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Optimized capillary zone electrophoretic separation of chlorinated phenols

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ABSTRACT

A mixture of thirteen chlorinated phenols was resolved within 16 min by using a 57 cm × 75 μm I.D. fused-silica tube with a 50 m*M* sodium phosphate buffer (pH 6.9) electrolyte, under an 18 kV potential. The electrophoretic behaviour of chlorophenol congeners was investigated in order to optimize their separation as a function of the running buffer pH, concentration and applied voltage. A migration order depending on both charge and size of the solutes was established. Selectivity is strongly affected by the electrolyte concentration, in a manner that cannot be easily predicted. Quantitative aspects of capillary zone electrophoresis are also discussed.

INTRODUCTION

Chlorinated phenols (CPs) in water can be degradation by-products of pesticides [1] or be formed from the chlorination of drinking water containing phenols originating from petroleum, steel or pulp and paper industry [2]. According to several workers, their toxicity and organoleptic properties are often manifested in the ppb range [3–5].

Chlorophenols are generally analysed by either GC [1] or HPLC [1–10]. Unfortunately, these separations are often time consuming or need derivatization of the solutes [5,9,10]. Moreover, the optimization of chromatographic methods, especially gradient HPLC [2,3,7,9], requires complex procedures or a large number of experiments.

The development of capillary zone electrophoresis (CZE) has permitted high-resolution separations of CPs faster than by conventional HPLC. Good results were achieved by Gaitonde and Pathak [11], who determined eleven CPs in less than 24 min using phosphate–borate buffer (pH 8.0) in a fused-silica capillary (65 cm × 25 μm I.D.) with an applied potential of 20 kV. Detection was performed with an on-column electrochemical detector in the picomole range. Efficiencies of the order of 320 000 theoretical plates were reached.

The use of micellar electrokinetic chromatography (MEKC) for the separation of chlorinated phenols was first reported by Otsuka *et al.* [12] the nineteen isomers were separated within 18 min, by using a 65 cm × 50 μm I.D. fused-silica tube with a 0.07 *M* sodium dodecyl sulphate (SDS) solution (pH 7.0). Eleven substituted phenols, listed by the US Environmental Protection Agency (EPA) as priority pollutants, were resolved by Ong and co-workers [13–15].

MEKC techniques have been widely used for the study of these compounds, whereas comparatively little attention has been paid to CZE. However CZE has several advantages, as follows. (1) With MEKC, the theoretical plate numbers are not as high as those which can be achieved by CZE, because of the resistance to

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mass transfer introduced by the solute distribution between the conducting buffer and the micelles. Consequently, the sensitivity in MEKC is lower than in CZE [16]. (2) CZE is a simpler technique than MEKC, which often requires extensive studies for optimizing the resolution [16]. (3) CZE is reported to be compatible with electrospray ionization mass spectrometry [17,18], whereas no such data have yet been published concerning MEKC. (4) CZE allows separations between neutral interferents and CPs. (5) Detection in CZE is likely to be enhanced by on-column sample concentration using field amplification [19,20]. On the other hand, MEKC gives greater selectivity than CZE for the separation of ionic solutes, which is influenced by differential partitioning and differential migration mechanisms.

The objective of this work was to demonstrate the suitability of CZE for the separation of chlorinated phenols. For this purpose, we tried to achieve a satisfactory optimization to obtain good resolution, based on three parameters: pH, buffer concentration and applied voltage. Observations on the migration behaviour of the CPs under different conditions were related to their physico-chemical properties and were used to predict CP migration and resolution.

EXPERIMENTAL

Apparatus

Measurements were carried out on an HPCE P/ACE system 2000 (Beckman, Palo Alto, CA, USA), equipped with a UV absorbance detector, an automatic injector, a thermostated column cartridge and an autosampler. A 57.0 cm long (50.0 cm to the detector cell) 75 mm I.D. fused-silica capillary column (Beckman) was used. All experiments were performed at 22.0°C at constant voltage. Samples were introduced pneumatically by application of pressure during 5 s. Solutes were monitored at 214 nm. Peak areas and electrophoretic mobilities were measured using Beckman GOLD 6.01 software.

Chemicals

Phosphate running buffers (pH 6.9) were prepared by mixing equimolar solutions of Na_2HPO_4 and NaH_2PO_4 (Sigma, St. Louis, MO, USA). HPLC-grade distilled water (Baker, Deventer, Netherlands) was used to prepare buffers and standard solutions. 2,3-, 2,4-, 2,5,-Di-,2,3,6-, 2,4,6-, 2,4,5-tri- and pentachlorophenol were obtained from Fluka (Buchs, Switzerland) and 2,6-, 3,5-di-, 2,3,4-, 2,3,5-tri-, and 2,3,5,6-tetrachlorophenol from Janssen (Noisy Le Grand, France). Standard solutions were prepared at a concentration of about 1.0 mg/l, except for *o*-chlorophenol (10.0 mg/l). All solutions were filtered through a 0.22-μm Nalgene filter (Sybron, Rochester, USA). Buffers could be stored for 3 days at 4°C.

