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Migration behaviour of catechols and catecholamines in capillary electrophoresis

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ABSTRACT

The migration behaviour of selected catechols and catecholamines in micellar electrokinetic chromatography (MEKC) was investigated. For the MEKC separation of the compounds, electrophoretic media with sodium dodecyl sulphate in phosphate–borate buffer were used. The migration behaviour of the catechols and catecholamines at different concentrations of micellar solution and at different pH of the electrophoretic media was investigated. The results successfully demonstrated the use of MEKC for the separation of a mixture of two catechols and six catecholamines.

INTRODUCTION

Catechols and catholamines play important roles as neurotransmitters in the central and peripheral autonomic nervous systems and as hormones exerting endocrine and exocrine effects. It is not surprising, therefore, that the lack of sufficient amounts of some of these compounds in the body can have a severe impact on both the quality and duration of life.

High-performance capillary electrophoresis (HPCE) is a fairly new technique and has become complementary to or a substitute for high-performance liquid chromatography (HPLC) for analytical purposes [1–8]. One of the reasons for its popularity is the exceptionally high separation efficiency achievable with this technique, which is based on a simple instrumental set-up. The characteristic of HPCE is that the separation takes place in a small capillary tube which is favourable for minimizing thermal zone distortion when high field strengths are required in order to obtain short run times and high resolution. At the same time, the capillary tube minimizes zone broadening generated by convection, which can be effectively suppressed in narrow-bore tubes.

Capillary electrokinetic chromatography was introduced by Terabe *et al.* [1] in 1984 by pumping micelles electroosmotically in a capillary electrophoresis system to effect the chromatographic separation of neutral compounds. In this system, ionic surfactants are added to the operating buffer at concentrations exceeding the critical micelle concentration. This technique, termed micellar electrokinetic capillary chro-

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matography (MEKC) by Burton *et al.* [2], has been used extensively for selective separations of both neutral and ionic compounds while retaining the advantages of the capillary electrophoresis format. Separation in MEKC is based on the chromatographic principle of partitioning the solute between the carrier (micelle) and the surrounding aqueous medium, and the differential migration of the two phases. MEKC is most frequently performed with anionic surfactants, *e.g.*, sodium dodecyl sulphate (SDS). In addition, experimental parameters, such as pH of the buffer solution, concentration of the micelle and the applied voltage, can help to increase the separation efficiency.

Although the capillary electrophoretic analysis of catechols and catecholamines have explored in previous investigations [3–8], the main emphasis was on the development of highly sensitive electrochemical techniques for capillary electrophoresis. The migration behaviour of catechols and catecholamines in MEKC has rarely been investigated. In this work, the separation of a mixture of two catechols and six catecholamines using the MEKC technique was studied. The effects of surfactant concentration and pH on the migration behaviour were investigated.

EXPERIMENTAL

The experiments were performed on a laboratory-built MEKC instrument. The power supply used was a Spellman (Plainview, NY, USA) Model RHR 30PN10/RVC capable of delivering up to 30 kV. A fused-silica capillary (45 cm effective length × 50 μm I.D.) obtained from Polymicro Technologies (Phoenix, AZ, USA) was used as the separating tube. On-column detection of the peaks was carried out on a Micro UVis detector (Carlo Erba, Milan, Italy) with the wavelength set at 210 nm. A Linear Instruments (Irvine, CA, USA) Model 252A/MM chart recorder was used to record the chromatograms. Sample solution was introduced by gravity feed. An injection time of 5 s at a height of 12 cm was used.

All chemicals were of analytical-reagent grade. The buffer solution was prepared by dissolving sodium dihydrogenphosphate dihydrate and sodium tetraborate in water purified with a Milli-Q system (Millipore, Bedford, MA, USA) electrophoretic medium consisting of SDS micelles in phosphate–borate buffer was prepared as described previously [9]. The structures of the two catechols and six catecholamines investigated are shown in Fig. 1. A standard solution of the catechol moieites and Sudan III was prepared in HPLC-grade methanol (J. T. Baker, Phillipsburg, NJ, USA) at a concentration of 1000 ppm for each of the species. All these chemicals were supplied by Aldrich (Milwaukee, WI, USA).

