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Review

Separation and sensing based on molecular recognition using molecularly imprinted polymers

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Abstract

Molecular recognition-based separation and sensing systems have received much attention in various fields because of their high selectivity for target molecules. Molecular imprinting has been recognized as a promising technique for the development of such systems, where the molecule to be recognized is added to a reaction mixture of a cross-linker(s), a solvent(s), and a functional monomer(s) that possesses a functional groups(s) capable of interacting with the target molecule. Binding sites in the resultant polymers involve functional groups originating from the added functional monomer(s), which can be constructed according to the shape and chemical properties of the target molecules. After removal of the target molecules, these molecularly imprinted complementary binding sites exhibit high selectivity and affinity for the template molecule. In this article, recent developments in molecularly imprinted polymers are described with their applications as separation media in liquid chromatography, capillary electrophoresis, solid-phase extraction, and membranes. Examples of binding assays and sensing systems using molecularly imprinted polymers are also presented.

Keywords: Reviews; Molecular imprinting; Molecular recognition; Template polymerization; Polymers

Contents

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1. Introduction

Since the Nobel Prize was awarded to Cram, Lehn, and Pederson in 1987, the term ‘molecular recognition’ has been recognized all over the world. The concept of molecular recognition and related chemistry [1] can be a powerful tool for the understanding of physiological and pharmacological phenomena, because the generation and maintenance of life is governed by combining many simple but specific chemical reactions based upon molecular recognition. Since molecular recognition is the origin of biological functions, the preparation and combination of synthetic molecules capable of molecular recognition may enable us to regenerate bio-functionalized artificial molecules. Such biomimetic molecules would be extremely useful as substitutes for biomolecules in biotechnological, medical, and bioanalytical fields. Therefore, the design and synthesis of artificial receptors have attracted a great deal of attention and experiments have been conducted in many laboratories.

Functionalized molecules can commonly be found in the biological processes of molecular recognition, transformation, and translocation. They are often composed of supramolecular structures which are formed by molecular assemblies based upon the integration of noncovalent intermolecular forces [1]. Therefore, it is acceptable to make use of supramolecular self-assembly for the preparation of artificial bio-functionalized molecules. As one such biomimetic strategy involving the formation of supramolecules, ‘molecular imprinting’ is recognized as a tailor-made way of preparing functionalized synthetic polymers capable of molecular recognition of given molecules [2–11].

The principle of molecular imprinting is illustrated in Fig. 1. It involves the following three steps. (1) Complex formation of a given target molecule (template molecules) with polymerizable monomers bearing functional group(s) capable of interacting with the target molecule (functional monomers) by covalent and/or noncovalent bonding. In this stage, pre-organization of the binding sites is achieved by assembling the functional monomers around the template. (2) Polymerization in order to maintain the alignment of the functional group(s) which are optimally set for binding the template molecule. (3) Removal of the template from the resulting polymer matrices, allowing ‘tailor-made’ binding sites for the template molecule to be generated. The technique can be characterized by its simplicity as a synthetic

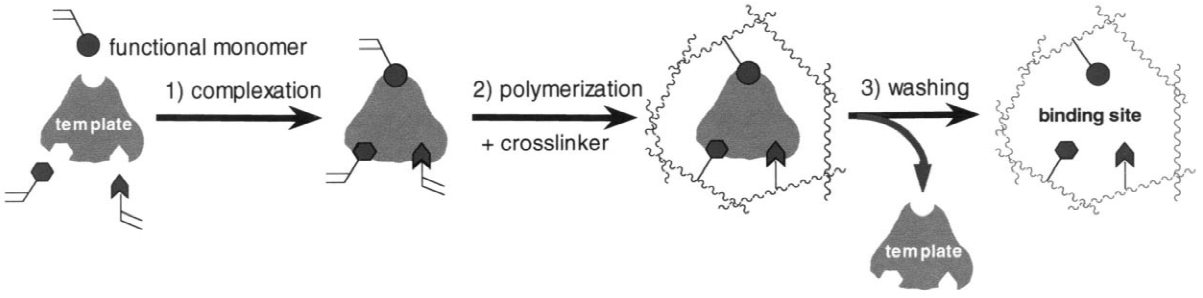

Fig. 1. Schematic diagram of molecularly imprinted polymer preparation.

strategy for designed binding sites, and the most significant advantage of the technique is that no detailed design of the binding sites is necessary before preparation, unlike the preparation of conventional artificial small molecule receptors. Surprisingly, you can even obtain artificial enzymes if substrates, products, or the transition state analogues of the desired enzyme reactions are used as template molecules in molecular imprinting, for example catalytic reactions of hydrolysis [12–15], dehydrohalogenation [16,17], aldol condensation [18] and the Diels–Alder reaction [19].

According to the chemical bonding involved in molecular imprinting, this technique can be classified into two systems, i.e. covalent bonding-based and noncovalent bonding-based molecular imprinting. Wulff and coworkers have made an intensive study of covalent molecular imprinting systems and their pioneering work has led to further development of novel molecular imprinting systems. In covalent systems, a template–monomer complex is formed through reversible covalent bonding such as ester [20,21], acetal/ketal [22–24], Schiff-base [25,26], metal coordination [27–32], etc. In order to obtain specific and homogeneous binding sites, it is crucial to maintain stable template–monomer complexes during the imprinting process, and these covalent systems are advantageous from this point of view, whereas several disadvantages should be considered such as slow binding kinetics that may not be suitable for chromatographic separations. Also, suitable covalent bonding can hardly be found, because the bonds should be cleaved easily and re-bound. Metal coordination, however, would be a good candidate for further development of covalent systems since the stability of the bonding is not too weak and ligand-exchange kinetics would be fairly fast compared to other covalent bonding, which could be controlled by the conditions employed.

Recently, a combination of covalent and noncovalent systems has been reported for cholesterol imprinting in which a covalently bonded template–functional monomer complex is used in the imprinting process and, after hydrolysis, the resulting complementary binding sites capable of hydrogen bond formation are generated [21]. This is an excellent way of producing more precise and homogeneous binding sites with high specificity.

Regarding noncovalent molecular imprinting using hydrogen bonding, electrostatic interactions, hydrophobic interactions, etc., Mosbach and coworkers have introduced this system to molecular imprinting [7–9]. The advantages of noncovalent molecular imprinting are that the procedure is simple and easy to perform because the complexation step can be achieved by mixing the template with functional monomers and polymerization is carried out without isolating the complexes formed. Furthermore, the template can easily be removed under mild conditions. However, noncovalent bonding may not be strong enough to maintain template–functional monomer complexes, therefore the population of the complex species is governed by equilibrium. Thus, an excess of functional monomers is usually added to the reaction mixture in order to complete the template–monomer complexation and to maintain the complex stably under the polymerization conditions, resulting in the presence of several species of template–functional monomer complexes in the reaction mixture. This results in a heterogeneous property of the binding sites in terms of affinity. Solvents such as porogen also affect the stability of the complexes, therefore solvents that can dissolve templates, functional monomers and other reagents necessary for the polymerization and do not interfere with the complexation should be employed. Several reports have been published on these pre-polymerization complexes [33–35].

Although both systems have their respective advantages, noncovalent bonding-based molecular imprinting is more attractive because self-assembly using biomimetic noncovalent bonding is utilized for the complexation of the template with functional monomers in the pre-polymerization stage, where host structures are constructed by the self-assembly of functional monomers which are to be the components of the binding sites in the resultant polymers. Many efforts have been made to develop various noncovalent bonding-based molecularly imprinted polymers, and excellent review articles on this topic have been published [2–11]. Therefore, in this article, more recent developments in noncovalent molecular imprinting are reviewed, including chromatographic separations, solid-phase extractions, membranes, binding assays, sensors, and other applications.

