergistic effects are responsible for the macromolecular memory: shape-specific cavities matching the template molecule, which provide stabilization of the chemistry in a crosslinked matrix; and functional groups oriented to form multiple interactions with the template (**Figure 1**). It should be considered that the selectivity of imprinted networks is dictated by the applications for which they are designed. Creating well-defined sites with high selectivity is crucial, for example, for applications where the separation of chiral entities is desired; however, other factors such as the ease of diffusion of analytes in and out of the polymer network also become important in applications such as drug delivery [2]. In practice, many parameters involved in the imprinting system and preparation process can affect the information associated with the binding sites, such as functional monomers/polymers, crosslinkers and solvents/porogens. Thus, both the feasibility of imprinting and the proper preparation conditions need exploration for the preparation of efficient imprinted materials [3].

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Article highlights.

- Molecularly imprinted polymers (MIPs) fully respond to the modern drug delivery systems (DDS) requirements due to their ability to sustain the release of a therapeutic agent, enhance the loading capacity, tailor the cross-linking type and amount, and intelligently release the therapeutic by responding to the environment.
- The ultimate aim of current studies was to obtain imprinted materials capable of selective recognition of the template in pure aqueous buffer with minimum nonspecific binding of the drug as well as other matrix components.
- Coupling the properties of hydrogels and MIPs, it is possible to obtain very useful systems to be applied in drug delivery field. The high swelling properties of these materials improved their recognition characteristics, because of the enhanced accessibility of template to the imprinted cavities.
- The combination of imprinting and stimuli-sensitivity may have considerable practical advantages in drug delivery field. The ability to respond to external stimuli contributes to modulate the affinity of the polymeric matrix for the target molecules, providing regulatory or switching capability of the loading/release processes.
- One of the most relevant challenges is the drug targeting. The selective recognition of MIPs could be useful, for example, to have drug targeting on cells with particular cellular receptor.

This box summarizes key points contained in the article.

MIPs are stable polymers with molecular recognition abilities and resistant to a wide range of conditions (pH, organic solvents, temperature, pressure). The behavior of MIPs emulates the recognition and binding properties of natural biomolecules, such as antibodies and enzymes [4]. MIPs, indeed, can mimic the interactions established by natural receptors to selectively retain a target molecule (i.e., antibody antigen) but without the associated stability limitations. Furthermore, their synthesis is relatively cheap and easy, making them a clear alternative to the use of natural receptors.

MIPs were used for several different applications, such as chromatographic stationary phases [5], enantiomeric separation [6], solid-phase extraction (SPE) [7] and catalysis [8]; they were also used as receptors [9], antibodies [10], enzyme mimics [11], affinity and sensing materials [12] and, in recent years, pharmaceutical applications, such as drug discovery, drug purification or drug delivery [13-16]. In particular, the enormous potential of molecular imprinting technology in biomedical field provided the tools for the creation of smart polymers capable of controlled drug delivery of therapeutic molecules, with the possibility to create macromolecular memory for a therapeutic within a flexible polymer network and delay the transport of drug from the matrix through interaction of the drug with functional groups organized within the network [17]. MIPs fully respond to the modern drug delivery systems (DDS) requirements due to their ability to sustain the release of a therapeutic agent, enhance the loading capacity, tailor the crosslinking type and amount, and intelligently release the therapeutic by responding to the environment [18].

DDS must be capable of regulating the rate of release (delayed- or extended-release systems) and/or targeting the drug to a specific site, to maximize the efficacy and safety of medicines. 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, minimize the dosing frequency and enhance patient compliance [17].

MIPs can help achieve sustained release because of the affinity of the template to the functional monomer, thereby increasing the residence time of the drug within the body. MIPs act as DDS by providing either the rate-limiting mechanism in controlled release systems or by acting as the trigger for the release of therapeutic beneficial agents in response to external stimuli, or even by being sensing elements to give feedback as part of a biological sensor [19]. If the drug has a narrow therapeutic window, MIPs as DDS can keep the plasma concentration below toxic levels while also above the minimum effective level. In addition, they possess the right physicochemical properties to protect the drug from degradation by enzymes or other proteins during systemic trafficking. Finally, in a recent study, the *in vivo* application of MIPs was proved [20]. MIPs designed for the specific recognized of the toxin melittin were found able to neutralize its activity while no detectable toxicity was observed histopathologically in tissue samples.

The main limitation in the use of traditional MIPs in biomedical field is that they demonstrate the best performance in hydrophobic organic solvents. This could be explained by the fact that apolar solvents eliminate the nonspecific hydrophobic interactions and create the best environment for the interactions involved in the molecular recognition mechanism [21]. There are some areas for MIPs application, for example, SPE of natural products, where the use of hydrophobic solvents could give an advantage; but when considering some of the others, full compatibility with water is required [22]. The capacity to utilize molecular imprinting for the preparation of synthetic receptors for water-soluble substances would be of great potential for the use of MIPs for biomedical applications and drug delivery in particular [23-25].

This review focused on the use of MIPs as DDS excipients, with particular attention to the different synthetic approaches to obtain water-compatible and stimuli-responsive materials.

