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CHEM. RES. CHINESE UNIVERSITIES 2012, 28(3), 415—418 Determination of Nicotine in Tobacco by Capillary Electrophoresis with Electrochemical Detection SUN Jin-ying¹, XU Xiao-yu^{1, 2}, YU Huan¹ and YOU Tian-yan^{1*} 1. State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China; 2. Ministry of Public Security of Jilin Province, Changchun 130051, P. R.

China Abstract A sensitive, simple and low-cost method based on capillary electrophoresis(CE) with electrochemical(EC) detection at a carbon fiber microdisk electrode(CFE) was developed for the determination of nicotine. Effects of detection potential, concentration and pH value of the phosphate buffer, and injection time as well as separation voltage were investigated. Under the optimized conditions: a detection potential of 1.20 V, 40 mmol/L phosphate buffer(pH 2.0), a sample injection time of 10 s at 10 kV and a separation voltage of 16 kV, the linear range obtained was from 5.0×10^{-7} mol/L to 1.0×10^{-4} mol/L with a correlation coefficient of 0.9989 and the limit of detection(LOD, S/N= 3) obtained was 5.0×10^{-8} mol/L. The method was also used to determine the nicotine in cigarettes. Nicotine amount ranged from 0.211 mg/g to 0.583 mg/g in the pipe tobacco of seven brands of cigarette and the amount in one cigarette varied from 0.136 mg/cigarette to 0.428 mg/cigarette. Keywords Capillary electrophoresis; Electrochemical detection; Nicotine; Tobacco Article ID 1005-9040(2012)-03-415-04 1 Introduction Nicotine accounts for about 98%(mass fraction) of the total alkaloids and presents in a concentration of 0.8% (mass fraction) in tobacco[1, 2]. And nicotine addiction is related with higher risk for many

kinds of diseases such as Alzheimer's, Parkinson's and even suicide[3, 4]. Thus it's necessary to control nicotine amount in tobacco products. Determination of nicotine is very important in both the tobacco industry and toxicology area[5]. A lot of analytical methods have been established for the analysis of nicotine and related alkaloids, such as radioimmunoassay[6], spectrophotometry[5], near-infrared spectroscopy[7], and recently flow injection(FI) with electrochemiluminescence(ECL) detection[8].

The most frequently used analytical techniques for nicotine and its relative compounds determination are high performance liquid chromatography(HPLC)[9? 15] and gas chromatography (GC)[16? 23] with mass spectrometry(MS). Besides, HPLC coupled with UV-visible absorption(UV)[24? 28] or GC with flame ionization detector(FID)[1, 29, 30] and atomic emission detector(AED)[31] have also been developed to determine nicotine and related alkaloids. Capillary electrophoresis(CE) is characterized by high separation efficiency, short analysis time and a small amount of reagent consumed.

Moreover, capillary column is flexible for use, easy to be treated with and cost effective. CE has been considered as an efficient alternative for HPLC technique[32]. Up to now, various detectors have been combined with CE separation for nicotine analysis, such as CE-MS[33, 34], nonaqueous CE(NACE)-MS[35], CE-UV[36? 38], microchip micellar electrokinetic chromatography(microchip MEKC)-UV[39] and CE with dual light-emitting diode induced fluorescence (LEDIF) and ECL detection[40]. Electrochemical(EC) detection has received more attention due to the simple manipulation and good selectivity.

Electrocatalytic oxidation properties of nicotine have been investigated at multi-walled carbon nanotube-alumina-coated silica nanocomposite modified glassy carbon electrode(MWCNTACS-GCE), MWCNT-GCE and pencil graphite electrode[3, 41, 42]. HPLC with EC detection has been used for nicotine assay in plasma and hair[43, 44]. NACE-EC was also established for tobacco nicotine detection[45]. In this paper, a simple CE-EC analytical procedure at a carbon fiber microdisk electrode(CFE) was developed. The linear range was 5.0×10^{-7} to 1.0×10^{-4} mol/L, with a correlation coefficient of 0.989. The limit of detection(LOD, S/N= 3) obtained was 5.0×10^{-8} mol/L. To evaluate the applicability of the proposed CE-EC method, seven different cigarette brands were tested. Nicotine amounts ranged from 0.211 mg/g to 0.583 mg/g in pipe tobacco of seven brands of cigarette and the amount in one cigarette varied from 0.136 mg/cigarette to 0.428 mg/cigarette.

2.2.1 Experimental Reagents All the reagents were of analytical grade that were used as received without further purification. Nicotine(purity 99.7%) was obtained from Alfa Aesar(USA). Stock solution of 1.0×10^{-3} mol/L nicotine was prepared in doubly distilled water _____ *Corresponding author. E-mail:jl.cn Received June 20, 2011; accepted November 24, 2011. Supported by the National Natural Science Foundation of China(No. 20875085). 416 CHEM. RES. CHINESE UNIVERSITIES Vol. 28 and stored at 4 °C. Na₂HPO₄, NaH₂PO₄, H₃PO₄ and NaOH were used for phosphate buffer solutions(PBS) preparation. All the solutions were prepared and diluted with doubly distilled water unless otherwise indicated. PBS was prepared daily with doubly distilled water.