2. Molecularly imprinted separation media

2.1. Liquid chromatography

2.1.1. Amino acids and peptides

Molecularly imprinted polymers for derivatized amino acids and peptides have been prepared using methacrylic acid, 4-vinylpyridine or 2-vinylpyridine/methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the crosslinker [36–45]. When methacrylic acid is used, the acid function of the monomer interacts (1) ionically with template molecules bearing an amine function and with the amide function of the template molecules and (2) with a variety of polar functionalities such as carboxylic acids, carbamates and carboxylic esters via hydrogen bonding. The former interactions were used for the chiral resolution of amino acid aromatic amide derivatives such as Phe-β-naphthylamide/Leu-β-naphthylamide and Phe-anilide, which bear an amino group. The latter interactions were used for the chiral resolution of N-protected amino acids and N-Ac-Phe–Trp-OMe (Ac, acetyl; OMe, methyl ester), which has no amino acid groups. On the imprinted polymer prepared for N-Ac-L-Phe–L-Trp-OMe, the racemic dipeptide was completely resolved with a separation factor of 17.8 in chloroform [41]. The increase in the water content of the acetonitrile-based buffer system resulted in a decrease of the retentivity and enantioselectivity of the racemic dipeptide [42]. This suggests that hydrogen bonding interactions play an important role in the chiral recognition of the dipeptide in this system. A basic functional monomer, 4-vinylpyridine, was utilized for the preparation of imprinted polymers for Cbz-L-Asp-OH, Cbz-L-Glu-OH, Cbz-L-Phe-OH and Cbz-L-Tyr-OH (Cbz, benzyloxycarbonyl), which have a carboxyl group in the molecule [36]. In this case, the amine function of the monomer could interact ionically with the acid function of the template molecule. Further, the π-electrons of the monomer may contribute to π–π or electrostatic interactions with the template molecule. Methacrylic acid and 2-vinylpyridine were used simultaneously as the functional monomers [39]. The obtained imprinted polymers performed better in the resolution of enantiomers bearing a carboxyl group than those prepared using the functional monomer individually. This result suggests that the interactions would work effectively for recognition of the imprint molecule by using two monomers simultaneously.

Since hydrophilic interactions played an important role in the recognition of the template molecule on the molecularly imprinted polymers described above, organic mobile phases have generally been used. However, the use of an aqueous–organic mobile phase resulted in an improved column performance of the molecularly imprinted chiral stationary phase for L-Phe-anilide [38]. The retention was controlled by a simple cation-exchange mechanism in this system, suggesting that hydrophobic interactions as well as ionic interactions work in an aqueous–organic mobile phase system. Thus, the use of an aqueous–organic mobile phase can widen the scope of molecular imprinting in that the retention of a solute can be adjusted by changing the pH and organic modifier content.

Usually, molecularly imprinted polymers are prepared by the bulk polymerization method. The disadvantage of the method is that the obtained block polymers should be crushed, ground and sieved to produce packing materials. Molecularly imprinted cross-linked methacrylate polymers for Boc-L-Phe (Boc, *tert*.-butyloxycarbonyl) were copolymerized with microparticulate particles of 2,2-bis(hydroxymethyl)butanol trimethacrylate (TRIM), which has a residual double bond [44]. The obtained composite polymer could be used after simple washing. Further, a suspension polymerization technique, based on the use of a liquid perfluorocarbon as the dispersing phase, was developed for the preparation of Boc-L-Phe-imprinted polymers [45]. The method produced polymer beads in quantitative yield which can be used after simple washing. These polymers gave similar enantioselectivity and resolution compared with those prepared with the traditional bulk polymerization method.

Molecularly imprinted polymers prepared with the trifunctional crosslinkers pentaerythritol triacrylate and TRIM were shown to be superior to those prepared with ethylene glycol dimethacrylate, in that higher load capacities and better resolution were achieved, where methacrylic acid was used as the functional monomer [46,47]. Enantiomeric resolution of the tripeptide Cbz-Ala–Gly–Phe-OMe and dipeptides Boc-Phe–Gly-OEt (OEt, ethyl ester) and Cbz-Ala–Ala-OMe was attained on the corresponding imprinted polymers for the L-forms. The

imprinted polymers for Boc-, Cbz- and Ac-amino acids were prepared using acrylamide and ethylene glycol dimethacrylate as a hydrogen-bonding functional monomer and a crosslinker, respectively [48,49]. The obtained imprinted polymers containing amide residues exhibited good enantiomeric recognition properties in aqueous media, where specific hydrophobic interactions should work between the enantiomeric species and the recognition sites of the imprinted polymers [48]. On the other hand, in organic mobile phases, functional groups capable of hydrogen bond formation and the shape and size of the derivatized amino acids were all important factors in determining the selectivity of the amide-based imprinted polymers [49].

With regard to enantiomeric resolution of underivatized amino acids, a ligand-exchange molecularly imprinted polymer was prepared based on methacrylate-derivatized silica for L-Phe using Cu(II)–*N*-(4-vinylbenzyl)iminodiacetic acid as an achiral monomer [50]. The obtained imprinted polymer could separate racemic Phe and Tyr, but could not resolve racemic Trp, Ala, Leu and Ile. The chiral recognition mechanism could be explained by the rebinding of the L-enantiomer proceeding through chelation of the metal ion, in addition to which the aromatic side chain fits into a cavity that selects for both the size and shape of this group, as shown in Fig. 2a. In contrast, metal chelation by the D-enantiomer would be sterically hindered (Fig. 2b). If the side group of the D-enantiomer fits into the cavity, binding to the metal would be obstructed (Fig. 2c). Racemic Trp and aliphatic amino acids could not be resolved on the imprinted polymer because of the loose fit in the cavity.

Recently, a polymerizable dipeptide that is expected to form multiple hydrogen bonds with peptide derivatives (Fig. 3) was synthesized, and a number of dipeptide derivatives were imprinted [51]. The resulting imprinted polymers exhibited high diastereoselectivity to the templates, and a remarkable imprinting effect was observed when a Phe–Ala derivative was imprinted. It was also demonstrated that the imprinted polymers possess amino acid sequence selectivity as well as diastereoselectivity.

2.1.2. Nucleotide bases

A nucleotide base analog, 9-ethyladenine, was imprinted using methacrylic acid and ethylene glycol dimethacrylate [52]. The association constant for the 9-ethyladenine binding to the polymer in chloroform was estimated to be $7.6\times10^4\ M^{-1}$, which is comparable to association constants reported for highly designed small molecule artificial receptors [53–56]. When the polymers were employed as chromatography supports, adenine and its derivatives were selectively retained over other purine and pyrimidine bases. The origin of 9-ethyladenine binding was investigated intensively [34,57], and it appeared that porogens and polymerization temperature are important variables during the polymerization, and factors that influence rebinding of the 9-ethyladenine imprinted polymer include pH and porogens used. In specific binding, the 2-aminopyridine substructure is important for the binding and specificity of polymers imprinted with 9-ethyladenine. As reported in this work, the importance of porogens in the rebinding of template molecules was pointed out for other molecular imprinting systems and it was proposed that the use of porogens structurally similar to the template gives a superior selectivity for the template [58,59].

As shown in Fig. 4, a 9-ethyladenine-imprinted polymer was synthesized using a metalloporphyrin as the functional monomer [32]. The retention of 9-

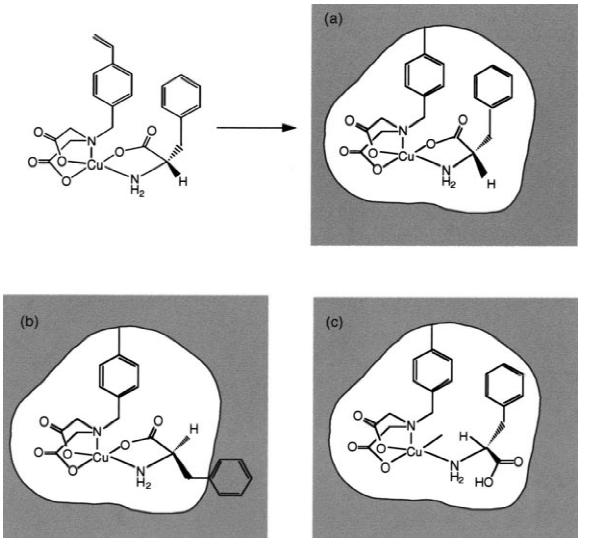

Fig. 2. Source of enantioselectivity in ligand-exchange molecularly imprinted polymers [50]. (a) L-Phe can simultaneously chelate to the metal ion and fit into the shape-selective cavity. (b) Rebinding of the D-isomer is hindered because chelation of the metal ion by the D-isomer is sterically unfavorable. (c) Alternately, if the molecule fits into the cavity, it cannot chelate Cu(II).

