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Journal of Chromatography B, 804 (2004) 3–12
JOURNAL OF CHROMATOGRAPHY B
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Review

Molecularly imprinted polymer formats for capillary electrochromatography

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Abstract

The research aimed towards the adaptation of molecularly imprinted polymers (MIPs) to the capillary format and the use of these highly selective matrices for capillary electrochromatography (CEC) is reviewed in this article. The MIP is prepared by incorporation of a template molecule into a polymerization protocol. After polymerization and extraction of the template from the resulting polymer a highly selective material with recognition cavities complementary to the template in size, shape and chemical functionality is obtained. MIPs have been used as recognition elements in several different analytical techniques. In combination with CEC a novel separation system with a unique selectivity towards a predetermined target (the template) is achieved. The merge of molecular imprinting technology (MIT) and CEC have introduced several interesting polymer formats, due to the adaptation of the MIP to the miniaturized capillary format. The polymer formats can be classified according to their preparation protocols and appearance into three conceptually different categories, i.e. the monolith, the coating and the nanoparticles. The preparation protocols, characteristics and applications of these formats will be discussed.

Keywords: Reviews; Molecularly imprinted polymers

Contents

1. Introduction

Molecular imprinting technology (MIT) allows synthesis of polymers with selectivity towards a predetermined target (Fig. 1) [1,2]. In short, the molecularly imprinted polymer (MIP) is prepared by mixing a template molecule with functional monomers, cross-linking monomers and a radical initiator in a proper solvent, most often an aprotic and non-polar solvent. Subsequently, this pre-polymerization mixture is irradiated with UV-light or subjected to heat in order to initiate polymerization. During polymerization, the complexes formed between the template molecule and the functional monomers will be stabilized within the resulting rigid, highly cross-linked polymer. Extraction of the template molecule reveals recognition cavities complementary to the template molecule in shape, size and chemical functionality.

MIPs have been applied in several different analytical techniques including high-performance liquid chromatography

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doi:10.1016/j.jchromb.2003.12.036

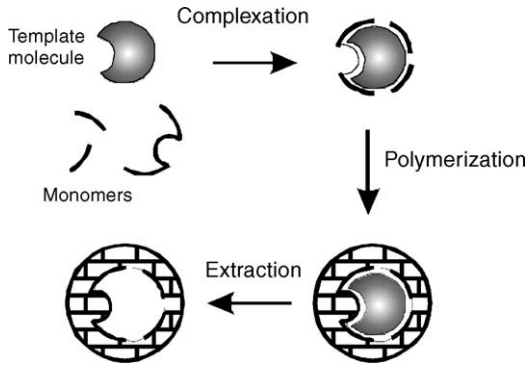

Fig. 1. Schematic description of MIP synthesis. A pre-polymerization solution is prepared by mixing the template with functional monomers, cross-linking monomers and initiator in a proper solvent. Complexes are formed between the functional monomers and the template molecule. Polymerization is initiated using either heat or UV-irradiation allowing the functional monomers to react with cross-linking monomers and form a rigid polymer network. Finally, the template molecule is extracted and a cavity, that is complementary to the template in size, shape and chemical functionality, is formed.

(HPLC) [3], solid-phase extraction (SPE) [4], binding assays [4], and as recognition elements on sensors [5]. In applications of MIPs as stationary phase sorbents in HPLC, a large amount of template is needed in order to synthesize the large amount of MIP that is required for packing of HPLC columns. The requirement for significant quantities of template can prove problematic, as the template in many cases can be both rare and expensive [6]. It has thus been essential to develop new miniaturized chromatographic methods based on MIPs. Capillary electrochromatography (CEC) met this desire, since the volume of the capillary column is magnitudes lower than the volume of the traditional HPLC column. Furthermore, higher separation efficiencies, faster analysis times, and lower sample consumption can be achieved using this technique [7].

Traditionally, the MIP has been synthesized as a bulk polymer, ground, sieved and slurry packed into columns [3]. This is a time-consuming and a somewhat tricky operation, due to the irregular geometry of the particles. The packing procedure becomes even more tedious when capillary columns are used. Thus, in order to circumvent capillary packing, several different MIP formats devoted to the capillary format have been developed. These formats can be classified according to their preparation protocols and appearance into three conceptually different categories, i.e. the monolithic MIP, the surface grafted MIP and the nanoparticle MIP. The monolithic format can either be synthesized in situ or constructed by entrapment of prefabricated MIP particles in different types of matrices. The surface grafted polymer format allows immobilization of the MIP stationary phase as a coating on the inside of the capillary for open-tubular (OT) CEC applications or on silica particles that subsequently can be packed into capillaries. The third format, i.e. the nanoparticle MIP, is used in a partial filling application of CEC. Protocols for synthesis of these novel MIP formats as well as their applications in CEC will be discussed.

2. MIP formats in CEC

Several different types of MIP-CEC formats have been reported in the literature during the past decade (Table 1). In the following text, these have been sorted into the three conceptually different categories, i.e. the monolithic format, the surface grafted format and the nanoparticle format.

2.1. Monolithic MIPs

This section, which is devoted to monolithic MIP formats for CEC, includes true monoliths or continuous beds as well as beds formed by entrapment of pre-fabricated MIPs in different types of matrices. In the first approach to incorporate MIPs in a CEC system an in situ dispersion polymerization protocol was used [8]. The polymerization, which was performed in mixtures of cyclohexanol–dodecanol and isopropanol–water, resulted in MIP agglomerates of 0.5–4 μm in size. The selectivity of this MIP was however low, for which two feasible explanations may be proposed. Firstly, the use of polar and protic solvent blends is usually considered disadvantageous for non-covalent MIP synthesis due to their possible interference with the template monomer complexes. Secondly, polymerization was thermally initiated, which means that on-column detection was performed through the MIP. Since detection was successfully performed on-column, the low selectivity could also be due to a low polymerization yield and thus a low amount of MIP present in the capillary column.

