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# Separation of the eleven priority pollutant phenols by capillary zone electrophoresis

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## Abstract

Capillary zone electrophoresis (CZE) provides highly efficient separation of the eleven US EPA priority pollutant phenols. All of these phenols are completely resolved in fewer than 15 min in a 100 cm  $\times$  75  $\mu$ m I.D. uncoated fused-silica capillary at 22.5 kV using a pH 9.8 phosphate–borate buffer. Buffer pH is the most critical parameter controlling resolution and separation time. A simple theoretical treatment greatly simplifies the pH optimization procedure. The effects on the separation of buffer concentration, applied voltage, and sample quantity injected were studied. Good calibration data were obtained for phenol concentrations up to 50 mg/l. Limits of detection for all phenols were less than 1 ppm.

## 1. Introduction

Phenols are important environmental pollutants due to their common use and toxicity. Among the substituted phenols, some chlorinated and nitrated phenols are of special concern. Eleven phenols appear on the US EPA priority pollutant list [1]. The analysis of these compounds has been widely studied using GC and HPLC [2–7].

Capillary electrophoresis has also been employed for the separation of certain phenols. Isomers of chlorinated phenols were separated by both MECC [8] and CZE [9,10]. The electrophoretic behavior of chlorophenol congeners was investigated and the separation was optimized as function of buffer pH, concentration and applied voltage by Gonnord and Collet [10]. Ong et al. [11] separated the eleven priority phenols by

MECC using a 180  $\mu$ m I.D. capillary at 10 kV. Very recently Chao and Whang [12] showed the separation of the eleven priority pollutant phenols as an application of CZE coupled with laser-induced indirect fluorimetry.

pH is a critical parameter in the separation of weakly acidic or basic solutes by CE. The extent of dissociation, which determines the overall electrical charge of the solute, is governed by the pH of the buffer. Thus the selection of buffer pH can greatly affect the separation, especially for mixtures of solutes with a wide range of  $pK_a$ , as is the case for the eleven priority pollutant phenols. Compared with techniques such as HPLC and MECC, a unique feature of CZE is the ability of the operator to predict the dependence of electrophoretic mobility on pH if a few simple parameters are known [10,13]. Thus it is possible to estimate the migration/pH profiles, which greatly simplifies the pH optimization process. Based on a few initial experiments,

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Smith and Khaledi [13] calculated the electrophoretic mobilities of several substituted phenols and optimized the separation pH using a window diagram. The objective of this work was to study the electrophoretic behavior of the eleven priority phenols and to establish a simple analytical method using CZE. The effects of pH, buffer concentration, and applied voltage on the separation were investigated. Under optimized conditions the eleven phenols can be completely resolved in less than 15 min. Quantitative calibration and detection limits were also investigated.

## 2. Experimental

### 2.1. Instrumentation

CZE was carried out on an Isco Model 3850 electropherograph with an adjustable-wavelength UV detector set at 210 nm. An uncoated fused-silica capillary 100 cm long (65 cm from injector to detector) and 75  $\mu\text{m}$  I.D. was used. Sample injection was carried out by applying a vacuum at the outlet buffer beaker for a controlled period of time. The electropherograms were recorded on a Spectra-Physics SP-4600 or a Shimadzu CR-6A integrator.

### 2.2. Chemicals

All the eleven phenols tested in this work were obtained from Aldrich (Milwaukee, WI, USA). HPLC grade water (Fisher Scientific) or distilled, deionized water was used to prepare buffers and samples. The buffers of different pH were prepared as follows:  $\text{NaH}_2\text{PO}_4$ , 47.1 mM and  $\text{Na}_2\text{HPO}_4$ , 2.9 mM, pH 5.6;  $\text{NaH}_2\text{PO}_4$ , 7 mM and  $\text{Na}_2\text{HPO}_4$ , 43 mM, pH 7.6;  $\text{Na}_3\text{PO}_4$ , 12.5 mM and  $\text{Na}_2\text{B}_4\text{O}_7$ , 37.5 mM, pH 9.3;  $\text{Na}_3\text{PO}_4$ , 25 mM and  $\text{Na}_2\text{B}_4\text{O}_7$ , 25 mM, pH 9.8;  $\text{Na}_3\text{PO}_4$ , 30 mM and  $\text{Na}_2\text{B}_4\text{O}_7$ , 20 mM, pH 10.5;  $\text{Na}_3\text{PO}_4$ , 37.5 mM and  $\text{Na}_2\text{B}_4\text{O}_7$ , 12.5 mM, pH 11.4;  $\text{Na}_3\text{PO}_4$ , 50 mM, pH 12.4. All buffers were filtered through 0.45- $\mu\text{m}$  syringe filters and degassed with high purity helium prior to analysis.

Phenol stock solutions were prepared separ-

ately by weighing and dissolving in HPLC grade methanol. A mixture of 11 phenols was prepared by diluting each of the stock solutions in methanol to give a concentration of 100 mg/l. This mixture was stored at 4°C and used to make test solutions by further dilution with the running buffer to the appropriate concentration.

