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# Separation of aniline derivatives by micellar electrokinetic chromatography

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ABSTRACT

The separation of ten aniline derivatives was investigated by micellar electrokinetic chromatography. The pH dependence of their migration behaviour was determined. The capacity factor for each aniline derivative was calculated and its pH dependence was elucidated from the $pK_a$ value of the aniline derivative. In the case of *p*-anisidine, the pH dependence can be explained by a simple model. The complete separation of ten aniline derivatives was achieved by optimizing the pH and the concentration of sodium dodecyl sulphate, and by adding γ-cyclodextrin. Separation of diphenylamine and N-nitrosodiphenylamine, which cannot be easily distinguished by gas chromatography because of thermal degradation, was achieved.

## INTRODUCTION

Micellar electrokinetic chromatography (MEKC) [1] is an attractive, recently developed analytical method. MEKC has a high separation efficiency in comparison with high-performance liquid chromatography, and unlike capillary zone electrophoresis can be used to separate electrically neutral substances. The principle of separation in MEKC is based on differential partition of the solute between ionic micelles and the surrounding aqueous phase [2,3]. MEKC has attracted much attention with regard to the separation of various substances [4–7]. We have previously reported preliminary studies on the analysis of environmental pollutants by MEKC [8,9].

Aniline derivatives are frequently found in environmental waters. In Japan, eight aniline derivatives have been detected in environmental water by capillary gas chromatography–mass spectrometry (GC–MS) [10]. In GC–MS analysis, however, diphenylamine and N-nitrosodiphenylamine cannot be directly distinguished because N-nitrosodiphenylamine decomposes at the temperatures of GC injection ports or columns to produce diphenylamine [11]. Therefore, these compounds are separated from the others by column chromatography prior to GC, and are identified and measured as diphenylamine. For other aniline derivatives, extraction with organic solvents is necessary [10]. These procedures are complex and time-consuming. High-performance liquid chromatography (HPLC) does not require prior separation and has also been used for analysis of aniline derivatives [12,13]; however, the separation efficiency is not as good as in capillary GC.

In this paper, conditions for separation by MEKC analysis of ten aniline derivatives, including eight aniline derivatives which have been found in environmental water, was studied. The separation of a series of aniline derivatives by

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MEKC has not been previously reported. In order to completely separate these ten aniline derivatives by MEKC in a single run, their migration behaviour under various analytical conditions was investigated. The pH of the micellar solution was the most significant factor affecting the migration behaviour of the aniline derivatives. The effects of the micellar concentration and the addition of cyclodextrin (CD) on the migration behaviour were also investigated.

## EXPERIMENTAL

### *Apparatus*

MEKC was performed with a Model 270A analytical capillary electrophoresis system (Applied Biosystems, San Jose, CA, USA). A fused-silica capillary tube (720 mm × 50 μm I.D., GL Sciences, Tokyo, Japan) was used as the separation tube. The migrating solute bands were detected at a position 500 mm from the positive end by on-column UV absorption measurements (210 nm). A Chromatopac C-R6A (Shimadzu, Kyoto, Japan) was used for data processing.

### *Reagents*

All aniline derivatives, CDs (α-CD, β-CD and γ-CD) and sodium dodecyl sulphate (SDS) were obtained from Nacalai Tesque (Kyoto, Japan). All reagents and solvents were of analytical grade and were used without further purification. SDS solutions were prepared by dissolving SDS in a mixture of 0.02 *M* sodium dihydrogenphosphate solution and 0.02 *M* sodium tetraborate solution adjusted to the appropriate pH.

### *Procedure*

Stock solutions contained 10 g/l of the aniline derivatives dissolved in methanol. A standard solution of a mixture of the ten aniline derivatives was made by diluting the stock solutions with SDS solution. The concentration of each aniline derivative was 50 mg/l. Samples were injected by vacuum injection [5 in.Hg (1 in.Hg = 3386.379 Pa), 0.2 s] and the injection volume was about 1.5 nl. The set-up voltage and temperature were 20 kV and 30°C, respectively, throughout all experiments.

