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The separation and determination of nitrophenol isomers by high-performance capillary zone electrophoresis

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Abstract

o-Nitrophenol, *m*-nitrophenol, and *p*-nitrophenol could well be separated by capillary zone electrophoresis (CZE) by only adjusting the run buffer with methanol. Efficiency up to 10^5 theoretical plates per meter was achieved. The effects of several important factors were investigated to find optimum conditions. The linear range, regression equation, and the recovery were given. This method possessed the advantages of simplicity, rapidity, and good reproducibility; it can be developed for the separation of practical samples in environment analysis.

Keywords: Capillary zone electrophoresis; *o*-, *m*-, *p*-Nitrophenols; Positional isomers

1. Introduction

Nitrophenols, coming from pesticide degradation products, car exhaust, and industrial wastes are listed as priority pollutions by the US Environmental Protection Agency [1,2]. They have great potential toxicities of cancinogenesis, teratogenesis, and mutagenesis [3]. Because of their detriment and vast scale distribution in the ecological environment, their separation and determination have been become one of the important study of environmental analysis. In the present, various methods have been reported for the separation and determination of nitrophenols. However, traditional spectrophotometry [4] and colorimetric method are easily interfered by related compounds. Gas chromatography [5–7] need beneficiation and derivatization before analysis, and it cannot be used directly to aqueous samples. High-performance liquid chromatography (HPLC) [8–15] is a good alternative method, but it need high cost to buy columns and waste more organic solvent.

High-performance capillary electrophoresis is a late-model separate and analytical technique developed in the 1980s. The greatest advantages are its diverse application range, the minimal sample volume requirement, short analysis time, and high separation efficiency. So, it has gained much attention in recent years. A survey of the current literature reveals that application of capillary zone electrophoresis (CZE) for the separation of nitrophenols is very few. Sudor et al. [16] reported the separation of nitrophenols and chlorophenols with a polyacrylamide-coated capillary. Kaniansky et al. [17] proposed an analytical method for the separation of nitrophenols on a fluorinated ethylene propylene (FEP) column and its running buffer containing β-cyclodextrin and polyvinylpyrrolidone (additives) and polyethyleneglycol (anticonvective additive). Another CZE method described by Zhao et al. [18] used *p*-sulfonic calixarene to the running buffer and separated isomers of nitrophenols, benzenediols, and aminophenols individually. It was reported that positional isomers of nitrophenols had not been separated without calyx[4]arene additive in a phosphate buffer [19]. Unfortunately, the detection of nitrophenols in capillary electrophoresis is influenced by the calixarene because of its high ultraviolet (UV) absorption.

In this paper, we reported the separations of nitrophenol isomers using methanol as organic additive in buffer solution without using of the costly calyxarene. This proposed method was successfully applied to separation of nitrophenol isomers with CZE technique. Efficiency up to 10^5 theoretical plates per meter was achieved. The effect of various parameters on the separations was investigated.

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Compared with the pioneer's work, this method not only separated the nitrophenol isomers completely, it could be developed for the separation and determination of nitrophenol isomers in environmental analysis, but also reduced the expense of operation.

2. Experimental

2.1. Apparatus

The CZE measurements were carried out with an HP^{3D} Capillary Electrophoresis System (Agilent Company, USA), equipped with a built-in diode-array detector. All CZE separations were performed in an uncoated silica capillary with 75 μm i.d. and 60.5 cm length, the effective length from the injection point to the detector was 52 cm (Yongnian Optical Fiber Factory, Hebei Province, China). 2001 Ultra-Pure Water System (SG Company, Germany), KQ-50DB Ultrasonic Washing Instrument (Kunshan Ultrasonic Instrument Company, China).

2.2. Reagents and solutions

Methanol was of HPLC grade (Scharlar Chemie S.A.). All other chemicals were of analytical reagent grade (Shanghai, China) and used without further purification. Standard stock solutions of nitrophenols at a concentration of 10^{-2} mol l^{-1} were prepared in a methanolic solutions in brown bottles and stored in a refrigerator at 4 °C. Nitrophenols working standard solutions were prepared by serial dilution of the stock standard solution.