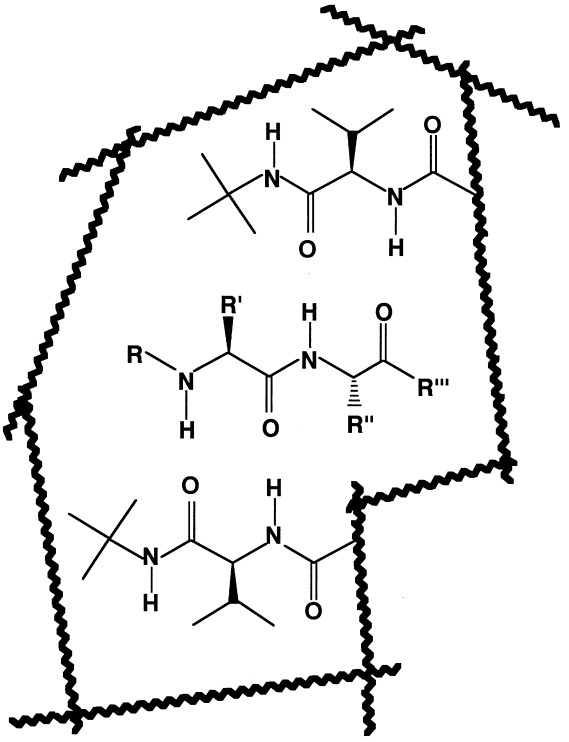

Fig. 3. Stereoselective recognition of dipeptide derivatives in molecularly imprinted polymers using an L-valine derivative as a functional monomer [51].

ethyladenine was decreased by adding methanol in chloroform, suggesting that 9-ethyladenine bound the polymers by metal coordination rather than hydrophobic interaction under the experimental conditions used. In the 9-ethyladenine imprinting process, a 1:1 complex of 9-ethyladenine and the polymerizable metalloporphyrin is supposedly formed, unlike in common molecular imprinting where excess functional monomers are used and optimally located for interacting with the template at multiple points. Therefore, the imprint effects on the retention characteristics observed here could be simply explained by cavity formation on the porphyrin plane by the template molecule, not by the cooperative interaction of several functional monomers. Interestingly, this polymer was capable of spectroscopic change based upon molecular recognition events. Such signaling binding would demonstrate the effectiveness of molecularly imprinted polymers on not only separation media but also sensing materials (see Section 3.2).

2.1.3. Drugs

Molecularly imprinted polymers for β-adrenergic blockers such as (*S*)-timolol [60] and (*S*)-propranolol [61–63] were prepared using methacrylic acid and ethylene glycol dimethacrylate as the functional monomer and crosslinker, respectively. Only a few imprinted polymers could be utilized as the separation medium [60,63]. The imprinted polymers prepared for β-adrenergic stimulants [64], ephedrine and pseudoephedrine, which have two chiral centers, could recognize the corresponding compounds, and show the moderate selectivity for epinephrine and several β-adrenergic blockers, which are structurally

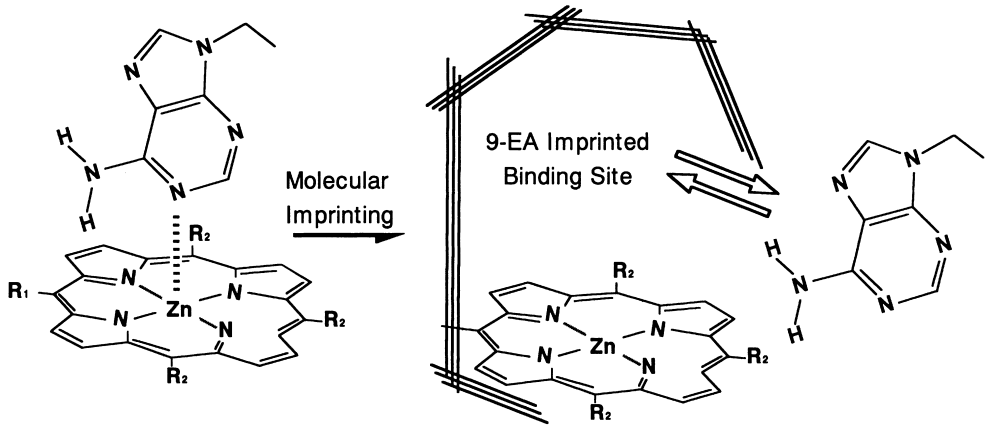

Fig. 4. 9-Ethyladenine imprinting using a metalloporphyrin-based functional monomer [32].

related to β-adrenergic stimulants. These molecularly imprinted polymers could separate the corresponding enantiomer from the antipode.

An imprinted polymer for (*S*)-naproxen (2-arylpropionic acid derivative), a non-steroidal anti-inflammatory drug, was prepared through bulk polymerization using 4-vinylpyridine and ethylene glycol dimethacrylate as the functional monomer and crosslinker, respectively, and its chiral recognition ability was evaluated using non-aqueous mobile phases [65]. A uniform-sized molecularly imprinted polymer was also prepared for (*S*)-naproxen by a two-step swelling and a redox polymerization method using the same functional monomer and crosslinker as described above with water as the suspension medium [66]. This imprinted polymer gave similar enantioselectivity for naproxen as that previously reported [67]. With regard to the comparison of the uniform-sized imprinted polymers for (*S*)-naproxen prepared by thermal and redox polymerization techniques, the latter materials gave higher retentivity and enantioselectivity than the former materials, while the former materials gave higher column efficiency than the latter materials [68]. Thus, naproxen enantiomers were resolved much better on the imprinted polymer prepared by a thermal polymerization method, as shown in Fig. 5. The two-step swelling and polymerization procedure can give monodisperse imprinted particles, as for the composite and suspension polymerization methods as described above. However, it is believed that water weakens the interaction between the template molecule and the functional monomers. Further studies are required to demonstrate the utility of the two-step swelling and polymerization procedure for the preparation of molecularly imprinted polymers.

Molecularly imprinted polymers for cinchona alkaloids, (−)-cinchonidine and (+)-cinchonine, were prepared using methacrylic acid and ethylene glycol dimethacrylate as functional monomer and crosslinker, respectively [68]. On the obtained imprinted polymer for (−)-cinchonidine, a separation factor of 31.7 was obtained for these two diastereomers, and structurally similar (−)-quinine and (+)-quinidine were also diastereoselectively discriminated, although the affinity was much less than that of (−)-cinchonidine. Further, a comparison of the utility of the functional monomers 2-(trifluoromethyl)acrylic acid and methacrylic acid was conducted for the imprinted polymers against the cinchona alkaloids [69]. The retention factors of the cinchona alkaloids dramatically increased on the imprinted polymer prepared with 2-(trifluoromethyl)acrylic acid compared with those with methacrylic acid. This is due to the fact that 2-(trifluoromethyl)acrylic acid is more acidic than methacrylic acid, i.e. a better hydrogen donor, allowing stronger hydrogen bonding. The imprinted polymer for (−)-cinchonidine

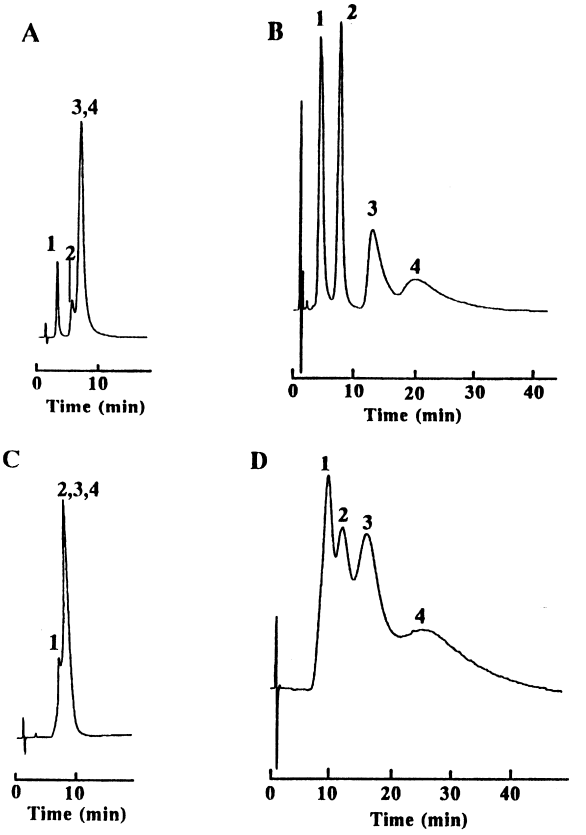

Fig. 5. Separation of 2-arylpropionic acid derivatives on non-imprinted and molecularly imprinted polymer materials prepared by thermal and redox polymerization methods. Reproduced from Ref. [66] with permission. (A) Non-imprinted polymer materials prepared by thermal polymerization. (B) Molecularly imprinted polymer materials prepared by thermal polymerization. (C) Non-imprinted polymer materials prepared by redox polymerization. (D) Molecularly imprinted polymer materials prepared by redox polymerization. Key: 1=racemic ketoprofen, 2=racemic ibuprofen, 3=(*R*)-naproxen, 4=(*S*)-naproxen. HPLC conditions: mobile phase, 20 m*M* phosphate buffer (pH 3.2)–CH_3CN=50/50 (v/v); flow-rate, 1.0 ml/min; loaded amount, 0.5 μg.

prepared with methacrylic acid gave higher diastereoselectivity than that with 2-(trifluoromethyl)acrylic acid, while the imprinted polymer for (+)-cinchonine prepared with methacrylic acid gave lower diastereoselectivity than that with 2-(trifluoromethyl)acrylic acid. This could arise from the differences in characteristics between methacrylic acid and 2-(trifluoromethyl)acrylic acid. Methacrylic acid may interact simultaneously with both the quinuclidine nitrogen and the hydroxyl group of (−)-cinchonidine. In contrast, such chelate-style binding may be unfavorable for (+)-cinchonine because of steric hindrance and, in this case, single but strong hydrogen bonding provided by 2-(trifluoromethyl)acrylic acid could give better performance.

