Short communication

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Role of the charge in continuous beds in the chiral separation of hydroxy acids by ligand-exchange capillary electrochromatography

This paper deals with the chiral separation of hydroxy acids using diallyl-dimethylammonium chloride as a positive charge-providing agent in the continuous bed. The chiral continuous bed was prepared by *in situ* copolymerization of monomers, including an L-4-hydroxyproline derivative as a chiral selector. This phase was applied to the chiral separation of hydroxy monocarboxylic acids and hydroxy dicarboxylic acids, respectively. The influence of both the selector concentration and the charge-providing agent on retention and separation was investigated.

Keywords: Capillary electrochromatography / Chiral continuous bed / Enantiomer separation / Ligand exchange / Monolithic phase DOI 10.1002/elps.200305525

The use of CEC for chiral separations has become more and more popular [1–5]. Three different modes have been used for chiral CEC separations: packed capillaries, open-tubular capillaries and monolithic columns. In the latter two approaches, no frits are necessary. The preparation of frits by sintering a zone of the packing represents a rather complicated procedure and the frits are sources of air bubbles. Open-tubular capillaries containing the chiral selector coated to the capillary wall are on one hand simple to prepare, on the other hand they are disadvantageous regarding sample capacity. Different types of monolithic phases have been described: phases prepared by sol-gel process starting from triethoxy-silane [6], by sintering a silica-based packing [7, 8] and by *in situ* copolymerization in the capillary ("continuous beds", CBs) [9, 10].

Recently, hydroxy acids were resolved by CEC using macrocyclic antibiotics as chiral selectors [11, 12]. Among several chiral separation principles in electromigration techniques, ligand exchange (LE) was found to be an efficient approach for the chiral resolution of chelate complex-forming analytes [13]. Thus, the preparation of a ligand-exchange chiral continuous bed (LE-CCB) for the enantioseparation of underivatized amino acids [14] and hydroxy acids [15] was described. In the case of amino acids vinylsulfonic acid (VSA) was used as a negative charge-providing agent for producing EOF. This reagent, however, was not suitable in the case of hydroxy acids, since the EOF was opposite to the migration direction of the hydroxy acids. Therefore, experiments without VSA were performed for enantioseparation of hydroxy acids [15]. In that case, these analytes migrated only according to their electrophoretic mobility to the anode. This paper deals with studies using diallyldimethylammonium chloride (DADMAC), a positively charged component in the CB with the goal of speeding up the separation of hydroxy acids.

All chemicals were of analytical grade. 2-Phenyllactic acid (atrolactic acid), 3-hydroxy-4-methoxymandelic acid, 4-hydroxy-3-methoxymandelic acid, 4-methoxymandelic acid, and citramalic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). 4-Hydroxymandelic acid, copper(II) acetate, mandelic acid, 3-hydroxymandelic acid, 4-bromomandelic acid, 3-phenyllactic acid and DADMAC were purchased from Fluka (Buchs, Switzerland). 3,4-Dihydroxymandelic acid was from EGA-Chemie (Steinheim, Germany), malic acid from Merck (Darmstadt, Germany). The mobile phase was prepared by dissolving 0.1–5 mM copper(II) acetate and 50 mM ammonium acetate in bidistilled water; pH was adjusted to 4.4 with glacial acid. Sample solutions were prepared by dissolving the analytes (1 mg) in 1 mL water. Samples were injected electrokinetically (−3 kV for 3 s). All solutions were filtered through a 0.20 μm pore size filter (Schleicher/Schuell, Dassel, Germany) and degassed

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Abbreviations: CB, continuous bed; **CCB**, chiral continuous bed; **DADMAC**, diallyldimethylamoonium chloride; **LE**, ligand exchange; **VSA**, vinylsulfonic acid

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with helium. The applied voltage was −5 kV (5 kV to the anodic end); separations were performed at ambient temperature. A fully automated ^{3D}CE system (Hewlett-Packard, Foster City, CA, USA) equipped with a CEC pressure device (12 bar) and a diode array detector was used. Detection was performed *via* on-column measurement of the UV absorption at 208 nm. The fused-silica tubing was purchased from Microquartz (Munich, Germany). The chiral continuous bed was synthesized as previously described [14], but DADMAC instead of vinyl sulfonic acid was used as charge-providing agent. The total length of the capillary was 34.5 cm × 0.075 mm, the length of the CCB was 26 cm.