2. MIPs as base excipients in sustained drug delivery: water compatibility

Norell *et al.* [26] proposed one of the first examples of MIPs as controlled release drug dosage form. Theophylline as a template molecule and non-covalent approach as a synthetic

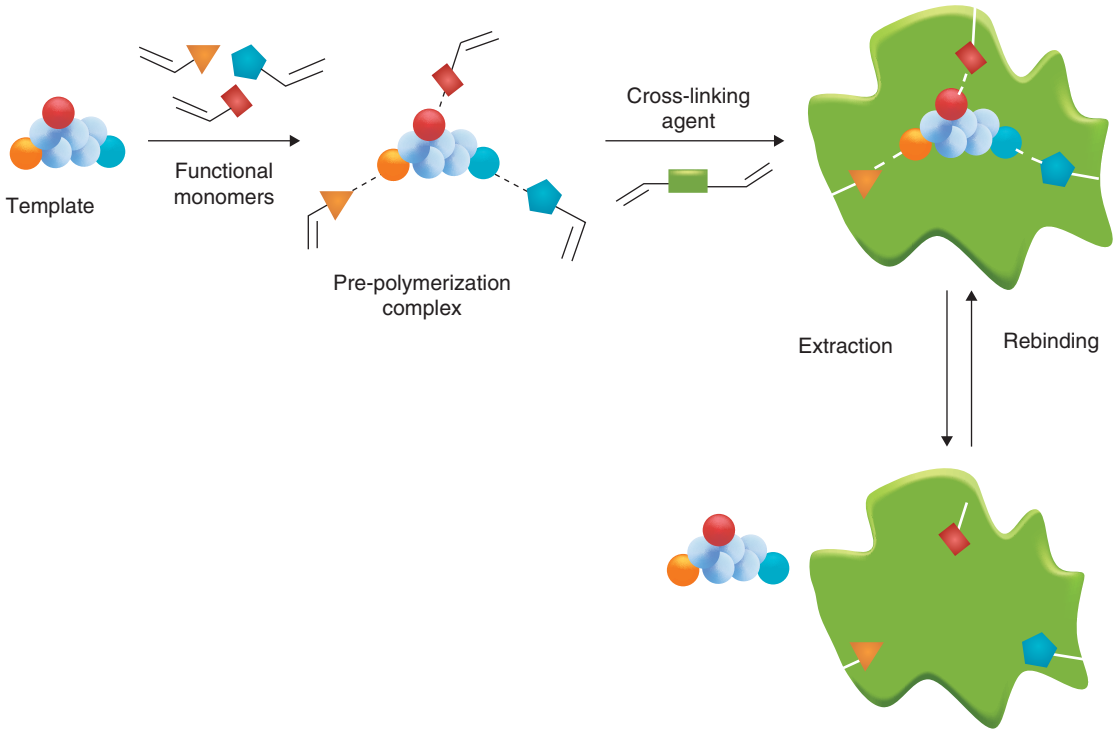

Figure 1. Schematic representation of molecular imprinting.

strategy were chosen to obtain microparticles able to sustain the release in pH 7.0 phosphate buffer for several hours. The non-imprinted polymers (NIPs) show faster release kinetics than MIPs, confirming the high specificity of the imprinted sites.

As these systems have to be employed in physiological fluids, their first requirement is the water compatibility. [27,28]. The main barrier preventing imprinting in aqueous media is due to the nature of hydrogen bonding, the most commonly exploited interaction for the non-covalent molecular imprinting, which is easily destroyed in aqueous media because aqueous solvents can compete with the template for the functional monomers. As a consequence, the recognition properties of networks prepared in organic solvents are stronger [29].

To date, several attempts were made to molecularly imprint water-soluble biomedical molecules, even if they have achieved only limited success [30].

A first approach is the use of water/alcohol mixtures. In a work in 2008 [31], ciprofloxacin-imprinted polymers were prepared in water-containing system for selective extraction and separation of ciprofloxacin from human urine. In another work [32], MIPs with high affinity and selectivity to nine quinolones in aqueous environment were prepared in water–methanol system. Under the optimal condition, the quinolones can be retained selectively and all matrices interferences were eliminated simultaneously. Currently, a few MIPs had been successfully obtained via bulk polymerization in water–methanol systems; however, other groups of target drugs cannot efficiently form the complex of template–monomer under aqueous conditions, thus the above-mentioned systems are not suitable for generic preparation of the water-compatible MIPs [33].

In order to avoid the disturbance caused by water during the polymerization, a solution is to introduce metal ions as mediator during the prepolymerization to form a complex consisting of template metal ion–monomer. In these systems, stronger ionic interactions are created, probably replacing hydrogen-bonding interactions with the template and functional monomer [4]. With the described approach, the interference of water was substantially reduced, and the tetracycline antibiotics were successfully imprinted in water using methacrylic acid (MAA) as a functional monomer with the formation of the ternary complex of tetracyclines/Fe^{2+}/MAA during prepolymerization [34]. A different kind of MIPs was prepared using a water-soluble crosslinking agent. An ionic complex was utilized as the assembly for the template molecule and the functional monomer, and water acts as porogen during the preparation of polymers [35].

A current trend to address the drawbacks of using MIPs in water media is based on the sol-gel process to synthesize imprinted materials by choosing appropriate metal alkoxide precursors. Different metal oxides (silica and mixed metal oxides) have been imprinted to produce materials with applications as sensing phases, catalysts and adsorbents. In a self-assembly imprinting approach, the template may be directly added to the metal alkoxide solution prior to acid- or

base-catalyzed hydrolysis and condensation. By using an adequate polar/nonpolar sol-gel functional precursor and a fairly polar solvent (e.g., ethanol), imprinted sites may be generated by electrostatic, π-stacking, van der Waals and so on interactions between the template and the sol-gel network. During the cascade of these events, the template molecule organizes itself onto the cavities of the amorphous silica [36].

2.1 MIPs and water affinity: restricted access materials approach

As reported, two main drawbacks have to be considered when using MIPs in aqueous solutions: the nonspecific interactions between small molecules and polymeric matrices due to the predominance of hydrophobically driven bonds; and the absorption of biological sample components, such as proteins and lipids, on the polymer surfaces [37]. The ultimate aim of current studies was to obtain imprinted materials capable of selective recognition of the template in pure aqueous buffer with minimum nonspecific binding of the drug as well as other matrix components [38,39].

