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Review

Recent applications of molecular imprinted polymers for enantio-selective recognition

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ABSTRACT

Molecular imprinted polymer (MIP) techniques have been increasingly used in a variety of fields including chromatography, sample pretreatment, purification, sensors, drug delivery, and catalysts, etc. MIP is a specific artificial receptor that shows favored affinity to the template molecule. The cavities of the template are produced by carrying out polymerization of a reaction mixture followed by eliminating the template molecules by washing. Various forms of MIP materials have been prepared for diverse applications including irregularly ground particles, regular spherical particles, nanoparticles, monoliths in a stainless steel or capillary column, open tubular layers in capillaries, membranes, surface attached thin layers, and composites, etc. When an enantiomer is used as the template, then the resulting MIP can show capability of enantiomeric recognition between the pair of enantiomers. In this review, progresses in applications of enantio-selective recognition by MIPs will be critically reviewed for the recent period since 2007.

Contents

Abbreviations: ACN, acetonitrile; AFM, atomic force microscopy; AIBN, 2,2′-azobis-isobutyronitrile; APS, ammonium persulfate; AM, acrylamide; AML, amlodipine; ATRP, atom-transfer radical polymerization; BMA, butyl methacrylate; Boc, *tert*-butoxycarbonyl; Boc-Trp, *tert*-butoxycarbonyl tryptophan; BP, brompheniramine; Cbz-L-Trp, carbobenzyloxy-L-tryptophan; CCD, charge-coupled device; CD, cyclodextrin; CDCA, chenodeoxycholic acid; CEC, capillary electrochromatography; CIT, citalopram; CL, chemiluminescence; CP-MAS, cross polaraization magic angle spinning; CP, chlorpheniramine; CS, camphorsulfoamide; CSA, camphorsulfonic acid; CTH, camphor-*p*-tosyl hydrazone; DA, dopamine; DCEE, 2,2′-dichlorodiethylether; DVB, divinylbenzene; DMAEMA, dimethylaminoethyl methacrylate; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; DPAP, 2,2-dimethoxy-2-phenyl acetophenone; EDMA, ethylene glycol dimethacrylate; ee, enantiomeric excess; EIS, electrochemical impedance spectroscopy; FE-SEM, field emission scanning electron microscopy; Glu, glutamic acid; HEMA, 2-hydroxyethylmethacrylate; IA, itaconic acid; ITO, indium–tin oxide; LC-MIP, liquid crystalline molecular imprinted polymer; LODs, limits of detection; L-THF, L-tetrahydropalmatine; L-Tyr, L-tyrosine; MAA, methacrylic acid; MAC, methyl-(*Z*)-a-*N*-acetamidocinnamate; MAM, 2-methacrylamidopropyl methacrylate; MBA, methyl benzylamine; Me_4Cyclam, 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane; MIP, molecular imprinted polymer; MIPCM, molecularly imprinted composite membrane; MIP-NOM, molecularly imprinted polymer nanoparticle-on-microspheres; MIP-SG, MIP on silica gel; MMA, methyl methacrylate; MISSM, molecularly imprinted self-supporting membrane; MPS, γ-methacryloylpropyl trimethoxysilane or 3-(trimethoxysilyl) propyl methacrylate; MQD, methacryloyl quinidine; MQN, methacryloyl quinine; *N*, number of theoretical plates; NEA, (1-naphthyl)-ethylamine; NHSG, nonhydrolytic sol–gel; NIP, non-imprinted polymer; NMMO, *N*-methylmorpholine-*N*-oxide; NMP, *N*-methyl pyrrolidone; NOM, nanoparticle-on-microspheres; NP, nanoparticle; o-PD, *o*-phenylenediamine; ONZ, ornidazole; OT, open tubular; PAA, phenylalanine anilide; pCEC, pressurized capillary electrochromatography; PCL-T, polycaprolactone-triol; PDMAEMA, poly(dimethylaminoethyl methacrylate); PEG, polyethyleneglycol; PFPS, perfluoro polymeric surfactant; Phe, phenylalanine; PIP, piperazine; PLOT, porous layer open tubular; PMC, perfluoro(methylcyclohexane); PO-CL, peroxyoxalate chemiluminescence; PPy, polypyrrole; PS, polystyrene; PSf, polysulfone; PVA, poly(vinyl alcohol); PVDF, poly(vinylidene fluoride); R, resolution; RSD, relative standard deviation; RTIL, room temperature ionic liquid; SBSE, stir bar sorptive extraction; SCC, 6-styrylcoumarin-4-carboxylic acid; SDS, sodium dodecyl sulfate; SEM, scanning electron microscopy; SPE, solid phase extraction; SSA, styrenesufonic acid; TCPO, bis(2,4,6-trichlorophenyl) oxalate; TEDMA, triethyleneglycol dimethacrylate; TEMED, *N*,*N*,*N*′*N*′-tetramethylethlenediamine; TFMAA, 2-(trifluoromethyl) acrylic acid; THF, tetrahydrofuran; THP, tetrahydropalmatine; TMC, trimesoyl chloride; TPD, temperature programmed desorption; TRIM, trimethylolpropane trimethacrylate; Trp, tryptophan; Tyr, tyrosine; UDCA, ursodeoxycholic acid; VCC, 6-vinylcoumarin-4-carboxylic acid; VPY, vinylpyridine

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1. Introduction

A MIP reaction mixture is commonly composed of a template, a functional monomer or monomers, a cross-linking monomer, a polymerization initiator, and a porogenic solvent. It is known that complex formation between the template and the functional monomer is very critical for fabrication of MIP, and the complex is surrounded by the surplus cross-linking monomer. A 3-dimensional polymer network with trapped template molecules is formed after completion of polymerization. The template molecules are taken off by exhaustive washing to give empty cavities complementary to the template in size, shape, and molecular interactions. The properties, physical appearance, morphology, and performance of the MIP are determined by polymerization conditions such as choice of functional monomer, cross-linking monomer, and porogen; mixing ratios of them; and reaction temperature and time, etc. The schematic illustration of MIP formation is given in Fig. 1.

There have been numerous review articles regarding MIPs. There have been several review articles on general and extensive introduction of MIPs [1–5]. Some of them are devoted to chiral recognition [2,3]. Reviews on characterization, evaluation and optimization of MIPs have also been published [6–10]. There have been many reviews on capillary electrochromatography (CEC) application of MIPs [11–19], an area of sharply increased attention. Most of them include discussion on enantiomeric separation. Reviews have also been made on specific forms of MIPs such as monoliths [18,19], particles [20–23], and membranes [6,24], etc. MIP has been incorporated in solid phase extraction (SPE), a powerful sample pretreatment/enrichment tool. Thus some reviews concerning SPE coupled with MIP have appeared in the literature [25–27]. The potential usefulness of MIPs in drug delivery has been examined in some review articles [28–30]. Application of MIPs as artificial enzymes or receptors for antibodies has also been reviewed in some articles [31–35]. There have been many reviews for MIP-based sensors [22,36–44] probably owing to their huge potential market. There are also some miscellaneous reviews on drug discovery with MIPs [45], MIPs incorporated with electrically conducting polymer [46], and template removal from the MIP matrix [47].

This review will focus on MIP applications only in enantiomeric recognition. Diverse studies of enantiomeric recognition by MIP methodologies in recent 5 years will be critically discussed. Applications in HPLC, CEC, SPE, sensors, and miscellaneous fields will be included in this review.

2. Enantiomeric recognition in HPLC

2.1. Bulk monolith MIPs

Monolithic MIP stationary phases have been simple and cost-effective compared to other types of MIPs [18,19]. Ou and Zou

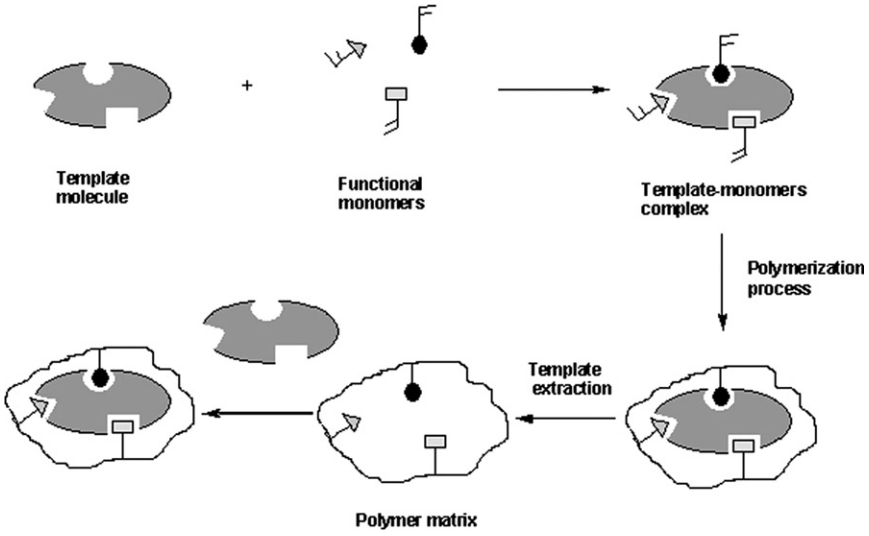

Fig. 1. The schematic illustration of preparation of molecule imprinted polymer (reproduced with permission from Ref. [1]).

et al. [48] prepared MIP derivatized silica monolith for HPLC and CEC application for chiral separation of tetrahydropalmatine (THP) and Tröger base. The emphasis of this work was rather on CEC, thus it will be reviewed in more detail in the relevant section later.

Use of a co-template was proposed to improve the MIP morphology by Haginaka et al. [49] who used *N*-carbobenzyloxy-L-tryptophan (Cbz-L-Trp) as a co-template for preparation of (+)-nilvaldipine MIPs by heating (45 °C, 24 h) the reaction mixture composed of (+)-nilvadipine, Cbz-l-Trp, 4-vinylpyrine (VPY), ethylene glycol dimethacrylate (EDMA), toluene, 1-dodecanol, and 2,2′-azobis-isobutyronitrile (AIBN). The optimum template: co-template mol ratio was 2:1. Sufficient macropores were formed in this MIP to give good chiral separation (α:1.4, R:1.1) while there were no macropores in the MIP prepared without co-template to give no separation (too broad peak bandwidths).

An enantio-selective MIP has been prepared by using supercritical fluid (CO_2) as a porogenic solvent [50] where *tert*-butoxycarbonyl (Boc)-L-tryptophan (Trp) was used as template, EDMA as cross-linker, *N*-isopropylacrylamide as functional monomer, and AIBN as initiator, and the reaction was carried out at 65 °C under 21 MPa CO_2 for 24 h. The resultant MIP was obtained as irregular particles although it was carried out in the bulk-like mode, thus they were slurry-packed in a column. The MIP showed some chiral separation performance in HPLC for Boc-Trp enantiomers. However, the peak bandwidths were rather broad, and the chromatographic resolution was only ca 0.5, certainly reflecting the limited porogenic power of supercritical fluid. Using supercritical fluid as porogenic solvent seems to be useful for production of MIPs of pharmaceutical application. Despite the demonstration for "green" production protocols for MIPs, further studies are required especially to enhance the porogenic power of the system for true realization of this approach. This study does not fit to the bulk monolith MIPs category well, but is included here since it was prepared in the bulk-like mode and there is no proper section for this phase otherwise.

