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Separation and determination of nitroaniline isomers by capillary zone electrophoresis with amperometric detection

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Abstract

In this paper, capillary zone electrophoresis with amperometric detection (CZE–AD) was firstly applied to the simultaneous separation and determination of nitroaniline positional isomers. The three analytes could be perfectly analyzed by using the buffer of extreme pH. The effects of several important factors were investigated to find optimum conditions. A carbon-disk electrode was used as working electrode. The optimal conditions were 40 mmol/L tartaric acid–sodium tartrate (pH 1.2) as running buffer, 17 kV as separation voltage and 1.10 V (versus saturated calomel reference electrode, SCE) as detection potential. Under the optimum conditions, *o*-, *m*- and *p*-nitroaniline were separated successfully and good linearity, reproducibility and recovery results were obtained. The detection limit for *m*-nitroaniline was as low as at 9.06×10^{-9} mol/L. This proposed method demonstrated long-term stability and reproducibility with relative standard deviations of less than 1.8% for migration time and 1.1% for peak areas. The utility of this method was demonstrated by monitoring dyestuff wastewater and the assay results were satisfactory.

Keywords: Capillary zone electrophoresis; Amperometric detection; *o*-, *m*-, *p*-Nitroaniline; Positional isomers; Extreme pH

1. Introduction

Nitroanilines are commonly encountered organic contaminants in environmental systems, as intermediates in the synthesis of dyestuffs, medicines, pesticides and rubber. They are strongly toxic and are now widely considered as potential carcinogens, and most of them have been included in the list of priority pollutants in many countries [1]. They can be released into the environment directly as industry waste or indirectly as breakdown products of herbicides and pesticides. Due to their solubility in water, anilines can readily permeate through soil and contaminate ground water. They can be taken up by humans via the skin, the respiratory tract and the gastrointestinal tract. Because of their toxicity, bioaccumulation and vast scale distribution in the ecological environment, their separation and determination have become one of the important studies of environmental analysis. Since isomers usually possess similar physical and chemical properties, their separation is one of the most challenging areas of separation science. Several methods including high performance liquid chromatography (HPLC) [2–6], planar electrochromatography [7] and liquid membrane permeator [8] have been employed to analyze these compounds. But planar electrochromatography and liquid membrane permeator are time-consuming and tedious; HPLC often requires large volumes of toxic organic solvent, which is expensive and hazardous to the environment.

In recent years, capillary electrophoresis (CE) has been developed as a highly effective analytical method in environmental areas because of its low sample consumption, short analysis time, high separation efficiency and relatively simple instrumentation. But the analysis of nitroaniline isomers by CE is rarely studied. Xu et al. [9] reported the separation of nitroanilines by capillary electrophoresis with cucurbit[7]uril as an additive. Ye et al. [10] used *p*-sulfonic calixarene to the running buffer and separated isomer of nitroanilines, unfortunately, the detection is influenced by the calixarene because of its high ultraviolet (UV) absorption. For UV detection, the sensitivity is somewhat low. The low sensitively of UV detection in combination with capillary electrophoresis makes it inappropriate for determining anilines in practical wastewater. It calls for a sample concentration step prior to analyses. This shortcoming can be overcome by using electrochemical detection

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(ED). ED shows higher sensitivity and selectivity than UV; especially the amperometric detection can remove the interferences caused by electro-inactive substances, so it is suited to be used with CE in environmental analysis. Because *o*-, *m*- and *p*-nitroaniline are electroactive substances, amperometric detection is very suitable to their analysis for above advantages. However, the method of simultaneous separation and determination of nitroaniline isomers with CE–ED technique has not been reported.

In our study, we developed a method to separate and determine nitroaniline isomers by CE–ED without any additives. Because the positional isomers of nitroaniline cannot be separated successfully by capillary zone electrophoresis in generic conditions as a result of their absorption, we restrained their absorption by using extreme pH and they were separated well. This method was also successfully used in monitoring dyestuff wastewater, and satisfactory assay results were obtained. Compared with the anterior works, this method was simple, reliable, convenient and very potential to be used in the environmental analysis.