In this direction, literature data report on the use of a hydrophilic co-monomer able to confer high water compatibility to the final system [40]. A pilot study [41] reports on a bupivacaine MIPs consisting of polyMAA-*co*-EGDMA (ethylene glycol dimethacrylate) containing 2-hydroxyethyl methacrylate (HEMA). As a result, a significant increase in the efficiency of the imprinted polymers in water was observed.

Although the above report represents a useful approach, several studies report that a considerable modification of the recognition properties of the polymeric matrices was often observed because of the formation of hydrogen bonds between various functionalities of template molecule and hydroxyl group of HEMA [42].

To improve the water compatibility of MIPs, the modification of a pre-formed MIPs surface after the polymerization step was proposed. In this direction, a useful approach involves the coupling of the MIPs properties with those of restricted access materials (RAM) [43]. In RAM–MIPs, the RAM component can exclude large molecules, such as serum proteins, by a molecular-weight cutoff, while MIPs selectively recognize small molecule because of the presence of specific recognition sites. Macromolecules are excluded and interact only with the outer surface of the particle support, which is coated with hydrophilic groups [44]. The basis of RAM is the simultaneous size exclusion of macromolecules and extraction/enrichment of low-molecular compounds into interior phase via partition [45]. The outer surface of the particles, which is in contact with biological matrix components, such as proteins and nucleic acids, possesses a special chemistry to prevent adsorption of these molecules [46]. Macromolecules can be excluded by a physical barrier by means of the pore diameter or by a chemical diffusion barrier created by a protein (or polymer) network at the outer surface of the particle [47].

The synthetic procedure consists of a multistep swelling and polymerization method followed by hydrophilic surface modification, in which hydrophilic monomers (generally a mixture of glycerol monomethacrylate and glycerol dimethacrylate) were added after the imprinting polymerization to form external layer [48].

A more recent approach is to employ a monomer [glycidilmethacrylate (GMA)] that less interferes in the prepolymerization complex formation, but able to impart water compatibility to the system after a post-polymerization straightforward modification [37]. Epoxy group of GMA has lower capacity to form hydrogen bonding than a free hydroxyl group, and a hydrophilic layer around polymeric microparticles could be created by opening GMA epoxide ring with hydroxyl and aminic reactants. In the experimental protocol, the formation of a hydroxyl shell around the microparticles was found to be the optimal solution [49].

2.2 MIPs and water affinity: cyclodextrins approach

Cyclodextrins (CDs) are a family of acyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. In the pharmaceutical field, they are used as complexing agent to surmount the problem of the poor solubility of some biological molecules in aqueous media [23]. CDs, indeed, are able to form inclusion complexes by taking up a whole drug molecule or rather some nonpolar part of it into the hydrophobic cavity, with a considerable alteration of the drug solubility [50]. When making MIPs for templates whose main body could be accommodated in the cavity of β-CD, the acrylate derivative of β-CD and another compound were used as combinatorial functional monomers [51,52]. By using this approach, MIP for the selective recognition of tryptophan was synthesized [53]. A unique polymer comprised a hydrophobic moiety-selective recognition element (bonded CDs) and a hydrogen bond interaction functional monomer (acrylic acid, AA) was prepared. CDs moiety could interact with the indole ring of the template molecule hydrophobicity and AA could form hydrogen bond with the amino and carboxyl groups of Trp. This approach is useful for both noncovalent and covalent imprinting methods. In this direction, in another report, cholesteryl acrylate as a template and CD derivatives as monomers were used to form novel hydrophilic MIPs in aqueous media via the covalent imprinting methodology [54].

The use of CDs is useful to imprint biologically active macromolecules primarily because template molecules have complex conformations, low solubility in organic monomers/crosslinkers solutions, slow diffusion rates, and suffer from interference with other components in the imprint systems [3].

The main difficulties associated with the imprinting of biomolecules include the large and flexible nature of the 'macrotemplates', low mass-transfer kinetics and the attempt to generate molecular recognition in water, where the weak non-covalent forces involved in the monomer–template complex formation may be severely suppressed [55]. In addition, macromolecules such as proteins dissolved in organic solvents exhibit distinctly different biological activity when separated

and re-dissolved in aqueous media. This change in activity is attributed to a conformational change in the protein due to unfolding and refolding in different media [56]. The main strategies to exploit CDs and its derivatives in this field are as follows: for those comparatively big templates, several CD molecules are assembled around the template and each CD molecule accommodates a part of the template, so that the assembled CD molecules can work as a whole to recognize the template precisely [27].

2.3 MIPs and water affinity: hydrogels

Hydrogels are insoluble, crosslinked polymer network structures composed of hydrophilic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water [57]. The development of 'conventional' controlled devices based on hydrogels or hydrophilic carriers that can swell in the presence of a biological fluid (osmotic-controlled and swelling-controlled release systems) has been described in several reviews [3]. The overall rate of drug release is controlled by the rate of water influx. In swelling-controlled systems, the drug, which is dispersed in the polymer, diffuses out as water uptake occurs and the polymer swells and the drug release rate is dependent both on water diffusion and polymer chain relaxation [4].

Coupling the properties of hydrogels and MIPs, it is possible to obtain very useful systems to be applied in drug delivery field [57]. First, due to their significant water content, hydrogels possess a degree of flexibility very similar to natural tissue, which minimizes potential irritation to surrounding membranes and tissues [58]. Moreover, the high swelling properties of these materials improved their recognition characteristics, because of the enhanced accessibility of template to the imprinted cavities [59]. Several reviews and research works report on the application of imprinted hydrogels as drug delivery devices or, in biomedical technologies, as sensing systems in point-of-care diagnostic devices [60]. Moreover, as reported by White and Byrne in a recent reviewing work [61], imprinted hydrogels were successfully employed as base materials in contact lenses to enhance loading and delay drug release and overcome limited patient compliance.