## RESULTS AND DISCUSSION

### *pH dependence of migration behaviour*

The separation of the ten aniline derivatives listed in Table I was investigated by MEKC. The effect of pH on their migration behaviour was investigated using 0.05 *M* SDS solutions whose pH values were 6.2, 7.0, 8.0 and 9.0. Separation could not be obtained with a solution of pH 5.0, due to broadening and delaying of the peaks. This may have been caused by the greatly decreased electroosmotic mobility. In order to investigate the pH dependence of the migration behaviour in detail, we calculated the capacity factors, $\tilde{k}'$, of the aniline derivatives from our experimental data.

In MEKC, the capacity factor of an electrically neutral solute is given by [2,3]:

$$\tilde{k}' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \quad (1)$$

where $t_R$, $t_0$ and $t_{mc}$ are the migration time for the solute, methanol (used as a tracer for the electroosmotic flow) and Sudan III (used as a tracer for the micelle), respectively. The dependence of the values of $\tilde{k}'$ calculated from eqn. 1 for each aniline derivative on the pH of the SDS solution is shown in Fig. 1. While $\tilde{k}'$ decreased significantly with increasing pH for *p*-anisidine

TABLE I

LIST OF THE INVESTIGATED ANILINE DERIVATIVES AND THEIR $pK_a$ VALUES

| Name of compound | $pK_a$ [a] |
|---|---|
| Aniline[b] | 4.63 |
| *o*-Anisidine[b] | 4.52 |
| *m*-Anisidine[b] | 4.23 |
| *p*-Anisidine | 5.34 |
| *o*-Chloroaniline[b] | 2.65 |
| *m*-Chloroaniline[b] | 3.46 |
| *p*-Chloroaniline | 4.15 |
| N-Methylaniline[b] | 4.848 |
| Diphenylamine[b] | 0.79 |
| N-Nitrosodiphenylamine[b] | – |

[a] Logarithm of the reciprocal of the conjugate acid dissociation constant taken from the literature [14].

[b] Detected in the environmental water of Japan [10].

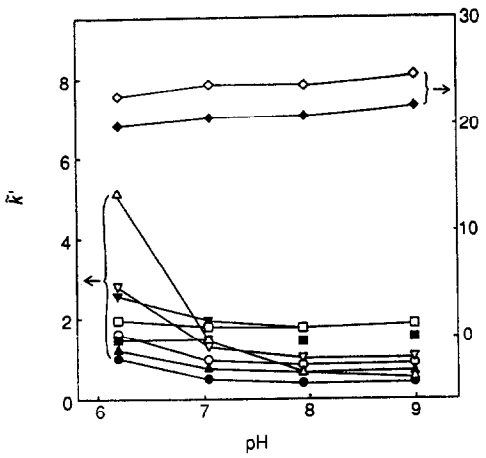


Fig. 1. Dependence of capacity factor $\tilde{k}'$ on pH at 0.05 *M* SDS. ● = Aniline; ○ = *o*-anisidine; ▲ = *m*-anisidine; △ = *p*-anisidine; ■ = *o*-chloroaniline; □ = *m*-chloroaniline; ▼ = *p*-chloroaniline; ▽ = N-methylaniline; ◆ = diphenylamine; ◇ = N-nitrosodiphenylamine.

and N-methylaniline, $\tilde{k}'$ was almost constant for *o*-chloroaniline and *m*-chloroaniline in the same pH range.

This behaviour can be considered to be related to the p$K_a$ values of the aniline derivatives, listed in Table I [14]. *p*-Anisidine and N-methylaniline have relatively high p$K_a$ values, whereas *o*-chloroaniline and *m*-chloroaniline have relatively low p$K_a$ values. The degree of protonation of the former derivatives is larger than that of the latter derivatives at the lower pH values investigated, so the former derivatives interact more strongly with the negatively charged SDS micelle.

