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Determination of sugars in Chinese traditional drugs by CE with amperometric detection

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Abstract

A simple, fast and reliable method, based on capillary zone electrophoresis with amperometric detection, for the separation and determination of sucrose, glucose, and fructose in Chinese traditional drugs, namely Astragalus Membranceus (Fish.) Bge, Angelica and Codonopsis pilosula (Franch.) Nannf. was described in this paper. A copper disk electrode was used as working electrode. The optimal conditions of separation and detection were 0.05 mol/l sodium hydroxide buffer (pH 12.7), 5 kV for the separation voltage and +0.65 V (vs. Ag/AgCl) for the detection potential. The linear ranges were from 5.0×10^{-6} to 5.0×10^{-4} mol/l for all three sugars. The all regression coefficients were more than 0.999. The detection limits were 1.0×10^{-6} mol/l for glucose and fructose, and 4.0×10^{-6} mol/l for sucrose. The method built in this paper was directly applied to the separation and determination of the three sugars in three Chinese traditional drugs without prior derivatization, and the content for every sugar in the drugs was first assayed. The assay results were satisfactory.

Keywords: Capillary electrophoresis; Electrochemical detection; Sugar; Angelica; Astragalus membranceus (Fish.) Bge; Codonopsis pilosula (Franch.) Nannf.

1. Introduction

Angelica, Astragalus Membranceus (Fish.) Bge and Codonopsis pilosula (Franch.) Nannf. have been used as Chinese traditional drugs for thousands of years. Chinese Angelica is the root of angelica belonging to the carrot family. It can be used to invigorate the circulation of blood; regulate the menstrual function and moisten the respiratory tract, skin, etc. [1]. Astrasalus Membranceus (Fish.) Bge is the root of A. mongholicus Bge belonging to milk vetch. Its function is to discharge pus, diuresis [2]. Codonopsis pilosula (Franch.) Nannf. is the root of codonopsis genus belonging to campanulaceae. It can be used to invigorate the function of the spleen and is good for the liver [3]. These three drugs are an important part of Chinese traditional drugs. However, each drug grown in different areas or different periods

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has different quality and this affects the cure efficiency of these drugs. So it is important and necessary to build a simple, fast and effective method to identify the qualities of these drugs. Bai et al. [4] and Han et al. [5] have found that these three drugs grown in different areas or different periods have different contents of sugars and the more sugars in the drugs, the better the qualities of the drugs. So the contents of sugars in these drugs can be used to identify their qualities. There are a few papers about the analysis of the sugars in these Chinese drugs [5–8] and traditional spectrophotometry method was used in all papers. But the total contents of the sugars, not the content for every sugar were determined by this method. Moreover, the sugars needed derivatization before determination due to the absence of chromophoric and/or fluorophoric groups in them. So, the operation is very complicated.

Now, capillary zone electrophoresis (CZE) has been widely used in analysis of sugars [9–12]. El Rassi has reviewed the carbohydrate analysis by capillary electrophoresis [13]. No papers have reported separating and determining the sugars in these Chinese traditional drugs by CZE.

In the present work, we developed a method to separate and determine three kinds of sugars in three Chinese traditional drugs, namely Angelica, Astragalus Membranceus (Fish.) Bge and Codonopsis pilosula (Franch.) Nannf. by using CZE with amperometric detection (CZE-AD) without derivatization before determination. Limits of detection, linearity, reproducibility and recoveries were presented for these analytes.

2. Experimental

2.1. Apparatus

The CZE system with wall-jet amperometric detection assembly was constructed in the laboratory and was similar to that described previously [14,15]. Electrophoresis in the capillary was driven by a $\pm$30 kV high-voltage supplier (Shanghai Institute of Nuclear Research, China). The cyclic voltammetry was carried out on CHI630 Electrochemical System (CH Instruments, USA). The separations were proceeded in a 45 cm long, o.d. 360 μm, i.d. 25 μm, polyimide-coated fused silica capillary (Polymicro Technologies, Phoenix AZ). The injector electrode was kept at high positive voltage, the electrochemical cell for detection was kept at ground and samples were all injected electro-kinetically, applying 5 kV for 10 s.

