The Analyst COMMUNICATION

Molecularly imprinted microparticles for capillary electrochromatographic enantiomer separation of propranolol

Leif Schweitz,* Peter Spégel* and Staffan Nilsson*

Technical Analytical Chemistry, Center for Chemistry and Chemical Engineering, Lund University, P O Box 124, SE-221 00 Lund, Sweden. E-mail: Staffan.Nilsson@teknlk.lth.se; Fax: +46 46 2224525

Received 6th September 2000, Accepted 20th September 2000
First published as an Advance Article on the web 16th October 2000

Molecularly imprinted microparticles imprinted against (*S*)-propranolol were synthesised and studied for use in capillary electrochromatographic separation of propranolol enantiomers. The imprinted microparticles were in the size range of 0.2–0.5 μm as determined by scanning electron microscopy. The microparticles were suspended, in high concentration, in the electrolyte and used to perform enantiomer separation by a partial filling technique.

Introduction

Capillary electrochromatography (CEC)[1–3] is a powerful separation method that theoretically provides improved efficiency over liquid chromatography with the same stationary phase. This is mainly an effect of the flat flow velocity profile generated by electro-osmosis, which is, besides the electrophoresis, the driving mechanism of the analytes through the column. Recently, much research effort has been put into the development of novel stationary phases for CEC.[4] In this direction, the use of molecularly imprinted polymers has drawn interest since this methodology enables the preparation of sorbents with a predetermined selectivity. Molecular imprinting[5,6] relies on the formation of complexes between functional monomers and template molecules, which, after co-polymerisation with crosslinking monomers and extraction of the template from the resultant polymer, yields affinity sites complementary to the template molecule. Molecularly imprinted polymers (MIPs) have been used as stationary phases for enantiomer separation of a number of compounds including drug compounds in LC or CEC mode.[7–11]

Previously, for use as CEC stationary phases, MIPs have been used in the format of superporous monoliths,[12,13] particles entrapped in different matrixes,[14,15] films or coatings covering the column wall;[16,17] they have also been used as pseudo-stationary phases.[18] Recently, preparation of molecularly imprinted monodisperse microspheres, used for competitive radioassay studies, has been reported.[19] The microspheres were prepared *via* a precipitation polymerisation protocol and the resultant microspheres were reported to be about 0.3 μm.

In this communication we report the use of molecularly imprinted microparticles for electrochromatographic enantiomer separation of propranolol using a partial filling technique.[20]

Experimental

Chemicals

Trimethylolpropane trimethacrylate (TRIM) was purchased from Aldrich (Gillingham, UK). 2,2'-Azobisisobutyronitrile (AIBN), and (*R*)-, (*S*)-, and *rac*-propranolol hydrochloride were obtained from Sigma (St. Louis, MO, USA). (*S*)-propranolol was converted to its free-base form by extraction in ethylacetate and saturated $NaHCO_3$ solution, and was subsequently washed once with water and evaporated. The free-base propranolol was stored at −20 °C until use. Water was purified by a MilliQ purification system (Millipore, Bedford, MA, USA). Methacrylic acid (MAA), acetonitrile (for chromatography) and all other chemicals were from Merck (Hohenbrunn, Germany) and were used as obtained.

Preparation of molecularly imprinted microparticles

(*S*)-propranolol (0.0135 mol L^{-1}) in its free-base form, AIBN (0.0012 mol L^{-1}), MAA (0.109 mol L^{-1}), and TRIM (0.109 mol L^{-1}) were dissolved in acetonitrile (normally 4 mL) in a borosilicate glass tube. This pre-polymerisation mixture was sonicated for 10 min followed by nitrogen purging for 6 min. The tube was sealed and put under a UV source (Type TL-900 UV lamp from Camag (Muttenz, Switzerland)) and irradiated (350 nm) at −26 °C. The polymerisation reaction was allowed to proceed overnight.The resultant polymer particles were collected and washed by successive centrifugation (4000 rpm, 30 min) and re-suspension (sonication for 10 min) twice in methanol–acetic acid (9 : 1 v/v) and once in methanol. Finally, the polymer particles were dried and stored at room temperature until use.

The microparticles were examined by scanning electron microscopy. The particles 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) set at 5 kV.

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 one vial or to both vials simultaneously.

Fused silica capillary (100 μm id; 375 μm od) obtained from Polymicro Technologies (Phoenix, AZ, USA) was derivatised with (methacryloxy)propyltrimethoxysilane. The derivatisation was performed by rinsing the capillary for 5 min with 1 mol L^{-1} NaOH, water, 0.1 mol L^{-1} HCl, and water, successively, followed by drying with a stream of nitrogen gas. The capillary was then rinsed and filled with a solution of toluene–(methacryloxy)propyltrimethoxysilane (85 : 15 v/v). This solution was kept in the capillary overnight and finally the capillary was rinsed with toluene and dried.