2.2. Ground and sieved MIPs

The MIP particles prepared by bulk polymerization followed by grinding and sieving have been known to show inferior separation performance to the stationary phases prepared by other methods due to the intrinsic heterogeneity of the MIP cavities in the bulk MIP. Thus the number of publication on application of such phases in HPLC tends to decay although this method seems to still have some feasibility in large scale separation.

MIPs with racemic or L-mandelic acid as template were recently prepared, ground, sieved, and packed in a HPLC column to examine retention behaviors of mandelic acid and its analogues [51]. 4-VPY or acrylamide (AM) was used as monomer. The MIP made of 4-VPY showed much greater retention for the template compared to the other analogues, but had no chiral separation ability. On the other hand, the MIP made with AM showed somewhat different retention times for L-and D-mandelic acid, but their bandwidths were very broad to result in poor resolution. Methanol was used as the polymerization solvent, and the reason was not explained. Use of a polar solvent in the MIP formation is well known to impose a negative effect on the molecular recognition.

(-)-Cathine (norpseudoephedrine) was used as a template to make a bulk MIP for separation of Cathine enantiomers [52]. The reaction mixture composed of (-)-cathine, MAA (methacrylic acid), EDMA, AIBN dissolved in chloroform, was polymerized at 5 °C under UV radiation. The resultant MIP was ground, sieved (5–10 μm), and packed in a HPLC column. A good baseline separation was obtained with separation factors (α) ranging 1.5–2.9 (Fig. 2) although the authors commented that elution

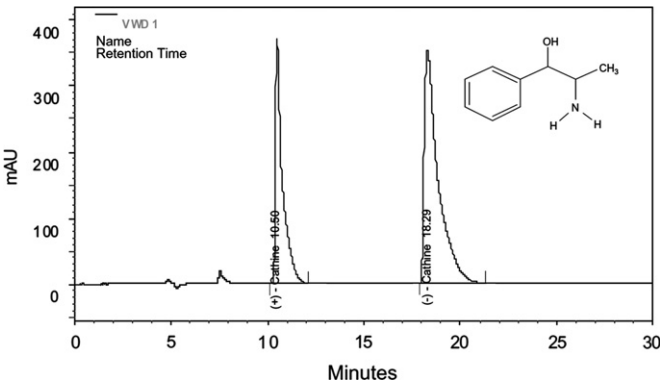

Fig. 2. The chromatogram monitored at 257 nm obtained with the (-)-Cathine MIP in a mobile phase composed of water, acetic acid and acetonitrile (2:7:91, v/v) at a flow rate of 0.5 mL/min at 20 °C. The Cathine structure is also given. Reproduced with permission from Ref. [52].

peaks were broad due to the heterogeneity of binding sites on MIP particles and the possible non-specific interaction.

2.3. Spherical MIP particles including uniformly sized particles

There have been some hopeful expectations for improved performances with particle MIP formats. Future industrial applications may be realized with MIPs prepared by suspension or precipitation polymerization coupled with surface imprinting [20,21]. Core-cross linked nanoparticles prepared by mini-emulsion polymerization show monoclonal binding behavior with enhanced recognition selectivity [22,23].

A new synthetic method was developed to control the size of uniformly sized *(S)*-propranolol MIP beads in the range of 130 nm to 2.4 μm in precipitation polymerization, where the particles size increased with a decrease of relative amount of trimethylolpropane trimethacrylate (TRIM) to divinylbenzene (DVB) [53]. The authors used the products for radioligand binding assays for *(S)*-propranolol but not for HPLC. The information on pore size distribution and total pore volume was not given. The MIP beads of 1.5–3 μm sizes could have been well used for HPLC purposes.

Uniformly sized MIPs for D-chlorpheniramine (D-CP) were prepared by a multi-step swelling and polymerization method [54] using MAA or 2-(trifluoromethyl) acrylic acid (TFMAA) as functional monomer and toluene, phenylacetonitrile, benzylacetonitrile or chloroform as porogen. The first step was dispersing polystyrene seed particles in water with dibutyl phthalate and sodium dodecyl sulfate. The second step was adding a dispersion of initiator, porogenic solvent, and polyvinyl alcohol (PVA) in water. The third step was adding a dispersion of D-CP (template), EDMA (cross-linker), TFMAA or MAA (functional monomer), and PVA in water. After polymerization at 50 °C under argon for 24 h, the polymer beads of ca 5–11 μm were obtained. Except for the nonporous MIP prepared with TFMAA and chloroform, all the other MIPs had proper surface area, pose size, and total pore volume. Nevertheless, all the MIPs showed rather disappointing enantio-selective properties. The typical chromatographic resolution was far less than 1. Serious heterogeneity of MIP cavity sites caused by severe agitation during MIP formation or improper pore size distribution (data was not shown in the article) might contribute to band broadening. Further study with emphasis on pore size distribution seems desirable.

Aiming to explore the feasibility for separation of ursodeoxycholic acid (UDCA) from its enantiomer chenodeoxycholic acid (CDCA) in a large-scale operation, a core–shell MIP with UDCA as template was prepared [55] with an emphasis on porogenic effects on the morphology and enantio-selectivity. Liquid solvents

like toluene, dodecyl alcohol/cyclohexanol, acetone and solid granules like $CaCO_3$, Na_2SO_4 were examined as porogens. Molecular simulation was used to predict the effect of liquid solvents. The computed absolute magnitudes of total interaction energy (ΔE) for the MIPs were in the following order: toluene > dodecyl alcohol/cyclohexanol > acetone > without porogen. Actually, toluene showed the best enantio-selectivity to UDCA with separation selectivity factor of 3.24. Incorporation of inorganic granules (*co*-porogen) in addition to toluene was found to significantly improve the MIP properties (surface area and porosity) and enatioselectivity. Thus a mixture of EDMA, AM, and UDCA, seed latex in toluene was admixed with an aqueous calcium carbonate suspension with an aid of sodium dodecyl sulfate. After nitrogen purging, an aqueous ammonium persulfate (APS) solution was added to initiate the copolymerization. The reaction was maintained for 6 h at 70 °C. The MIP prepared with the combined toluene–$CaCO_3$ porogen had large pores volume (0.46 mL/g) in addition to high specific surface area (138.88 m^2/g). This MIP showed higher adsorption capacity and fast adsorption kinetics. The improved enantiomeric recognition performance of this MIP was monitored in a chromatogram with a chromatographic resolution of ca 4. This study adds to the hope for development of enantio-selective MIPs toward large scale separation.

Molecularly imprinted polymers for CP, D-CP, brompheniramine (BP) and D-brompheniramine (D-BP) were prepared by dissolving template, MAA, TRIM and AIBN in chloroform [56]. Each de-oxygenated pre-polymerization solution was suspended in water at 9500 rpm, and polymerized by UV at 4 °C for 6 h. Imprinted polymers were also prepared using dispersion speeds of 13,500 and 24,000 rpm. The higher the agitation speed, the smaller particles size and the narrower size distribution (also increased BET surface area and pore volume) were obtained. Controlled polymer beads in the low micron range were made. Some enantio-selectivity was observed with the columns of the MIP particles with single enantiomer as template (D-CP or D-BP) and cross-selectivity (enantomeric separation of racemic CP with D–BP MIP, for example) was also found although the chromatographic resolution in any case was less than 1 with broad bandwidths. It seems difficult to compensate for the heterogeneity of MIP cavities caused by the vigorous agitation during suspension polymerization even by significant improvements in particle size and morphology.

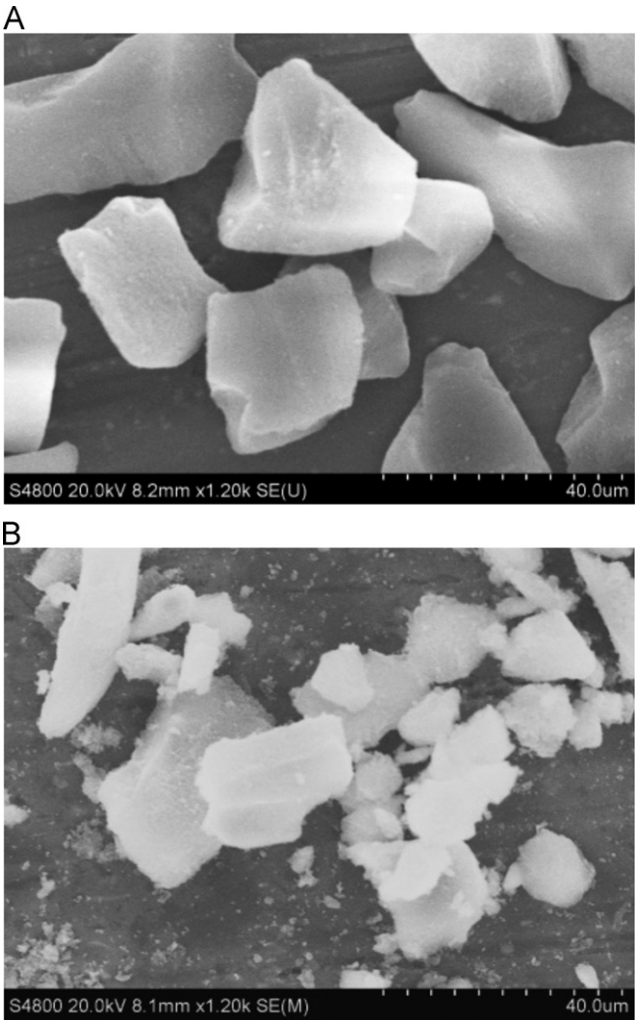

Fig. 3. Scanning electron micrograph images of (A) bare silica and (B) MIP-SG (polymerization time, 24 h). Reproduced with permission from Ref. [57].

2.4. Silica gel based MIPs

An enantiomer of Boc-Trp was used as a template, and a MIP layer was grown from the surface of silica gel particles via atom-transfer radical polymerization (ATRP) [57]. 3-Amimopropyl dimethylethoxysilane was reacted with silica to give amino groups. Then the initiator ligand, (4-chloromethyl) benzoyl chloride was reacted to give terminal chlorine through amide bond formation. Cu(I)Br, Cu(II)Br_2, and 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam) were used to form the organometallic catalyst. 2-VPY was used as functional monomer, EDMA as cross-linker, and acetonitrile, as solvent. The resultant MIP on silica gel (MIP-SG) had improved mass transfer properties and higher binding capacity per unit mass of polymer as well as better chromatographic resolution compared to MIPs prepared by the conventional method. However, disappointingly, the chromatographic chiral resolution for the Boc-Trp enantiomers was far less than 1. A possible reason for this is deformation of the silica particles in the course of ATRP as shown in Fig. 3 although the author stated that such deformation was negligible.

