Short Communication

Monitoring Pyridoxine by Capillary Zone Electrophoresis with Electrochemical Detection

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Abstract
Capillary zone electrophoresis was employed for the determination of pyridoxine using end-column amperometric detection with a carbon fiber microelectrode at a constant potential of 0.72 V (vs. SCE). The optimum conditions of separation and detection are 1.0×10^{-2} mol/L NH_4Cl–1.0×10^{-2} mol/L NH_3 for the buffer solution, 10 kV for the separation voltage, 5 kV and 10 s for the injection voltage and the injection time, respectively. The limit of detection is 1.2×10^{-6} mol/L or 3.5 fmol ($S/N = 2$). The relative standard deviation is 1.3 % for the migration time and 3.4 % for the electrophoretic peak current. The method was applied to determining pyridoxine in human urine.

Keywords: Electrophoresis, Electrochemical detection, Pyridoxine, Drug

Pyridoxine is an important and indispensable vitamin for human beings. Extreme deficiency can cause convulsions similar to epilepsy and indicates a profound disturbance in the central nervous system [1]. A multitude of methods has been developed for the assay of pyridoxine. The most common techniques are titrimetric method [2], high-performance liquid chromatography [3, 4] and single sweep oscilloscopic voltammetry [5, 6]. Capillary zone electrophoresis (CZE) has emerged as a powerful new method for rapid separation and detection of charged analytes [7, 8]. Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE, while offering a high degree of selectivity toward electroactive species and low cost [9]. In our laboratory this technique has been applied to cysteine [10], glutathione [11], purine bases [12–14], bovine serum albumin [15] and cytochrome c [16]. Yik et al. [17] used micellar electrokinetic capillary chromatography to determine pyridoxine. The method of detection of pyridoxine has two disadvantages. First, this kind of amperometric detector needs a conductive connection. Secondly, the working electrode must be inserted into the detection capillary. Recently, a new design for CZE-amperometric detector called the end-column amperometric detector has been described [18–21]. In this design, no conductive connection was used to decouple the electrophoretic and the detection current, and the working electrode was placed directly at the outlet of the separation capillary, and not inserted into the detection capillary. This kind of design is convenient, though the coulometric efficiency is slightly smaller [22]. The theory concerning the current for the end-column amperometric detector in CZE has been investigated as well [23, 24].

In this study we developed a CZE method for the detection of pyridoxine with the end-column amperometric detection at a carbon fiber microelectrode. The electrochemical detection was carried out by using potentiostatic control of the electrode potential by means of a three-electrode system. The method has been used to determine pyridoxine in human urine.

Optimum conditions of CZE with end-column amperometric detection: It was found that pyridoxine can be oxidized at the carbon fiber electrode in NH_4Cl–NH_3. Figure 1 shows the relationship between the detected peak current, i_p and the applied potential, E_d. When $E_d < 0.6$ V, i_p increases slowly with increasing E_d. When $E_d > 0.6$ V, i_p increases rapidly. When $E_d > 0.7$ V, i_p is almost constant. When an E_d of 0.72 V is applied, the base line of detection current is getting lower and the shape of the peak on the electropherogram also becomes better. E_d of 0.72 V is suitable for the detection because of the smooth base line and fine shape of the electropherograms. The effect of the buffer concentration, C_B, on the migration time, t_m, peak current, i_p, and the number of theoretical plates, N, in NH_4Cl–NH_3 is listed in Table 1. In Table 1, C_B indicates the value of the concentration of NH_4Cl, the ratio of the concentration of NH_4Cl to the concentration of NH_3 is 1 : 1. t_m, N, and i_p increase slowly with increasing C_B. In our experiments 0.01 mol/L NH_4Cl–0.01 mol/L NH_3 was used.

