Journal of Chromatography, 474 (1989) 441–446
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 526

Note

Separation of optical isomers by capillary zone electrophoresis based on host–guest complexation with cyclodextrins

S. FANALI
Istituto di Cromatografia del C.N.R., Area della Ricerca di Roma, Casella Postale 10, 00016 Monterotondo Scalo, Rome (Italy)
(Received March 21st, 1989)

Sympathomimetic drugs with substitution at either the α or β carbon atoms exhibit optical isomerism. When the drug is lavorotatory arising from substitution at the β carbon, it has greater peripherical activity, *e.g.*, L-epinephrine and L-norepinephrine are ten times more potent than their dextrorotary isomers[1].

Optical isomer resolution is an important and attractive field of research; sympathomimetic amine racemates are resolved by high-performance liquid chromatography (HPLC) and gas chromatography (GC) using optically active compounds in the stationary phase or by derivatization[2–5]. Recently, capillary zone electrophoresis (CZE) has been used to resolve dansyl-amino acid racemates using either cyclodextrins or the aspartame copper complex[6,7].

The optical isomer resolution of ephedrine and related compounds was studied by capillary isotachophoresis[8] but the resolution of ephedrine and norephedrine was not achieved. There appears to be no report of enantiomer resolutions of epinephrine, norepinephrine and isoproterenol with electrophoretic techniques. Being interested in optical resolution by electrophoretic techniques[9–11], we used CZE for the separation of ephedrine, norephedrine, epinephrine, norepinephrine and isoproterenol enantiomers. Cyclodextrins were added as chiral agents to the background electrolyte (BGE) and the influence of the shape of the cyclodextrin and its concentration on the effective mobility was studied. Complete resolution of the five racemates by using low pH and 18 m*M* heptakis(2,6-di-O-methyl-β-cyclodextrin) were obtained.

EXPERIMENTAL

Apparatus

The experiments were performed with a Bio-Rad HPE 100 apparatus (Richmond, CA, U.S.A.) equipped with an UV detector with a deuterium lamp (190–380 nm). The volumes of the electrode vessels were about 1 ml and 100 μl; for electrophoretic sampling the vessel with the lower volume was used. The apparatus was equipped with a power supply able to deliver up to 12 kV. Sampling and electrophoresis were controlled by a microprocessor.

Separations were performed in a Bio-Rad 148-3002 HPE capillary cartridge (20 cm × 0.025 mm, coated). The capillary was filled with the BGE by using an

0021-9673/89/$03.50

Hamilton microsyringe of 50 μl. Electropherograms were recorded with an LKB 2210 line recorder at a chart speed of 10 mm/min.

Chemicals

Tris(hydroxymethyl)aminomethane (Tris) and phosphoric acid were obtained from Carlo Erba (Milan, Italy), β-cyclodextrin (β-CD), ephedrine (E), norephedrine (nor-E), epinephrine (Ep), norepinephrine (nor-Ep), isoproterenol(i-P) and their enantiomers from Sigma (St. Louis, MO, U.S.A.). Heptakis(2,6-di-O-methyl-β-cyclodextrin) (di-OMe-β-CD) and heptakis(2,3,6-tri-O-methyl-β-cyclodextrin) (tri-OMe-β-CD) were kindly supplied by Dr. D. Sybilska, Institute of Physical Chemistry, Warsaw, Poland.

RESULTS AND DISCUSSION

The resolution of the sympathomimetic drugs is based on the host–guest complexation between the enantiomers and cyclodextrins. Cyclodextrins are neutral polymers with different units of D(+)-glucopyranose and the most commonly CD used in such separation techniques are α, β and γ with six, seven and eight glucose units respectively. Modified CDs, *e.g.*, di- and tri-O-methyl-β-CD are also available.

The enantioselectivity of the CDs arises from the chiral atom present in the glucose units and depends on the stability of the complexes formed with the compounds studied.

Snopek *et al.*[8] in their study by capillary isotachophoresis achieved good enantiomeric resolution of organic bases by using cyclodextrins at pH 5.4. At this pH no resolution of ephedrine and norephedrine was obtained.

We investigated the effect of β-CD, di-OMe-β-CD and tri-OMe-β-CD added to the BGE at low pH on the effective electrophoretic mobility of ephedrine, norephedrine, epinephrine, norepinephrine and isoproterenol.