### 2.3. Capillary conditioning

Each day the capillary was first conditioned by filling with 1 M NaOH and soaking for 10 min. The capillary was then washed with deionized water followed by running buffer. At the end of the day the column was washed with water followed by 0.1 M NaOH. The column was left filled with 0.1 M NaOH overnight for the next day's use.

## 3. Results and discussion

### 3.1. Optimization of buffer pH

All the phenols in this study are weak acids. In aqueous solution the phenols are partially dissociated:



$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \quad (1)$$

If such a solution is subject to electrophoresis in the absence of electroosmotic flow, the ionic species  $\text{A}^-$  will migrate at the electrophoretic mobility  $\mu_{\text{ep},\text{A}^-}$  under the electrical field. The electrophoretic mobility of the neutral species  $\mu_{\text{ep},\text{HA}}$  is zero. If the dissociation equilibrium is fast, the overall mobility of this solute can be considered as an weighted average of the mobilities of the two species:

$$\mu_{\text{ep}} = \frac{[\text{HA}]}{[\text{HA}] + [\text{A}^-]} \mu_{\text{ep},\text{HA}} + \frac{[\text{A}^-]}{[\text{HA}] + [\text{A}^-]} \mu_{\text{ep},\text{A}^-} \quad (2)$$

$$= \frac{[\text{A}^-]}{[\text{HA}] + [\text{A}^-]} \mu_{\text{ep},\text{A}^-} \quad (3)$$

$$= \frac{K_a}{K_a + [H^+]} \mu_{ep,A^-} \quad (4)$$

The usefulness of Eq. (4) is that it provides the possibility of calculating the overall mobility of a particular solute, and subsequently predicting separation profiles of the mixture. If the values of  $pK_a$  and  $\mu_{ep,A^-}$  of a solute remain constant over the operating pH range, the overall mobility of the solute is the sole function of pH. If the  $pK_a$  and  $\mu_{ep,A^-}$  of each solute in a mixture are known, the overall mobilities of each solute at any pH can be calculated and separation of the mixture can be predicted.

In aqueous buffers the value of  $pK_a$  can be considered constant if the capillary is well thermostatted to keep the buffer temperature constant. The electrophoretic mobility,  $\mu_{ep,A^-}$ , depends on the charge and size of the solute as well as the concentration and viscosity of buffer [10,14], and can be considered essentially constant if buffers with similar ion compositions and concentrations are used. The  $pK_a$  values are generally available from the literature. The electrophoretic mobilities,  $\mu_{ep,A^-}$ , on the other hand, usually have to be determined experimentally. Thus, as suggested by Smith and Khaledi [13], if the value of  $pK_a$  is available, only one buffer solution is needed to determine values of  $\mu_{ep,A^-}$  which can be used subsequently to predict

the migration behavior of each solute over the entire pH range.

In this study, the relationship between the electrophoretic mobility and pH for each phenol was calculated as discussed above. The values of  $pK_a$  were taken from the literature [11,15]. The  $\mu_{ep,A^-}$  of each solute was determined by measuring the apparent migration time ( $t_{app}$ ) in the buffer of pH 11.4 and subtracting the electroosmotic migration time,  $t_{MeOH}$ . The following equation was used:

$$\mu_{ep,A^-} = -(1 + [H^+]/pK_a)[(1/t_{app}) - (1/t_{MeOH})]lL/V \quad (5)$$

where  $l$  is 65 cm, the effective column length;  $L$  is 100 cm, the total column length; and  $V$  is 20 kV, the applied voltage. The negative sign accounts for the fact that the direction of electrophoretic flow of anions is opposite that of electroosmotic flow. The migration time of methanol,  $t_{MeOH}$ , was used to calculate the electroosmotic flow. The values of  $\mu_{ep,A^-}$  and  $pK_a$  are listed in Table 1. This measured  $\mu_{ep,A^-}$  of each solute was then used with Eq. (4) to calculate the overall electrophoretic mobilities at other pH values.

To verify the calculated results, the electrophoretic mobility/pH relationship was determined experimentally using running buffers of

Table 1  
 $pK_a$  Values of phenols and the measured electrophoretic mobilities of A

Peak I.D.	Compound	$pK_a^a$	$\mu_{ep,A^-} (\times 10^{-4} \text{ cm}^2/\text{V s})^b$
a	2,4-Dimethylphenol	10.59	3.98
b	Phenol	9.89	3.64
c	4-Chloro-3-methylphenol	9.54	3.17
d	Pentachlorophenol	4.50	3.35
e	2,4,6-Trichlorophenol	7.42	3.64
f	2,4-Dichlorophenol	7.85	4.04
g	2-Methyl-4,6-dinitrophenol	4.70	3.55
h	2-Chlorophenol	8.48	3.47
i	2,4-Dinitrophenol	4.00	3.87
j	4-Nitrophenol	7.15	4.10
k	2-Nitrophenol	7.23	3.95

<sup>a</sup> From Refs. [11,15].