Further, this was proved by nicotine imprinting [70–72]. Because nicotine has no vicinal atoms capable of hydrogen bond formation, 2-(trifluoromethyl)acrylic acid, a stronger hydrogen bonding provider, could be a better functional monomer than methacrylic acid. As expected, the 2-(trifluoromethyl)acrylic acid-based polymer exhibited a higher affinity for nicotine than the methacrylic acid-based polymer. Recently, similar results were obtained in solid-phase extraction using a nicotine-imprinted polymer [73].

A new class of functional monomer, 2,6-bis(acrylamido)pyridine, was reported for cyclobarbital imprinting (Fig. 6), which can form simultaneous multiple hydrogen bonding with barbiturate drugs [74]. In the structure–affinity study, it appeared that the 1-*N*-methyl group of hexobarbital inhibited hydrogen bond formation and the binding seems to be less affected by substitution at the 5-position of the malonylurea structure [75].

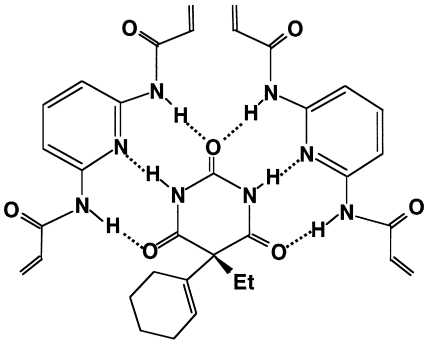

Fig. 6. Schematic representation of cyclobarbital recognition via multiple hydrogen bond formation [74]. Functional monomer, 2,6-bis(acrylamido)pyridine.

In situ molecular imprinting, in which polymers are prepared directly inside the column, allowing tedious and time-consuming steps of crushing and sieving of bulk polymers to be avoided, was demonstrated for enantioseparation and constitutional isomer separation [76]. This polymer-rod preparation technique may be advantageous because a ready-to-use column is easily prepared, however the presence of cyclohexanol and 1-dodecanol as porogens in the reaction mixture sometimes interferes with hydrogen bond formation between the templates and functional monomers, resulting in reduced affinity and selectivity in the resultant polymers.

A non-stabilizing dispersion polymerization was combined with in situ molecular imprinting to prepare pentamidine-imprinted polymers [77,78]. Unlike the polymer rods described above, this method can produce agglomerates of globular micron-sized particles in a column. This particle-type in situ method has the merit of the replacement of the particles when the performance decreases after long-term operation.

Other in situ molecular imprinting for drugs was reported for theophylline [79], nicotine [72] and cinchona alkaloids [69]. Fig. 7 shows a typical chromatogram of the theophylline-imprinted polymer. Because this technique can be a powerful tool for the preparation of imprinted polymer-packed columns including capillaries, further investigation is necessary for the establishment as a more feasible technique.

2.1.4. Sugars

Molecularly imprinted polymers for sugars were prepared using covalent molecular imprinting by forming reversible boronate esters between *cis*-diol groups on the sugar molecule and 4-vinylbenzeneboronic acid (Fig. 8) [2,20,80]. The phenyl-α-D-mannopyranoside-imprinted polymers could be used for racemic resolution of free sugars, mannose and fructose, as well as phenyl-α-mannopyranoside, which is enantiomerically separated with near baseline resolution. These results suggest that the orientation of the functional groups inside the cavity is the dominating factor, and the shape selectivity is

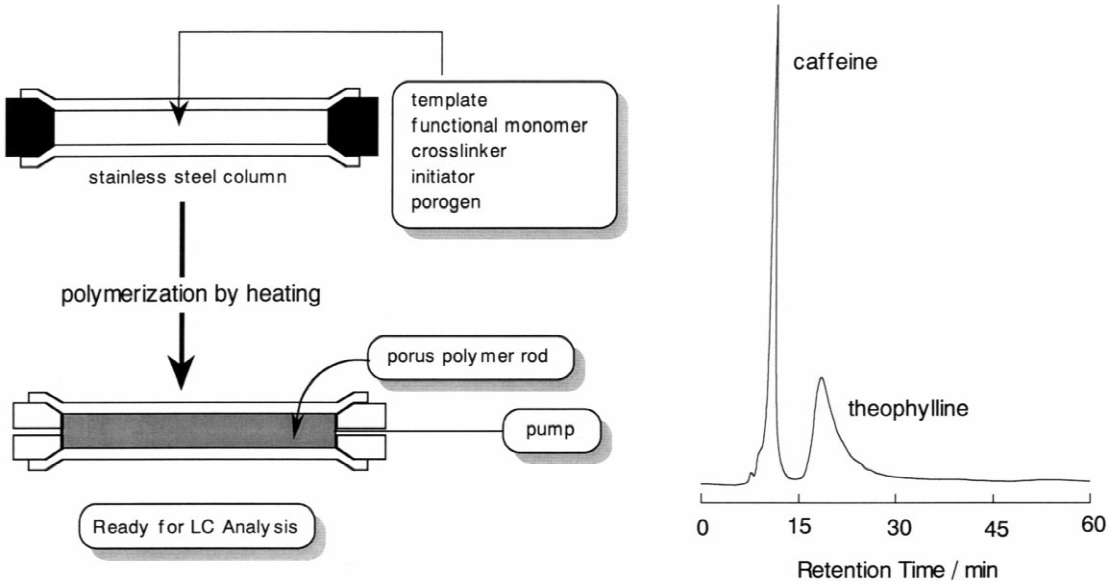

Fig. 7. In situ molecular imprinting of theophylline [79]. Column size, 150 mm×4.6 mm I.D.; eluent, acetonitrile, 0.25 ml/min.

only of secondary importance. On the other hand, molecularly imprinted polymers for derivatized sugars showing high anomeric and epimeric discrimination were prepared by noncovalent molecular imprinting using methacrylic acid as a functional monomer [81,82]. An imprinted polymer prepared with *p*-nitrophenyl-α-D-galactoside could separate anomers of the same compound with near baseline resolution [81]. Further, on the imprinted polymer for *p*-nitrophenyl-α-D-galactoside, the glucoside (one epimeric change from galactoside) was only moderately recognized and the mannoside (two epimeric changes) was not specifically recognized at all. Thus, the recognition sites should involve hydrogen bonding interactions between both the C-2 and C-4 hydroxyl groups and the methacrylic acid residue. An imprinted polymer prepared using peracetylated *p*-aminophenyl-β-galactoside gave high selectivity for the β-galactoside over the α-galactoside [82]. Ionic interactions between an amino group of the derivatized galactoside and a carboxylic group of methacrylic acid could play an important role in recognition of these anomers.

