Capillary electrochromatography with molecular imprint-based selectivity for enantiomer separation of local anaesthetics

Leif Schweitz[a], Lars I. Andersson[b], Staffan Nilsson[a],*

[a]*Division of Technical Analytical Chemistry, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[b]*Department of Bioanalytical Chemistry, Astra Pain Control, SE-151 85 Södertälje, Sweden*

Abstract

Preparation of stationary phases of predetermined selectivity for use in capillary electrochromatography by molecular imprinting was studied. Molecular imprinting of the local anaesthetic (*S*)-ropivacaine in methacrylate-type polymers was done using an in situ photo-initiated polymerisation process. Such stationary phases could separate the enantiomers of ropivacaine and the enantiomers of the structural analogues mepivacaine and bupivacaine. The influence of several parameters on the ability of the resultant imprinted capillary column to resolve *rac*-ropivacaine was investigated. These parameters included the type and amount of functional and cross-linking monomers, the molar ratio of the imprint molecule to the monomers and the type of the porogen. The polymer-based, monolithic capillary columns could be rendered super-porous by the use of 1–25% isooctane as a porogenic agent. The best resolution was obtained for polymers made using trimethylolpropane trimethacrylate as the cross-linking monomer. The enantiomer separation increased with increasing molar ratio of the functional monomer methacrylic acid to (*S*)-ropivacaine, however, at the expense of peak broadening. Initial studies on the optimisation of the electrolyte composition showed that the separation increased for electrolytes as the volume ratio of acetonitrile to buffer increased, the temperature raised and the pH became higher.   © 1997 Elsevier Science B.V.

Keywords: Electrochromatography; Enantiomer separation; Molecular imprinting; Selectivity; Stationary phases, LC; Ropivacaine; Mepivacaine; Bupivacaine

1. Introduction

Capillary electrochromatography (CEC) has become recognised as a powerful tool for enantiomer separation. A chiral selector, which can either be employed as an additive to the electrolyte, be as micelles in the electrolyte or be immobilised on a stationary phase, is used to effect the enantiomer separation [1]. The chiral selectors most widely used include cyclodextrins or derivatives thereof [2,3], proteins such as albumins, cellulases and α_1-acid glycoprotein [4–7], crown ethers [8] and chiral surfactants [9]. The selectivity of such chiral selectors, however, is difficult to predict. Accordingly, the choice of a chiral selector for a specific separation problem must be based on trial and error. In contrast, molecular imprinting methodology [10–12] provides a means for the preparation of stationary phases of pre-determined selectivity. The polymerisation of functional monomers in the presence of an imprint species is the key step. The monomers are selected for their ability to interact by non-covalent interactions with the imprint molecule. The polymerisation reaction embeds the imprint molecules in a solid, highly cross-linked polymer network in which the interactions between the imprint species and the

*Corresponding author.

monomers are preserved. This process gives rise to imprints possessing steric and chemical complementarity to the imprint species. Subsequent removal of the imprint molecules leaves recognition sites with affinity for the original imprint species (cf. Fig. 1). Such molecularly imprinted polymers (MIPs) have been employed in the liquid chromatographic enantiomer separations of a number of substances including amino acid derivatives, sugars and drugs [13–15].

We have recently presented a technique for the in situ preparation of super-porous MIPs inside fused-silica capillary columns [16]. Such MIP-based capillary columns can be used as stationary phases in CEC for the efficient separation of the enantiomers of the β-blockers used as imprint species [16,17]. The use of MIPs prepared by a dispersion polymerisation process [18] or by the immobilisation of MIP particles in an acrylamide gel [19] in capillary electroseparation systems have also been reported. However, the interest in continuous porous separation media [20] is emerging, which further moti-

Fig. 2. Structure of the compounds studied. (1) Ropivacaine, (2) mepivacaine and (3) bupivacaine.

vates the development of our approach. A positive feature of molecular imprinting is the possibility of designing a stationary phase with selectivity predetermined to meet ones demands. Hence, MIP-based stationary phases have the potential to become a valuable complement to the selectors presently employed in capillary electrochromatographic enantiomer separations. In the present study, molecular imprinting of the *S*-enantiomer of ropivacaine (Fig. 2), a local anaesthetic, was performed. Several factors such as the molar ratio of the functional monomer to the imprint molecule, the isooctane content of the porogen and the type of functional and cross-linking monomers affecting the enantiomer separation ability of the resultant MIP capillary column were investigated.

