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High-Sensitivity Analysis of Cyanide by Capillary Electrophoresis with Fluorescence Detection

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A capillary electrophoretic method for a high-sensitivity analysis of cyanide has been developed. Cyanide was derivatized with 2,3-naphthalenedialdehyde and taurine to give a fluorescent product of 1-cyanobenz[*f*]isoindole. This compound was detected with high sensitivity by fluorescence detection. The detection limit was 0.1 ng/mL, and the calibration curve was linear over the range 0.1 - 200 ng/mL. The precision of the migration time of within-run assays (n = 6) of 1 ng/mL cyanide standard solution was 0.14%. The precision of the peak area for the same runs was 1.0%. This method was applicable to blood analysis. Detection of the cyanide derivative by UV was also examined.

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Introduction

Cyanide is often used as a toxicant in cases of suicide, homicide and indiscriminate killing by spiked drinks or food. Furthermore, in fire, hydrogen cyanide is formed from polymers that contain nitrogen, and can be the cause of death. Therefore, cyanide analysis is important in forensic analyses, especially in analyses of the blood of victims. The analysis of trace cyanide in blood is also important for clinical medicine, because cyanide acts not only as an acute toxicant, but also as a chronic toxicant.[1]

In forensic science, a microdiffusion-spectrophotometric method using the König reaction[2] or headspace GC[3,4] is commonly used for determining cyanide in blood. We previously reported a highly sensitive cyanide analysis method using HPLC or ion chromatography (IC) with fluorescence detection.[5,6]

Recently, capillary electrophoresis (CE) has become widely used in various fields. CE is a powerful and practical tool because of its high resolution, ability to analyze impure samples, low reagent consumption, short analysis time and low running cost. A few CE methods for free cyanide using indirect UV detection[7] or indirect fluorescence detection[8] have been developed, with detection limits of 8 μg/mL and 130 ng/mL, respectively. However, a CE method with high sensitivity and high selectivity for cyanide has not yet been developed.

This report describes a new CE method which we have discovered using fluorometric cyanide derivatization by 2,3-naphthalenedialdehyde (NDA) and taurine, as shown in Fig. 1. Efforts were made to apply it to blood cyanide analysis.

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Experimental

Reagents and chemicals

NDA was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Taurine, potassium cyanide, sodium borax and phosphoric acid were of analytical reagent grade (Wako Pure Chemical Industries, Tokyo, Japan). All other reagents used were of analytical reagent grade. Ultrapure water, provided by a Milli-RX12α and Milli-Q SP system (Millipore, Bedford, MA, USA), was used for all procedures. A borate-phosphate buffer was prepared by mixing a 25 mM sodium borate solution and a 50 mM potassium dihydrogenphosphate solution (46.5:53.5, v/v). NDA was dissolved in methanol to give an 8 mM solution, and then the solution was diluted with the borate-phosphate buffer to give a 2 mM NDA solution. A 50-mM taurine solution was prepared by dissolving taurine in the borate-phosphate buffer.

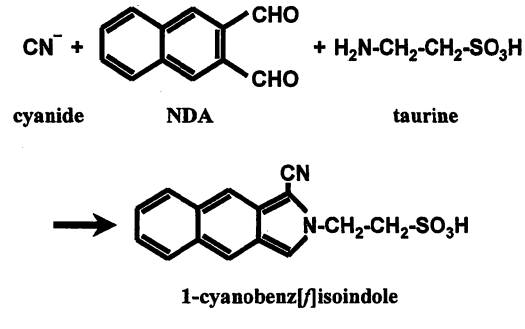

Fig. 1 Reaction of cyanide with 2,3-naphthalenedialdehyde (NDA) and taurine.

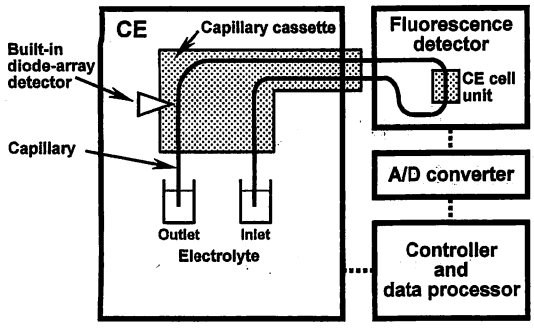

Fig. 2 CE system with fluorescence detection.

