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Short communication

Separation of biogenic amines by micellar electrokinetic chromatography with on-line chemiluminescence detection

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Abstract

A method of on-line chemiluminescence detection with capillary electrophoresis for biogenic amines (diaminopropane, putrescine, cadaverine and diaminohexane) labeled with *N*-(4-aminobutyl)-*N*-ethylisoluminol is reported for the first time. Two separation modes, capillary zone electrophoresis and micellar electrokinetic chromatography (MEKC), were studied. The results show that excellent resolution was achieved in MEKC. Parameters affecting separation process and chemiluminescence detection have been examined in detail. Under the optimum conditions, the baseline separation of four amines was obtained within 7.5 min. The detection limits ($S/N=3$) of diaminopropane, putrescine, cadaverine and diaminohexane are $3.5\cdot10^{-8}$, $3.5\cdot10^{-8}$, $3.9\cdot10^{-8}$ and $1.2\cdot10^{-7}$ *M*, respectively. The method was applied to the analysis of biogenic amines in lake water.
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Keywords: Detection, electrophoresis; Chemiluminescence detection; Biogenic amines

1. Introduction

Biogenic amines are naturally occurring compounds in diverse biological systems. They are formed as a consequence of metabolic processes and by the action of microorganisms [1]. They can be found in biological fluid, environmental samples and industrial process streams, often at trace levels. Amines are also emitted from animal wastes and biological activities. In the analysis of food, polyamines especially for putrescine and cadaverine were widely determined. Gas chromatography [2,3], thin-layer chromatography [4,5] and high-performance liquid chromatography (HPLC) methods [5–11] were usually used to determine biogenic amines, and HPLC is the most widely used. Capillary electrophoresis (CE) has been proved to be a powerful separation technique. Recently, CE has been used successfully in the separation of amines. So far, several detection methods, such as fluorescence [1,12–18], UV–Vis [19–24] and conductivity [25,26], have been developed in the analysis of amines in CE. Chemiluminescence (CL) detection, as a highly sensitive mode of detection with simple and cheap optical systems, has been demonstrated in conventional CE [27–32] and microchip [33,34]. The chemiluminescence detection with CE separation for arginine and glycine derivatized with *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI) was carried out by Dadoo et al. [35]. Most recently, the determination

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of diphenhydramine by capillary electrophoresis with tris(2,2′-bipyridyl)ruthenium(II) electrochemiluminescence detection was performed by Liu et al. [36].

In this study, we report the micellar electrokinetic chromatographic (MEKC) separation of polyamines (e.g., 1,3-diaminopropane, 1,4-putrescine, 1,5-cadaverine, and 1,6-diaminohexane) labeled with ABEI with on-line CL detection. Parameters affecting electrophoretic separation and chemiluminescence detection have been investigated in detail. The proposed method was used in the determination of biogenic amines in lake water.

2. Experimental

2.1. Reagents and solutions

ABEI and diaminopropane were obtained from Fluka (Buchs, Switzerland). *N*,*N*′-Disuccinimidyl carbonate (DSC) was purchased from Aldrich (Milwaukee, WI, USA). Putrescine, cadaverine, diaminohexane, and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, MO, USA). 30% Hydrogen peroxide solution, anhydrous sodium tetraborate and potassium ferricyanide from Shanghai Chemical Factory (Shanghai, China). All chemicals were of analytical-reagent grade or better. Water purified with a Water PRO PS system (Labconco, Kansas City, KS, USA) was used to prepare all solutions. The electrophoretic buffer was borate buffer modified with SDS and was filtered through a 0.22-μm membrane prior to use. Stock solutions of ABEI and DSC were prepared in methanol and acetonitrile, respectively. Stock solutions of the amines were prepared in methanol and diluted as needed.

