ANALYSIS OF PRIORITY SUBSTITUTED PHENOLS BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract. The use of micellar electrokinetic capillary chromatography (MECC) for the separation of eleven substituted phenols listed by the United States Environmental Protection Agency as priority pollutants was demonstrated. The effects of the pH and ionic strength of the buffer, and the length of the separation column on resolution were investigated. The retention characteristics of the eleven phenols in MECC are discussed.

Introduction

Micellar electrokinetic capillary chromatography (MECC) is a new and powerful type of liquid chromatography based on micellar solubilization phenomena and electrokinetic migrations (Terabe *et al.*, 1984, 1985, 1989). This technique utilizes the difference in the solute distribution between the aqueous phase and the micellar phase to effect separation. It can be used to separate a mixture of ionic and non-ionic substances by changing some of the parameters such as the pH of the buffer solution, the applied voltage, the diameter and the length of the capillary column and the type of modifiers (Terabe *et al.*, 1985). In addition to these fundamental studies, MECC has also been employed as an efficient means for separating a wide variety of compounds of both biological and environmental significance (Otsuka *et al.*, 1985; Otsuka *et al.*, 1985; Burton *et al.*, 1986; Swaile *et al.*, 1988). However, the use of this technique in the analysis of important environmental pollutants has been rarely studied thus far.

Substituted phenols are of great environmental concern due to their high toxicity and wide distribution. They are generated by a number of processes, such as insecticide applications and the chlorination of water. Due to their high toxicity, the USEPA lists eleven of them as priority pollutants. The structure of the eleven phenols are shown in Table I.

Separations of phenolic compounds have been widely studied. The more common separation technique used for the analysis of the eleven phenols is high performance liquid chromatography (HPLC) of which both reversed-phase isocratic and gradient elution analyses have been reported (Abrahamsson *et al.*, 1983; Buckman *et al.*, 1984; Bigely *et al.*, 1985; Li *et al.*, 1988). In this paper, the analysis of the eleven priority phenols by MECC is reported. The effects of varying the pH of the buffer and the length of the capillary on the resolution of the eleven phenols are studied. The retention behaviour of the phenols in MECC is also investigated.

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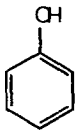
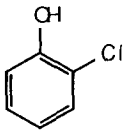
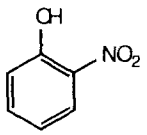
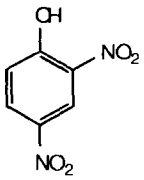
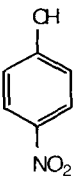
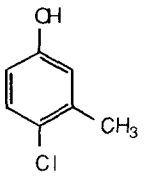
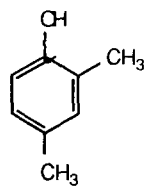
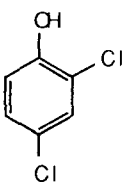
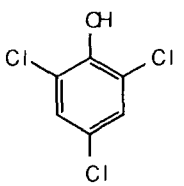
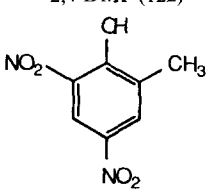
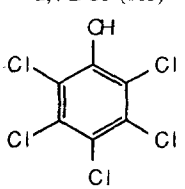
TABLE I

Structures of the eleven phenols studied in this work.

		
PHENOL (94)	2-CHLOROPHENOL 2-CP (128)	2-NITROPHENOL 2-NP (139)
		
2,4-DINITROPHENOL 2,4-DNP (184)	4-NITROPHENOL 4-NP (139)	4-CHLORO-3-METHYLPHENOL 4C,3-MP (142)
		
2,4-DIMETHYLPHENOL 2,4-DMP (122)	2,4-DICHLOROPHENOL 2,4-DCP (163)	2,4,6-TRICHLOROPHENOL 2,4,6-TCP (197)
		
2-METHYL-4,6-DINITROPHENOL 2-M-4,6-DNP (198)	PENTACHLOROPHENOL PCP (266)	

Experimental

The experiments were performed on a home-built MECC instrument. The on-column detection of peaks was carried out on a micro UV detector (Carlo Erba Instruments, Italy) with wavelength set at 254 nm. Fused silica capillary tubes of I.D. 50 μm and 180 μm were used. Two different lengths of the 50 μm I.D. tubes were used for this investigation: 50 cm and 60 cm. The power supply used was a Spellman Model RM 15 KPD unit capable of delivering up to 15 kV. Chromatographic data were collected using a Linear Instruments Corporation (U.S.A.) Model 252A MM chart recorder. All chemicals were of reagent

grade. As a chromatographic solution, 0.05 M sodium dodecyl sulphate (SDS) in phosphate/borate buffer was prepared as previously described (Terabe *et al.*, 1984). The buffer/SDS solutions were filtered before experimental runs. A schematic diagram of the MECC system is shown in Figure 1.

