Journal of Chromatography, 538 (1991) 385–391
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 802

Improvement of resolution in the capillary electrophoretic separation of catecholamines by complex formation with boric acid and control of electroosmosis with a cationic surfactant

TAKASHI KANETA, SHUNITZ TANAKA and HITOSHI YOSHIDA*
Department of Chemistry, Faculty of Science, Hokkaido University, Kita-ku, Sapporo 060 (Japan)
(Received August 22nd, 1990)

ABSTRACT

In order to improve the resolution of catecholamines, the control of both the electroosmotic and the electrophoretic mobilites were carried out. The former was controlled by the addition of borate ion and a change in pH. The control of the latter was carried out by addition of a cationic surfactant. Ten catecholamines were sufficiently separated.

INTRODUCTION

Capillary electrophoresis (CE) [1–3] is used to separate mixtures of ionic species. Many attempts have been made to improve the resolution of CE. The factors governing the resolution of CE include the electrophoretic mobilities of sample species and the electroosmotic mobility, the diameter and length of the capillary and the applied voltage. The electroosmotic and electrophoretic mobilities are most important factors for achieving a higher separation resolution in CE.

The conventional technique for the control of electrophoretic mobilities is the selection of the pH of the background electrolyte. For the separation of ionic species such as weak acids or bases, the pH should be selected near the dissociatioa constant (pK_a) of the species. Then the separation can be easily accomplished on the basis of the difference in their values [4–6].

Another approach is the use of a complex formation reaction. For example, amino acid enantiomers have been separated by using ligand exchange with copper(II)–aspartame complexes [7]. Addition of cyclodextrin to the background electrolyte allowed the separation of optical isomers of catecholamines [8]. Micellar electrokinetic capillary chromatography (MECC) is also an effective technique for controlling the mobilities and improving the resolution [9,10]. The utility of MECC combined with complex formation has been reported, *e.g.*, for the separation of catecholamines [11] and oligonucleotides [12].

The electroosmotic flow, which is another important factor with regard to

0021-9673/91/$03.50

resolution, is generated by the negative charge on the inner wall of the glass capillary, resulting from the dissociation of silanol groups. The electroosmotic velocity is dependent on several factors, which include the origin of the capillary, the nature and concentration of background electrolyte and their pH. One of the methods for controlling the electroosmotic mobility is to add surfactants to the background electrolyte [5,13–15]. It is effective not only for the suppression of electroosmotic flow but also for its reversal. Tsuda obtained flow reversal by using cetyltrimethylammonium ion [15]. Huang *et al.* [5] reported the separation of a six-component carboxylic acid mixture using the tetradecyltrimethylammonium ion.

In this work, to improve the resolution of catecholamines, the control of both the electroosmotic and the electrophoretic mobilities was investigated. The effective mobilities of catecholamines could be controlled by complex formation with borate ion. The control of electroosmotic flow was performed by the addition of cationic surfactants to the background electrolyte. The electroosmotic mobility decreased with increasing concentration of surfactant and the length of its alkyl chain. The electroosmotic flow was reversed by a cationic surfactant with a longer alkyl chain. Using a background electrolyte containing borate buffer and a cationic surfactant, ten components of catecholamines were sufficiently separated.

EXPERIMENTAL

Apparatus

A fused-silica capillary tube (70 cm × 100 μm I.D.) was obtained from Gasukuro Kogyo (Tokyo, Japan). A high-voltage power supply of a Shimadzu (Kyoto, Japan) IP-1B isotachophoretic analyser was used. A Model CV[4] variable-wavelength absorbance detector (ISCO, Lincoln, NE, U.S.A.) was employed. Detection was carried out by measuring the absorbance at 217 nm on the column at a position 20 cm from the negative end of the capillary tube. A sample was injected by moving the injection end of the capillary to the sample reservoir and raising it higher than the other end for a constant time. The electroosmotic velocity was measured by the peak of methanol or toluene. A new capillary was flushed with 0.1 *M* potassium hydroxide solution overnight before the experiments, and according to the study by Lauer and McManigill [16], a 15-min flush with water followed by a 15-min flush with operating buffer was applied new experiments. When the experiments were completed, the capillary was flushed and filled with 0.1 *M* potassium hydroxide solution. All investigations were performed at room temperature.

