Quantitation of Caffeine by Capillary Zone Electrophoresis with End-Column Amperometric Detection at a Carbon Microdisk Array Electrode

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Abstract

Capillary zone electrophoresis is employed for the determination of caffeine using end-column amperometric detection with a carbon fiber microdisk array electrode at a constant potential of 1.45 V versus a saturated calomel electrode. The optimum conditions of separation and detection are 0.152mM NaH_2PO_4–0.648mM Na_2HPO_4 for the buffer solution, 20 kV for the separation voltage, 5 kV for the injection voltage, and 10 s for the injection time. The limit of detection is 2.9×10^{-4}mM or 1.2 fmol (signal-to-noise ratio = 2). The relative standard deviation is 0.68% for the migration time and 2.3% for the electrophoretic peak current. The method is applied to determining caffeine in human serum and a cola drink.

Introduction

7-Methyltheophylline (caffeine) is an important analgesic and stimulant drug that is commonly used and combined in pharmaceutical formulations (1). Many methods have been developed for the determination of caffeine involving the use of chromatographic techniques, such as thin-layer chromatography (2), gas chromatography (3–5), high-performance liquid chromatography (6–9) and flow injection analysis with Fourier transform infrared (10,11). Nevertheless, there are no reports on the determination of caffeine by capillary zone electrophoresis (CZE).

Recently, CZE has rapidly become an important instrumental technique suitable for the rapid separation and detection of complex mixtures (12–14). The primary strength of CZE is its ability to provide extremely high separation efficiencies in short times with relatively simple instrumentation. Amperometric detection provides excellent sensitivity for the small dimensions associated with CZE and also offers a high degree of selectivity toward electroactive species and low cost (15). In our laboratory, this technique has been applied to cysteine (16), glutathione (17), purine bases (18–20), bovine serum albumin (21), and cytochrome *c* (22). The theory concerning the current for the end-column amperometric detector in CZE has been investigated (23,24).

A method for the detection of caffeine by CZE with the end-column amperometric detection at a carbon fiber microdisk array electrode has been developed. The separation was performed in a 25-µm i.d. fused-silica capillary. Potentiostatic control of the electrode potential by means of a three-electrode system aided the detection. The method has been used to determine caffeine in human serum and a cola drink. The samples can be directly injected. No pretreatment of the samples is needed. The migration time is also short (approximately 2 min for 35-cm long capillary). The main advantage of the method is its excellent selectivity for the determination of caffeine in both sorts of samples. Only one peak of caffeine appears on the electropherograms.

Experimental

Apparatus

Linear sweep voltammetry

A commercial polarograph (model 83-2.5, Ningde Analytical Instruments, Ningde, China) coupled with an X-Y recorder (model 3086-11, Yokogawa Hokuskin, Tokyo, Japan) was used. It was used in connection with a cell using potentiostatic control of the electrode potential by means of a three-electrode system that consisted of a carbon fiber array electrode as the working electrode, a Pt wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. The reference electrode was connected to the analyte via a salt bridge filled with the same supporting electrolyte as the cell.

Capillary zone electrophoresis

A reversible high-voltage power supply (model GDY, Shandong Institute of Chemical Engineering and School of Chemistry, Shandong University, Jinan, China) provided a variable voltage of 0~30 kV across the capillary with the outlet of the capillary at ground potential. Fused-silica capillaries (360-µm o.d., 25-µm

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i.d.) were purchased from Yongnian Optical Conductive Fiber Plant (Yongnian, China). They were cut to a length of 35 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 20 kV.

The electrochemical detection at a constant potential with CZE was performed using the end-column amperometric approach with a voltammetric analyzer (model JF-01, Shandong Institute of Chemical Engineering and School of Chemistry). The detection cell and detector were housed in a faraday cage in order to minimize interference from external sources of noise. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber microdisk array electrode 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 detail in the literature (21).