In a previous paper, we described the preparation of a CB for the chiral separation of amino acids using the principle of LE. As charge-providing agent VSA was added to the CB for producing EOF. Since the amino acids are positively charged at the pH applied (pH 4.4), their velocity is superimposed to that of the EOF. Under these conditions, however, the EOF is not strong enough to carry the hydroxy acids, which tend to move towards the anode, to the cathode; therefore, extremely long retention times resulted. When VSA was omitted, hydroxy acids migrated with their own electrophoretic mobility to the anode. Good separations were obtained under these conditions, however, retention times were not satisfying yet [15]. With the goal of further reducing retention times, we checked the influence of a positive charge providing agent in the CB. In this respect, we copolymerized DADMAC instead of VSA with the monomers. As a result, the EOF was reversed and thus superimposed to the electrophoretic migration of the hydroxy acids, resulting in decreased retention times. A further decrease of retention times can be achieved by applying either higher voltage or pressure-supported CEC. The elution order was determined for mandelic acid and 3-phenyllactic acid, for which the enantiomers were available, and was found to be (+) before (−). In addition to hydroxy monocarboxylic acids, also hydroxy dicarboxylic acids were resolved under these conditions.

The selector concentration in the CB was found to be crucial for the resolution of hydroxy acids. We investigated concentrations in the range from 10 to 25%, whereby 10% showed an optimal ratio of retention times and resolution (Fig. 1). The influence of the concentration of DADMAC in the CB was studied by adding 0–3% to the monomer solution. Compared to a CB without charge-providing agent, a significant decrease in retention times was observed, connected with an improvement in peak shape. It turned out, however, that the concentration does not significantly effect retention times and resolution (Fig. 2).

We studied also the influence of the concentration of copper(II) acetate adding amounts between 0.1 and 5 mM to the mobile phase; a concentration of 5 mM was found to be optimal, whereas a further increase of the Cu(II) concentration did not result in improvement of resolution and efficiency. Ammonium acetate buffer was found to be su-

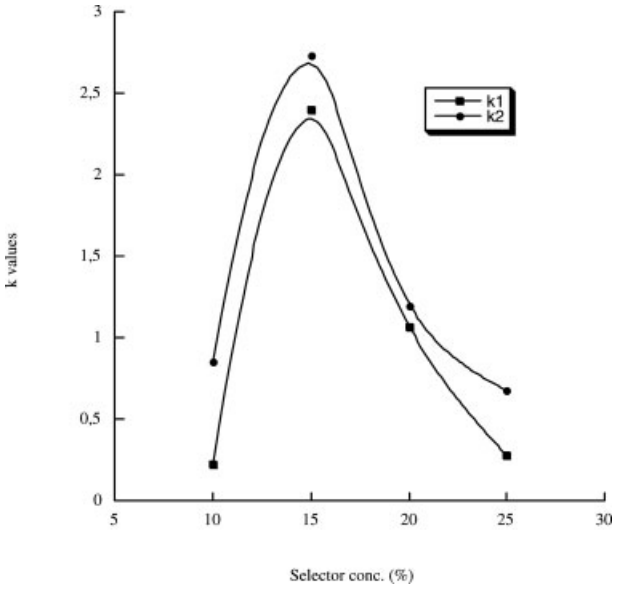

Figure 1. Dependence of *k*-values of the enantiomers of DL-3-phenyllactic acid on the selector concentration in the CB. Conditions: LE-CCB, 26 cm × 75 μm, 5 mM $Cu(CH_3COO)_2$, 50 mM NH_4CH_3COO, pH 4.4: injection, −3 kV × 3 s; run, −5 kV.

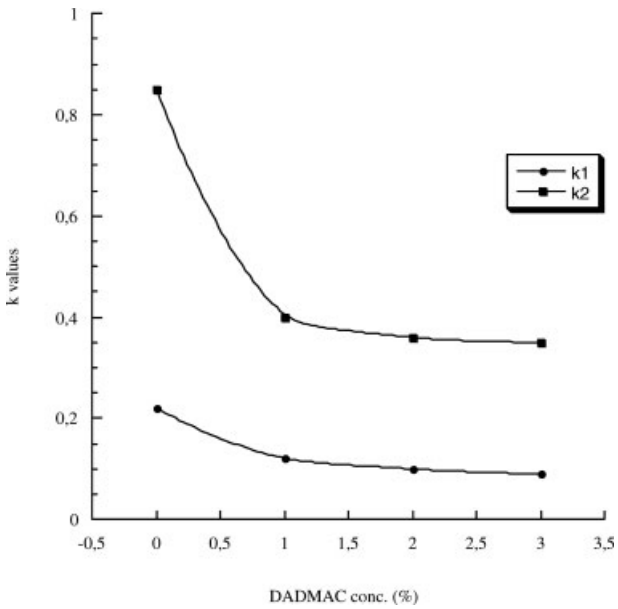

Figure 2. Dependence of *k*-values of the enantiomers of DL-3-phenyllactic acid on the concentration of DADMAC in the CB. Conditions as in Fig. 1, except DADMAC from 0–3%.

