Separation of phthalates by micellar electrokinetic chromatography

C. P. ONG, H. K. LEE and S. F. Y. LI*

Department of Chemistry, National University of Singapore, Kent Ridge Crescent, 0511 (Republic of Singapore)

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ABSTRACT

Micellar electrokinetic chromatography (MEKC) of priority phthalate esters was investigated. A commercial photodiode-array ultraviolet–visible detector was modified for on-column detection in an MEKC system. For the separation of phthalate esters, electrophoretic media with sodium dodecyl sulphate in phosphate–borate buffer were used. The retention behaviour of the phthalate esters at different concentrations of micellar solution and at different pH values of the electrophoretic media was investigated. In addition, the effect of different voltages across the capillary tubing was examined. The results successfully demonstrated the application of MEKC for the separation of a group of six phthalate esters, five of which are priority pollutants.

INTRODUCTION

Interest in the use of high-performance capillary electrophoresis (CE) in separation science has been tremendous in recent years. The rapid developments in this area could be largely attributed to the many advantages of this technique, including its exceptionally high efficiency, rapid rate of separation and relatively simple instrumentation.

An example of the CE techniques is micellar electrokinetic chromatography (MEKC), first developed by Terabe *et al.* [1]. The instrumental set-up is the same as that in conventional capillary electrophoresis, but a micellar solution is usually employed as the electrophoretic medium. The main advantage of MEKC is that both neutral analytes and charged solutes can be separated. There have been numerous reports in which MEKC has been successfully used for separating chemical and biological compounds [2,3]. However, to the best of our knowledge MEKC has not been employed to separate phthalates.

Phthalates are used extensively as plasticizers in the formulation of polymers [4]. As these plasticizers are not chemically bonded to the polymer, they can migrate from the plastics into the environment under suitable conditions [5]. The widespread use of these compounds and their presence in the environment have promoted great interest in the development of chromatographic methods for their detection [6,7].

This paper describes the use of MEKC for the separation of six phthalate esters,

five of which are listed by the United States Environmental Protection Agency (USEPA) as priority pollutants. In a previous study [8], five of the phthalates investigated were successfully separated using isocratic high-performance liquid chromatography (HPLC). However, the sixth phthalate, bis(2-ethylhexyl) phthalate, was found to be satisfactorily separated only with gradient elution [9].

In this application of MEKC, an on-column photodiode-array detector was used for detection. Sodium dodecyl sulphate (SDS) in borate–phosphate buffer was used as the electrophoretic medium. The effects of various experimental parameters, including the pH of the buffer, the SDS concentration in the electrophoretic medium and the voltage used, on the retentions of the phthalates were investigated.

EXPERIMENTAL

The experiments were performed on a laboratory-built MEKC instrument. The on-column detection of the peaks was effected on a Shimadzu (Kyoto, Japan) Model SPDM6A detector. The detector cell was modified according to the procedures described elsewhere [10]. Briefly, the laboratory-built cell was erected on two metal blocks between which the separation column was mounted. The window for the UV light path was made by removing a small portion of the polyimide layer on the capillary tubing. A fused-capillary tube, 50 cm × 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, U.S.A.), was used as the separation column. The power supply was a Spellman Model RHR 30PN10/RVC capable of delivering up to 30 kV.

All chemicals were of analytical-reagent grade unless specified otherwise. The buffer solution was prepared by dissolving sodium dihydrogenphosphate dihydrate and sodium tetraborate in water purified with a Millipore system. The electrophoretic medium consisting of SDS micelles in phosphate–borate buffer was prepared as described previously [1]. The structures of the six phthalates studied are shown in Fig. 1. The standard solution of the phthalates and Sudan III was prepared in HPLC-grade methanol (J. T. Baker, Phillipsburg, NJ, U.S.A.) at a concentration of 500 ppm for each of the species. All of these chemicals were supplied by Fluka (Buchs, Switzerland).