Samples were introduced manually by gravity feed. This was done 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 reservoirs. The injection time was 5 s. The capillary end was subsequently rinsed by dipping into a solution similar to that of the buffer reservoir. It was then transferred back to the buffer reservoir before the power was switched on. The standard mixture consists of the eleven priority phenols whose structures are shown in Table I. The concentration of each phenol in the sample mixture was 1000 ppm. Sudan III was used as the marker for the micelles (Terabe *et al.*, 1984) and its concentration was 100 ppm.

Results and Discussion

The capacity factor used in this investigation, unlike the usual definition in most chromatographic techniques, is calculated using Equation (1) (Otsuka *et al.*, 1985):

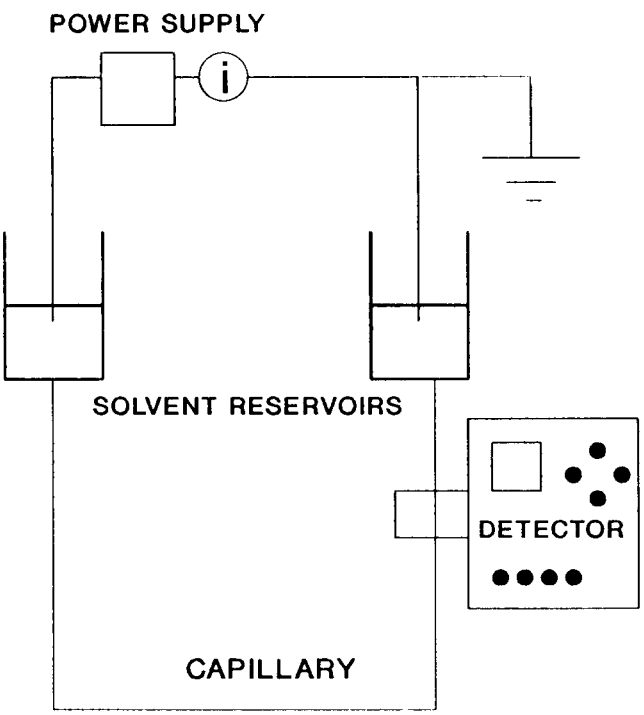


Fig. 1. Schematic diagram of MECC system.

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

where t_r, t_0 and t_{mc} are the elution times of the solute, the insolubized solute and the micelle, respectively. In the experiments, t_0 was regarded as the retention time of methanol and t_{mc} that of Sudan III.

EFFECT OF PH

For the investigation of the effect of pH on the resolution of the phenols in MECC, the experimental conditions listed in Table II were used.

Results were obtained for three pH values: 6.6, 7.0 and 7.5. Figure 2 shows a graphical representation of the experimental values of the capacity factor, $\tilde{k}'$, obtained using the conditions listed in Table II. In Table III, the capacity factors, together with the pK_a values for the phenols are listed. For the sake of clarity in subsequent discussions, the eleven phenols are divided into groups I, II and III as shown in Table III, based on their retention characteristics.

It was found that satisfactory separation could be achieved using pH 6.6. A typical chromatogram for this pH is shown in Figure 3. From Figure 3 it can be observed that the capacity factors for the phenols at pH 6.6 are in general very much higher than those at the other two pHs, i.e. 7.0 and 7.5. We can assume that the degree of ion dissociation is approximately the same at pH 7.0 and 7.5 for the eleven phenols. Therefore, the difference in $\tilde{k}'$ values between them would not be so significant. However, this is not the case for phenols at pH 6.6. At this pH, most phenols are in the neutral state and, therefore, are highly solubilized by SDS. Hence they are retained in the micelles for a longer period of time and this accounts for the large $\tilde{k}'$ values. It was noted that group I phenols has a fairly high pK_a value (6.23–8.55) and thus, at pH 6.6, the compounds in this group were either neutral or partially ionised. In group II, the pK_a values are smaller (4.07–4.70). These compounds can be assumed to be completely ionised at this pH. Being ionised, they were less solubilized by the micelles, and, hence, the $\tilde{k}'$ values for this group would be expected to be smaller than those of group I. However, from Table III, it was observed that the order was reversed. The reason could be due to the fact that group II, being ionised, would be negatively charged. Subsequently, they tend to be attracted to the positive electrode under electrophoretic migration. The direction of this electrophoretic pull is opposed to that of the electroosmotic flow. Hence, the compounds in group II were detected at a

TABLE II

Experimental conditions for the investigation on the effect of pH on resolution.