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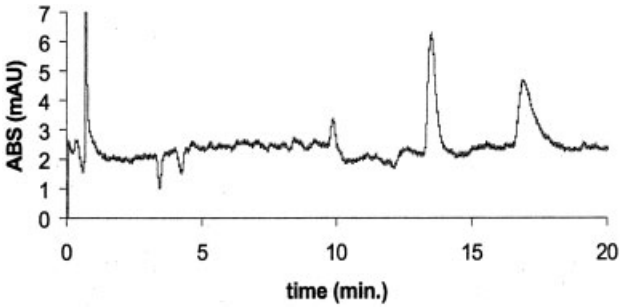

Figure 3. Chiral separation of DL-3-phenyllactic acid. Conditions as in Fig. 1.

perior to phosphate buffer, because of reduced electric current and thus less Joule heating. Figure 3 shows the enantioseparation of 3-phenyllactic acid. In Table 1 the separation data for a selection of hydroxy acids are given. Plate numbers were checked for the enantiomers of 3-phenyllactic acid and were found to be 11 000 and 13 000, respectively.

Table 1. Separation data of hydroxy acids

Analyte	k_1	k_2	α	R_s
Mandelic acid	0.28	0.35	1.25	1.12
3-Hydroxymandelic acid	0.30	0.45	1.51	1.21
4-Hydroxymandelic acid	0.36	0.50	1.37	1.29
4-Brommandelic acid	0.28	0.44	1.58	1.09
4-Methoxymandelic acid	0.29	0.40	1.36	1.12
3-Hydroxy-4-methoxy-mandelic acid	0.21	0.35	1.69	1.46
3-Hydroxymandelic acid	0.23	0.31	1.35	1.32
3,4-Dihydroxymandelic acid	0.25	0.49	1.98	1.70
Atrolactic acid	0.10	0.22	2.21	2.48
3-Phenyllactic acid	0.22	0.85	3.84	5.34
Citramalic acid	0.14	0.28	2.00	1.15
Malic acid	0.25	0.32	1.28	0.77

Conditions as in Fig. 1

In conclusion, the chiral separation of hydroxy acids by LE-CEC using a chiral CB was optimized. The influence of selector concentration and the charge-providing agent on retention and resolution was studied. It has been shown that a positive charge-providing agent, such as DADMAC reduces retention and improves peak shape.

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References

[1] Gübitz, G., Schmid, M. G., *Biopharm. Drug Disp.* 2001, *22*, 291–336.

[2] Chankvetadze, B., *J. Sep. Sci.* , 2001, *24* 691–705.

[3] Fanali, S., Catarcini, P., Blaschke, G., Chankvetadze, B., *Electrophoresis* 2001, *22*, 3131–3151.

[4] Wistuba, D., Schurig, V., *Electrophoresis* 2000, *21*, 4136–4158.

[5] Kang, J., Wistuba, D., Schurig, V., *Electrophoresis* 2002, *23*, 4005–4021.

[6] Tanaka, N., Nagayama, H., Kobayashi, H., Ikegami, T., Hosoya, K., Ishizuka, N., Minakuchi, H., Nakanishi, K., Cabrera, K., Lubda, D., *J. High Resolut. Chromatogr.* 2000, *23*, 111–116.

[7] Wistuba, D., Schurig, V., *Electrophoresis* 2000, *21*, 3151–3159.

[8] Wistuba, D., Schurig, V., *J. Chromatogr. A* 2000, *875*, 255–276.

[9] Hjertén, S., Végvári, A., Srichaiyo, T., Zhang, H.-X., Ericson, C., Eaker, D., *J. Capil. Electrophor.* 1998, *5*, 13–26.

[10] Peters, E. C., Lewandowski, K., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Commun.* 1998, *35*, 83–86.

[11] Fanali, S., Catarcini, P., Presutti, C., Stancanelli, R., Quaglia, M. G., *J. Chromatogr. A* 2003, *990* 143–141.

[12] Fanali, S., Catarcini, P., Presutti, C., Quaglia, M. G., Righetti, P. G., *Electrophoresis* 2003, *24*, 904–912.

[13] Schmid, M. G., Grobuschek, N., Lecnik, O., Gübitz, G., *J. Biochem. Biophys. Methods* 2001,*48*, 143–154.

[14] Schmid, M. G., Grobuschek, N., Tuscher, C., Gübitz, G., Végvári, Á., Machtejevas, E., Maruska, A., Hjertén, S., *Electrophoresis* 2000, *21*, 3141–3144.

[15] Schmid, M. G., Grobuschek, N., Lecnik, O., Gübitz, G., Végvári, Á., Hjertén, S., *Electrophoresis* 2001, *22*, 2616–2619.

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