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Application of ligand-exchange capillary electrophorersis to the chiral separation of α-hydroxy acids and β-blockers

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Abstract

The application of the principle of ligand-exchange capillary electrophoresis to two substance classes is described. As chiral selector *N*-(2-hydroxyoctyl)-L-4-hydroxyproline–copper(II) complex was used. This principle was applied to the chiral separation of α-hydroxy acids and drugs containing amino alcohol structure such as β-blockers. The enantioselectivity was found to be strongly dependent on pH corresponding to the optimal conditions for complex formation for each structure class.

Keywords: Ligand-exchange capillary electrophoresis; Enantiomer separation; Hydroxy acids; Beta-blockers

1. Introduction

Ligand-exchange chromatography, introduced in the early 1970s by Davankov and Rogozhin [1] has been shown to be a powerful tool for the chiral separation of amino acids and other chelate complex forming compounds [2].

This basic separation principle was also found to be applicable to capillary electrophoresis (CE) by simply using a chiral selector–metal complex as an additive to the electrolyte. The first application of this technique in CE was reported by Zare and co-workers using histidine– [3] or aspartame–copper(II) complexes [4] as chiral selectors for the resolution of dansyl amino acids. Cohen et al. [5] and later Sundin et al. [6] described the use of *N*,*N*-didecyl-L-alanine–Cu(II) in combination with sodium dodecyl sulfate (SDS) for the chiral separation of dansyl amino acids. Desiderio et al. succeeded in resolving α-hydroxy acids using the copper(II) complexes of L-4-hydroxyproline (L-Hyp) or aspartame as chiral selectors [7]. Krasensky et al. used the same selectors for the chiral resolution of α-hydroxy acids in a column coupling system in order to prevent interferences with the detection [8].

In a previous paper we reported the first direct resolution of underivatized amino acids using L-proline (L-Pro)– or L-Hyp–copper(II) complexes as additives to the electrolyte. The separations were carried out either using capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC) using SDS as a micelle forming additive [9]. The latter approach was recently applied by Chen et al. for the separation of optical and positional isomers of tyrosine and fluorophenylalanine [10] as well as tryptophan derivatives [11]. We could not detect aliphatic amino acids using L-Pro or L-Hyp because of the high selector concentration in the electrolyte. A significant improvement in enantioselectivity was achieved by using *N*-alkyl derivatives of L-Hyp as chiral selectors [12,13]. In addition to aromatic amino acids, these selectors showed enan-

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tioselectivity for several aliphatic amino acids and dipeptides. The required selector concentration was significantly lower compared to underivatized L-Hyp thus eliminating interferences at the detector. Recently, we showed that this principle is also applicable to the chiral separation of sympathomimetics having an amino alcohol structure [14].

The present paper deals with the application of this basic principle to the chiral resolution of α-hydroxy acids and β-blockers using *N*-(2-hydroxyoctyl)-L-4-hydroxyproline (HO-L-Hyp) as a chiral selector.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical grade. Dimethylsulfoxide (DMSO), atrolactic acid, 3-hydroxy-4-methoxymandelic acid, 4-hydroxy-3-methoxymandelic acid and 3-(4-hydroxyphenyl)-lactic acid were purchased from Aldrich. 4-Hydroxymandelic acid, tropic acid and β-blockers were from Sigma (St. Louis, MO, USA). L-4-Hydroxyproline, copper(II) sulfate, 1,2-epoxyoctane, 3-hydroxymandelic acid, 4-bromomandelic acid, 3-phenyllactic acid and methanol (twice distilled) were purchased from Fluka (Buchs, Switzerland). Sodium hydroxide, phosphoric acid and triethylamine were from E. Merck (Darmstadt, Germany). 3,4-Dihydroxymandelic acid was from EGA (Steinheim, Germany). *N*-(2-Hydroxyoctyl)-L-4-hydroxyproline was synthesized as described previously [12].