All the solutions were filtered through a 0.22 μ m membrane before use. Different brands of cigarette were purchased from local market. high detection sensitivity. As shown in Fig. 2, with the increase of the applied detection potential, the current response increased slowly between 0.70 and 1.00 V, and then increased quickly between 1.00 and 1.20 V. Higher detection potential than 1.20 V led to a peak current decrease and background noise increase. To achieve high detection sensitivity, 1.20 V was selected as the optimum applied detection potential.

2.2 Apparatus

EC experiment was conducted with a Voltammetric Analyzer(CHI 800, USA). A conventional three-electrode system was employed with a 33- μ m CFE as working electrode, a Pt wire as counter electrode and an Ag/AgCl electrode as reference electrode. An uncoated fused-silica capillary with i. d. of 25 μ m and length of 45 cm(Ruifeng Chromatogram Equipment Co. , Ltd. , Hebei, China) was used for sampling and separation. Capillary was rinsed in 0.1 mol/L NaOH overnight before use. Every day before experiments, it was flushed with doubly distilled water for about 10 min and balanced with running buffer for about 15 min.

CE-EC was conducted on a self-assembly instrument including a Voltammetric Analyzer(CHI 800, USA) and a high voltage supplier(MPI-A, Remax Electronic Co. , Ltd. , Xi'an, China). Sample injection was performed electrokinetically for 10 s at 10 kV. Fig. 2 HDV investigation of nicotine c(Nicotine)= 1.0 \times 10⁻⁵ mol/L; sample injection: 10 s at 10 kV; separation voltage: 20 kV; CE buffer: 40 mmol/L PBS(pH 2.0); cell buffer: 0.1 mol/L PBS(pH 8.0).

3.3 Optimization of CE-EC Conditions

3.3.1 Results and

Discussion Cyclic Voltammetry(CV) CV was used to investigate the electrochemical behavior of nicotine.

A dramatic current increased from 0.70 V was observed for nicotine(Fig. 1, curve b) compared with that of background electrolyte(Fig. 1, curve a), indicating that nicotine had high electroactivity at CFE. The adsorption property of CFE for nicotine was also investigated under CV experiment, however, no adsorption phenomenon of nicotine was observed. Since the oxidation potential of nicotine was not high at CFE, CE coupled with EC at CFE is practical for nicotine determination. Some other important factors including buffer concentration and buffer pH as well as separation voltage were investigated.

Running buffer pH value influences the charge-mass ratio of the analyte and then influences the electrophoresis behavior of the analyte. We investigated the effect of pH values on the detection between pH 2.0 and pH 10.0 as shown in Fig. 3. Fig. 3 Effect of pH of CE buffer on detection of nicotine pH: a. 2; b. 4; c. 6; d. 8; e. 10. Separation voltage: 14 kV; other conditions were the same as those in Fig. 2. Fig. 1 Cyclic voltammetry curve of nicotine a. Background electrolyte, 0.1 mol/L PBS(pH 8.0); b. 1.0×10^{-3} mol/L nicotine; scan rate: 0.05 V/s. 3. Hydrodynamic Voltammogram(HDV) Investigation Since applied detection potentials influence the detection sensitivity, thus we investigated the HDV of nicotine to achieve At pH 2.0, nicotine was fully protonated and electroosmotic flow(EOF) was well restrained. EC response was the highest at pH 2.0, and then decreased with the increase of pH value. With the increase of pH value, the migration time decreased correspondingly. Strong acidic CE buffer pH is more beneficial to sensitive

and selective determination of nicotine. In consideration of the detection sensitivity, pH 2. was selected as the proper CE buffer pH value. Separation voltage is an important factor that influences the detection sensitivity and the migration time. When the separation voltage was changed from 10 kV to 20 kV, the migration time decreased from 14 min to 7 min correspondingly. No. 3 SUN Jin-ying et al. 417 As for EC response, when the separation voltage increased from 10 kV to 18 kV, the EC intensity of nicotine increased quickly, after that it decreased quickly from 18 kV to 20 kV (shown in Fig. 4). Detection sensitivity at 16 kV was higher than that at 18 kV.

In consideration of the detection sensitivity, 16 kV was selected as the optimum separation voltage. supermarket. Pipe tobacco of 0.1 g was weighed and placed in a polyethylene tube, in which 10 mL of solvent was added for nicotine extraction. Then, 10 μ L of the extraction solution was transferred into a new polyethylene tube and diluted 100 times by doubly distilled water and the diluted extraction solution was used for analysis.

4. 2 Investigation of Extraction Solvents Fig. 4 Effect of separation voltage on detection of nicotine Applied detection potential: 1. 20 V; other conditions are as those in Fig. . 3. 4 Linear Range and LOD To obtain high extraction efficiency, solvents including water, methanol, chloroform and ethyl acetate were investigated. Current response of nicotine extracted with different solvents is shown in Fig. 5. Current response of nicotine was the highest when water was used as solvent. Methanol was also efficient for the extraction of nicotine from pipe tobacco. However, when methanol was used as solvent, migration time prolonged and the baseline shifted. When

chloroform was used for extraction, current response was about 36% of that when water was used for extraction.