2. Experimental

2.1. Chemicals

Trimethylolpropane trimethacrylate [1,1,1-tris-(hydroxymethyl)propanetrimethacrylate; TRIM], pentaerythritol triacrylate (PETRA), and pentaerythritol tetraacrylate (PETEA) were purchased from Aldrich (TRIM and PETRA from Gillingham, UK, and PETEA from Milwaukee, WI, USA). 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from Sigma (St. Louis, MO, USA). (*R*)-Ropivacaine, (*S*)-ropivacaine, *rac*-mepivacaine and *rac*-bupivacaine,
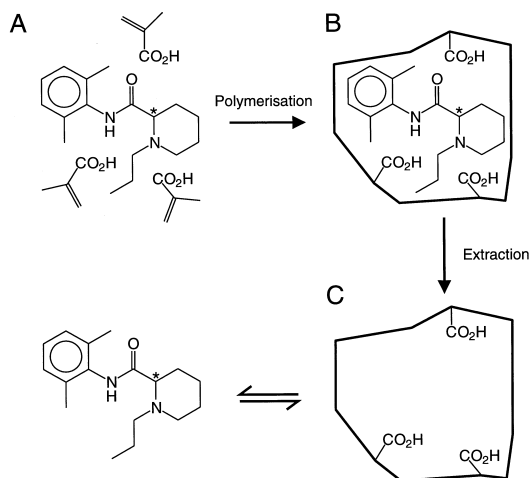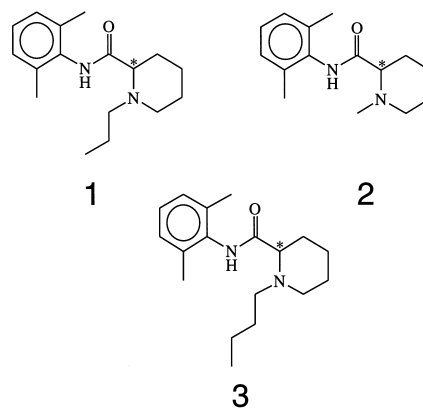

Fig. 1. Scheme of molecular imprinting of (*S*)-ropivacaine using methacrylic acid as the functional monomer (A). Crosslinking monomer is added followed by initiation of polymerisation by addition of AIBN and irradiation of UV light at −20°C (B). The imprint molecule is extracted, leaving recognition sites in the polymer matrix complementary to the imprint molecule in terms of chemical functionality, size and shape (C). In this study, the capillary column is inserted in the instrument and the extraction was done on line followed by equilibration of the CEC system. The molecularly imprinted polymer is then ready to selectively rebind the imprint molecule.

all as hydrochlorides monohydrates, were obtained from Medicinal Chemistry, Astra Pain Control (Södertälje, Sweden). The toluene (HPLC grade), from Labscan (Dublin, Ireland), was dried and stored over 4 Å sieves after delivery. All the other chemicals were obtained from Merck (Hohenbrunn, Germany) and were used as received.

2.2. Preparation of capillary columns containing molecularly imprinted polymers

Fused-silica capillary with a transparent protecting polymer coating (75 μm I.D.×375 μm O.D.; TSU075375) obtained from Polymicro Technologies (Phoenix, AZ, USA) was derivatised with [(methacryloxy)propyl]trimethoxysilane, as described elsewhere [16]. A detection window was prepared by removing about 0.5 cm of the polymer layer 8.5 cm from one end by burning. The detection window was covered by a piece of paper to prevent polymerisation in this area of the capillary column. Typically, the capillary column was 35 cm in length (unless stated otherwise). (S)-Ropivacaine was converted to its free-base form by extraction in ethylacetate and saturated NaHCO₃-solution, and was subsequently washed once with water and evaporated. The (S)-ropivacaine in free-base form was then stored at −20°C until use.

A pre-polymerisation mixture containing (S)-ropivacaine free base, functional monomer(s) [methacrylic acid (MAA) and/or 2-vinyl pyridine (2VPy)], cross-linking monomer [TRIM, PETRA, PETEA or ethyleneglycol dimethacrylate (EDMA)] and radical initiator (AIBN) dissolved in toluene–isooctane, composed as described in Table 1, was prepared. The pre-polymerisation mixture was introduced into the capillary using a syringe (BD U100 Micro Fine plus, Becton Dickinson, Franklin Lakes, NJ, USA) and a piece of plastic tubing connected to the capillary. The capillary ends were then sealed by soft plastic rubber. To perform the polymerisation, the capillary was illuminated in a freezer (−20°C) by a Type TL-900 UV lamp from Camag (Muttenz, Switzerland) set at 350 nm for about 16 h.

