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Review

Molecular imprint-based stationary phases for capillary electrochromatography

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Abstract

The combination of capillary electrochromatography, which provides a high degree of separation efficiency and short separation times, with molecular imprinting, which provides a means for preparing stationary phases of predetermined selectivity, is discussed in this review. Polymerisation around a templating ligand, using monomers that are selected for their ability to participate in non-covalent interactions, constitute the key step of molecular imprinting. Imprints possessing a permanent memory for the imprint species are formed, enabling the resultant polymer selectively to rebind the imprint molecule from a mixture of closely related compounds. Levels of binding affinity and selectivity typical of antibodies are achievable in the best cases. Several studies have focused on imprinted polymers being used as chiral stationary phases in liquid chromatography (LC) where the enantioselectivity of a given polymer is predetermined by the enantiomeric form of the templating ligand. The use of imprinted LC columns, however, is hampered by their poor chromatographic performance, evidenced by peak broadening and tailing. Being a more efficient chromatography technique, the use of capillary electrochromatography greatly improves the performance of imprinted polymer-based separations. The most successful approach utilises capillary columns filled with a monolithic, superporous imprinted polymer obtained by an in situ photo-initiated polymerisation process. This technique enables imprint-based separation systems to be operational within 3 h from the start of capillary preparation. Enantiomer separations with baseline resolution have been carried out in less than 2 min. Other approaches to capillary electrochromatography (CEC) and capillary electrophoresis (CE) involve immobilisation of imprinted polymer particles inside capillaries using a polyacrylamide gel, the use of imprinted particles as a chiral mobile phase additive and the use of an imprinted polymer prepared by a dispersion polymerisation process.

Keywords: Stationary phases, LC; Reviews; Molecular imprinting

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1. Introduction

The ever ongoing research into novel stationary phases for the chromatographic separation of chemical entities has sparked interest in molecular imprinting [1–5] as a facile technique for the synthesis of phases with predetermined selectivity. The imprinted polymers can be made selective for one of the enantiomers of a chiral compound, a specific compound or for a class of compounds. Most molecularly imprinted polymer (MIP) studies have focused on chiral separation problems and many different racemic compounds have been successfully resolved, including drugs [6–8], amino acid derivatives [1–3] and sugars [1–3]. Often, good separations can be achieved and separation factors as high as 17.8 have been reported [9]. The severe peak broadening and tailing that frequently has been observed, however, hampers the wider use of imprinted phases. This is in part due to the MIPs being prepared as bulk polymers, which are then ground and sized into irregular particles of <25 μm before packing into liquid chromatography (LC) columns. Another factor claimed to contribute to peak broadening is imprint heterogeneity, with a distribution of sites from high to low affinity for the ligand and variable association and dissociation kinetics [2]. The recent development of a novel suspension polymerisation technique by which uniformly shaped beads of a defined size range can be made [10] have only partially overcome the problem of poor performance of imprinted columns. Also, separation systems based on continuous rods of imprinted polymer inside LC stainless steel columns have still to show an advantage over packed particle columns [11,12]. Discussions on the use of MIPs in liquid chromatography can be found in some recent reviews [1–3]. Being inherently more efficient chromatography techniques [13], it is believed that the use of capillary electrochromatography (CEC) and capillary electrophoresis (CE) would greatly improve the performance of imprinted polymer-based separations. The combination of molecular imprinting and CEC is the focus of this review.

2. Preparation of molecular imprints

Polymerisation around a templating ligand constitutes the key step of molecular imprinting (Fig. 1). The monomers are selected for their ability to interact, either by noncovalent interactions [2,3], such as hydrogen bonding, electrostatic interactions, etc., reversible covalent interactions [1] or metal ion-mediated interactions [5], with the imprint species. Due to its greater simplicity and wider applicability to a large number of compounds relative to the other approaches, the noncovalent strategy