A 1 mg/mL standard solution of cyanide was prepared with potassium cyanide in 1% sodium hydroxide. Working standard solutions were prepared by diluting stock solutions with ultrapure water.

Apparatus and conditions

CE with the fluorescence detection system is shown in Fig. 2. Experiments were carried out using an Agilent CE system (Agilent Technologies, Waldbronn, Germany), an FP-1520 fluorescence detector (JASCO, Tokyo, Japan), a capillary electrophoresis cell unit (JASCO) and a 35900E A/D converter (Agilent Technologies). A small remodeled capillary cassette for the CE/MS interface (Agilent Technologies) was used to route the capillary from the CE through the fluorescence detector and back to the CE. Derivatized samples were injected by applying a pressure of 50 mbar for 30 s. The separations were performed in an uncoated fused-silica capillary of 75 μm i.d. × 117 cm (55 cm to the fluorescence detector, 108.5 cm to the built-in diode-array detector). The applied voltage was +30 kV and the capillary cassette temperature was maintained at 25°C. The fluorescence detection wavelengths were 418 nm excitation and 460 nm emission. The built-in diode-array detector of the CE system was used for UV detection at 250 nm. The electrolyte was a 20 mM borax buffer (pH 6 adjusted by phosphoric acid)–methanol (8:2, v/v), which was filtered with a 0.45 μm filter before use. Between consecutive runs, the capillary was washed for 8 min with the electrolyte.

Blood sample and pretreatment

A blood sample was collected from a healthy individual (one of the authors) by venipuncture into heparinized tubes on the day of analysis. The pretreatment procedure was as follows: 0.5 mL of water was added, followed by the addition of 2 mL of methanol to a 0.1-mL aliquot of blood. After vortex-mixing, the mixture was centrifuged at 1600*g* for 10 min, and the supernatant was used for the derivatization. All other conditions were the same as described previously.[6]

Derivatization procedure

A 0.5-mL aliquot of the standard solution or the above supernatant was placed in a 1.6-mL brown-colored sample vial, and 0.1 mL each of 2 mM NDA and 50 mM taurine solution were added. After standing for 5 min (30 min for the supernatant) at room temperature, the mixture was applied to the CE system. All other conditions were the same as described previously.[6]

Results and Discussion

Initial examinations

The initial examinations were performed using UV detection at 250 nm, which was the wavelength of the maximum absorbance of the cyanide derivative. The capillary was 75 μm i.d. × 80.5 cm (effective length 72 cm). Because the cyanide derivative had a sulfo group in its structure, we first tried to separate it in the negative charge mode (*i.e.*, the inlet electrode was negative). In this mode, the addition of a cationic surfactant to the electrolyte is required in order to reverse the electro-osmotic flow (EOF). Cetyltrimethylammonium bromide and hexamethonium bromide were used as cationic surfactants. However, the cyanide derivative reacted with both surfactants, and no peak appeared using an electrolyte that included either surfactant. This was confirmed by a separate experiment: the fluorescence reaction was performed in a test tube, and when the surfactant was added, the fluorescence disappeared. Thus, the negative charge mode could not be adopted.

Next, the positive charge mode (*i.e.*, the inlet electrode was positive) was examined. Under these conditions, the cyanide derivative has an electrophoretic mobility toward the inlet. Therefore, the EOF mobility must be higher than the electrophoretic mobility of the cyanide derivative in order to detect the cyanide derivative. To do this, the pH of electrolyte must be greater than three. Under borax–phosphate buffer (pH 6) conditions, the derivative peak appeared after the peaks of neutral compounds. Hence, the positive charge mode was used in all following experiments.

The detection limit of cyanide under these conditions using UV detection was *ca.* 20 ng/mL. This value was better than the values obtained with other CE methods for cyanide analysis.[7,8] However, it was significantly worse than the value obtained with our previous HPLC and IC methods (0.1 ng/mL).[5,6] Therefore, CE with UV detection was not suitable for blood cyanide analysis. Fluorescence detection was needed for trace cyanide determination. Thus, an external fluorescence detector was adopted for this analysis.