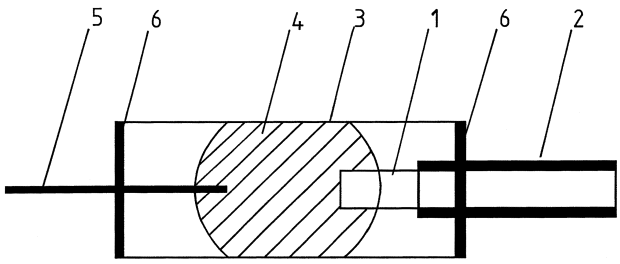

Figure 1. A top view of the carbon fiber microdisk array electrode: 1, carbon fiber microdisk array with ethyl α-cyanoacrylate adhesive; 2, fused-silica capillary; 3, glass capillary; 4, mercury; 5, copper wire; 6, epoxy resin.

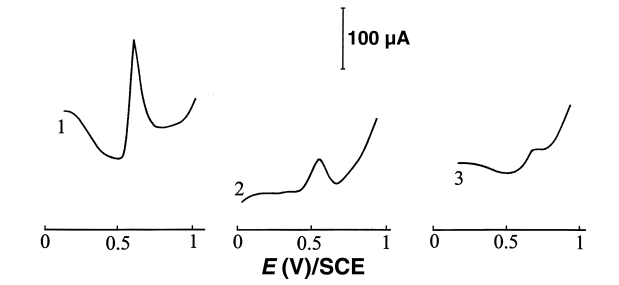

Figure 2. Typical linear sweep voltammograms of caffeine at the carbon fiber array electrode in NaH_2PO_4–Na_2HPO_4 with different pH (v = 100 mV/s). 1, pH = 6.0; 2, pH = 7.0; 3, pH = 8.0.

Carbon fiber electrodes

For linear sweep voltammetry, the carbon fiber array electrode was used. A small amount of mercury was drawn into the glass capillary (approximately 0.5-mm i.d., 1-mm o.d., and 5-cm length). Approximately 60 carbon fibers (6-µm diameter) soaked in acetone were carefully inserted into the glass capillary at the other end. The role of the acetone was to gather together the fibers as an array for convenience of operation. The carbon fiber array was connected to a copper wire (0.4-mm diameter, 12-cm length) via the mercury junction by pushing a copper wire down. The other end of the copper wire and the carbon fiber array (after drying) were bonded to the glass capillary using a low-viscosity ethyl α-cyanoacrylate adhesive. The carbon fibers and the adhesive were lightly touched with a glass bar to mix them. A glass tube (1.5-mm i.d., 8-mm o.d., 6-cm length) was put around the glass capillary in order to protect it. The copper wire was bonded to the glass tube using epoxy. The carbon fiber array was bonded at the other end of the glass tube and protruded approximately 1 cm from the end. Then, the carbon fibers were cut to 4 mm in length.

For CZE, the carbon fiber microdisk array electrodes were constructed using 6-µm carbon fibers. The process of manufacture was similar to the carbon fiber array electrode previously described. An approximately 30-carbon fiber array soaked with acetone was inserted into a fused-silica capillary (approximately 250-µm i.d., 375-µm o.d., 1.5-cm length). Next, the carbon fiber array was immersed into the ethyl α-cyanoacrylate adhesive, and the adhesive was allowed to pass through the whole carbon fiber array into the fused silica capillary. Then, the fused-silica capillary with the carbon fiber array was inserted into a glass capillary (approximately 0.5-mm i.d., 1-mm o.d., 2.5-cm length) and was bonded together. Finally, the carbon fiber array protruding from the fused-silica capillary was trimmed. The carbon fiber microdisk array electrode is illustrated in Figure 1.

Before use, all carbon fiber microdisk array electrodes were cleaned in alcohol and washed with double-distilled water for 5 min by a supersonic wave cleaner. During electrophoresis, the electrodes can be directly washed with alcohol and water in the detection cell.

Table I. Values of t_m, i_p, $W_{1/2}$, and N in NaH_2PO_4–Na_2HPO_4 at Different pH*

Buffer	pH	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	10^{-3} N
0.051mM NaH_2PO_4–0.049mM Na_2HPO_4	6.8	119.6	4.62	3.3	7.7
0.039mM NaH_2PO_4–0.061mM Na_2HPO_4	7.0	111.4	4.78	3.2	6.7
0.028mM NaH_2PO_4–0.072mM Na_2HPO_4	7.2	107.8	5.49	3.3	5.9
0.019mM NaH_2PO_4–0.081mM Na_2HPO_4	7.4	101.1	7.78	3.2	5.5
0.013mM NaH_2PO_4–0.087mM Na_2HPO_4	7.6	98.3	7.43	3.2	5.2
0.0085mM NaH_2PO_4–0.092mM Na_2HPO_4	7.8	92.5	4.68	3.2	4.6

* 0.100mM caffeine; capillary, 35-cm length, 25-mm i.d.; injection, 5 kV for 10 s; separation voltage, 20 kV; detection potential, 1.45 V.