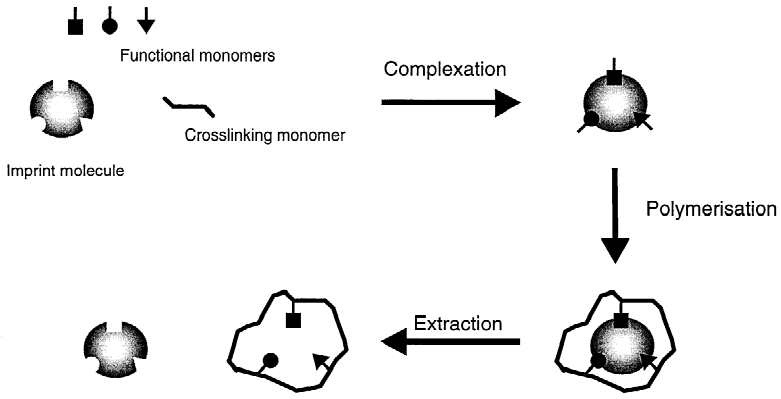

Fig. 1. Schematic depiction of the preparation of molecular imprints. In the prepolymerisation mixture, the functional monomers interact with the imprint molecule forming complexes, which, during polymerisation, are 'frozen' into the resultant polymer. This process gives rise to an imprint (memory) that is complementary in size, shape and chemical functionality to the template molecule. Subsequent removal of the imprint species exposes these memory sites, which enables the polymer to rebind the imprint species selectively from a mixture of closely related compounds.

is the one being most widely employed [2]. Imprints possessing a permanent memory for the imprint species are formed, enabling the resultant polymer selectively to rebind the imprint molecule from a mixture of closely related compounds. For polymers made in the presence of one of the enantiomers of a chiral compound, enantiomer separation is possible, always with a predictable elution order (Fig. 2). Parameters requiring attention in the planning of a molecular imprinting experiment include the choice of functional and crosslinking monomers, solvent and the temperature of polymerisation (for more thorough discussions on imprint preparation, see refs. [1–5]). The most widely used functional monomer is