The copper disk electrode was constructed with a 140-μm-diameter copper wire whose sides were covered with a non-conductive coating. One end of the fine copper wire was attached to another piece of copper wire (5–7-cm length, 0.5–1-mm diameter) by welding. A glass pipette of about 0.4 mm tip diameter drawn from borosilicate glass tube (o.d. 0.4 cm, i.d. 0.2 cm) was gently cut to allow passage of the fine copper wire. The fine copper wire was carefully introduced into the pipette through the end opposite to the micro-tip until it protruded approximately 0.3 cm from the pipette tip. Non-conductive gel was applied at both ends of the pipette to seal the two ends. Prior to use, the surface of the copper disk electrode was gradually polished with emery paper and 0.05 μm alumina powder, then ultrasonicated in de-ionized water, and finally positioned carefully opposite the capillary outlet with the aid of a micromanipulator to minimize the gap between the electrode tip and the capillary outlet.

The electrochemical cell consisted of a platinum auxiliary electrode, a copper disk working electrode and an Ag/AgCl (3 mol/l KCl) reference electrode. A BAS LC-3D amperometric detector (Bioanalytical System, West Lafaytte, IN) provided potential control and current output.

2.2. Materials and procedures

Sugars were purchased from Sigma Chemical Co. and were used as received without further purification. Angelica was grown in Sichuan province, Astragalus Membranceus (Fish.) Bge and Codonopsis pilosula (Franch.) Nannf. were grown in Shanxi province. All of them were purchased from local drug store.

All the other chemicals including carrier electrolytes were of analytical reagent grade and bought from local commercial sources, de-ionized water was used throughout. Stock solutions with the

concentration of 1.0×10^{-3} mol/l for each sugar and the separation electrolyte were prepared in de-ionized water. The required sugar solutions were obtained by serial dilutions in the separation electrolyte. These dilutions were freshly prepared to avoid sample degradation with time in the hydroxide solutions. In addition, the electrolyte solutions at both buffer cells were replaced every 2 h. This procedure was necessary since the separation current was observed to decrease by approximately 10% during 1 day of continuous running. This decrease in current made the separation less reproducible. Preparation of samples: the three drugs were washed with water, dried at 70 °C in oven, and then ground. Accurately weighed drug powders (0.20 g for each drug) were separately immersed in 25 ml methanol for about 15 h, then supersonicated for 30 min, filtered and diluted with de-ionized water to 50 ml. The required sample solutions were obtained by serial dilutions in the separation electrolyte.

All experimental solutions were filtered through a polypropylene filter (0.22 μm), and degassed by ultrasonication prior to their use. The capillary was pretreated for 10 min with 0.5 mol/l NaOH before the first run and then for 10 min with 0.05 mol/l NaOH by using a laboratory-built high-pressure system.

The optimum standard separation conditions were voltage 5 kV (positive polarity), and a 0.05 mol/l NaOH buffer solution (pH 12.7), detection potential 0.65 V (vs. Ag/AgCl, 3 mol/l KCl), sampling time 10 s at 5 kV. In addition, all experiments were performed at room temperature (22 °C).

3. Results and discussion

3.1. Selection of working electrode and voltammogram

Carbohydrates are not considered electro-active compounds under normal amperometric conditions at the surface of carbon electrodes [16] (at which they exhibit a large over-potential for oxidation). So, carbon electrodes are not suitable. Luo et al. have developed several electrode materials for the catalytic oxidation of carbohydrates at constant applied potentials [17]. One of these electrodes is copper electrode [18].

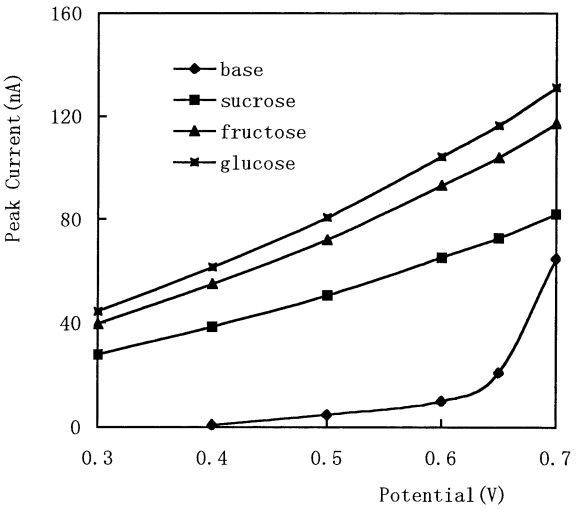

Fig. 1. Hydrodynamic voltammograms for the three standard sugars in CZE. Fused-silica capillary: 25 μm i.d. × 45 cm; working electrode: 140 μm-diameter copper disk electrode; separation medium and voltage: 50 mmol/l NaOH, 5 kV; injection: 5 kV × 10 s; detection potential: +0.65 V (vs. Ag/AgCl, 3 mol/l KCl); room temperature. The concentration of each sugar was 0.10 mmol/l.

