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Molecularly Imprinted Polymer Coatings for Open-Tubular Capillary Electrochromatography Prepared by Surface Initiation

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Molecularly imprinted polymer coatings were synthesized in fused-silica capillary columns by the use of a surface-coupled radical initiator. The coatings were prepared using either toluene, dichloromethane, or acetonitrile in the prepolymerization mixtures and were 0.15−2 μm thick as determined by scanning electron microscopy. Solvent-dependent differences in appearance were observed. All the molecularly imprinted polymer-based open-tubular capillary columns were able to separate the enantiomers of propranolol by means of electrochromatography. Electrochromatographic performance was found to be dependent on the type of solvent used during the synthesis.

Molecular imprinting[1,2] has during the past five years become an interesting approach to the preparation of stationary phases for use in capillary electrochromatography (CEC).[3,4] Since CEC provides a high degree of separation efficiency and short analysis times, it is recognized as a powerful tool for analytical separations.[5,6] Preparation protocols of capillary columns with stationary phases based on monolithic superporous molecularly imprinted polymers (MIPs) prepared in situ have been developed.[7−9] In addition, preparation protocols in which MIP particles were supported by acrylamide gel[10] or by a silica matrix[11] as well as prepared as a macroporous polymer monolith[12] have been reported. Recently, capillary columns packed with MIP-covered silica particles were reported.[13]

The selectivity of the MIP is predetermined by the choice of template molecule used for the molecular imprinting. Monomers are selected for their ability to interact by noncovalent interactions with the template molecule. The polymerization reaction embeds the template molecules in a solid, highly cross-linked polymer network in which the interactions between the template species and the monomers are maintained. This process gives rise to imprints possessing chemical and steric complementarity to the template. Subsequent washing steps leave recognition sites, within the polymer matrix, with affinity for the original template molecule.

MIP-based CEC separation systems have still to show the separation efficiency characteristic of CEC. The main probable reason for this lack of separation efficiency is unfavorable association and dissociation kinetics of the analyte with the MIP stationary phase. An interesting approach that may overcome this problem is to prepare the stationary phase as a film coating at the capillary wall. Recently, approaches to obtain MIP film-coated capillaries have been reported.[14,15] These approaches have only partially showed improved separation efficiency, and the MIP preparation variability was limited due to factors necessary to obtain polymer films.

The present study reports on a novel preparation protocol for MIP film coatings in capillary columns. The MIP coatings were synthesized in situ under conditions that allow a wide variability of the MIP composition. By immobilizing a photolabile radical initiator to the capillary surface prior to the introduction of the prepolymerization mixture, the polymerization reaction was confined to the vicinity of the capillary surface, resulting in a covalently attached MIP coating. The MIP capillary columns made were evaluated according to their enantiomer separation ability and separation efficiency when used in CEC.

EXPERIMENTAL SECTION

Chemicals. Trimethylolpropane trimethacrylate (1,1,1-tris-(hydroxymethyl)propane trimethacrylate; TRIM) was purchased from Aldrich (Gillingham, U.K.). 3-(Aminopropyl)triethoxy-

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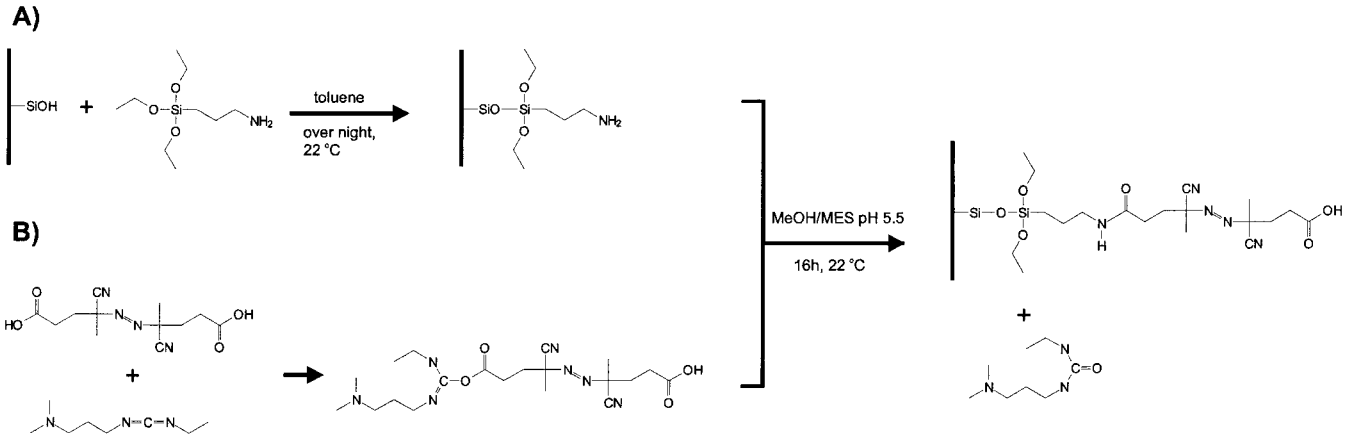