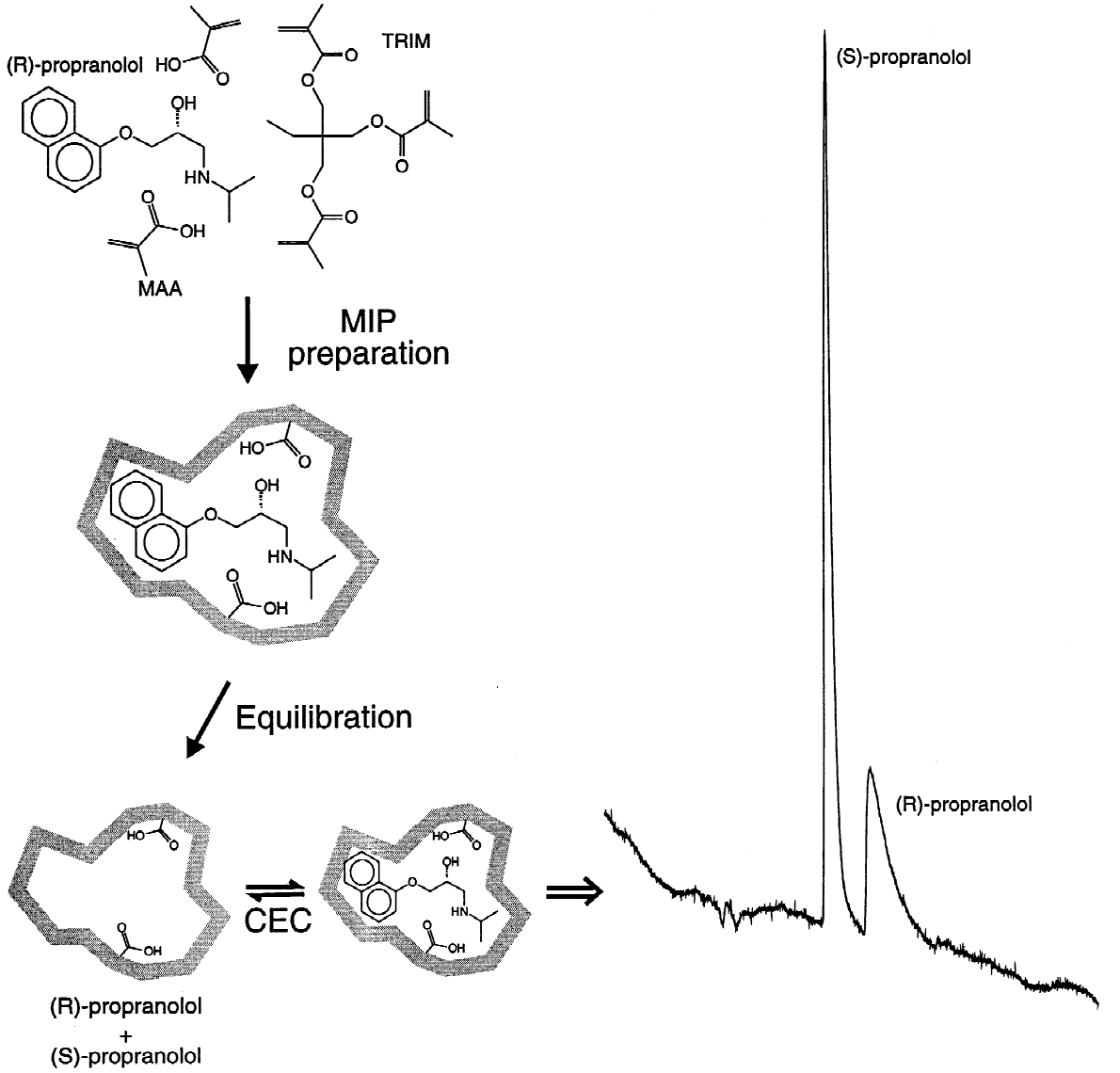

Fig. 2. Molecular imprinting of (*R*)-propranolol using methacrylic acid (MAA) as the functional monomer and trimethylolpropane trimethacrylate (TRIM) as the crosslinking monomer. The enantioselectivity of a given polymer is predetermined by the enantiomer of the ligand that was present during its preparation, here the *R*-form of propranolol. Since the imprinted enantiomer possesses the higher affinity for the polymer, the separation is done with a predictable elution order of the enantiomers.

methacrylic acid, which forms hydrogen bonds to a number of chemical structures. Others are trifluoromethyl acrylic acid, vinylpyridines and methacrylamide. Combinations of these monomers can be employed. To preserve the integrity of the imprints in the resultant MIP, high crosslinking of the polymer network is required [1]. Ethylene glycol dimethacrylate and trimethylolpropane trimethacrylate are, thus far, the most extensively utilised crosslinking monomers, due to their mechanical and thermal stability, ease of removal of the imprint molecule and the high selectivities that have been observed [1]. The latter crosslinker is claimed to be the superior one for chromatographic applications, due to its higher load capacity, increased selectivity and better resolving capability [14]. The polymerisation mixture is prepared by dissolving the imprint compound in an appropriate organic solvent and adding the crosslinking monomer and one or more functional monomers. For maximal efficiency of imprint formation, at least for noncovalent type MIPs, the polymerisation reaction should be performed using a solvent that is as apolar as possible without compromising solubility of the imprint species [2]. Furthermore, the solvent also affects the polymer morphology, which, in turn, affects the chromatographic properties of the MIP. It has been demonstrated repeatedly that MIPs prepared at lower temperatures (−20 to 0°C) using photo-initiation exhibit higher molecular recognition capabilities [1]. The prepared material can be stored in a dry state at ambient temperatures for a long time (years) before use. In the best cases, levels of binding affinity and selectivity that are typical of antibodies are achievable. In the analytical area, MIPs have been applied to a range of applications requiring selective ligand binding, including chromatography, solid-phase extraction and immunoassay.