Imprinted hydrogels coated on a material surface are widely explored in producing materials by surface imprinting techniques for the selective recognition of bio-macromolecules [56,62]. In this regards, polyampholytes, polymers containing both positive and negative charges on the polymer backbone, were successfully explored [63]. MIPs synthesized in these conditions were explored for the selective recognition of Bovine hemoglobin (Bhb). The presence of both positively and negatively charged monomers in the imprinted hydrogels could enable increased template molecule recognition for two reasons. First, the presence of two oppositely charged monomers in the pre-polymerization mixture could result in imprinted hydrogels with cavities containing highly specific functional group orientation. The Bhb protein template contains a distribution of positively and negatively charged functional groups on its surface, and therefore an imprinted hydrogel containing both positively and negatively charged monomers should result in a more accurate complementary structure for Bhb recognition. Second, the polyampholyte hydrogels should exhibit decreased swelling when compared with their polyelectrolyte counterparts. Repulsive interactions between similarly charged monomers are shielded within the hydrogels, resulting in decreased swelling and a lower degree of cavity deformation.

Finally, the use of natural polymers was also explored for the synthesis of highly water compatible hydrogel [64]. Chitosan, a natural mucopolysaccharide with similar structural characteristics to cellulose, has been considered as one of the most promising materials to be used in biomedicine due to its biodegradability, biocompatibility and non-toxicity [65]. Being a natural chiral compound, chitosan is also a multifunctional polymer containing large numbers of amine groups together with hydroxyl groups capable of assembling with template molecules such as amino acids through forming hydrogen bond [66]. Based on these considerations, in literature, different MIPs based on this polysaccharide were developed for the recognition of aspartic acid [64], bovine serum albumin (BSA) [65] and hemoglobin [66,67], while calcium phosphate/alginate hybrid polymer microspheres were synthesized for the selective recognition of BSA [68].

3. Stimuli-responsive MIPs in drug delivery

The combination of imprinting and stimuli-sensitivity may have considerable practical advantages in drug delivery field [69]. Polymers that modify their structure and their properties in response to small 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 [70]. The ability to respond to external stimuli contributes to modulate the affinity of the polymeric matrix for the target molecules, providing regulatory or switching capability of the loading/release processes [1]. In addition, it is also interesting to study the ability of the polymeric material to memorize a specific conformation after a dramatic change in swelling degree [24]. In **Table 1**, the most recent works on stimuli-responsive MIPs are summarized.

3.1 pH-responsive MIPs

pH responsiveness is one of the most frequently adopted external stimuli because it is convenient to apply and easy to control. The pH-responsive DDS have been targeted for oral-controlled drug delivery, taste-masking of bitter drugs and intravascular drug release during increased blood pH in certain cardiovascular defects [1].

Suedee *et al.* [71] developed enantioselective-controlled DDS for selective release of the required (*S*)-omeprazole in a dose formulation containing a racemic drug in response to pH. The recognition system was obtained from a nanoparticle-on-microsphere MIPs with a multifunctional

Table 1. Summary of the most recent works on stimuli-responsive MIP.

Ref.	Stimulus	Drug	Functional monomer/polymer	Cross-linker	Solvent	Polymerization
[71]	pH	(*S*)-Omeprazole	Methacryloyl quinine (MQN) and methacryloyl quinidine (MQD)	Ethylene glycol dimethacrylate (EGDMA)	Chloroform	UV
[72]	pH	Bisphenol A (BPA)	Polyethersulfone/acrylic acid	*N*,*N*-methylenebis (acrylamide)	Water	Thermo
[73]	pH	Dexamethasone-21 phosphate disodium (DXP)	2-Hydroxyethyl methacrylate (HEMA), 2-(diethylamino) ethyl methacrylate (DEAEMA)	EGDMA	Acetonitrile/water mixture 3:1 (v/v)	UV
[74]	pH	5-Fluorouracil (5-FU)	Methacrylic acid (MAA)	EGDMA	Dimethyl formamide (DMF)	Thermo
[75]	pH	α-Tocopherol (α-TP)	MAA	EGDMA	Chloroform	UV
[76]	pH	Glycyrrhizic acid (GL)	MAA, HEMA, 2-(dimethylamino) ethyl methacrylate (DMAEMA)	EGDMA	DMF	Thermo
[79]	Photo	Caffeine	4-[(4-methacryloyloxy)phenylazo] benzoic acid (MPABA)	Trimethylolpropane trimethacrylate (TRIM)	Acetonitrile/DMF mixture 5:1 (v/v)	Thermo
[80]	Photo	bis(TBA)-*N*-*Z*-L-glutamate (methotrexate analog)	Di(ureidoethylenemethacrylate) azobenzene monomer	EGDMA	Dimethyl sulfoxide (DMSO)	UV
[81]	Photo	5-(3,5-Dioctyloxyphenyl)-10,15,20-tri-4-carboxyphenyl-porphyrin	4-{4-[2,6-bis(*n*-butylamino) pyridine-4-yl]-phenylazo}-phenyl methacrylate, styrene	Divinylbenzene (DVB)	Toluene/DMSO mixture 9:1 (v/v)	Thermo
[82]	T	Dopamine hydrochloride	MAA, acrylamide (ACM)	*N*,*N*′-methylene-bisacrylamide (MBAA), EGDMA	Methanol/water mixture 4:1 (v/v)	Thermo
[83]	T	Divalent ions	*N*-isopropylacrylamide (NIPA), lead methacrylate ($PbMAA_2$)	*N*,*N*′-bis-(acryloyl) cystamine (BAC), *N*,*N*′-methylene-bisacrylamide (BIS)	Dioxane	Thermo
[86]	T	Divalent ions	MAA, NIPA, $PbMAA_2$	BIS	Dioxane	Thermo
[87]	T	Pyrene derivatives	NIPA, methacrylamide-propyl-trimethyl-ammonium chloride (MAPTAC)	BIS	DMSO	Thermo
[88]	T	4-Aminopyridine (Apy)	MAA, *N*-isopropylacrylamide (NIPAAm)	EGDMA	DMF	Thermo
[89]	T	L-Pyroglutamic acid (Pga)	MAA, NIPAAm	EGDMA	Methanol	Thermo
[90]	T	Adenine (Adn)	MAA, *N*-isopropylacylamide (NIPA)	EGDMA	DMSO	UV
[91]	T	2,4-Dichlorophenoxyacetic acid (2,4-D)	4-Vinylpyridine (4-VP) *N*-isopropylacrylamide (NIPAAm)	EGDMA	Methanol/water mixture 4:1 (v/v) DMF	Thermo Surface-initiated RAFT polymerization
[94]	T	2,4-Dichlorophenol (2,4-DCP)	Methacrylamide (MAAM)	DVB	Chloroform/ acetonitrile mixture 2:1 (v/v)	Thermo-initiated RAFT polymerization
[95]	T	2,4-D	4-VP	EGDMA	Methanol/water mixture 4:1 (v/v)	Thermo-initiated RAFT polymerization
[96]	T	L-phenylalanine anilide (L-PA)	MAA	EGDMA	Toluene	UV-initiated RAFT polymerization
[97]	Electromagnetic	L-glutamate	Overoxidized polypyrrole (oPPy)		Aqueous solution	
[98]	Electromagnetic	Aspirin (ASP)	MAA	TRIM	Chloroform	Thermo