Reagents and solutions

A 10.0mM stock solution of caffeine was prepared by dissolving an appropriate amount of caffeine in water and storing them 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.

Procedure

For linear sweep voltammetry, the carbon fiber array electrode was directly inserted into the

experimental solution containing caffeine, and a linear sweep voltammogram was recorded.

For CZE, the carbon fiber microdisk array electrode was cemented onto a microscope slide that was placed over a homemade XYZ micromanipulator and glued in place. The position of the carbon fiber microdisk array electrode was adjusted (under a microscope) against the end of 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 removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with double distilled water, then 0.1M NaOH, then double-distilled water, and then 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 (~ 20 min), electromigration injection was carried out at 5 kV for 10 s, and the electropherogram was recorded. The separation electrolyte in the capillary was replaced after 5 or 6 runs.

All potentials were measured against SCE.

Results and Discussion

Linear sweep voltammograms of caffeine

The voltammetric characteristics of caffeine have been reported using the glassy carbon electrode (25) and the chemically modified electrode (26). It was found that caffeine can also be oxidized at the carbon fiber array electrode in NaH_2PO_4–Na_2HPO_4 buffers. Figure 2 shows typical linear sweep voltammograms in solutions at different pH. An oxidation peak of caffeine at approximately 0.6~0.8 V is observed.

Optimum conditions of CZE with end-column amperometric detection

In the pH range of 6~8, there is one oxidization peak of caffeine on the linear sweep voltammograms shown in Figure 2. Therefore, the electrophoretic behavior of caffeine in solutions of pH near 7 was researched. The peak current (i_p), the migration time (t_m), the width at half-height ($W_{1/2}$) on the electropherograms, and the number of theoretical plates (N) at different pH is listed in Table I. N was calculated according to the following equation:

$$N = 5.54 \times (t_m/W_{1/2})^2 \qquad \text{Eq. 1}$$

t_m decreases with increasing pH, but $W_{1/2}$ is almost constant. So N decreases with increasing pH. i_p is the highest at pH 7.4. Therefore pH 7.4 was selected. The effect of the concentration of the buffer (CB) on t_m, i_p, $W_{1/2}$, and N in NaH_2PO_4–Na_2HPO_4 is listed in Table II. In Table II, C_B indicates the value of the concentration of NaH_2PO_4 (the ratio of the concentration of NaH_2PO_4 to

Table II. Values of t_m, i_p, $W_{1/2}$, and N at different concentrations of CB

C_B (mM)	t_m (s)	i_p (nA)	$W_{1/2}$ (s)	10^{-3} N
0.019	101.1	7.78	3.2	5.5
0.038	101.8	12.6	3.2	5.6
0.095	105.3	14.4	3.2	6.0
0.152	108.5	17.1	3.2	6.4
0.190	112.4	17.5	3.2	6.8

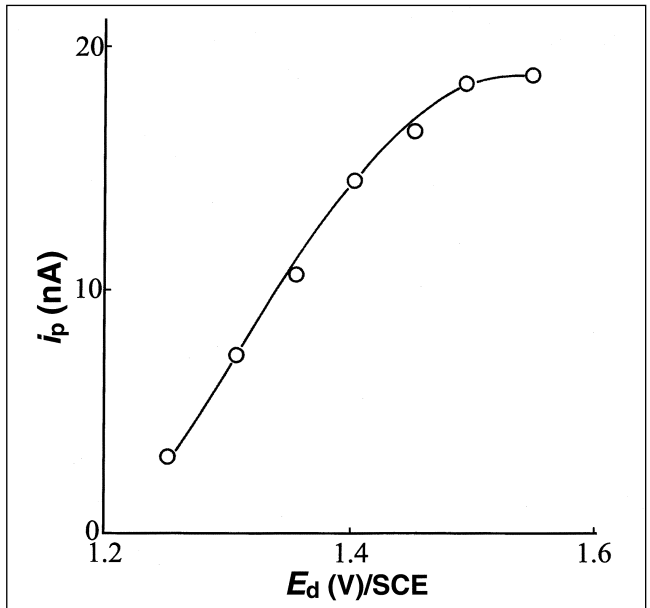

Figure 3. Relationship between detected peak current and detection potential (0.152mM NaH_2PO_4–0.648mM Na_2HPO_4). Other conditions in Table I.