All buffers were prepared from 50 mM sodium borate and 50 mM sodium carbonate stock solutions in different proportion and the appropriate amount of organic solvent. The pH was adjusted by the addition of 0.1 mol l^{-1} NaOH. All solutions were filtered through a 0.45 μm membrane filter and degassed by ultrasound before use. Ultra-pure water was used throughout to prepare solutions.

2.3. Electrophoretic procedure

The new fused silica capillary was first treated by rinsing with methanol for 30 min, followed by 1 mol l^{-1} NaOH for 1 h, then by 1 mol l^{-1} HCl for 1 h, and then by ultra-pure water for 1 h. Every day before experiments, the capillary was flushed with 0.1 mol l^{-1} NaOH for 15 min, then with ultra-pure water for 10 min, and finally with buffer for 20 min. At the end of each day, the capillary was rinsed with 0.1 mol l^{-1} NaOH and ultra-pure water for 10 min each. In between two consecutive runs or when any poor performance (such as poor peak shape or noisy baseline) was observed, the capillary was flushed with running buffer for 5 min in order to keep the capillary wall in good condition.

The capillary was packed in a standard Agilent cassette. Data collection and analysis were conducted using Agilent Chemstation Software. Injection was accomplished by application of 50 mbar pressure to the inlet vial for 3 s. Analyses were conducted in the positive polarity mode at 25 kV. The detection wavelength was set at 191 nm. All experiments were thermostated at 20 °C and measurements were run at least in triplicate.

3. Results and discussion

3.1. Selection of detection wavelength

A suitable detection wavelength was important to detect these nitrophenols. The UV spectra of *o*-, *m*-, and *p*-nitrophenols showed that they all have maximum absorptivity at 191 nm or so. In order to get equivalent sensitivity, 191 nm was selected as experimental detection wavelength.

3.2. Effect of background electrolyte (BGE) and its concentration

The composition and concentration of the BGE plays a central role in CE methods as it determines the fundamental migration behavior of the analytes. A good BGE must guarantee suitable electrophoresis behavior of all individual analytes. In this work, the influence of several kinds of buffers on separation was studied, and sodium borate–sodium carbonate was found to be favorable for this experiment as BGE.

Because the buffer concentration influences the viscosity of the solution, the diffusion coefficient of the analytes, and the zeta-potential of the inner surface of capillary tube, it affects not only the resolution and migration time of the analytes, but also the peak current. The influence of the concentration of running buffer in the range of 10–40 mmol l^{-1} on the separation was examined. It was found that *o*- and *p*-nitrophenol cannot be separated completely when the buffer concentration less than 15 mmol l^{-1}. But higher buffer concentration also have a negative effect on the separation. With the concentration of buffer increasing, high electrophoretic current generated and the effect of Joule heating becomes more pronounced, this in turn results in peak broadening and migration time lengthening. Fig. 1 indicates that migra-

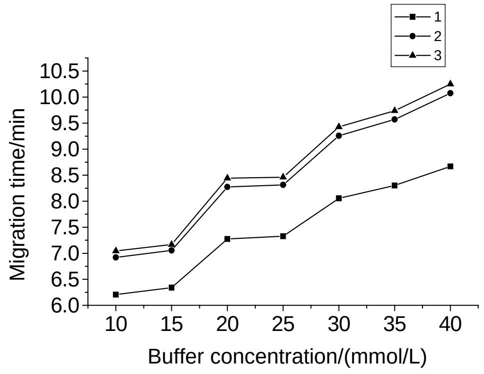

Fig. 1. Influence of buffer concentration on migration time: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol.

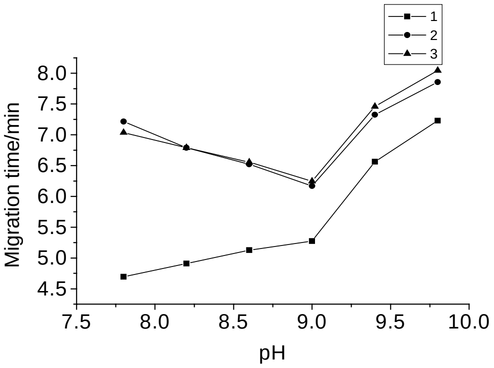

Fig. 2. Effect of pH on the migration time: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol.

tion time increases with increasing buffer concentration. So 20 mmol l^{-1} was chosen as the buffer concentration in this work, on the basis of the peak current, resolution, and analysis time.