An alternative approach to the in situ preparation is to synthesize the MIP as a bulk polymer, crush, grind, and sieve it to obtain the appropriate particle size. These particles can subsequently be packed or entrapped in a matrix inside the capillary column. Lin et al. [9] reported the use of polyacrylamide gel as an entrapping matrix for prefabricated 1–15 μm MIP particles. Polymerization of the acrylamide matrix was achieved using a thermal in situ initiation protocol. Although not clearly stated it can be assumed that the detection window was free from polymer since no detection problems occurred. Since the soft polyacrylamide gel cannot be hydrodynamically flushed, the capillary column cannot be regenerated once current breakdown has appeared. In another design of this format, Lin et al. [10–12] used retaining frits of polyacrylamide to keep the MIP particles in a specified section of the capillary column. In order to enable on-column detection, a Teflon sleeve connection was utilized to connect the MIP filled capillary with a buffer filled capillary, as shown in Fig. 2.

Chirica and Remcho [13] prepared silicate-entrapped capillary columns by trapping MIP particles, as well as reversed phase chromatographic packing materials, in a network of silica. Retaining frits were initially needed in order to keep

Table 1
MIP formats used in CEC

Type of column	Separated species	Template molecule	Monomers used functional/ cross-linking	References
Dispersion polymerized MIP	Selective retardation of template molecule	Benzamidine	MAA/EDMA	[8]
	Selective retardation of template molecule	Pentamidine		[8]
	No enantiomer separation	L-Phenylalanine anilide		[8]
Superporous MIP monolith	*rac*-Propranolol, enantiomers of β-blockers	(*R*)-Propranolol (*S*)-Propranolol	MAA,2VPy/ TRIM, PETRA, PETEA, EDMA	[16,25]
	rac-Propranolol, *rac*-metoprolol	(*S*)-Metoprolol		[16,20,22]
	Enantiomers of local anaestethics	(*S*)-Ropivacaine		[17]
	rac-Atenolol	(*S*)-Atenolol		[17]
Multiple template MIP	*rac*-Atenolol, *rac*-metoprolol	(*S*)-Atenolol, (*S*)-metoprolol	MAA/TRIM	[17]
MIP monolith	Enantiomers of amino acids	L-Phenylalanine anilide	MAA,2VPy/ EDMA	[14,15]
MIP particles entrapped in acrylamide	Enantiomers of amino acids	L-Phenylalanine anilide	MAA, 2VPy/ EDMA	[9–11]
	Enantiomers of amino acids	L-Phenylalanine		[11]
	Enantiomers of amino acids	L-Dansyl-leucin, L-dansyl-phenylalanine		[12]
Silicate entrapped MIP particles	*rac*-Dansyl-phenylalanine	L-Dansyl-phenylalanine	Not stated	[13]
Open-tubular	*rac*-2-Phenylpropionic acid	(*R*)- or (*S*)-2-phenylpropionic acid	*trans*-3-(3-Pyridyl)-acrylic acid/EDMA, DVB	[32]
	rac-Dansyl phenylalanine	Dansyl-L-phenylalanine	MAA, 2VPy/TRIM, EDMA	[33]
	rac-Propranolol	(*S*)-Propranolol	MAA/TRIM	[29]
Surface grafted particles	*rac*-Phenylalanine anilide	L-Phenylalanine anilide	MAA/EDMA	[27,28]
MIP as electrolyte additive	*rac*-Propranolol	(*S*)-Propranolol	*N*-Acryloyl-alanine/EDMA	[42]
MIP nanoparticles in partial filling	*rac*-Propranolol	(*S*)-Propranolol	MAA/TRIM	[44]
	rac-Propranolol, enantiomers of β-blockers	(*S*)-Propranolol	MAA/TRIM, PETEA, EDMA	[46,47]
	rac-Ephedrine and *rac*-salbutamol	(+)-Ephedrine	MAA/TRIM	[48]

Abbrevations: DVB, divinylbenzene; EDMA, ethylene glycol dimethacrylate; MAA, methacrylic acid; PETEA, pentaerythritol triacrylate; TRIM, trimethylolpropane trimethacrylate; and 2VPy, 2-vinylpyridine.

the packing material in a defined section of the capillary whilst the entrapment mixture was introduced and polymerized. Following the entrapment procedure, the frits could be removed and an on-column detection window created.

As an alternative to the above described entrapment techniques, in situ MIP preparation protocols have been developed. The benefits of an in situ preparation protocol include the complete absence of retaining frits as well as a means of circumventing time consuming packing procedures. Lin et al. [14,15] developed a thermally initiated in situ polymerization protocol that yielded dense MIP monoliths. Prior to polymerization, the capillary inner surface was derivatized using thionyl chloride followed by vinylization with a vinyl magnesiumbromide Grignard reagent. This derivatization procedure resulted in surface bond vinylic groups that were able to participate in the polymerization reaction. Thus, the MIP monolith became covalently linked to the capillary, circumventing detachment of the monolith upon polymer shrinkage. Due to the absence of larger pores in the monolith, ammonium acetate had to be added to the polymerization mixture in order to facilitate electrophoretic exchange of the polymerization solvent for electrolyte. Once more, the MIP monolith filled capillary had to be connected to another buffer filled capillary using a Teflon sleeve to facilitate on-column detection. A separation of D,L-phenylalanine performed on a dense L-phenyl alanine anilide templated MIP monolith is shown in Fig. 3.

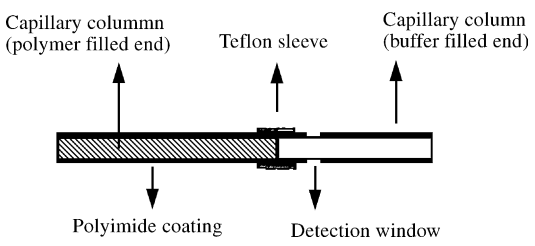

Fig. 2. Schematic description of the Teflon sleeve connection between an MIP monolith filled capillary and an open buffer filled capillary used for on-column detection. Reprinted from [15] with permission.

The gel entrapped columns and the dense monoliths all have in common the impossibility to regenerate the columns after current breakdown, due to the lack of means

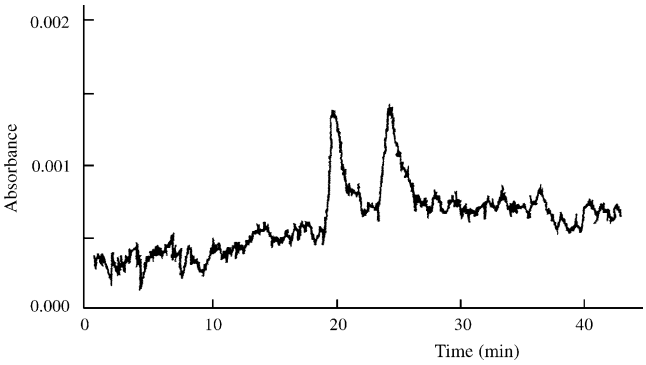

Fig. 3. CEC separation of DL-phenylalanine using the cross-selectivity of an L-phenylalainen anilide templated MIP monolith. Underivatized amino acids are most often not applicable to non-covalent molecular imprinting due to their low solubility in the commonly used organic solvents. Reprinted from [15] with permission.