Sialic acid-imprinted polymers were prepared using three different methods [83–85]: (1) a covalent imprinting method using 4-vinylbenzeneboronic acid [83], (2) a noncovalent imprinting method using 4-vinylpyridine or *N*,*N*,*N*-trimethylaminoethyl methacrylate chloride [84], and (3) a combined method using 4-vinylbenzeneboronic acid and *N*,*N*,*N*-trimethylaminoethyl methacrylate chloride [85]. The latter two polymers were prepared with 2-hydroxy-

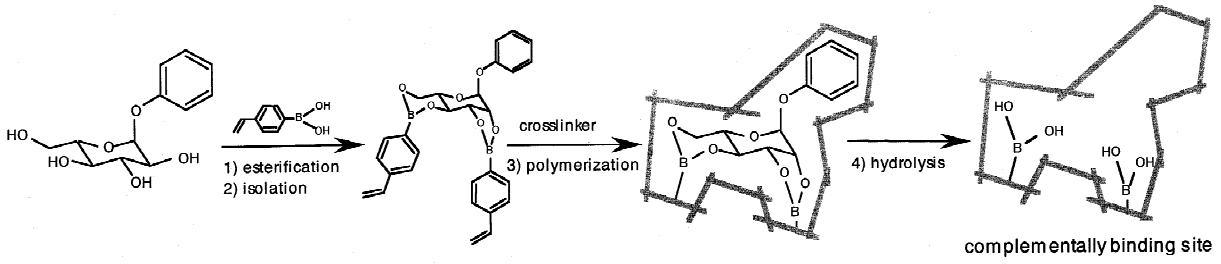

Fig. 8. Sugar-imprinted polymers based on covalent bonding [20].

ethyl methacrylate (2-HEMA) and ethylene glycol dimethacrylate. The introduction of hydrophilic 2-HEMA into the polymer resulted in good performance in both a buffer and a 50% (v/v) methanolic buffer solution, while without 2-HEMA the polymer only worked in the methanol/buffer system, suggesting that a somewhat hydrophilic property is necessary for the molecular recognition in aqueous media, which may facilitate the penetration of sialic acid.

It is expected that the methods described above could be applicable to the molecular recognition of sugar oligomers and sugar chains of glycoconjugates.

2.1.5. Steroids

A number of molecularly imprinted polymers were prepared for steroids such as cholesterol [21,86,87], corticosteroids [88,89], testosterone [87,90] and castasterone [91], however only a few imprinted polymers were applied as separation medium. Imprinted polymers for 11-α-hydroxyprogesterone and corticosterone, which have Δ^4-androsten-3-one structures, were prepared using methacrylic acid as functional monomer [89]. The imprinted polymer prepared could be employed to select the target steroid from a combinatorial library. Fig. 9 shows the separation of 11-α-hydroxyprogesterone from closely related steroids on the imprinted (Fig. 9a and b) and non-imprinted polymers (Fig. 9c). As shown in Fig. 9a,b, 11-α-hydroxyprogesterone was efficiently separated from other steroids on the imprinted polymer by isocratic and gradient elution, respectively, while the non-imprinted polymer showed no significant selectivity.

A testosterone-imprinted polymer was synthesized using methacrylic acid and ethylene glycol dimethacrylate as a functional monomer and crosslinker, respectively [90]. The most selective polymer bound testosterone over four times more strongly than did a non-imprinted polymer and at least three times more selectively than other steroids such as β-estradiol, estrone and progesterone. However, the imprinted polymer prepared by a covalent imprinting method using testosterone methacrylate and ethylene glycol dimethacrylate gave less selectivity for testosterone than that prepared by a noncovalent imprinting method. The authors suggested that this is due to the resistance to hydrolysis of the esterified polymer. It is concluded that the noncovalent imprinting method is vastly superior for this substrate.

Imprinted polymers for a brassinosteroid, castasterone, which is a plant hormone having a steroidal skeleton with a *cis*-diol group, were prepared by covalent and noncovalent imprinting methods using *p*-vinylbenzeneboronic acid and methacrylic acid, respectively, as the functional monomers [91]. Both imprinted polymers showed excellent selectivity for the target molecule.

2.2. Capillary electrophoresis (CE) and capillary electrochromatography (CEC)

Methacrylate-based imprinted dispersion polymers for pentamidine were prepared in situ using methacrylic acid and ethylene glycol dimethacrylate, and used for CE separation [92]. Recently, the in situ preparation conditions for methacrylate-based imprinted polymers for CE and CEC were optimized to obtain superporous and flow-through polymers inside fused-silica capillaries; important factors are polymerization time, solvent (porogen) and concentration of crosslinker [93–96]. A too long polymerization time results in a dense polymer, through which rapid solvent and electrolyte exchange, as well as easy regeneration of the capillaries, are impossible by hydrodynamic pumping. The density and structure of the final polymer are highly dependent on the chemical nature of the porogens used. The thickness of the polymer and the crosslinker concentration gave a linear relation. Fig. 10 shows a schematic illustration of superporous three-dimensional molecularly imprinted polymers for CE and CEC separation [93]. Using capillaries coated with the imprinted polymers, enantioseparations of 2-phenylpropionic acid [93], aromatic amino acids [94] and local anesthetics [95] such as ropivacaine, mepivacaine and bupivacaine were obtained. The enantioseparations of β-adrenergic blockers such as propranolol and metoprolol were also attempted, where the separation systems were operational within 3 h of the start of capillary preparation, and chiral separation with baseline resolution could be achieved in less than 120 s [96].

Molecularly imprinted polymers prepared by a bulk polymerization method were crushed, sieved and used for CE and CEC separations as well as LC

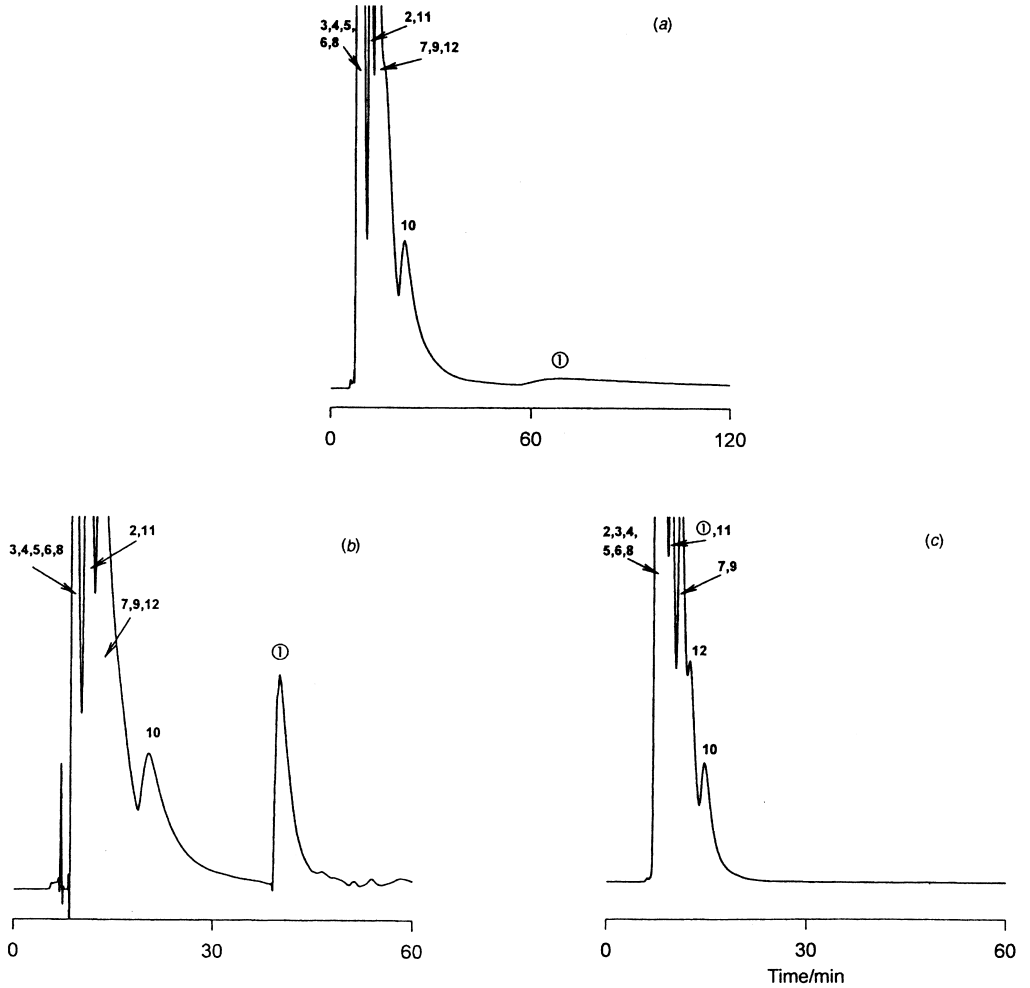

Fig. 9. Screening of a steroid library. Reproduced from Ref. [89] with permission. (a) An imprinted polymer prepared for 11-α-hydroxyprogesterone. Isocratic elution, dichloromethane (DCM) 0.1% acetic acid (v/v), 0.5 ml/min. Sample, 20 μl; concentration, 0.8 m*M* of each component. (b) An imprinted polymer prepared for 11-α-hydroxyprogesterone. Gradient elution, 0–25 min, DCM 0.1% acetic acid (v/v); 25–30 min, DCM 0.1–5% acetic acid (v/v); 30–40 min, DCM 5% acetic acid (v/v); 40–45 min, DCM 5–0.1% acetic acid (v/v), 0.5 ml/min. Sample, 20 μl; concentration, 0.8 m*M* of each component. (c) A control polymer prepared in the absence of template molecule. Isocratic elution, DCM 0.1% acetic acid (v/v), 0.5 ml/min. Sample, 20 μl; concentration, 0.8 m*M* of each component. Key: 1=11-α-hydroxyprogesterone, 2=11-α-hydroxyprogesterone, 3=17-α-hydroxyprogesterone, 4=progesterone, 5=4-androsten-3,17-dione, 6=1,4-androstadiene-3,17-dione, 7=corticosterone, 8=cortexone, 9=11-deoxycortisol, 10=cortisone, 11=cortisone-21-acetate, 12=cortisol-21-acetate.