As shown previously[12], in order to obtain highly efficient separations, electromigration dispersion must be minimized, *e.g.*, by using a co-ion in the BGE having similar effective mobility to that of the analyte. On the other hand the BGE should not adsorb at the wavelength used and should not form inclusion complexes with cyclodextrins. Tris, with a relatively low mobility, was selected as the cation in the BGE; phosphate was the anion at a pH of 2.4.

In order to obtain high separation efficiencies and short analysis time, the electrophoretic experiments were carried out by using a short capillary with a relatively small I.D. The dissipation of the heat generated during electrophoresis is greater when capillaries with small I.D.s are used[13,14].

The column length plays no rôle in separation efficiency but influences the migration time. The capillary used in the experiments was a commercially available coated one that allowed the electroosmotic flow to be minimized to the benefit of the resolution. The resolution of compounds with very similar mobilities can be achieved by balancing the electroomotic flow and effective mobility[15].

Fig. 1 shows the complete separation of the five racemic sympathomimetic drugs. The analysis was carried out in only 4 min. In this experiment it was possible to obtain a relatively high electric field (400 V/cm) by using a relatively low voltage (8 kV).

In the separation shown in Fig. 1 the amount injected was estimated by the formula proposed by Jorgenson and Lukacs[15]

$$Q = (u + u_{os})VACt_i/L \quad (1)$$

where Q is the quantity injected in mole, u the effective mobility, u_{os} the electroosmotic flow, V the injection voltage, A the cross-sectional area of the capillary, C the concentration of the sample, t_i the injection time and L the length of the capillary.

The quantity of epinephrine injected in the separation shown in Fig. 1 was found to be approximately $45 \cdot 10^{-15}$ mole and the volume injected was about 2 nl.

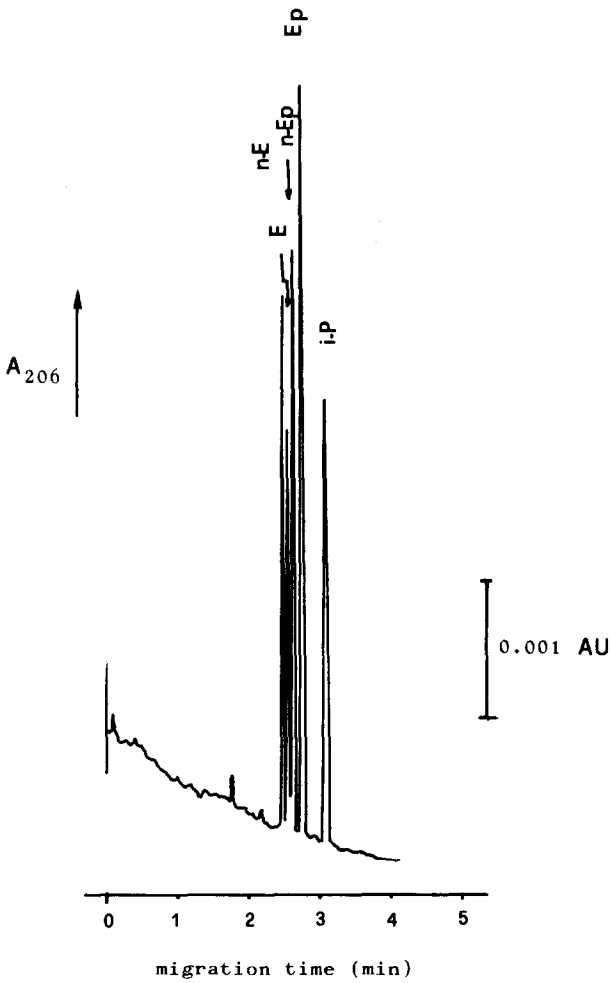

Fig. 1. Electropherogram of the separation of the racemic amines: n-E = norephedrine; E = ephedrine; n-Ep = norepinephrine; Ep = epinephrine and i-P = isoproterenol. BGE: 10 mM Tris–H_3PO_4, pH 2.4. Sampling: electrophoresis at 6 kV for 6 s. The mixture contained $2 \cdot 10^{-5}$ M for each racemic compound. Electrophoretic experiment: 8 kV; I = 6.8 μA.