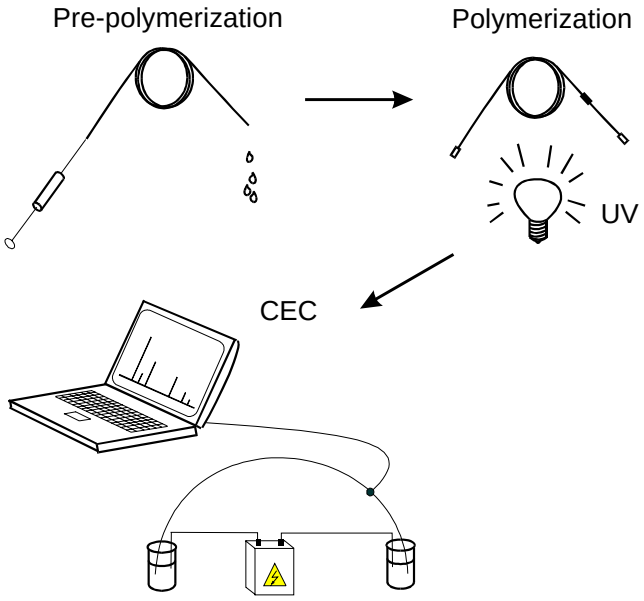

Fig. 4. Schematic illustration of in situ MIP monolith preparation. The capillary is filled with pre-polymerization solution containing the template molecule, functional monomers, cross-linking monomers, radical initiator and solvent. Both ends are sealed and a detection window is created on-column by masking the capillary with a UV-impermeable coating. UV-irradiation is used to initiate polymerization and after completed polymerization, the capillary can be hydrodynamically flushed in order to remove unreacted monomers, polymerization solvent, remaining radical initiators and the template molecules.

to hydrodynamically flush them. Furthermore, all of the described entrapment techniques require multi step synthetic routs in order to obtain a MIP capillary column. Thus, a one-step synthetic route yielding a super porous monolithic MIP was desirable. Schweitz et al. [16,17] developed two different protocols for fabrication of super porous MIP monoliths. Both protocols yielded MIP monoliths that could be hydrodynamically flushed, thus facilitating both regeneration of the capillary as well as the exchange of polymerization solvent for electrolyte. In both protocols, the inner surface of the capillary was derivatized with (methacryloxy-propyl)-trimethoxysilane, according to the procedure first described by Hjertén [18], in order to covalently link the polymer to the capillary. Furthermore, UV-irradiation was applied for initiation of the polymerization, which has two advantages over thermal initiation. Firstly, UV-initiation can be applied at very low temperatures which have been shown to favor MIP synthesis [16,19]. This effect is probably due to the formation of strong complexes between the template molecules and the functional monomers at low temperatures. Secondly, UV-initiation also enables a convenient method for preparation of a detection window [16] as well as a means for controlling the length of the monolith [20]. Masking the areas where polymer is unwanted using a UV-impermeable material realizes a simple photochemical masking procedure. A schematic description of the preparation of a monolithic MIP capillary column is shown in Fig. 4.

The first technique applied in order to obtain super porous MIP monoliths was interrupted polymerization [16]. This technique can be applied in order to generate super pores in polymers that are synthesized in solvents that otherwise would yield dense polymers. Basically, the polymerization is not allowed to proceed to completeness, but is stopped after a certain time before the polymer has grown dense. To stop the polymerization, the capillary was simply hydrodynamically flushed with electrolyte to remove unreacted monomers and remaining radical initiators. This technique required some optimization in polymerization time, but once this was optimized the success rate was 100%. The major advantage of the technique is its independence of the pore forming properties of the polymerization solvent. In the second technique, pore generating solvents were applied in order to generate super pores in the MIP monolith [17,20–22]. The pore generating solvent is a thermodynamically poor solvent for the polymer, i.e. the solubility of the resulting polymer decreases with the addition of this solvent. Several commonly applied solvents with pore generating properties have polar and protic properties, which are considered unfavorable for efficient molecular imprinting due to their interference with the functional monomer-template complexes [23]. However, Schweitz et al. [17,20–22] used apolar and aprotic isooctane-toluene blends as pore generating solvent (Fig. 5). The porosity of the MIP monolith could easily be altered by varying the concentration of isooctane, a higher concentration of isooctane yielding more porous polymer structures. Enantiomer separations of propranolol performed on a super porous MIP monolith prepared using toluene-isooctane as pore generating solvent are shown in Fig. 6. Just recently, Schweitz et al. [20] managed to synthesize monolithic MIP capillary columns with a very short sorbent length. By altering the concentration of monomers and the composition of the pore generating solvent, an MIP monolith of only 8.5 cm in length could be prepared using a photochemical masking procedure with a preparation time

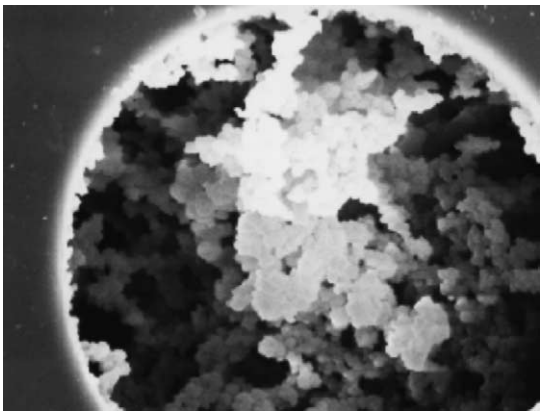

Fig. 5. Scanning electron micrograph of the cross section of a capillary column containing a super-porous monolith. Micrometer sized interconnected globular particles are surrounded by micrometer sized super-pores that gives the capillary a low flow resistance.

of only 1 h. This short MIP monolith, that was restricted to the volume between the outlet end of the capillary and the detection window, allowed enantiomer separations in 30 s to be achieved successfully. This study illustrated the possibilities to direct the polymer synthesis to any defined compartment and also, due to the short length of this sorbent, the potential application of MIT to the chip format.