separations. An imprinted polymer was prepared for L-Phe-anilide using methacrylic acid and ethylene glycol dimethacrylate as the functional monomer and crosslinker, respectively, and used for the separation of underivatized aromatic amino acids such as Phe, Tyr and phenylglycine using a polyacrylamide-supported molecularly imprinted polymer [97] or molecularly imprinted polymer-filled capillary column [98]. The obtained enantioselectivity using L-Phe-anilide as a template molecule was higher than that using L-Phe. The enantiomer of propranolol was resolved using an imprinted polymer prepared by *N*-acryloyl-Ala and ethylene glycol dimethacrylate, where the imprinted polymer is present in the

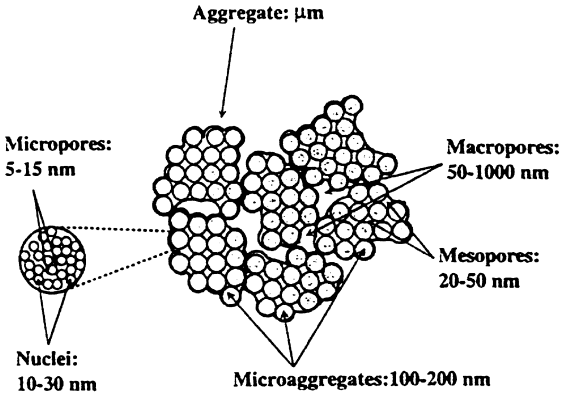

Fig. 10. Schematic illustration of superporous three-dimensional molecularly imprinted polymers for CE and CEC separation. Reproduced from Ref. [93] with permission.

background electrolyte as a suspension of particles [99].

CE and CEC combined with molecular imprinting technique could be a promising analytical tool for efficient and selective separation of given target molecules.

2.3. Affinity-based solid-phase extraction

Molecularly imprinted polymers sometimes possess too strong affinity for their target molecules, making the mass transfer rate of elutes slow in the chromatography process and leading to low separation efficiency, as well as natural antibody-based affinity media [100]. However, this feature is very suitable for an on–off-type separation such as traditional affinity chromatography where the substance of interest is first absorbed, then undesirable substances are washed out and, finally, the bound substance is recovered. Common affinity chromatography relies on biospecific interactions in which natural affinity ligands including proteins, antibodies, substrates and inhibitors of enzymes, nucleic acids, carbohydrates, ligands for receptors, etc., are immobilized on various supports [101]. The preparation of affinity sorbents, i.e. immobilization of natural ligands, which are normally precious and expensive, is tedious and time-consuming. Furthermore, care should be taken in the conservation of affinity sorbents because some natural ligands are not sufficiently stable for long-term storage.

Molecularly imprinted polymers are capable of molecular recognition and are stable, easy to prepare and inexpensive, thus they could be considered as artificial affinity media, and applied to specific on–off separation. In practice, the effectiveness of molecularly imprinted polymers for affinity separation has been demonstrated in applications to solid-phase extraction (SPE), which involves an on–off separation protocol for pre-concentration or pre-treatment of analytes.

The first molecularly imprinted SPE (MI-SPE) was reported for pentamidine, which is used for the treatment of AIDS-related pneumonia [78]. The high selectivity of the polymer allowed the drug to be sufficiently enriched to be analyzed directly even when present in low concentration in a urine sample. In this system, 20% (v/v) phosphate buffer in acetonitrile was used and the adsorption and desorption were switched by changing the pH of buffer: for adsorption, pH 5, and for desorption, pH 3. This means that electrostatic interaction is the main factor for the specific extraction of pentamidine. Sometimes a non-specific hydrophobic interaction reduces the selectivity of MI-SPE, however, in this case, by contriving the properties and content of the eluents used, the system gave good performance.

MI-SPE of triazine herbicides was reported using an atrazine-imprinted polymer [102]. This procedure

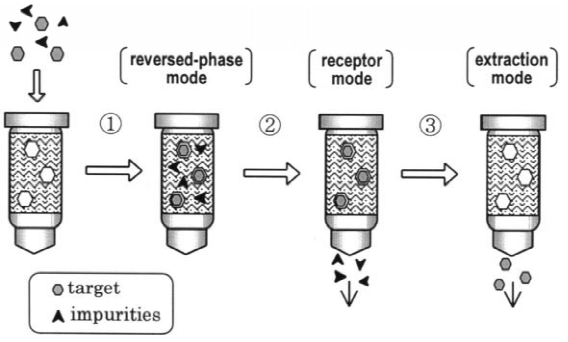

Fig. 11. Principle of the molecular imprint-based solid-phase extraction for triazine herbicides [102]. (1) Aqueous sample loading (reversed-phase mode); (2) selective washing with dichloromethane (receptor mode); (3) extraction with methanol (extraction mode).

consists of three steps (Fig. 11): (1) sample loading where the system works as a reversed-phase mode because an aqueous sample is loaded on the column; (2) washing with dichloromethane where the system was changed to a hydrogen bonding-based affinity mode in which triazine herbicides can be selectively retained in the polymer while other structurally unrelated impurities are washed off; (3) recovery of triazine herbicides with methanol where hydrogen bonding is significantly weakened because of the interference of hydrogen bond formation by methanol. By employing such MI-SPE, simazine (0.1 ppm), one of the typical herbicides commonly used on golf courses in Japan, was selectively concentrated to approximately 60 times with >90% recovery from a mixture of simazine, asulam, mecoprop, propyzamide and iprodione (0.1 ppm each, 500 ml aqueous solution).

Cleaning up of biological sample extracts by MI-SPE was demonstrated for the determination of triazine herbicides [103]. In this system, crude chloroform tissue extracts of beef liver were applied to a column filled with the atrazine-imprinted polymer. After the column was washed with chloroform to eliminate fats, lipids and other hydrophobic impurities, atrazine was typically recovered with 10% (v/v) acetic acid in acetonitrile. Although crude tissue sample extracts interfered with both reversed-phase chromatographic assay and enzyme-linked immunosorbent assay (ELISA) methods, the method using MI-SPE followed by HPLC or ELISA improved the accuracy and precision of quantification of atrazine at a tolerance level of 0.02 ppm in meat products.

Propranolol (β-blocker) selective MI-SPE was performed, in which propranolol was extracted from aqueous media including biological samples using methanol–water 1% triethylamine as eluent [104]. A low recovery was observed when the elution was performed with methanol or acetonitrile alone, whereas the use of triethylamine improved the recovery to >90% propranolol.

As another example, MI-SPE of sameridine, which is provided for anesthesia during surgery and prolonged post-operative analgesia, was reported, in which MI-SPE was performed prior to gas chromatographic analysis [105]. A non-steroidal estrogen antagonist, tamoxifen, was extracted from biological samples by MI-SPE [106]. More recently, this technique has been extended to the extraction of nicotine and its oxidation products in chewing gum [73] and theophylline in serum [107].

As can be seen, molecularly imprinted polymers have provided a new tool in affinity separation-based SPE. Because conventional affinity sorbents are expensive, it is no wonder that no disposable affinity sorbent for SPE is available. The development of SPE based on inexpensive imprinted polymers allows disposable-type affinity-SPE, making the availability of affinity separation with excellent selectivity widespread. Many applications would be expected in clinical, pharmaceutical, biochemical and environmental analyses.

2.4. Molecularly imprinted membranes

With respect to the applications of molecularly imprinted polymers, membrane applications could be one of the most desirable features because many analytical and preparative techniques involve membrane-type materials, such as membrane separation, sensors, etc.