<sup>b</sup> Measured at pH 11.4.

various pH values. The phenol mixture was separated in each buffer solution under the same experimental conditions. Electropherograms at each pH are shown in Fig. 1. The experimental electrophoretic mobilities were calculated from migration times of each solute using the following equation.

$$\mu_{ep} = -[(1/t_{app}) - (1/t_{MEOH})]lL/V \quad (6)$$

The calculated and the measured curves of  $\mu_{ep}$  versus pH for the eleven phenols are given in Fig. 2. It can be seen that in the pH range of about pH 9–11 all the phenols are separated; the best separation of all phenols was obtained at pH 9.8, and this buffer was used for all following experiments.

Comparison of the calculated and the experimental  $\mu_{ep}$  versus pH plots shows that the electrophoretic behavior of most of the phenols follows Eq. (4). The calculated and experimental order of decreasing mobilities, i.e. the order of the peaks in the electropherogram, are the same at most pH values, although some differences in the absolute mobilities exist. The discrepancies are more obvious in the lower pH region, probably because either the actual  $\mu_{ep,A^-}$  values differ more at low pH values from the values used for calculation, or the migration time reproducibilities are relatively not as good at lower pH values. For the purpose of pH optimization, however, these simple calculations can serve as useful guidelines. The prediction of the electrophoretic mobility/pH relationship can be considered a general method for optimizing pH for the separation of weakly acidic and basic solutes, as long as the ionization equilibrium occurs over a practical pH range. For solutes of unknown  $pK_a$ , measurement of  $\mu_{ep}$  in two buffers of different pH values should allow determination of both  $pK_a$  and  $\mu_{ep,A^-}$ .

For the separation of a complex mixture, both  $pK_a$  and  $\mu_{ep,A^-}$  need be considered, as shown in Eq. (4). At pH near  $pK_a$ , selectivity is determined mainly by the difference in  $pK_a$  between solutes. At  $pH \gg pK_a$ , separation is mainly based on differences in  $\mu_{ep,A^-}$  of each solute. The relative contribution of  $pK_a$  and  $\mu_{ep,A^-}$  to

the mobility at different pH values is easily seen in Fig. 2 for the four chlorinated phenols. Because of the electron-withdrawing effect of chlorine, the order of  $pK_a$  for these phenols is pentachloro- < trichloro- < dichloro- < chlorophenol. Thus in the lower pH range (6–7.5), the order of the electrophoretic mobilities is opposite to that of the  $pK_a$  (i.e. same order as  $K_a$ ). At  $pH > 9.5$ , the order of electrophoretic mobilities changes completely to the same order as that of the  $\mu_{ep,A^-}$ . Presumably, the more chlorine atoms on the benzene ring, the bulkier the molecule and thus the smaller  $\mu_{ep,A^-}$ . This change in migration order with pH is also observed for the nitrophenols. To achieve separation of a pair of solutes, there must be sufficient difference in the  $pK_a$  or the  $\mu_{ep,A^-}$ . If both parameters are similar for the two solutes, adjustment of pH will not significantly improve the separation of the two solutes. For example, a baseline separation of 2-nitrophenol and 4-nitrophenol was rarely achieved over the entire pH range studied. In this case, other means must be employed to differentiate the values of either  $pK_a$  or  $\mu_{ep,A^-}$ , e.g. by the addition of organic buffer modifiers [16] and surfactants.

### 3.2. Effect of buffer concentration

The concentration of buffer can affect the CZE separation in several ways. The electroosmotic flow increases with decreasing buffer concentration, producing a shorter analysis time. On the other hand, a certain minimum buffer concentration is necessary for uniform distribution of the electrical field and for adequate buffering capacity. Higher buffer concentrations are also required to minimize solute adsorption on the capillary wall. However, increasing the buffer concentration is limited by Joule heating. The optimum buffer concentration has to be determined experimentally.

In this study, pH 9.8 buffers of various concentrations in the range 10–50 mM were prepared by diluting the stock pH 9.8 buffer solution. At pH 9.8, the slowest migrating three pairs, 2,4-dichlorophenol/2-methyl-4,6-dinitrophenol, 2-chlorophenol/2,4-dinitrophenol, and