Optimization of CE conditions

The CE conditions were optimized for fluorescence detection using borax buffer as the electrolyte. First, the effect of the pH was examined by varying the pH of the electrolyte from 3 to 10. Under pH 5, the cyanide derivative peak did not appear within 60 min; pH values of 6 and 10 gave sharper peaks. However, the peak (1 ng/mL cyanide) at pH 10 was larger, and it became even larger as time passed. This phenomenon was also observed in an analysis of the reagent blank. This was due to dissolution of trace hydrogen cyanide in the atmosphere into the electrolyte. The pH of the electrolyte should be one or more units lower than the pK_a value of hydrogen cyanide (9.21) to avoid the dissolution of hydrogen cyanide. Therefore, pH 6 was selected. Different combinations of borax (10 - 50 mM) and methanol (0 - 30%) were then examined. A combination of 20 mM borax and 20% methanol gave the best resolution and the best separation from other matrix peaks in the analysis of both the cyanide standard and the blood sample. Finally, the sample injection volume was examined. The standard sample was injected by applying a pressure of 50 mbar for 6 - 120 s. The peak area increased in proportion to the injection time, but when the injection time was more than 30 s, the peak resolution declined. Moreover, the signal-to-noise ratio of the reagent blank peak, which was a minor cyanide derivative peak appearing in the analysis of the reagent blank,[5,6,9] was three and

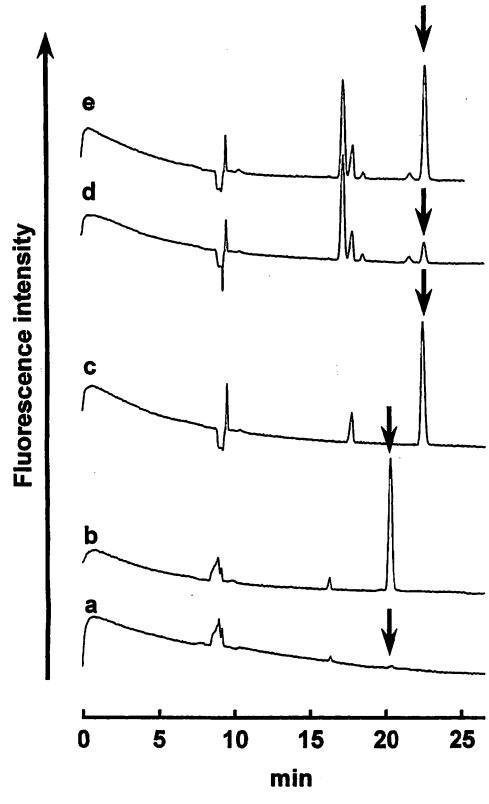

Fig. 3 Fluorescence electropherograms of (a) the reagent blank, (b) the cyanide standard solution (3.8 ng/mL), (c) the cyanide standard solution containing methanol (3.8 ng/mL), (d) a blood sample (with a calculated cyanide concentration of 9.5 ng/mL) and (e) a blood sample spiked with 100 ng/mL cyanide. Conditions: capillary, 75 μm i.d. × 117 cm (effective length 55 cm) uncoated fused-silica; applied voltage, +30 kV (current, *ca.* 23 μA); capillary cassette temperature, 25°C; fluorescence detection wavelengths, 418 nm (excitation), 460 nm (emission); sample injection, 50 mbar for 30 s; electrolyte, 20 mM borax buffer (pH 6)-methanol (8:2, v/v).

the peak area (corresponding to *ca.* 0.04 ng/mL of cyanide) was almost constant at an injection time of 30 s. Hence, an injection time of 30 s was selected. Electropherograms of the reagent blank and the cyanide standard using the final CE conditions are shown in Figs. 3a and b, respectively.

Calibration curve, detection limits and reproducibility

The reagent blank always showed a minor peak of cyanide corresponding to *ca.* 0.04 ng/mL, which was the same value as that reported previously.[5,6] Thus, the detection limit was estimated to be 0.1 ng/mL, which was the concentration that gave a peak height 2.5 times that of the reagent blank peak. The calibration curve was linear in the range 0.1 - 200 ng/mL (y = 74.60x + 6.04, r^2 = 0.999). The intermediate precisions of within-run assays for the migration time and peak area are summarized in Table 1. This method gave good reproducibility.