To explain these considerations, we have applied a simple model for the migration behaviour of the aniline derivatives. The model shown in Fig. 2 is a simplified version of the complex model for cationic solutes in MEKC [15,16]. In Fig. 2, "B" represents the base form of the aniline derivative and "$BH^+$" represents its conjugate acid. $\mu_{eo}$ is the electroosmotic mobility and $\mu_{mc}$ and $\mu_{BH^+}$ are the electrophoretic mobility of the micelle and $BH^+$, respectively. Both B and $BH^+$ are partitioned between the micelle and the solvent (surrounding aqueous phase). The partition coefficients of B and $BH^+$ ($K_{mw,B}$ and $K_{mw,BH^+}$) differ because of the difference in their charges.

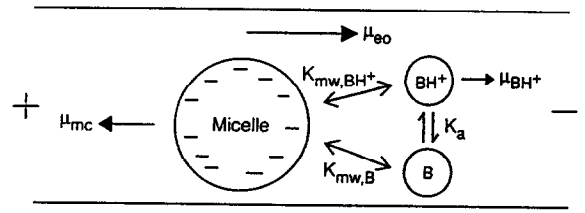


Fig. 2. Interaction of a solute acid/base pair with a micelle in MEKC.

The capacity factor of the aniline derivative was taken as the weighted average of the capacity factors of the base $\tilde{k}'_{BH^+}$ and its conjugate acid $\tilde{k}'_B$ as follows [16]:

$$\tilde{k}' = F^{aq}_{BH^+}\tilde{k}'_{BH^+} + F^{aq}_{B}\tilde{k}'_{B} \quad (2)$$

The *F* values are the mole fractions of the base and conjugate acid in the aqueous phase. The *F* values can be expressed in terms of the concentration of $H^+$ and the acid dissociation constant $K_a$:

$$F^{aq}_{BH^+} = \frac{[H^+]}{[H^+] + K_a} \quad (3)$$

and

$$F^{aq}_{B} = \frac{K_a}{[H^+] + K_a} \quad (4)$$

Substitution of eqns. 3 and 4 into eqn. 2 yields an equation expressing the pH dependence of $\tilde{k}'$:

$$\tilde{k}' = \frac{\tilde{k}'_{BH^+}[H^+] + \tilde{k}'_B K_a}{[H^+] + K_a} \quad (5)$$

Eqn. 5 predicts sigmoidal plots of $\tilde{k}'$ *vs.* pH, as shown schematically in Fig. 3. If the observed $\tilde{k}'$ values show this type of behaviour, $\tilde{k}'_{BH^+}$, $\tilde{k}'_B$ and $K_a$ can be estimated from the experimental data and eqn. 5.

However, calculation of $\tilde{k}'$ by eqn. 1 is valid only for electrically neutral solutes, whereas the degree of ionization of aniline derivatives increases with a decrease in pH. $\tilde{k}'$ can be expressed for ionized solutes by the electrophoretic mobility of the solute $\mu$ as follows [5,16]:

$$\tilde{k}' = \frac{\mu - \mu_0}{\mu_{mc} - \mu} \quad (6)$$

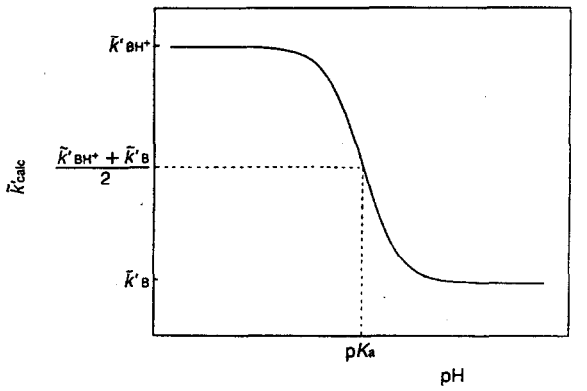


Fig. 3. Illustration of pH dependence of capacity factor $\tilde{k}'$ calculated from eqn. 5.