chiral cinchona anchor, synthesized by suspension polymerization, using EGDMA as a crosslinker. The ability of the prepared recognition polymers to selectively rebind (*S*)-omeprazole was evident at different pH levels, with the highest being at pH 7.4. At this value, indeed, the MIP/NIP-binding ratios of (*S*)-enantiomer of omeprazole were of 2.5 and 1.5 for the two formulations synthesized. The partial selective-release phenomenon of the (*S*)-enantiomer in MIP-containing composite cellulose membranes with increased vehicular racemic omeprazole concentrations was highly pH-dependent. Cinchona-bonded polymers imprinted with (*S*)-omeprazole could recognize the moldable contact site of (*S*)-omeprazole independently of its chirality; this is responsible for the delivery of (*S*)-enantiomer from racemic omeprazole. The controlled-release drug devices were fabricated with synthesized composite latex, and consisted of a pH stimuli-responsive polyHEMA and polycaprolactone-triol blend, and MIPs with preloaded drug, along with pH 7.4 buffer in the device's interior.

Zhao *et al.* [72] proposed a simple way to prepare pH-responsive molecularly imprinted materials by pore-filling poly(acrylic acid) (PAA) gels into bisphenol-A (BPA)-imprinted polyethersulfone particles. The adsorbed BPA amount (or rate) decreased after filling the PAA gels both for the imprinted and non-imprinted particles. Due to the swelling of the PAA gels in the particles, it was confirmed that the change in the acidity of the solution reversibly controls the rebinding ability toward BPA (the adsorbed BPA amount in 4 h decreased from 0.47 μmol/g at pH 2 to 0.25 μmol/g at pH 7) and that the BPA uptake of the pore-filled particles exhibited chemical valve behavior at a pH between 3 and 6.

Wang *et al.* [73] developed implantable biosensors based on a pH-sensitive MIP nanospheres/hydrogel composite. The molecularly imprinted pH-sensitive nanospheres were prepared by UV-initiated precipitation polymerization using dexamethasone-21 phosphate disodium (DXP) as a template molecule. MIP nanospheres exhibited a higher loading level and slower release rate than non-imprinted nanospheres due to the interaction of DXP with the DXP-imprinted cavities within the MIP nanospheres. For instance, when the initial DXP concentration was 500 mg/ml, the DXP loading content for the MIP and NIP nanospheres was 9.65 and 5.27%, respectively. Furthermore, the MIP nanospheres exhibited a faster DXP release rate at a lower pH value within the pH range tested (i.e., 6.0 – 7.4), which is desirable for suppressing inflammation because inflammation induces an acidic microenvironment. In contrast, the NIP nanospheres did not show a notable pH-responsive DXP release behavior. The hydrogel poly(HEMA)-*N*-vinyl-2-pyrrolidinone–2-methacryloyloxyethyl phosphorylcholine was prepared by UV polymerization. The MIP nanospheres were successfully incorporated into the hydrogel. The equilibrium water content and swelling kinetics of the MIP nanospheres/hydrogel composite were similar to those of pure hydrogel. It was observed that DXP was completely released from the NIP nanospheres within 5 weeks, while the release of DXP from the MIP nanospheres was only ~ 65% after 5 weeks.

Over the past few years, in several papers, in order to mimic the path that an oral therapeutic might encounter when taken by a patient, the release studies were conducted at two conditions, simulated gastric fluid (pH 1.0) for the first 2 h after which sodium phosphate was added to simulate intestinal fluid (pH 6.8).

Using MAA as a functional monomer and EGDMA as a crosslinking agent, 5-fluorouracil-imprinted materials were obtained [74]. In this study, three systems were synthesized varying the template to monomer ratio (1:4 to 1:8) and evaluating the drug release in gastrointestinal simulating fluid. The results obtained from the *in vitro* release studies indicated that these polymeric matrices are suitable for a controlled/sustained delivery of the anticancer drug, which is completely released within 5 h from NIP, while for MIP samples, even after 30 h the release is not yet complete.

By employing radical photopolymerization technique, α-tocopherol (α-TP) imprinted particles were synthesized [75]. The release properties were studied, once again, in simulated gastrointestinal fluid. For MIPs materials, the complete α-TP release was recorded in 40 h while, because of the absence of the binding cavities, the control polymer release the entire loaded drug in 4 h.