3. Preparation of imprinted polymer-based capillaries for CEC

Recently, the use of polymer-based stationary phases as an alternative to conventional modified silica particles has rendered great interest. The main driving forces for this development were problems often encountered with packed bed capillaries in CEC, such as the difficult fabrication of frits within a capillary, the packing of beads into a tube with very small diameter, the limited stability of packed columns and the formation of bubbles within the capillary during runs. The polymer-based phases are prepared by in situ polymerisation procedures, yielding polymer monoliths [15–18] or film coatings [19]. In general, the polymerisation of monomers into polymer monoliths is less laborious than the packing of particles into capillary columns. Furthermore, since the polymer is covalently attached to the inner surface of the capillary, frits can be avoided completely. Silica surface derivatisation chemistries similar to those described for the preparation of MIP-based capillaries below have been used. In some instances, however, CEC could be run without any requirement for anchoring the polymer monolith to the capillary surface. Whereas studies on polymer phases have led to novel reversed-phase-, ion-exchange-, etc., type separation systems [15–19], MIP-based CEC studies have focused on chiral separations (Table 1).

The first reported preparation of capillary columns containing MIPs utilised a thermally initiated dispersion polymerisation procedure [20]. Agglomerates of micrometer-sized globular polymer particles could be prepared in situ in the capillary. Molecular imprinting of L-phenylalanine anilide, pentamidine and benzamidine was undertaken. A pH-dependent retardation of pentamidine over benzamidine in the pentamidine capillary was observed, while the opposite, i.e. retardation of benzamidine over pentamidine in a benzamidine imprinted capillary, was not. Enantiomer separation of *rac*-phenylalanine anilide could not be achieved using the L-phenylalanine anilide imprinted capillary. This lack of enantioselectivity may be a consequence of the polymerisation method, necessitating the use of a dispersion medium, such as cyclohexanol and 1-dodecanol. Protic solvents, however, interfere with the ionic and hydrogen bonds, giving rise to complexation between the imprint molecule and the functional monomers. Hence, the formation of well-defined imprints with such a method is less efficient (see above).

The, as yet, most successful approach to MIP-based CEC utilises capillary columns filled with a monolithic, superporous imprinted polymer. Using this system, enantiomer separations with baseline

Table 1
Types of molecularly imprinted polymers used in capillary electrochromatography

Type of column	Imprint species	Separated species	References
Dispersion polymerised MIP	Benzamidine	Selective retardation of imprint species	[20]
	Pentamidine	Selective retardation of imprint species	[20]
	L-Phenylalanine anilide	No chiral separation	[20]
Superporous MIP monolith	(*R*)-Propranolol	Enantiomers of β-blockers	[21,22]
	(*S*)-Metoprolol	*rac*-Metoprolol	[23]
	(*S*)-Ropivacaine	Enantiomers of local anaestethics	[22]
MIP monolith	L-Phenylalanine anilide	Enantiomers of amino acids	[24,25]
MIP particles entrapped in acrylamide	L-Phenylalanine anilide	Enantiomers of amino acids	[26–28]
	L-Phenylalanine	Enantiomers of amino acids	[27]
MIP as electrolyte additive	(*S*)-Propranolol	*rac*-Propranolol	[29]
MIP as film coating	(*S*)-2-Phenylpropionic acid	*rac*-2-Phenylpropionic acid	[30]
	(*R*)-2-Phenylpropionic acid	*rac*-2-Phenylpropionic acid	[30]

resolution have been carried out in less than 2 min. The MIP-filled capillaries are obtained by an in situ photo-initiated polymerisation process (Fig. 3). Briefly, the capillary is filled with a prepolymerisation mixture of imprint molecule, functional and crosslinking monomers (methacrylic acid and trimethylolpropane trimethacrylate, respectively), radical initiator (2,2′-azobis(isobutyronitrile, AIBN)) and solvent (toluene). Both ends of the capillary are sealed and the polymerisation is performed by placing the capillary under a UV source (350 nm) at −20°C. At the end of the polymerisation, the solvent is exchanged for electrolyte by hydrodynamic pumping, simultaneously flushing the remaining monomer, radical initiator and imprint molecule out of the capillary. After a short time for equilibration, the capillary column is then ready for use. The inner surface of the capillary is derivatised with methacryloxypropyltrimethoxysilane, which participates in the polymerisation reaction. In this way, the polymer monolith is covalently attached to the inner wall of the capillary (Fig. 4), thereby preventing elution of the polymer during electrochromatography. The technique is simple and quick, and enables imprint-based separation systems to be operational within 3 h from the start of capillary preparation. The success of this approach relies on the polymer-filled capillaries possessing good flow-through properties, since, before use, the organic solvent employed for polymerisation must be replaced by an electrically conducting electrolyte. The MIPs could be rendered superporous by the use of 1–25% iso-octane as a porogenic agent [21,22] or by careful timing of the polymerisation reaction [23]. The introduction of superpores permits rapid exchange of the solvent of polymerisation for electrolyte by hydrodynamic pumping at low pressures. Molecular imprinting of the β-adrenergic antagonists metoprolol and propranolol [23] and of the local anaesthetic ropivacaine [22] have been done.