Procedure

The capillary column was first conditioned with the separation buffer (1 min) immediately prior to injection. The column was systematically washed with 0.1 *M* NaOH (1 min), then rinsed with water (1 min) between runs.

RESULTS AND DISCUSSION

Theoretical

According to Giddings [21], the resolution (R_s) of two zones in electrophoresis can be expressed as

$$R_s = \tfrac{1}{4} N^{1/2} \Delta\nu/\bar{\nu} \quad (1)$$

where N is the theoretical plates number, $\Delta\nu/\bar{\nu}$ is the relative velocity difference of the two ions to be separated and, $\bar{\nu}$ is the average velocity of these two ions.

Jorgenson and Lukacs [22] derived the following expression for resolution in CZE:

$$R_s = \tfrac{1}{4}\sqrt{2}(\mu_{ep,1} - \mu_{ep,2}) V^{1/2} D^{-1/2} (\bar{\mu}_{ep} + \mu_{eo})^{-1/2} \quad (2)$$

where V is the applied voltage, D the analyte average diffusion coefficient, $\mu_{ep,1}$ and $\mu_{ep,2}$ the electrophoretic mobilities of the two solutes, $\bar{\mu}_{ep}$ their average electrophoretic mobility and μ_{eo} the electroosmotic flow coefficient. Eqn. (2) suggests that optimization of resolution can be achieved by controlling either (1) the difference in the electrophoretic mobilities of the solutes,

$\Delta\mu_{ep} = \mu_{ep,1} - \mu_{ep,2}$, which represents the selectivity factor, or (2) the electroosmotic flow coefficient (μ_{eo}).

μ_{eo} and μ_{ep} are related through the relationships [23]

$$\mu_{eo} = -\epsilon\zeta_c/\eta \quad (3)$$

$$\mu_{ep} = 2\epsilon\zeta_a f(\kappa r)/3\eta \quad (4)$$

where ϵ is the permittivity of the bulk electrolyte, ζ_c and ζ_a are the zeta potentials of the capillary inner wall and of the analyte, respectively, η is the viscosity of the electrolyte and $f(\kappa r)$ is a function dependent on the analyte double layer thickness ($1/\kappa$) and the solute "radius" (r), assuming that CPs can be considered as spherical particles.

Whereas D is determined for each analyte, V can be selected by the operator and $\mu_{ep,1}$, $\mu_{ep,2}$ or μ_{eo} can be modified by using different running buffers, by varying the pH and electrolyte type or concentration.

Effect of buffer pH on resolution

Different buffer pH values have been used for the analysis of CPs [11–13], and the optimum pH for the separation of CPs has been reported to range from 6.6 to 8.0. Terabe and co-workers succeeded in resolving the nineteen isomers of CPs using phosphate–borate buffer (pH 7.0). In fact, in most instances, the buffer pH has been chosen in a very empirical manner. This is particularly true when performing MEKC, for which the elution order of the solutes is difficult to predict. On the other hand, in CZE, it is possible to estimate the migration order, based on the solute pK_a values, using the following model.

Hunter [24] defined the zeta potential of an analyte (ζ_a) as

$$\zeta_a = \frac{Q_a}{4\pi\epsilon r(1+\kappa r)} \quad (5)$$

where Q_a is the analyte charge. Q_a may be further defined as the product of the electron charge (e) and the degree of dissociation (α) of the CP in the medium

$$Q_a = e\alpha \quad (6)$$

where

$$\alpha = 1/1 + 10^{pK_a - pH} \quad (7)$$

Combining eqns. 4, 5 and 6 yields the following expression for the electrophoretic mobility:

$$\mu_{ep} = \frac{e\alpha f(\kappa r)}{6\pi\eta r(1+\kappa r)} \quad (8)$$

Therefore, as the ionic mobility of an analyte is dependent on its charge, manipulation of the buffer pH becomes one of the key strategies in optimizing a separation. As can be seen from Fig. 1, the optimum pH corresponds to larger values of α. In practice, this pH is obtained by calculating the mean value of the solute pK_a values. Consequently, the useful range of pK_a available for the separation is limited by the electrolyte buffer pH ± 2 units, leading to a degree of dissociation between 1% and 99%. Then, it can be assumed that compounds with a pK_a that is not included in this pratical interval of pH (4 units wide) will not be successfully resolved. This is exemplified in Table I, where the pK_a values of CPs range from 4.74 to 9.37. In that case, the wide distribution of pK_a values adds to the difficulty in resolving the nineteen isomers using a single buffer pH. Hence, different runs at different pH values could be performed in order to overcome such difficulties. The feasibility of this approach will be discussed later.

The thirteen CPs isomers tested in this work exhibit an average pK_a value of 6.82. Separa-

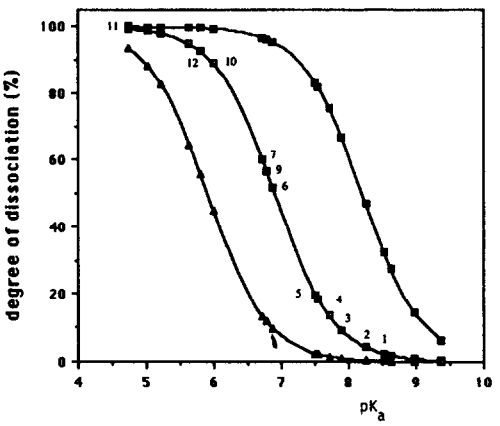

Fig. 1. Calculated degree of dissociation of CPs as a function of pK_a, at different constant pH values: (▲) 5.9; (□) 6.9; (■) 8.2. For solute numbers, see Table I.