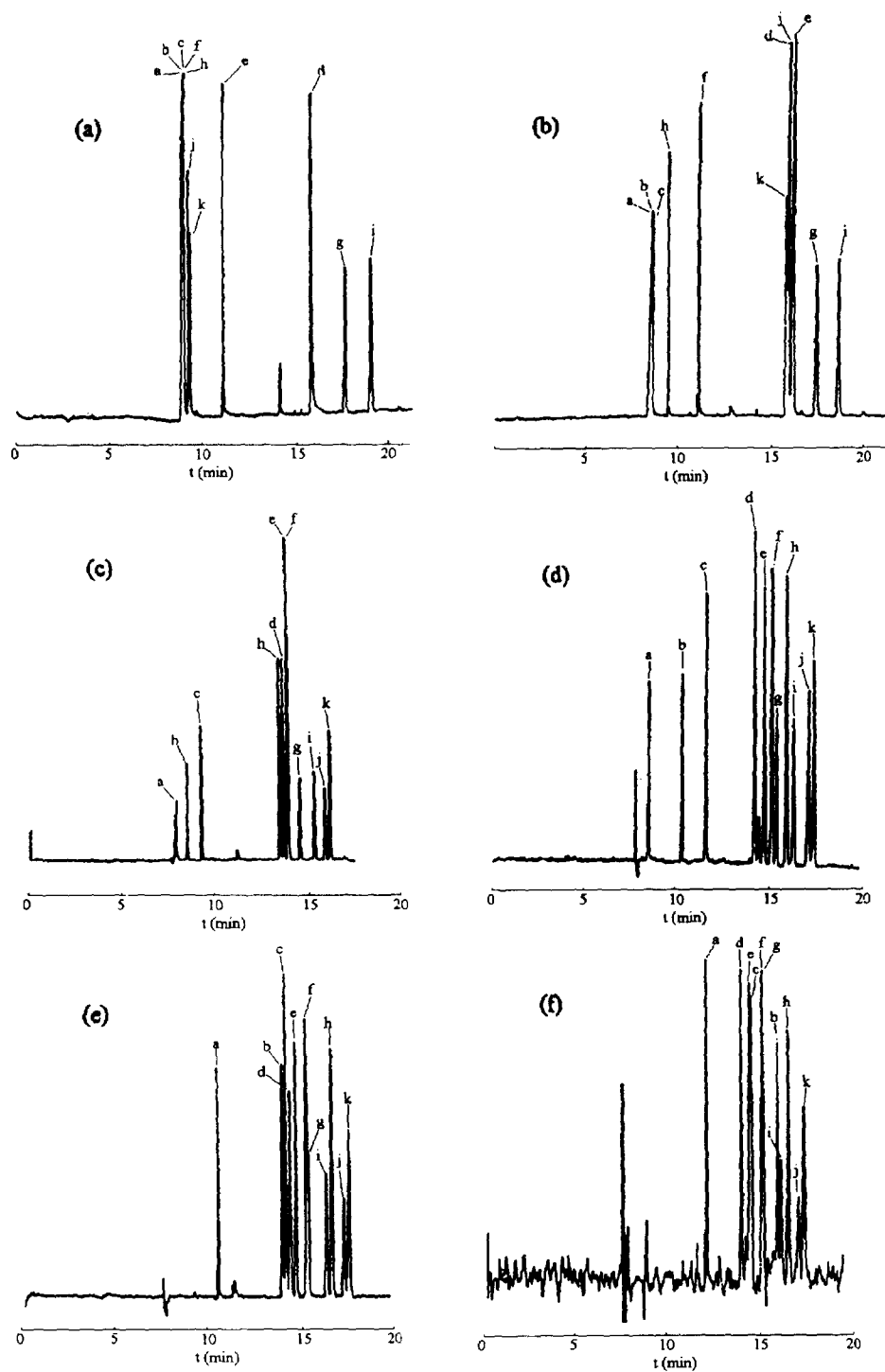


Fig. 1. Electropherograms of the eleven priority phenols by CZE at six different pH buffers: (a) pH 5.6; (b) pH 7.6; (c) pH 9.1; (d) pH 9.8; (e) pH 10.5; (f) pH 11.4. All buffer concentrations are half of the stock buffer concentrations given in the Experimental section. Peak identifications as in Table 1.