Figure 1. Schematic of the derivatization chemistry for the coupling of the radical initiator initiator 4,4′-azobis(4-cyanopentanoic acid) to the capillary inner surface. The fused silica was first derivatized with (3-aminopropyl)triethoxysilane (A), which was subsequently coupled to the ACPA via an carbodiimide-coupling using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (B).

silane, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC), and (*R*)-, (*S*)-, and *rac*-propranolol hydrochloride were obtained from Sigma (St. Louis. MO). (*S*)- or (*R*)-propranolol was converted to its free-base form by extraction in ethyl acetate and saturated $NaHCO_3$ solution and was subsequently washed once with water and evaporated.[7] The free-base propranolol was then stored at −20 °C until use. The 4,4′-azobis(4-cyanopentanoic acid) (ACPA) was from Fluka (Buchs, Switzerland). The toluene (HPLC grade), from Labscan Ltd. (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 delivered.

Initiator Derivatization of the Capillary Surface. Fused-silica capillary (25- or 50-μm i.d.; 375-μm o.d.) obtained from Polymicro Technologies (Phoenix, AZ) was subsequently flushed with 0.1 M sodium hydroxide, water, and acetone followed by drying in a stream of nitrogen. The capillary was then treated with 15% (v/v) (3-aminopropyl)triethoxysilane in toluene, which was kept in the capillary at room temperature overnight. The capillary was emptied and subsequently flushed with acetone followed by water and again emptied by a flow of nitrogen. A solution of 10 mM ACPA and 20 mM EDAC in methanol/0.05 mol/L MES buffer, pH 5.5 (1/1 v/v), was introduced into the capillary and kept at room temperature for 16 h (Figure 1). The capillary was washed with methanol/water (1/1 v/v), dried and stored at +4 °C until use.

Synthesis of MIP Coating. A detection window was prepared by removing about 0.5 cm of the protecting polymer layer 8.5 cm from one end. The detection window was covered by a piece of paper to prevent polymerization in this area of the capillary column. Typically, the capillary column was 35 cm in length (unless stated otherwise).

Prepolymerization mixtures were prepared by dissolving template molecule ((*S*)-propranolol free base, 0.060 mol/L), functional monomer (methacrylic acid (MAA), 0.48 mol/L), and cross-linking monomer (TRIM, 0.48 mol/L) in either toluene, dichloromethane, or acetonitril. The prepolymerization mixture was degassed by sonication for 5 min and then introduced into the capillary. The capillary ends were sealed by soft plastic rubber. To perform the polymerization, the filled capillary was illuminated by a type TL-900 UV lamp from Camag (Muttenz, Switzerland) set at 350 nm. The polymerization reaction was allowed to proceed for 3 h at 22 °C.

After polymerization, the capillary column was flushed with several column volumes of acetonitrile/acetic acid (9/1 v/v) in order to wash the template molecules and remaining monomers out of the capillary column. The MIP capillary columns were then emptied and stored at room temperature until use.

Scanning Electron Microscopy of the MIP Coating. MIP-coated capillaries (about 1−3 mm) were cut and examined by scanning electron microscopy. The pieces of capillary were sputter coated with gold (about 10 nm) using a SCD004 Sputter Coater (Balzers, Lichtenstein), and images were obtained by a JSM-840A scanning microscope (JEOL, Japan) operating at 5 or 10 kV.