Different amounts of β-CD were added to the BGE and the migration times of the compounds studied were measured. By increasing the concentration of β-CD the migration times of the five racemic compounds analyzed were reduced. This indicates that all the analytes form inclusion complexes with the CD used. Ephedrine and norephedrine showed the highest reduction in the effective mobility. Despite being a good complexing agent towards racemic compounds, β-CD was not able to resolve the sympathomimetic drugs into their enantiomers. Very poor resolution was obtained at a relatively high concentration of β-CD (20 m*M*) for ephedrine and isoproterenol.

Fig. 2A and B shows the effect of the concentration of heptakis(2,6-di-O-methyl-β-CD) on the migration time of E, nor-E, Ep, nor-Ep and i-P. From these results di-OMe-β-CD seems to be a very good enantioselective complexing agent towards all five compounds studied by CZE.

Complete enantiomer resolution was achieved for nor-E, Ep and i-P when 9 m*M* of di-OMe-β-CD was added to the BGE. To resolve completely the racemic E and nor-Ep, 18 m*M* of the chiral agent was used. In all cases the (+) isomer shows the lowest migration time and this indicates that the inclusion complexation is higher than that obtained with the (−) isomer.

From the data shown in Fig. 2 it is evident that E and nor-E fit closely the cavity of the di-OMe-β-CD and thus are more complexed than the catecholamines studied. In this case the shape of the guest (analytes) plays an important rôle in the complexation: E and nor-E possess the aromatic group without any substitution.

For optical resolution of the compounds studied, di-OMe-β-CD was found to be a very good resolving agent for all samples but the best discriminating effect was observed in the optical resolution of catecholamines.

Fig. 3a and b shows the resolution of racemic nor-E and E, nor-Ep, Ep and i-P respectively.

In order to explain the different complexation of the enantiomers and modified CD is necessary to consider that CDs contain five chiral atoms for each glucose[16]. Furthermore, the complex is influenced by the hydroxyl and O-methyl groups present on the rim of the cavity of the CD. The rim is relatively hydrophobic and can offer the possibility to form hydrogen bonds.

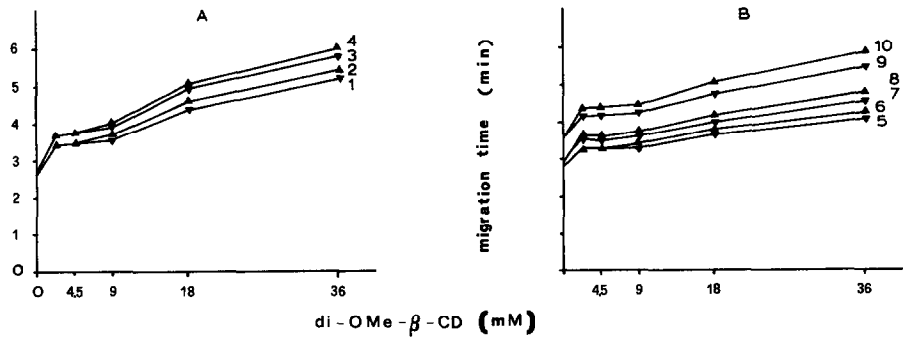

Fig. 2. Effect of the concentration of heptakis(2, 6-di-O-methyl-β-CD) in the BGE on the migration time of: (A) 1 = (−)norephedrine; 2 = (+)norephedrine; 3 = (−)ephedrine; 4 = (+)ephedrine; (B) 5 = (−)norepinephrine; 6 = (+)norepinephrine; 7 = (−)epinephrine; 8 = (+)epinephrine; 9 = (−)isoproterenol; 10 = (+)isoproterenol.