2. Experimental

2.1. Apparatus

CZE–AD system was laboratory-built [11,12]. Electrophoresis was driven by a high-voltage supplier (±30 kV, Shanghai Institute of Nuclear Research, Shanghai, China). All separations were performed in an uncoated silica capillary (Hebei Yongnian Laser-Fiber Factory, China) with 25 μm i.d. and 60 cm long. Electrochemical experiments were carried out by a CHI 830 electrochemical analyzer (CH Instruments, Austin, TX, USA) connected to a computer. A three-electrode system, which consisted of a carbon disk working electrode (Ø 300 μm), a saturated calomel reference electrode (SCE) and a platinum wire counter electrode, was used in both electrochemistry and detection experiments.

2.2. Reagents and solutions

o-, *m*- and *p*-Nitroaniline were provided by Shanghai SSS Reagent Co. Ltd., China. Methanol was of HPLC grade (Shanghai Jianxin Reagent Co. Ltd., China). Other chemicals were of analytical grade.

Standard stock solutions of nitroanilines at a concentration of 10^{-2} mol/L were prepared in methanolic solutions in brown bottles and stored in a refrigerator at 4 °C. Nitroanilines working standard solutions were prepared by serial dilution of the stock standard solution with double distilled water. All buffers were prepared from 100 mmol/L tartaric acid and 100 mmol/L sodium tartrate stock solutions in different proportion. Double distilled water was used throughout to prepare solutions.

Before CZE experiments, all used solutions were filtered through 0.22 μm polypropylene acrodisc syringe filter and sonicated for 5 min to remove bubbles.

2.3. Electrophoretic procedure

The new fused silica capillary was first treated by rinsing with 1 mol/L NaOH for 1 h, followed by 1 mol/L HCl for 1 h, then by double distilled water for 1 h. Every day before experiments, the capillary was flushed with 0.1 mol/L NaOH for 15 min, then with double distilled water for 10 min, and finally with buffer till the inside current of the capillary reached stability. This was important to get a reproducible electroosmotic flow. 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 three-electrode system was fixed in the corresponding holes of the electrochemical cell and the carbon disk electrode was positioned straight, opposite to the capillary outlet, as close as possible by a three-dimension positioner.

CZE was performed at the separation voltage of 17 kV with 40 mmol/L tartaric acid–sodium tartrate (pH 1.2) used as running buffer. The potential applied to the working electrode was 1.1 V (versus SCE). Samples were electrokinetically injected at 17 kV for 10 s.

2.4. Preparation of carbon working electrode

The carbon disk electrode was constructed by using a 300 μm diameter pencil lead (produced by Mitsubishi Pencil Co., Ltd.) with one end winded by copper wire. A glass pipette of about 0.4 mm tip diameter drawn from a borosilicate glass tube (o.d. 0.4 cm, i.d. 0.2 cm) was cut to the desired length, ca. 18 cm. The unwired end of the pencil lead was introduced into the pipette until it protruded approximately 0.3 cm from the pipette tip. Non-conductive gel was applied at both ends of the pipette to seal the lead and copper wire. Prior to use, the surface of the carbon disk electrode was gradually polished with emery paper and 0.05 μm alumina powders, then ultrasonicated in deionized water, and then transferred to the electrochemical cell for activating by cyclic voltammetry between −1.10 and +1.10 V (versus SCE) in 40 mmol/L tartaric acid–sodium tartrate, until a stable CV profile was obtained, and finally carefully positioned opposite the capillary outlet with the aid of a micromanipulator to minimize the gap between the electrode tip and the capillary outlet.

2.5. Sample preparation

Samples of dyestuff wastewater from Shengtian and Sanlian dyestuff industry were collected in brown bottles and kept in the dark at 4 °C until analysis. The samples were first filtered through a paper filter and then through a 0.22 μm filter, before use. For recovery studies of nitroanilines, the accurate amounts of three anilines were added to the wastewater samples after they were filtered. Peak identification was performed by standard addition method.