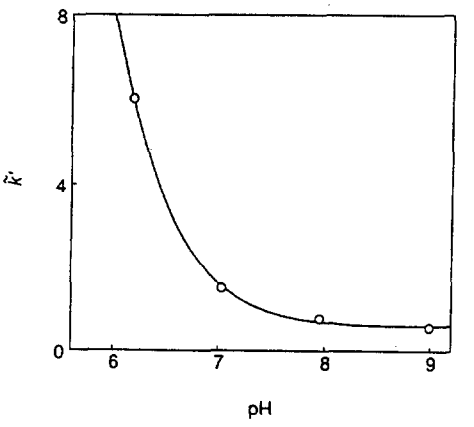


Fig. 4. Least-squares fitting of experimental data of *p*-anisidine for eqn. 7. O = $\tilde{k}'_{obs}$; —— = $\tilde{k}'_{calc}$.

where $\mu_0$ is the observed overall mobility of the solute in the aqueous phase, given by:

$$\mu_0 = \frac{[H^+]}{[H^+] + K_a} \mu_{BH^+} \quad (7)$$

For neutral solutes, $\mu_0 = 0$ and eqn. 6 is equivalent to eqn. 1. The contribution of $\mu_0$ to $\tilde{k}'$ is negligible in the pH range of this experiment except for the case of *p*-anisidine at pH 6.2. According to Otsuka *et al.* [5], it is difficult to estimate the value of $\mu_0$ for ionized solutes in micellar solutions, and it is reasonable to assume that the value of $\mu_0$ is the same as that in the buffer solution containing 5 m*M* SDS.

For *p*-anisidine, the least-squares fitting of the experimental data for eqn. 5 using the above assumption is shown in Fig. 4. The resulting estimated values of $\tilde{k}'_{BH^+}$, $\tilde{k}'_B$ and p$K_a$ are shown in Table II, along with the literature value of p$K_a$. The good fit of the curve and the closeness of the estimated p$K_a$ value to the literature value indicate that the model can be considered to be valid for this case. For the other aniline derivatives, such fitting was unsuccessful, because the experimentally acceptable pH variation is restricted to a narrow range, and the p$K_a$ values of the aniline derivatives are too small for the estimation to be carried out over the entire range of pH values investigated.

*Optimization of the analytical conditions for the complete separation of ten aniline derivatives*

Optimization of the pH of the SDS solution was carried out by observing the migration behaviour of ten aniline derivatives. Complete separation of eight of the aniline derivatives was achieved at pH 6.2, but the separation of two other compounds, diphenylamine and N-nitrosodiphenylamine, was incomplete. In order to separate these last two compounds completely, the effects of SDS concentration and addition of CD were investigated as follows.

The capacity factor $\tilde{k}'$ is related to the overall partition coefficient, $K_{mw}$, by the following equation [3]:

$$\tilde{k}' = K_{mw} \frac{V_{mc}}{V_{aq}} \quad (8)$$

$$\approx K_{mw} \bar{v} (c_{sf} - CMC) \quad (9)$$

where $V_{mc}/V_{aq}$ is the volume ratio of the micelle and the aqueous phase, $\bar{v}$ is the partial molar volume of the micelle, $c_{sf}$ is the total concentration and CMC is the critical micellar concentration of the surfactant.