Reagents

All reagents were of analytical-reagent grade and used without further purification. Epinephrine (EP), isoproterenol (IP), 3,4-dihydroxyphenylalanine (Dopa) and vanillylmandelic acid (VMA) were obtained from Wako (Osaka, Japan), metanephrine (MN) and normetanephrine (NM) from Nacalai Tesque (Kyoto, Japan), dopamine (DA) and 3,4-dihydroxybenzylamine (DBA) from Aldrich (Milwaukee, WI, U.S.A.) and norepinephrine (NE) and deoxyepinephrine (DEP) from Sigma (St. Louis, MO, U.S.A.). The solutions were stored in a refrigerator. Decyltrimethylammonium bromide (DeTAB), dodecyltrimethylammonium bromide (DoTAB) and tetradecyltrimethylammonium bromide (TTAB) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan).

RESULTS AND DISCUSSION

Control of effective mobilities of catecholamines

The effective mobilities of catecholamines are too close for them to be separated. Therefore, the resolution cannot be improved simply by controlling the pH (8–10) of the background electrolyte containing ammonium chloride. Previously, we have reported the utility of complex formation with boric acid for the separation of catecholamines on CE [17]. By adding boric acid to the background electrolyte, the retention time of each solute is increased as they react with boric acid to form borate complexes with no charge or a negative charge, except for MN and NM. The resolution increased with increasing concentration of boric acid.

When borate buffer containing 100 m*M* potassium hydroxide was used as the background electrolyte, the retention times of catecholamines and the electroosmotic flow were varied under the influence of the pH, as shown in Fig. 1. The retention time of electroosmotic flow became shorter with increasing pH and hardly changed above

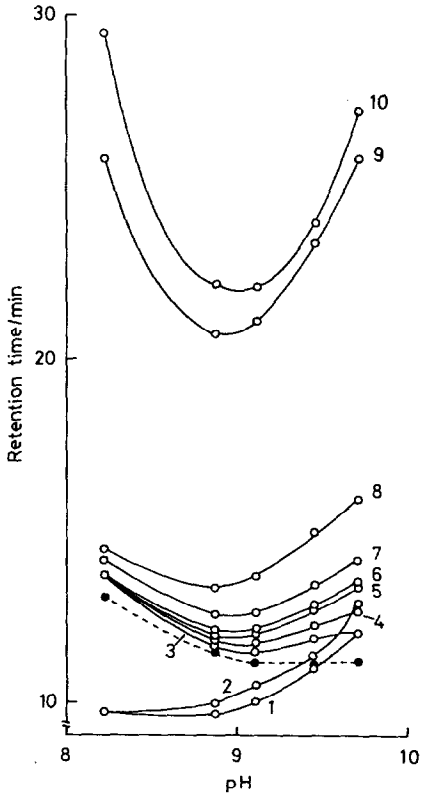

Fig. 1. Effect of pH of borate buffer on retention times of catecholamines: (1) MN; (2) NM; (3) DEP; (4) DA; (5) DBA; (6) IP; (7) EP; (8) NE; (9) Dopa; (10) VMA. The dashed line represents electroosmotic flow; buffer solution, 100 m*M* KOH–boric acid; separation tube, 700 mm × 100 μm I.D.; length of the tube used for separation, 500 mm; total applied voltage, *ca.* 10 kV; current, 100 μA; detection wavelength, 217 nm; concentration of test mixture, 0.5 m*M* of each catecholamines; injection time, 10 s.

pH 9.1; the retention times of catecholamines also became shorter up to pH 9.1. Above pH 9.1, the retention times of catecholamines increased with increasing pH. The reason may be that catecholamines may be dissociated via the amino group and complexed with borate efficiently to produce anions of higher charged states, except MN and NM, which exist as cations. Therefore, as shown in Fig. 1, pH 9.0–9.5 was suitable for the separation of the ten catecholamines.

Fig. 2 illustrates the electropherogram obtained with 100 mM potassium hydroxide–200 mM boric acid solution (pH 9.1). The resolution of catecholamines was improved better than when using ammonia buffer. It seems reasonable to assume that sample ions except for MN and NM migrated in the opposite direction to the electroosmotic flow. However, the separation of DBA and IP was not sufficient even under these condition. In order to improve the resolution further, the control of the electroosmotic flow was investigated.