The electrolyte was composed of acetonitrile and 25 mmol L^{-1} phosphoric acid adjusted to the desired pH by triethanolamine. MIP particles were suspended in the electrolyte (5 mg mL^{-1}). The samples were prepared from 10 mmol L^{-1} water solutions diluted with electrolyte to 25 or 50 μmol L^{-1} concentration. All solutions were degassed by sonication.

Prior to CEC analysis the capillary was rinsed with 0.1 mol L^{-1} sodium hydroxide, water and electrolyte. The MIP

DOI: 10.1039/b007221o

electrolyte was then introduced into the separation capillary column hydrodynamically to partially fill the capillary with MIP particles. The sample was then injected electrokinetically. The separation voltage was set to 5–30 kV (143–857 V cm^{-1}) and the capillary column was thermostated to 60 °C. During the separation an overpressure of 5 bar was applied to both the inlet and the outlet vial. UV detection was performed at 215 nm (10 nm bandwidth).

The effect of the amount of MIP electrolyte introduced into the capillary, the influence of separation voltage, and the influence of electrolyte composition on the enantiomer separation of propranolol were studied. 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 the peak maximum and $t_{R,1}$ is the retention time of the first eluted enantiomer. When appropriate, a resolution factor, f/g, was calculated. 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]

Results and discussion

Preparation of MIP microparticles

The preparation of (*S*)-propranolol imprinted microparticles yielded spherical beads in the range of 0.2–0.5 μm diameter as determined from scanning electron microscopy (Fig. 1). The preparation protocol used followed the principle reported by Ye *et al*.[19] but was modified in the amount and ratio of monomers, initiator and template molecule. In the preparation of the (*S*)-propranolol imprinted microparticles used in this study, the template concentration was well below the saturation concentration of the imprinting solvent. The polymerisation reaction was performed at low temperature (−26 °C) since it was previously found that a low temperature was beneficial for the enantiomer separation on superporous MIP monoliths.[12]

The benefit of using the precipitation polymerisation protocol is that molecularly imprinted microparticles of sub-micron size are obtained without any need for stabilising surfactants. Conventional monomers and porogenic solvents for molecular imprinting may be used as well. The reduced size of the MIP particles, as compared to larger ones, may potentially improve the mass transfer kinetics and result in improved electrochromatographic performance.

It was found that microparticles prepared without any template molecule were smaller in size (approximately 0.1 μm) than those prepared with a template. This demonstrates the importance of the composition of the pre-polymerisation mixture, also in the case of template molecule concentration.

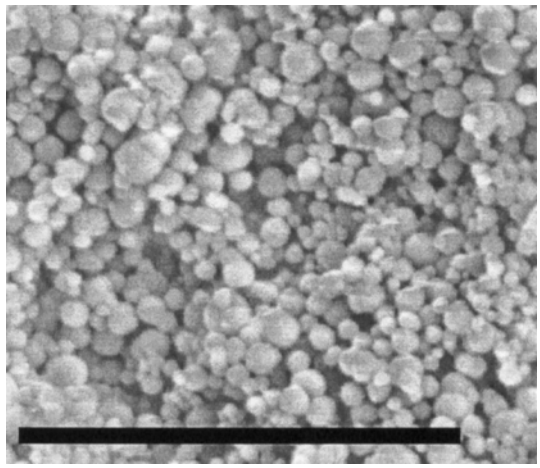

Fig. 1 Scanning electron micrograph of (*S*)-propranolol imprinted microparticles used for enantiomer separation of propranolol. The particles are 0.2–0.5 μm in diameter. The length of the bar is 10 μm.

CEC enantiomer separation

Enantiomer separation of propranolol was achieved using microparticles of MIP prepared from the monomers MAA and TRIM, which are commonly used in molecular imprinting protocols. (*R*)- and (*S*)-propranolol were baseline separated in less than 75 s with the expected elution order of (*S*)-propranolol (the template molecule) being the most retained (Fig. 2).

To be able to use MIP particles as a pseudo-stationary phase, the so-called partial filling technique was utilised since the particles would absorb and scatter the light from the UV detection source making detection difficult if the whole capillary was filled with MIP electrolyte. The partial filling technique allows the user to alter the amount of MIP used for a certain separation, which is advantageous for fast optimisation. It also allows different MIPs to be used in the same capillary column since after each CEC run the used MIP electrolyte is discarded and the capillary is rinsed and filled with new MIP in a few minutes. Also, a minute amount of MIP is used (in the present study 3.5–5.8 μg per run), which may realise the use of precious or expensive chemicals for the MIP preparation protocols.