TABLE I

pK_a VALUES OF CHLOROPHENOL CONGENERS

Peak No.	Congener	pK_a [2] (25°C)
1	2-Chlorophenol	8.52
[a]	3-Chlorophenol	8.97
[a]	4-Chlorophenol	9.37
4	2,3-Dichlorophenol	7.71
3	2,4-Dichlorophenol	7.90
5	2,5-Dichlorophenol	7.51
9	2,6-Dichlorophenol	6.78
[a]	3,4-Dichlorophenol	8.62
2	3,5-Dichlorophenol	8.25
6	2,3,4-Trichlorophenol	6.87
8	2,3,5-Trichlorophenol	?
12	2,3,6-Trichlorophenol	5.80
7	2,4,5-Trichlorophenol	6.72
10	2,4,6-Trichlorophenol	5.99
[a]	3,4,5-Trichlorophenol	7.55
[a]	2,3,4,5-Tetrachlorophenol	5.64
[a]	2,3,4,6-Tetrachlorophenol	5.22
13	2,3,5,6-Tetrachlorophenol	5.03
11	Pentachlorophenol	4.74
Average, monoCPs + diCPs		8.18
Average, triCPs + tetraCPs + pentaCP		5.95
Average, thirteen congeners tested		6.82

[a] Not tested in this study.

tions were therefore performed using a phosphate buffer at pH 6.9 corresponding to the pK_a of $H_2PO_4^- - HPO_4^{2-}$. Sodium salts were preferred to potassium salts, in agreement with Issaq *et al.* [25], who demonstrated that sodium phosphate buffers allow shorter migration times and better resolution and selectivity than potassium phosphate buffers of the same concentration and pH. Further, among commonly used buffer systems, phosphate buffer is the best known in capillary electrophoresis [11–13].

Fig. 2 illustrates the separation of thirteen CPs at pH 6.9. The reciprocals of the migration times of each ion were further plotted *versus* α for five different chlorophenols, at seven different buffer concentrations (Fig. 3). The experimental plots consist of straight lines with good correlation coefficients, as can be seen in Table II. The extrapolated values of $1/t_m$ at $\alpha = 0$ were used to calculate the migration times of a theoretical

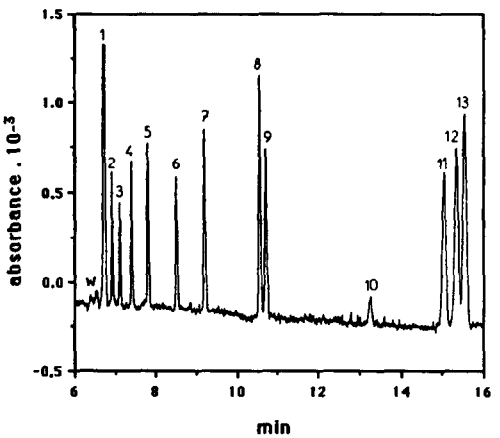

Fig. 2. Zone electrophoretic separation of thirteen CPs. Column, 57 cm × 75 μm I.D. fused-silica tube; buffer, $[Na_2HPO_4] = [NaH_2PO_4] = 50$ m*M* (pH 6.9); applied voltage, 18 kV; current 172 μA. For peak identification see Table I (w = water).

electroosmotic flow marker (t_{eo}). The extrapolated values of t_{eo} are listed in Table II and experimental values of t_{eo} are given for comparison. From these results, it is readily apparent that the experimental t_{eo} has been systematically underestimated, the meaning of which is not clear. In this study, the electroosmotic flow marker was pure water, also contained in the samples: the water plug is detected by the UV cell as a result of local variation of the refractive

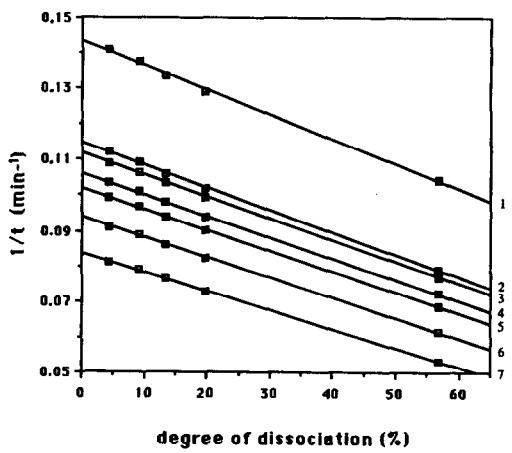

Fig. 3. Reciprocal of migration times as a function of the degree of dissociation of five different dichlorophenols at seven different buffer concentrations: $[Na_2HPO_4] = [NaH_2PO_4]$ = (1) 10; (2) 25; (3) 30; (4) 35; (5) 40; (6) 50; (7) 75 m*M*. Constant pH, 6.9; constant applied voltage, 12 kV.