In a most recent research article, glycyrrhizic acid (GL) imprinted microparticles were synthesized by employing acidic (MAA), neutral (HEMA) and basic [2-(dimethylamino) ethyl methacrylate-DMAEMA] functional monomers [76]. The most promising matrices, containing HEMA and MAA, were applied as GL-controlled delivery devices in gastrointestinal simulating fluid. Also in this case, the imprinted materials showed a more controlled release profile in comparison with control polymers. In fact, the release from NIP is almost completed in acidic pH conditions, while the release from both MIPs is completed in 15 h with different kinetics of ionization referable to the stronger interaction between MAA and GL comparing to HEMA.

3.2 Photoresponsive MIPs

Photoresponsive polymers are a promising route to switchable drug delivery devices with molecular recognition characteristics. Over the last decades, azobenzene has been the most widely used optical trigger for the design and synthesis of a large variety of photoresponsive systems [77]. The azobenzene chromophore group exists in two isomeric states, a thermodynamically more stable *trans* and a metastable *cis*. When irradiated with the light of appropriate wavelength, azobenzene and its derivatives undergo photoisomerization: the *trans*-form is converted into the *cis* isomer by UV light irradiation, and the *cis*-isomer can return to the *trans*-form photochemically under visible light irradiation or thermally in the dark. Quantum yields of the process are generally high and there are no significant competing reactions. Also, *trans–cis* photoisomerization of azobenzene brings about large changes

in the geometry and dipole moment to the chromophore. Incorporation of azobenzene moieties into polymers creates materials with photocontrolled mechanical and optical properties and has also found extensive applications in the search for innovative photoresponsive materials [78].

Gong *et al.* [79] fabricated photoresponsive imprinted polymers from azobenzene-based functional monomers, using caffeine as a template molecule. The binding and release experiments, carried out upon different wavelengths, demonstrated the reversibility of the receptor-site configuration and substrate affinity, due to the photoswitching of the azobenzene chromophores. The *trans–cis* photoisomerization of azobenzene, indeed, causes significant changes to its geometry, which, in theory, could be used as an on/off switch for specific recognition. Upon irradiation at 365 nm, 58.3% of bound caffeine is released from the MIP, while subsequent irradiation at 440 nm causes 96.4% of the released caffeine to be rebound by the MIP.

Gomy and Schmitzer [80] prepared photoresponsive MIP from a di(ureidoethylenemethacrylate)azobenzene monomer, using a methotrexate analog as a template. Photoisomerization of the crosslinked polymer matrix allowed switching the substrate affinity by altering the geometry and spatial arrangement of the receptor-binding sites. As a result, controlled release (irradiation at 440 nm) and uptake (irradiation at 365 nm) of the template (or analogous ligands) were obtained.

In 2007, Takeuchi *et al.* [81] reported a newly designed photoresponsive functional monomer having diaminopyridine and azobenzene moieties, 4-{4-[2,6-bis(*n*-butylamino)pyridine-4-yl]-phenylazo}-phenyl methacrylate (FM) for preparing photoresponsive imprinted polymers (IP) for porphyrin derivatives with carboxylic acids. As a result, the association constants (K_a) and the maximum numbers of binding sites (B_{max}) in *trans*-IP (1.8×10^5 M^{-1} and 3.82 μmol/g) were higher than those in *cis*-IP (7.9×10^4 M^{-1} and 3.19 μmol/g).

3.3 Temperature-sensitive MIPs

Combining the properties of a thermosensitive polymer with molecular imprinting techniques may provide a promising strategy for ensuring that the system responds more rapidly to an external temperature change [82].

This research field came from the exciting challenge to mimic, in synthetic polymers, the protein abilities to reversibly capture target molecules and release them by obeying certain 'molecular signals' [83,84]. Recently, several efforts were made to achieve this goal by using weakly crosslinked polymer gels that can reversibly swell and shrink in response to environmental changes. [85]. *N*-isopropylacylamide (NIPAAm) monomer was used as the major component that allowed swelling and shrinking of the gels in response to temperature changes. Tanaka can be considered the father of these kinds of materials, which were largely studied in several works. The general principles for the design and synthesis of gels with the memory of monomer pair assembly were demonstrated in 2000 [86] by careful selection of monomer components, crosslinks, target molecules and solvent. MAA was chosen as the adsorbing monomers with calcium ions as the target. In another paper, the possibility that breaking and reforming reversible crosslinks in the presence of quenched ones can distort an imprinted polymer network and create frustrations for the imprinted groups to adsorb the target was investigated [83]. Furthermore, the affinity of a thermosensitive heteropolymer gel for target molecules was found to depend very strongly on the salt concentration [87].

In two different articles, Liu *et al.* have reported the synthesis of thermoresponsive MIP by using 4-aminopyridine (Apy) [88] and L-pyroglutamic acid (Pga) [89] as template molecules. In both cases, NIPAAm was employed as a smart element, MAA as a functional monomer, able to recognize target molecule by hydrogen bonds, and EGDMA as a crosslinking agent.

The loading studies were conducted at a temperature above the LCST where the gel was in its collapsed state to retain the binding sites, while the release studies were held at a temperature below the LCST (swollen state) both at pH 9.6 in an aqueous solution. In both systems, ~ 80% of the drug loaded was released in the MIPs (compared with 60% in the control) and this process was repeatable as the same amount was released for three subsequent loading/release cycles.

Li *et al.* [90] proposed the synthesis of a temperature-sensitive adenine-imprinted polymer using PNIPAAm as a thermosensitive element. The prepared MIPs exhibited a temperature-responsive molecular recognition behavior. At a relatively low temperature (such as 20°C), the obtained MIPs presented highly specific recognition (70%) for the imprint species. However, the MIPs did not show any significant resolution for the template and its analog above the transition temperature.