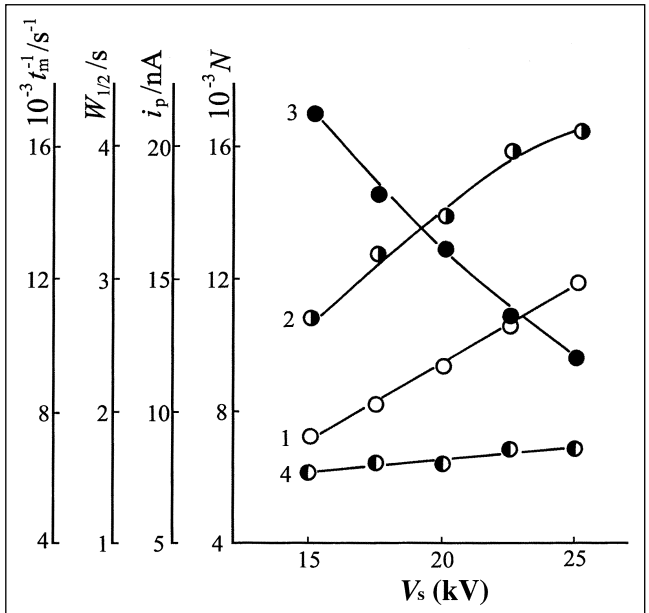

Figure 4. Dependence of the reciprocal migration time (1), the peak current detected (2), the width at half-peak (3), and the number of theoretical plates (4) on the separation voltage. Conditions as in Figure 3.

the concentration of Na_2HPO_4 is 1:4.26). t_m, N, and i_p increase with increasing C_B, because the migration velocity of the substance depends mainly on the electroosmotic velocity (v_{eo}) of buffer, which is proportional to the ζ potential (27). With increasing buffer concentration, the thickness of the electrical double layer is reduced, and the ζ potential becomes smaller. Therefore, v_{eo} decreases and t_m increases. N increases because t_m increases and $W_{1/2}$ is constant in the range of the concentrations tested with increasing buffer solutions (Equation 1). In the concentration range, t_m is short, and its change is only 11 s, so the difference in $W_{1/2}$ that is mainly contributed to by the molecular diffusion is not obvious. In addition, when v_{eo} decreases, caffeine has more time to be in contact with the surface of the working electrode, which allows more caffeine molecules to be oxidized on the surface of the electrode. Therefore, i_p increases with increasing CB. In these experiments, 0.152mM NaH_2PO_4–0.648mM Na_2HPO_4 was used because of higher i_p, larger N, and lower noise.

Figure 3 shows the relationship between the detected peak current (i_p) and the applied potential (E_d). i_p increases with increasing E_d. When E_d is between 1.25 and 1.50 V, i_p increases rapidly. When E_d > 1.50 V, i_p increases slowly. When E_d > 1.45 V, the baseline current and noise increase. Therefore, an E_d of 1.45 V is suitable for detection because of the good reproducibility, lower baseline noise, and fine shape of the electropherograms.

The separation voltage (V_s) exerts an influence on t_m and N (28). Figure 4 shows the dependence of $1/t_m$, i_p, $W_{1/2}$, and N on V_s. $1/t_m$ is proportional to V_s. There is a linear relationship between N and V_s . i_p increases with increasing V_s. Nevertheless, the noise increases with increasing V_s. Therefore, 20 kV for V_s was chosen because of larger i_p and N and lower noise.