TABLE II

REGRESSION PARAMETERS FROM FIG. 3

Buffer concentration (mM)	r	Slope (10^{-4} min^{-1})	t_{eo} (min) (extrapolated value)	t_{eo} (min) (experimental)
10	−0.99901	−6.9242	6.980	7.002
25	−0.99955	−6.3169	8.719	8.537
30	−0.99968	−6.0860	8.957	8.697
35	−0.99966	−5.9710	9.421	9.197
40	−0.99972	−5.8627	9.810	9.477
50	−0.99976	−5.6780	10.655	10.293
75	−0.99964	−5.3243	11.968	11.330

index of the medium. Hence this water plug appears as a small peak on the electropherogram. Finally, the suitability of this marker is made questionable by these last results, and one should be cautious when using this method. Moreover, it must be remembered that a small error in t_{eo} determination may greatly influence the electrophoretic mobility (μ_{ep}) calculation. This is more critical when t_m is closed to t_{eo}

$$\mu_{ep} = \mu_{app} - \mu_{eo} = (1/t_m - 1/t_{eo})L'L/V \quad (9)$$

where μ_{app} is the apparent mobility of the analyte, L' is the distance along the capillary between the sample introduction point and the detector and L is the total length of the capillary.

On the basis of eqn. 8 and 9, the linear plots observed in Fig. 3 should be interpreted as a linear relationship between μ_{ep} and α, as the radii (r) and $f(\kappa r)$ functions of the different diCPs are assumed to be nearly identical.

In addition, it is of interest to compare the behaviour of the diCPs and the triCPs. For example, it can be seen in Fig. 4 that 2,6-di-CP ($pK_a = 6.78$) has a larger migration time than 2,4,5-tri-CP ($pK_a = 6.72$). It is readily apparent that the two solutes are well resolved, despite a very minor discrepancy in their pK_a values. This can be explained by the fact that, in this case, the separation is primarily based on size rather than on charge.

Finally, it can be recognized from the results presented here that the migration order of CPs is based on both the solute pK_a value and size, that is, on the number of chlorine atoms on the aromatic ring.

Effect of buffer concentration on selectivity and resolution

Tsuda *et al.* [26] demonstrated ten years ago that the electrophoretic mobility and its complementary electroosmotic mobility should be inversely proportional to the square root of ionic strength. These results were later confirmed by Issaq *et al.* [27] and Atamna *et al.* [28]. Theoretical justifications for the dependence of μ_{ep} on buffer concentration can be summarized by the following equations.

According to Hunter [24], the reciprocal of the

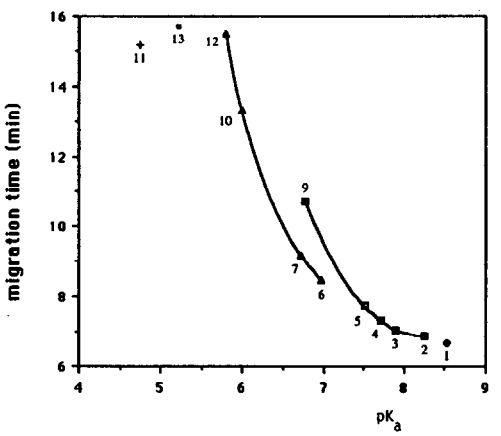

Fig. 4. Dependence of migration times of twelve CPs on their pK_a values. For solute numbers, see Table I. Conditions as in Fig. 2. ◇ = 2-CP; □ = diCPs; ▲ = triCPs; ■ = 2,3,5,6-tetraCP; + = pentaCP.

analyte double layer thickness (κ) may be given as

$$\kappa = (2000F^2/\epsilon RT)^{1/2} I^{1/2} \quad (10)$$

where F is the Faraday constant, R is the perfect gas constant, T is the temperature and I is the ionic strength, defined by

$$I = \tfrac{1}{2} \sum C_i Z_i^2 \quad (11)$$

where C_i is the concentration of ion i with a charge Z_i.

From eqns. 10 and 11, κ is proportional to the square root of the buffer concentration, if T and ϵ are kept constant: $\kappa = aC^{1/2}$, where a is a constant. As a result, eqn. 8 may be rewritten to express μ_{ep} as a function of C as

$$\mu_{ep} = \frac{e\alpha f(aC^{1/2}r)}{6\pi\eta r(1 + aC^{1/2}r)} \quad (12)$$

In practice, one could make the assumption that $f(\kappa r)$ is relatively unaffected by concentration changes [24]. It can then be expected that the plot of α/μ_{ep} ratio *versus* $C^{1/2}$ should be linear, assuming that r is constant for a given series of CPs, *e.g.*, dichlorophenols:

$$\alpha/\mu_{ep} = m + nC^{1/2} \quad (13)$$

where m and n are constants.

In Fig. 5, the experimental plot consists of a straight line with a correlation coefficient of 0.99814, providing evidence that the previous assumptions were reasonable. Additionally, it can be shown that the five diCPs tested in this experiment show the same electrophoretic behaviour, that is, m and n are identical for these five congeners.