Capillary Electrochromatography. Capillary electrochromatographic experiments were performed on an HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany), comprising a diode array detector, ChemStation software for data processing, and a high-pressure unit allowing pressures up to 12 bar to be delivered to the inlet vial or to both vials simultaneously.

The electrolyte was composed of acetonitrile and different ratios of 2 M acetic acid, adjusted to the desired pH by triethanolamine or 5 mol/L sodium hydroxide. The samples were prepared from 10 mM water solutions diluted with water to the desired concentration. All the buffer and sample solutions were prepared using water from a Milli-Q purification system (Millipore, Bedford, MA) and were degassed by sonication prior to use. Sample injections were made electrokinetically, typically by applying 5 kV (143 V/cm) for 5 s (unless stated otherwise). The separation voltage was set to 5−30 kV (143−858 V/cm), and the capillary column was thermostated to 60 °C (unless stated otherwise). UV detection was performed at 215 nm (10-nm bandwidth). When appropriate, a resolution factor, f/g, was calculated according to Meyer.[16] f being the distance from a line connecting the peaks of the eluting bands to the valley between the bands and g being the corresponding distance to the baseline. The degree of enantiomer separation was represented by a normalized separation index $\Delta t_R/t_{R1}$, where Δt_R is the difference

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in the elution times of the enantiomers at peak maximum and t_{R1} is the retention time of the first eluted enantiomer.

RESULT AND DISCUSSION

Preparation Protocol for MIP-Coated Capillaries. Successful MIP coatings could be prepared using the reported preparation methodology. In the present study, molecular imprinting of the amino alcohol (*S*)-propranolol was used as model substance since this compound previously has been used in efficient molecular imprinting for CEC applications.[7,8,17] Likewise, the functional monomer MAA and the cross-linking monomer TRIM were used. The MIP coating is covalently attached to the fused-silica capillary surface via the initiator that is attached to the capillary surface prior to MIP synthesis. The use of a surface-coupled initiator seems a very attractive approach for the preparation of MIP coatings. Upon radical formation, initiated by illumination of UV light, the polymerization reaction will be limited to the vicinity of the capillary surface. The extent of propagation, and thus the thickness of the MIP coating, is probably affected by several factors including functional and cross-linking monomer type and concentration, template molecule, temperature, initiator concentration, and solvent (porogen) type of polymerization.

The effect of using different solvents (porogens) for the MIP coating synthesis was studied. Toluene, acetonitrile, and dichloromethane were used as solvents in otherwise identical prepolymerization mixtures. In all cases, a MIP coating was formed. The MIP synthesized using toluene (TOL-MIP) as solvent produced thin coatings with rough appearance with a variable thickness of about 0.15−0.45 μm (Figure 2A). Using dichloromethane (DCM-MIP) instead also produced rough coatings but the MIP appeared to consist of clusters of larger units than in the case of toluene. The thickness was about 0.45−2 μm, and local clusters extending 5 μm from the surface were seen (Figure 2B). The MIP coatings obtained employing acetonitrile (ACN-MIP) as the solvent had a smooth appearance with an approximate thickness of 1 μm. Local spherical units of polymer in the size of 3−4 μm were also observed (Figure 2C).

The results indicate that MIP coatings can be prepared using solvents of diverse properties that may be advantageous in terms of MIP synthesis. Thus, the solvent can be chosen to optimize solubility of monomers and template molecule, to govern optimal monomer−template interaction in the prepolymerization mixture resulting in well-defined molecular imprints of high yield, and the possibility to alter the morphology of the resultant MIP coating by the solvent's (or mixture of solvents') porogenic properties. The ACPA initiator seems to be efficiently coupled to the capillary surface by the carbodiimide coupling (Figure 1) and is indeed able to initiate the polymerization reaction to produce a MIP coating. It should be noted that the ACPA may be coupled to the capillary surface via one or both of its carboxylic acid moieties.

Thus, MIP coatings of variable thickness and appearance can be synthesized using surface initiation. The MIP coating characteristics are dependent on the choice of solvent used for its preparation. The resultant open-tubular capillary columns are easily flushed by low pressure facilitating fast regeneration and electrolyte exchange. The physical stability of the coatings was good. During the coarse of this study, the capillaries were used for several weeks under variable conditions, including different electrolytes, pH, temperatures, and electric fields, and no leakage or extrusion of the MIP coatings was observed.