The electrolyte solution was prepared by dissolving copper(II) sulfate and HO-L-Hyp in water or 5 m*M* phosphoric acid; pH was adjusted by 2 *M* NaOH. Ammonia or triethylamine (TEA) was added to prevent precipitation of copper hydroxide at high pH. Sample solutions were prepared by dissolving the analytes (1 mg) in double distilled, deionized water (1 ml). Samples were injected hydrodynamically (10–50 mbar) for 6 s (about 10–40 nl injection volume). All solutions were filtered through a 0.20-μm pore size filter (Schleicher and Schuell, Dassel, Germany) and degassed with helium prior to use. The applied voltage was between 15 and 28 kV. Mobility of the electroosmotic flow (EOF) was determined by injecting DMSO under the same conditions. Separations were carried out at ambient temperature.

2.2. Instrumentation

A PrinCE capillary electrophoresis system (PrinCE Technologies, Emmen, The Netherlands) equipped with a Lambda 1000 UV–Vis detector (Bischoff Analysentechnik, Leonberg, Germany) was used. Fused-silica capillaries (70 cm×50 μm I.D.) were purchased from Composite Metal Services (Hallow, UK). UV detection was performed at 208 nm. An Axxiom Chromatography 737 system, v 3.91 (Moorpark, CA, USA) was used to process data.

2.3. Separation conditions

2.3.1. Conditions (a), for α-hydroxy acids

Electrolyte: 10 m*M* HO-L-Hyp, 5 m*M* copper(II) sulfate, 5 m*M* phosphate solution, pH 4.3, sample injection: 10 mbar×6 s, capillary: 70 cm (effective length 26 cm)×0.050 mm, voltage: 25–28 kV.

2.3.2. Conditions (b), for β-blockers

Electrolyte: 20 m*M* HO-L-Hyp, 10 m*M* copper(II) sulfate, 100 m*M* TEA, adjusted to pH 12 with NaOH, sample injection: 10 mbar×6 s, capillary: 70 cm (effective length 26 cm)×0.050 mm, voltage: 15 kV.

2.4. Calculation of separation data

Effective mobility (μ_{eff}), selectivity factor (α) and resolution (R_s) were calculated by the following equations:

$$\mu_{\text{eff}} = \mu_{\text{app}} - \mu_{\text{EOF}}$$

$$\alpha_{\text{eff}} = \mu_{\text{eff2}} / \mu_{\text{eff1}}$$

$$R_s = \frac{2(t_2 - t_1)}{w_1 + w_2}$$

where w is the width of the peak.

3. Results and discussion

The separation mechanism of ligand-exchange CE is based on the formation of diastereomeric ternary mixed metal complexes between the chiral selector ligand and the analytes. Resolution is due to the difference in complex stability constants of the two mixed complexes with the analyte enantiomers. The following equilibria are to be taken into account:

$Cu(L\text{-Sel})_2 + S\text{–}A \leftrightharpoons Cu(L\text{-Sel})(S\text{–}A) + L\text{-Sel};$
$Cu(L\text{-Sel})_2 + R\text{–}A \leftrightharpoons Cu(L\text{-Sel})(R\text{–}A) + L\text{-Sel};$
Sel: selector (HO-L-Hyp)
A: analyte

3.1. Resolution of α-hydroxy acids

The chiral separation of α-hydroxy acids is of interest in biochemistry and pharmaceutical chemistry as well as in the course of metabolism studies. Ligand-exchange chromatography has already successfully been applied to the chiral resolution of hydroxy acids using either chiral stationary phases [15,16] or chiral selectors as additives to the mobile phase [17–21]. More recently, ligand-exchange capillary electrophoresis was shown to be also applicable to the chiral separation of hydroxy acids using the copper(II) complexes of L-Hyp or aspartame as chiral selectors [7,8]. HO-L-Hyp was found to show improved enantioselectivity for α-hydroxy acids compared to L-Hyp.

Fig. 1 shows possible structures of the ternary mixed complexes. The structures have not yet been confirmed by spectroscopic studies, however, they are in good accordance with proposed structures of similar complexes described in the literature [2,22].