In contrast, it can be demonstrated that triCPs and tetraCPs or pentaCP exhibit different changes in electrophoretic mobilities with buffer concentration. The observed variations could not be accounted for solely by eqn. 12 as CP isomers are not influenced by C to the same extent.

The discrepancy in the behaviour of the different CPs results in a significant improvement of the separation selectivity, especially for the last three peaks (peaks 11, 12 and 13). Similar series of data, obtained at two different voltages, are

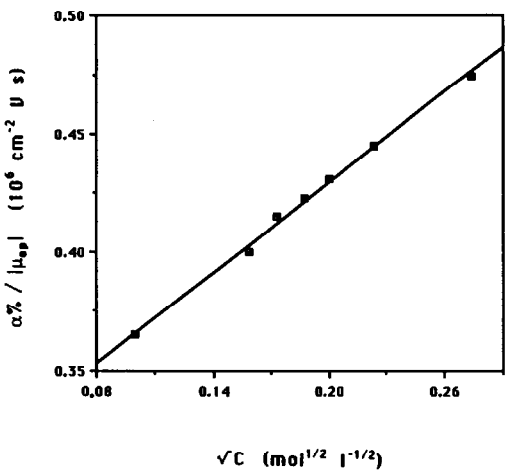

Fig. 5. $\alpha\%/|\mu_{ep}|$ ratio *versus* the square root of buffer concentration. The ratio values were obtained from the slopes of the experimental plots shown in Fig. 3. The extrapolated values of t_{eo} (listed in Table II) were used for calculations. Conditions as in Fig. 3.

illustrated in Figs. 6 and 7. Mobility differences $\Delta\mu$ have been calculated using the equation

$$\Delta\mu = \mu_{app,1} - \mu_{app,2}$$

$$= (\mu_{ep,1} + \mu_{eo}) - (\mu_{ep,2} + \mu_{eo})$$

Hence

$$\Delta\mu = \mu_{ep,1} - \mu_{ep,2}$$

which can be modified to

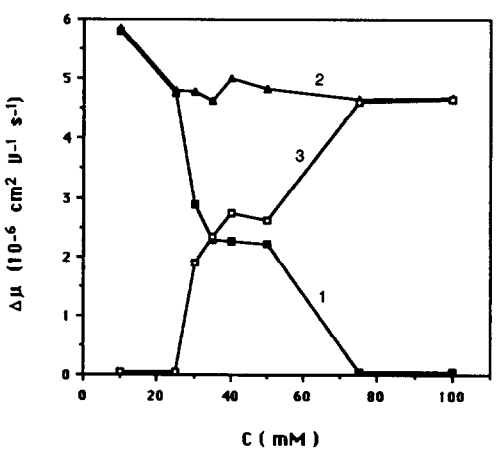

Fig. 6. Mobility difference *versus* buffer concentration. Constant applied voltage, $V = 12$ kV. Other conditions as in Fig. 2. $1 = \mu_{11} - \mu_{12}$; $2 = \mu_{11} - \mu_{13}$; $3 = \mu_{12} - \mu_{13}$ (subscript numbers refer to peak numbers in Table I).

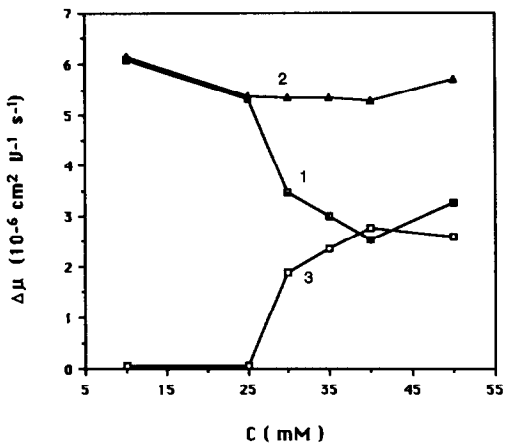

Fig. 7. Mobility difference *versus* buffer concentration. Constant applied voltage, $V = 18$ kV. Other conditions as in Fig. 2. Symbols as in Fig. 6.

$$\Delta\mu = (1/t_{m,1} - 1/t_{m,2})L'L/V \quad (14)$$

It can be noted that $\Delta\mu$ is insensitive to t_{eo} determination.

As shown in Fig. 6 or 7, the $\mu_{11} - \mu_{13}$ difference is independent of the buffer concentration although the migration of solute 12 is greatly influenced. At low concentrations, solutes 12 and 13 co-migrate ($\Delta\mu = 0$) whereas, as the concentration increases, peak 12 moves towards peak 11. It can thus be demonstrated that the best separation can be achieved at an optimum buffer concentration 40 mM at $V = 12$ kV and 50 mM at $V = 18$ kV.