RESULTS AND DISCUSSION

Effect of pH

The results obtained for the migration times at different pH values are shown in Fig. 2. With the exception of 3,4-dihydroxyphenylacetic acid (DHPAA) and DL-3,4-dihydroxyphenylglycol (DHPG), a general trend of a decrease in migration times with increase in pH was observed. It should be noted that with a decrease in pH, a substantial decrease in electroosmostic velocity, v_{eo}, and migration velocity of the SDS micelles, v_{mc}, would be expected [10]. In fact, our attempt to study the migration

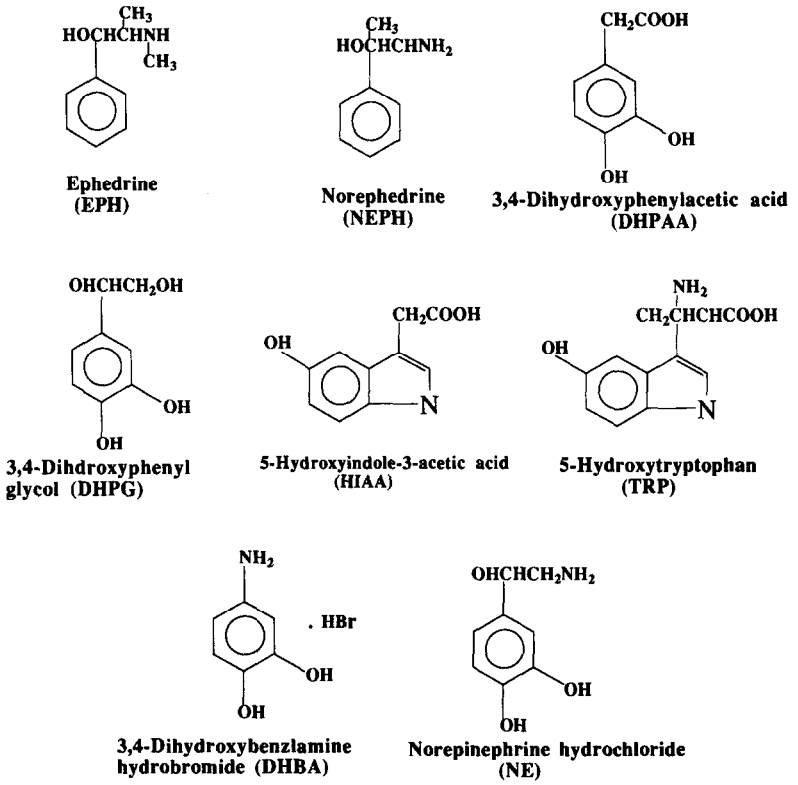

Fig. 1. Structures of the catechols and catecholamines investigated.

behaviour of the catechols and catecholamines at pH 5 failed to provide any satisfactory results. Sudan III, ephedrine (EPH), norepinephrine (NEPH) and 5-hydroxytryptophan (TRP) were not detected even after a prolonged analysis time of more than 1 h. At this pH, it is believed that v_{mc} approaches zero and subsequently solutes which have interacted with the SDS micelles, together with Sudan III, would not migrate within a reasonable time.

In addition to being affected by the changes in v_{mc} and v_{eo} due to variations in pH, another possible reason for the variation of migration times could be the differences in the extent of ionization of the various compounds in the pH range investigated. In fact, from the results shown in Fig. 2, it seems that this effect plays a more important role than the former.