Control of electroosmotic flow

In CE, the resolution between a pair of adjacent components is calculated as follows, as shown by Jorgenson and Lukacs [1]:

$$R_S = 0.177\,(m_{eff1} - m_{eff2})[V/D(m_{av} + m_{eo})]^{1/2} \quad (1)$$

where R_S is the resolution, V is the applied voltage, D is the diffusion coefficient, m_{av} is the average mobility of the two components and m_{eff1}, m_{eff2} and m_{eo} are the effective mobilities of the two components and the electroosmotic mobility, respectively. From the above equation, the resolution becomes better when the sum of the electroosmotic mobility and the average electrophoretic mobility is smaller. If the value of electroosmotic mobility is the same as, but of the opposite sign to, the average

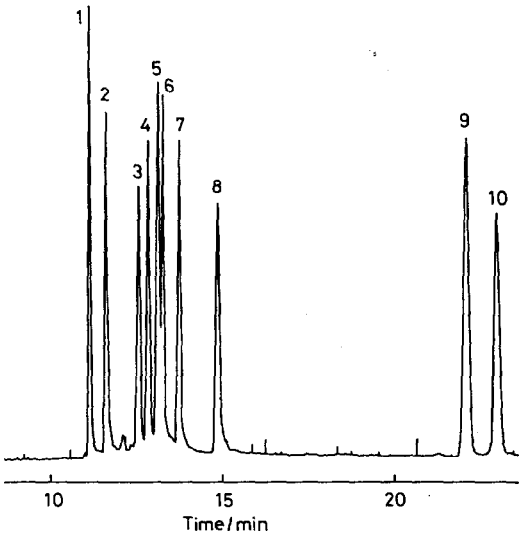

Fig. 2. Electropherogram of test mixture of catecholamines. pH of buffer solution, 9.1. Solutes and other conditions as in Fig. 1.

electrophoretic mobility of sample ions and $m_{eff1} \neq m_{eff2}$, two components will migrate in the opposite direction to each other.

To control the electroosmotic flow, electrically neutral surfactants such as poly(vinyl alcohol) (PVA), Triton X-100 and hydroxyethylcellulose have usually been used in isotachophoresis [13] and CE [14]. These surfactants suppress the electroosmotic flow by adsorption on the capillary inner wall or by increasing the viscosity of the solution. In our study, the electroosmotic flow was suppressed slightly by the addition of PVA or Triton X-100 at the final concentration of 0.01–0.1%, but not sufficiently to improve the resolution. Especially PVA was complexed with borate, and it made the solution very viscous. Nevertheless, the effect of suppressing the electroosmotic flow was small.

Control of electroosmotic flow by a cationic surfactant

Addition of cationic surfactants such as the long-chain ternary alkylammonium type would result in the suppression or reversal of the electroosmotic flow. Depending upon the concentration of the surfactant, the inner wall of the capillary can have varying degrees of negative or positive charge or be neutral. We therefore investigated the influence of three cationic surfactants with different lengths of the alkyl chain, DeTAB, DoTAB and TTAB. Huang *et al.* [5] have reported the effect of TTAB on electroosmosis in low pH buffer. However, the effect will be different in alkaline media. In fact, in alkaline media, with increasing concentration of cationic surfactant the electroosmotic velocity was changed more than in acidic media. The silica surface of the capillary tube probably become more negatively charged in alkaline media owing to the dissociation of the silanol groups. Therefore, addition of a cationic surfactant in alkaline media will have a much greater effect on the electroosmotic flow than in a more acidic medium, where the silanol group are not as fully dissociated.

Fig. 3 shows the effect on the electroosmotic mobility, m_{eo}; m_{eo} is positive when

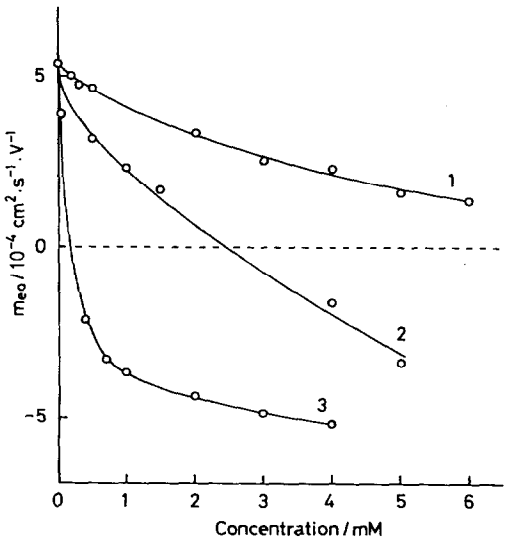

Fig. 3. Effect of concentration of cationic surfactants on electroosmotic mobility: (1) DeTAB; (2) DoTAB; (3) TTAB. pH of buffer solution, 9.1; other conditions as in Fig. 1.