A specific MIP was prepared by surface molecular imprinting of L-Trp on functionalized silica (40–60 μm, 150 A°) with γ-methylacryloxypropyltrimethoxysilane (MPS, a double bond supplying ligand) and a beta-cyclodextrin (CD) moiety [58] using AM as functional monomer, *N*,*N*′-methylenebiacrylamide as crosslinker, *N*,*N*,*N*′,*N*′-tetramethylethlenediamine (TEMED) and APS as catalytic initiator, and pH 6.2 aqueous phosphate buffer

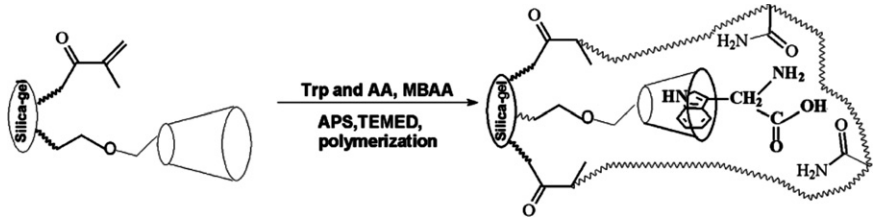

Fig. 4. The procedure of the immobilization of imprinted polymer on CD-modified silica gel formation. Reproduced with permission from Ref. [58].

as solvent. The polymerization was carried out at 37 °C for 1 h. For comparison purpose, another MIP was prepared on plain silica functionalized with only MPS (without CD moiety) maintaining the other conditions the same, and still another MIP on the CD modified silica but without using AM in the polymerization. The selectivity of the MIP synthesized with bonded –CD and AM, was superior to those obtained with only AM or bonded –CD. The authors explained that the CD moiety could interact with the indole ring of the template molecule hydrophobically and AM could form hydrogen bond with the amino and carboxyl group of tryptophan (Fig. 4). Nevertheless, the chiral separation resolution of the best MIP was less than 1 owing to broad bandwidths. A few factors may be responsible for such poor separation performance. First, the particle size of silica gel was too large. The results could have been much better if silica particles of 5–10 μm had been used. Second, an aqueous media was used as the solvent. Such a polar solvent might reduce the functional monomer-template interaction. Finally, the MIP formed should narrow the pores of silica to cause retarded mass transfer kinetics. Improvements with considering these factors might have resulted in MIPs of much better performance.

Most recently, a sophisticated core/shell type L-phenylalanine (Phe) imprinted polymer was synthesized on 1 μm hybrid silica particles using acryloyl-β-cyclodextrin and MMA as binary functional monomers, EDMA as crosslinker, AIBN as initiator, and 17/3 (v/v) acetonitrile/water as porogenic solvent [59]. The hybrid silica beads with $-CH=CH_2$ groups were prepared by a sol–gel process in aqueous media using tetraethylorthosilicate and MPS as the precursors. Although a baseline chiral separation of Phe enantiomers was obtained, the separation efficiency (peak bandwidths) was far behind the expected value based on the particle size and morphology of the phase. The authors did not provide the pore size distribution data of the hybrid silica and the core/shell type MIP phase. It seems that the authors assumed that nonporous hybrid silica was prepared. How about the MIP shell? The broad bandwidths might be caused by poor pore size distribution in the MIP layer (0.5 μm thickness) causing retarded mass transfer kinetics or serious heterogeneity of MIP sites probably caused by vigorous agitation during MIP formation.

3. Enantiomeric recognition in CEC

The general trends in CEC were well summarized in recent review articles. CEC coupled with MIP significantly enhances column efficiencies compared to MIP-HPLC [11–18]. Monolithic MIP-type porous layer open tubular (PLOT) columns showed superior separations [18]. The MIP nanoparticle partial filling CEC technique seems to be a promising tool for screening applications [13–16].

3.1. Bulk monolith CEC

Ou and Zou et al. [48] prepared very sophisticated MIP capillary columns where silica monolith was first formed, then copolymerization and anchoring of the MIP layer was carried out. The optimized mixture of L-THP, MAA, and AIBN in acetonitrile was incubated at 55 °C for 40 min. Baseline enantio-separations were achieved in 4 min with the effective column length of 8.5 cm. The authors claimed that the separation performance on MIP-derivatized silica monolith was superior to that on the organic monolith either in CEC or LC, but the difference was not pronounced. Despite the efforts and sophistication of their work, the separation performance of the resultant MIP columns was rather unsatisfactory still showing broad bandwidths and tailing. The strategy of this work (bulk silica monolith-graft MIP composite) seems insufficient to significantly overcome the slow mass transfer kinetics in MIP.

S-zolmitriptan MIP monolith was synthesized in a silica capillary by the room temperature ionic liquid (RTIL)-mediated nonhydrolytic sol–gel (NHSG) protocol for chiral separation of zolmitriptan enantiomers [60]. RTIL was incorporated to reduce gel shrinkage and also to act as the pore template. Three different acids (TFMAA, cinnamic acid, and MAA) were used as functional monomer and a catalyst for the NHSG condensation of MPS. Typically, a mixture composed of *S*-zolmitriptan, MPS, AIBN, MAA, and $BMIM^+PF_6^-$ dissolved in acetonitrile was filled in a capillary and incubated at 54 °C for 6 h. The resultant MIP showed a good chiral separation performance in CEC, R being as high as 4.26. Increased porosity was also observed for the RTIL mediated MIP compared to the non-RTIL mediated MIP, contributing to enhanced mass transfer kinetics and selectivity. This study showed the best level of chiral separation performance among the MIP phases reported up to the date of this study (2008).

A L-tyrosine (Tyr) imprinted monolith was fabricated in a silica capillary by in situ thermo-initiated copolymerization and used to separate tyrosine enantiomers and analogous amino acids in pressurized capillary electrochromatography (pCEC) [61]. The mixture of the template (L-Tyr), functional monomer (MAA and 4-VPY), cross-linker (EDMA), and initiator (AIBN) dissolved in toluene–dodecanol was deoxygenated and filled into the pretreated capillary. The capillary was sealed and submerged into a bath at 50 °C for 12 h. The optimum template:monomer ratio was 1:3. The enantiomers were rapidly separated in less than 10 min by pCEC. The chiral chromatographic resolution as high as 2.64 was obtained. The cross-selectivity study leaded to the conclusion that molecularly imprinted polymer recognizes the template molecule by its molecular shape defined binding cavity.

Positively charged monolithic MIP capillary columns were prepared and evaluated in the CEC mode for separation of the enantiomers of the template (*S*-ibuprofen) [62]. Pre-polymerization mixtures were prepared by mixing functional monomer (2-VPY), co-monomer (MMA), cross-linker (TRIM or EDMA), initiator (AIBN), and template molecule in toluene–isooctane. Polymerization was initiated by UV (350 nm) at −26 °C. The vinylpyridine based MIP becomes protonated and positively charged at a low pH. It was found that the composition of porogen had significant effects on polymerization yield and polymer density and such effects were dependent on the type of cross-linker. Briefly, high porosity polymers with good flow properties were obtained upon either decreasing the concentration of monomers or increasing the concentration of isooctane in the porogen. Efficiencies as high as 30,000 plates per meter with an asymmetry factor below 4 were obtained for the imprinted enantiomer. However, the separation performance was quite disappointing since the maximum resolution was only 0.7. It seems that optimization of the formulation of the reaction mixture was not completely achieved in this study.

A new monolithic stationary phase for the rapid chiral separation of antiparasitic drugs by pCEC was designed and prepared. The poly (MAA-*co*-ethylene dimethacrylate-*co*-4-VPY) monolith was fabricated with *S*-ornidazole (ONZ) as the template by the simple thermal polymerization of MAA, 4-VPY, and ethylene dimethacrylate in the presence of a binary porogenic mixture of toluene and dodecanol [63]. Optimal porous properties and good selectivities were claimed to be obtained. The enantiomers were rapidly base-line separated within 9 min. Chiral separation of enantiomers of a template analogue (secnidazole) was obtained within 14 min.

Monolithic (-)-norepinephrine MIP capillary columns were prepared using a variety of prepolymerization mixtures for CEC separation of a group of structurally related neutrotransmitter compounds [64]. The MIP of the best performance was obtained from the mixture composed of (-)-norepinephrine (template), itaconic aid

(IA, functional monomer), EDMA (crosslinker), AIBN (initiator), and dimethylformamide (porogenic solvent). Polymerization was carried out at 65 °C for 17 min. The MIP column was successfully used simultaneously for chiral separation of template enantiomers and nonchiral/chiral separation of structurally related compounds with a mobile phase including SDS. Addition of a surfactant (SDS) to the eluent was found to improve the CEC separations. The best chromatographic resolution for norepiephrine enantiomers was ca 1.

3.2. Partial filling CEC

Successful use of *(S)*-propranolol MIP nanoparticles prepared by a new microemulsion polymerization approach in chiral separation by the partial filling CEC technique was demonstrated [65] based on the advantages of the partial filling technique over the conventional CEC modes as follows: (1) use of a fresh and new MIP nanoparticle phase each time eliminating sample carryover; (2) easy control of the length and type of the particle phase for each separation; (3) no need for time-consuming and tedious packing or troublesome frit preparation. Very specific nanoparticles were made by incorporation of a surfactant functional monomer (sodium *N*-undecenoyl glycinate). Baseline separations with plate numbers of 25,000–60,000/m were obtained without apparent tailing owing to reduction of the MIP site heterogeneity by means of peripheral location of the core cross-linked nanoparticles and formation of MIP-binding sites with the same ordered radial orientation. The MIP nanoparticles were also compatible with aqueous CEC mobile phase owing to their micellar character. However, the above achievement is not satisfactory enough if it is considered that *(S)*-propranolol MIPs have shown good results almost exclusively.

Molecular crowding is a new concept to obtain MIPs with greater capacity and selectivity by shifting the equilibrium of template-functional monomer complexing toward complex formation. A molecular crowding agent was, for the first time, applied to the preparation of MIP microparticles by precipitation polymerization [66]. The molecular crowding system was made by dissolving 40 mg polystyrene (PS) in 1 mL tetrahydrofuran (THF). The partial substitution for MAA with weaker functional monomers, butylmetnacrylate (BMA), was beneficial. Thus the pre-polymerzation mixture that showed the best chiral separation resolution (1.53) was composed of *S*-ofloxacin, AIBN, EDMA, and 1:1 mixture of MAA and BMA dissolved in PS-THF. The mixture was heated at 53 °C. With PS present, MIP particles were synthesized in a short time (1.5–2 h). Interestingly, MIP particles were not generated in 24 h without PS. Partial filling CEC was used to evaluate the prepared microparticles. Baseline separation of ofloxacin (R=1.53) was obtained under optimized conditions. A troublesome strategy of this study should be noted. An identical CEC elution mobile phase was used throughout to compare separation performances of the prepared MIPs, which might hamper the derived conclusions. Different conclusions could have been made if a different mobile phase had been used. For example, a MIP prepared by using the conventional porogen (acetonotrile, ACN) had much greater specific surface area (128 m^2/g), total pore volume (0.4537 mL/g), and average pore size (16.84 nm) than the MIP prepared by using PS-THF (5.6 m^2/g, 0.00242 mL/g, 4.85 nm, respectively), which would, in common sense, show much better enantiomeric separation performance for the ACN based MIP than for the PS-THF based MIP. In this study, the better chiral separation performance (R=1.53, α=1.07) was reported for the latter than for the former (R=1.13, α=1.04), and no persuasive explanation was given for this.

3.3. Open tubular CEC

A series of open tubular (OT) MIP-CEC studies have been carried out by Zaidi and Cheong et al. [67–74] demonstrating the usefulness of the OT-MIP CEC approach in MIP study by showing dramatically improved chiral separation selectivity and efficiency.