As can be seen from Fig. 2, the pH optimum for complexation varies for different substance classes. As in the case of amino acids, the optimal pH for the resolution of hydroxy acids was found to be 4.3. Unlike amino acids, which are positively charged at pH 4.3, α-hydroxy acids show a negative mobility. There is an equilibrium between the free analyte and the mixed complex. The mobility of amino acids is superimposed on the EOF. Upon forming the mixed complex with the selector, hydroxy acids are transported to the cathode, but with significantly lower velocity compared to amino acids. The analytes are detected in form of the copper(II) complexes. That has been confirmed by the diode-array spectra.

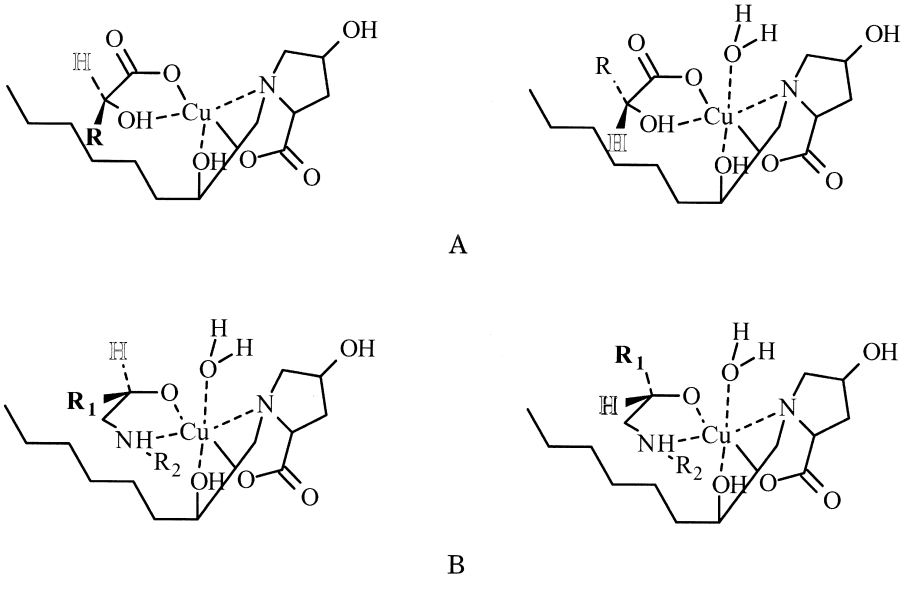

Fig. 1. Possible structures of the ternary mixed complexes between HO-L-Hyp and the two enantiomers of a α-hydroxy acid (A) and an amino alcohol (B).