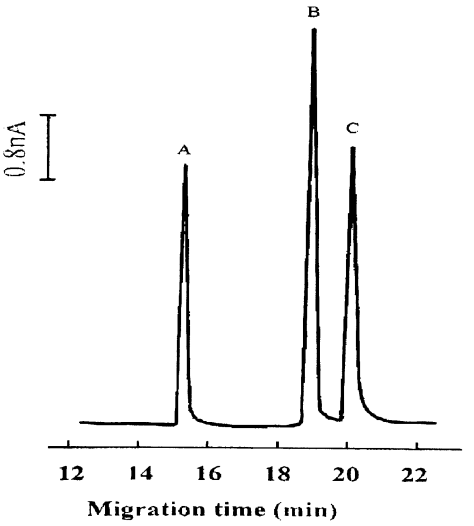

Fig. 2. Electropherogram of the three standard sugars. (A) Sucrose, (B) glucose, (C) fructose. The concentration of each sugar was 5.0×10^{-5} mol/l. Other conditions as in Fig. 1.

Table 1
The regression equation and detection limit

Analyte	Regression equation C (mmol/l) I (nA)	Correlation coefficient	Linear range (mmol/l)	Detection limit (mmol/l)
Sucrose	$I=47.021C+0.5301$	0.9989	$5.0\times10^{-3}\sim0.50$	4.0×10^{-3}
Glucose	$I=74.731C+0.6823$	0.9997	$5.0\times10^{-3}\sim0.50$	1.0×10^{-3}
Fructose	$I=66.692C-0.03305$	0.9992	$5.0\times10^{-3}\sim0.50$	1.0×10^{-3}

CZE-AD consideration as in Fig. 2. Detection limit was estimated based on a signal-to-noise ratio of three.

In our study, we selected copper disk electrode acting as working electrode for the determination of the three sugars.

In order to select a proper potential applied to the working electrode, we performed the hydrodynamic voltammograms (HDV) for the three sugars. The response of the three sugars was monitored after separation at different applied potentials. The HDVs of the three sugars obtained under CZE conditions were nearly identical in shape and exhibited a good response in the range of 0.30–0.68 V as shown in Fig. 1. The peak currents of the three sugars increased with the increase of potential applied to the working electrode, but at the same time, the base current increased, too. Considering the detection sensitivity of the studied analyzes and the baseline noise, a potential of 0.65 V (vs. Ag/AgCl, 3 mol/l KCl) was chosen for detection.

3.2. Effect of the background electrolyte

The separation of carbohydrates by CZE is based on their degrees of dissociation [19]. The pKs of the three sugars are in the vicinity of 12–13, so alkali-metal hydroxide solution as the separation electrolyte (pH 12–13) provided a degree of dissociation of the sugars. This permitted the separation of negatively charged carbohydrates on the basis of their electrophoretic mobilities without any prior derivatization. These alkaline conditions also satisfied the pH requirements for the proper performance of the Cu microelectrode in the electrocatalytic detection of sugars [17]. So we chose sodium hydroxide (NaOH) as the electrolyte. The effect of the concentration of the background electrolyte was examined by using 0.01, 0.02, 0.04, 0.06 and 0.08 mol/l NaOH. The experimental result showed that the use of low hydroxide concentration (i.e. lower pH) did not accomplish a complete separation. As the hydroxide ion concentration was increased, the sugars became more negative (dissociated), leading to an improved separation. When the concentration of NaOH was above 0.05 mol/l, the three sugars were basically separated, the migration time increased, too. This is because the ionic strength of the running solution increases with the increase of the concentration of the sodium hydroxide, which results in the decrease of the mobility of the solute in the capillary. So the migration time was prolonged. The electric current in the capillary also increased, which will cause Joule heating and it will make the peak shape broadening. Considering the sensitivity, the time of analysis and the resolution, 0.05 mol/l NaOH (pH 12.7) was chosen as the running solution in our experiments.

3.3. Effect of separation voltage and sampling

The effect of separation voltage on the separation of the three sugars was examined for various

Table 2
The precision of the present method ($n=5$)

Sugar	Migration time (min)		Peak Current (nA)	
	Mean	RSD (%)	Mean	RSD (%)
Sucrose	16.42	0.17	5.01	2.81
Glucose	18.50	0.19	8.13	2.59
Fructose	19.56	0.24	6.68	2.32

CZE-AD consideration as in Fig. 2. The concentrations of the three sugars were all 0.1 mmol/l.