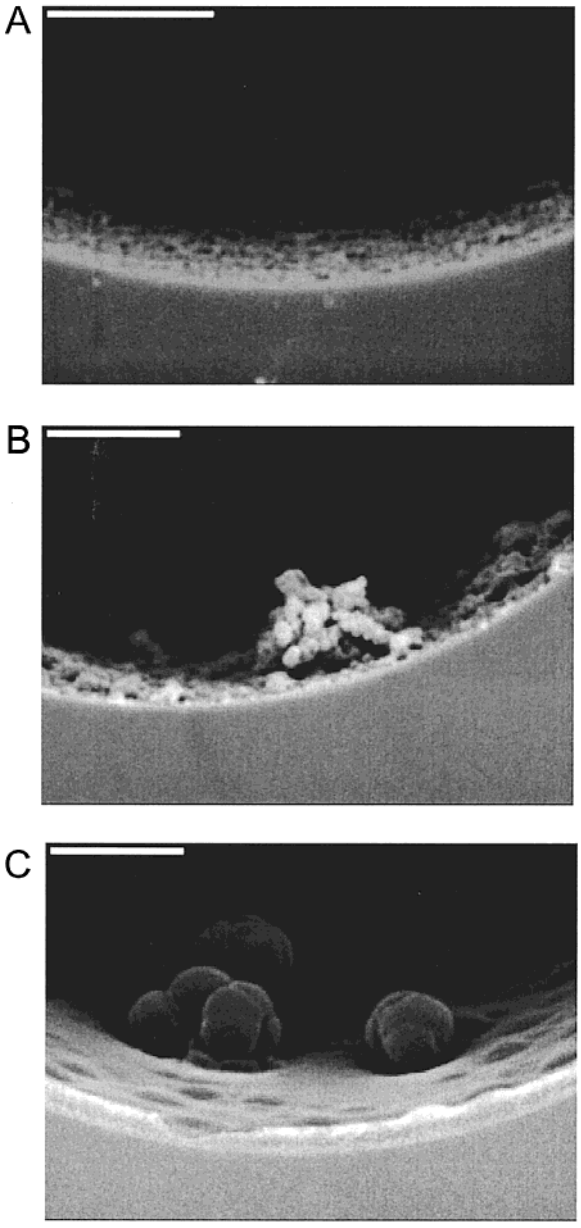

Figure 2. Scanning electron micrographs of the MIP coatings inside 50-μm fused-silica capillaries showing solvent-dependent differences in appearance. The MIP coatings were synthesized using different solvents (porogens) with otherwise identical prepolymerization mixtures and conditions; (A) toluene, (B) dichloromethane, and (C) acetonitrile. The scale bars indicate 5 μm.

Capillary Electrochromatographic Enantiomer Separation. *MIP Type.* Separation of the template molecule (*S*)-propranolol from its optical antipode (*R*)-propranolol was used in the evaluation of the MIP-coated capillaries and to characterize the electrochromatographic behavior at different conditions. All three MIP-type capillary columns were able to separate the enantiomers of propranolol with the expected elution order where the imprinted (*S*)-propranolol was the most retained (Figure 3). The characteristic band broadening for MIP-based separations was noted, especially the tailing of the imprinted analyte, which is considered to arise from imprint heterogeneity and slow on−off kinetics,

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Table 1. Resolution and Normalized Separation Index of Propranolol Enantiomers Separated in Different OT-MIP Capillary Columns[a]

OT-MIP column	solvent of polymerization	t_{R1} (min)	t_{R2} (min)	normalized separation index ($\Delta t_R/t_{R1}$)	resolution (f/g)	μ_{EOF}[b] (1×10^{-8} m^2 V^{-1} s^{-1})
TOL-MIP	toluene	3.4[c]	3.8[c]	0.098[c]	0.77[c]	1.63
DCM-MIP	dichloromethane	6.2	7.2	0.166	1	1.40
ACN-MIP	acetonitrile	4.3	5.4	0.256	0.9	2.25

[a] Data recorded by electrokinetic injections at 3kV, 3 s from a 50 μM *rac*-propranolol solution. Separation, 15 kV, 60 °C; electrolyte, acetonitrile/2 M acetic acid-triethanol amine buffer pH 3.0 (80/20 v/v). [b] Recorded using an acetonitrile/buffer ratio of 90/10 (v/v) as electrolyte. [c] The temperature was 29 °C.