Length/cm	100
Tubing I.D./μm	180
Power/kV	10
Electrophoretic solution	50 mM SDS in 0.01 M borate/0.005 M phosphate buffer
pH	6.6, 7.0, 7.5
current/μA	130, 145, 158

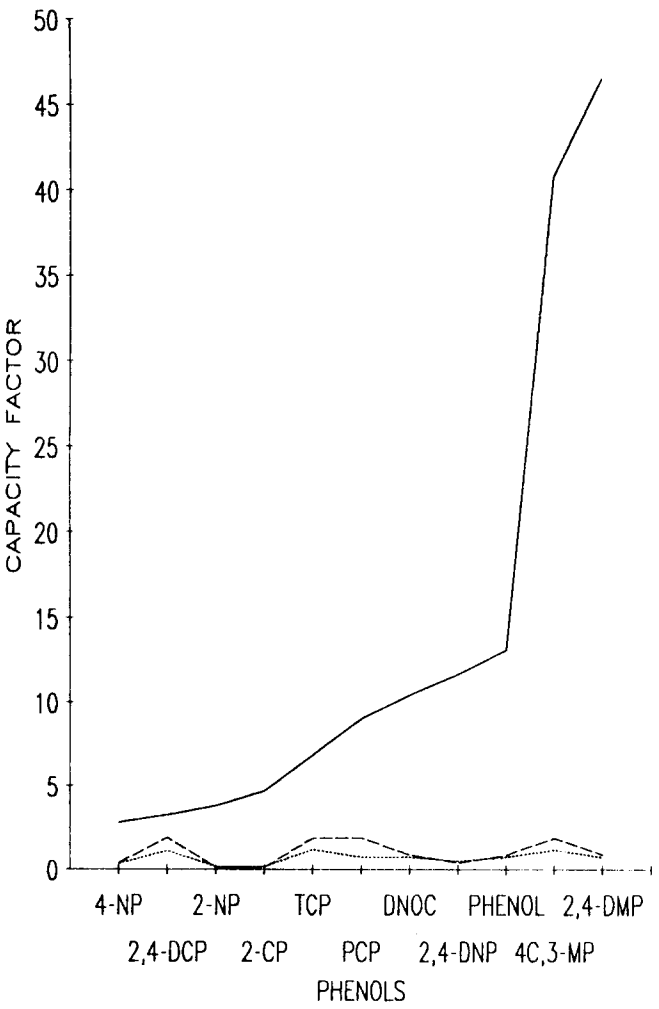


Fig. 2. Variation of capacity factor with pH for each phenol. Experimental conditions as in Table II. Legend: ____ pH 6.6; - - - - pH 7.0; ..... pH 7.5.

later time than compounds in group I.

The compounds in group III (peaks numbered 10–12), having fairly high pK_a values, are in the neutral form. Elution of solutes in group III showed unusual retention order. From their pK_a values, it is expected that their retention time should be comparable to that of group I. At the same time, the retention order for phenol does not seem to be similar to the work carried out by Terabe (Terabe *et al.*, 1984, Otsuka *et al.*, 1985). A possible reason could be that the fused silica tube in this experiment carried undeactivated hydroxyl groups which tend to form hydrogen bonds with phenol which subsequently resulted in its longer retention time. For the other phenols, due to steric hindrance contributed by the

TABLE III

Capacity factors obtained at pH 6.6, 7.0 and 7.5 using the conditions listed in Table II and the pK_a values for the eleven phenols.