At low pH (*i.e.*, pH 6), protonation of the amino group in some of these species is possible. These positively charged protonated species would form ion pairs with the anionic micelles. As a result of the strong electrostatic attraction of the SDS micelles towards the anode, an increase in migration times would be expected for these species. Among the catechols and catecholamines investigated, only EPH, NEPH, TRP and norepinephrine (NE) seemed to be affected. This effect is more pronounced for EPH as the protonation of its secondary amino group is highly favourable. Therefore, a large variation of migration time was observed for EPH. On the other hand,

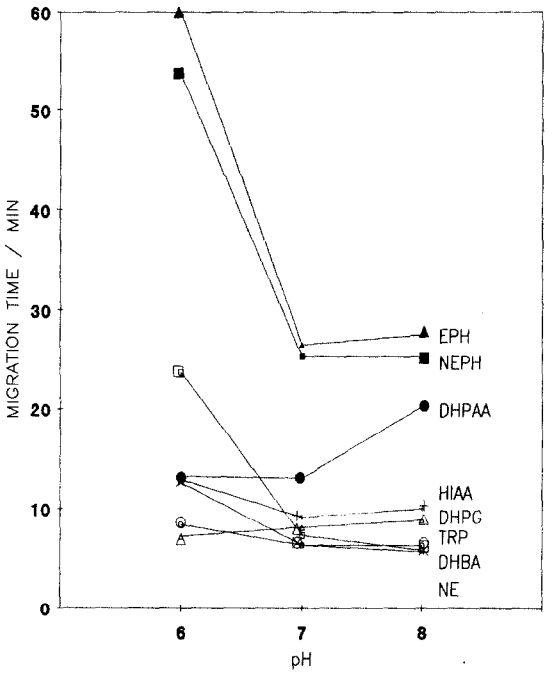

Fig. 2. Plot of migration times of the catechols and catecholamines with variation of pH. Experiments were carried out at 80 m*M* SDS.

for NE, as a result of possibility of bond rotation, the amino group can readily form intrahydrogen bonds with the phenolic OH groups, which consequently render effective protonation of this species. Therefore, this effect is less significant for NE. A similar observation is noted for TRP; because of the close proximity of the amino group with its carboxylate group, protonation to a certain extent would also be affected. However, owing to the presence of the basic pyrrole ring, protonation is still possible and migration behaviour as shown in Fig. 2 is observed.

As the pH of the electrophoretic media increases, protonation of these amino groups would be inhibited. Therefore, it would be reasonable to expect to observe a decrease in migration times as shown in Fig. 2 for species that tend to protonate less at higher pH. With DHPAA and DHPG, instead of decreases in their migration times with increasing pH, the reverse trend was observed. From their structures, it can be seen that both species possess an acidic hydrogen (i.e., the carboxylate in DHPAA and the OH in DHPG). These acidic hydrogens at higher pH would tend to ionize and thus result in negatively charge species. These negatively charged species, like the anionic SDS micelles, would be electrophoretically attracted to the anode. However, it should be noted that larger variations in migration times were observed for DHPAA than DHPG with changes in pH. This can be explained by the fact that as the pK_a for DHPG (aliphatic alcohol) is larger than that for DHPG, the extent of ionization would be higher in the latter. As a result, DHPAA was more affected than DHPG by pH changes. In contrast to DHPAA and DHPG, even though TRP pos-

sesses a carboxylate group, owing to the close proximity of the two basic groups (namely the amino group and the pyrrole ring) ionization of this carboxylate group would be highly unfavourable. Thus no significant increase in the migration time was observed for TRP.

For DHBA and HIAA, because of the possibility of intrahyrogen bonding, either between the two adjacent phenolic OH groups (as in DHBA), or between the carbonyl group and the phenolic OH group (as in HIAA), the hydrogen is now no longer available for ionization. Therefore, marginal decreases in migration times were observed. This is in response to the increase in the v_{eo} for the system at higher pH.