3.3. *Influence of buffer pH*

The pH of the electrolyte had a significant impact on the electroosmotic flow. The influence of the pH values on the migration time was examined in the pH range 7.8–9.8, as shown in Fig. 2. The methanolic peak and *m*-nitrophenol peak could not separate as the pH < 7.8, and *o*-nitrophenol overlapped seriously with *p*-nitrophenol by peak broadening as the pH > 9.8. So it was not applicable as the pH $<$ 7.8 or >9.8. Experiment illustrated that when the running buffer pH was in the range of 7.8–8.0 and 9.0–9.6, three nitrophenol isomers could separate completely. But in the former, the peak current was low and the peak shape was broad, relatively. Hence, the pH 9.4 of buffer was found to be optimal, and selected to perform the separation of nitrophenols in the following study.

3.4. *Influence of organic modifier*

For CZE, organic solvent being commonly used to the buffer for improving separation and resolution. In this paper, the methanol as an organic modifier was added to the buffer electrolyte, and the proportion of methanol varied from 5 to 20% (v/v) on the separation was examined. Fig. 3 showed the variation of electrophoretic mobilities of these nitrophenols as a function of the different proportion of methanol modifier. As can be seen, when the proportion of methanol was increased, the resolution improved and the analysis time lengthened. Because the conductivity of the buffer was decreased with increasing of the content of methanol, and therefore a higher separation voltage can be applied without significantly increasing Joule heat during the analysis, thus favored high separation efficiency. However, the electroosmotic flow (EOF) decreased with the addition of methanol. The migration time was very sensitive to the change of EOF, under this operation mode the analytes migrated in the direction opposite to the EOF and they were drawn out by EOF, from our observation, the analysis time increased with increasing the content of methanol. The objective of this work was to find a balance between the resolution and the analysis time. The content of methanol had been optimized at 10% (v/v).

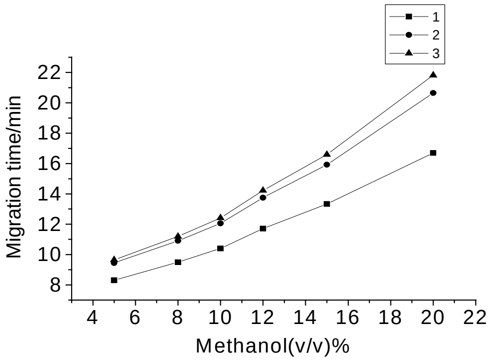

Fig. 3. Effect of the content of methanol on the migration time: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol.

3.5. *Influence of separation voltage*

The separation voltage determines the migration time and resolution of analytes. Fig. 4A depicted the effect of separation voltage on migration time and Fig. 4B illustrated the effect of voltage on the resolution of the least resolved peak pair. The resolution became poor when the separation voltage was less than 15 kV or exceeded 27 kV. Increasing the voltage resulted in shorter migration times, but also increased the base line noise, resulting in poorer detection limits. It was found that too high separation voltages were not

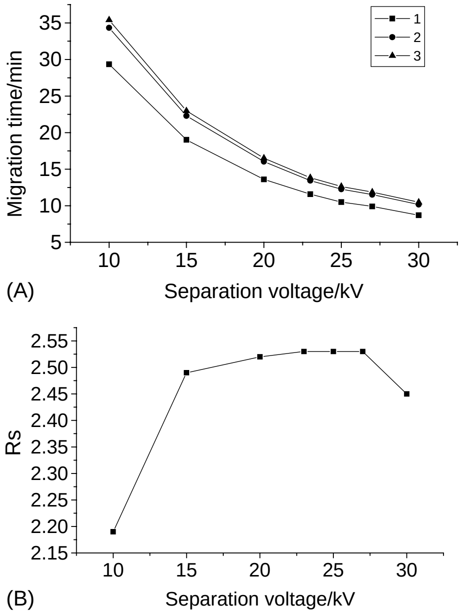

Fig. 4. (A) Effect of separation voltage on migration time: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol. (B) Influence of voltage on the resolution of the least resolved peak pair.