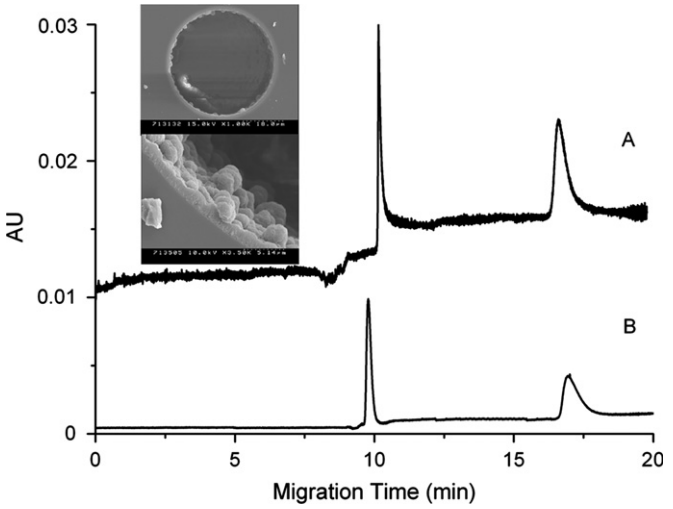

Fig. 5. Comparison of chiral separation of ketoprofen enantiomers in different buffer systems. (A) 60/40 acetonitrile/pH 3.0 50 mM sodium acetate buffer, R: 9.5. (B) 70/30 acetonitrile/pH 3.5 50 mM sodium formate buffer, R: 10.5. The migration times of EOF marker (acetone) were 7.6 and 7.4 min, respectively, for (A) and (B). The SEM photos of the cross-section of PLOT column and the enlarged view are also included. Reproduced with permission from Ref. [67].

In the first study of the series, very impressive chiral separation performance of *S*-ketoprofen MIP was obtained for enantioseparation of *R*- and *S*-ketoprofen by fabrication of OT-MIP on the inner surface of silica capillary using a reaction mixture of specific formulation [67]. The polymerization mixture was prepared by mixing *S*-ketoprofen 5.16 mg, MAA 8.2 μL, EDMA 59 μL, 4-styerenesulfonic acid (4-SSA) 2 mg and AIBN 3.5 mg in 1 mL 9/1 (v/v) acetonitrile/2-propanol, and incubated at 50 °C for 4 h. The particular features of the polymerization mixture to enable stable formation of a thin porous OT-MIP layer with sufficient ruggedness on the inner wall of silica capillary as shown in Fig. 5, were addition of 4-SSA in the reaction mixture, use of 9/1 acetonitrile/2-propanol as the reaction solvent, and the relatively low composition of monomer mixture (10.5% in weight) in the solvent. Various parameters such as buffer pH, organic modifier composition, salt concentration, and applied potential were optimized for CEC chiral separation of ketoprofen enantiomers. The separation performance of the column was demonstrated in Fig. 5.

The above preparation protocol was employed to make long (1, 2, and 3 m) OT-MIP CEC columns, too [68]. Optimization in elution conditions was carried out to achieve the best separation efficiency. The optimized eluent was 92/8 (v/v) acetonitrile/60 mM sodium acetate (pH 3.5). Excellent chiral separation of racemic ketoprofen as well as non-chiral separation of other profen drugs was achieved to give the number of theoretical plates (N) over a million per column.

The same MIP preparation protocol has been successfully employed for chemically different templates, too. For example, an *S*-ofloxacin OT-MIP CEC column was prepared to show far better separation efficiency and selectivity than those obtained by other methods for chiral separation of *R*- and *S*-ofloxacin [69]. The optimized chromatographic eluent was 85/15 (v/v) acetonitrile/60 mM sodium acetate at pH 7. The separation efficiency of the 4-SSA MIP column was also found quite better than that of the MIP column without 4-SSA.

The above developed preparation protocol of OT-MIP CEC capillary columns was found to be used generally for a variety of templates. It was also found that the resultant MIP would show

good chiral separation performance only if the template is of good chiral recognition susceptibility. For example, the MIPs made with camphor derivatives having some polar functional groups near the chiral center showed quite good chrial separation performance for the template enantiomers [70] while the MIP made with camphor showed no chiarl separation capability. The camphor derivatives were 10-camphorsulfonic acid, 10-camphorsulfonamide and camphor-*p*-tosyl hydrazone. The optimized elution conditions were found different for the three MIPs. The different morphologies were also observed for them. The best morphology and enantioselectivity were obtained for the 10-camphorsulfonamide MIP.

The generalized preparation protocol was examined for a large group of templates (mostly acidic) [71]. The preparation procedure was exactly the same for all the MIPs except for the selection of template. The morphologies of the MIP layers were markedly variant depending upon the choice of template. Different optimized conditions were also obtained for different OT-MIP columns although a unified eluent could also be used to obtain still quite satisfactory results. Non-chiral separation of the MIP columns was also examined. The crucial factors for good chiral separation of the MIP columns were intrinsic susceptibility of the template to enatio-recognition, morphology control of the MIP layer, and optimization of chromatographic conditions. The three points interaction rule (enough polar, π–π interaction, and steric functional groups near the chiral center) and the template molecular structure enabling some spatial space around the chrial center were found to be critical factors to give good chrial recognition susceptibility of the resultant MIP.

The above argument was confirmed in an additional study [72] where phenylcarboxylic acids and their derivatives of similar structures were comparatively examined as templates. It was found that prevention of spatial congestion around the chiral center as well as fulfillment of the three-points interaction rule are required for good chiral recognition susceptibility. Subtle variation of the template molecular structure was crucial to enhance chiral recognition ability of the MIPs while intramolecular hydrogen bond in the template molecule degraded chiral recognition. There were strong correlations among template chiral susceptibility, MIP morphology, and chiral separation performance.

The generalized preparation protocol of OT-MIP CEC capillary columns was successfully expanded to basic templates [73]. The basic templates examined were atenolol, sulpiride, methyl benzylamine (MBA) and (1-naphthyl)-ethylamine (NEA). Atenolol and sulpiride that fulfilled the requirements as good template yielded MIPs of nice morphology and nice chiral separation performance, NEA of less fulfillment, a MIP of moderate chiral separation capability, and MBA of the least fulfillment, poor chiral separation ability.

Expansion of the generalized preparation protocol to a genuine neutral template was also carried out [74] although such application has been quite limited so far. Anyhow, such trial of establishment of a generalized preparation protocol of MIPs was of great importance since it will require a huge amount of time to develop a useful MIP for chiral separation of a new pair of enantiomers if optimization is mandatory in both MIP formation and chromatographic elution. Thus, foundation of a generalized preparation protocol regardless of the types of template will reduce such burden dramatically. In addition, some empirical rules of eluent optimization were proposed for the OT-MIP CEC columns as follows [73]. The eluent composed of acetonitrile and an aqueous buffer (either acetate or phosphate) will be useful and the optimized acetonitrile composition is in the range of 70–92% (v/v). The optimized buffer pH is 3–5 for weak acid templates, 2–3 for strong acid templates, 6–8 for neutral templates, and ca 9 for basic templates.

The novel results of the above series of studies were claimed to be primarily owing to the superiority of OT-CEC columns in analytical separation and the success in formation of thin porous and rugged MIP layers. CEC combines the partitioning effect of HPLC and high separation efficiency of CE to take advantage of both systems. OT-CEC MIP columns are also easy to prepare, free of bubble formation, and easy to wash for removal of template molecules. In addition, eluent optimization is easily realized without any restriction since there is negligible pressure drop across the OT-CEC column. It should be noted that the above generalized MIP preparation protocol was valid only for fabrication of OT-CEC MIP columns since their similar trials for generalization in preparation of bulk monolithic MIPs or precipitated MIP particles have failed [73].

A microfluidic device with a MIP incorporated (coated) microchannel was for the first time prepared for fast chiral separation of enantiomers of Boc-Trp [75]. The microchannel (25 μm × 80 mm) was filled with an optimized mixture composed of Boc-L-Trp, AM, EDMA, and AIBN dissolved in ACN–isooctane, and incubated at 60 °C for 3.5 h. The channel was blocked when the reaction time was 4 h. The resultant MIP was able to achieve baseline separation of Boc-Trp enantiomers within 75 s by amperometirc dectection with a home-made carbon fiber electrode. The chromatographic resolution (R) could be slightly over 1 based on the reported chromatograms. Such performance is rather disappointing for the MIP morphology (open tubular and porous thin MIP layer) shown in the SEM photos. It seems that degradation of chromatographic performance in a microfluidic device is not due to intrinsic performance of separation channel but due to extra-column band broadening related to sample injection and/or detection.

A special ternary porogen system was employed to fabricate a specific OT-MIP layer for a very polar template (D-zopiclone) [76]. The porogen system was composed of a mixture of toluene, isooctane and dimethyl sulfoxide (DMSO). An interesting observation was that the MIP morphology and separation performance were dramatically varied with a little change of DMSO composition in the porogen. The best result was obtained when the DMSO composition was 30.0%. The capillary was clogged when the DMSO content was 33.3%. The pore size distribution was also varied a lot in the small range of DMSO composition. The optimized reaction mixture was composed of template, AIBN, MAA, EDMA, toluene, isooctane, and DMSO (30.0% composition in the whole porogen). The mixture was filled in a silica capillary and heated at 53 °C for 1.5 h. The resultant MIP showed quite good chiral separation performance for zopiclone enantiomers. The highest column efficiency of D-zopiclone was 21,400 plates m^{-1}.

An interesting OT-MIP-CEC capillary column was prepared using 2-methacrylamidopropyl methacrylate (MAM) as single functional/crosslinking monomer [77]. The polymerization mixture was composed of imprinted molecule, MAM, MPS, AIBN dissolved in toluene or 7/3 (v/v) toluene–isooctane. MPS was incorporated in copolymerization to give ionizable functional groups (silanol groups) upon hydrolysis after completion of MIP formation. The mixture was filled in a pretreated silica capillary and incubated (temperature and time not given) to yield a very thin MIP layer. Excellent chiral separation performance was reported. The resolution of enantio-separation achieved on *S*-amlodipine (AML) imprinted capillary was up to 16.1. The strong recognition ability (selectivity factor was 3.23) and high column performance (N=26,053 plates m^{-1}) of template were obtained.

The same research group of the prior article [77] reported another OT MIP of low level cross-linking (ca 20%) that showed excellent separation performance such as chromatographic resolution as high as 13.8 for AML enantiomers, selectivity factor of 1.81, and high separation efficiency (N) as high as 23,300 m^{-1} [78]. A large amount of liquid crystalline monomer (4-methyl phenyl dicyclohexylpropylene) was incorporated. Thus reaction mixture was prepared by dissolving S-AML, liquid crystalline monomer, MAA, EDMA and AIBN in toluene–isooctane (7/3, v/v). The mixture

was incubated at 53 °C for 1.75 h. It is interesting that the MIP obtained by cooking for 1.5 h did not show any chiral separation while the MIP obtained by cooking for 2 h was clogged. The thickness of the MIP layer was only 0.1–0.2 um. The authors declared that the good chiral separation performance was owing to the flexible liquid crystalline structure enabling fast mass transfer kinetics. This argument is contradictory to that of the prior article [77] that had shown even better chiral separation performance with the MIP of rigid structure prepared with single cross-linking monomer (near 100% cross-linking).

4. Enantiomeric recognition in SPE

SPE-MIP techniques have been extensively employed in either on-line or off-line modes, in the formats of mini-columns, membranes, disks, knotted reactors, renewable beads or cartridges [25–27]. Today there are already SPE-MIP cartridges commercially available for selective extraction of several molecules [1,26]. There have been numerous articles on use of MIP sorbents for solid phase extraction [79]. However, examples of enantiomeric recognition by MIP-SPE are very rare. A couple of recent examples are given below.