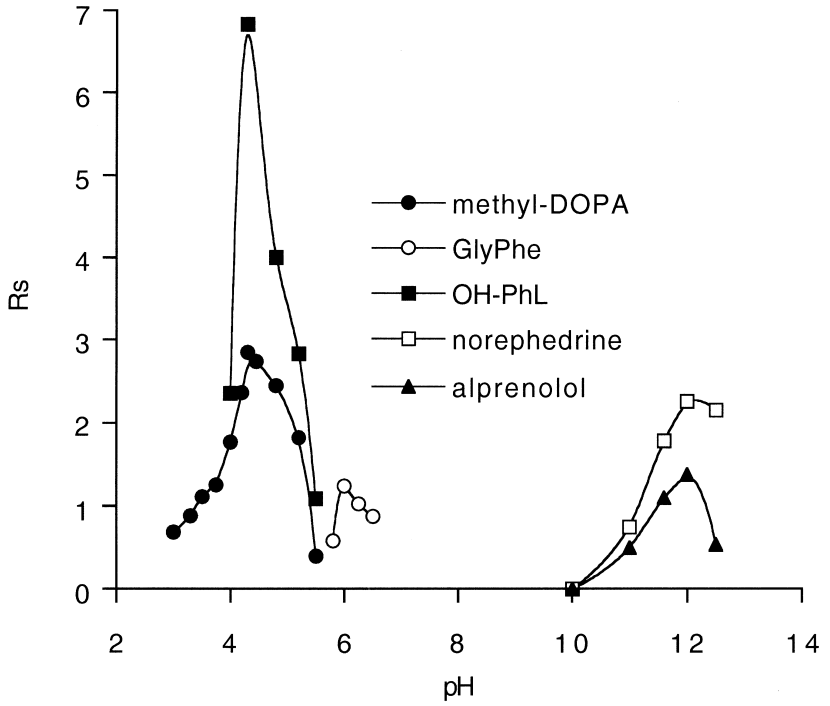

Fig. 2. Dependence of resolution on pH using methyl-DOPA, glycyl-phenylalanine (GlyPhe), 3-(4-hydroxyphenyl)lactic acid (OH-PhL), norephedrine and alprenolol as model compounds. Conditions: 10 or 20 m*M* HO-L-Hyp, 5 or 10 m*M* Cu(II).

As can be seen from Fig. 3, the velocity order is: amino acid>EOF/selector>α-hydroxy acid. The selector complex is neutral and co-migrates with the EOF. A simultaneous separation of asparagine and 3-(4-hydroxyphenyl)lactic acid using DMSO as an EOF marker is shown in Fig. 4. Since the separation

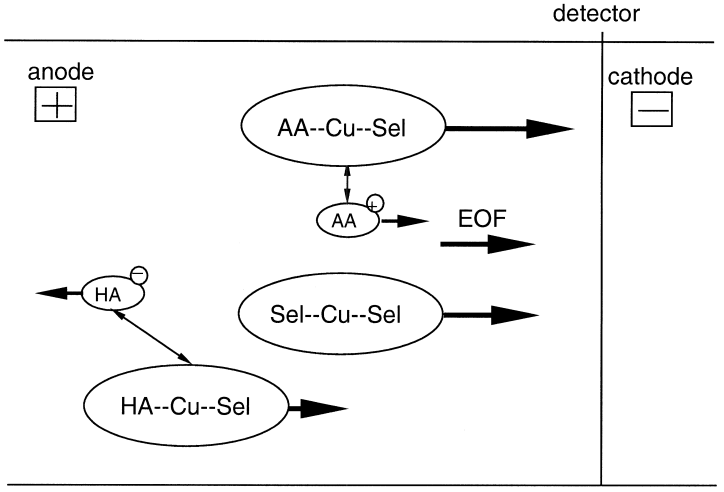

Fig. 3. Mobilities of the selector (Sel) complex and the mixed complexes with amino acids (AAs) and α-hydroxy acids (HAs).