A nucleotide base-imprinted polymer membrane was reported, in which methacrylic acid was used as a functional monomer for the imprinting of an adenine derivative, 9-ethyladenine [108]. A free-standing film was prepared by polymerizing a DMF solution containing methacrylic acid and ethylene glycol dimethacrylate on a silanized glass slide at 65–70°C under nitrogen atmosphere. Transport studies conducted by an H-shaped two-compartment cell with continuous stirring showed that the transport rate of adenine was higher than those of thymine and cytosine. In competitive transport studies, adenine again gave a higher rate than thymine. The steady-state flux of adenine was 1.62×10^{-6} mol. cm^{-2} h^{-1} and the selectivity factor, defined as the ratio of the flux of adenine to that of thymine, was 1.37. A non-imprinted polymer prepared without the template showed comparable rates for adenine, thymine and cytosine. A lower methanol content gave higher selectivity factors, suggesting the formation of more stable membrane-bound adenosine complexes in the less polar media. For adenosine, the membrane gave a faster flux rate than for guanosine, and the highest separation factor was observed to be

3.4 for the transport of adenosine over that of guanosine in methanol–chloroform (6:94, v/v). These results suggest that the membrane recognized the adenine structure and this allows selective acceleration for adenine and adenosine transportation.

A series of enantioselective imprinted membranes for amino acid derivatives was prepared using oligopeptides as functional monomers [109–112]. A tetrahydrofuran solution containing Boc-L-tryptophan, a template molecule as well as a porogen, a functional monomer of a tetrapeptide derivative attached to polystyrene resin that is commonly used in solid-phase peptide synthesis [H-Asp(OcHex)–Ile–Asp(OcHex)–Glu(OBzl)–CH_2–; cHex, cyclohexyl; Bzl, benzyl], a copolymer of acrylonitrile and styrene was poured into a flat laboratory dish and left for 24 h to remove the solvent. After the removal of the template molecule by washing with methanol, a membrane with thickness 140–150 μm was obtained. The adsorption selectivity showed a preference for the L-form of the template molecule. Enantioselective permeation was attained with the membrane and Boc-D-Trp was preferentially permeated through the membrane and the permeation rate was 1.4 times faster than that of Boc-L-Trp. This technique was applied to enantioselective electrodialysis. Although the enantioselectivity has not been satisfactory and the imprinting process and specific binding mechanisms have not yet been fully clarified, such easily prepared enantioselective membranes capable of molecular recognition could find new applications.

By using a phase-inversion technique, a theophylline imprinted membrane was prepared with acrylonitrile–acrylic acid copolymer [113–115]. The copolymer and the template molecule, theophylline, dissolved in dimethylsulfoxide was spread on a glass plate at about 100 μm thickness and coagulated in water. After the removal of theophylline by washing with methanol containing 0.1% (v/v) acetic acid, a theophylline-imprinted polymer membrane was obtained. The membrane showed selective uptake of theophylline; the amount of theophylline uptake was 52, 3.9 and 3.2 times higher than that of caffeine, 2-hydroxyethyltheophylline and uracil, respectively. According to IR analysis, the selective binding of theophylline was found to be due to hydrogen bond formation between theophylline and the hydroxy group of non-dimerized carboxylic acid segments. This membrane was prepared without crosslinking and this might be merit for attaining 'induced fit' in polymers, allowing the binding to be more biomimetic compared with conventional robust crosslinked polymers.

Recently, an ultrathin film composite membrane selective for theophylline was reported [116]. The technique entails the preparation of a theophylline-imprinted polymer inside the pores of a microporous alumina support membrane (thickness, 500 nm; pore size, 20 nm), in which pores of the membrane were filled by the polymerization solution containing the template theophylline, methacrylic acid, and ethylene glycol dimethacrylate, and the membrane was illuminated with UV light for 1 h, followed by immersion in methanol containing 10% (v/v) acetic acid to remove the template and any excess monomer. The selective transport of theophylline was observed in permeation experiments in methanol. The rate of transport for theophylline was faster than that of caffeine and the selectivity for theophylline over caffeine was 2.6, while it was 0.73 in the non-imprinted membrane prepared without the template. Because the membrane is extremely thin, the flux rate is relatively high compared to the 9-ethyladenine-imprinted membrane described previously [108]; it was at least two orders of magnitude more than the flux in the 9-ethyladenine-imprinted membrane.

3. Assays and sensing systems

3.1. Molecularly imprinted sorbent assays

Molecularly imprinted polymers have attracted a great deal of attention as biomimetic sensing materials for the supplemental use of biosensing because biomolecule-based reagents and elements are unstable and not so easy to operate, though the performance is known to be excellent. Molecularly imprinted sorbent assays are the most typical application of biomimetic use. Usually, this system involves competitive binding of an analyte with a certain amount of labeled ligand, in which the labeled ligand unbound is proportional to the analyte added. Because the dissociation constants of com-

mon imprinted polymers are around 10^{-6} to10^{-9} *M*, competitive binding assays could be easily performed. In practice, many molecularly imprinted sorbent assays have been developed for biologically active compounds, including theophylline [117], diazepam [117], methyl-α-glucoside [81], morphine [118], Leu-enkephalin [118], atrazine [119,120], corticosteroid [88], yohimbine [121], *S*-propranolol [61,122], cyclosporin A [123], and 2,4-D [124].

The results for this technique appear to be correlated with those in an enzyme immunoassay [117] (Fig. 12) and may be useful as a supplement for antibody-based assays because imprinted polymers can be stable under severe conditions which natural molecules cannot tolerate, such as organic solvents, acidic/basic solutions, high temperature, etc. Molecularly imprinted polymers would resemble antibodies in their tailor-made fashion, therefore imprinted polymers could be called 'artificial antibodies', although the polymers are not constructed by biomolecules and they are different in nature. Currently, molecularly imprinted sorbent assays are usually performed with radio-ligands. Non-isotopic assays are desired, however they cannot be established easily, because binding sites in imprinted polymers are fit to the template and labeled templates are not usually suitable for the binding sites. Efforts in the development of non-isotopic molecularly imprinted sorbent assays have been made and several studies have been reported [125–128], however further development should be addressed to developing more reliable methods for practical use.

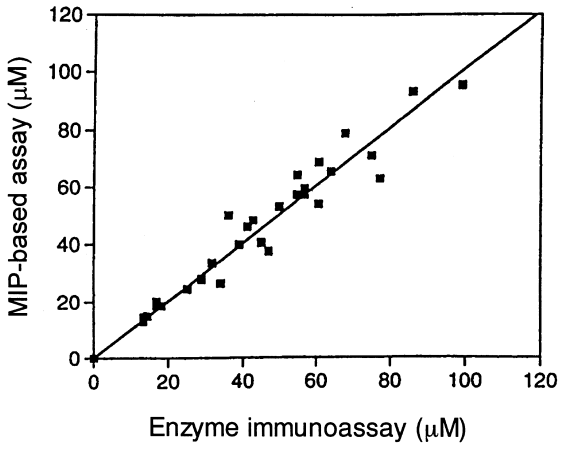

Fig. 12. Correlation between a molecularly imprinted polymer-based competitive binding assay and an enzyme immunoassay for 32 samples of serum theophylline [117]. The correlation coefficient was calculated to be 0.98.

3.2. Sensing systems

Various imprinted polymer-based sensors have been developed [129]. Preliminary experiments have been reported on an L-phenylalanine anilide-imprinted polymer membrane field-effect capacitor, in which similar capacitance changes were given for L-phenylalanine anilide and structurally similar tyrosinanilide; however, for the less structurally related phenylalaninol, only a small change was observed [130].

Membrane-based conductometric sensors for various target compounds have been reported [131–133]. Interestingly, an opposite sensor response was observed between a covalent bonding-based imprinted polymer and a noncovalent bonding-based imprinted polymer; in the covalent system, the conductivity decreased with increasing target compound, while the noncovalent systems showed opposite behavior. These phenomena were explained as being due to the difference in the number of binding sites available for specific binding between both systems, which leads to the difference in the degree of shrinking when the template was added. In the covalent system the number of binding sites available for specific binding would be greater than that in the noncovalent system and the binding sites could be more homogeneous. When the target molecule was bound to the polymer, the covalent bonding-based polymer shrank more, reducing the polymer's micropores. This may cause a decrease of electroconductivity based on ion transfer.