The capillary column was then flushed with several column volumes of acetonitrile–acetic acid (9:1, v/v) by a syringe or a HPLC-pump (Jasco 880-PU, Jasco, Tokyo, Japan) in order to wash the imprint molecules, the unreacted monomers, and the remainder of the initiator out of the capillary column, which were then stored at room temperature until use.

2.3. Capillary electrochromatography

CEC experiments were performed on an HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany), consisting of a diode array detector, ChemStation software for data processing, and a high-pressure facility allowing pressures up to 12 bar to be delivered to the inlet vial or to both vials simultaneously. The electrolyte was composed of acetoni-

Table 1
Composition of the pre-polymerisation mixture

MIP	Imprint molecule (mol/l)	MAA (mol/l)	Crosslinking monomer Type/(mol/l)	Isooctane content in porogen (%, v/v)
A	0.020	0.24	TRIM/0.24	1
B	0.030	0.24	TRIM/0.24	1
C	0.060	0.24	TRIM/0.24	1
D	0.120	0.24	TRIM/0.24	1
E	0.240	0.24	TRIM/0.24	1
F	0.120	0.48	TRIM/0.48	10
G	0.120	0.48	TRIM/0.48	20
H	0.120	0.48	TRIM/0.48	25
I	0.240	0.48	PETRA/0.48	25
J	0.240	0.48	PETEA/0.48	25
K	0.240	0.48	EDMA/0.96	25
L	0.240	0.24+0.24[a]	TRIM/0.48	25

[a] In this instance MAA and 2VPy were used as functional monomers.

trile, and different ratios of buffer, either 0.1 M phosphoric acid, 0.025 M phosphoric acid, or 0.025 M citric acid adjusted to the desired pH by triethanolamine. The samples were prepared from 10 mM water solutions diluted with water or electrolyte–water (1:1, v/v) to the desired concentration. All the buffer and sample solutions were made using water from a Milli-Q purification system (Millipore, Bedford, MA, USA). The samples were degassed by sonication, injections being made electrokinetically, typically by applying 5 kV (143 V/cm) for 5 s. The separation voltage was set to 5 kV (143 V/cm) and the capillary column was thermostatted to 60°C (unless stated otherwise). UV detection was performed at 195 nm (10 nm bandwidth). When appropriate, a resolution factor, R_s or f/g was calculated. R_s was calculated as $R_s = \Delta t_R / 0.5[W_{(1)} + W_{(2)}]$, where Δt_R is the difference in migration times for the peaks and W is the width at the baseline between tangents drawn to the inflection points for the peaks. f/g is defined as the ratio of the distances from a line connecting the peaks to the valley between the peaks (f) and the corresponding line to the baseline (g) [21,22]. The degree of enantiomer separation was represented by a normalised separation index $\Delta t_R / t_R(1)$, where Δt_R is the difference in the elution times of the enantiomers at peak maximum and $t_R(1)$ is the retention time of the first eluted enantiomer.

3. Results

3.1. Preparation of MIP monolith-based capillary columns

A number of MIP columns were produced and their ability to separate the enantiomers of the imprint species (ropivacaine) in the CEC mode was recorded. The degree of enantiomer separation was represented by a normalised separation index, which is the difference in elution times of the enantiomers divided by the elution time of the first eluting enantiomer. This normalised separation index was used to account for the sometimes variable MIP densities in the capillary columns and the differences in the morphologies of the MIPs that may cause variations in bulk flow. In some cases, a resolution factor, R_s or f/g was calculated and was used in the

evaluation of the separation abilities of a MIP column.

Optimisation of the volume ratio of toluene to isooctane in the porogen was found to be important for obtaining columns with optimal flow-through properties. If no isooctane was added, the polymer became very dense, and hydrodynamic pumping was not possible. Too much isooctane, however, led to the polymer having a soft gel-like appearance rather than being a stable monolith. The flow-through properties of a MIP capillary were judged to be good if hydrodynamic pumping of liquid through the capillary column, using either a syringe or an HPLC pump, was possible. If a MIP column was dense to a degree where it was not possible to exchange the porogen for an electrolyte, the column was discarded and not evaluated further. The formation of an even polymer monolith inside the capillary column was controlled visually, after the exchange of solvent for electrolyte followed by equilibration, by inspection in a microscope.