The preparation of MIP-monoliths in capillaries utilising thermally induced polymerisation at 60°C have been described [24,25]. The result is a dense polymer inside the whole capillary. The resulting MIP capillary is then connected to an electrolyte-filled open capillary via a PTFE tube, and a detection window is prepared on the open capillary to facilitate detection. Ammonium acetate is added to the prepolymerisation mixture at a concentration of 1–2 m*M* and the exchange of the solvent of polymerisation to electrolyte is done electrophoretically by increasing the electric field stepwise until a stable baseline is obtained. The imprint species was phenylalanine anilide.

A different approach to CEC involves immobilisation of MIP particles, of the same type as those that have been used for LC, inside capillaries [26–28]. The MIP was prepared in bulk and subsequently crushed into small (<10 μm) irregular polymer particles. These were suspended in a solution of acrylamide monomers and introduced into the capillary column where a polyacrylamide gel was formed

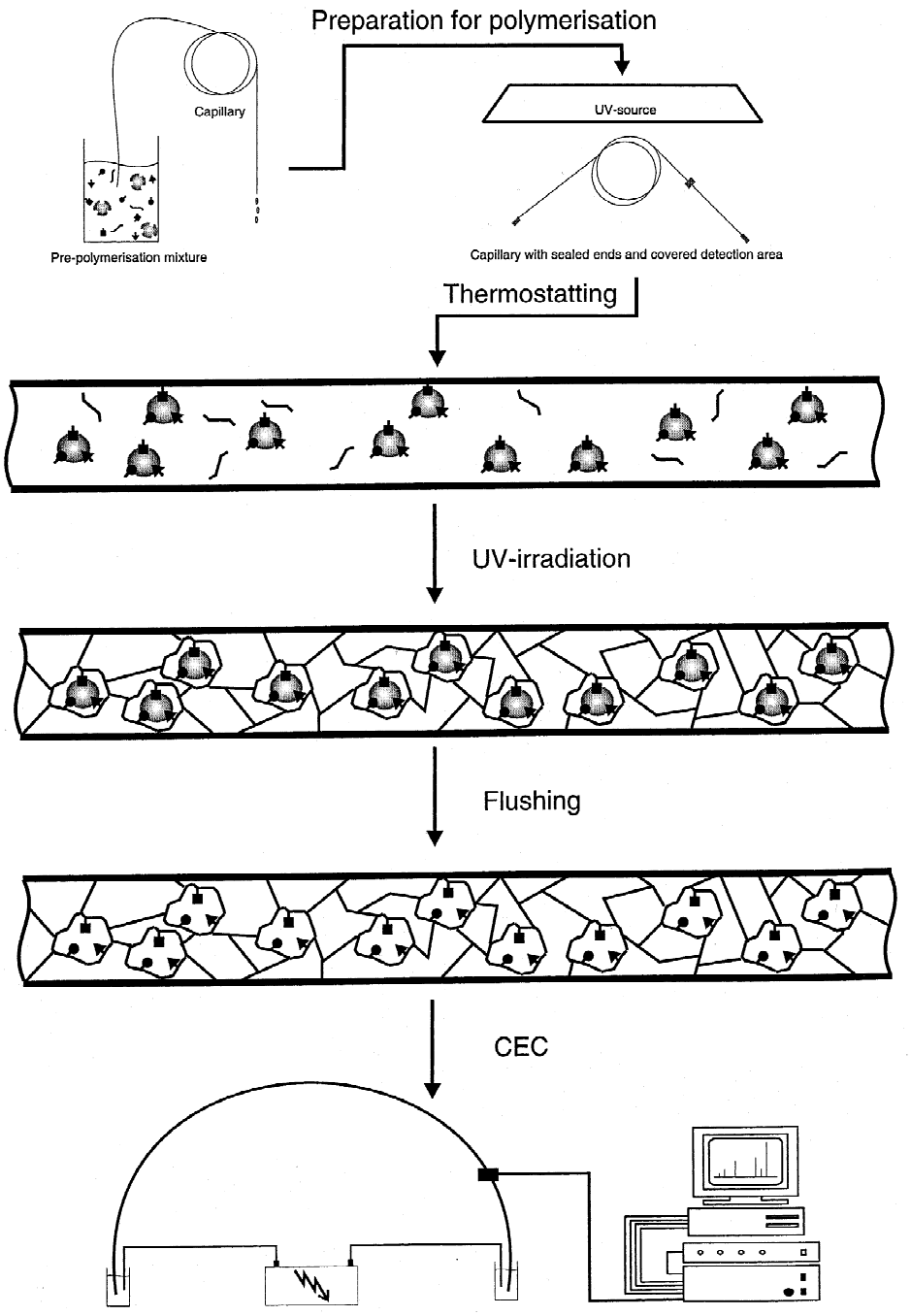

Fig. 3. Typical procedure for the preparation of a capillary column filled with a molecularly imprinted polymer. A mixture of imprint molecule, functional and crosslinking monomer, radical initiator and a solvent is prepared and poured into the capillary. Both ends of the capillary are sealed and the polymerisation reaction is initiated either by placing the capillary under a UV source (350–366 nm) at −20–+4°C or by heating it to 45–65°C. After completion of the polymerisation reaction, the solvent used for polymerisation is exchanged for electrolyte. The flushing also extracts imprint molecules, and traces of remaining unreacted monomer and radical initiator out of the polymer. The capillary column is then ready for CEC.

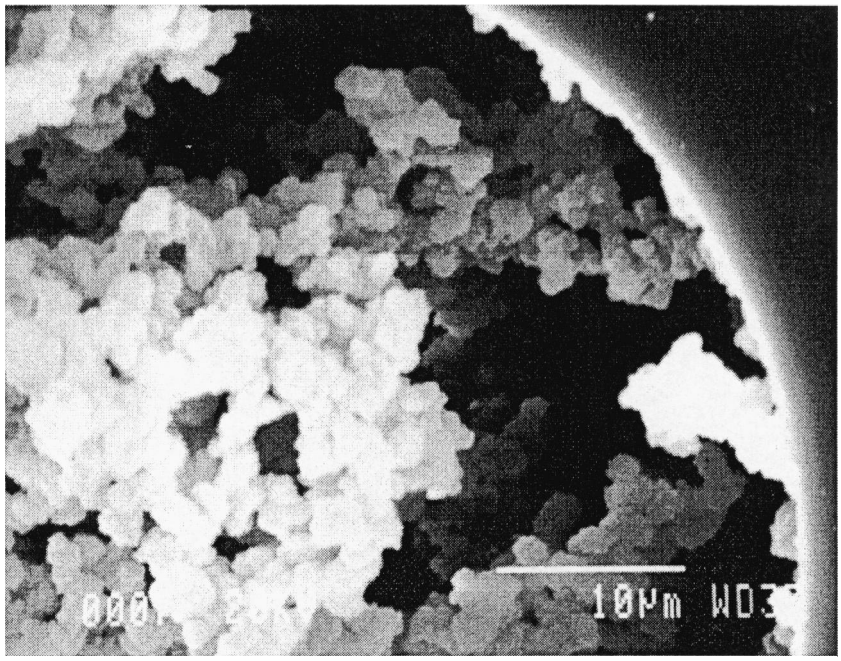

Fig. 4. Scanning electron micrograph of a polymer-filled capillary column. μm-sized globular units of macroporous molecularly imprinted polymer are surrounded by interconnected superpores. The covalent attachments of the polymer to the capillary wall can be seen.