2.2. Apparatus

The CE–CL apparatus was built in the laboratory. A 0–30 kV power supply (Peking University, China) provided the high separation voltage. Separation capillaries of 50 μm I.D.×375 μm O.D. were from Yongnian Optical Fiber Factory (Hebei, China). A 5 cm coating section of one end of the separation capillary was burned and then etched with hydrofluoric acid for 2.5 h to about 200 μm O.D. (before etch the tip of capillary is sealed by wax to avoid the inner wall is etched). The hydrofluoric acid treated end of the separation capillary was then inserted into a reaction capillary of 530 μm I.D. These two capillaries were held in a plexiglass four-way joint. The post-column reagents (the mixture of potassium ferricyanide and sodium hydroxide solution) were delivered by gravity through a reagent capillary of 320 μm I.D. The outlet of the reagent capillary was also led to the four-way joint. Plexiglass nuts and polyimide ferrules were used to fix the above mentioned three capillaries inside the four-way joint. The grounding electrode was also put into the joint to complete the CE electrical circuit. The outlet of the reaction capillary was 2 cm lower than the other end to make the solution flow out of the reaction capillary more easily and quickly. A 1-cm detection window was formed on the reaction capillary by burning off the polyimide coating. The CL emission was collected with a type R928 photomultiplier tube (PMT; Hamamatsu Photonics, Japan). In order to collect the most intensive CL signal, the detection window was situated just in front of the PMT. The photocurrent was fed to a type HX-2 signal magnifier (Institute of Chemistry, Chinese Academy of Sciences, Beijing, China) and then recorded using a 3066-type chart recorder (The Fourth Instrumental Factory of Sichuan, China). The whole CL detection system was held in a large light-tight box to exclude stray light.

2.3. Procedures

2.3.1. Derivatization of amines with ABEI

Labeling of analytes with ABEI followed the procedure described by Kawasaki et al. [6]. First, a 5 m*M* solution of ABEI in methanol was added to an equal volume of a 5 m*M* DSC solution. The mixture was allowed to stand at room temperature for 2 h. Then, the desired amines were added to 50 μl solution of the ABEI–DSC solution. After vortex mixing, the mixture was allowed to stand at room temperature for 2 h. The derivatized amines solutions were diluted in 5 m*M* borate buffer (pH 8.5), if necessary. The effect of the molar ratios of ABEI–DSC to amines was investigated. The optimum

molar ratio of ABEI–DSC to amines was found to be 10.

The new capillaries were rinsed sequentially with 2 *M* NaOH–CH_3OH, 1 *M* NaOH, 1 *M* HCl, water for 30 min, and were then equilibrated with the running buffer solution for 30 min. Hydrogen peroxide solution was added to the running buffer. The separation capillary was filled with running buffer while the four-way joint and reaction capillary were filled with post-column reagents. After each run, the separation capillary was treated with running buffer for 3–5 min. Injection was performed hydrodynamically at a height difference of 10 cm.

3. Results and discussion

3.1. Choice of separation mode

It seems very difficult to discriminate diaminopropane, putrescine, cadaverine, and diaminohexane in capillary zone electrophoresis only based on their intrinsic mobility. The experimental results show that four amines could not been separated in capillary zone electrophoresis using borate buffer. Addition of SDS can effectively improve the resolution and peak shape. MEKC provides a sophisticated means for achieving a very high selectivity of solutes with closely related structure [37]. The optimum concentration of SDS was found to be 80 m*M*. However, addition of higher concentration of SDS resulted not only in longer migration times but larger electrophoretic current. The effect of concentration of SDS on migration times of four amines was shown in Fig. 1.

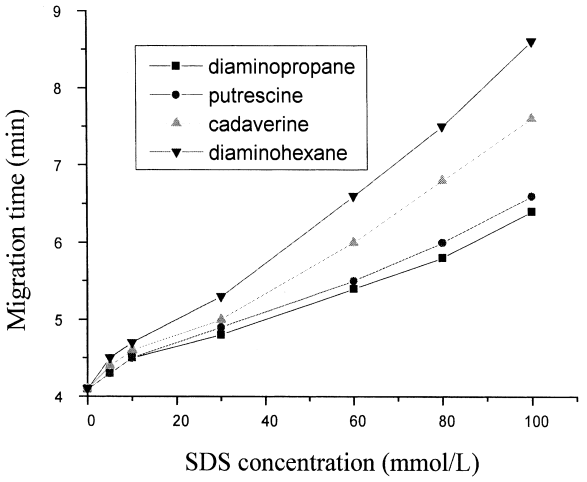

Fig. 1. Effect of SDS concentration on migration times. Conditions: electrophoretic buffer, 10 m*M* borate+100 m*M* H_2O_2+80 m*M* SDS (pH 9.3); post-column CL reagent, 3 m*M* $K_3Fe(CN)_6$+ 0.8 *M* NaOH; injection, hydrodynamic injection for 15 s, separation voltage, 22 kV.