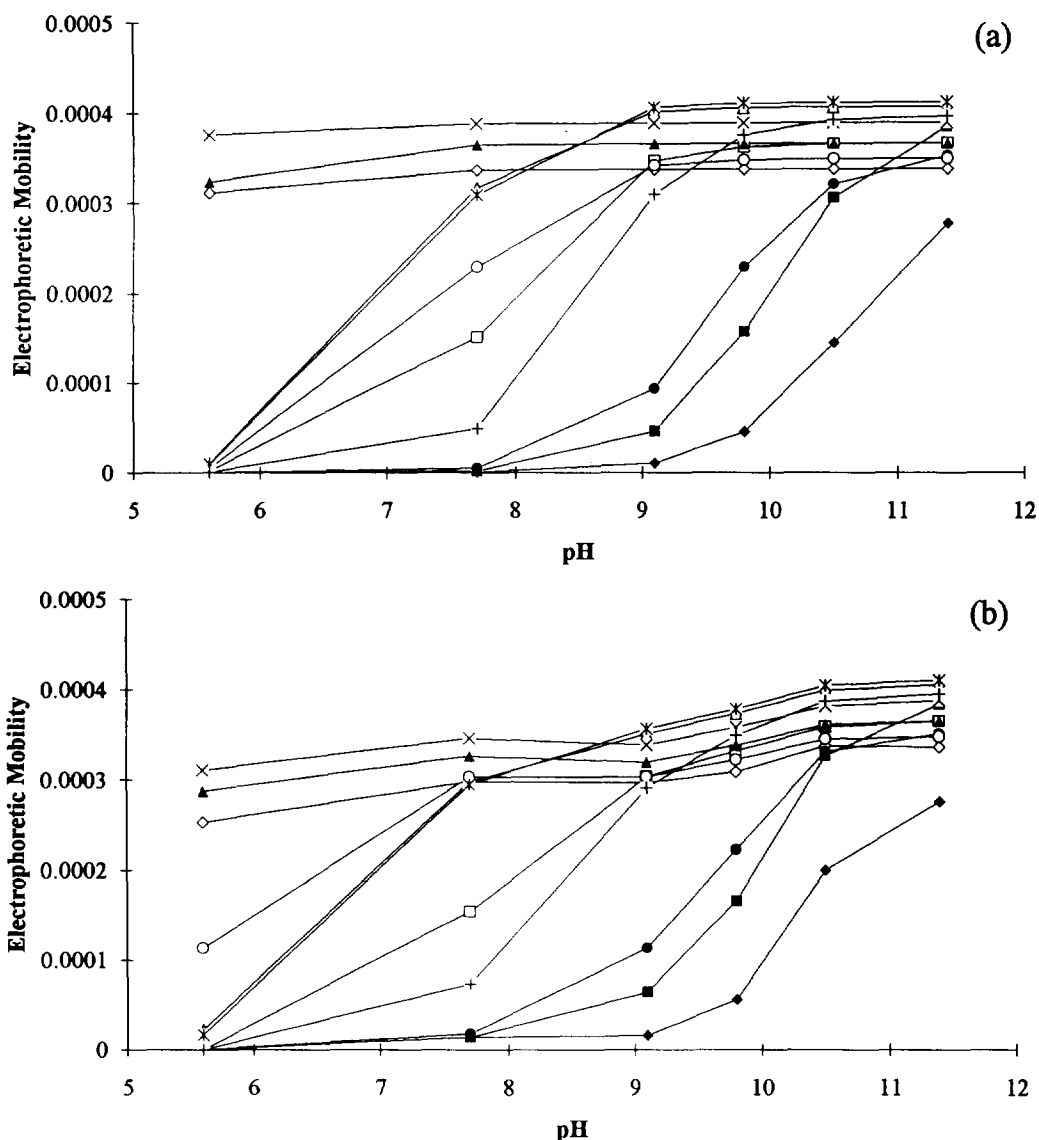


Fig. 2. Dependence of overall mobility on pH: (a) calculated; (b) experimental. Units of mobility:  $\text{cm}^2/\text{V s}$ . (■) Phenol, (□) 2,4-dichloro-, (◆) 2,4-dimethyl-, (◇) pentachloro-, (▲) 2-methyl-4,6-dinitro-, (△) 4-nitro-, (●) 4-chloro-3-methyl-, (○) 2,4,6-trichloro-, (×) 2,4-dinitro-, (\*), (+) 2-chloro-.

4-nitrophenol/2-nitrophenol, have relatively small resolution values,  $R_s$ . The resolution of these three pairs was studied as a function of buffer concentration. Fig. 3a shows a plot of  $R_s$  versus buffer concentration.  $R_s$  decreases with increasing buffer concentration, more rapidly at concentrations higher than 20 mM. However, all

three pairs show baseline separation ( $R_s > 1.4$ ) at buffer concentrations up to 30 mM.

The relationship of current and buffer concentration is shown in Fig. 3b. Theoretically the current should increase linearly with buffer concentration. However, positive deviation of current from linearity was observed at buffer con-