The separation voltage, V_s, exerts an influence on t_m and N [25]. Figure 2 shows the dependence of $1/t_m$, i_p and N on V_s. $1/t_m$ is proportional to V_s. N decreases with increasing V_s. There

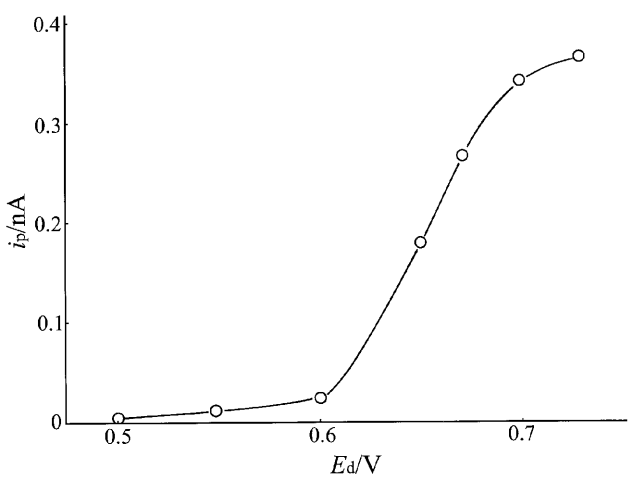

Fig. 1. Relationship between detected peak current and detected potential. 0.01 mol/L NH_4Cl–0.01 mol/L NH_3; 9.80×10^{-5} mol/L pyridoxine; capillary: 34.5 cm × 25 μm ID; injection, 5 kV for 10 s; separation voltage, 10 kV.

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Table 1. The values of t_m, N and i_p at different concentrations of C_B ($E_d = 0.72$ V, other conditions as in Figure 1).

C_B [mol/L]	t_m [s]	$10^{-3}N$	i_p [pA]
1.0×10^{-3}	170	6.40	301
2.0×10^{-3}	196	8.51	352
5.0×10^{-3}	247	13.5	352
8.0×10^{-3}	285	18.0	357
1.0×10^{-2}	303	20.3	380

is a maximum for i_p at $V_s = 10$ kV. Therefore 10 kV for V_s was chosen.

Reproducibility, limit of detection and linear range: The response for a series of six injections of 9.80×10^{-5} mol/L pyridoxine resulted in a relative standard deviation of 1.3 % for t_m and 3.4 % for i_p, respectively. The limit of detection is 1.2×10^{-6} mol/L or 3.5 fmol for the injected volume calculated (according to a signal-to-noise ratio of 2). A linear relationship holds between the peak current detected and concentration in the range of 4.90×10^{-6} to 4.5×10^{-4} mol/L. Least-squares treatment of these data yielded a slope 5.45 pA μmol^{-1}L and a correlation coefficient of 0.9995.

Determination of pyridoxine in human urine: Pyridoxine concentration in human urine can be readily determined by using this CZE-electrochemical detection system. A human urine sample containing 2.00×10^{-3} mol/L pyridoxine was used to verify the possibility of the standard addition method. The fresh urine sample of 500 μL and a 100 μL of solution containing 4×10^{-3} mol/L Na_2EDTA and 2 mol/L $HClO_4$ were combined in a 5 mL centrifuge tube, causing the proteins to separate. After standing for 5 min to precipitate proteins, the sample was centrifuged for 20 min at 4000 rpm. Ten mL of 0.01 mol/L NH_4Cl–0.01 mol/L NH_3 was added into the supernatant liquid of 100 μL. After homogenizing, the sample solution was injected into the CZE system by electromigration. The electropherograms of the human urine sample without and with the standard solution of pyridoxine are shown in Figure 3. The average concentration in the human urine samples obtained by the standard addition method is 2.07×10^{-3} mol/L, which agrees with the value in the human urine sample. The recovery is between 97 % and 99 %.

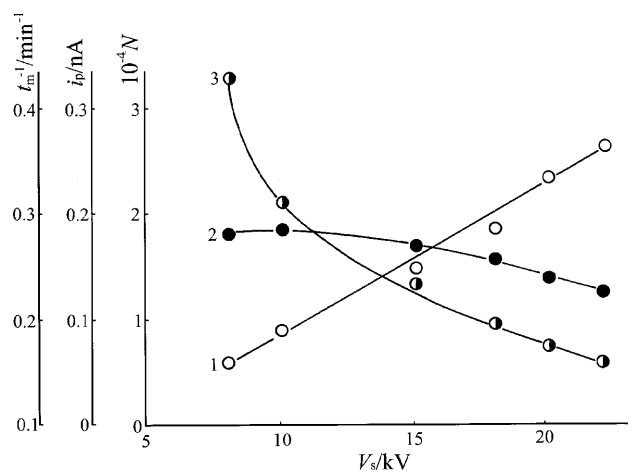

Fig. 2. Dependence of the reciprocal migration time (1), the number of theoretical plates (2) and the peak current detected (3) on the separation voltage. $E_d = 0.72$ V, 4.90×10^{-5} mol/L pyridoxine. Other conditions as in Figure 1.