3.2. Optimization for MEKC

We examined the effects of electrophoretic buffer pH (in the range of 8.0 to 11.0), separation voltage, and injection time on resolution and CL intensity. The results show that the pH of electrophoretic buffer markedly influences electroosmotic flow, resolution and sensitivity. When pH≤8.5, resolution of diaminopropane and putrescine is unsatisfactory, meanwhile, the peaks of cadaverine and diaminohexane also broaden. When pH reached the 11.0, several analytes crowd together and are difficult for the identifications. The optimum pH was found to be 9.3. Separation voltage influences not only the migration times but also the resolution and sensitivity. With the increase in voltage, migration time decrease and the number of theoretical plates increased. Hence it seemed that a high voltage lead to the optimization of separation. However, further increase in voltage was limited by the increase of electrophoretic current levels associated Joule heating, which resulted in decrease of separation efficiency. Peaks of diaminopropane and putrescine partially overlapped when separation voltage was over 24 kV. Therefore, 22 kV was chosen as the optimal separation voltage. CL intensity increases with an increase of the injection time, however, longer injection times would result in unsatisfactory resolution, especially for diaminopropane and putrescine. The compromise between the signal intensity and the resolution is 15 s at a height difference of 10 cm.

3.3. Optimization of CL detection

We examined effect of concentration of H_2O_2, $K_3Fe(CN)_6$, and NaOH in post-column solution on CL detection. Hydrogen peroxide, used as oxidant in the chemiluminescence reaction, has greatly influ-

ence on sensitivity. The CL emission was found to increase with increasing H_2O_2 concentration. However, the intensity of emitted light reaches a plateau at the H_2O_2 concentration of 100 m*M*. A concentration of 100 m*M* H_2O_2 was used for further studies. In this work, the reaction of ABEI and hydrogen peroxide can be catalyzed by $K_3Fe(CN)_6$. CL intensity was found to be markedly affected by the concentration of $K_3Fe(CN)_6$. As shown in Fig. 2, maximum light emission occurred at 3 m*M*, and the sensitivity decreased sharply on either side of this concentration. At the below 2 m*M*, the CL intensity is proportional to the concentration of $K_3Fe(CN)_6$. At higher concentration of $K_3Fe(CN)_6$, the decrease of CL intensity is attributed to the deeper color of $K_3Fe(CN)_6$. Therefore, 3 m*M* $K_3Fe(CN)_6$ was adopted for the method. The effect of concentration of NaOH in post-column solution on sensitivity was also examined, because the chemiluminescent reaction is pH dependent. It can be seen from the results that the concentration of NaOH has evident influence on CL intensity. The optimal NaOH concentration was found to be 0.8 *M*.

3.4. Quantitation analysis

Fig. 3 shows an electropherogram of a mixture of four aliphatic diamines standards obtained using the optimum conditions. The baseline separation was achieved within 7.5 min. In addition, the electrophoretic peaks obtained are all very sharp and symmetrical. Precision (RSD) in peak height and migration time, linear range, and the detection limits (DLs) are listed in Table 1.

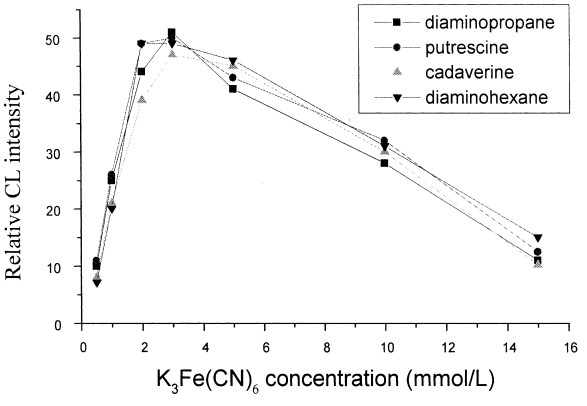

Fig. 2. A plot of CL intensity vs. $K_3Fe(CN)_6$ concentration. Conditions as in Fig. 1.