[26]. A MIP-particle slurry could also be contained within the capillary by closing each end of the capillary with a small plug of polyacrylamide gel [27,28]. An open capillary with a detection window was connected to the MIP-filled capillary in the same manner as mentioned above. The imprint molecule was L-phenylalanine anilide.

In a preliminary study, MIP particles made selective for (*S*)-propranolol were used as a mobile phase additive in the CE separation of (*R*)- and (*S*)-propranolol [29]. Baseline separation of the enantiomers was achieved. The use of a chiral functional monomer, *N*-acryloyl–L-alanine, may be a key component in the high selectivity observed.

Preparation of coatings of imprinted polymer inside capillaries has been reported [30]. Investigation into the effect of several parameters, including the type of porogen, the type and concentration of crosslinker and silanization of the inner surface of the capillary, on the synthesis of MIP-coatings of controllable thickness was undertaken. Although preliminary, the results obtained may open up the interesting technique of open tubular CEC for molecular imprinting.

4. Electrochromatographic separations

As yet, research into the field of MIP–CEC has focused on the adaptation of imprinted polymers to electrochromatography, and the preparation of MIP stationary phases within fused-silica capillaries (Table 1). Optimisation of separation parameters has been addressed less. Most often, the electrolyte is composed of 70–90% of an organic modifier, such as acetonitrile, and an aqueous buffer at low pH. In many instances, the aqueous component is merely a mixture of water and acid. In one study, using (*S*)-ropivacaine imprinted capillaries, it was found that an electrolyte composed of 80% acetonitrile and 20% aqueous buffer was suitable for the CEC separation of the enantiomers of ropivacaine [22]. The enantiomeric separation increased with increasing pH, at least in the pH range 2–6.5, as well as

with an increasing volume ratio of acetonitrile to aqueous buffer. In most instances, however, larger separations occurred at the expense of peak broadening. It was observed that the resolution could be improved by increasing the temperature of the capillary. Similar observations were made in a study on the separation of the enantiomers of amino acids in a capillary with L-phenylalanine anilide MIP [27,28]. The optimal composition of the electrolyte was found to be a mixture of 90% acetonitrile, 5% water and 5% acetic acid. Omitting the acetic acid resulted in a complete loss of enantiomer separation. Again, the resolution was found to be improved at elevated temperatures.

Pure enantiomer imprinting of L-phenylalanine anilide, (*R*)-propranolol, (*S*)-metoprolol and (*S*)-ropivacaine has been undertaken and these MIP capillaries have been used in the CEC mode for chiral separations [21–28] (Table 1). Baseline separations of the enantiomers of propranolol and metoprolol could be carried out in less than 2 min. The propranolol column was shown to be able to resolve several other β-blockers, including prenalterol, atenolol, pindolol, etc. [21], and the ropivacaine column could separate the enantiomers of structurally similar local anaestethics, including mepivacaine and bupivacaine [22]. An interesting strategy was illustrated by the molecular imprinting of phenylalanine anilide and the use of the resultant capillary columns for the separation of the enantiomers of phenylalanine [26]. Due to their low solubility in the prepolymerisation mixture, amino acids are normally not amenable to noncovalent imprinting. Instead, a structurally similar compound, one that gives rise to imprints that have the ability to bind phenylalanine and is soluble in the polymerisation mixture, was used as the imprint molecule.