Pan *et al.* [91] have reported a new methodology to develop advanced MIP materials with water-compatible and/or stimuli-responsive binding properties. In particular, reversible addition-fragmentation chain transfer (RAFT) polymerization was employed to grafting of PNIPAAm brushes onto the preformed MIP particles [92]. The polymers generated via RAFT polymerization generally contain a dithioester end group, which makes their further chain modification possible. In recent years, RAFT polymerization has been utilized for the controlled preparation of MIPs with tailor-made structures and improved properties [93], including surface-imprinted core–shell particles [94,95].

The introduction of PNIPAAm brushes onto the MIP microspheres has proven to significantly improve their surface hydrophilicity at ambient temperature and impart stimuli-responsive properties to them, leading to their pure water-compatible and thermoresponsive binding properties [96].

3.4 Electromagnetic MIPs

In a recent study [97], a biocompatible electrochemical device consisting of a gold electrode coated with molecularly imprinted, overoxidized polypyrrole (oPPy) was prepared.

This system was successfully employed for the voltage-dependent uptake and release of the neural transmitter L-glutamate in neutral pH solution.

To investigate the change in mass of the polymer layer as a result of the applied voltage, EQCM measurements were performed. It was found that L-glutamate was taken up by the film for positive potentials, and release for negative potentials. This indicates that oPPy can behave as an anion exchanger in neutral pH solutions. The selectivity of the oPPy for L-glutamate over D-glutamate in neutral pH was demonstrated. These results are promising for further work to investigate the selectivity of the device in physiological solutions. The biocompatibility of the oPPy layer is demonstrated using retinae from young rats, showing a good tolerance degree.

Kan *et al.* [98] described the development of core–shell structural magnetic molecularly imprinted polymers (magnetic MIPs) for the selective uptake and controlled release of aspirin (ASP). Magnetic MIPs were synthesized by the copolymerization of MAA and trimethylolpropane trimethacrylate (TRIM) around the template ASP, at the surface of double-bond-functionalized Fe_3O_4 nanoparticles in chloroform. The obtained spherical magnetic MIPs with diameters of ~ 500 nm had obvious superparamagnetism and could be separated quickly by an external magnetic field. The magnetic MIPs exhibited good special binding and selectivity capacities to the template molecule (imprint factor of 2.39 for the template and 1.48 and 1.74 for analog molecules). The ASP-loaded magnetic MIPs or NIPs dispersed in pH 6.8 PBS showed a controlled-release ASP property. This study indicated that the prepared magnetic MIPs possess the combined properties of selective recognition and controlled release.

4. Conclusion

In the last years, the number of research articles about stimuli-responsive MIPs is considerably growing. The presence of imprinted cavities allows to obtain materials with a defined affinity for a specific molecule, while the insertion of a comonomer able to respond to the variation of the surrounding environment makes the polymers suitable to be used as smart drug delivery devices. The obtained polymers are very effective materials able to recognize and release the template in response to the variation of different stimuli, such as pH, temperature, light, electrochemical and magnetic fields.

The second point treated in this review is the water compatibility of MIPs as indispensable requirement of materials suitable for application in biomedical and pharmaceutical fields. In this regard, the most recent approaches are presented.

5. Expert opinion

Despite the large number of reports in the literature of MIPs as chromatography phases (SPE, HPLC stationary phases, etc.), there are relatively few examples of DDS based on imprinted polymers. Generally, in this last field, MIPs are used to modify the drug delivery because of their ability to promote a slow release. However, molecular imprinting is only in its infancy in the DDS area and its huge potential has yet to be realized. One of the most promising application in DDS area is the stimuli-responsiveness, which refers to the release, in a predictable way, of a therapeutic agent in response to specific stimuli such as the presence of another specific molecule or small changes in temperature, pH, solvent composition, ionic strength, electric field or incident light. The ability of polymers to reversibly respond to small environmental changes mainly depends on different interactions between functional segments of the polymer network. The combination of stimuli-sensitivity and imprinting may have considerable practical advantages: the imprinting provides a high loading capacity for specific molecules, while the ability to respond to external stimuli contributes to modulating the affinity of the network for the molecules, providing regulatory or switching capability of the loading/release processes.

One of the most relevant challenges in this area is of course the drug targeting. In principle, an ideal drug carrier is injected into the body and transports itself to the correct target, such as a tumor, and delivers the required dose at this target. This idealized concept was first proposed by Paul Ehrlich at the beginning of the 20th century and was nicknamed the 'magic bullet' concept. The selective recognition of MIPs could be useful, for example, to have drug targeting on cells with particular cellular receptor (**Figure 2**).

Although several articles described this potential opportunity using MIPs [99], to date no example of this application is reported. To achieve this goal, indeed, some critical points have to be optimized, including the interaction MIPs–cell (size and hydrophilicity) and the recognition properties of selective sites in the macromolecular system for the surface-cellular receptors [100]. This last aspect plays a crucial role for the real targeting: the receptor is a long peptide chain characterized by intercalating segments in the cell membrane (**Figure 3**). Thus, the ideal specific recognition site should involve the complete peptide sequence and its tridimensional geometry, but it is quite difficult to achieve, almost impossible. The epitope approach is the only applicable strategy; in this case the epitope, a particular aminoacidic segment, is used as a template [101]. However, this method carried out the formation of binding sites that could be no highly selective. Anyway, this is the only possibility and it means that a very deep study of the surface receptor and the right choice peptide sequence in order to have the effective targeting is necessary (**Figure 4**).

As mentioned above, another critical aspect of the drug targeting based on MIPs is the interaction MIPs–cell: in particular, the size of the particles and the hydrophilicity of the polymeric device are the most important parameters to be considered. The particle size of the MIPs has to be smaller than cell and able to interact with the receptors (5 – 10 nm).