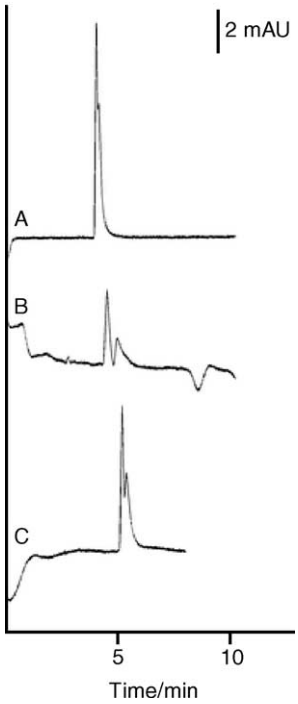

Fig. 6. CEC separation of *rac*-propranolol on a superporous MIP monolith prepared using (*S*)-propranolol as template and toluene-isooctane as pore-forming solvent. These separations also illustrate the effect of surfactant addition to the electrolyte (SOAP-MIP-CEC). The electrolyte was composed of 80 vol.% acetonitrile and 20 vol.% 25 mM phosphate acid + TEA pH 3.0. (A) 1 mM SDS added to the electrolyte; (B) 1 mM CTAB added to the electrolyte; and (C) no modifier added to the electrolyte. Reprinted from [22] with permission.

The cross-selectivity of the MIP allows, in resemblance with the biological antibodies, it to recognize also structural analogues of the template [24,25]. Thus, a MIP prepared using one of the enantiomers of a chiral substance as template cannot only be used for separations of the enantiomers of that particular substance but also of several of its structural analogues. This property can be of great usefulness in, e.g. applications of the MIP as SPE sorbent [26]. For trace amount analysis using MIP-SPE, template molecules remaining in the MIP can cause significant problems as they might leak from the MIP during operation. To circumvent this problem, an alternative template approach can be applied. This approach makes use of a structural analogue of the intended analyte as template during the preparation of the MIP [26]. Several studies on MIP cross-reactivities have been published during recent years. These works, when applied to chiral compounds, indicates that a lower resolution is obtained for the structural analogues than for the raceme corresponding to the template. One explanation for the lower resolution is that the structural analogues fit worse into the imprinted site when compared to the template. Schweitz et al. [22] prepared a monolithic MIP using two structural analogues, i.e. (*S*)-metoprolol and (*S*)-atenolol, as template molecules simultaneously. An interesting outcome of this study was that the degree of enantiomer separation, represented by a normalized separation index [17], was higher for both of the analogues using the multiple templated MIP (MIP^n) monolith compared to the separation index achieved on the single templated MIP monoliths. The authors speculated that the reason for this finding could be related to template–template interactions during the imprinting process as well as during electrochromatography. Also, in this work a comprehensive study on the monomer composition of the MIP as well as the electrochromatographic parameters was conducted. Monomers that were supposed to interact only weakly with the template molecule were introduced in the polymerization protocol. These monomers were butyl (BMA) and methyl (MMA) esters of the functional monomer methacrylic acid (MAA). Improvements in both separation efficiency, resolution and enantiomer separation were achieved on MIP monoliths prepared using a one-to-one molar ratio between the functional monomer and the esterified monomers. The reason for these improvements is not clear, whether it was related to the formation of more well defined imprints, improved electrochromatographic performance, polymer appearance and morphology, or less unspecific sample adsorption to the MIP monolith. Furthermore, also in this study, Schweitz et al. studied the effect of detergent addition to the electrolyte on the enantiomer separation (SOAP-MIP). It was shown that the addition of sodium dodecylsulfate (SDS) decreased the resolution whereas polyoxyethylene sorbitanmonolaurate (Tween 20) and cetyltrimethylammonium bromide (CTAB) increased the resolution in a concentration dependent manner. An interesting finding in this study was the ability to decrease the concentration of organic modifier in the electrolyte upon

addition of Tween 20 and still obtain enantiomer separations. The author speculated that the ability to use more water rich electrolytes could be an effect of dynamic surface modifications on the MIP caused by surfactant adsorption. Enantiomer separations achieved using different types of detergents as electrolyte modifiers are shown in Fig. 6.

2.2. Surface grafted MIPs

Several different techniques have been applied in order to produce MIP coatings. Just recently, several MIP formats have been prepared utilizing a surface initiation methodology [27–29]. In common for these approaches is the ability to define the areas on which polymer is desired utilizing a surface bond radical initiator. Thus, upon initiation the polymer will start to grow from these surfaces whilst no polymer growth will appear on surfaces lacking the surface bond radical initiator. The technique has been applied to prepare MIP surface coatings for OT-CEC applications [29–32] as well to prepare MIP coatings on silica based packing materials [27,28,33].

2.2.1. MIP coatings for OT-CEC

Several different types of MIP coatings designed for OT-CEC applications have been described in the literature. The obvious benefit of MIP coatings for OT-CEC is the lack of backpressure, allowing simple regeneration and exchange of electrolyte, and the elimination of problems with clogging and trapped air bubbles during electrochromatography.

Brüggeman et al. [31] prepared MIP coatings covalently bond to the capillary inner surface using (methacryloxy-propyl)-trimethoxysilane derivatized capillaries. The polymerization protocol was studied in terms of alterations in porogen type and monomer composition and resulted in, when successfully performed, a thin MIP coating on the inner surface of the capillary. Since polymerization was thermally initiated, the MIP coating was present in the entire capillary, including the detection window. Thus, as detection was performed on-column, the presence of polymer in the detection window might have influenced the results.

Tan and Remcho [32] developed a similar protocol for synthesis of MIP coatings in thin 25 μm capillaries. The capillaries were pretreated with (methacryloxy-propyl)-trimethoxysilane in order to covalently link the MIP coating to the capillary. Following thermally initiated polymerization, the capillaries were connected to a vacuum system in one end and a pressurized gas flow in the other end in order to remove remaining monomers, solvents and radical initiators and to shrink the MIP to a thin film. Several different types of MIP coated capillary columns were prepared out of which about 50% were discarded as they were occluded and thus filled with dense monolithic MIP.