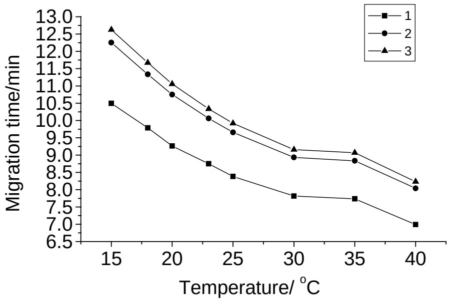

Fig. 5. Influence of temperature on migration time: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol.

beneficial to the resolution and can result in higher Joule heating, which directly affected the separation efficiency of this method. But lower separation voltages would increase the analysis time considerably; which in turn caused peak broadening, and thus the resolution was deteriorated by peak broadening. On the basis of these, 25 kV was chosen as the optimum voltage to accomplish a good compromise.

3.6. *Influence of electrophoretic temperature*

Because the electrophoretic temperature influences the viscosity of the solution, this in turn causes the change of EOF, and thus affects the separation. Fig. 5 indicates the effect of applied temperature on migration time for three analytes. As illustrated, increasing the temperature gave shorter migration time, but also enhanced the Joule heating, which directly reduced the separation efficiency. On the other hand, the electroosmotic flow increased slightly with increasing in temperature. This may have been due to the decreased viscosity with higher temperatures. The resolution decreased slightly with an increase in temperature. Moreover, lower temperature may resulted in peak broadening and longer analysis time. With regard to a short analysis time and good resolution, 20 °C was chosen as the electrophoretic temperature in this work.

3.7. *Influence of injection time*

In capillary electrophoresis, the volume of injection directly influences the sensitivity of determination and the resolution of the analytes. In order to investigate the effect of injection time on the separation of the analytes, 50 mbar pressure was kept unchanged, and the injection time was varied from 1 to 8 s. As can be seen, longer injection time, that is to say, bigger injection volume can result in higher sensitivity, whereas increasing the injection time can also causes peak broadening, which in turn results in resolution decreasing. All things considered, 3 s is selected as the optimum injection time. And typical electropherogram obtained under the optimum conditions for a standard solution of 10^{-3} mol l^{-1} nitrophenols is shown in Fig. 6.

4. Characteristic of quantitative analysis

4.1. *Calibration curves and detection limit*

A series of the standard solutions of a mixture of nitrophenols in the concentration range 10^{-6} to 10^{-3} mol l^{-1} were

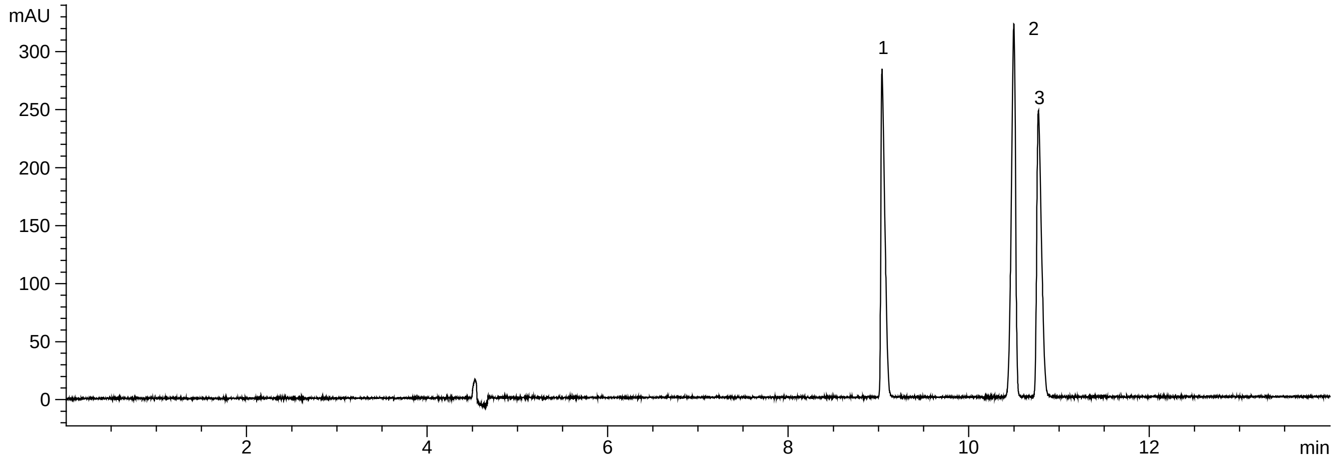

Fig. 6. Capillary electropherogram of three phenols: (1) *m*-nitrophenol, (2) *p*-nitrophenol, (3) *o*-nitrophenol.