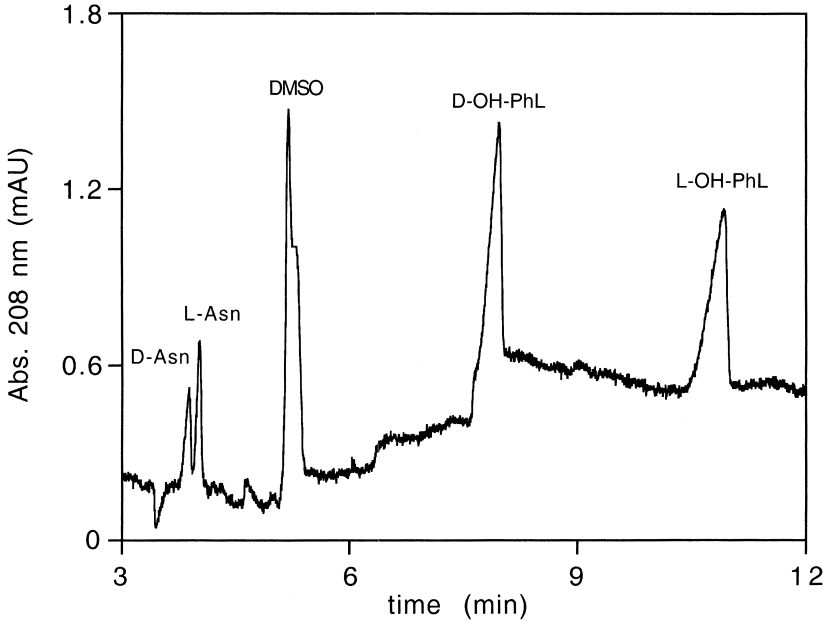

Fig. 4. Electropherogram of the chiral separation of asparagine and 3-(4-hydroxyphenyl)lactic acid (OH-PhL) using DMSO as an EOF marker. Conditions: 20 m*M* HO-L-Hyp, 10 m*M* copper(II) sulfate, 5 m*M* phosphate solution, pH 4.3, voltage 30 kV.

conditions chosen for this model separation are optimal for α-hydroxy acids and not for amino acids, asparagine is not baseline resolved under these conditions.

Out of 11 α-hydroxy acids tested, six were baseline separated. Table 1 gives the separation data for α-hydroxy acids. The highest enantioselectivity was observed with α-hydroxy acids containing an aromatic moiety. Under the same conditions hexahydromandelic acid was also resolved. Aliphatic α-hydroxy acids did not show resolution. Only at higher selector concentration some aliphatic α-hydroxy acids were partially resolved; however, in this case serious detection problems occurred. Tropic acid, a β-hydroxy acid, showed a slight resolution.

The resolution was found to be dependent on the nature of the substituents at the aromatic ring. Mandelic acid showed high resolution, however, connected with a high migration time. Migration time decreased with mandelic acid derivatives containing a hydroxy group in the ring. Additional introduction of a methoxy group again resulted in an increase in migration time. Compared to 2-phenyllactic acid (atrolactic acid), 3-phenyllactic acid showed a significantly higher resolution ($R_s = 5.2$). The highest resolution was obtained with 3-(4-hydroxyphenyl)lactic acid ($R_s = 6.8$).

The migration times were generally rather high. A reduction in migration time was obtained by increasing the voltage and shortening the effective length of the capillary to 26 cm. Since the resolution was excellent with most of the hydroxy acids the use of shorter capillaries would certainly solve this problem. With this equipment, however, it was not possible to use capillaries with an effective length less than 26 cm.

The migration order determined for 3-phenyllactic acid by injecting the authentic enantiomers was found to be D before L, which is in agreement with results obtained by Desiderio et al. using L-4-hydroxyproline as a selector in a coated capillary [7]. Recently, Chen et al. reported the migration order to be L before D for some selected hydroxy acids using *trans*-L-Hyp as a selector in an uncoated capillary [25]. Since in the case of HO-L-Hyp the hydroxy group in the side chain is assumed to participate in complex formation, a different mechanism is to be expected. Contrary to our observations with amino

Table 1
Separation data for α-hydroxy acids and β-blockers by ligand-exchange CE[1]

Analyte	Condition	t_1	t_2	α_{eff}	R_s
α-Hydroxy acids					
Mandelic acid	a	16.04	17.33	1.03	1.52
3-Hydroxymandelic acid	a	9.40	9.72	1.03	1.00
4-Hydroxymandelic acid	a	11.21	11.76	1.04	1.13
3,4-Dihydroxymandelic acid	a	12.54	13.07	1.03	1.01
3-Hydroxy-4-methoxymandelic acid	a	14.81	15.45	1.03	1.01
4-Hydroxy-3-methoxymandelic acid	a	15.96	17.40	1.05	2.12
4-Bromomandelic acid	a	13.72	14.42	1.21	1.13
3-Phenyllactic acid	a	7.79	9.62	1.29	5.26
Atrolactic acid	a	11.1	11.38	1.02	0.77
3-(4-Hydroxyphenyl)lactic acid	a	7.96	10.93	1.51	6.83
Tropic acid	a	12.54	12.74	0.51	0.46
β-Blockers					
Alprenolol	b	4.93	5.53	1.39	1.11
Atenolol	b	4.12	4.12	1.00	0.00
Bupranolol	b	4.87	5.04	1.13	0.72
Metoprolol	b	4.15	4.23	1.12	0.67
Oxprenolol	b	5.17	5.66	3.15	2.04
Propranolol	b	5.70	7.03	2.16	1.84
Sotalol	b	5.07	5.37	1.20	2.22
Timolol	b	4.63	4.63	1.00	0.00