A specific Nylon-6 based MIP film on a magnetic bar was reported for differential chiral recognition of glutamine enantiomers [80]. In this MIP system, the template molecule was incorporated into the polymer matrix in solution, and the film was cast from this solution, thus no polymerization step was necessary. A MIP mixture composed of Nylon-6 (30 wt%), L-glutamine (template, 3 wt%), and formic acid (67 wt%) was vortexed to yield a clear colloid solution. A stir bar (30-mm) pre-coated with 200 mg polydimethylsiloxane was submerged in the solution, then the stir bar was put into a pure water for gelation of the coated layer. The sol–gel L-glutamine imprinted Nylon-6 film possessed porous structure, while the nonimprinted Nylon-6 film had not. The MIP layer showed remarkable enantioselectivity to L-glutamine. For example, the concentration of L-glutamine was 9.64 μmol/L, and that of D-glutamine, 24.1 μmol/L, respectively after sorption equilibrium for the solution of the same concentration of 25.0 μmol/L. The thickness of dry coated films was ca 160 μm. The reproducibility of film thickness was better than 10%. The stir bars could be used for up to 100 extractions.

A *S*-citalopram (CIT) MIP was synthesized by polymerization initiated by UV onto magnetic stir-bars to obtain stir bar sorptive extraction (SBSE) devices capable of selective enantiomeric recognition [81]. The computational modeling was used to decide the functional monomer (AM) and monomer/template ratio (3:1). Thus the polymerization mixture consisted of tri(ethylene glycol) dimethacrylate, AM, S-CIT, polyethyleneglycol (PEG), 1,3-divinyltetramethyl disiloxane, and ACN-benzophenone. The MIP was synthesized in the presence (10 wt%) of a linear polymer (PEG 20,000) to obtain a porous coating. A magnetic bar pretreated by encapsulating in a glass container followed by silanizing with MPS, was placed in a Teflon tube previously closed at one end. The tube was filled with the polymerization mixture, and the remaining open end was closed with another Teflon tube. Then it was irradiated by UV for 30 min. The developed MIP stir-bar was able to recognize the target enantiomer with a high specificity level between 25 and 500 $\mu g\,L^{-1}$ where the amount of bound *R*-enantiomer was below the detection limit. At higher analyte levels (500–3000 $\mu g\,L^{-1}$) the specificity factor (*K*; peak area *S*/peak area *R*) was around 4. The specificity factor decreased to 3 at 5 $mg\,L^{-1}$, and to 1.5 at 10 $mg\,L^{-1}$.

5. Enantiomeric recognition in sensor applications

MIP sensors are the subject of a really huge future market in various fields such as clinical, bioanalytical, process control, and environmental applications [22,36–44]. Unfortunately, practical application in commercialized formats is very limited so far. MIP based sensor analysis should compete with present commercialized analytical tools such as HPLC, mass spectrometry, spectroscopic methods, and immunoassay methods, etc. MIP-based biomimetic sensors are regarded to be promising among all at present since they have a number of merits such as low cost, easy storage, applicability in harsh conditions, and long lifetime over their competitors such as enzymes, antibodies, and proteins. Thus some commercial protein sensor array systems are already available although MIP-based biomimetic sensors are still rather inferior to biosensors [41,44]. However, studies of enantioselective sensors have been rather rare.

L- (or D-)phenylalanine anilide (PAA) imprinted poly(EDMA-*co*-MAA) was grafted on an indium-tin oxide (ITO) to serve as an enantio-selective sensor device [82]. The mixture of MAA, EDMA, L- or D-PAA, and AIBN was dissolved in toluene, cyclohexane, pyridine, or DMF. Toluene was found to give the best chiral selectivity for the resultant MIP. The methacrylated ITO glass plate was soaked in this solution, and polymerized by heating to 60 °C for 12 h. Conventional cyclic voltammetry of ferrocene in organic solvents was carried out using the grafted ITO as the working electrode. Selectivity was defined as the ratio of the relative change in maximum anodic current of the template to that of the other enantiomer. The anodic current decreased in the presence of the template to a greater extent than in the presence of the other enantiomer. Interestingly, the best chiral selectivity (4.83) of cyclic voltammetry was obtained with dichloromethane as the solvent when the template was L-PAA, while it (9.47) was obtained with chloroform when the template was D-PAA.

A capacitive sensor device for enantio-selective recognition of glutamic acid (Glu) was developed by fabricating a MIP layer on a gold electrode in one step by electrochemical copolymerization [83]. Electrochemical polymerization was performed in a deoxygenated buffer containing *o*-phenylenediamine (*o*-PD) and dopamine (DA) in the presence of L- or D-Glu with cyclic voltammetry scanning from −0.5 to 0.8 V. The optimal *o*-PD:DA molar ratio was 3:2. The obtained copolymer modified electrode was immersed in an ethanol solution of 1-dodecanethiol for 12 h to fill the pinholes or defects, and washed with 50 mM HCl and water alternately to remove the template. The capacitance of this system was measured with the potentiostatic time scan method. The rebinding of the L-Glu (or D-Glu) molecules decreased the dielectric constant of the MIP film due to the exclusion of water and buffer species, thus decreased the capacitance. The capacitance response of the sensor for L-Glu or D–Glu was proportional to their concentration in the range of 16.7 to 250 μM. The enantiometric selectivity coefficients for L-Glu and D-Glu imprinted films against their respective enantiomers were 24 and 15, respectively.

Fabrication of MIP arrays on a chip by infrared laser pulse initiated polymerization was introduced as the first report of such technology [84]. The work of combinatorial monomer selection was carried out by microfluidic technology (the miniaturized reactor with an inlet and outlet, Fig. 6) and laser technology combined with charge-coupled device (CCD) camera and computer control (spot reaction). The reactor was filled with a reaction mixture, and a MIP spot was made by illumination of IR laser. After removal of the residual reaction mixture by washing, another MIP reaction mixture was filled and another spot was made by IR illumination, and the reactor was washed again. This procedure was repeated until complete formation of all the MIP spots (Fig. 6). A sensor chip bearing both non-imprinted as well as

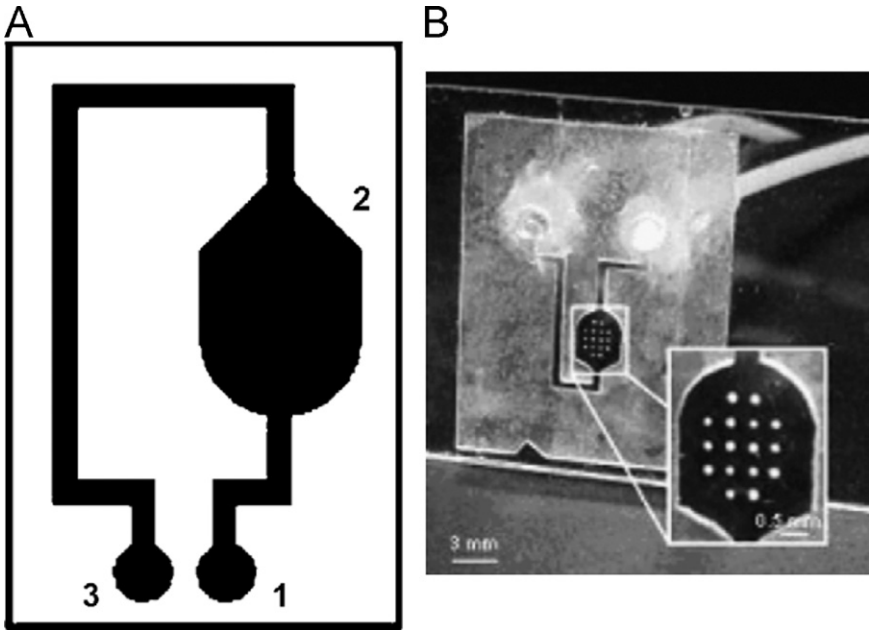

Fig. 6. (A) Schematic of laser cut microfluidic system. 1: inlet, 2: polymerization chamber, 3: outlet and (B) heterogeneous polymer microarray prepared in situ via CO_2 laser initiated polymerization. Reproduced with permission from Ref. [84].

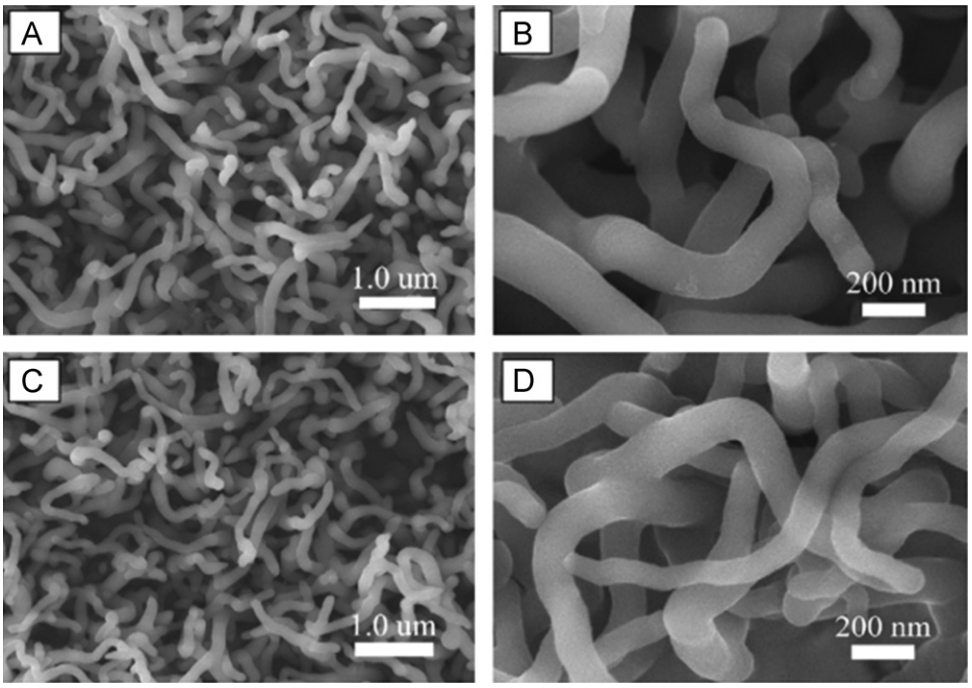

Fig. 7. Typical SEM images of CSA doped PPy nanowires prepared by electrochemical polymerization. (A) Low and (B) high magnification images of D-CSA doped PPy nanowires. (C) Low and (D) high magnification of L-CSA doped PPy nanowires. From these SEM images we can observe that PPy nanowires prepared by different chiral dopant have same nanostructures. The average diameter of these nanowires is approximately 100 nm with length ranged from one to several micrometers. Reproduced with permission from Ref. [85].

imprinted polymers was also prepared, thus 3×3 microarrays of both MIP and NIP were fabricated on a single chip and used in a dip-and-read format. Instead of the above microfluidic device, a simple reservoir of 300 μm thickness was used. The imprinted polymerization mixture (dansyl-L-Phe) was deposited in the reservoir and a chip was immersed and the MIP microarray was fabricated using the CO_2 laser. The chip was then removed from the reservoir, washed and dried. The reservoir was filled with the non-imprinted polymerization mixture, and the vacant part of the chip was immersed and the NIP microarray was fabricated. For the assay, the chip bearing the MIP/NIP microarrays was soaked in solutions of ACN/deionized water (2:8) containing 1–500 μg mL^{-1} of dansyl- L- or dansyl- D-Phe for 10 min without agitation, rinsed with deionized water, dried in a stream of argon and their fluorescence image taken immediately. At a sample concentration of 500 μg mL^{-1}, the fluorescence response measured for the template (L-enantiomer) was only 1.6 times higher than the response measured upon binding of the D-enantiomer. In contrast to the success of micro-fabrication of MIP dots, formation of MIPs of good chiral selectivity in such a format

was not successful, thus the enantio-selectivity of the MIP dots was only marginal.