Just recently, Schweitz [29] developed a novel technique for fabrication of MIP coatings for OT-CEC utilizing a surface initiation methodology. The radical initiator 4,4′-azobis(4-cyanopentanoic acid) (ACPA) was covalently linked to aminopropyltrimethoxysilane (APS) derivatized capillaries using a carbodiimide coupling reagent. Following immobilization of ACPA, the capillary was filled with the polymerization mixture, the detection window was masked and polymerization was initiated using UV-irradiation. The thickness of the polymer coating was determined by the polymerization time, and thus careful timing was required in order not to fill the entire capillary with polymer. However, once optimized, MIP surface coatings could be prepared reproducible. The flow through properties of these columns is not dependent upon the porosity of the MIP but rather on the thickness of the coating. Thus, a large spectrum of polymerization solvents can be applied without having to consider their pore forming properties. Different types of polymerization solvents were tested for their applicability in MIP coating synthesis and the results obtained indicated noticeable differences in both appearance and electrochromatographic performance (Figs. 7 and 8).

2.2.2. Surface grafted MIP particles

An interesting approach for preparation of particle based MIP materials with well defined shape and size was developed by Quaglia et al. [27,28] and Sulitzky et al. [33]. Utilizing a surface bond radical initiator, MIPs could be grafted on silica seed particles with well defined size, shape and porosity. A great advantage of grafting the MIP to pre-fabricated particles is the ability to control both shape and size of the resulting MIP particles and thus facilitate packing of these particles into HPLC columns or capillary columns. The majority of MIP HPLC columns previously prepared have been prepared by packing of highly irregular MIP particles obtained by grinding and sieving of a bulk polymerized MIP [3]. These columns are difficult to prepare reproducible and have poor flow through properties due to the irregularity of the packing material. The silica templated MIP particles thus offer a new very interesting approach for the preparation MIP-HPLC [33] columns as well as packed columns for CEC [27,28]. In CEC, these MIP-silica composite particles have an additional advantage as the frits can be burned directly on the packing material. Fig. 9 shows a scanning electron micrograph of the cross-section of a capillary packed with MIP-silica composite particles prepared from ACPA modified APS derivatized 10 μm silica particles with 100 Å average pore diameter. In a thorough investigation, Sulitzky et al. [33] varied the type of radical initiator, the pore size of the silica seed particle, the monomer content and the chromatographic evaluation. From this study, it was concluded that an increased MIP film thickness led to improvements in selectivity and capacity. However, problems with mass-transfer kinetics hampered the separation efficiency. Furthermore, when evaluated in CEC the highest separation efficiency was achieved when large porous 1000 Å silica particles were used as a shape template, followed by 100 Å particles and non-porous particles [27,28]. The authors were not able to explain these results as the reversed order was obtained when the particles were applied

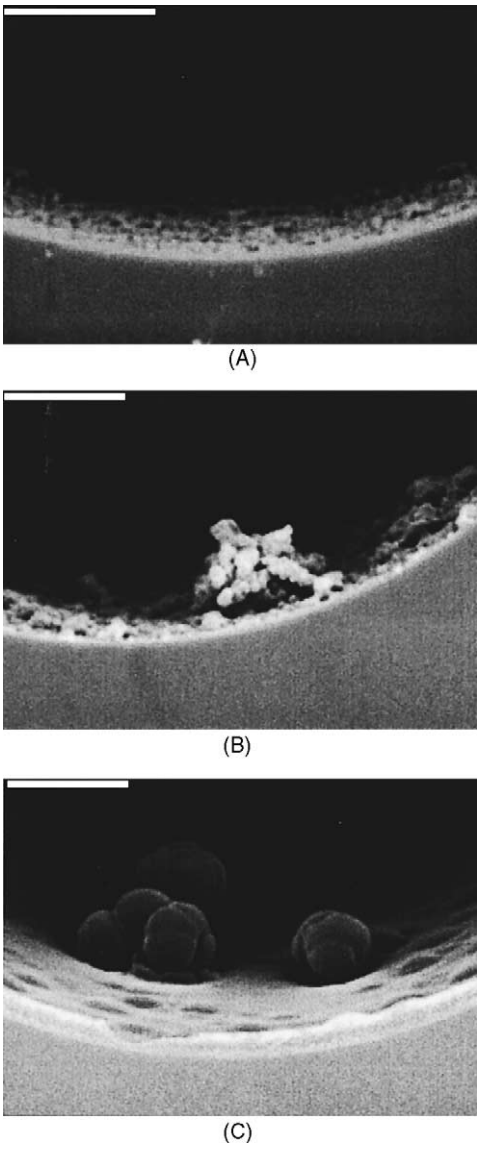

Fig. 7. Scanning electron micrograph of the cross section of surface coated MIP capillary columns for OT-CEC. The surface prepared using toluene as solvent (A) has a rough surface of approximately 0.15–0.45 μm thickness. The surface synthesized using dichloromethane as solvent (B) tended to yield a slightly thicker coating, about 0.45–2 μm, with larger aggregates extending about 5 μm from the surface. MIP coatings prepared using acetonitrile as solvent (C) had a smoother appearance and a layer thickness of about 1 μm with larger almost spherical clusters of 3–4 μm in diameter connected to the surface. Reprinted from [29] with permission.

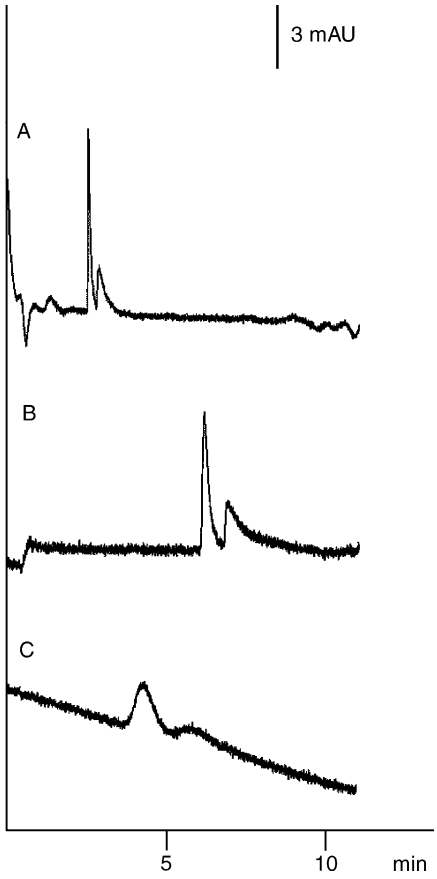

Fig. 8. Enantiomer separations of *rac*-propranolol using the MIP coated capillaries in OT-CEC. The coatings were synthesized using different polymerization solvents: (A) toluene; (B) dichloromethane; and (C) acetonitrile. Reprinted from [29] with permission.

in HPLC. However, a plausible explanation is the presence of an intra-particle electroosmotic flow that can provide considerable gain in efficiency due to electroosmotic perfusion [34,35]. Separations performed using a capillary packed with MIP-silica composite particles are shown in Fig. 10.