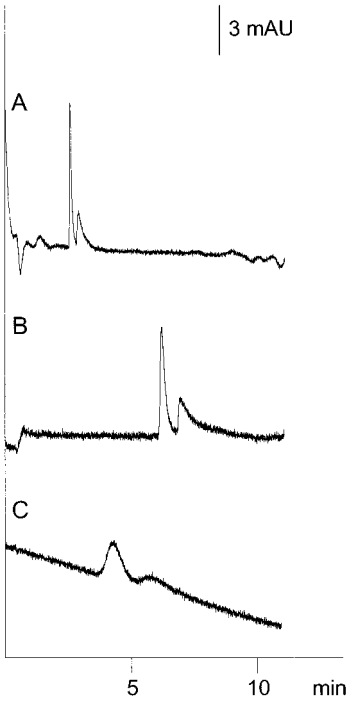

Figure 3. Electrochromatograms of the enantiomer separation of propranolol using MIP coatings synthesized in different solvents: (A) toluene ; (B) dichloromethane ; (C) acetonitrile . Elution order: (*R*)-propranolol followed by (*S*)-propranolol. Conditions: capillary, 50-μm inner diameter, 35-cm total length, and 26.5-cm effective length; separation, 15 kV; temperature, 60 °C. Other conditions as stated in the Experimental Section.

which in turn results in column overloading.[18] This was also indicated by the fact that the retention time decreased upon injection of higher sample amount.

Under the conditions used, it was observed that the MIP-coated capillary columns synthesized using toluene (TOL-MIP) and dichloromethane (DCM-MIP) performed generally better than those synthesized using acetonitrile (ACN-MIP). Highest plate numbers were recorded for the TOL-MIP, whereas for the ACN-MIP, the peaks was extremely broadened often to an extent that detection was problematic. The selectivity (in terms of normalized separation index) was superior in the ACN-MIP though. Resolution and selectivity was slightly higher in the DCM-MIP than in the TOL-MIP (Table 1) which may be an effect of there being more MIP material in the DCM-MIP and thus a greater number of imprints. The overall morphology of the MIP coatings may also influence the accessibility of the imprints in the coatings.

There was a considerable difference in electroosmotic flow (EOF) in the respective OT-MIP capillaries. Highest EOF was recorded in the ACN-MIP, which were about 1.6 times that of the DCM-MIP and 1.4 times that of the TOL-MIP (Table 1). Thus, the EOF seem to be dependent on the MIP coating morphology controlled by the different solvents used during synthesis. It may be speculated that a more stable EOF is obtained in MIP coating-based CEC than in MIP monolith CEC since electric double-layer overlap is unlikely in the former case. Also, the coating approach permits the use of capillary columns with a smaller inner diameter than normally employed in CEC. The Joule heating can thus be decreased, which allows the use of higher field strengths, which leads to shorter analysis times. The smaller capillary dimensions may also be favorable in terms of mass transfer from the mobile phase to the stationary phase.

Electrolyte. The effect of the electrolyte composition was studied. In the present study, an electrolyte composed of acetonitrile and a low-pH buffer (acetic acid/triethanolamine at pH 3.0) was used since this type of electrolyte was shown previously to be adequate in monolithic or microparticle-based MIP-CEC systems.[7,9,17] It was found, as a general trend, that the normalized separation index and resolution was increased when the acetonitrile concentration increased, which is in agreement with similar monolithic MIP-based CEC systems studied previously.[19] The highest normalized separation index as well as resolution was achieved at 80% acetonitrile (by volume) in the electrolyte (Figure 4). At the highest level studied (90% acetonitrile), the band broadening was immense to a degree that required a higher sample amount to be injected for detection, which may explain the decrease in normalized separation index and resolution found at this level. Enantiomer separation was recorded at acetonitrile concentrations down to 30% (by volume), which demonstrates that rather water-rich electrolytes can be used and still provide enantiomer separation, at least in OT-capillary columns of the DCM-MIP type. The retention times for the analytes decreased on increasing acetonitrile concentration, again with the exception of the highest concentration (90%), where the longest retention times were found. Also, the highest EOF was observed using the high acetonitrile concentration electrolyte. In fact, with this electrolyte, the analytes were eluted after the EOF, hich indicates a strong interaction with the MIP for both