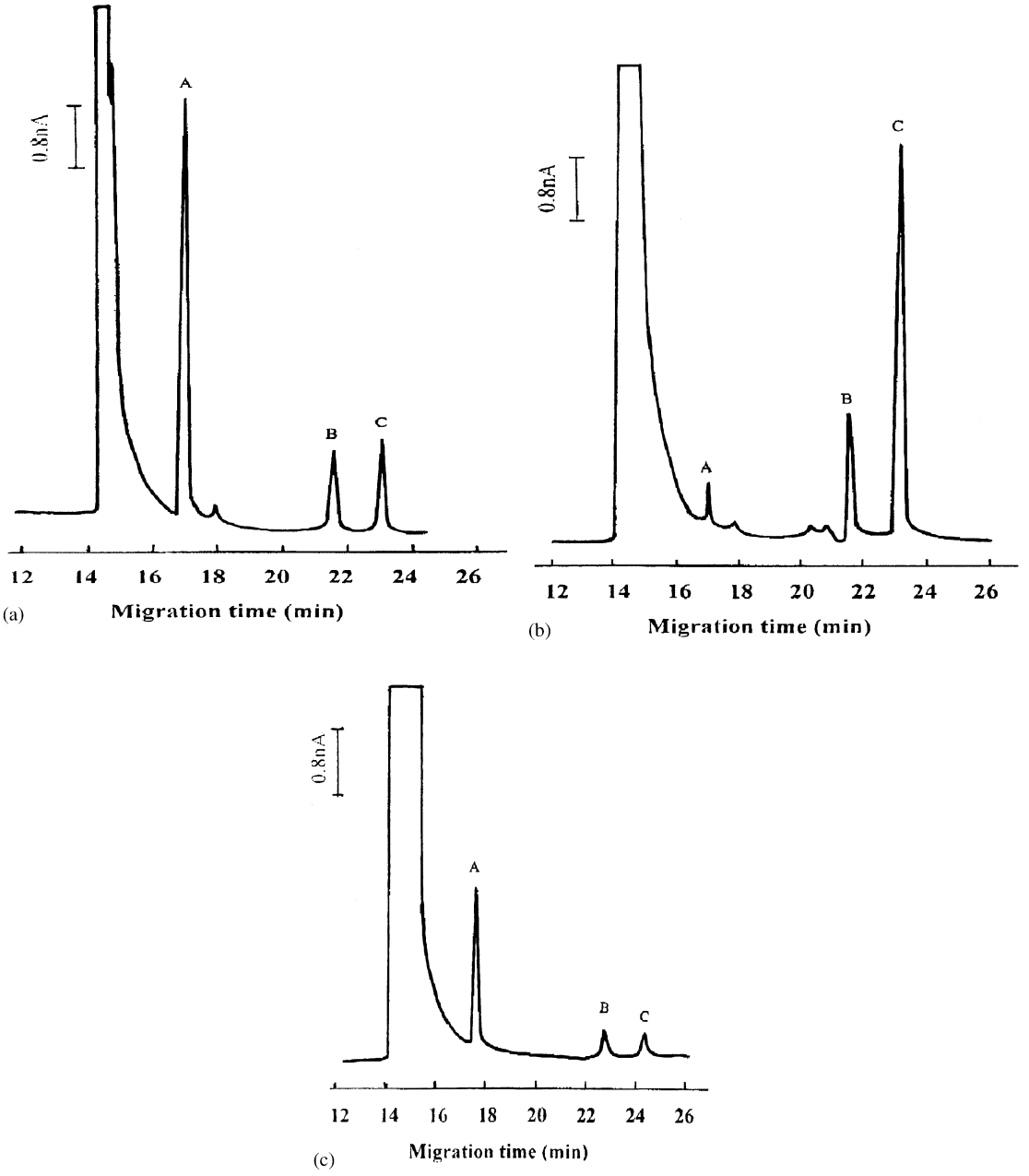

Fig. 3. (a) Electropherogram of angelica. (A) Sucrose, (B) glucose, (C) fructose. Other conditions as in Fig. 2. (b) Electropherogram of codonopsis pilosula (Franch.) Nannf. (A) sucrose, (B) glucose, (C) fructose. Other conditions as in Fig. 2. (c) Electropherogram of astragalus membranceus (A) sucrose, (B) glucose, (C) fructose. Other conditions as in Fig. 2.

voltages. Voltages from 2 to 6 kV with 1 kV interval were tested. The result showed that with the increase of the separation voltage, the peak shapes became sharper and the electric current in the capillary increased from about 16 to 50 μA, respectively. However, the peaks of the three

sugars would overlap when the separation voltage was over 5 kV. If the separation voltage was too low, the analysis time would be too long, and the sample in the capillary would diffuse leading to the broadening of the peaks in the electropherogram. In order to obtain higher efficiency and save analysis time, 5 kV was used as the separation voltage.