The best enantiomer selectivity was obtained for MIP F, which was prepared using a toluene to isooctane ratio of 9:1 (v/v) (Table 2). The MIP D, prepared using less isooctane, and MIPs G and H, prepared using more isooctane, were shown to be less selective than MIP F. An increase in isooctane content made it possible to use larger amounts of functional and cross-linking monomers in the pre-polymerisation mixture. MIPs F, G and H were prepared using a pre-polymerisation mixture with a higher monomer content than MIP D (Table 1). The overall morphology of the MIPs is probably altered by a change in the toluene–isooctane composition.

Table 2
Enantiomer separation on MIP capillary columns using varying isooctane content in the porogen

MIP	Isooctane % (v)	$t_R(R)$ (min)	$t_R(S)$ (min)	$\Delta t_R / t_R(R)$
D	1	7.50	7.93	0.057
F	10	12.06	13.92	0.154
G	20	9.50	10.16	0.069
H	25	4.64	4.97	0.071

Generating these data, an electrolyte composed of acetonitrile–0.100 M phosphoric acid adjusted to pH 3.0 with triethanol amine in the ratio 80:20 (v/v) was used. 200 µM *rac*-ropivacaine was injected. Other conditions as described in Section 2.

Further investigation here is warranted. In this context, the column containing MIP F, which showed a significantly higher enantiomer selectivity than those containing MIPs D, G and H, had a very high flow resistance. This MIP CEC column could only be flushed using an HPLC pump set at a pressure of 450 bar. Its high selectivity may thus, at least in part, be explained by this column containing a larger amount of MIP and thus having a higher density of imprinted sites.

Super-porous MIP monoliths were obtained for all of the combinations of cross-linking and functional monomers used. The cross-linking monomers include TRIM, PETRA, PETEA and EDMA and the functional monomers include MAA and 2VPy. The highest selectivity was recorded for the EDMA-based MIP K (Table 3). The peaks were extremely broadened, however, to the extent that the last eluting peak [(S)-ropivacaine] was difficult to detect. The enantiomer selectivity of the MIPs decreased in the order of H>I>J, which were made using TRIM, PETRA and PETEA, respectively (Table 3). MIP L, in which two functional monomers MAA and 2VPy were incorporated, did not show any improved selectivity compared with the MIPs prepared using only MAA as the functional monomer, at least not for the molecular imprinting of (S)-ropivacaine.

The molar ratio of MAA to imprint molecule was varied by adjusting the amount of imprint molecule added to an otherwise constant pre-polymerisation mixture. An increased molar ratio resulted in increased enantiomer selectivity of the resultant MIP (Table 4). It should be noted, however, that the best resolution (calculated as resolution factor R_s) was recorded for MIP column E, which was the least

Table 4
Enantiomer separation on MIP capillary columns prepared in the presence of varying concentration of imprint molecule

MIP	Molar ratio MAA/ imprint molecule	$t_R(R)$ (min)	$t_R(S)$ (min)	$\Delta t_R/t_R(R)$
A	12	7.62	8.25	0.083
B	8	8.41	8.98	0.068
C	4	7.63	8.16	0.069
D	2	7.50	7.93	0.057
E	1	10.98	11.38	0.036

Conditions as in Table 2.

enantiomer selective. The R_s ranged from 0.82 to 1.10. Thus, the differences in the resolution factor recorded for the MIP columns A–E were small. It was also observed that a decrease in the molar ratio of MAA to imprint molecule increased the flow resistance through the resultant MIP CEC column. This was shown by the observation that columns containing MIP A, B and C could be flushed using a syringe, whereas MIPs D and E required an HPLC pump. This indicates an alteration of the MIP morphology in relation to the amount of the imprint molecule present in the pre-polymerisation mixture.