the solution flows from the anode to the cathode. As the alkyl chain increases, the change in electroosmotic flow increased and a reversed electroosmotic flow was observed at a lower concentration of cationic surfactant with a longer alkyl chain. Reversed electroosmotic flow was observed at less than the critical micellar concentration with both DoTAB and TTAB. However, the increase in the concentration of the cationic surfactant makes the conductivity of the background electrolyte larger. To minimize the increase in the conductivity, it will be effective for a cationic surfactant with a longer alkyl chain to be used at a lower concentration for the control of electroosmotic velocity. Fig. 4 shows the electropherogram of catecholamines with buffer containing 0.1 m*M* TTAB (its critical micellar concentration is about 3 m*M*). Each peak was extended slightly, but the resolution between DBA and IP increased and the ten catecholamines could be sufficiently separated under these conditions. The electroosmotic mobility in Fig. 4 was $3.9 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} and in Fig. 2 $5.3 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1}.

To improve the resolution in CE, it is important to be able to control both the electrophoretic mobilities of sample ions and the electroosmotic mobility. Control of the electrophoretic mobilities of many sample ions can be achieved by using not only the adjustment of the pH of the background electrolyte but also complex formation. Complex formation makes it possible to change the charge of sample ions and then to increase the differences between the effective mobilities of sample ions. Many catecholamines exist as positively charged species even in a background electrolyte of pH *ca.* 9. However, on complex formation with borate ion they are converted into the negatively charged species. Therefore, the direction of migration of catecholamines is changed to the opposite direction of the electroosmotic flow and the resolution is improved. Further, the control of the electroosmotic velocity can be achieved by the addition of a cationic surfactant. Cationic surfactants with long chains such as tetraalkylammonium salts were effective for achieving changes at less than the critical micellar concentration. A longer chain surfactant is preferred because the electroosmotic flow can be suppressed at lower concentration, and the influence on the constitution of the background electrolyte, *i.e.*, the increase in the conductivity and the association of ions, can be reduced. Addition of a longer aklyl chain cationic surfactant is one of the easiest techniques for controlling the electroosmotic flow to improve the resolution of CE.

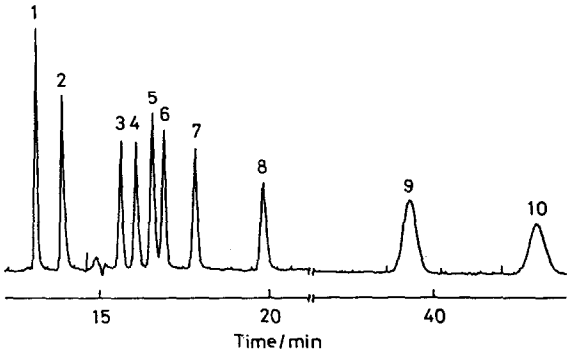

Fig. 4. Electropherogram of test mixture of catecholamines. Buffer, 100 m*M* KOH–200 m*M* boric acid containing 0.1 m*M* TTAB (pH 9.1). Solutes and other conditions as in Fig. 1.

REFERENCES

1 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
2 J. W. Jorgenson and K. D. Lukacs, *J. Chromatogr.*, 218 (1981) 209.
3 J. W. Jorgenson and K. D. Lukacs, *Science (Washington, DC)*, 222 (1983) 266.
4 W. G. Kuhr and E. S. Yeung, *Anal. Chem.*, 60 (1988) 2642.
5 X. Huang, J. A. Luckey, M. J. Gordon and R. N. Zare, *Anal. Chem.*, 61 (1989) 766.
6 S. Terabe, T. Yashima, N. Tanaka and M. Araki, *Anal. Chem.*, 60 (1988) 1673.
7 P. Gozel, E. Gassmann, H. Michelsen and R. N. Zare, *Anal. Chem.*, 59 (1987) 44.
8 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
9 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
10 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
11 R. A. Wallingford and A. G. Ewing, *J. Chromatogr.*, 441 (1988) 299.
12 A. S. Cohen, S. Terabe, J. A. Smith and B. L. Karger, *Anal. Chem.*, 59 (1987) 1021.
13 J. C. Reijenga, G. V. A. Aben, Th. P. E. M. Verheggen and F. M. Everaerts, *J. Chromatogr.*, 260 (1983) 241.
14 S. Terabe, H. Utsumi, K. Otsuka, T. Ando, T. Inomata, S. Kuze and Y. Hanaoka, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1986) 666.
15 T. Tsuda, *Nippon Kagaku Kaishi*, 7 (1986) 937.
16 H. H. Lauer and D. McMannigill, *Anal. Chem.*, 58 (1986) 166.
17 S. Tanaka, T. Kaneta and H. Yoshida, *Anal. Sci.*, 6 (1990) 467.