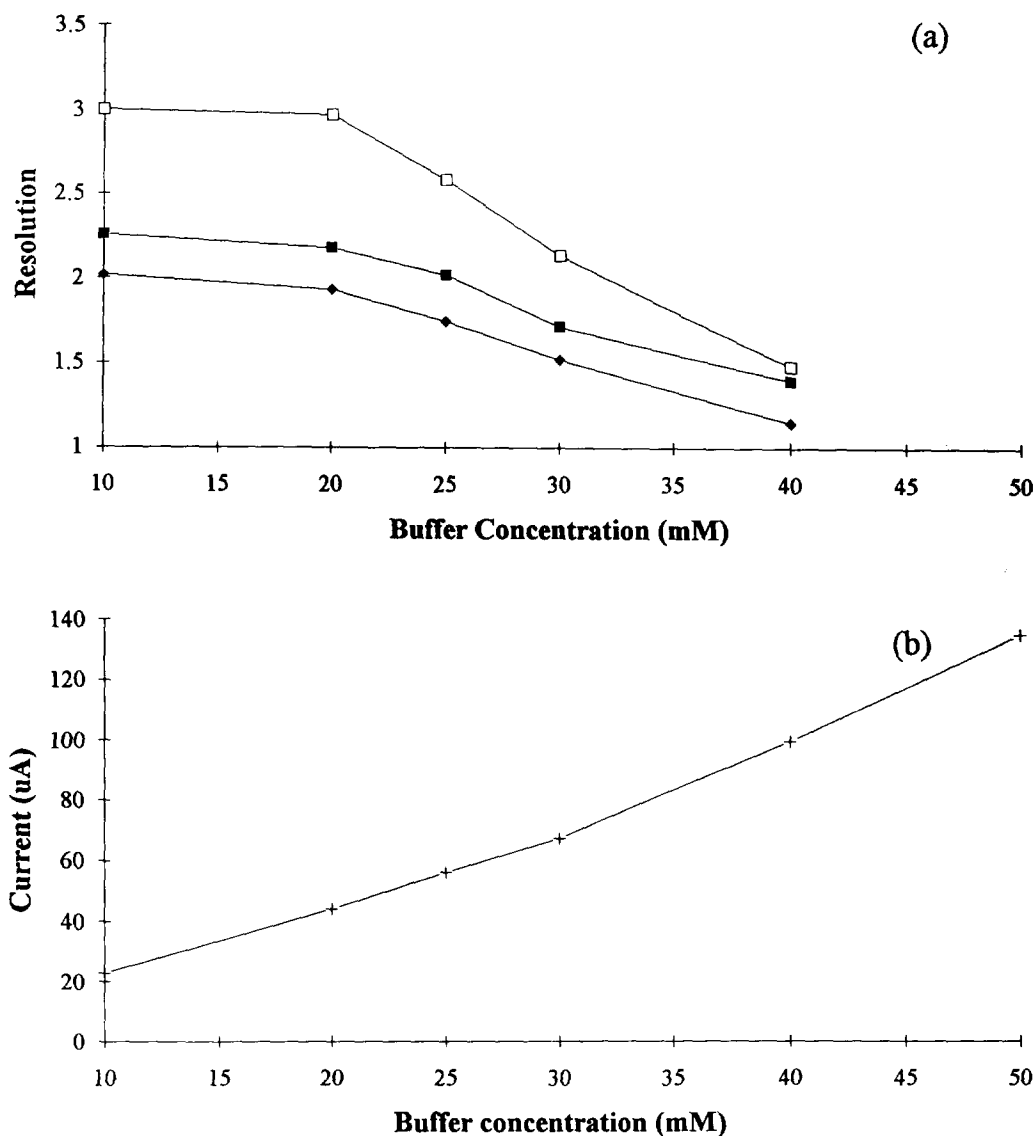


Fig. 3. The effect of buffer concentration on resolution and current: (a) resolution versus buffer concentration; (b) current versus buffer concentration. (■) 2,4-Dichloro-/2-methyl-4,6-dinitro-, (□) 2-chloro-/2,4-dinitro-, (◆) 4-nitro-/2-nitro-.

centrations higher than 30 mM, probably because of Joule heating.

### 3.3. Effect of applied voltage

In principle the plate number in CE is proportional to the applied voltage [17]. However, increase in the applied voltage is limited by Joule

heating which results in zone broadening and decrease of efficiency. An optimum voltage range exists within which higher efficiency and shorter analysis times can be achieved without generation of detrimental amounts of heat.

For the phenol separation, the voltage range studied was 10–30 kV. The theoretical plate number for each solute was measured for each