The D- or L-camphorsulfonic acid (D- or L-CSA) doped polypyrrole (PPy) nanowires were fabricated by electrochemical polymerization on a platinum electrode (Fig. 7) [85]. The CSA molecule acted as both the dopant and a pseudo-template. The typical electrolyte was an aqueous solution of 0.145 M distilled pyrrole and 0.04 M D- or L-CSA. A constant current of 0.3 mA was applied on the electrode for 30 min, and the working electrode was rinsed by water. Acidic surfactants, such as CSA, have been widely used as the dopant to produce conducting polymer nanostructures. CSA molecules could form micelles in cooperation with pyrrole and also act as protecting agent to prevent the overgrowth of PPy nanowires. Phe was a model analyte in this case due to its similar molecular size and conformation to CSA. The enantio-selective interactions between Phe enantiomers and D- (or L-) CSA imprinted PPy nanowires were demonstrated by both electrochemical impedance spectra (EIS) and circular dichroism spectra. For example, a dramatic change was observed between the impedance values before and after adsorption of the template-matching enantiomer while an insignificant change was observed for adsorption of the other enantiomer.

A molecularly imprinted self-supporting membrane (MISSM) of 50 μm thickness that can be used as a biomometic enantio-selective sensor was developed by copolymerization of MAA and 2-VPY (functional monomers) using TEDMA (crosslinker) in the presence of L- (or D-) Phe (template) [86]. This MISSM showed selective adsorption characteristics to the template with negligible adsorption of the other enantiomer and solute permeability in the MISSM increased in the presence of the template (gate effect), but was insensitive to the other enantiomer.

Two novel polymerizable coumarins, 6-styrylcoumarin-4-carboxylic acid (SCC) and 6-vinylcoumarin-4-carboxylic acid (VCC), were used to serve as suitable fluorescent moieties of a MIP which responded to the binding event with significant fluorescence changes [87]. Such changes are owing to hydrogen bond formation between the fluorescence moiety and the template upon insertion of template in the MIP. (-)-Ephedrine was used as the template. Polymerization of a mixture composed of (-)-ephedrine, SCC (or VCC), EDMA, MMA dissolved in ACN was carried out at 60 °C for 17 h and at 80 °C for additional 2 h. The hard bulk polymers were ground into fine particles (<50 μm). In acetonitrile, the polymers exhibited a decrease of fluorescence in response to amines, with some selectivity for the template over its enantiomer (+)-ephedrine and other structural analogues.

A new MIP-chemiluminescence (CL) imaging detection method for chiral recognition of dansyl-Phe was developed, where in the presence of dansyl-Phe, bis(2,4,6-trichlorophenyl) oxalate (TCPO) reacted with hydrogen peroxide (H_2O_2) to emit peroxyoxalate chemi-luminescence (PO-CL) [88]. 4-VPY and MAA were used as functional monomers. Thus the reaction mixture composed of dansyl-L-Phe (template), 4-VPY, MAA, EDMA, AIBN, and ACN, was incubated at 50 °C overnight. MIP microspheres (0.7 μm) were immobilized in microtiter plates (96 wells) using poly(vinyl alcohol) (PVA) as glue. The analyte was selectively adsorbed on the MIP microspheres. Test compound solution (10 μL) was added to the well of MIP microspheres coating and incubated for 30 min. After rinsing and drying, the solution of chemi-luminescent substrate (TCPO+H_2O_2) was immediately added. After vortex shaking, the plate was imaged with a sensitive CCD camera. The background value was obtained outside the region of interest, and was subtracted from each measurement. The LODs for the L and D analytes were 0.025 and 0.075 μmol L^{-1}, respectively. This approach was found to serve as a faster and less tedious CL imaging method for quantitative enantio-selective determination if the total analyte concentration is available.

6. MIP characterization and thermodynamics/kinetics in enantio-selective MIPs

BET (surface area) and BJH (pose size distribution) methods, FT-IR and solid state NMR methods (functional group incorporation, degree of polymerization), and volumetric methods (MIP swelling) are commonly used for characterization of MIP materials [1,7–10]. The peak properties of a MIP column, including peak broadening and tailing, can be well interpreted by the theory of nonlinear chromatography considering heterogeneous adsorption sites [10].

A work on kinetically studying the enantio-selective adsorption and recognition by MIP was reported [89]. A reaction mixture composed of *S*-naproxen, EDMA, AIBN, AM dissolved in THF was irradiated under UV (365 nm) at 0 °C for 24 h. The final polymer was ground into 40–50 mesh particles. Then the change of substrate concentration before and after adsorption was monitored at 348 nm. The adsorption amount per gram MIP was obtained from the mass balance of substrate. The result indicated that the prepared polymer showed a more complicated sorption toward *S*-naproxen than toward its enantiomer *R*-naproxen.

Incorporation of a coordination bond in the complex formation between the template and functional monomer during MIP fabrication was found very effective for stabilizing the complex [90]. Li and Hao et al. used cobalt ion as pivot for such purpose in fabrication of S-naproxen MIP. Thus *S*-naproxen, EDMA, AIBN, 4-VPY, and cobalt acetate were dissolved in ACN-DMSO. After deoxygenation, the reaction mixture was irradiated by UV (365 nm) at 0 °C for 72 h. The resulted polymer was crushed and washed. The enhanced enantio-specific recognition of the Co-pivoted MIP for *S*-naproxen was demonstrated by adsorption isotherms and temperature programmed desorption (TPD) profiles where desorption of each adsorbed species was observed with respect to temperature, and the largest discrimination for *R*- and *S*-enantiomer was shown in the Co-pivoted MIP (MIP-Co). The difference in desorption temperature between *R*- and *S*-enantiomer for the regular MIP (13 °C) was much lower than that of the Co-pivoted MIP (24 °C), and the difference for the NIP was negligible. A new approach, TPD was introduced as a powerful tool for evaluating enantio-selectivity of MIPs, and pivoting was proposed as a promising strategy for improving enantio-selectivity.

MIP microparticles with good enatio-selectivity were synthesized via suspension polymerization [91]. When TRIM was used as cross-linker, the produced MIP microparticles exhibited a higher rebinding capacity and selectivity towards the template molecule despite the decrease in the overall specific surface area. The optimum reaction mixture composed of Boc-L-Trp (template), MAA, TRIM, AIBN, and chloroform was dispersed into an aqueous PVA solution (1% w/w), and the polymerization was carried out at 60 °C for 24 h. Batch binding experiments were performed to determine the rebinding capacity of the MIP microparticles. The rebinding capacity (selectivity) of the MIP microparticles to the Boc-L-Trp molecules was higher than that of the NIPs and that of Boc-D-Trp. A quantitative description of the experimentally measured rebinding isotherms was obtained using the well-known Freundlich–Langmuir models.

A specific method using Raman spectroscopy was reported for quantification of a template in a MIP [92]. The MIPs were prepared by UV photopolymerization from a mixture containing TRIM, MAA, *R*-propranolol (template), 2,2-dimethoxy-2-phenyl acetophenone (DPAP), diglyme, and PVA. The MIPs and NIPs were mechanically ground to 1–10 μm particles. For Raman measurements, the MIPs and NIPs were incubated in a series of propranolol solutions in toluene for 3 h. The solutions were centrifuged, then the supernatant was removed and the powder was dried.

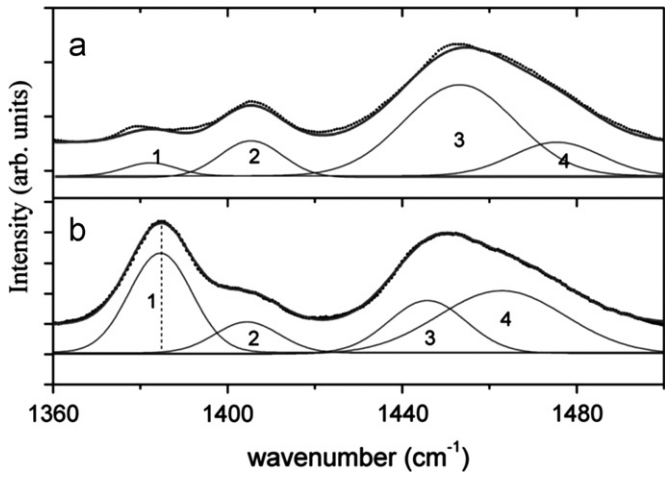

Fig. 8. Deconvolution of the peak in the 1380 cm^{-1} region for (a) NIP and (b) MIP. The lower traces (1–4) show the four Gaussians used to obtain the fitted curve. The upper dots and solid lines represent the measured and fitted spectra, respectively. The dashed line indicates the peak used to quantify *R*-propranolol presence. Reproduced with permission from Ref. [92].

Specific Raman peaks originating in the template (*R*-propranolol) enabled quantification of bound target molecule. The predominant peak in the spectrum of propranolol was in the 1380 cm^{-1} region, and this strong propranolol peak overlaps weaker peaks originating in other components of the mixture. To correctly account for propranolol only, the broad peaks in the 1380 cm^{-1} region were de-convoluted into four Gaussian contributions as shown in Fig. 8 where the leftmost peak's area represents the relative amount of propranolol. Using this procedure, the amount of adsorbed *R*-propranolol or *S*-propranolol by the *R*-propranolol imprinted MIP and the corresponding NIP, was quantified. The specific binding was calculated by subtracting the value measured for NIP from the value measured for MIP. The dissociation constants were estimated to be 0.4 and 1 mM for *R*-propranolol (the imprinted target) and its enantiomer, respectively, demonstrating the MIP selectivity. This study adds to the diversity of available techniques of MIP characterization.

Submicron/nanoscale MIP beads with higher adsorption capacity and selectivity were prepared by modified suspension polymerization at higher rotation speed under N_2 [93]. The reaction mixture composed of D-Phe or L-Phe (template), MAA, EDMA, AIBN, toluene, acetic acid, water, PVA, and SDS was well mixed, purged with N_2 for 5 min, and stirred at 650 rpm for 60min. Polymerization was carried out at 60 °C for 24 h under N_2. The prepared submicron/nanoscale beads had regular spherical shapes with rough and porous surfaces and very low agglomeration. FT-IR, ^{13}C CP-MAS NMR, and FE-SEM studies supported the presence of higher content of functional sites as well as the larger sizes of the prepared MIP beads than that of NIPs. L- and D-Phe imprinted beads were larger (100 nm to 1.5 μm) than non-imprinted nanobeads (100–800 nm). L-Phe-imprinted beads showed enhanced adsorption capacity and selectivity over D-Phe imprinted and non-imprinted beads although the difference was only marginal.