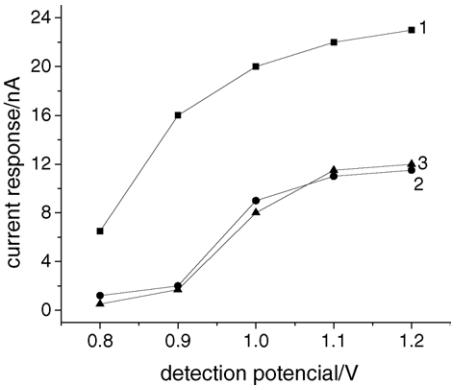

Fig. 1. The hydrodynamic voltammograms of the three analytes. (1) *m*-Nitroaniline (7.24×10^{-5} mol/L); (2) *p*-nitroaniline (9.60×10^{-5} mol/L); (3) *o*-nitroaniline (1.17×10^{-4} mol/L). The CZE conditions are as the optimal.

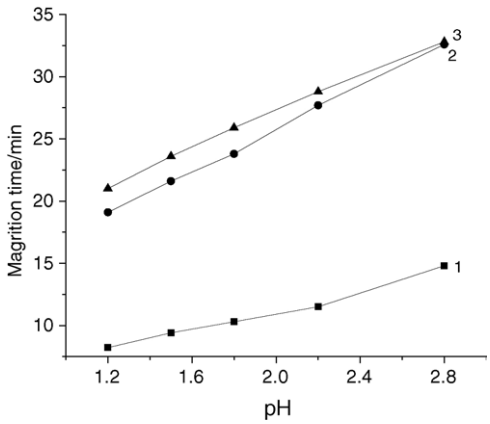

Fig. 2. Effect of pH on the migration time. Working electrode potential is +1.10 V (vs. SCE); other conditions are the same as in Fig. 1 except pH value.

3. Results and discussion

3.1. Conditions of amperometric detection

Since there is an amino group in nitroaniline moleculars, the three isomers can be electrochemically oxidized at a carbon electrode and produce current responses. Because the potential applied to the working electrode greatly affects the response of analytes, it is very important to select a suitable potential when AD acts as a detection method in CE. Fig. 1 shows the hydrodynamic voltammograms (HDVs) of the *o*-, *m*- and *p*-nitroaniline. When the potential was lower than 0.8 V, the peak currents for *p*-nitroaniline and *o*-nitroaniline were relatively small. While the potential was greater than 0.8 V, the response currents of the three analytes increased with the increase of the potential applied to the working electrode. At the same time, the base current increased too. Considering the detection sensitivity of the studied analytes and the baseline noise, 1.10 V was chosen as the optimum working potential.

3.2. Influence of buffer pH

The pH of the electrolyte had a significant impact on the electro-osmotic flow. The positional isomers of nitroaniline cannot be separated successfully by capillary zone electrophoresis in generic conditions as a result of their absorption. In this paper, we restrained their absorption by using extreme pH. The influence of the pH values on the migration time was examined in the pH range 1.0–4.2. The experiment results showed that with an increase of the pH, the migration time increased, the resolution decreased and the peak became broader. The peak shape was bad as the pH < 1.2, and *o*-nitroaniline overlapped seriously with *p*-nitroaniline by peak broadening as the pH > 2.4. Experiment illustrated that three nitroaniline isomers could separate completely when the pH was in the range of 1.2–2.4. The influence of pH values in the range of 1.2–2.8 on the migration time were showed in Fig. 2.

Considering the sensitivity, the time of analysis and the resolution, the pH 1.2 of buffer was found to be optimal, and selected to perform the separation of nitroanilines in the following study.