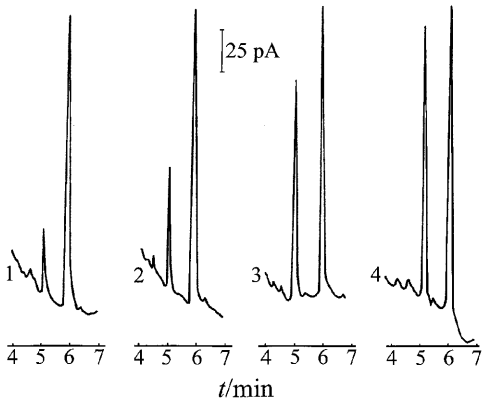

Fig. 3. Electropherograms of pyridoxine in the sample of human urine. The added concentration of pyridoxine (mol/L): 1) 0; 2) 1.98×10^{-5}; 3) 5.94×10^{-5}; 4) 7.92×10^{-5}. $E_d = 0.72$ V. Other conditions as in Figure 1.

Experimental

Apparatus: A reversible high-voltage power supply (Model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, China) provided a variable voltage of 0–30 kV across the capillary with the outlet of the capillary at ground potential. Fused-silica capillaries were purchased from Yongnian Optical Conductive Fiber Plant, China. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. The electrochemical detection at a constant potential was performed using the end-column amperometric approach with a microcurrent voltammeter (Model 901-pA, Ningde Analytical Instruments, China). The detection cell and detector were housed in a Faradaic cage in order to minimize the interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system, which consisted of a carbon fiber microelectrode as the working electrode, a coiled Pt wire as the auxiliary electrode, which also served as the ground for the high potential drop mentioned above across the capillary and a SCE as the reference electrode. The arrangement of the electrochemical detection cell was illustrated in [16] in detail. We took a glass capillary (ca. 0.5 mm ID, 1 mm OD and 5 cm length) with a funnel-shaped inlet at one end. A droplet of mercury was put into the glass capillary from the funnel-shaped inlet. Then the "funnel" was removed. A carbon fiber of 8 μm diameter soaked with acetone was carefully inserted into the glass capillary at the other end. The carbon fiber was connected to a copper wire of 0.1 mm diameter via the mercury junction by pushing a copper wire down. After drying the other end of the copper wire and the carbon fiber were bonded to the glass capillary using a low viscosity ethyl α-cyanoacrylate adhesive. The carbon fiber and the adhesive were lightly touched with a glass bar. The carbon fiber protruding was cut to about 300 μm. Before use all carbon fiber electrodes were washed with alcohol and immersed in water for 4–5 hours.

A 5.00×10^{-3} mol/L stock solution of pyridoxine was prepared by dissolving an appropriate amount of pyridoxine (pharmaceutical grade, Shanghai Xinya Pharmaceutical Co., Ltd) in water and stored at 4 °C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with water. All reagents were of analytical grade. All solutions were prepared with double distilled water.

The carbon fiber microelectrode was cemented onto a microscope slide, which was placed over a homemade XYZ micromanipulator and glued in place. The position of the carbon fiber microelectrode was adjusted (under a microscope) against the end of the capillary, so that the electrode and the capillary were in contact. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, 0.1 mol/L NaOH and double distilled water, respectively, and then filled with the corresponding separation electrolyte by means of a syringe. In addition, the electrolyte solution at the electrochemical cell was also replaced before each run. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic current reached a constant value (after 10 min), the electromigration injection was carried out and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after 5 or 6 runs.

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