Several (-)-ephedrine imprinted MIPs were prepared based on predictions from NMR data for template-functional monomer complex formation and their chiral recognition capabilities were comparatively examined by HPLC [94]. NMR data were measured to account for 1:1, 2:1, and 3:1 template/ functional monomer complexes and monomer self-association using several different functional monomers. A commercial NMR fitting program was used to derive corresponding association constants and to predict imprinting characteristics among the complex, porogen, and cross-linker. TFMAA and itaconic acid (IA) were found to interact more strongly with ephedrine than MAA. However, TFAAA and IA based MIPs showed poorer chrial separation performance in HPLC than the MAA based MIP. This study demonstrates that there could be various factors in the procedure of MIP formation affecting MIP performance.

Dimethylaminoethyl methacrylate (DMAEMA) was graft-polymerized onto the surfaces of micron sized silica gel particles to give the grafted particles PDMAEMA/SiO_2 [95]. Silica gel particles were first surface-modified with coupling agent MPS to introduce double bonds on the surface (MPS–SiO_2). The aqueous suspension of MPS–SiO_2 including DMAEMA (monomer) and APS (initiator) was subject to graft polymerization under N_2 at 75 °C for 10 h. After Soxhlet extraction and drying under vacuum, the obtained PDMAEMA/SiO2 particles were then saturated with L-Glu, and placed in 7/3 water–ethanol mixture containing L-Glu and 2,2′-dichlorodiethylether (DCEE, crosslinker) was added. The crosslinking reaction was performed at 35 °C for 24 h to give L-Glu imprinted material MIP-PDMAEMA/SiO_2. The selectivity coefficient of MIP-PDMAEMA/SiO_2 for L-Glu with respect to D-Glu was 3.30, demonstrating a good enantio-recognition. This study certainly adds to the variety of preparation methods for MIPs with improved enantio-selectivity.

A combined theoretical and experimental study on optimization of *S*-naproxen MIPs was carried out via UV/IR spectral analysis and computer simulation [96]. MAA, AM and 4-VPY were the functional monomers employed. The MIPs were prepared by irradiating the reaction mixture composed of template, functional monomer, EDMA, LR8893 (2,4,6-trimethylbenzoylphenyl phosphinic acid ethyl ester) in chloroform under UV light (365 nm) at 4 ° C for 24 h. In FT-IR study, the specific peak of carboxyl group of S-naproxen appeared at 1730 cm^{-1}, and the peak shifted to 1691 cm^{-1} after combining with MAA, and to 1680 cm^{-1} after mixing with AM, suggesting a stronger interaction between template and AM. When using the 4-VPY as monomer a new band near 1610 cm^{-1} (COO dissymmetry stretching) emerged, indicating the formation of carboxylate ions (COO^-) and the existence of ion interaction between *S*-naproxen and 4-VPY. The order of imprinting effect of the MIPs with three monomers is 4-VPY > AM > MAA, which confirmed the results of spectral analysis and computer modeling. The chiral separation factor monitored by TLC on the 4-VPY based MIP reached as high as 2.42, and the MAA based MIP showed a minimum value of 1.35.

7. Miscellaneous application of enantiomeric recognition by MIP

The environments for MIP application in membranes are rather poor in view of the sophistication of necessary algorithms, methodology, and the level of MIP fabrication techniques [1]. MIP application in drug delivery is still at the stage of early development and its realization in clinical application may be achieved far later since the adaptability of molecular imprinting technology for drug delivery requires convincing answers to safety and toxicological concerns [28–30]. MIP application in catalysts is still at the stage of early development, too [1].

7.1. Membranes

Poly(vinylidene fluoride) (PVDF) hollow-fiber membranes modified by a thin layer MIPs were developed for the selective separation of levofloxacin (*S*-ofloxacin) [97]. The mixture composed of levofloxacin, MAA, EDMA, and AIBN in chloroform, was cast onto the membrane and heated in vacuum at 60 °C for 48 h. Permeability level could be controlled by repeated polymerization cycles. The weight of the imprinted membranes was increased by 14 mg/cm^2 after a five-cycle polymerization. Levofloxacin imprinted MIP membranes showed highly selective binding of levofloxacin. Selective

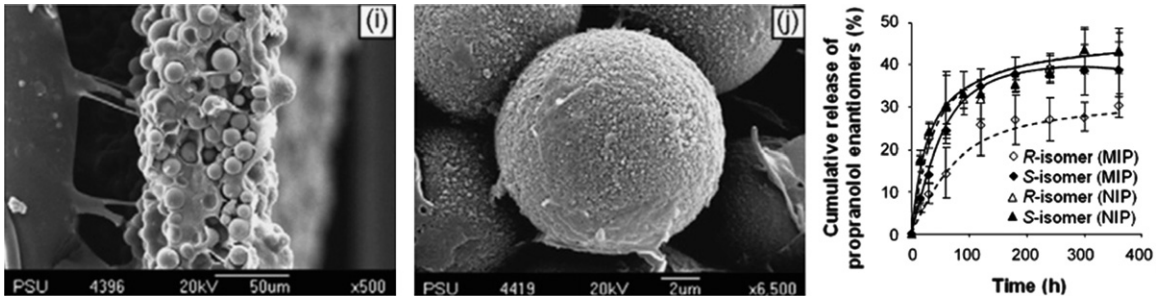

Fig. 9. SEM images of (i) the MIP nanoparticle-on-microsphere (NOM) composite cellulose membrane and (j) its enlargement image, and the release profiles of propranolol enantiomers from MIP and NIP of NOM composite bacterial cellulose membranes at the drug:polymer loading ratios of 1:100. Reproduced with permission from Ref. [99].

permeation seemed to be held through multisite binding to the template via ionic, hydrogen-bond, and hydrophobic interactions. Variable solvent systems in the permeation experiments were used to understand the hydrophobic interaction. Chiral recognition property of this MIP was confirmed by monitoring the higher permeation results for levofoxacin (*S*-ofloxacin) than for racemic ofloxacin.

D-Serine imprinted MIP composite membranes were prepared using conventional interfacial polymerization technique [98]. A *N*-methy pyrrolidone (NMP) solution of 15 wt% polysulfone (PSf) was cast on the polyester nonwoven fabric and phase-inversed in water to form microporous PSf support. Then the surface of PSf support was coated with an aqueous solution of piperazine (PIP) well mixed with D-serine. The composition of PIP/D-serine/water was 5/1/94 (wt%). It was then immersed into a hexane solution of the 1 wt% trimesoyl chloride (TMC) for 10 s for the interfacial polymerization of PIP with TMC to form the active layer, and kept in water at 50 °C for 50 h to remove the template. The resultant MIP membranes proved to be effective for the selective permeation of D-serine. When serine racemate was used for optical resolution, the diffusion rate of D-serine appeared to be faster than that of L-serine, and in permeates, the concentration of D-serine increased with operation time almost linearly while only limited amount of L-serine was accumulated under the optimized operation.

7.2. Drug delivery

Molecularly imprinted polymer (MIP) nanoparticle-on-microspheres (NOM) selective for *S*-propranolol were prepared by suspension polymerization with high speed agitation [99]. The perfluoro polymeric surfactant (PFPS) was prepared by mixing 95% acryloyl-2-*N*-ethylperfluoroalkyl sulfonamide and 5% acryloyl PEG 2000 monomethyl ether. The MIP reaction mixture composed of *S*-propranolol, MAA, EDMA, AIBN, PFPS and perfluoro(methylcyclohexane) (PMC) in chloroform was deoxygenated and vigorously stirred at 1000 rpm. Polymerization was done by UV (365 nm) for 4 h. MIP granules and microspheres were also prepared for comparison purpose. The MIP-NOM could be successfully fabricated into self-assembled porous bacterial cellulose membrane using a phase inversion method. Cellulose sheet (96 mg) was dispersed in an aqueous solution of *N*-methylmorpholine-*N*-oxide (NMMO, 50%, w/w), and heated at 80 °C to yield a clear, brown solution. The drug polymer mixture (100 mg, drug: polymer ratios 1:10, 1:50, 1:100, 1:200 and 1:500) and 0.3 mL of polycaprolactone-triol (PCL-T) were added into the cellulose solution. The resulting suspension was poured into a Petri-dish (10 cm). After 5 min, generated membranes were transferred into a beaker containing water for 12 h. The membrane was recovered, dried in air and stored in a desiccator. The MIP-NOM within the membrane was easily accessible for binding of the imprinted molecule and appeared to maintain high selectivity. The proposed MIP-NOM composite membrane controlled release system (Fig. 9) had better enantioselectivity than the composite membranes based on MIP granules or microspheres, and showed its potential in the enantioselective-controlled transdermal delivery of the *S*-isomer of racemic propranolol and its prodrug analogs through rat skin.

A MIP thin-layer composited cellulose membrane with selectivity for *S*-propranolol was employed as the enantioselective-controlled release system [100]. A transdermal MIP patch was also prepared. In the preparation of the MIP composite membrane, the cellulose membrane (5 μm thickness) was activated with MPS. A mixture of *S*-propranolol HCl, MAA, EDMA and AIBN dissolved in DMF was poured onto the surface of the functionalized cellulose membranes to give MIP membranes after polymerization at 60 °C for 20 h. The results from confocal laser scanning microscopy study, carried out with the *R*- and *S*-propranolol enantiomers labeled with a 1-pyrenebutyric acid probe as fluorescent markers, suggested that the MIP composite membrane selectively regulated the release of the recognized *S*-enantiomer. The selected gel formulation (used for membrane permeation study and production of MIP patch) was prepared with chitosan. Racemic propranolol HCl was added in this gel to obtain the reservoir formulation. The MIP patch was prepared by laminating a sheet of MIP membrane with an adhesive-coated liner, attaching a spacer on the membrane, filling the spacer with the gel reservoir, and attaching a backing laminate with epoxy resin. These patch devices were evidently enantio-selcetive in uptake and release of propranolol when attached to the skin, based on pharmacokinetic studies and plasma concentration profiles of *S*-propranolol obtained with the transdermal patch in the in vivo study with rats.