R_s is proportional to both $(\mu_{ep,1} - \mu_{ep,2})$ and $(\bar{\mu}_{ep} + \mu_{eo})^{-1/2}$ (eqn. 2). This is the reason why many workers [22,29,30] have predicted that maximum resolution is obtained when the the electroosmotic flow is opposite and equal to ion migration. This can be carried out by lowering electroosmosis, by increasing the buffer ionic strength. An improvement in resolution will be obtained, however, at the expense of a large increase in analysis time, as the migration time (t_m) is defined as

$$t_m = \frac{L'L}{(\mu_{ep} + \mu_{eo})V} \quad (15)$$

Other workers [27,28] have recently pointed out that this argument cannot be taken as a systematic approach to improving resolution. In the present case, Fig. 6 clearly shows that an increase in buffer concentration results in co-migration of solutes 11 and 12. Hence the use of the more concentrated buffer does not systematically provide a significant improvement in resolution.

Applied voltage and heat dissipation

In most CZE separations, it can be observed that the ideal situation is obtained by applying as high a voltage as possible. This leads to the highest separation efficiency in the shortest time. However, the principal difficulty with this approach lies in the limited ability to dissipate the heat generated during the electrophoretic process. Indeed, numerous workers [22,26,27,31–33] have reported that μ_{app} (or V_{app} or $1/t_m$) deviates positively at high values of E. This effect is attributed to temperature-induced buffer viscosity changes, as a consequence of Joule heat dissipation. According to a generally accepted value of 2%/C for the decrease in water viscosity [22], in-column temperature increases (ΔT) have been estimated (Table III) from the non-linear dependence of the current on the applied voltage (Fig. 8). By analogy with the expression developped by Vindevogel and Sandra [33], ΔT can be calculated from the following expression

$$\Delta T(°C) = \frac{(I_m/I_{th}) - 1}{2\%} \quad (16)$$

where I_m is the current measured for voltage V and I_{th} is the theoretical current, calculated from the assumption of a linear relationship between I and V. With a 50 mM phosphate buffer, I_{th} can be expressed as

$$I_{th} = I_{9\,kV}V_{kV}/9\,kV \quad (17)$$

where $I_{9\,kV}$ is the experimental current corresponding to $V = 9$ kV, taken as a reference value (ΔT is there considered to be insignificant).

Estimated values of ΔT, calculated from data in Fig. 8, are listed in Table III. Results obtained with a borate buffer are given for comparison, but it should be remembered that the ionic strength is different in phosphate and borate buffers. Dissipated powers were calculated using the equation $P = VI/L$. As the upper power limit

TABLE III

ESTIMATED IN-COLUMN TEMPERATURE INCREASE AND DISSIPATED POWER AS A FUNCTION OF APPLIED VOLTAGE

Conditions as in Fig. 8.

V (kV)	Phosphate buffer		Borate buffer	
	ΔT (°C)	P (mW/cm)	ΔT (°C)	P (mW/cm)
9	0	11.5	0	1.4
12	2.6	21.5	–	–
15	5.3	35.3	0	3.9
18	9.2	54.4	–	–
20	13.9	72.6	0.51	6.9
22	18.9	94.7	–	–
25	–	–	1.2	11.0
30	–	–	2.2	16.2

recommended by the manufacturer is about 50 mW/cm [34], it can be concluded from the data in Table III that, using a 50 mM phosphate buffer, the applied voltage must be kept below 18 kV. Under extreme conditions, *i.e.*, at $I = 245$ μA, the temperature rise ($\Delta T = 18.9$°C) will become high enough to cause zone broadening, or affect the μ_{app} reproducibility. Another undesirable consequence of the increase in the running buffer temperature will be instabilities that can affect the baseline, probably due to solvent degassing. Finally, from these results, it can be suggested that the actual in-column temperature is likely to be enhanced with an increase in the buffer concentration. However, this temperature rise should not be responsible for the variations of selectivity that are observed in Figs. 6 or 7. As the phosphate buffer has a relatively small temperature coefficient (*ca.* −0.0028 pH/°C) [35], it can be concluded that the difference in pH of the buffers of various concentrations (10–100 mM) can be considered to be insignificant. Additionally, temperature-induced shifts in the solutes pK_a values is not a dominant factor for acting on the selectivity {*e.g.*, $d(pK_a)/dT = -0.013°C^{-1}$ for the phenol [36]}. In fact, it can be seen from ref. 35 that changes in electromigration times of CPs with temperature should be predominantely due to temperature-induced viscosity changes of water. All the solutes being influenced to the same extent, resolution will not be modified by minor temperature shifts.

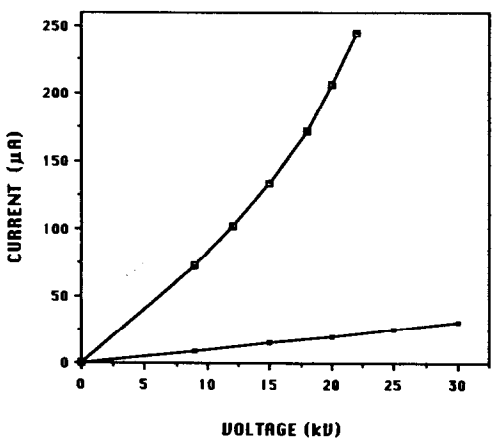

Fig. 8. Current as a function of applied voltage for two different buffers; □ = phosphate buffer (50 mM); ■ = borate buffer (100 mM). Column, 57 cm × 75 μm I.D. fused-silica tube.