Introduction of sample was made manually by gravity feed, by placing the tip of the capillary at the high-potential end into a sample vial at a level 5 cm higher than the buffer reservoir. The time for each injection was 5 s. The amount of sample injected by this method was typically about 1.5 nl. The capillary end was subsequently rinsed by dipping it in a rinsing solution similar to that of the reservoir. It was then returned to the buffer reservoir before the power was switched on.

RESULTS AND DISCUSSION

All six phthalates were successfully analysed in a single run by MEKC. The separation was achieved by optimizing the pH of the buffer, SDS concentration and voltage across the separating column.

Preliminary experiments were first conducted at pH 6.0 and 7.5 without SDS in the electrophoretic medium. These conditions are similar to those used in conventional capillary zone electrophoresis (CZE). In both instances, a single broad peak was observed for all the phthalates, indicating that all the solutes possess similar charges
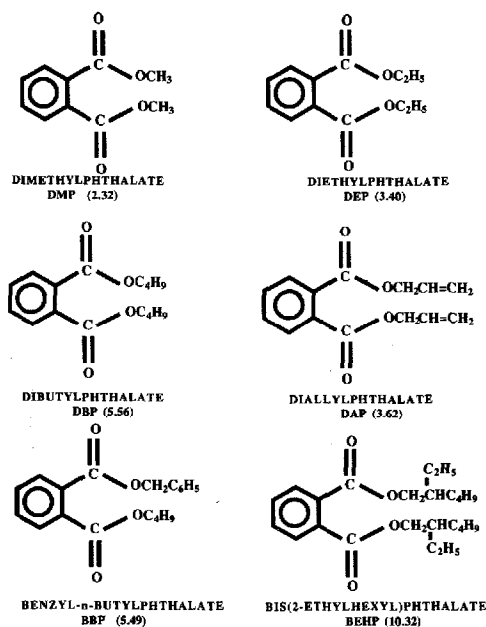
Fig. 1. Structures of the phthalates studied and their log P values.

under these pH conditions. The electrophoretic medium in the absence of the micelles does not seem to provide sufficient selectivity to separate the six phthalates. On the other hand, with SDS, the components in the mixture sample can be separated on the basis of the relative affinity for the micellar environment or the bulk aqueous phase. The more hydrophobic phthalates would tend to be strongly associated with the micelles and thus be eluted later than the hydrophilic species. This is one of the unique features of the MEKC system which enables the phthalates in the mixture to be separated.

Effect of pH

The experimental conditions used for the investigation of the effect of pH on the migration of the phthalates are listed in Table I. The results obtained are shown

TABLE I

EXPERIMENTAL CONDITIONS EMPLOYED TO INVESTIGATE THE EFFECT OF pH ON THE SEPARATION OF PHTHALATES AND EXPERIMENTAL t_0/t_{mc} RATIOS

Parameter	Experiment No.			
	1	2	3	4
pH	6.0	6.6	7.0	7.5
Applied voltage (kV)	15	15	15	15
Length (cm)	50	50	50	50
Tubing I.D. (μm)	50	50	50	50
Electrophoretic solution	10 mM SDS in 0.1 M borate–0.05 M phosphate buffer			
t_0/t_{mc}	0.21	0.21	0.20	0.22

graphically in Fig. 2. The capacity factor, k', used in this investigation was calculated using the equation [11]

$$k' = \frac{t_r - t_0}{t_0[1 - (t_r/t_{mc})]} \tag{1}$$

where t_r, t_0 and t_{mc} are the migration times for the solute, the insolubilized solute (methanol) and the micelles (measured using Sudan III as the marker), respectively.

Fig. 2 indicates that there was no change in the migration order for the six phthalates throughout the whole range of pH examined. The migration times for the phthalates increased in the order DMP < DEP < DAP < BBP < DBP < BEHP, and their hydrophobicities (log P) (see Fig. 1) in the order log P_{DMP} < log P_{DEP} < log P_{DAP} < log P_{BBP} < log P_{DBP} < log P_{BEHP}. By correlating these two series, it can be deduced that the phthalates might be in the neutral form in the pH range investigated. Consequently, their migration order would be dominated by their log P values, *i.e.*, the more hydrophobic phthalates are eluted later.