Effect of SDS concentration

The results obtained for the investigation of the effect of SDS concentration on the migration of the catechols and catecholamines are shown in Fig. 3. For EPH, NEPH and DHPAA, with an increase in SDS concentration their migration times increased correspondingly. The increase in migration times for these compounds is explained in terms of their high lipophilicity [11]. This observation is in agreement with the trend observed above. As protonation of NEPH and EPH may be still possible at pH 7, ion pairing with the SDS micelles would be likely to occur. Thus, with increase in SDS concentration, there would be a corresponding increase in the probability of interaction. Therefore, the migration times were found to increase. For the other compounds, only marginal changes with increase in SDS concentration were observed. These observations suggest that there is minimum interaction between these species and the SDS micelles. One of the possible reasons could be the presence of polar substituent groups in these species. All these species possess, in addition to the two phenolic OH groups, additional polar substituent groups, which makes them

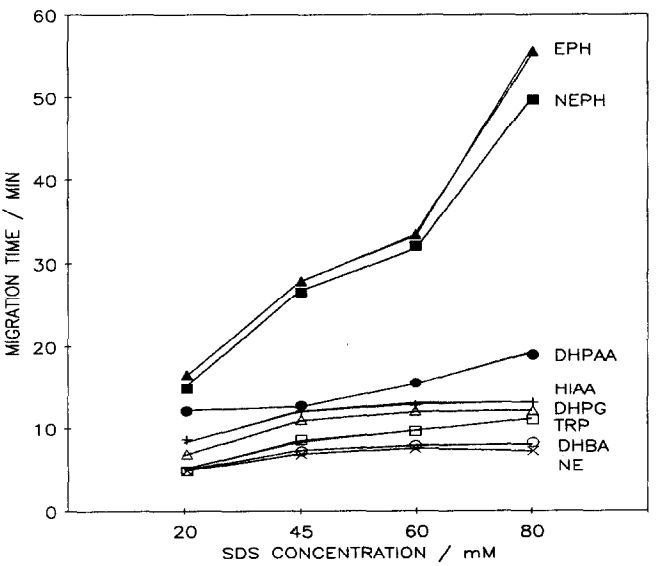

Fig. 3. Plot of migration times of the catechols and catecholamines with variation in SDS concentration. Experiments were carried out at pH 7.00.

hydrophilic. These species would tend to be solvated more by the aqueous phase and effective solubilization by the SDS micelles would be affected. As a result, their migration times would be shorter.

Migration order

Optimum separation for this group of catechols and catecholamines was obtained using an 80 m*M* SDS concentration and the corresponding chromatogram is shown in Fig. 4. It can be seen that all the peaks are satisfactorily separated. The usual peak broadening and tailing of peaks observed in the HPLC separation of this group of compounds are not seen here. In Table I, the ratios t_0/t_{mc}, where t_0 and t_{mc} are the migration times of an insolubilized species and that of the micelle respectively, obtained at various SDS concentrations are listed. From Table I, it is worth noting that the t_0/t_{mc} ratio obtained using 80 m*M* SDS was the smallest among the four sets of conditions. It is known that a smaller t_0/t_{mc} ratio usually offers a wider elution range which, to a certain extent, would enhance the separation of peaks [12].

Many complex equilibria are thought to occur in this system [5,8], including ion-pair formation in solution, penetration of the ion pair into the micelle interior, solubilization of the ion pair by insertion of the monomer end ino the micelle, solubilization of the ion pair with the catechols or catecholamines penetrating into the micelle, solubilization of the free cation at the anionic surface of the micelle and partitioning of catechols and catecholamines through complexation with boric acid present in the buffer. Therefore, elucidation of the solubilization mechanism would be