Key strategies of optimization

As discussed previously, combined effects of buffer concentration and applied voltage must be taken into consideration for optimizing a separation. Depending on the purpose of the study, a compromise between resolution, power consumption, speed and sensitivity must be achieved.

When coupling CZE with a mass spectrome-

ter, the objectives of the separation will be totally different. Indeed, the resolution of peaks 11, 12 and 13 will no longer be useful, as these three compounds have different molecular masses and will be easily discriminated by MS. In comparison with Fig. 1 ($C = 50$ mM; $V = 18$ kV), it can be deduced from Fig. 9 ($C = 10$ mM; $V = 30$ kV) that a decrease in buffer concentration permits a maximum voltage (the limit of the power supply) to be applied while the current drifts from 172 to 65 μA. This results in a loss of resolution between peaks 12 and 13, but a substantial improvement in both speed and efficiency is obtained. In addition, the baseline in Fig. 9 is more stable than that in Fig. 1.

Quantitative aspects

In Fig. 10, the standard deviations (σ) of corrected peak area, calculated as the average of five successive runs, are plotted *versus* the analyte concentration of various CPs. For $\sigma/\bar{x} = 33\%$ the detection limits are found to be between 60 and 100 μg/l, depending on the CPs tested; for $\sigma/\bar{x} = 10\%$, they are found between 80 and 340 μg/l. Therefore, it must be concluded that the sensitivity of the measurement is poor. This must be accounted for by the diameter of the capillary (75 μm) and the limited performance of the UV detector. Finally, the linearity of the calibration graphs is fairly good between 0 and 1 mg/l (Fig. 11).

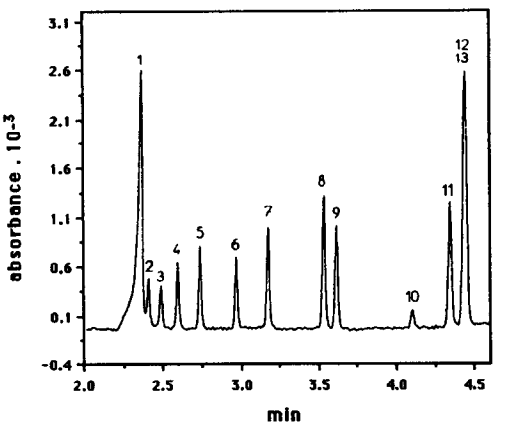

Fig. 9. Zone electrophoretic separation of thirteen CPs. Column, 57 cm × 75 μm I.D. fused-silica tube; buffer: $[Na_2HPO_4] = [NaH_2PO_4] = 10$ mM (pH 6.9); applied voltage, 30 kV; current, 65 μA. For peak identification, see Table I.

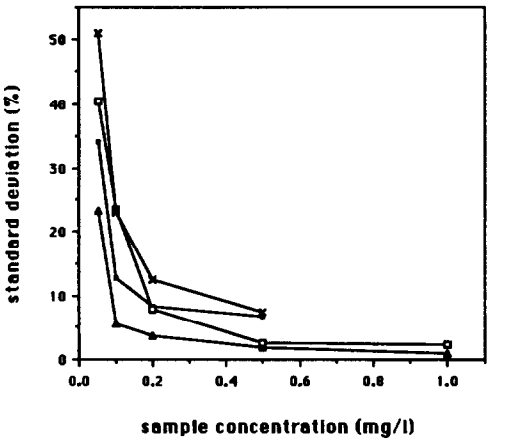

Fig. 10. Standard deviation of corrected peak area *versus* sample concentration. Corrected peak areas (A_c) were calculated from electropherograms, using the Gold software, by the equation $A_c = A_m V_{app}$, where A_m is the measured area and V_{app} is the apparent velocity of the solute. Standard deviations were calculated on the basis of five succeeding runs. Experimental conditions as in Fig. 2. △ = Peak 2; ■ = peak 3; □ = peak 5; × = peak 7 (see Table I).

CONCLUSIONS

The separation of thirteen chlorophenols has been satisfactorily optimized to obtain maximum peak separation in less than 16 min. By coupling CZE with electrospray ionization mass spec-

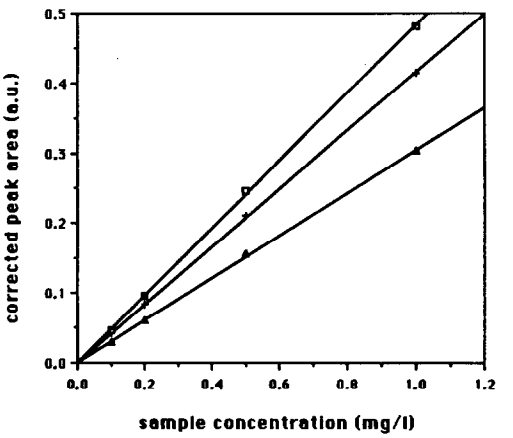

Fig. 11. Calibration graphs: corrected peak area (arbitrary units) *versus* sample concentration. △ = Peak 2; + = peak 4; □ = peak 5 (see Table I).