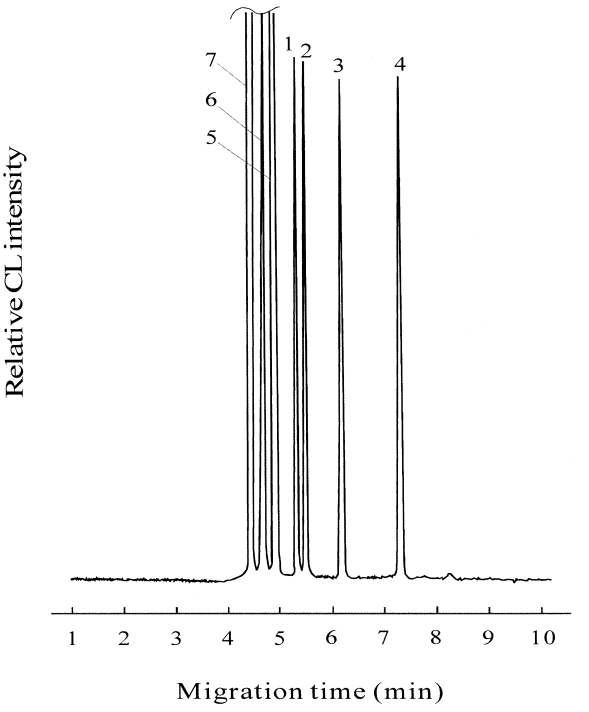

Fig. 3. Capillary electrophoretic separation of a mixture of ABEI-amines. Conditions: electrophoretic buffer, 10 m*M* borate+100 m*M* H_2O_2+80 m*M* SDS (pH 9.3); post-column reagents, 3 m*M* $K_3Fe(CN)_6$+0.8 *M* NaOH. Peaks: 1=diaminopropane, 2=putrescine, 3=cadaverine, 4=diaminohexane, 5, 6 and 7 show derivatization reagents peaks (ABEI or ABEI–DSC), identified in the blank sample. Concentration of each peak is $2.0 \cdot 10^{-6}$ *M* except diaminohexane of $6.0 \cdot 10^{-6}$ *M*.

3.5. Application to real-world sample

The developed method was applied to the determination of amines in the lake water sample. The fresh sample was immediately acidified to pH 5.0 by hydrochloric acid, then homogenized and filtered through a 0.22-μm membrane filter. The sample was derivatized with ABEI–DSC. There is a small amount of putrescine (2.98 μ*M*) and cadaverine (2.25 μ*M*) in the tested sample (as shown in Fig. 4). This phenomenon was believed to be a result of fish and shrimp corpse. In Fig. 4, 8 and 9 are unknown peaks in the present work. The recoveries of amine analysis (94.2% for diaminopropane, 106.8% for

Table 1
Relative standard deviations ($n=5$) for migration time and peak height, linear ranges and the detection limits (DLs)

Analyte	RSD (%)		Linear range (μM)	DL ($\cdot 10^{-8}$ M)
	Migration time	Peak height		
Diaminopropane	1.0	3.1	0.1–50	3.5
Putrescine	0.9	3.3	0.1–50	3.5
Cadaverine	0.8	4.1	0.1–50	3.9
Diaminohexane	1.1	6.0	0.1–20	12

putrescine, 95.2% for cadaverine and 92.8% for diaminohexane) in lake water were obtained by spiking standards.

4. Conclusion

A method of on-line CL detection with MEKC separation of four aliphatic diamines was described for the first time. Excellent separation efficiency was achieved using borate buffer modified with SDS. This work demonstrates that CE with CL detection provide a simple and new method for sensitive detection of small organic ions. Further application of this system to the determination of other amines, amino acid and protein, etc., is expected.

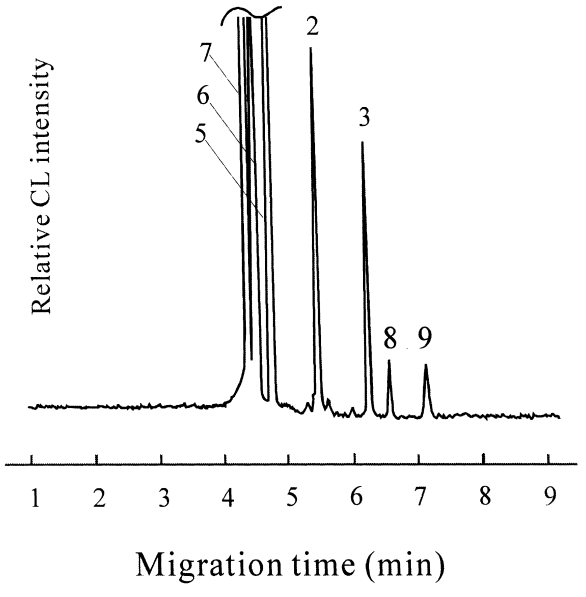

Fig. 4. Electropherogram of lake water. Conditions and peak identifications as in Fig. 3 (8 and 9 are unknown peaks).

Acknowledgements

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