2.3. MIP nanoparticles

An elegant alternative approach to use particle based MIPs in CEC is to simply ad them to the electrolyte and introduce the resulting slurry into the capillary. This approach elegantly circumvents time consuming and tedious packing procedures as well as a new MIP phase is used in every separation. Several different techniques have been reported for the preparation of spherical MIPs [36–41]. However, so far only MIP particles prepared by crushing and sieving of bulk polymerized MIPs and MIP nanoparticles

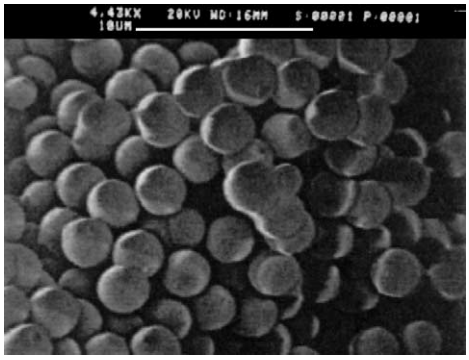

Fig. 9. Scanning electron micrograph of the cross section of a capillary packed with MIP coated silica particles. Reprinted from [27] with permission.

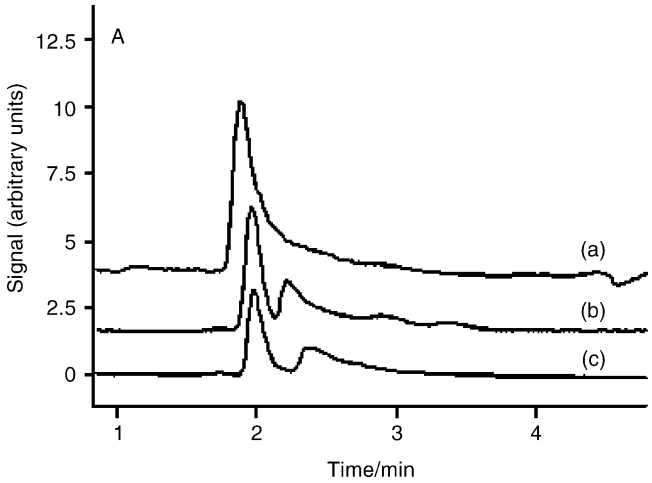

Fig. 10. Enantiomer separation of DL-phenylalanine anilide performed on a capillary packed with 10 μm 1000 Å silica particles coated with L-phenylalanine anilide templated MIP. Assuming a homogeneous coverage of the grafted MIP layer, the carbon content corresponds to polymer thicknesses of: (a) 0.9 nm (1.6%), (b) 2.2 nm (4%), and (c) 3.8 nm (7%). Reprinted from [28] with permission.

prepared using a precipitation polymerization protocol have been applied as pseudo stationary phases in CEC. Walshe et al. [42] prepared 20–30 μm irregular MIP particles by crushing and sieving a bulk polymerized MIP. The MIP was prepared using (*S*)-propranolol as template and the chiral monomer *N*-acryloyl-alanine as functional monomer. The resulting MIP particles were slurried in electrolyte and introduced continuously into the capillary column. Even though these particles had a considerable size, the authors claimed that they were stable in electrolyte suspension and that they did not scatter light nor hamper detection. The reference particles, prepared in absence of template molecule, were not properly evaluated as the authors claimed these to be insoluble in the electrolyte. Furthermore, the authors did not succeed in preparing MIP particles using the achiral monomer MAA. It is thus questionable if the separation shown in this work is based on a true imprinting effect or if it is a result of the presence of a chiral monomer in the polymer.

Precipitation polymerization has been introduced as an elegant technique for synthesis of MIP nanoparticles (Fig. 11) [41,43,44]. This technique allows sub micrometer sized spherical particles to be prepared from diluted monomer solutions without addition of surfactants. Monomers and solvents applicable to MIT can straightforwardly be used. It is believed that the smaller size of the MIP nanoparticles, in comparison with other MIP formats, can have a positive effect on the mass transfer restrictions. MIP nanoparticles prepared using a precipitation polymerization protocol have been applied in radio ligand binding assay [41], chemiluminescence imaging assay [45] and CEC [44,46–48]. Schweitz et al. [44] used a precipitation polymerization protocol to prepare nanoparticle MIPs for application in CEC. To circumvent detection problems related to the light scattering properties of the nanoparticles, a partial filling technique

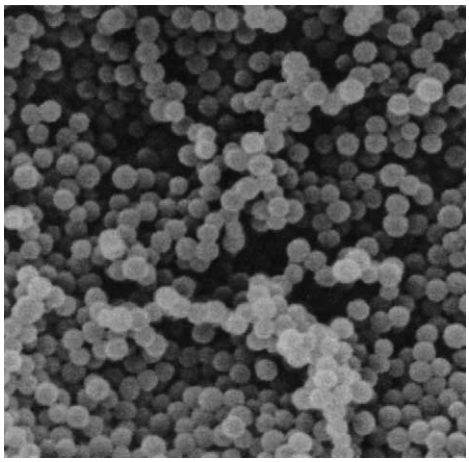

Fig. 11. Scanning electron micrograph of anti-17β-estradiol nanoparticles of approximately 300 nm in diameter. Reprinted from [41] with permission.