Since the MIP plug will migrate through the capillary it is important to optimise the system so that the analytes will reach the detector window of the capillary prior to the MIP. The migration of the MIP plug is determined by the electro-osmotic flow (EOF) of the system and the charge of the MIP. In the present study, the MIP plug was mainly migrating due to EOF. It was noted that even though a low pH electrolyte was used (in 90% acetonitrile), the EOF was 1.5×10^{-7} m^2 V^{-1} s^{-1} using an uncoated capillary column. Therefore, the use of a capillary column with a (methacryloxy)propyltrimethoxysilane-derivatised surface was used, this reduced the EOF 3-fold to 4.9×10^{-8} m^2 V^{-1} s^{-1}. The use of a capillary column with even further reduced EOF may realise the introduction of a larger amount of MIP.

Electrolyte composition

The electrolyte was composed of acetonitrile and low pH buffer since it was previously shown that this type of electrolyte was suitable for CEC separation of propranolol enantiomers on superporous monolithic MIP filled capillary columns.[13] Using 80% (by volume) of acetonitrile resulted in co-elution of the enantiomers. In the case of the superporous monolithic MIP based system, this electrolyte composition was found to be optimal. Effective enantiomer separation was achieved by increasing the acetonitrile content to 90%. An even further increase of acetonitrile to 95% resulted in higher resolution but the last eluting enantiomer was co-eluted with particles migrating with the EOF. Therefore an electrolyte composition

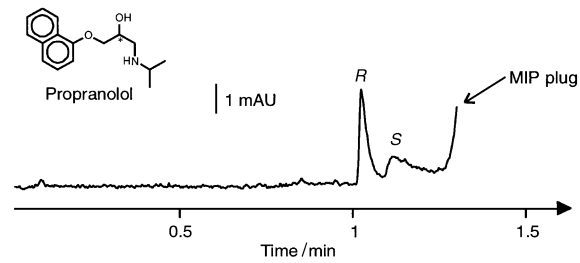

Fig. 2 Electrochromatogram of a typical enantiomer separation of propranolol enantiomers. The resolution, f/g = 1. The electrolyte was acetonitrile+25 mmol L^{-1} phosphoric acid–triethanolamine buffer of pH 3.5 (90+10 v/v). MIP microparticles were suspended in the electrolyte to 5 mg mL^{-1} and were introduced by applying 50 mbar for 4 s, which corresponds to 11.8 cm of the capillary length. The capillary was of 100 μm id, 35 cm total length, and 26.5 cm effective length. Sample (25 μmol L^{-1}) was injected electrokinetically at 5 kV for 4 s. The separation was performed by applying 15 kV (429 V cm^{-1}) at 5 bar overpressure.

of 90% acetonitrile and 10% 25 mmol L^{-1} phosphoric acid–triethanolamine buffer (pH 3.5) was used in the following studies.

MIP-microparticle-modified electrolyte

The MIP microparticles were suspended in the electrolyte to a maximum amount of 5 mg mL^{-1} to get stable suspensions. Increasing the MIP content further resulted in sedimentation, probably caused by aggregation of the particles. This may cause differences in the amount of MIP introduced onto the capillary column and may even cause blockage of the capillary. Accordingly, a MIP concentration of 5 mg mL^{-1} was used throughout this study.

The effect of the amount of MIP introduced onto the capillary on the enantiomer separation of propranolol was examined by varying the plug length of the MIP, *i.e.*, the time of the applied pressure (50 mbar). To obtain a detectable enantiomer separation, 33% (8.8 cm) of the effective length of the capillary (26.5 cm) had to be used. Increasing the MIP plug length resulted in an increased normalised separation index, and increased elution times for both enantiomers. Increasing to 55% (14.7 cm) of the effective length of the capilllary resulted in partial co-elution of the last eluting enantiomer ((*S*)-propranolol) with the EOF and the MIP plug. It was thus found that a MIP plug of 44% of the effective length (11.8 cm) was suitable. This corresponds to only 4.6 μg of MIP.

Since enantiomer separation of *rac*-propranolol could be performed using microparticles imprinted with (*S*)-propranolol and the fact that the (*S*)-enantiomer was always the last enantiomer eluted strongly indicates that (*S*)-propranolol was efficiently imprinted into the polymer network. This was further emphasised since microparticles prepared without any template molecule failed to perform any separation. By using the partial filling method, high concentrations of MIP may be used without hampering detection. In a study by Walshe *et al.*,[18] (*S*)-propranolol was imprinted using a chiral functional monomer. The particles used were about 20–30 μm in diameter but were claimed not to disturb detection at a concentration of 0.05% w/v. However, it was reported that the reference particles made without the template molecule were not soluble in the electrolyte and were not properly tested. Also, the use of MAA as the functional monomer failed to provide baseline separation of *rac*-propranolol. The particles investigated in the present study were much smaller than those used by Walshe *et al.*, however, they hampered detection even at low concentrations. Using the partial filling technique we realised the use of the MIP particles in CEC separation of *rac*-propranolol.