3.3. 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, four kinds of buffers, namely hydrochloric acid–potassium chloride, tartaric acid–sodium tartrate, hydrochloric acid–sodium citrate and acetic sodium–hydrochloric acid were tested, and tartaric acid–sodium tartrate 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 20–60 mmol/L on the separation was examined. It was found that *o*- and *p*-nitroaniline could not be separated completely when the buffer concentration was less than 20 mmol/L. But higher buffer concentration also has a negative effect on the separation. With the increasing concentration of buffer, high electrophoretic current is generated and the effect of Joule heating becomes more pronounced, this in turn results in peak broadening and lengthening of migration time. For a comprehensive thought, 40 mmol/L was chosen as the buffer concentration in this work.

3.4. Influence of separation voltage

The separation voltage determines the migration time and resolution of analytes. Increasing the voltage not only resulted in shorter migration times, but also increased the baseline noise, resulting in poorer detection limits. It was found that too high separation voltages were not 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 obviously, which in turn caused peak broadening and, thus, the resolution was deteriorated. On the basis of these, 17 kV

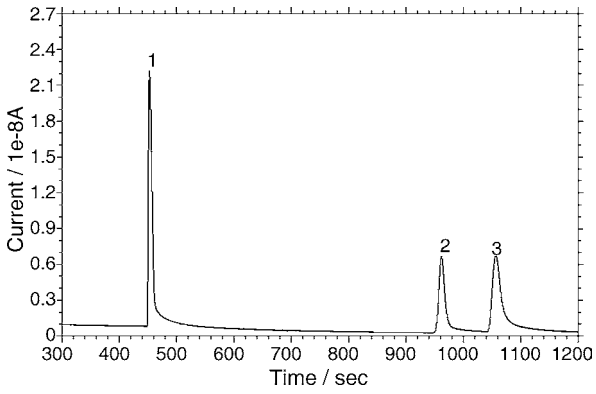

Fig. 3. Capillary electropherogram of three nitroanilines under the optimal CZE–ED conditions. (1) *m*-Nitroaniline (7.24×10^{-5} mol/L); (2) *p*-nitroaniline (9.60×10^{-5} mol/L); (3) *o*-nitroaniline (1.17×10^{-4} mol/L).

was chosen as the optimum voltage to accomplish a good compromise.

3.5. Influence of injection time

In capillary electrophoresis, the volume of injection directly influences the sensitivity of determination and the resolution of the analytes. The amount of sampling was also tested by changing the sampling time for 5, 8, 10, 12 and 14 s at 17 kV. As can be seen, longer injection time 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, 10 s is selected as the optimum injection time.

Through the experiments above, the optimum conditions of CZE–AD for separating and determining nitroaniline isomers were detection potential 1.10 V (versus SCE), separation voltage 17 kV, electrokinetic sampling time 10 s at 17 kV and 40 mmol/L tartaric acid–sodium tartrate (pH 1.2). And typical electropherogram obtained under the optimum conditions for a standard solution of 10^{-5} mol/L nitroanilines is shown in Fig. 3.

3.6. Calibration curves and detection limit

A series of the standard solutions of a mixture of nitroanilines in the concentration range 10^{-9} to 10^{-3} mol/L were 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 signal-to-noise ratio of 3.

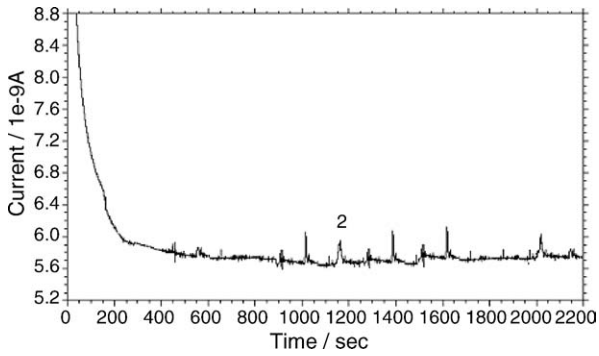

Fig. 4. Capillary electropherogram of Shengtian wastewater under the optimal CZE–ED conditions, (2) *p*-nitroaniline.