Recently, there was a report on development of enantioselective-controlled drug delivery systems for selective release of *(S)*-omeprazole in response to pH stimuli. The recognition system was obtained from a nanoparticle-on-microsphere (NOM) MIP with a multifunctional chiral cinchona anchor synthesized by suspension polymerization [101]. *S*-omeprazole, methacryloyl quinine (MQN) or methacryloyl quinidine (MQD), EDMA, AIBN, and PMC were dissolved in chloroform with an emulsifier (PFPS) to make MIP beads. Polymerization was performed by UV (366 nm) at room temperature for 4 h with constant stirring. MIP composite cellulose membranes, both with and without racemic omeprazole, were prepared by the phase inversion method as follows: Racemic omeprazole loaded MIP beads were thoroughly mixed with an aqueous NMMO solution (50 wt%) involving cellulose and a plasticizer (PCL-T). This mixture was poured into a 9 cm Petri dish. The generated membrane was transferred into water, and left for 12 h. The membrane was recovered and dried. In-vitro release studies of *(S)*- and *(R)*-omeprazole, were carried out by placing the loaded membranes in pH 7.4 phosphate buffer under constant stirring. Samples were withdrawn at appropriate time intervals up to 6 h. The controlled-release drug devices consisted of a pH stimuli-responsive poly(hydroxyethyl methacrylate) (HEMA) and PCL-T blend, and a MIP membrane with preloaded drug, along with pH 7.4 buffer inside. The SEM photo of the surface of this system was given in Fig. 10. The results demonstrated that drug delivery systems containing *(S)*-omeprazole

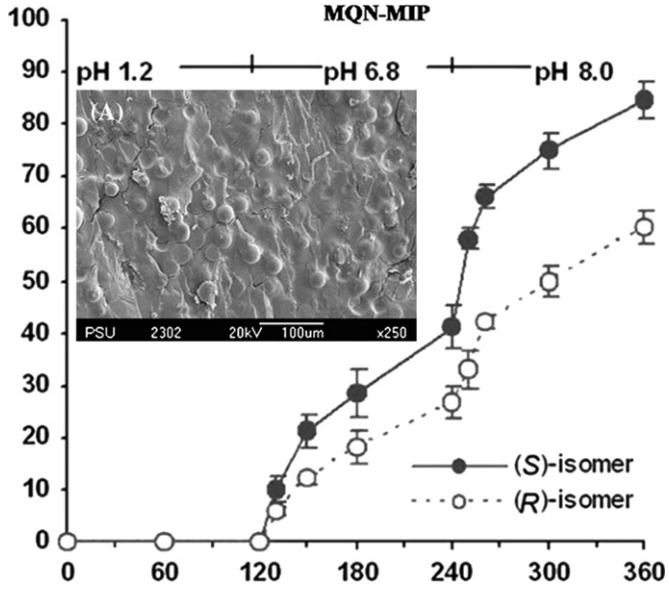

Fig. 10. In vitro dissolution profile of omeprazole enantiomers from MQN-MIP loaded delivery system in dissolution medium changed every 2 h with pH 1.2, 6.8 and 8.0, respectively (mean ± SD, $n=6$). The SEM image of the surface of this delivery system is shown, too. Reproduced with permission from Ref. [101].

imprinted cinchona-polymer nanoparticle-on-microspheres may maximize efficacy while minimizing dose frequency. The enantio-selective and pH sensitive delivery of this system was well demonstrated in Fig. 10 although the enantio-selectivity is only marginal.

7.3. Catalysts

Liquid crystalline (LC) based MIPs with low cross-linking ratios have shown the greater molecular trapping capacity than most of the previously studied non-mesomorphous systems. It was found that mesomorphic order provides significant enhancement to the bonding between the template and the liquid crystalline network and reinforces the shape memory of the imprinted cavities. Several liquid crystalline imprinted materials have been synthesized from polysiloxanes or polyacrylates bearing mesogenic side-chains [102]. Some of these materials were used to catalyze the isomerization of benzisoxazole. Chiral LC-MIPs were synthesized, based on polysiloxane polymers. Depending on the system, either *R*- or *S*-methylbenzylamine or (*N*-carbobenzyloxy)-L-phenylalanine were used as template. Recognition properties for LC-MIPs for *R*- or *S*-methylbenzylamine were well characterized by using a microbalance technique. Sample was separately exposed to each enantiomer of the imprint molecule and the quantity of amine was recorded with time. The imprinted material presented a high recognition, and an enantiomeric excess of 35% was obtained.

A new class of heterogeneous catalysts for asymmetric hydrogenation of enamides was synthesized using molecular imprinting technology. These new catalysts were MIPs made from rhodium (I) and copper (II) complexes with a chiral ligand [103]. The ligand was 2,2′-[2-(4-vinylphenyl)-1-(4-vinylbenzyl)ethylidene] bis[(4S)-4-phenyl-4,5-dihydro-2-oxazole]. For the enamide, methyl-*(Z)*-a-*N*-acetamidocinnamate (MAC), the hydrogenation products are Ac-L-Phe-OMe and Ac-D-Phe-OMe (Fig. 11). The non-chiral reactant and two product enantiomers were used as templates to make MAC-MIP, L-MIP, and D-MIP, respectively. To make MAC-MIP, MAC, MAA, EDMA, styrene, the Rh–ligand or Cu–ligand complex, and dichloromethane were placed into a 25-mL test tube in a globe box. The test tube was put in the fridge at 4 °C for 2 h for prearrangement. After addition of AIBN and deoxygenation, polymerization was done in an incubator/ shaker at 69 °C overnight. The polymer was ground into small particles (ca. 10 μm), and washed with methanol. To make L-MIP (or D-MIP), the same procedure as the above was used except that Ac-L-Phe-OMe or Ac- D-Phe-OMe was used in place of MAC as the template. The enantioselective (asymmetric) hydrogenation capabilities of the MIPs were examined in comparison with the conventional free catalysts. One of the most effective catalysts available for asymmetric hydrogenation is [(S)–BINAPINE(cod)Rh]BF_4 which produced good yield and enantiomeric excess (ee) of 77% with the preference for D-enantiomer. When L-MIP was used, a dramatically enhanced ee, 87% (42% ee with MAC-MIP) for L-enantiomer, was observed. This is among the best MIPs in terms of ee. While the Rh-L-MIP showed 87% ee toward L-enantiomeric product, the Cu-MAC-MIP showed 82% ee toward D-enantiomeric product. The Rh-MIP was found to be re-used five times without loss of enantioselectivity while the Cu-MIP retained half of its enantio-selectivity for the first reuse.

8. Concluding remarks

In application of MIP in enantio-selective HPLC, MIP particles seem to be the ultimate prototype for industrial chiral HPLC application. There have been several successful preparation protocols for monodispersed MIP particles where nevertheless the resultant particles have shown much lower separation efficiencies than the commercial C18 particles of similar sizes. The major reason for this may be heterogeneity of the MIP sites inevitably happened by heavy agitation during polymerization. One way to reduce such a problem is to minimize the physical thickness of MIP, thus approaches such as surface-imprinted MIPs, MIP nanoparticles, inert core/ shell MIP type particles have been developed, and their applications in HPLC are the general trends nowadays. However, despite incorporation of such approaches, the separation performances of the resultant MIPs in HPLC have been rather unsatisfactory so far. It is suspected that in most of such studies, the improvement in site heterogeneity might be cancelled to a large extent by degradation of other elements such as retarded mass transfer kinetics or ruined morphology (reduction of mesoporous pores). The MIP should be hard enough to maintain the template cavity structure and also soft enough to enable fast template uptake/release equilibrium. The MIP should also have enough meso-pores for fast mass transfer kinetics. Fulfilling such requirements in applying the above approaches regarding monodisperive MIP particles should be the key strategy in developing new promising systems in the future.

Use of MIP-CEC in analytical enantiomeric determination has been gaining more and more attraction owing to enhanced separation efficiency and simple inexpensive preparation procedures. Especially, the progresses in enantio-selective separation by OT-MIP CEC in very recent years are amazing. OT-MIP-CEC will surely become the prototype of enantio-selective determination by MIP and numerous related studies are expected to prosper.

There is no serious demand for enantio-selective SPE since quantitative enantiomeric determination can be well carried out by using HPLC or CEC MIP (or even non-MIP) columns and large scale separation can also be done by preparative scale HPLC. Thus not much enantio-selective SPE studies are expected in the near future. Nevertheless, perfect enantio-selective SPE will be beneficial if possible. Thus, researchers are encouraged to carry out SPE studies, too, when a new excellent enantio-selective HPLC MIP phase is developed.

Development of enantio-selective MIP sensors is far behind that of achiral MIP sensors since quantitative de-convolution of the mixed signal into separate signals of individual enantiomers is difficult and essential. Such de-convolution is possible so far only when a fluorephore incorporated MIP is used and the total sample

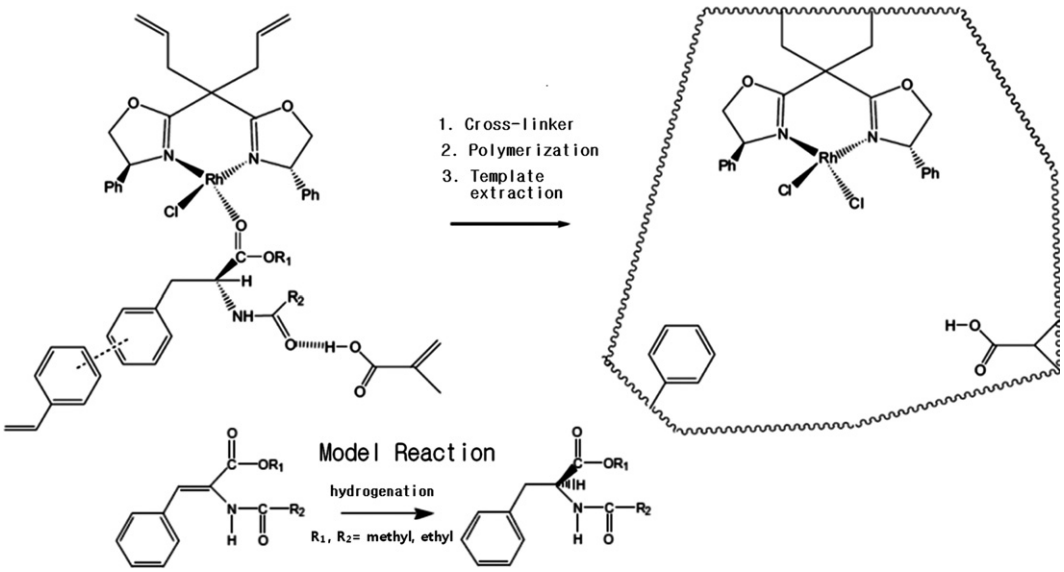

Fig. 11. Preparation of metal-containing asymmetric heterogeneous catalysts by molecular imprinting against the substrate (R_1, R_2=Me, Et; Cl=one ligand example). The model reaction held in the MIP cavity is asymmetric hydrogenation of an enamide. Reproduced with permission from Ref. [103].

concentration ($R+S$) is known with an aid of calibration curves, and this approach is expected to lead to development of the first competitive sensor device in the future for quantitative enantiomeric determination in comparison to chiral HPLC.

Based on the related studies so far it seems that complete enantio-separation of a racemic mixture through one step operation with a MIP membrane will be impossible ever. In other words, 100% ee cannot be achieved by a single operation. Multi-stage membrane permeation process will be the prototype of future industrial application of enantio-selective MIP membranes. The number of stages is directly related to cost, and the goal of enantio-selective MIP membrane studies should be to increase the ee as high as possible to minimize the required number of stages. Meanwhile, many MIP membranes will be continually developed, and their enantio-selectivities will be estimated by measuring the concentrations of the template and the other enantiomer from the separate permeates.

Enatioselective drug delivery is necessary when one enantiomer is an efficient drug and the other enantiomer is not or far less efficient and if the cost of pre-enantio-separation is more expensive than the cost of enantio-selective drug delivery. Considering the incomplete enantio-selectivity, imperfect clinical safety, and sophisticated multi-step fabricating protocols regarding present MIP drug delivery technology, realization of useful, safe and cost-effective enantio-selective MIP drug delivery devices is unlikely to happen in the near future. A huge amount of time and efforts should be devoted.

Making use of MIPs as new catalytic systems is at the early stage of development. Although some success has been reported in developing an efficient asymmetric MIP catalytic system to give high ee for the product, it is usually accompanied by reduction of reaction yield despite the increased cost in comparison to conventional free catalysts. Thus future relevant studies should pursue for high yield in addition to high ee.

Briefly, extensive and productive studies of enantiomeric recognition by MIPs have been observed in HPLC and CEC, but similar studies in other application fields have been rather limited, and such trends will continue for a while.

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