Analysis of blood

Electropherograms of a blood sample of a healthy person and a blood sample spiked with cyanide (100 ng/mL) are shown in Figs. 3d and e, respectively. The cyanide peaks appeared at a migration time of 21.5 min, and were free from interference. The migration times were 2.5 min later than the migration time of the cyanide standard solution. This was due to methanol in the injected sample, which was added during the pretreatment. Methanol interfered with the electric current, resulting in a

Table 1 Intermediate precisions of within-run assays for migration time and peak area

Cyanide/ ng mL^{-1}	Precision (RSD, %)[a]	
	Migration time	Peak area
0[b]	0.19	3.11
1	0.14	1.00
10	0.14	1.12

a. n=6.
b. Reagent blank.

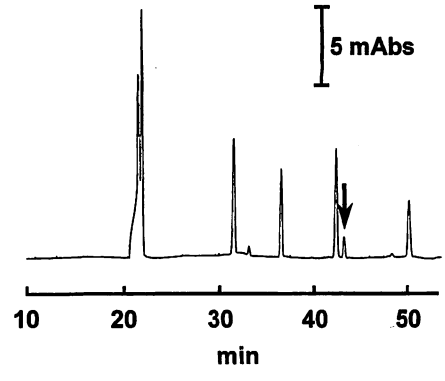

Fig. 4 UV electropherogram of the cyanide standard solution (38.5 ng/mL). Conditions: capillary effective length, 108.5 cm; UV detection wavelengths, 250 nm; other conditions, as in Fig. 3.

delay of the migration time. The electropherogram of the cyanide solution including methanol (the concentration was the same as that in the pretreated blood sample) is shown in Fig. 3c. The migration time of cyanide was the same as that in the blood sample.

During successive blood analyses, the migration time of all peaks gradually increased. This may have been due to the blood matrix binding to the fused-silica surface, resulting in a degradation of the EOF mobility. However, the capillary could be completely regenerated by washing it with methanol and 0.1 M NaOH.

UV detection

Throughout this study, data were simultaneously acquired by the fluorescence detector and the built-in diode-array detector. The UV electropherogram of the standard cyanide solution obtained from the diode-array detector is shown in Fig. 4. The detection limit, defined as the concentration which produced a signal equal to five times the background noise level, was 5 ng/mL. This value was only 50 times worse than that obtained by fluorescence detection, because the detection limit of fluorescence detection is based on the reagent blank peak.

The migration time of the cyanide derivative was rather long, because the conditions were optimized for CE with fluorescence detection. If only the diode-array detector is used without the external fluorescence detector, only about half the capillary length is needed. In this case, the cyanide derivative can be detected in about half the time. However, in blood analyses, the cyanide derivative overlapped with the blood matrix. Therefore, a different electrolyte is needed to analyze cyanide in blood using UV detection.

Conclusions

A new CE method for trace cyanide analysis using NDA-taurine derivatization has been developed. The proposed method is highly sensitive and highly selective, and is applicable for the analysis of cyanide in blood from a healthy person.

References

1. J. L. Way, *Ann. Rev. Pharmacol.*, **1984**, *24*, 451.
2. The Pharmaceutical Society of Japan, "*Yaku Dokubutsu Kagaku Shikenho To Chukai* (Standard Methods of Chemical Analysis in Poisoning —With Commentary—, in Japanese)", 4th ed., **1992**, Nanzando, Tokyo, 80.
3. Y. Seto, N. Tsunoda, H. Ohta, and T. Shinohara, *Anal. Chim. Acta*, **1993**, *276*, 247.
4. Proceedings of the 115th Annual Meeting of the Pharmaceutical Society of Japan, "*Koshu Eisei Kyougikai Siryo* (Data of Public Health Conference, in Japanese)", March **1995**, 76.
5. S. Chinaka and N. Takayama, *Jpn. J. Sci. Tech. Iden.*, **1997**, *2*, 27.
6. S. Chinaka, N. Takayama, Y. Michigami, and K. Ueda, *J. Chromatogr. B*, **1998**, *713*, 353.
7. T. Soga, I. Tajima, and D. N. Heiger, *Am. Lab.*, **2000**, *32*, 124.
8. V. Martí, M. Aguilar, and E. S. Yeung, *J. Chromatogr. A*, **1995**, *709*, 367.
9. A. Sano, M. Takezawa, and S. Takitani, *Biomed. Chromatogr.*, **1989**, *3*, 209.