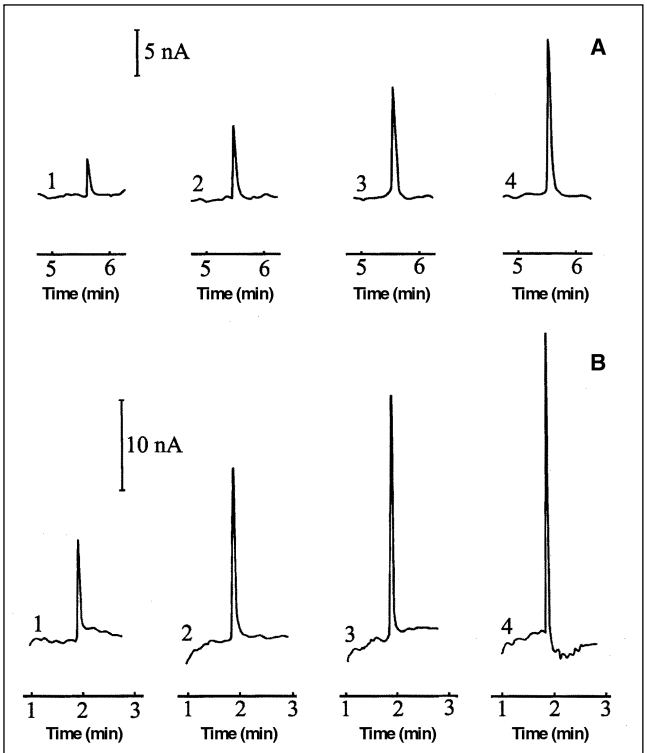

Figure 5. Electropherograms of caffeine in samples of human serum (A) and cola drink (B). The concentration of caffeine (in mM): 1, sample; 2, sample + 2.00×10^{-5}; 3, sample + 4.00×10^{-5}; 4, sample + 6.00×10^{-5}; 5, sample; 6, sample + 5.00×10^{-5}; 7, sample + 1.00×10^{-4}; 8, sample + 1.50×10^{-4}. Capillary length: 70 cm (A) or 35 cm (B). Other conditions as in Figure 3.

Reproducibility, limit of detection, and linear range

The response for a series of six injections of 0.100mM caffeine resulted in a relative standard deviation of 0.68% for t_m and 2.3% for i_p, respectively. The limit of detection is 2.9×10^{-4}mM (at a signal-to-noise ratio of 2), which was estimated from the electropherograms obtained for 1.00×10^{-3}mM caffeine or 1.2 fmol for the injected volume.

A linear relationship exists between the peak current detected and concentration in the range of 1.00×10^{-3} to 1.00mM. Least-squares treatment of these data yielded a slope of 169 pA/µmol and a correlation coefficient of 0.9997.

Determination of caffeine in human serum and cola drink

A synthetic human serum sample containing 2.00mM caffeine that consisted of serum from an adult volunteer and the standard caffeine was used to verify the possibility of standard addition method. A 100-µL amount of 1.52mM NaH_2PO_4–6.48mM Na_2HPO_4 was added to a 10-µL serum sample. After the sample solution was diluted to 1 mL, it was injected into the CZE-electrochemical system. The electropherograms of the human serum sample without and with the addition of the standard solutions of caffeine are shown in Figure 5A. The concentrations of caffeine in the human serum sample obtained by the standard addition method for two injections were 1.96 and 2.03mM, respectively, which agree with the value in the human serum sample. The recovery is between 98% and 102%.

Caffeine concentration in cola drinks can be readily determined by using this CZE-electrochemical detection system. A 100-µL amount of 1.52mM NaH_2PO_4–6.48mM Na_2HPO_4 was added to a 100-µL cola sample. After dilution to 1 mL, it was injected and determined by CZE. Figure 5B shows the electropherograms without and with addition of the standard solution. The concentrations of caffeine in the sample obtained by the standard addition method for two injections were 119 and 121 mg/L, respectively. The recovery is between 98 and 104%. According to literature (29), the normal caffeine content in cola drinks is approximately 100 mg/L. Therefore, this result should be satisfactory.

From the electropherograms of the human serum sample and cola drink sample, it can be seen that only one peak of caffeine appears on their electropherograms. This indicates that the main advantage of the method is its excellent selectivity. In addition, the samples can be directly injected, and no pretreatment of the samples is needed. The migration time is also short (approximately 2 min for a 35-cm long capillary).

Acknowledgments

This project was supported by the National Science Foundation of China, the Science Foundation of Shandong Province, and the Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

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Manuscript accepted November, 17, 1999.