TABLE II

ESTIMATED VALUES OF $\tilde{k}'_{BH^+}$, $\tilde{k}'_B$ AND p$K_a$ OF *p*-ANISIDINE BY LEAST-SQUARES FITTING OF THE EXPERIMENTAL DATA

| | Estimated value | Literature value |
|---|---|---|
| $\tilde{k}'_{BH^+}$ | 26 | – |
| $\tilde{k}'_B$ | 0.58 | – |
| p$K_a$ | 5.6 | 5.34 |

In the case of neutral solutes, as can be seen from eqn. 3, the order of their capacity factors is independent of the SDS concentration. Some of the aniline derivatives, however, are partially ionized at low pH. Accordingly, the dependence of capacity factor on SDS concentration at pH 6.2 was investigated; the results are shown in Fig. 5. The capacity factor of the aniline derivatives varies with SDS concentration, and some peaks are overlapped at high (0.08–0.15 *M*) SDS concentration. This variation in capacity factor seems to be caused by the partial ionization of some of the aniline derivatives. At 0.03 *M*, the separation behaviour was almost the same as that observed at 0.05 *M*, but slight peak broadening was observed. Consequently, the optimum concentration of SDS was taken to be about 0.05 *M*.

However, the separation of diphenylamine and N-nitrosodiphenylamine was incomplete at all SDS concentrations. Since diphenylamine and N-nitrosodiphenylamine are relatively hydrophobic compounds, their partition into the micelle is too large to separate them completely. Therefore, three kinds of CD ($\alpha$-, $\beta$- and $\gamma$-CD) were added to the SDS solutions in order to aid in achieving complete separation of the ten aniline derivatives, especially diphenylamine and N-nitrosodiphenylamine. CD-modified MEKC has been used in order to separate hydrophobic compounds that are almost totally partitioned into the micelle [17]. Addition of CD reduces

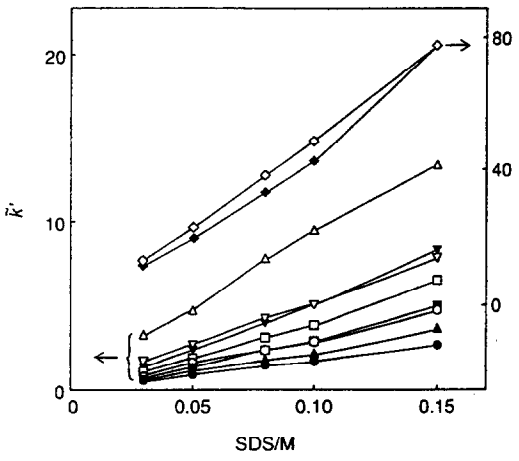


Fig. 5. Dependence of capacity factor $\tilde{k}'$ on SDS concentration at pH 6.2. Symbols as in Fig. 1.

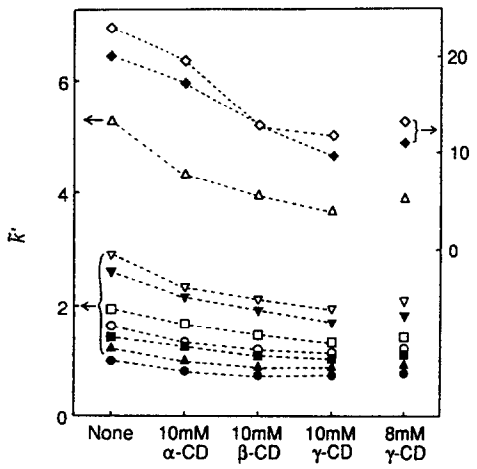


Fig. 6. Effect of CD addition on the capacity factor $\tilde{k}'$ at pH 6.2, 0.05 *M* SDS. Symbols as in Fig. 1.

partition into the micelle because of inclusion in the cavity of CD, which migrates with the electroosmotic velocity.