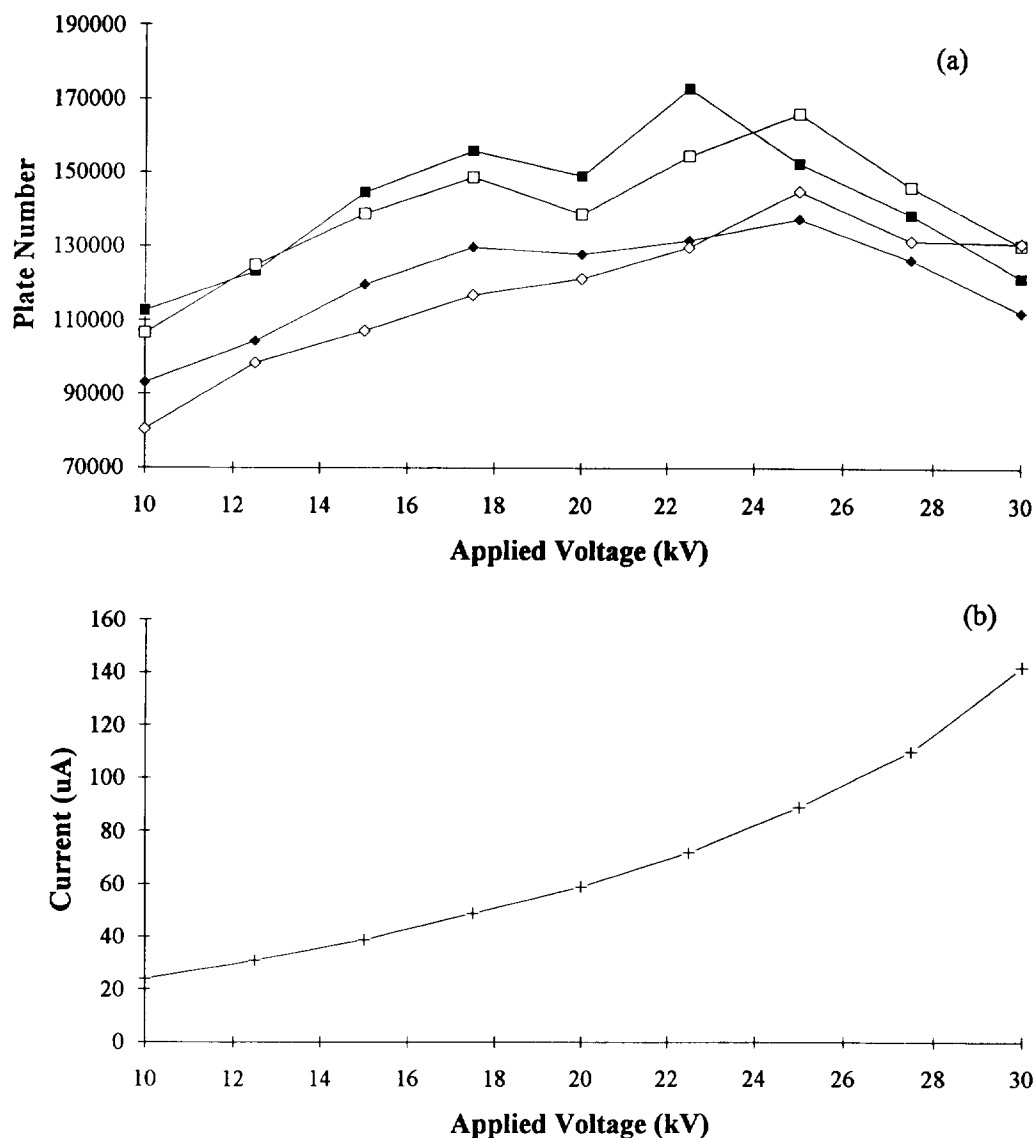


Fig. 4. The effect of applied voltage on column efficiency: (a) plate number versus applied voltage; (b) current versus applied voltage. (□) Pentachloro-, (■) 4-chloro-3-methyl-, (◆) 4-nitro-, (◇) 2-nitro-.

phenol at each applied voltage. Representative results are shown in Fig. 4a. The theoretical plate numbers roughly increase with increasing applied voltage, pass through a maximum near 22.5 kV, and then decrease with a further increase in voltage. Again, the current–applied voltage curve in Fig. 4b shows a nonlinear increase in current for voltages higher than about 20 kV because of Joule heating.

### 3.4. Effect of sample injection time

Samples were injected by applying vacuum at the column outlet buffer beaker. The amount injected depends on the strength of the applied vacuum, the capillary length and I.D., and the sample viscosity. Within limits, peak height increases with the absolute amount of sample introduced; however, the maximum allowable