Only very low response was found when ethyl acetate was used as solvent. Water was selected as nicotine extraction solvent for the highest nicotine current response obtained. Under the selected conditions: an applied detection potential of 1.20 V; sample injection for 10 s at 10 kV; a separation voltage of 16 kV; 40 mmol/L PBS(pH 2.0) as running buffer and 0.1 mol/L PBS(pH 8.0) as detection buffer, EC response of nicotine was linear with concentration from 5.0×10^{-7} mol/L to 1.0×10^{-4} mol/L ($81 \times 16200 \mu\text{g/L}$) ($y = -0.2566 + 0.4884x$, $R^2 = 0.9978$) and LOD ($S/N = 3$) of nicotine was determined to be 5.0×10^{-8} mol/L ($8 \mu\text{g/L}$). The proposed CE-EC method was compared with HPLC-MS[9, 10], HPLC-UV[24, 28], CE-UV[38, 39], CE-MS[34], CE-LEDIF-ECL[40], microchip CE-UV[39], NACE-MS[35] and NACE-EC[45] methods (Table 1). From Table 1 we can know that linear range and LOD of CE-EC are nearly comparable with those of HPLC-MS[9] and UV[24] or NACE-EC[45] methods and CE-EC is almost 2000 times more sensitive than microchip CE method.

Table 1 Method HPLC-MS HPLC-MS FI-ECL HPLC-UV HPLC-UV CE-MS NACE-MS CE-UV Micorchip CE-UV CE-LEDIF-ECL NACE-EC CE-EC

Fig. 5 Extraction solvent comparison a. Water; b. methanol; c. chloroform; d. ethyl acetate.

Separation voltage: 16 kV; injection: 10 s at 10 kV; applied detection potential: 1.20 V; cell buffer: 0.1 mol/L PBS(pH 8.0); CE buffer: 40 mmol/L PBS(pH 2.0).

4.3 Extraction Time Investigation Comparison of CE-EC with other methods for nicotine determination

Method	Linear range ($\mu\text{g}\cdot\text{L}^{-1}$)	LOD ($\mu\text{g}\cdot\text{L}^{-1}$)
HPLC-MS	10×10000	1×100
HPLC-UV	0×16000	25×500
CE-UV	250×100000	500×100000
NACE-EC	1724×17240	—
CE-EC	—	—

100? 10000 81? 16200 LOD/(? g·L⁻¹) 10 1 0. 19 8 100 0. 55 20 — 16000
259. 2 13 8. 1 Ref. [9] [8] [22] [24] [28] [34] [35] [38] [39] [40] [45] Our
method The influence of extraction time on nicotine detection was
investigated between 2 and 7 h.

When extraction time was increased from 2 h to 4 h, the nicotine response kept increasing. But when extraction time continued to increase, the current response decreased, which may be due to the nicotine decomposition under room conditions. We chose 4 h as proper extraction time based on the experiment result. 4. 4 Tobacco Analysis 4 4. 1 Cigarette Analysis Extraction Procedure Seven brands of cigarettes were purchased from local Pipe tobacco of 0. 1 g of each seven brands of cigarettes was weighed respectively and 10 mL of doubly distilled water was used for nicotine extraction.

Nicotine amounts ranged from 0. 211 mg/g to 0. 583 mg/g in the pipe tobacco of each of seven brands of cigarettes. Nicotine amount in one cigarette varied from 0. 136 mg/cigarette to 0. 428 mg/cigarette(as listed in Table 2). The results obtained are slightly less than the amounts reported in the literature[46]. With the increase of nicotine concentration, the recovery decreased. For 5. 0? 10⁻⁶, 5. 0? 10⁻⁵ and 5. 0? 10⁻⁴ mol/L of nicotine, the recoveries were 80%, 75% and 418 CHEM. RES. CHINESE UNIVERSITIES [20] [21] [22] [23] [24] [25] [26] [27] B, 2006, 844, 322 Vol. 28 72%, respectively(n= 3).

Table 2 Nicotine amounts in pipe tobacco and cigarette obtained by CE-EC analysis
Number of tobacco sample 1 2 3 4 5 6 7 Nicotine amount in pipe tobacco/(mg·g⁻¹) 0. 518 0. 502 0. 454 0. 211 0. 583 0. 454 0. 421 Nicotine

amount in cigarette/ (mg·cigarette⁻¹) 0. 337 0. 355 0. 316 0. 136 0. 428 0. 327 0. 276 Man C. N. , Gam L. H. , Ismail S. , Lajis R. , Awang R. , J. Chromatogr. Lafay F. , Vulliet E. , Flament-Waton M. M. , Anal. Bioanal. Chem. , 2010, 396, 937 Shrivastava K. , Patel D. K. , Food Chem. , 2010, 122, 314 Bao M. L. , Joza P. , Rickert W. S. , Lauterbach J. H. , Anal. Chim. Acta, 2010, 663, 49 Sharp M. P. , Hale T.

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