trometry, it can be expected that the analysis time could be reduced to 5 min. Although this approach seems to be promising, it should be remembered that the theory predicts that the nineteen congeners cannot be easily resolved using a single buffer pH. Therefore, an alternative method is to use two different buffer pH values, yielding two group separations penta-, tetra- and triCPs could be separated at pH 5.9 while mono- and diCPs could be conveniently resolved at pH 8.2. Further, the increase of selectivity due to the pH shift will allow two short and efficient separations, with extreme voltage (30 kV) and minimum buffer concentration (10 m*M*). Because the pH is a dominant factor that could be manipulated to control resolution, this last method should be applicable to complex samples, where inadequate separation is obtained at any single buffer pH.

REFERENCES

1 E.M. Lores, T.R. Edgerton and R.F. Moseman, *J. Chromatogr. Sci.*, 19 (1981) 466.
2 K. Ugland, E. Lundanes, T. Greibrokk and A. Bjorseth, *J. Chromatogr.*, 213 (1981) 83.
3 O. Busto, J.C. Olucha and F. Borrull, *Chromatographia*, 32 (1991) 423.
4 J. Czuczwa, C. Leuenberger, J. Tremp, W. Giger and M. Ahel, *J. Chromatogr.*, 403 (1987) 233.
5 F. Dondi, A. Betti and C. Bighi, *J. Chromatogr.*, 257 (1983) 69.
6 H. A. McLeod and G. Laver, *J. Chromatogr.*, 244 (1982) 385.
7 E. Burtscher, H. Binder, R. Concin and O. Bobleter, *J. Chromatogr.*, 252 (1982) 167.
8 N.G. Buckman, J.O. Hill, R.J. Magee and M.J. McCormick, *J. Chromatogr.*, 284 (1984) 441.
9 F.P. Bigley and R.L. Grob, *J. Chromatogr.*, 350 (1985) 407.
10 C. de Ruiter, J.F. Bohle, G.J. de Jong, U.A.Th. Brinkman and R.W. Frei, *Anal. Chem.*, 60 (1988) 666.
11 C.D. Gaintonde and P.V. Pathak, *J. Chromatogr.*, 514 (1990) 389.
12 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 348 (1985) 39.
13 C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 516 (1990) 263.
14 C.P. Ong, C.L. Ng, N.C. Chong, H.K. Lee and S.F.Y. Li, *Environ. Monit. Assess.*, 19 (1991) 93.
15 Y.F.K. Yik, C.L. Ng, C.P. Ong, S.B. Khoo, H.K. Lee and S.F.Y. Li, *Bull. Sing. N. I. Chem.*, 18 (1990) 91.
16 G.M. Janini and H.J. Issaq, *J. Liq. Chromatogr.*, 15 (1992) 927.
17 F. Garcia and J.D. Henion, *Anal. Chem.*, 64 (1992) 985.
18 M.A. Moseley, J.W. Jorgenson, J. Shabanowitz, D.F. Hunt and K.B. Tomer, *J. Am. Soc. Mass Spectrom.*, 3 (1992) 289.
19 R.L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 489A.
20 R.L. Chien and D.S. Burgi, *Anal. Chem.*, 64 (1992) 1046.
21 J.C. Giddings, *Sep. Sci.*, 4 (1969) 181.
22 J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
23 T.T. Lee and E.S. Yeung, *Anal. Chem.*, 63 (1991) 2842.
24 R.J. Hunter, *Zeta Potential in Colloid Science—Principles and Applications*, Academic Press, New York, 1981, pp. 27, 32 and 71.
25 H.J. Issaq, I.Z. Atamna, C.J. Metral and G.M. Muschik, *J. Liq. Chromatogr.*, 13 (1990) 1247.
26 T. Tsuda, K. Nomura and G. Nakagawa, *J. Chromatogr.*, 264 (1983) 385.
27 H.J. Issaq, I.Z. Atamna, G.M. Muschik and G.M. Janini, *Chromatographia*, 32 (1991) 155.
28 I.Z. Atamna, H.J. Issaq, G.M. Muschik and G.M. Janini, *J. Chromatogr.*, 588 (1991) 315.
29 A.G. Ewing, R.A. Wallingford and T.M. Olefirowicz, *Anal. Chem.*, 61 (1989) 292 A.
30 J.W. Jorgenson and K.D. Lukacs, *Science*, 222 (1983) 266.
31 H.T. Rasmussen and H.M. McNair, *J. Chromatogr.*, 516 (1990) 223.
32 K.D. Altria and C.F. Simpson, *Chromatographia*, 24 (1987) 527.
33 J. Vindevogel and P. Sandra, *J. High Resolut. Chromatogr.*, 14 (1991) 795.
34 *P/ACE System 2000 Instrument Manual*, Beckman Instruments, Palo Alto, CA, 1989.
35 C.W. Whang and E.S. Yeung, *Anal. Chem.*, 64 (1992) 502.
36 D.D. Perrin, B. Dempsey and E.P. Serjeant, in pK_a *Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981, p. 7.