3.2. Capillary electrochromatography

The electrolytes were composed of mixtures of acetonitrile and buffer. The separation of the enantiomers of ropivacaine was favoured by high contents of acetonitrile in the electrolyte. As the amount of acetonitrile became less the separation decreased. The retention times of the peaks were also reduced. For an electrolyte composed of 30% acetonitrile and 70% buffer pH 3.0 (v/v), the separation of *rac*-ropivacaine could only be detected as a shoulder of

Table 3
Enantiomer separation on MIP capillary columns prepared using varying functional and crosslinking monomer types

MIP	Functional monomer	Crosslinking monomer	$t_R(R)$ (min)	$t_R(S)$ (min)	$\Delta t_R/t_R(R)$
H	MAA	TRIM	4.64	4.97	0.071
I	MAA	PETRA	9.43	9.69	0.028
J[a]	MAA	PETEA	9.30	9.48	0.019
K[a,b]	MAA	EDMA	5.68	7.73	0.361
L[a]	MAA+2VPy	TRIM	11.16	11.51	0.031

Conditions as described in Table 2.
[a] The electrolyte used had an acetonitrile–pH 3.0 buffer ratio of 75:25 (v/v).
[b] The injection was made at 10 kV (286 V/cm) for 10 s. The separation was performed at 10 kV (286 V/cm) and an over-pressure of 7 bar was applied. All other parameters were as described above.

the almost co-eluting (*R*)- and (*S*)-ropivacaine peaks. Electrolytes containing 80% (v/v) of acetonitrile were used in all investigations presented in this study. Furthermore, it was found that high temperatures were beneficial for the separation of the ropivacaine enantiomers. In one example, the R_s increased from 0.39 to 0.73 and f/g increased from 0.21 to 0.75 when the temperature of the capillary column was raised from 25 to 60°C. An increase in the normalised separation index from 0.021 to 0.048 and a decrease in retention time was also observed when the temperature was raised. At 60°C the plate number recorded for (*S*)-ropivacaine, the last eluted peak, was twice as high and for the (*R*)- ropivacaine peak 1.4 times higher, than that recorded at 25°C. All other separation parameters remained constant. Raising the temperature even higher may increase resolution still further, but since the CE instrument used was limited to 60°C, this temperature was maintained in the subsequent studies.

The influence of the pH of the buffer component of the electrolyte on enantiomer separation was likewise studied and the results are shown in Table 5. The apparent pH values (pH_{app}), which are the pH values recorded for the mixtures of acetonitrile and buffer, are also reported. There was a general trend for the normalised separation index, $\Delta t_R/t_R(1)$, to increase as the pH of the electrolyte increased. A decrease in separation was observed, however, when the pH was raised from 3.0 to 4.0. It should be noted that this involves a change from a phosphate buffer to a citrate buffer, which may explain the observed dip in the trend. The shorter retention times observed for the pH 4.0 buffer electrolyte may be explained by electroosmotic flow (EOF) through the column raising and interactions with the stationary phase being altered less. A general increase in EOF with an increased pH was observed. In contrast, interactions of the analytes with the stationary phase were stronger at pH 5.0 and 6.5, since the retention times and the selectivities were found to increase despite the rise in EOF. It should be noted that the analytes

Table 5
Enantiomer separation at various electrolyte pH values

Buffer pH[a]	pH_{app}	Compound	$t_R(1)$ (min)	$t_R(2)$ (min)	$\Delta t_R/t_R(1)$
2.0	3.3	Ropivacaine	11.59	11.81	0.019
		Mepivacaine	11.42	11.42	0
		Bupivacaine	12.26	12.54	0.023
3.0	4.5	Ropivacaine	11.33	12.20	0.077
		Mepivacaine	10.99	11.24	0.023
		Bupivacaine	11.48	12.17	0.060
4.0	5.4	Ropivacaine	9.42	9.88	0.049
		Mepivacaine	9.10	9.10	0
		Bupivacaine	9.80	10.21	0.042
5.0	6.4	Ropivacaine	15.15	17.14	0.131
		Mepivacaine	13.84	14.27	0.031
		Bupivacaine	15.17	16.88	0.113
6.5	6.8	Ropivacaine	24.65	>30[b]	>0.217[b]
		Mepivacaine	21.96	23.29	0.061
		Bupivacaine	23.85	28.02	0.175

A 100 cm capillary column containing MIP C. Samples were 1 m*M* in concentration and injected electrokinetically at 10 kV (100 V/cm), 3 s. The separations were performed at 60°C by applying 30 kV (300 V/cm) and an over-pressure of 7 bar.
[a] The electrolytes were composed of acetonitrile–buffer (80:20, v/v). Buffers were prepared either from 25 m*M* phosphoric acid (pH 2.0, 3.0 and 6.5) or 25 m*M* citric acid (pH 4.0, 5.0), the pH adjusted by addition of triethanol amine.
[b] The second peak was co-eluted with unidentified species migrating with the EOF.

were positively charged, thus migrating through the columns towards the cathode both by electrophoresis and electroosmosis, at all pH values studied.