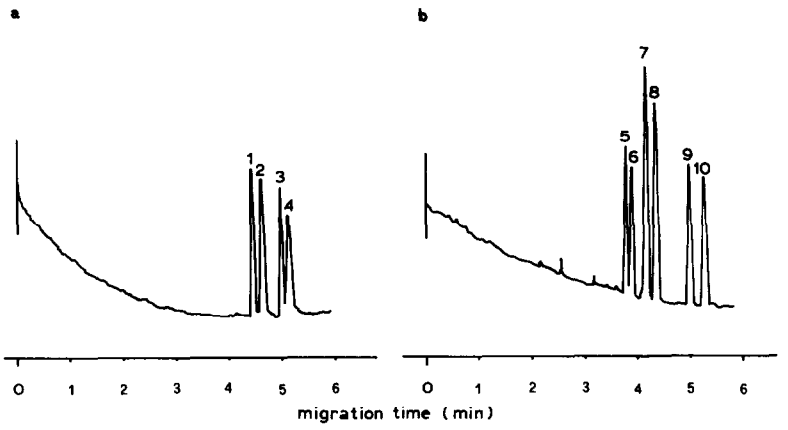

Fig. 3. Electropherograms of the optical isomer resolution of: (a) norephedrine and ephedrine; (b) norepinephrine, epinephrine and isoproterenol. BGE: 10 m*M* Tris-H_3PO_4, pH 2.4 and 18 m*M* of di-OMe-β-CD. Other conditions as in Figs. 1 and 2.

Experiments carried out by adding different amounts of tri-OMe-β-CD to the BGE showed retardation of all the five racemic compounds studied but a chiral resolution was not obtained.

CONCLUSIONS

Our experiments show that the resolution of racemic mixtures of sympathomimetic drugs can be obtained rapidly with CZE by adding to the BGE a chiral host–guest complexing agent. The resolution of the optical isomers studied depends on the type of cyclodextrin. The hydroxyl and the O-methyl groups on the rim of the cavity of CDs influence the resolution power; the presence of both groups in the entrance of the hydrophobic cavity improves the optical resolution. Very poor resolution is obtained when the CD possesses the same groups in the 2, 3 and 6 positions either OH (β-CD) or OCH_3 (tri-OMe-β-CD). The optical resolution is also influenced by the alkyl group bonded to the nitrogen atom of the sample. Isoproterenol with an isopropyl group is the best resolved racemic compounds. An increase in the amount of CD in the BGE improves the resolutions.

CZE is a very promising analytical technique for the optical isomer resolution of the compounds studied.

The drawbacks of other techniques such as HPLC where sophisticated stationary phases and/or the relatively high quantity of the chiral agent in the mobile phase do not exist in CZE. In fact the separations are generally performed in free solutions and the volume of the BGE in each experiment is relatively low. In this study only 2 ml of BGE was used for each run.

ACKNOWLEDGEMENT

Thanks are due to Bio-Rad Laboratories (Segrate, Milan, Italy) for lending the apparatus HPE-100 used in these studies.

REFERENCES

1 I. R. Innes and M. Nickerson, in L. S. Goodman and A. G. Gilman (Editors), *The Pharmacological Basis of Therapeutics*, Macmillan, New York, 5th ed., 1975 p. 477.
2 H. G. Kicinski and A. Kettrup, *Fresenius' Anal, Chem.*, 320 (1985) 51.
3 I. W. Wainer, T. D. Doyle, Z. Hamidzadeh and M. Aldridge, *J. Chromatogr.*, 261 (1983) 123.
4 W. A. Köning and K. Ernst, *J. Chromatogr.*, 280 (1983) 135.
5 L. R. Gerber, B. L. Karger, J. L. Neumeyer and B. Feibush, *J. Am. Chem. Soc.*, 106 (1984) 7729.
6 A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg and B. L. Karger, *J. Chromatogr.*, 448 (1988) 41.
7 P. Gozel, E. Gassmann, H. Michelsen and R. N. Zare, *Anal. Chem.*, 59 (1987) 44.
8 J. Snopek, I. Jelínek and E. Smolková-Keulemansová, *J. Chromatogr.*, 438 (1988) 211.
9 S. Fanali, V. Cardaci and L. Ossicini, *J. Chromatogr.*, 265 (1983) 131.
10 S. Fanali, M. Lederer, P. Masia and L. Ossicini, *J. Chromatogr.*, 440 (1988) 361.
11 S. Fanali, L. Ossicini, F. Foret and P. Boček, *J. Microcolumn Separations*, in press.
12 F. Foret, S. Fanali, L. Ossicini and P. Boček, *J. Chromatogr.*, 470 (1989) 299.
13 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 11.
14 K. D. Lukacs and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 407.
15 J. W. Jorgenson and K. D. Lukacs, *Science (Washington, D.C.)*, 222 (1983) 266.
16 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.