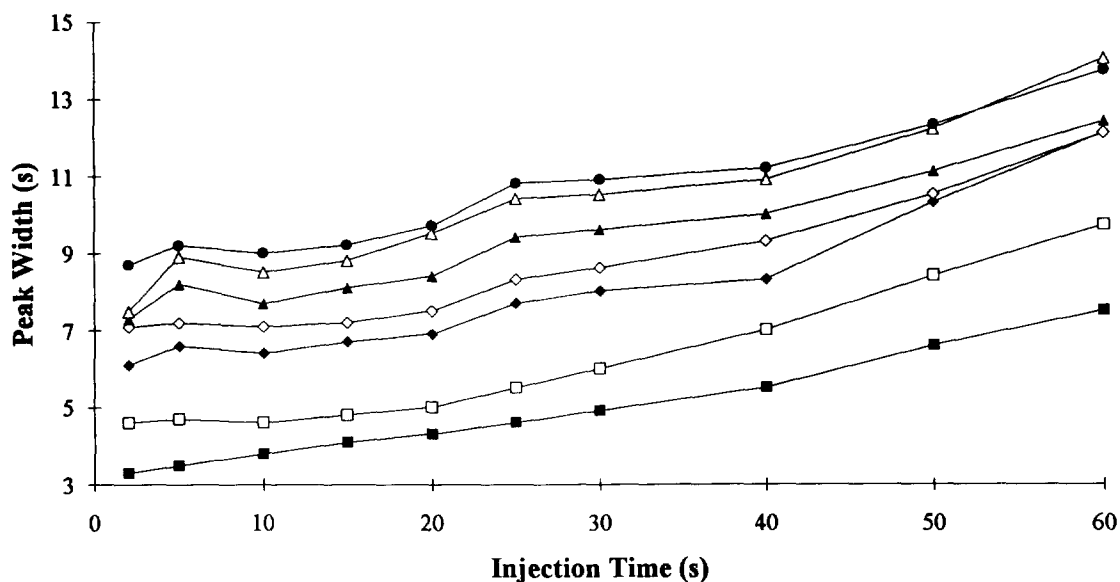


Fig. 5. The dependence of peak width on sample injection time. (■) 2,4-Dimethyl-, (□) 4-chloro-3-methyl-, (◆) pentachloro-, (◇) 2,4-dichloro-, (▲) 2,4-dinitro-, (△) 4-nitro-, (●) 2-nitro-.

sample volume introduced using vacuum or pressure injection is limited by the initial band width. When this initial band width becomes a significant factor in overall band broadening, any further increase in injection time will be detrimental to the separation.

Vacuum injection times were varied from 2 to 60 s. The resulting peak widths of several phenols are plotted in Fig. 5. The peak widths of all solutes increase with injection time. However, the rate of increase of peak widths with injection time was relatively small for injection times less than 20 s. With some sacrifice of peak width, the injection time could be made as long as 40 s. For injection times larger than 40 s, dramatic increases of peak widths were found. Thus injection times were restricted to 20 s or less.

### 3.5. Optimized separation conditions

Taking into consideration the experimental results described above, the optimum conditions for the separation of the eleven phenols were determined to be pH 9.8 buffer, with

$[\text{Na}_3\text{PO}_4] = [\text{Na}_2\text{B}_4\text{O}_7] = 10 \text{ mM}$ , applied voltage 22.5 kV, current  $53 \mu\text{A}$ , solute concentration 25 mg/l, and vacuum injection time 10 s. The electropherogram obtained using these conditions is shown in Fig. 6. The eleven phenols are separated in less than 15 min.

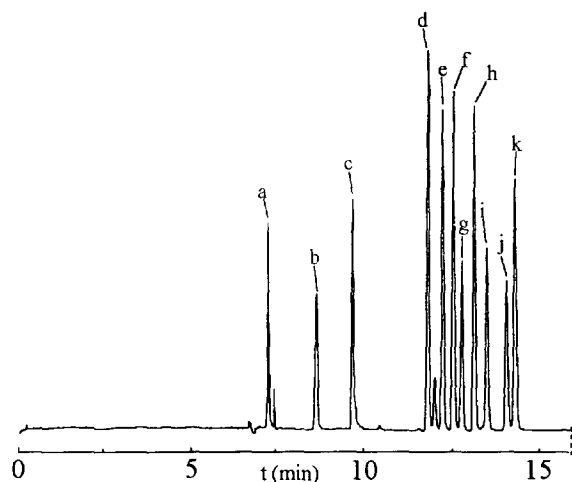


Fig. 6. The CZE separation of eleven priority phenols under optimized conditions. Buffer:  $[\text{Na}_3\text{PO}_4] = [\text{Na}_2\text{B}_4\text{O}_7] = 10 \text{ mM}$  (pH 9.8); applied voltage 22.5 kV; current  $53 \mu\text{A}$ ; solute concentration 25 mg/l; vacuum injection time 10 s.

Table 2  
Regression coefficients ( $r$ ) for calibration curves and limit of detection (LOD)

Compound	$r^a$	LOD (mg/l) <sup>b</sup>
2,4-Dimethylphenol	0.9980	0.33
Phenol	0.9995	0.38
4-Chloro-3-methylphenol	0.9995	0.34
Pentachlorophenol	0.9996	0.27
2,4,6-Trichlorophenol	0.9994	0.29
2,4-Dichlorophenol	0.9993	0.27
2-Methyl-4,6-dinitrophenol	0.9996	0.59
2-Chlorophenol	0.9999	0.27
2,4-Dinitrophenol	0.9970	0.54
4-Nitrophenol	0.9998	0.51
2-Nitrophenol	0.9996	0.32

<sup>a</sup> At five concentration levels ranging from 5 to 50 mg/l, the average of three replicate injections at each level.

<sup>b</sup> Signal-to-noise ratio of 3, the average of three replicate injections.

### 3.6. Quantitation

Phenol mixtures at 5 different concentration levels ranging from 5 mg/l to 50 mg/l were injected in triplicate. The calibration curves for each solute were obtained using the averaged peak areas. The correlation coefficients ( $r$ ) are listed in Table 2. The results show that good linearities can be obtained for concentrations up to at least 50 mg/l. The limits of detection at the wavelength used (210 nm) were found to be between 0.3 mg/l and 0.6 mg/l (signal-to-noise ratio of 3), and are also listed in Table 2.

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