3.3. Enantiomer separations of structural analogues

The MIP CEC columns, which were imprinted with (*S*)-ropivacaine, were found to separate the enantiomers of other local anaesthetics, such as the structural analogues bupivacaine and mepivacaine (Fig. 2), as well. A typical enantiomer separation of ropivacaine is shown in Fig. 3. The separation of the enantiomers of bupivacaine was efficient, whereas mepivacaine was only poorly resolved (cf. Fig. 4). In general, the enantiomer separations $[\Delta t_R / t_R(1)]$ and

Fig. 4. Enantiomer separation of *rac*-mepivacaine and *rac*-bupivacaine on a MIP capillary column containing imprints of (*S*)-ropivacaine. Conditions as described in Fig. 3.

resolutions (measured as the R_s and/or f/g value) recorded were found to be best for ropivacaine and worst for mepivacaine (Table 5).

4. Discussion

The successful in situ preparation of MIP monoliths in fused-silica capillaries and their use in CEC requires that several conditions be fulfilled. (1) The MIP monolith must possess good flow-through properties for efficient exchange of the porogen (the solvent of polymerisation) for the electrolyte, which is a necessary prerequisite for a CEC experiment, by hydrodynamic pumping. (2) The MIP preparation needs to be done under conditions that are optimal for imprint formation and (3) the electrochromatography must be carried out under conditions that favour the rapid association and dissociation of the analyte-imprint binding and disfavour non-specific interactions.

The use of toluene–isooctane blends as porogen has here been proven successful in the preparation of molecularly imprinted super-porous polymer monoliths for use in CEC. The use of apolar porogens such as toluene have been shown previously to increase the yield of high affinity imprints [23,24].
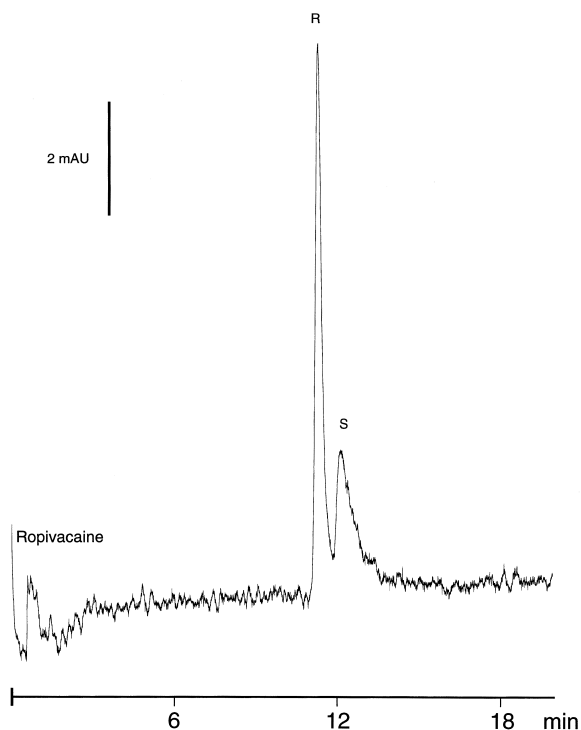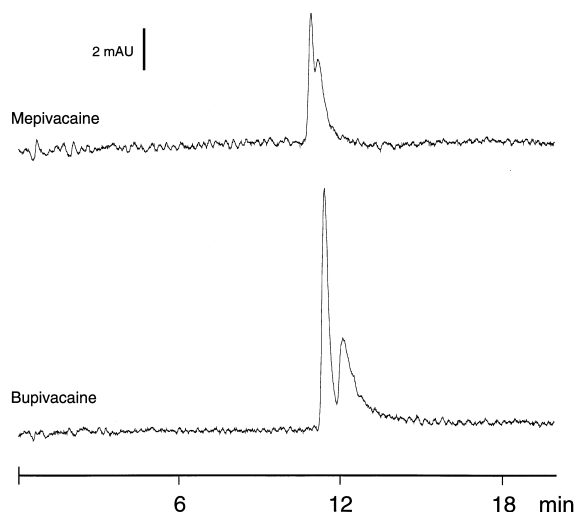

Fig. 3. Enantiomer separation of *rac*-ropivacaine on a MIP capillary column, 100 cm in length with a distance of 91.5 cm to the detection point, containing imprints of (*S*)-ropivacaine. The sample, 1 m*M* in concentration, was injected electrokinetically at 10 kV (100 V/cm) for 3 s. Separation was performed by applying 30 kV (300 V/cm) and an over-pressure of 7 bar. The electrolyte used was composed of acetonitrile–25 m*M* phosphoric acid adjusted to pH 3.0 with triethanol amine (80:20, v/v).