A competitive amperometric morphine sensor was constructed, where the sensor was prepared as follows: a platinum electrode was dipped into morphine-imprinted polymer suspended in aqueous agarose solution, followed by crosslinking of agarose with treatment of epichlorhydrine and sodium borohydride in an alkaline solution [125]. The procedure involves two steps: (1) morphine binding to the sensor for 2 h; (2) an electroinactive competitor, codeine, is added in excess and the released morphine can be selectively detected by amperometry

after 20 min. The sensor could measure morphine in the concentration range 0.1–10 μg ml^{-1}.

A fluorescence optical sensor for dansyl-L-phenylalanine was reported [134]. In the optical sensor, the imprinted polymer was placed in front of a fiber-optic device and held in place by a nylon net. Although the system worked well, there are some inherent problems that should be addressed: the time required for a steady response was 4 h which appears to be too long, and only fluorescent analytes could be applied to this system.

Besides the sensors described above, a benzyltriphenylphosphonium-selective conductometric sensor was demonstrated [135]. A fluorescence detection system for sialic acid was also reported [136]. In sialic acid detection, *o*-phthalaldehyde reagent was used for the fluorescence measurement of the polymer matrix containing an amine residue. When sialic acid was bound to the polymer, the fluorescence intensity increased, because the permeability of the *o*-phthalaldehyde reagent would increase due to the swelling change by the binding of the template. The fluorescence increase was proportional to the sialic acid bound.

The optical detection of an antibiotic, chloramphenicol, based on competitive displacement of a chloramphenicol–methyl red conjugate bound to a chloramphenicol-imprinted polymer with free chloramphenicol was demonstrated [126]. A flow injection system in conjunction with a 10 cm stainless steel column packed with the imprinted polymer and acetonitrile as a carrier solution containing chloramphenicol–methyl red conjugate was constructed. The dye conjugate released due to the displacement by free chloramphenicol was monitored at 460 nm. The signals were proportional to the concentration of free chloramphenicol injected, and the calibration range of this system included the therapeutic range of chloramphenicol. This concept of flow displacement systems could be applicable not only for chloramphenicol determination but also for other template molecules.

An interesting glucose-sensing system based on a metal-complexing glucose-imprinted polymer, involving ligand-exchange on a triazacyclononane–Cu^{2+} complex was reported [31] (Fig. 13). The polymer was prepared by the crosslinking polymerization of a copper (II) complex of a new chelating

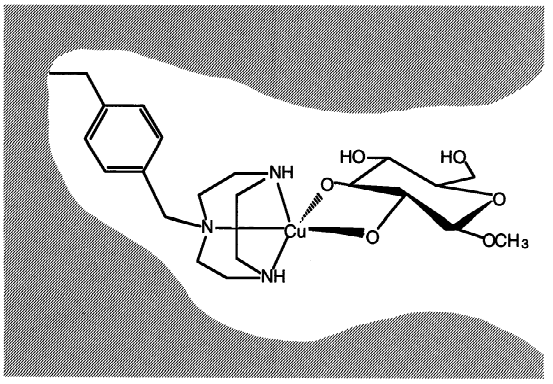

Fig. 13. Schematic representation of a binding site in the glucose-sensing polymer [31]. Functional monomer, 1-(4′-vinylbenzyl)-1,4,7-triazacyclononane.

functional monomer, 1-(4′-vinylbenzyl)-1,4,7-triazacyclononane, and methyl-β-D-glucopyranoside. After the removal of methyl-β-D-glucopyranoside, the resultant polymer bound glucose selectively at alkaline pH, with the release of protons in proportion to the concentration of glucose. By operating in an appropriate alkaline pH region where the buffer capacity of biological samples is small, interference with the measurement of protons released in biological samples was minimized. Equilibration of the complexation is very rapid, which therefore suggested that this system would be suitable for continuous glucose monitoring in clinical and bioprocess applications.

A combination of polyurethane-based molecularly imprinted polymers with mass-sensitive transducers such as quartz microbalances and surface acoustic wave resonators was attempted [137]. Although the selectivity and affinity seem to be insufficient because the main force of the binding is derived from Van der Waals interactions, the sensor appeared to detect vapors of polar solvents, aromatic solvents and halogenated hydrocarbons, suggesting that this type of sensor could be applicable for volatile compounds. A dye-doped imprinted polymer was also reported, in which a substituted 3,3-diphenylphthalimide was embedded in an acidic polyurethane-based polymer. Because the dye forms a colored carbenium ion under acidic conditions, if the polymer interacts with a compound, the absorbance could be reduced due to the interference

from the interaction between the dye and the acidic portion of the polymer. It is difficult to evaluate this system at the current stage, because selectivity tests have not been fully addressed, however the concept would be applicable to other molecular imprinting systems.

A fluorescent molecularly imprinted polymer for aqueous adenosine 3′,5′-cyclic monophosphate (cAMP) was prepared [138]. *trans*-4-[*p*-(*N*,*N*-Dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride was used as the functional monomer for interacting with the template molecules by electrostatic and aryl stacking. In addition to the functional monomer, a large amount of 2-hydroxyethyl methacrylate (HEMA) was mixed in order to increase the hydrophilicity of the polymer and for supplemental hydrogen bond formation with the template, because it has been reported that the use of HEMA improves the recognition ability of sialic acid-imprinted polymer in aqueous solution [84,85]. When cAMP was bound to the polymer, the fluorescence of the polymer was quenched, and was proportional to the amount of cAMP added. In contrast, adding cAMP did not change the fluorescence of a control polymer that was prepared without the template, cAMP. For a structurally similar molecule, guanosine 3′,5′-cyclic monophosphate (cGMP), fluorescence quenching was not observed and it appears that cAMP is selectively bound to the imprinted polymer and the binding event is recorded by the quenching measurement. It should be noted that the association constant of the free functional monomer with cAMP is reported to be 13.8 M^{-1}, whereas that of the imprinted polymer is 3.5×10^{5} M^{-1}, suggesting that the three-dimensional polymer network affects the specific binding and enhances the affinity.

More recently, a nucleobase derivative, 9-ethyladenine-imprinted polymer capable of spectroscopic change based upon molecular recognition was prepared using the metalloporphyrin-based functional monomer [32] shown in Fig. 4. The polymer strongly bound adenine, 9-ethyladenine and 4-aminopyridine; however, it did not bind thymine, cytosine, or 2-aminopyridine at all. When the concentration of 9-ethyladenine was increased, the visible absorbance spectra of the polymer dispersed in chloroform showed a red shift and the degree of the shift was dependent upon the concentration change.

As can be seen there are three ways to achieve signaling molecularly imprinted polymer systems: (1) detection of signals due to the inherent properties of the target molecules; (2) detection of labeled molecules; (3) detection of the changing properties of polymers by the binding of target molecules. These recordable molecular recognition phenomena will open potential applications, including biomimetic sensors and molecularly imprinted sorbent assays. Regarding the response time of sensors, this should be improved in order to achieve the rapid determination that is essential in practical use. Ultrathin membranes, fine particles and other material engineering techniques should be developed for the feasible and reliable use of molecularly imprinted polymers in practical applications.

4. Conclusion

The origin of molecular imprinting may go back to Fischer's 'lock and key concept' [139], Pauling's 'production of antibodies in vitro' [140–142], and Dickey's 'specific adsorbents' [143,144]. However, modern molecular imprinting was clearly established by Wulff and Mosbach, and their pioneering work has led to the current flourishing of molecular imprinting, although many investigations are required for the improvement of imprinted polymers for practical use, such as the improvement of the mass transfer rate in imprinted polymers, the development of new functional monomers necessary for stronger affinities, higher selectivities, signaling properties for the readout of specific binding events, and the acceleration of catalytic activities. Much effort has been made in the development of new artificial systems comparable to biomolecules using the molecular imprinting approach, in which knowledge of biomimetic chemistry and supramolecular chemistry would be helpful and their combination could generate new ideas and open a new way of preparing the next generation of bio-functional polymers applicable to specific separation media, artificial antibodies, sensors, and catalysts.

The recent advancement of high-throughput screening methods in combinatorial chemistry may also contribute to the further development of molecular imprinting if appropriate polymer synthesis and

assay systems could be established. Because the factors that affect molecular imprinting are not yet fully understood, a continual series of trial-and-error experiments is necessary. Therefore, the best method currently for acquiring high-performance molecularly imprinted receptors seems to be to examine thousands of various polymers prepared using different combinations and amounts of agents under different polymerization conditions. For this purpose, combinatorial techniques would be most suitable, and efforts at such combinatorial molecular imprinting are currently ongoing [145].

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