Group	Compound	Capacity Factor $\tilde{k}$'			
		pH 6.6	pH 7.0	pH 7.5	pK_a
	Methanol	0.00	0.00	0.00	NA
I	4-NP	2.76	0.359	0.310	7.16
	2,4 DCP	3.23	1.838	1.100	7.89
	2-NP	3.76	0.083	0.107	7.23
	2-CP	4.67	0.083	0.107	8.55
	2, 4, 6-TCP	6.82	1.838	1.174	6.23
II	PCP	8.98	1.838	0.711	4.50
	DNOC	10.39	0.841	0.711	4.70
	2, 4 DNP	11.64	0.355	0.447	4.07
III	PHENOL	13.11	0.841	0.711	10.00
	4C, 3MP	40.72	1.838	1.100	9.54
	2, 4 DMP	46.44	0.841	0.711	10.59

NA: Not Applicable.

substituent groups, hydrogen bonding is not favourable. As a result, they are not affected. As for 4-chloro-3-methylphenol and 2, 4-dimethylphenol, their migration times were found to be exceptionally high. Because each of these two phenols contains a methyl group, there is a possibility of strong interaction between the long alkyl chains of the micelles and the methyl group of the two compounds. As a result, these two phenols were highly solubilized and thus showed high $\tilde{k}$' values.

EFFECT OF COLUMN LENGTH

For the investigation of the effect of the length of the capillary tube on the resolution of the phenols, two different lengths were used. The experimental conditions used are listed in Table IV. The capacity factors and log P values for each phenol are listed in Table V. A plot of capacity factor against each individual phenol for the two lengths is shown in Figure 4. Satisfactory separation was achieved using the 60 cm capillary column. A typical chromatogram is shown in Figure 5.

By comparing the capacity factor values for the two lengths, it is noted that the use of the 60 cm capillary column offers slightly better resolution which was sufficient to separate the last pair of peaks (2, 4, 6-TCP and PCP). Furthermore, it is observed that the retention order is largely governed by the log P values, i.e. hydrophobicity. The larger the log P value, the greater is the extent of solubilization with the micelles. For example, pentachlorophenol (PCP), which is the most hydrophobic among all the eleven phenols, was retained the longest by the micelles and thus, it was the last compound to be eluted.