of CEC was applied [44,49]. Using the partial filling technique, a plug of nanoparticles suspended in electrolyte, is injected prior to the sample. Provided that there is a mobility difference between the analytes and the nanoparticles, the analytes will start to migrate through the nanoparticle plug as a voltage is applied over the capillary. During its passage through the capillary, the analytes will interact with and become separated on the MIP nanoparticles, and finally elute prior to the MIP nanoparticle plug (Fig. 12). The partial filling technique possesses several benefits when compared to traditional CEC techniques. Firstly, a fresh, unused MIP nanoparticle phase is used for every new analysis. This can be of great advantage when samples in complex matrices are analyzed, for instance blood plasma or cell lysates. Secondly, the length and type of the MIP nanoparticle phase can easily be varied and optimized for a certain separation without column switching and purchasing of new expensive columns. Thirdly, new types of phases can be screened very fast without time consuming packing procedures, and

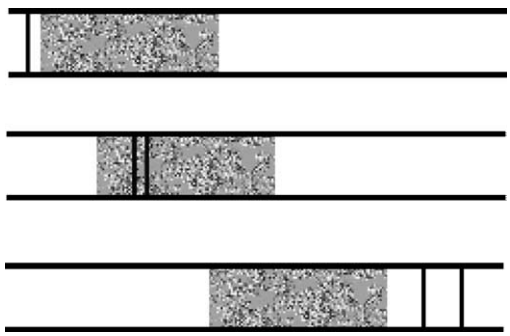

Fig. 12. Schematic description of the partial filling technique: (Top) A plug of MIP nanoparticles slurried in electrolyte is injected into the capillary prior to the sample. (Middle) As a voltage is applied the sample starts to migrate through the MIP plug. The analyte that binds more tightly to the nanoparticle phase will be most retained. (Bottom) The sample has passed through the MIP nanoparticle plug and reached the detection window prior to the light scattering nanoparticles.

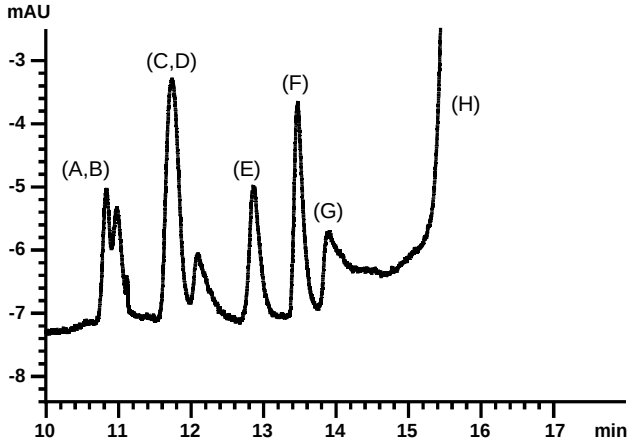

Fig. 13. Simultaneous enantiomer separation of β-blockers utilizing the cross-selectivity of (*S*)-propranolol templated nanoparticles in a partial filling application of CEC. (A, B) Enantiomers of pindolol; (C,D) enantiomers of atenolol; (E) impurity; (F) (*R*)-propranolol; (G) (*S*)-propranolol; and (H) eluting MIP nanoparticle plug.

fourthly, there is no need for incorporation of retaining frits, which sometimes can be disadvantageous from an electrochromatographic point of view [50]. Schweitz et al. [44] found that the template had a tremendous influence on the size of the resulting MIP nanoparticles. Nanoparticles prepared in the absence of a template molecule had a diameter around 100–200 nm. The corresponding MIP nanoparticles were considerably larger, with sizes ranging from 500 to 700 nm. A thorough investigation on the preparation and electrochromatographic behavior of MIP nanoparticles has been conducted by Spégel et al. [46]. In this study, the synthesis protocol was varied in terms of template concentration, addition of weakly interacting monomers and type of cross-linker. In the optimization and alterations of the electrolyte, an additional parameter needed to be considered, i.e. the stability of the nanoparticle electrolyte suspension. The monomers traditionally applied in MIT are relatively hydrophobic in nature and thus results in a relatively hydrophobic MIP nanoparticle. The ability to create a stable slurry of the nanoparticles in a water rich electrolyte is thus limited. Even though the majority of published MIP-CEC separations utilize an electrolyte composed of a low pH buffer and a high concentration of organic modifier this is probably an issue that needs to be addressed in the future. Nevertheless, the nanoparticle MIP format has a great potential in the future owing to its many advantages over traditional CEC. Fig. 13 shows a simultaneous enantiomer separation of pindolol, atenolol and propranolol on (*S*)-propranolol templated MIP nanoparticles utilizing the cross-selectivity of the MIP.

Just recently, de Boer et al. [48] prepared MIP nanoparticles for partial filling CEC using (+)-ephedrine as template. Using a highly diluted pre-polymerization solution, MIP nanoparticles of approximately 100 nm in diameter were prepared using a thermally initiated precipitation polymerization protocol. The authors claimed that simultaneous enantiomer separation of ephedrine and its structural analogue salbutamol could be achieved using these nanoparticles in a partial filling application of CEC (Fig. 14).

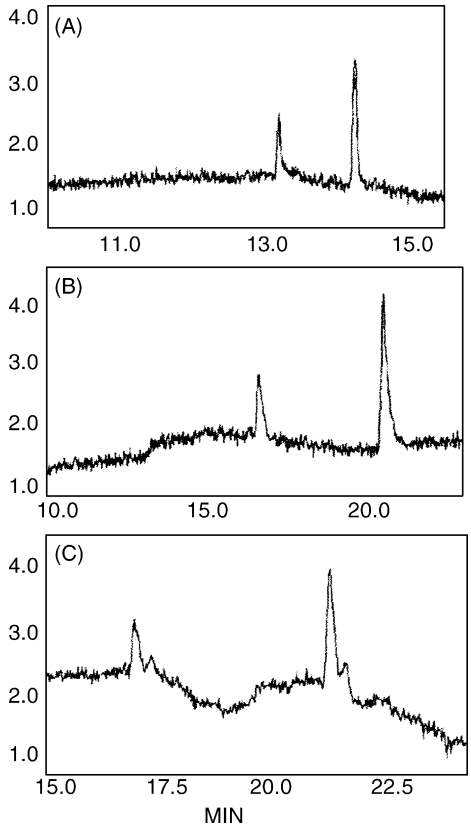

Fig. 14. Separation of ephidrine and salbutamol on (+)-ephedrine templated MIP nanoparticles in a partial filling application of CEC. (A) Capillary electrophoretic separation in absence of nanoparticles. (B) Separation using non-imprinted nanoparticles. (C) Separation achieved on MIP nanoparticles templated with (+)-ephedrine. Reprinted from [48] with permission.