The effect of CD addition on the migration behaviour of the aniline derivatives is shown in Fig. 6. The capacity factor of all aniline deriva-

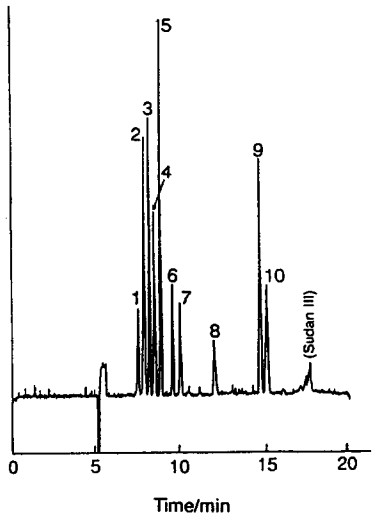


Fig. 7. Complete separation of ten aniline derivatives in MEKC. Peaks: 1 = aniline; 2 = *m*-anisidine; 3 = *o*-chloroaniline; 4 = *o*-anisidine; 5 = *m*-chloroaniline; 6 = *p*-chloroaniline; 7 = N-methylaniline; 8 = *p*-anisidine; 9 = diphenylamine; 10 = N-nitrosodiphenylamine. Micellar solution, 8 m*M* $\gamma$-CD and 0.05 *M* SDS in 0.02 *M* borate–phosphate buffer (pH 6.2); separation column, 720 mm × 0.05 $\mu$m I.D.; effective length to the detector, 500 mm; applied voltage, 20 kV; current, 20 $\mu$A; detection wavelength, 210 nm; temperature, 30°C.

tives decreased upon addition of 10 m*M* of any type of CD, and the separation behaviour of diphenylamine and N-nitrosodiphenylamine depended on the type of CD. This dependence may be due to differences in their cavity sizes. Complete separation was achieved only when γ-CD was used. The migration times of some aniline derivatives were too close with 10 m*M* γ-CD, and better separation was obtained with 8 m*M* γ-CD.

The chromatogram of ten aniline derivatives in MEKC obtained using optimum analytical conditions is shown in Fig. 7. The complete separation of all of the aniline derivatives was achieved in a single run, and the peaks show high separation efficiency (their theoretical plate numbers are about 100 000). This demonstrates that MEKC is a powerful technique for the analysis of these aniline derivatives. Their concentration in environmental waters, however, is at the ppb level or less. Therefore, in order to apply MEKC to the analysis of real samples of environmental waters, more sensitive and qualitative detection methods are required.

## REFERENCES

1 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
2 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
3 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
4 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 332 (1985) 219.
5 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 348 (1985) 39.
6 A.S. Cohen, S. Terabe, J.A. Smith and B.L. Karger, *Anal. Chem.*, 59 (1987) 1021.
7 H. Nishi and S. Terabe, *Elecrophoresis*, 11 (1990) 691.
8 S. Takeda, S. Wakida, M. Yamane, A. Kawahara and K. Higashi, *Anal. Sci.*, 7, Suppl. (1991) 1113.
9 S. Wakida, S. Takeda, M. Yamane, A. Kawahara and K. Higashi, *Anal. Sci.*, 7, Suppl. (1991) 1109.
10 *Kagakubusshitsu to Kankyo*, Office of Health Studies, Environmental Health Department, Environment Agency of Japan, Tokyo, 1991.
11 J.W. Eichelberger, E.H. Kerns, P. Olynyk and W.L. Budde, *Anal. Chem.*, 55 (1983) 1471.
12 A. Di Corcia and R. Samperi, *Anal. Chem.*, 62 (1990) 1494.
13 J.S. Ho, T.A. Bellar, J.W. Eichelberger and W.L. Budde, *Environ. Sci. Technol.*, 24 (1990) 1748.
14 R.C. Weast, M.J. Astle and W.H. Beyer (Editors), *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, FL, 67th ed., 1986–87, p. D-159.
15 J.K. Strasters and M.G. Khaledi, *Anal. Chem.*, 63 (1991) 2503.
16 M.G. Khaledi, S.C. Smith and J.K. Strasters, *Anal. Chem.*, 63 (1991) 1820.
17 S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya and N. Tanaka, *J. Chromatogr.*, 516 (1990) 23.