[1] Migration times of the enantiomers (t_1 and t_2), effective separation factor α_{eff} and resolution (R_s) are given. For conditions see Experimental.

acids [9], no inversion of the migration order of the hydroxy acid enantiomers was observed by these authors when adding SDS, probably due to the fact that both the analytes and the micelles have the same migration direction. However, addition of cetyltrimethylammonium bromide (CTAB) instead of SDS resulted in a reversal of the migration order due to a reversed EOF. Furthermore, these authors observed a reversed migration order when changing from *trans*- to *cis*-L-Hyp. The influence of the addition of surfactants on the migration order of hydroxy acid enantiomers using HO-L-Hyp was not yet investigated and will be subject of further studies.

3.2. Resolution of amino alcohols

Several drugs show amino alcohol structure such as sympathimimetics and β-blockers.

The chiral resolution of such compounds is of great interest since pharmacological activity is mainly restricted to one of the enantiomers. In the case of sympathomimetics the *R*(−) enantiomers are the pharmacologically active enantiomers (eutomers) with β-blockers the *S*(−) forms. In several cases the ''inactive'' enantiomer (distomer) shows unwanted side effects.

Even if the side effects are not drastic, the distomer has to be metabolized and represents an unnecessary burden on the organism.

Using CE with HO-L-Hyp as a chiral selector, we reported the resolution of some sympathomimetics [14]. Out of 13 compounds investigated, nine were resolved with baseline separation.

The second group of drugs investigated containing amino alcohol structure are β-blockers.

Contrary to amino acids and hydroxy acids, the complexation optimum for amino alcohols is at a rather high pH (Fig. 2). HPLC separations of amino alcohols by means of ligand exchange at pH 6 were reported [23,24]. Although the pH optimum for complexation of amino alcohols is in the high pH range (Fig. 2), additional supporting interactions with the stationary phase can enable separation also at lower pH in HPLC. Since in CE such additional interactions are not present, separation of amino alcohols is only possible at high pH. To prevent

precipitation of copper hydroxide, small amounts of ammonia or triethylamine, which do not compete with the analyte were added to the electrolyte.

The selector itself is negatively charged at this pH and shows a negative mobility. There is an equilibrium between the free selector and the selector complex. The selector complex is neutral and migrates with the EOF. The analytes migrate with lower velocity than the EOF. Ion pair formation between the basic analyte and the anionic selector might be an additional mechanism superimposed to ligand exchange.

The influence of selector concentration on the resolution of oxprenolol is demonstrated in Fig. 5. The resolution improves with increasing selector concentration. There are no solubility problems with the selector at high pH, since the sodium salt is formed.

No separation was obtained using L-Hyp instead of HO-L-Hyp under these conditions. The hydroxyl group in the side chain of the selector is assumed to participate in complex formation, as indicated in Fig. 1B, thus supporting the separation. Another supporting effect might be based on hydrophobic interactions between the long alkyl chain of the selector and the aromatic moiety of the analyte.

The separation data for some examples of β-blockers are given in Table 1. For propranolol the migration order was found to be *S*(−) before *R*(+). Fig. 6 shows the resolution of metoprolol.

4. Conclusion

We have shown that ligand-exchange CE represents a rapid and simple approach for the chiral resolution of chelate complex forming compounds. The application of this basic principle to the chiral resolution of α-hydroxy acids and β-blockers containing amino alcohol structure using an *N*-(2-hydroxyoctyl)-L-4-hydroxyproline–copper(II) complex as a chiral selector is demonstrated. This selector showed an excellent enantioselectivity for these compound classes. A representative number of these compounds showed baseline separations.