Table 1
The linear ranges, regression equations, correlation coefficients, and detection limits of three nitrophenols

Component	Linear range (mol l^{-1})	Linear regression equation	Correlation coefficient	Detection limit (mol l^{-1})
m-Nitrophenol	$3.06 \times 10^{-5} \sim 4.08 \times 10^{-3}$	Area = 670.32 × amount + 0.0363	0.9996	1.02×10^{-5}
p-Nitrophenol	$2.03 \times 10^{-5} \sim 4.06 \times 10^{-3}$	Area = 712.31 × amount + 0.0387	0.9998	4.06×10^{-6}
o-Nitrophenol	$2.08 \times 10^{-5} \sim 4.16 \times 10^{-3}$	Area = 683.47 × amount + 0.0331	0.9998	1.04×10^{-5}

Table 2
Reproducibilities of migration times and peak areas

Component	R.S.D. of peak areas (%)		R.S.D. of migration times (%)	
	In a day	Day-to-day	In a day	Day-to-day
m-Nitrophenol	1.3	1.4	0.3	0.9
p-Nitrophenol	1.2	1.3	0.2	0.4
o-Nitrophenol	1.6	1.9	0.4	0.7

Table 3
Determination results of recoveries

Component	Added concentration ($\mathrm{mol\,l^{-1}}$)	Detected concentration ($\mathrm{mol\,l^{-1}}$)	Recovery (%)
m-Nitrophenol	2.72×10^{-3}	2.68×10^{-3}	98.5
	8.16×10^{-4}	8.24×10^{-4}	100.9
	5.10×10^{-5}	4.94×10^{-5}	96.8
p-Nitrophenol	2.71×10^{-3}	2.65×10^{-3}	97.8
	8.12×10^{-4}	8.01×10^{-4}	98.6
	5.08×10^{-5}	5.11×10^{-5}	100.6
o-Nitrophenol	2.77×10^{-3}	2.68×10^{-3}	96.7
	8.32×10^{-4}	8.36×10^{-4}	100.4
	5.20×10^{-5}	4.97×10^{-5}	95.6

tested to determine the linearity of the method. The regression equations, correlation coefficients, linear ranges, and detection limits were listed in Table 1. The detection limits were evaluated on the basis of a single-to-noise ratio of 3.

4.2. Reproducibility

The reproducibilities of peak areas and migration times in this experiment were determined by injecting a standard solution of a mixture of three nitrophenols ($10^{-3}\,\mathrm{mol\,l^{-1}}$) into the system under the optimum conditions. The relative standard deviations (R.S.D.) of peak areas and migration times in a day were no more than 1.6 and 0.4% ($n = 8$), and R.S.D. of peak areas and migration times between day-to-day were no more than 1.9 and 0.9% ($n = 7$). The results were shown in Table 2. The high reproducibility indicates that this method is suitable for analysis of practical samples.

4.3. Recovery

The recovery of the method was studied using the polluted water as practical samples which were collected from a local river. Accurate amounts of three phenols in different concentration were added to the water, and recovery values were obtained by use of their peak areas from the calibration curve under the same conditions. Average recoveries for the analytes are listed in Table 3.

5. Conclusion

CZE with methanol as additive has been shown to be suitable for the separation and determination of nitrophenol isomers. The main advantage of this work for the separation and determination of nitrophenol isomers is that its higher resolution and sensitivity, excellent stability and reproducibility, low expense of operation, and less amount of sample. It is concluded that CZE technique is a simple and powerful technique, it can be developed for the determination of nitrophenols in environment analysis.

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