An interesting trend observed was that at higher pH (7.5), there was a marked increase in the migration times for some of the phthalates (DBP and BEHP in particular). This observation seems to contradict the fact that if the phthalates are in the neutral form they should not be affected by any changes in pH. A possible reason for this discrepancy could be that preferential dissociation of some of the phthalates
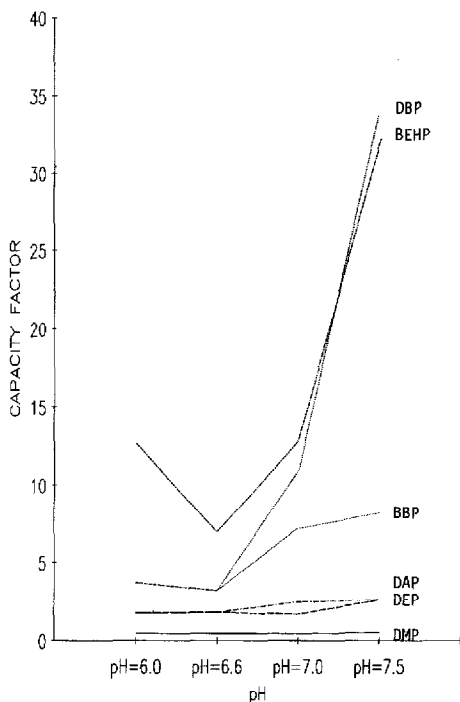

Fig. 2. Plot of capacity factors *versus* pH. Other conditions are given in Table I.

might occur at pH 7.5. These negatively charged phthalates would then be affected by the electrophoretic attraction towards the anode. Therefore, it is expected that there will be competition between the micelles and the anode for these partially negatively charged species. The electrophoretic attraction is a much stronger electrostatic type of interaction than the Van der Waal's type of interaction for micellar solubilization. Therefore, in spite of the fact that the SDS concentration remained constant in all these experiments, an increase in the migration times was observed for these species at pH 7.5.

Effect of SDS

Experiments at three different SDS concentrations were performed. The experimental conditions for these three experiments are listed in Table II. The results obtained are given in Fig. 3, where the capacity factors are plotted against SDS concentration.

There was an increase in the migration times of the phthalates when the SDS concentration in the electrophoretic solution increased. This increase can be accounted for by the fact that at higher SDS concentrations the phase ratio of the micelle to the aqueous phase would be larger. Hence the probability of solubilization of the phthalates by the micelles would be higher, resulting in an increase in the migration times for these compounds, as observed. This trend is also consistent with those observed previously by Terabe and co-workers [12,13].

Effect of voltage

The application of a higher voltage increases the resolution in CE [14]. As our attempts to optimize the separation of the phthalates based on SDS concentration and pH failed, the effect of higher voltages on the separation was examined. The experimental conditions for this investigation are listed in Table III.

The results obtained are shown in Fig. 4. The migration order was observed to be the same for all four sets of experiments and the order is governed by the log P values. Sharper peaks were observed at higher voltages. The increase in efficiency due to the higher voltages seems to improve the overall peak shape and resolution. It is worth

TABLE II

EXPERIMENTAL CONDITIONS EMPLOYED TO INVESTIGATE THE EFFECT OF VARYING SDS CONCENTRATION ON THE SEPARATION OF PHTHALATES AND EXPERIMENTAL t_0/t_{mc} RATIOS