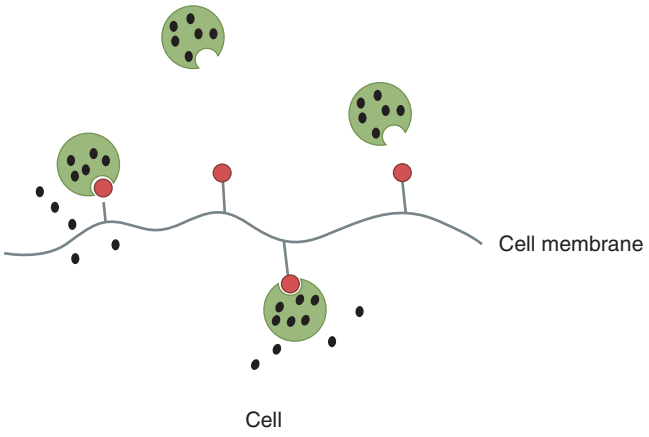

Figure 2. Interaction between MIPs and cellular receptor.

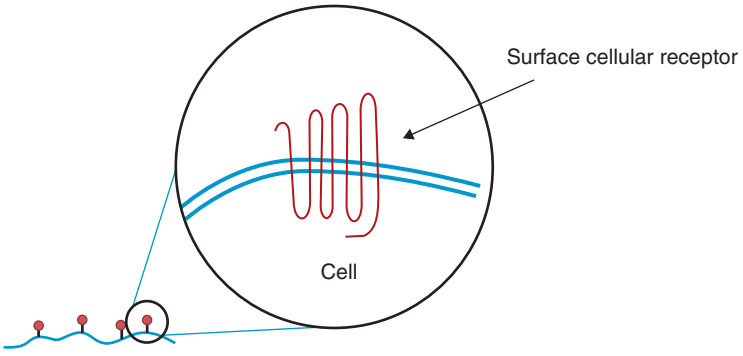

Figure 3. Schematic representation of a receptor on cell membrane.

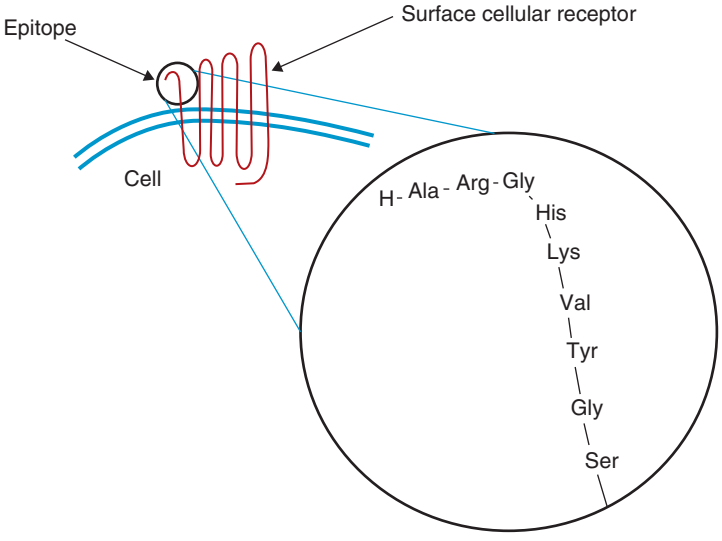

Figure 4. Schematic representation of epitope.

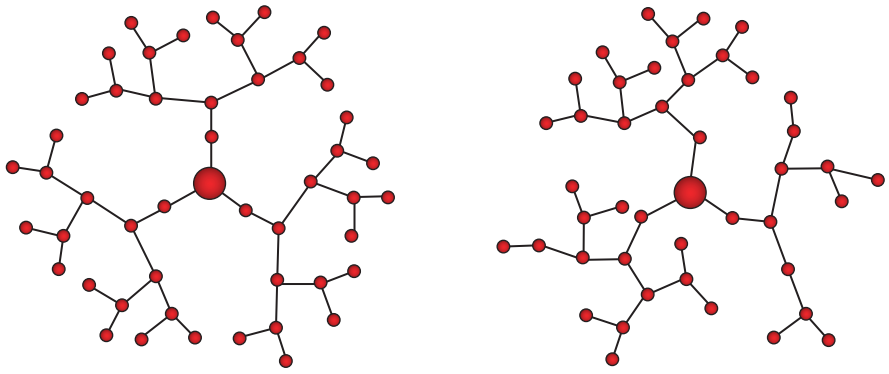

Figure 5. Dendrimers and hyperbranched polymers.

Furthermore, a rigid structure, such as the nanoparticles, is not the more suitable drug carrier because of stiffness and poor ability to undergo the tridimensional structure deformation. The model drug carrier should have spaces created within the structure of the network having the necessary size to transport the pharmaceutical drug, a very good hydrophilicity, and a water dispersibility similar to biological systems such as enzymes, proteins and so on.

Nanostructured hydrogels could be a good key strategy for the development of 'magic bullet based on MIPs'. In particular, dendritic structures (**Figure 5**), we think, fit better than traditional systems the real needs of effective drug-targeting device. They are polymers with densely branched structure and a large number of end groups. These polymers include dendrimers, which have completely branched star-like topologies, and hyperbranched polymers, which have imperfectly branched or irregular structures. Both dendrimer and hyperbranched polymer molecules are composed of repeating units emanating from a central core. The physical properties of these polymeric device, including their monodispersity, water solubility, encapsulation ability, large number of functionalizable peripheral groups and their flexible structures, make these macromolecular appropriate candidates for evaluation as drug-targeting vehicles. In any case, the preparation of these carriers has to be changed in order to get imprinted sites in the network for the selective recognition of the cell surface epitope.

In conclusion, although it is easy to imagine imprinted polymers for biomedical applications, several aspects have to be investigated, such as the *in vivo* studies, efficiency and biocompatibility.

However, we think that this area will grow very fast and, in few next years, it will be possible to see unprecedented progress in the preparation of such systems and the translational application of these intelligent structures in medicine.

Declaration of interest

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