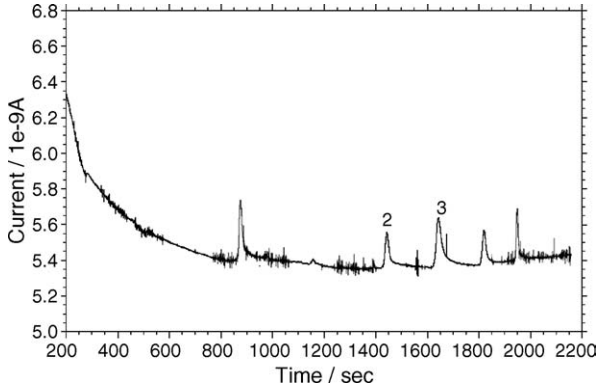

Fig. 5. Capillary electropherogram of Sanlian wastewater under the optimal CZE–ED conditions. (2) *p*-Nitroaniline and (3) *o*-nitroaniline.

Table 2
The concentration of the analytes in the samples

Sample	*m*-Nitroaniline	*p*-Nitroaniline	*o*-Nitroaniline
Shengtian	–	4.57×10^{-7}	–
Sanlian	–	5.41×10^{-7}	8.80×10^{-7}

3.7. Reproducibility

The reproducibilities of peak areas and migration times in this experiment were determined by injecting a standard solution of a mixture of three nitroanilines (10^{-6} mol/L) into the system under the optimum conditions. The relative standard deviations (R.S.D.) ($n = 7$) of peak areas and migration times were 0.9 and 1.3% for *m*-nitroaniline, 1.1 and 1.5% for *p*-nitroaniline, 0.9 and 1.8% for *o*-nitroaniline. The high reproducibility indicates that this method is suitable for analysis of environmental samples.

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

Component	Linear range (mol/L)	Linear regression equation C (mol/L); A (nQ)	Correlation coefficient	Detection limit (mol/L)
m-Nitroaniline	4.53×10^{-8} to 1.81×10^{-3}	$A = 5.05 \times 10^5 C + 10.57$	0.9996	9.06×10^{-9}
p-Nitroaniline	1.20×10^{-7} to 2.40×10^{-3}	$A = 2.71 \times 10^5 C + 6.166$	0.9999	2.40×10^{-8}
o-Nitroaniline	1.96×10^{-7} to 3.91×10^{-3}	$A = 1.12 \times 10^5 C + 11.82$	0.9989	3.92×10^{-8}

Table 3
Determination results of recoveries ($n = 4$)

Component	Sample concentration (mol/L)	Added concentration (mol/L)	Detected concentration (mol/L)	Recovery (%)	R.S.D. (%)
m-Nitroaniline	–	4.53×10^{-6}	4.49×10^{-6}	99.1	0.26
p-Nitroaniline	4.57×10^{-7}	6.01×10^{-6}	6.53×10^{-6}	101.0	0.97
o-Nitroaniline	–	9.78×10^{-6}	9.63×10^{-6}	98.4	1.43

3.8. Sample analysis and recovery

Under the optimum conditions, CZE–AD was applied for the determination of two dyestuff wastewater samples which were obtained from Shengtian and Sanlian dyestuff industry. Electropherograms of the two samples are shown in Figs. 4 and 5.The assay results are summarized in Table 2.

The recovery of the method was studied using Shengtian dyestuff industry wastewater as practical samples. Accurate amounts of three anilines in different concentration were added to the sample solution, and recovery values were obtained by the use of their peak areas from the calibration curve under the same conditions. Average recoveries for the analytes are listed in Table 3.

4. Conclusion

The experimental results demonstrated that CZE–ED was a practical method for simultaneous determination of nitroaniline isomers in dyestuff wastewater. This method has many merits such as short analysis time, low sampling volume, high sensitivity and reproducibility, especially compared with the common UV detection, this method is more sensitive. This method is easy to manipulate and can be applicable to measure other analogous substances in environment analysis.

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