Super-porous MIP monoliths possessing good flow-through properties can be obtained without any porogenic agent (isooctane) by careful timing of the polymerisation reaction as reported previously [16]. When isooctane is added to the solvent as a porogenic agent the preparation is easier to perform since the polymerisation reaction is simply allowed to proceed to completion, although in this study we used a fixed polymerisation time of 16 h. This approach has previously been applied in the preparation of propranolol MIP monoliths [17] and has been investigated in detail in this study. The formation of super pores is attributed to toluene being a good solvent for the growing polymer chains whereas isooctane being a poor one [25]. This leads to precipitation of the growing polymer which in turn leads to the formation of super pores in the polymer matrix. Several types of functional and cross-linking monomers are amenable to this preparation technique, including the cross-linking monomers TRIM, PETRA, PETEA and EDMA and the functional monomers MAA and 2VPy. All of these monomers have previously been used in non-covalent imprinting protocols for the preparation of enantiomer-selective MIPs in bulk [23,26,27].

In the present study all polymerisation reactions were carried out at $-20°C$ and was initiated by UV light irradiation. It has been demonstrated repeatedly that molecularly imprinted polymers prepared at lower temperatures using photo-initiation exhibit higher enantiomer separation capabilities [28]. This is attributed to the complexes of monomers with imprint molecule in the pre-polymerisation mixture being more stable at low temperatures due to a more favourable entropy term, leading to more well-defined imprints in the resultant polymer.

We believe that use of an appropriate porogen composition, one giving rise to super-pores in the MIPs, and of a photo-initiated polymerisation process that can be carried out at very low temperatures, may be two key factors to obtain MIPs showing good performance in chromatographic applications. Isooctane is a useful porogenic agent since it is an apolar and aprotic solvent, that does not interfere with the imprint formation. Hence, the preparation procedure presented here increases the applicability of MIPs as stationary phases for CEC. Certain benefits of the MIP phases compared with capillary columns prepared by conventional packing techniques are the less tedious preparation they require and the lack of any need for frits. The MIP monoliths are covalently attached to the inner walls of the capillary columns due to the presence on the inner surface of the capillary column of (methacryloxy)-propyl trimethoxysilane units which participate in the polymerisation reaction [16]. These covalent attachments prevent the MIP monolith from being eluted during hydrodynamic pumping or during CEC.

The composition of the electrolyte is the single most important parameter in the optimisation of the CEC performance. The electrolyte used in the present study, composed of a mixture of acetonitrile and of an aqueous buffer, was chosen because of its proven usability in previous molecular imprinting based CEC experiments [16,17]. Optimisation studies involved investigation of the effects of acetonitrile content, temperature and pH of the buffer on the extent of separation of the ropivacaine enantiomers. The separation was found to increase for electrolytes as the volume ratio of acetonitrile to buffer increased, the temperature raised and the pH became higher. High separations occurred, however, at the expense of peak broadening and an optimal situation needs to be found. Further studies on the optimisation of the electrolyte composition may be worthwhile, both for new MIPs and for the type of MIPs presented here.

The ability of the MIP CEC-columns that were imprinted with (S)-ropivacaine to separate the enantiomers of other local anaesthetics, such as the structural analogues bupivacaine and mepivacaine (Fig. 2), were studied as well. In general, the enantiomer separations and resolutions recorded were found to be best for ropivacaine followed by bupivacaine and mepivacaine. This observation can be explained in terms of the slightly more close structural resemblance of ropivacaine to bupivacaine than to mepivacaine (Fig. 2). This is a confirmation and extension of previous findings that, for a series of compounds, the best enantiomer separation is always obtained for the imprint species [29].