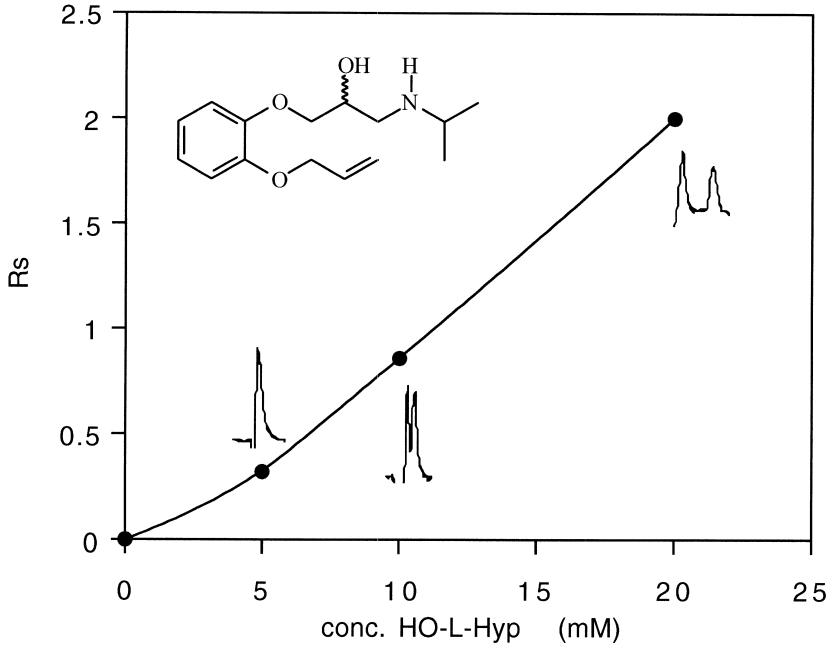

Fig. 5. Influence of selector concentration on the resolution of oxprenolol. Conditions: 5–20 m*M* HO-L-Hyp, 2.5–10 m*M* Cu(II), 100 m*M* TEA, adjusted to pH 12 with NaOH, voltage 15 kV.

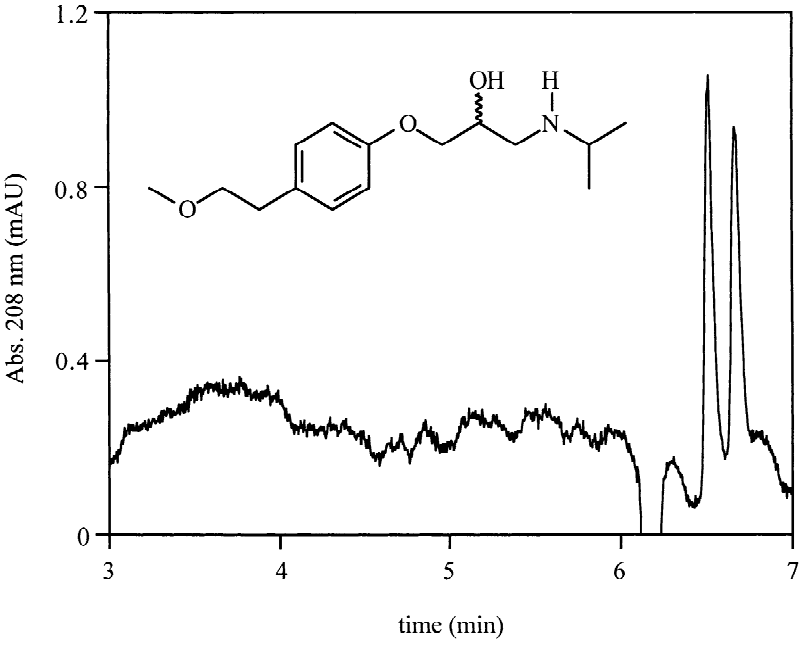

Fig. 6. Electropherogram of the chiral separation of metoprolol. Conditions: 20 m*M* HO-L-Hyp, 10 m*M* copper(II) sulfate, 100 m*M* TEA, adjusted to pH 12 with NaOH, voltage 15 kV.

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