The amount of sampling also affects the peak height of the three sugars. We have tested the effects by changing the sampling time for 6, 8, 10 and 12 s at 5 kV. The result showed that the peak current was relatively higher when injection time was longer. When injection time was more than 10 s, the height of the peak current changed slowly, but the peak exhibited a significant broadening, too. In our experiments, 10 s was chosen as the sampling time.

The suggested analytical conditions were therefore: working potential 0.65 V (vs. Ag/AgCl, 3 mol/l KCl), separation voltage 5 kV, sampling time 10 s at 5 kV, and a 0.05 mol/l NaOH running buffer (pH 12.7).

Under the selected optimum conditions, the electropherogram for the mixture of the three standard sugars is shown in Fig. 2. It is clear that the three analytes are basically separated in less than 22 min.

3.4. Linearity and detection limit of the method for the three sugars

Under the optimized conditions, a series of concentrations of the three sugars were tested to determine the linearity for the three sugars at the copper disk electrode in CZE-AD. The linear range, regression equation, correlation coefficient and detection limit are listed in Table 1. Good linear relationships between peak heights and the concentrations of the three sugars were obtained in the concentration range of $5.0 \times 10^{-6} - 5.0 \times 10^{-4}$ mol/l for all the three sugars. Based on a three ratio of signal to noise, the detection limits were 1.0×10^{-6} mol/l for glucose and fructose and 4.0×10^{-6} mol/l for sucrose. Reproducibility was determined by making five repetitive injections of the three sugars at 1.0×10^{-4} mol/l. The result was shown in Table 2. The precisions of migration time and peak current (in terms of relative standard deviation (RSD)) were found to be $0.17 \sim 0.24$ and $2.32 \sim 2.81\%$, respectively (Table 2).

Table 3
Assay results of sugars in Chinese traditional drugs (%, mass/mass)

Sample	Sucrose	Glucose	Fructose
Angelica	65.60	2.09	3.36
Codonopsis pilosula	5.66	4.99	24.06
Astragalus membranceus	26.43	0.95	0.98

CZE-AD consideration as in Fig. 2.

3.5. Sample analysis and recovery

In order to demonstrate the applicability of the CZE-AD method for the determination of the three sugars in practical samples, this method was applied to three kinds of Chinese traditional drugs, namely angelica, codonopsis pilosula (Franch.) Nannf and Astragalus Membranceus (Fish.) Bge. Fig. 3a–c showed the electropherogram of the samples, respectively. We identified peak A as sucrose, peak B as glucose and peak C as fructose. Each migration time of the sugars for the three samples was longer than that of the standard

Table 4
The results of the determination of the recovery in this method ($n = 5$)

Analyte	Added amount (mmol/l)	Found amount (mmol/l)	Recovery (%)	RSD (%)
Sucrose	5.0×10^{-2}	5.0×10^{-2}	100.0	1.3
Glucose	5.0×10^{-2}	5.1×10^{-2}	102.0	2.3
Fructose	5.0×10^{-2}	4.8×10^{-2}	96.0	2.4

CZE-AD consideration as in Fig. 2.

sugars. This is because there was methanol in the sample solutions, which was added when processing the three drugs. Quantification of the three sugars in samples was carried out by standard addition method. Table 3 shows the results of this analysis.

Recovery experiments were performed by accurately adding several amounts of the three sugar standard solutions into samples under the same conditions stated above. The analytical data are given in Table 4. The results demonstrated that this method had both a high accuracy and a good precision for the analytes tested.

4. Conclusion

For the first time we developed a method to separate and determine three kinds of sugars in Angelica, Astragalus Membranceus (Fish.) Bge and Codonopsis pilosula (Franch.) Nannf. by using CE-AD. And first gave the content of every sugar in these drugs. The method built is simple, fast, sensitive and reliable. Samples do not need derivatization before determination. It may be a very effective method to determine the sugars in plants.

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