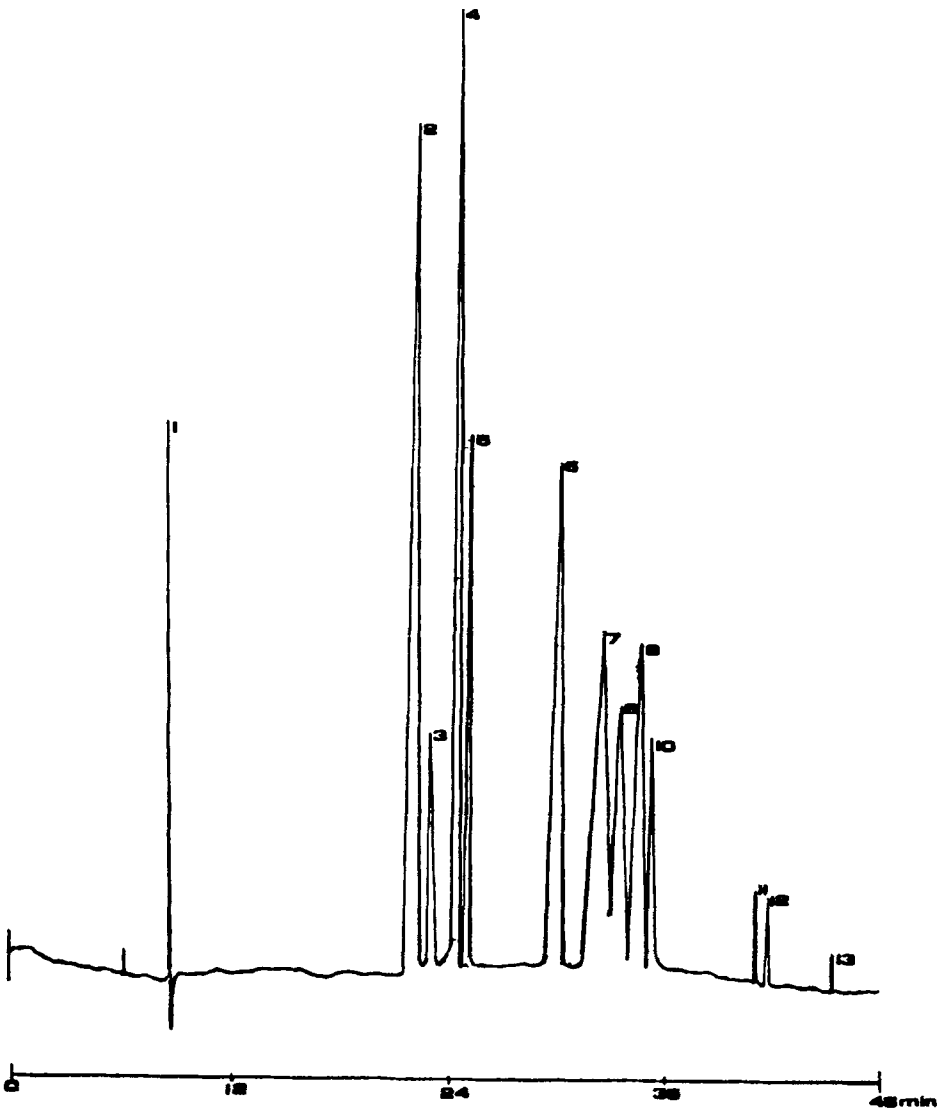


Fig. 3. Electrokinetic chromatogram of the eleven phenols: (1) Methanol; (2) 4-NP; (3) 2, 4-DCP; (4) 2-NP; (5) 2-CP; (6) 2, 4, 6-TCP; (7) PCP; (8) DNOC; (9) 2, 4-DNP; (10) Phenol; (11) 4c, 3-MP; (12) 2, 4-DMP; (13) Sudan III. Electrophoretic solution: 0.05M SDS in 0.01M borate/0.005M phosphate buffer; pH = 6.6; separation tube: 100 cm × 180 μm I.D. fused silica capillary; voltage: 10 kV; current: 130 μA; detection wavelength: 254 nm.

EFFECT OF IONIC STRENGTH

Although no further experiments were carried out to illustrate the effect of ionic strength on resolution, it was noted that when comparing the results in Column 3 of Table III (pH=6.6) and Columns 2 and 3 of Table V (50 and 60 cm), large differences in the retention orders were observed. This is very peculiar since these experiments were carried

TABLE IV

Experimental conditions for the investigation on the effect of length of capillary on resolution.

Tubing I.D/μm	50
Power/kV	15
pH	6.5
Electrophoretic solution	50 mM SDS in 0.1 M Borate/0.05 M Phosphate buffer
Length/cm	50, 60
Current/μA	34, 20

out at the same pH which would therefore suggest that the degree of ionisation for the phenols would be the same. Although the length and applied voltage were different, these factors may not cause large changes in the retention order. The most likely reason for the anomalous retention behaviour could be due to the higher ionic strength used in the latter case (i.e. experiments for the 50 cm and 60 cm length tubes). The greater ionic strength would result in an increase in the concentration of ions in the buffer. Consequently, the higher H^+ concentration in the buffer would tend to suppress the ionization of the weakly ionizable phenols by the common ion effect. As a result, all the phenols are in the neutral form and would be solubilised by the micelles. Hence, their hydrophobicity values dominate their retention order as observed in Table V.

Our results show that micellar electrokinetic chromatography is a relatively simple technique and is one that gives high resolution. This technique also offers great flexibility in that by just varying one parameter, e.g., pH or length of separation column, satisfactory separation of the priority phenols can be obtained. It is believed that the inherently high resolving power of this technique can be exploited for the analysis of other environmental pollutants.

TABLE V

Capacity factors obtained using the conditions listed in Table IV and the log P values for the eleven phenols.

	Capacity factor for length		
Solute	50 cm	60 cm	Log P
Methanol	0.00	0.00	NA
Phenol	0.56	0.53	1.46
4 NP	1.37	1.35	1.96
2 NP	1.59	1.46	1.79
2-CP	1.82	1.77	2.15
DNOC	2.02	1.97	2.77
2, 4-DNP	2.40	2.30	2.29
2, 4-DMP	3.91	3.60	2.42
4C, 3-MP	9.10	7.79	2.95
2, 4-DCP	10.70	9.32	3.08
2, 4, 6-TCP	13.12	11.54	3.77
PCP	13.12	12.82	5.85

NA: Not Applicable.