5. Conclusions and future outlook

The combination of CEC, which provides a high degree of separation efficiency and short separation times, and molecular imprinting, which provides a means for preparing stationary phases of predetermined selectivity, may well lead to efficient and highly selective separation systems. Molecular imprinting technology represents a widely applicable strategy for producing stationary phases with selectivity for a predetermined ligand, or class of compounds. Its utility has been shown repeatedly in the imprinting of a range of functionally distinct compounds. The chemical resistance of the polymer permits the use of organic solvents, of strong acidic and basic buffers, and of high voltages, which may lead to short separation times. The microcolumn format of CEC leads to a minimal consumption of chemicals, including the imprint species. Since the concentration of imprint species in the prepolymerisation mixture is high, the large scale production of MIP-based microcolumns may be more easily realised compared with conventional LC columns. Typically, 10–100 nmol of imprint species are required for a polymer the size of a capillary column, which is about 1–10 μl. Although the peak performance is better than that obtained in LC, separation efficiencies characteristic of CEC are still to be achieved. For MIP-based stationary phases to become a true complement to the selectors presently employed in CEC, further improvements in peak shape are required. To achieve this, further research into both the methodology for the preparation of MIP-based columns and optimisation of the conditions for MIP-based electrochromatography is warranted. The combination of MIPs and CEC is still in its infancy, however, and its present performance should be judged accordingly.

References

[1] G. Wulff, Angew. Chem., Int. Ed. Engl. 34 (1995) 1812–1832.
[2] K. Mosbach, O. Ramström, Bio/Technology 14 (1996) 163–170.
[3] I.A. Nicholls, L.I. Andersson, K. Mosbach, B. Ekberg, Trends Biotechnol. 13 (1995) 47–51.
[4] J. Steinke, D.C. Sherrington, I.R. Dunkin, Adv. Polym. Sci. 123 (1995) 81–125.
[5] S. Mallik, S.D. Plunkett, P.K. Dhal, R.D. Johnson, D. Pack, D. Schnek, F.H. Arnold, New J. Chem. 18 (1994) 299–304.
[6] M. Kempe, K. Mosbach, J. Chromatogr. A 664 (1994) 276–279.
[7] L. Fischer, R. Müller, B. Ekberg, K. Mosbach, J. Am. Chem. Soc. 113 (1991) 9358–9360.
[8] J. Matsui, I.A. Nicholls, T. Takeuchi, Tetrahedron Asymmetry 7 (1996) 1357–1361.
[9] O. Ramström, I.A. Nicholls, K. Mosbach, Tetrahedron Asymmetry 5 (1994) 649–656.

[10] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769–3774.
[11] J. Matsui, T. Kato, T. Takeuchi, K. Yokoyama, E. Tamiya, I. Karube, Anal. Chem. 65 (1993) 2223–2224.
[12] J. Matsui, T. Takeuchi, Anal. Chem. 34 (1997) 199–200.
[13] J.H. Knox, I.H. Grant, Chromatographia 32 (1991) 317–328.
[14] M. Kempe, Anal. Chem. 68 (1996) 1948–1953.
[15] C. Fujimoto, Y. Fujise, E. Matsuzawa, Anal. Chem. 68 (1996) 2753–2757.
[16] J.-L. Liao, N. Chen, C. Ericson, S. Hjertén, Anal. Chem. 68 (1996) 3468–3472.
[17] E.C. Peters, M. Petro, F. Svec, J.M.J. Fréchet, Anal. Chem. 69 (1997) 3646–3649.
[18] A. Palm, M.V. Novotny, Anal. Chem. 69 (1997) 4499–4507.
[19] Z.J. Tan, V.T. Remcho, Anal. Chem. 69 (1997) 581–586.
[20] K. Nilsson, J. Lindell, O. Norrlöw, B. Sellergren, J. Chromatogr. A 680 (1994) 57–61.
[21] S. Nilsson, L. Schweitz, M. Petersson, Electrophoresis 18 (1997) 884–890.
[22] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 792 (1997) 401–409.
[23] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179–1183.
[24] J.-M. Lin, T. Nakagama, X.-Z. Wu, K. Uchiyama, T. Hobo, Fresenius' J. Anal. Chem. 357 (1997) 130–132.
[25] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Pharm. Biomed. Anal. 15 (1997) 1351–1358.
[26] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Chromatographia 43 (1996) 585–591.
[27] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 1489–1506.
[28] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Biomed. Chromatogr. 11 (1997) 298–302.
[29] M. Walshe, E. Garcia, J. Howarth, M.R. Smyth, M.T. Kelly, Anal. Commun. 34 (1997) 119–122.
[30] O. Brüggemann, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43–53.