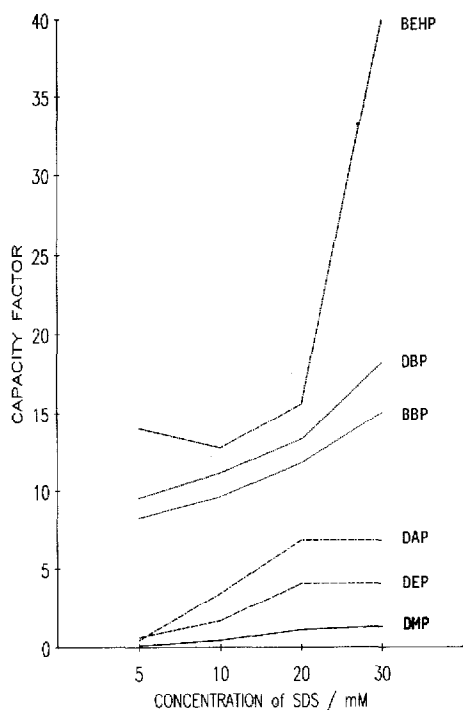| Parameter	Experiment No.		
	3	5	6
SDS concentration (mM)	10	20	30
Applied voltage (kV)	15	15	15
Length (cm)	50	50	50
Tubing I.D. (μm)	50	50	50
pH	7.0	7.0	7.0
Electrophoretic solution	0.1 M borate–0.05 M phosphate buffer		
t_0/t_{mc}	0.20	0.19	0.17

Fig. 3. Plot of capacity factors *versus* SDS concentration. Other conditions are given in Table II.

noting that the values of the ratio t_0/t_{mc}, obtained for experiments conducted at higher voltages (see Table III) are generally smaller than those shown in Tables I and II. The ratio t_0/t_{mc} indicates the elution range for the solute under a given set of experimental conditions. A smaller t_0/t_{mc} ratio offers a wider elution range, which to a certain extent enhances the separation of peaks [12]. Therefore, it is expected that the experiments performed at higher voltages would give better separations. In fact, the two experiments conducted at 25 and 30 kV have the smallest t_0/t_{mc} values and complete separation of all six phthalates was achieved.

The corresponding chromatograms are shown in Figs. 5 and 6. From Fig. 5, it can be seen that all six phthalates are satisfactorily separated within 40 min. However, at 30 kV, even though all six phthalates were satisfactorily separated, an additional peak (peak 7 in Fig. 6) was observed. This could be due to the stronger electric field at the higher voltage, which tends to produce excessive Joule heat [14]. The excessive heat generated at such a high potential could have promoted the hydrolysis of the phthalate ester to give an alcohol and an acid (probably phthalic acid).

Amongst the six phthalates, it seems that bis(2-ethylhexyl) phthalate is the one most prone to hydrolysis at the higher voltage. This is probably due to its bulky substituent groups which make it very unstable. After further investigation, it was found that the extra peak was indeed due to phthalic acid, the hydrolysed product of bis(2-ethylhexyl) phthalate. Hence, even though the higher voltage of 30 kV resulted in a higher efficiency, because of the possibility of side-reactions (hydrolysis) occurring

TABLE III

EXPERIMENTAL CONDITIONS EMPLOYED TO INVESTIGATE THE EFFECT OF VOLTAGE ON THE SEPARATION OF PHTHALATES AND EXPERIMENTAL t_0/t_{mc} RATIOS

Parameter	Experiment No.			
	1	7	8	9
Applied voltage (kV)	15	20	25	30
Length (cm)	50	50	50	50
Tubing I.D. (μm)	50	50	50	50
pH	6.0	6.0	6.0	6.0
Electrophoretic solution	10 mM SDS in 0.1 M borate–0.05 M phosphate buffer			
t_0/t_{mc}	0.21	0.18	0.14	0.12

during the separation, the optimum voltage for the separation of the phthalates was found to be 25 kV in these experiments.