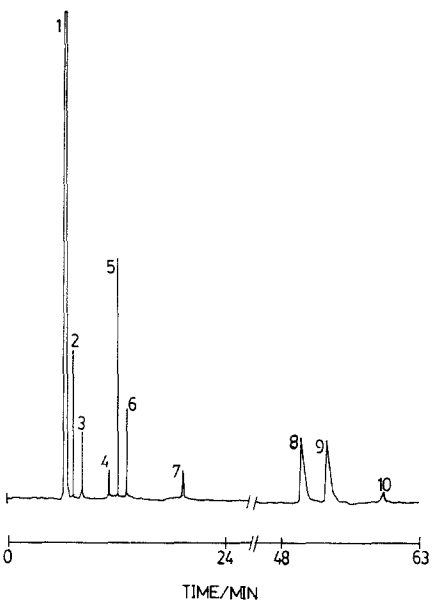

Fig. 4. Electrokinetic chromatogram of the catechols and catecholamines. Peaks: 1 = methanol; 2 = Ne; 3 = TRP; 4 = DHBA; 5 = DHPG; 6 = HIAA; 7 = DHPAA; 8 = NEPH; 9 = EPH; 10 = Sudan III. Electrophoretic solution, 80 m*M* SDS in 0.1 *M* borate–0.05 *M* phosphate buffer (pH 7.0); separation tube, 45 cm × 50 μm I.D. fused-silica capillary; voltage, 15 kV; detection wavelength, 210 nm.

TABLE I

EXPERIMENTAL t_0/t_{mc} RATIOS OBTAINED AT VARIOUS SDS CONCENTRATION

SDS concentration (m*M*)	t_o/t_{mc}
20	0.19
45	0.19
60	0.19
80	0.11

difficult. However, the migration order observed in Fig. 4 can be briefly explained by noting the dominant interactions.

At pH 7, protonation of the amino group in EPH, NEPH and TRP would be likely to occur. Consequently, long migration times are observed for these species. For the other compounds, because of the presence of the polar groups, they tend to be solvated more by the aqueous phase and therefore they migrate faster. With DHPAA and 5-hydroxyindole-3-acetic acid (HIAA), because of the orientation of the subsituent groups, intrahydrogen bonding is possible (*i.e.*, for DHPAA between the carboxylate group and the phenolic OH, and for HIAA between the carbonyl and the phenolic groups). As a result, they are solvated less by the aqueous phase. Hence they migrate more slowly than all the other compounds except EPH and NEPH.

Two of the cationic species (NE and DHBA) show relatively short migration times. It has been documented by Wallingford and Ewing [5] that cations interact with free SDS monomers (DS^-) to form ion pairs. The neutral ion pairs would be very hydrophobic and can be solubilized by the micelles, and hence longer migration times would be expected for the cations. However, the two cationic compounds under invesigation did not seem to have formed ion pairs with the SDS. The reason could be that owing to the presence of *o*-dihydroxyl groups, the compounds may form complexes with boric acid present in the buffer solution [5,8]. Consequently, NE and DHBA exist in the form of net-neutral complexes, which do not interact strongly with the SDS micelles. Therefore, they show very short migration times.

On the other hand, for the other two cationic compounds, NEPH and EPH, formation of the borate complexes did not take place as they do not possess *o*-dihydroxyl groups. As a result, they interact with SDS through the formation of ion pairs as expected, thus resulting in the longer migration times obtained for these two species.

For the neutral compounds, TRP and HIAA, there is no tendency to form complexes with borate or to interact with the SDS micelles. They are polar and would remain in the aqueous phase. Hence they have short migration times. It was also noted that TRP migrated faster than HIAA. This can be attributed to the fact that the primary amine group in TRP increases its hydrophobicity.

With DHPG, which is non-ionic, the dominant factor that determines its migration order may be its ability to form a net-anionic complex with boric acid. This net-anionic complex was found to migrate more slowly than the neutral species (borate complexes of NE and DHBA, and TRP). This could be due to the fact that electrophoretic effects predominate as the anions were attracted by the positive electrode and hence the migration time would be longer for this species. As for DHPAA,

although there is also a tendency to form a net-anionic complex with boric acid, the effect due to intrahydrogen bonding may be dominant, as discussed earlier. Consequently, DHPAA shows an even longer migration time than that of DHPG.

The results obtained in this work successfully demonstrated the use of MEKC for the separation of catecholamines. The interesting trends observed in their migration behaviour at different pH values and SDS concentrations demonstrated the versatility of the MEKC technique. From the results obtained, it is believed that this technique possesses immense potential for the separation of other groups of complicated mixtures.

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