5. Conclusions

(S)-Ropivacaine could be successfully imprinted

into methacrylate-based polymers and provide selective stationary phases for CEC by use of a simple in situ polymerisation procedure. The imprinted polymers can be made super-porous by the use of isooctane as a porogenic agent in mixtures of toluene. The super-porous structure of the MIP facilitates easy regeneration and electrolyte exchange, at the same time as an adequate amount of MIP is present in the capillary column.

Factors affecting the results of the MIP column preparation include the choice and amount of monomers, amount of the imprint molecule and amount of the isooctane used in the porogen. An increase in the monomer or imprint molecule content in the pre-polymerisation mixture results in the MIP stationary phase being more dense. An increase in the isooctane content, in contrast, results in the polymer stationary phase being less dense. An optimal ratio of monomers, imprint molecule and isooctane should be aimed at so as to obtain MIP CEC columns possessing good flow-through characteristics.

In order to obtain enantiomer separations, the composition of the electrolyte used during CEC should be considered, particularly the pH and organic solvent content. For the enantiomer separation of the local anaesthetics that were studied, a high amount of acetonitrile should be used in the electrolyte. It was found that raising the pH led to an increase in selectivity but to a decrease in separation efficiency, and to much broader peaks being obtained. The use of triethanol amine phosphate buffer of low pH was found to be suitable since this electrolyte provides stable UV baseline and enantiomer separations are achieved.

Acknowledgements

References

[1] S. Fanali, J. Chromatogr. A 735 (1996) 77–121.
[2] S. Cladrowa-Runge, R. Hirz, E. Kenndler, A. Rizzi, J. Chromatogr. A 710 (1995) 339.
[3] F. Lelièvre, C. Yan, R.N. Zare, P. Gareil, J. Chromatogr. A 723 (1996) 145–156.
[4] S. Birnbaum, S. Nilsson, Anal. Chem. 64 (1992) 2872–2874.
[5] H. Ljungberg, S. Nilsson, J. Liq. Chromatogr. 18 (1995) 3685–3698.
[6] L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjertén, J. Chromatogr. 638 (1993) 263–267.
[7] S. Li, D.K. Lloyd, Anal. Chem. 65 (1993) 3684–3690.
[8] R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem. 64 (1992) 2815.
[9] S. Terabe, H. Shibata, Y. Miyashita, J. Chromatogr. 480 (1989) 403.
[10] K. Mosbach, Trends Biochem. Sci. 19 (1994) 9–14.
[11] G. Wulff, Angew. Chem., Int. Ed. Engl. 34 (1995) 1812–1832.
[12] T. Takeuchi, J. Matsui, Acta Polymer. 47 (1996) 471–480.
[13] L.I. Andersson, K. Mosbach, J. Chromatogr. 516 (1990) 313–322.
[14] G. Wulff, K. Minarik, J. Liq. Chromatogr. 13 (1991) 2987–3000.
[15] M. Kempe, K. Mosbach, J. Chromatogr. A 664 (1994) 276–279.
[16] L. Schweitz, L.I. Andersson, S. Nilsson, Anal. Chem. 69 (1997) 1179–1183.
[17] S. Nilsson, L. Schweitz, M. Petersson, Electrophoresis 18 (1997) 884–890.
[18] K. Nilsson, J. Lindell, O. Norrlöw, B. Sellergren, J. Chromatogr. A 680 (1994) 57–61.
[19] J.M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Chromatographia 43 (1996) 585–591.
[20] F. Svec, J.M.J. Fréchet, Science 273 (1996) 205–211.
[21] R.E. Kaiser, Gaschromatographie, Geest and Portig, Leipzig, 1960.
[22] V.R. Mayer, Chromatographia 24 (1987) 639–645.
[23] L.I. Andersson, Anal. Chem. 68 (1996) 111–117.
[24] G. Vlatakis, L.I. Andersson, R. Müller, K. Mosbach, Nature 361 (1993) 645–647.
[25] T. Hargitai, P. Reinholdsson, B. Törnell, R. Isaksson, J. Chromatogr. 630 (1993) 79–94.
[26] M. Kempe, Anal. Chem. 68 (1996) 1948–1953.
[27] O. Ramström, L.I. Andersson, K. Mosbach, J. Org. Chem. 58 (1993) 7562–7564.
[28] D.J. O'Shannessy, B. Ekberg, K. Mosbach, Anal. Biochem. 177 (1989) 144–149.
[29] L.I. Andersson, D.J. O'Shannessy, K. Mosbach, J. Chromatogr. 513 (1990) 167–179.