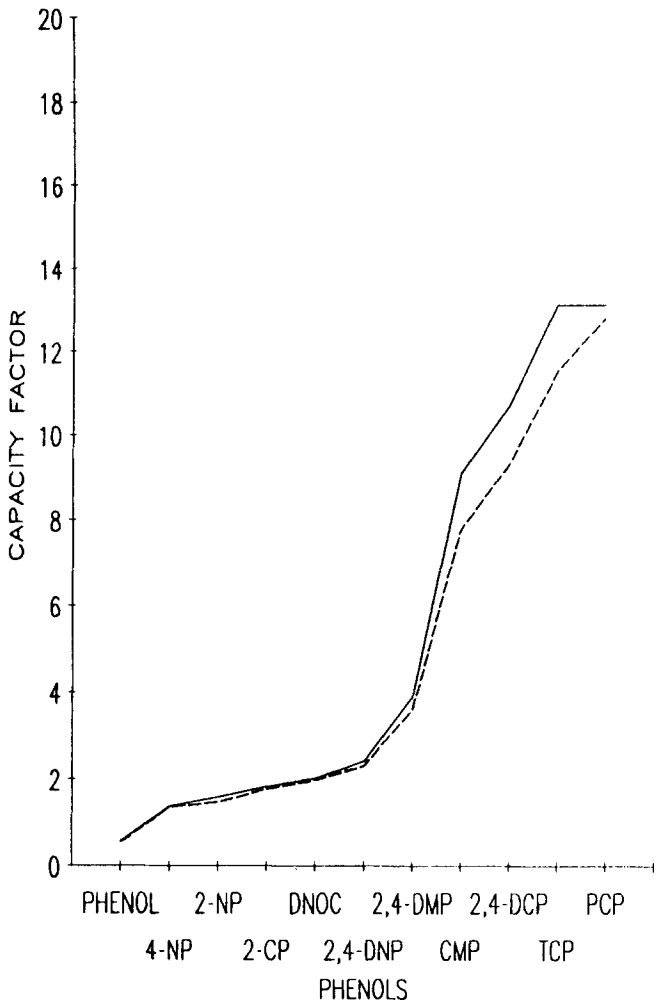


Fig. 4. Variation of capacity factor with length for each phenol. The experimental conditions are stated in Table IV.
Legend: ____ 50 cm; - - - - 60 cm.

Acknowledgment

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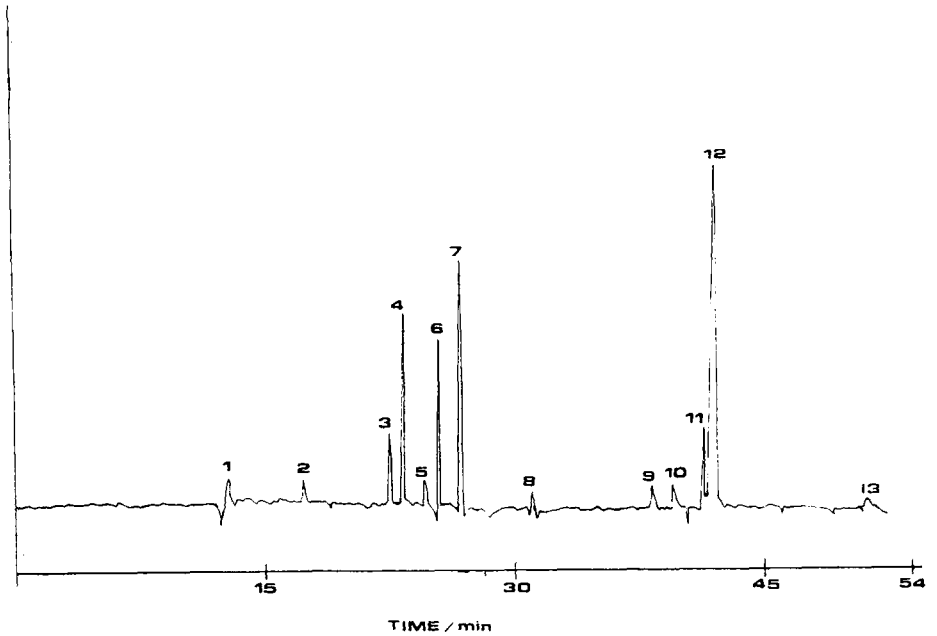

Fig. 5. Electrokinetic chromatogram of the eleven phenols: (1) Methanol; (2) Phenol; (3) 4-NP; (4) 2-NP; (5) 2-CP; (6) DNOC; (7) 2, 4-DNP: (8) 2, 4-DMP; (9) 4C, 3-MP; (10) 2, 4-DCP; (11) 2, 4, 6-TCP; (12) PCP; (13) Sudan III. Electrophoretic solution: 0.05M SDS in 0.1M borate/0.05M phosphate buffer; pH = 6.5; separation tube: 60 cm × 50 μm I.D. fused silica capillary; voltage = 15kV; current = 20 μA; detection wavelength = 254 nm.

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