3. Conclusion and future outlook

The variety of MIP-CEC formats available allows tailor-made separation system with a unique selectivity for a predetermined target to be prepared in a fast and reproducible manner. The number of applicable polymerization solvents, which hampered the use of the earlier formats, has been enlarged due to the introduction of new types of MIP formats. A large spectrum of solvents can now be applied for synthesis of MIPs for CEC. A common problem in chromatography is contamination caused by adsorption of sample components to the stationary phase. This is especially crucial when samples of biological nature in complex matrices are analyzed, for instance blood plasma and cell lysates. A promising tool in MIP-CEC, which circumvents problems with contamination, is the partial filling application of CEC using MIP nanoparticles. It is our strong

believe that the knowledge in MIP synthesis gained in this area can be fruitful for the MIP area in general as well as in other applications of CEC.

References

[1] K. Mosbach, Trends Biochem. Sci. 19 (1994) 9.
[2] P. Spégel, L. Schweitz, S. Nilsson, J. Anal. Bioanal. Chem. 372 (2002) 37.
[3] B. Sellergren, J. Chromatogr. A 906 (2001) 227.
[4] L.I. Andersson, J. Chromatogr. B 739 (2000) 163.
[5] K. Haupt, K. Mosbach, Chem. Rev. 100 (2000) 2495.
[6] E. Yilmaz, K. Mosbach, K. Haupt, Anal. Commun. 36 (1999) 167.
[7] L.A. Colon, G. Burgos, T.D. Maloney, J.M. Cintron, R.L. Rodriguez, Electrophoresis 21 (2000) 3965.
[8] K. Nilsson, J. Lindell, O. Norrlöw, B. Sellergren, J. Chromatogr. A 680 (1994) 57.
[9] J.M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Chromatographia 43 (1996) 585.
[10] J.-M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Biomed. Chromatogr. 11 (1997) 298.
[11] J.M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 1489.
[12] J.M. Lin, K. Uchiyama, T. Hobo, Chromatographia 47 (1998) 625.
[13] G. Chirica, V.T. Remcho, Electrophoresis 20 (1999) 50.
[14] J.-M. Lin, T. Nakagama, X.-Z. Wu, K. Uchiyama, T. Hobo, Fresenius J. Anal. Chem. 357 (1997) 130.
[15] J.M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, J. Pharm. Biomed. Anal. 15 (1997) 1351.
[16] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179.
[17] L. Schweitz, L.I. Andersson, S. Nilsson, J. Chromatogr. A 792 (1997) 401.
[18] S. Hjertén, J. Chromatogr. 347 (1985) 191.
[19] D. O'Shannessy, B. Ekberg, K. Mosbach, Anal. Biochem. 177 (1989) 144.
[20] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chim. Acta 435 (2001) 43.
[21] L. Schweitz, L.I. Andersson, S. Nilsson, Chromatographia 49 (1999) S93.
[22] L. Schweitz, L.I. Andersson, S. Nilsson, Analyst 127 (2002) 22.
[23] L.I. Andersson, Anal. Chem. 68 (1996) 111.
[24] B.J. Hart, D.J. Rush, K.J. Shea, J. Am. Chem. Soc. 122 (2000) 460.
[25] S. Nilsson, L. Schweitz, M. Petersson, Electrophoresis 18 (1997) 884.
[26] L.I. Andersson, Analyst 125 (2000) 1515.
[27] M. Quaglia, E. De Lorenzi, C. Sulitzky, G. Caccialanza, B. Sellergren, Electrophoresis 24 (2003) 952.
[28] M. Quaglia, E. De Lorenzi, C. Sulitzky, G. Massolini, B. Sellergren, Analyst 126 (2001) 1495.
[29] L. Schweitz, Anal. Chem. 74 (2002) 1192.
[30] K. Jinno, H. Sawada, Trends Anal. Chem. 19 (2000) 664.
[31] O. Brüggeman, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.
[32] J.Z. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.
[33] C. Sulitzky, B. Rückert, A.J. Hall, F. Lanza, K. Unger, B. Sellergren, Macromolecules 35 (2002) 79.
[34] P.T. Vallano, V.T. Remcho, J. Phys. Chem. B 105 (2001) 3223.
[35] A.I. Liapis, B.A. Grimes, J. Chromatogr. A 877 (2000) 181.
[36] R.J. Ansell, K. Mosbach, J. Chromatogr. A 787 (1997) 55.
[37] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769.
[38] K. Hosoya, K. Yoshizako, Y. Shirasu, K. Kimata, T. Araki, N. Tanaka, J. Haginaka, J. Chromatogr. A 728 (1996) 139.
[39] M. Lehman, H. Brunner, G.E.M. Tovar, Desalination 149 (2002) 315.
[40] N. Pérez-Moral, A.G. Mayes, in: K.R. Brain, C.J. Allender (Eds.), MIP2002, La Grande Motte, Languedoc, France, 2002.
[41] L. Ye, P.A.G. Cormack, K. Mosbach, Anal. Commun. 36 (1999) 35.
[42] M. Walshe, E. Garcia, J. Horwarth, M.R. Smyth, M.T. Kelly, Anal. Commun. 34 (1997) 119.
[43] L. Ye, P.A.G. Cormack, K. Mosbach, Anal. Chim. Acta 435 (2001) 187.
[44] L. Schweitz, P. Spégel, S. Nilsson, Analyst 125 (2000) 1899.
[45] I. Surugiu, B. Danielsson, L. Ye, K. Mosbach, K. Haupt, Anal. Chem. 73 (2001) 487.
[46] P. Spégel, L. Schweitz, S. Nilsson, Electrophoresis 22 (2001) 3833.
[47] P. Spégel, S. Nilsson, Am. Lab. 34 (2002) 29.
[48] T. de Boer, R. Mol, R.A. de Zeeuw, G.J. de Jong, D.C. Sherrington, P.A.G. Cormack, K. Ensing, Electrophoresis 23 (2002) 1296.
[49] A. Amini, U. Paulsen-Sorman, D. Westerlund, Chromatographia 50 (1999) 497.
[50] B. Behnke, J. Johansson, E. Bayer, S. Nilsson, Electrophoresis 21 (2000) 3102.