It should be noted that generally with increasing applied voltage, a corresponding decrease in migration time would be expected for most solutes. This observation can be accounted for by the fact that at a higher voltage, the electroosmotic velocity (V_{os}) would be increased. At the same time, a corresponding increase in the electrophoretic velocity (V_{ep}) would also be observed. However, it was found that in most instances, under normal circumstances the increase in V_{os} is often
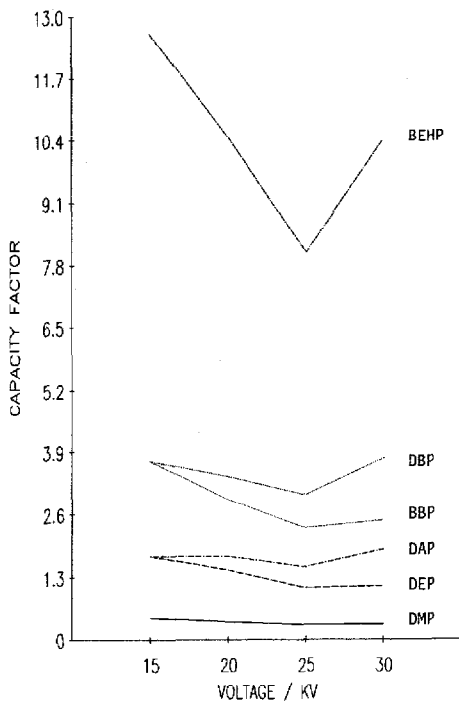

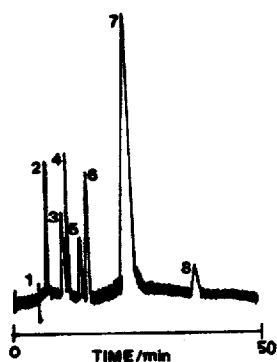Fig. 4. Plot of capacity factors *versus* voltage. Other conditions are given in Table III.

Fig. 5. Electrokinetic chromatogram of the six phthalate esters: 1 = methanol; 2 = DMP; 3 = DEP; 4 = DAP; 5 = BBP; 6 = DBP; 7 = phthalic acid; 8 = BEHP; 9 = Sudan III. Conditions as in Fig. 5, except voltage = 30 kV.

more pronounced than V_{ep}. Consequently, an overall decrease in migration time is expected. The results of our study, with the exception of those obtained using 30 kV, seem to be in agreement with the above trend. At 30 kV, a reversal in this trend was observed for most of the phthalates. A possible reason could be the heat generated at the relatively higher applied voltage. It is known that in such a system, V_{ep} would be affected such that the electrophoretic mobility would be increased. In fact, it was found that V_{ep} increases at a rate of ca. 2%/°C [15]. Therefore, in this instance, the increase in V_{os} in the system can no longer compensate for the larger increase in V_{ep}. Consequently, there is an overall increase in the migration times for the phthalates at 30 kV. From Fig. 4 it was noted that the increase is less apparent for those phthalates with shorter migration times. This is expected, as the fact that these phthalates are eluted earlier suggests that they are not much influenced by the electrophoretic interaction. Hence it would be reasonable to expect that any changes in V_{ep} would not be significant enough to cause any drastic change in their migration times.
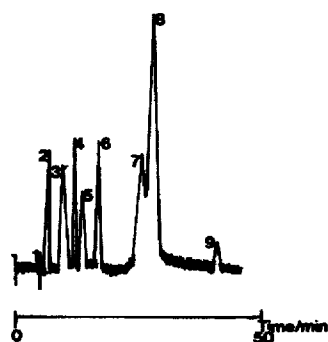

Fig. 6. Electrokinetic chromatogram of the six phthalate esters: 1 = methanol; 2 = DMP; 3 = DEP; 4 = DAP; 5 = BBP; 6 = DBP; 7 = BEHP; 8 = Sudan III. Electrophoretic solution, 10 mM SDS in 0.1 M borate–0.05 M phosphate buffer; pH, 6.0; separation tube, 50 cm × 50 μm I.D. fused-silica capillary; voltage, 25 kV; detection wavelength, 210 nm; volume of sample injected, 1.5 nl.

This work has successfully demonstrated the use of MEKC for the separation of priority phthalates. By optimizing parameters such as the pH and SDS concentration of the electrophoretic media and the voltage across the capillary tubing, high-resolution separations can be easily achieved. From the promising results obtained, it is believed that this technique can be extended to the analysis of other groups of priority pollutants.

ACKNOWLEDGEMENT

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