Effect of applied electric field

The effect of the electric field applied on the enantiomer separation was investigated. The electrolyte used was 90% acetonitrile and 10% 25 mmol L^{-1} phosphoric acid–triethanolamine buffer (pH 3.5). The MIP (5 mg mL^{-1}) was introduced at a constant amount (44% (11.8 cm) of the effective capillary length). The separation voltage was varied between 10 and 30 kV (the limit of the instrument). The highest normalised separation index was found at 25 kV (714 V cm^{-1}) and the separation was done in less than 1 min. Enantiomer separation was obtained at all electric fields investigated. The voltage (U/kV) to current (I/μA) ratio was linear in the range examined ($I = 0.2076U + 0.0421$, $R^2 = 0.9997$), indicating that minimal excessive Joule heating occurred.

Conclusion

The preparation and use of molecularly imprinted microparticles against (*S*)-propranolol were used to separate the enantiomers of propranolol in the CEC mode. The use of a partial filling method realised the use of a high concentration of MIP particles in the electrolyte. The microparticles were synthesised using chemicals, including monomers and solvents, that are commonly used in molecular imprinting. It was found that the presence of a template molecule in the polymerisation mixture is important for the final size, shape, and morphology of the microparticles.

The use of capillary columns with suppressed EOF characteristics would increase the amount of MIP particles that could be used. Also, chemical modification of the MIP or modifications of the electrolyte may result in the MIP migrating countercurrently to the analyte, which may improve the demonstrated strategy to use MIP microparticles with the partial filling technique in CEC. It would thus also be possible to separate negatively charged or uncharged analytes.

The method reported here allows altering of the amount of MIP used for a certain separation which is advantageous for fast optimisation. It also allows different MIPs to be used in the same capillary column since after each CEC run the used MIP electrolyte is discarded. Thus, an entirely fresh column is used for every analysis. We believe that the methodology has potential for use in applications of CEC mass spectrometry.

Acknowledgement

The financial support from TFR, NFR, Fysiografiska sällskapet i Lund, and the Carl Trygger foundation is acknowledged.

References

1 G. Blaschke and B. Chankvetadze, *J. Chromatogr., A*, 2000, **875**, 3.
2 K. D. Altria, N. W. Smith and C. H. Turnbull, *Chromatographia*, 1997, **46**, 664.
3 M. G. Cikalo, K. D. Bartle, M. M. Robson, P. Myers and M. R. Euerby, *Analyst*, 1998, **123**, 87R.
4 M. Pursch and L. C. Sander, *J. Chromatogr., A*, 2000, **887**, 313.
5 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812.
6 K. Mosbach and O. Ramström, *BioTechnology*, 1996, **14**, 163.
7 M. Kempe, *Anal. Chem.*, 1996, **68**, 1948.
8 B. Sellergren, *Int. Lab.*, 1997, 10A.
9 V. T. Remcho and Z. J. Tan, *Anal. Chem.*, 1999, **71**, 248A.
10 L. Schweitz, L. I. Andersson and S. Nilsson, *J. Chromatogr., A*, 1998, **817**, 5.
11 P. T. Vallano and V. T. Remcho, *J. Chromatogr., A*, 2000, **887**, 125.
12 L. Schweitz, L. I. Andersson and S. Nilsson, *Anal. Chem.*, 1997, **69**, 1179.
13 L. Schweitz, L. I. Andersson and S. Nilsson, *J. Chromatogr., A*, 1997, **792**, 401.
14 J.-M. Lin, T. Nakagama, K. Uchiyama and T. Hobo, *J. Liq. Chromatogr. Relat. Technol.*, 1997, **20**, 1489.
15 G. Chirica and V. T. Remcho, *Electrophoresis*, 1999, **20**, 50.
16 O. Brüggemann, R. Freitag, M. J. Whitcombe and E. N. Vulfson, *J. Chromatogr., A*, 1997, **781**, 43.
17 Z. J. Tan and V. T. Remcho, *Electrophoresis*, 1998, **19**, 2055.
18 M. Walshe, E. Garcia, J. Howarth, M. R. Smyth and M. T. Kelly, *Anal. Commun.*, 1997, **34**, 119.
19 L. Ye, P. A. G. Cormack and K. Mosbach, *Anal. Commun.*, 1999, **36**, 35.
20 A. Amini, U. Paulsen-Sorman and D. Westerlund, *Chromatographia*, 1999, **50**, 497.
21 V. R. Meyer, *Chromatographia*, 1987, **24**, 639.