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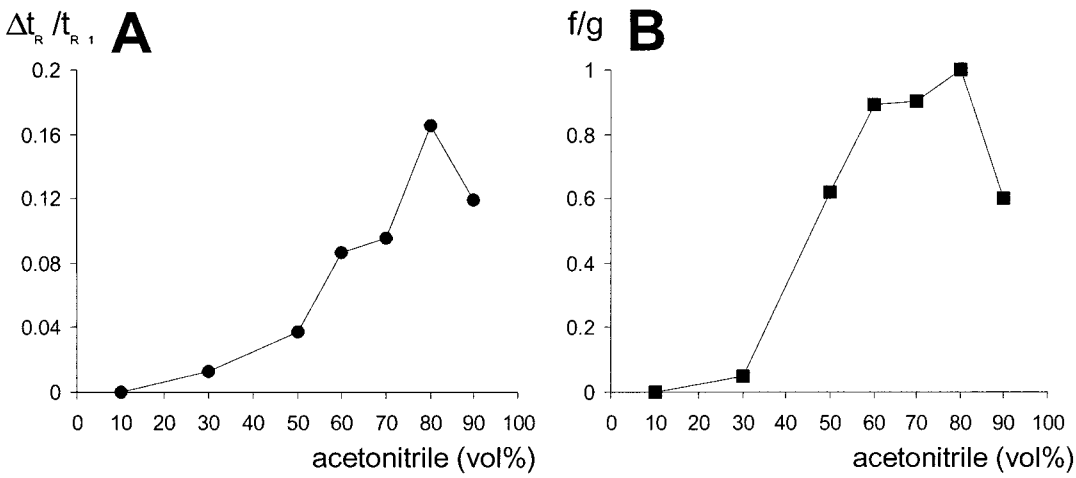

Figure 4. Effect of acetonitrile concentration in the electrolyte on normalized separation index ($\Delta t_R/t_{R1}$) and resolution (*f*/*g*) of propranolol enantiomers. conditions: electrolyte, acetonitrile/2 M acetic acid−triethanolamine, pH 3.0; sample, 50 μM *rac*-propranolol; injection, 3 kV, 3 s (except at 90 vol % acetonitrile, where injection was performed at 15 kV, 10 s); separation, 15 kV, 60 °C; capillary, DCM-MIP (total length, 35 cm; effective length, 26.5 cm; inner diameter, 50 μm).

enantiomers. In all other electrolytes studied, the analytes were eluted prior to the EOF.

CONCLUSION

It can be concluded from these preliminary results that thin MIP coatings can be synthesized in fused capillary columns by the use of a surface-coupled radical initiator. The formation of a surface coating is obtained because the polymerization reaction is initiated at the surface and the MIP will be limited to the vicinity of the capillary surface. The derivatization of the ACPA initiator via a carbodiimide coupling reaction seems adequate for MIP coating synthesis using different prepolymerization protocols. The use of different solvents (porogens) during the polymerization reaction facilitates control of the MIP coating in terms of morphology and appearance. The MIP coatings synthesized using the solvents tested in this study, toluene, dichloromethane, and acetonitrile, all provided enantiomer separation of the amino alcohol propranolol when the *S* enantiomer was used as template for the MIP. Performance comparable to that recorded for superporous monolithic MIPs[7−9] was observed. Differences in the electrochromatographic behavior were noted for the different MIP coatings studied. The highest normalized separation index and resolution of the enantiomers was found in the acetonitrile- and dichloromethane-based MIP coating, respectively. The use of toluene is normally considered to give imprints of higher efficiency since this solvent is the most apolar. The observed effects may be due to differences in imprint concentration, accessibility of the imprints due to morphology differences, effects associated with the electrochromatographic conditions, or a combination of these and suggest further investigation. The method reported here is further suited for MIP coating preparation directly at any surface, including in chip-based platforms.

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