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Determination of carbohydrates by capillary zone electrophoresis with amperometric detection at a nano-nickel oxide modified carbon paste electrode

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Abstract

In this paper, a nano-NiO modified carbon paste electrode in capillary zone electrophoresis with amperometric detection (CZE-AD) was firstly applied to the determination of carbohydrates. Effects of several important factors such as detection potential, the concentration of running buffer, separation voltage and injection time were investigated to acquire the optimum conditions. Under the selected optimum conditions, three carbohydrates: glucose, sucrose and fructose could be perfectly separated within 20 min. The relationships between peak current and concentration of three carbohydrates were linear over about 3 orders of magnitude with detection limits (S/N = 3) ranging from 3.0×10^{-7} to 6.0×10^{-7} mol L^{-1}. The electrode was stable, and can be used for at least one week. The proposed method has been successfully applied to monitor carbohydrates in the real samples with satisfactory assay results.

Keywords: Capillary zone electrophoresis; Amperometric detection; Nano-NiO modified carbon paste electrode; Carbohydrates

1. Introduction

Honey has always been regarded as a food which provides health advantages and as a product which has 'hea ling qualities'. Its composition is highly complicated, but the major components are fructose, glucose, and water. According to Chinese government standard, the proportion of fructose and glucose in natural honey products is at least more than 60%, while the amount of sucrose must be less than 5%, except for some kinds of honey. However in the market, honey frauds have appeared resulting from the deliberate addition of sucrose for extra profit. In this sense, fructose, glucose and sucrose are usually detected to justify the quality of honey.

The quantitation of carbohydrate compounds is currently of great interest, and still requires for more efficient analytical methods (Lee & Chen, 2004). The difficulty arose from the lack of chromophores for photometric detection. Derivatization with chromophore (Rassi & Mechref, 1996) or use of indirect UV detection can help, but the former is time-consuming while the latter lacks the necessary sensitivity. Sensitive techniques such as fluorescence often limit their usefulness by inherent selectivity (Damm & Overklift, 1994). Hence, much attention has been devoted to electrochemical detection (EC) for liquid chromatography (LC), capillary electrophoresis (CE) or flow injection analysis (FIA) (Baldwin, 1999; Wang & Fang, 2004).

Common amperometric detectors, based on glassy carbon or carbon paste electrodes, exhibit no response for carbohydrates because of a high overpotential. Metallic electrodes have thus been utilized in place of them. The first employed gold (Neuburger & Johnson, 1987) or platinum (Hughes & Johnson, 1982) electrodes on which the carbohydrates adsorbed and subsequently underwent relatively facile dehydrogenation/oxidation. However, because of the surface cleaning and regeneration steps necessary to obtain stable repetitive response, simple constant potential

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operation was impossible, and double or triple pulsed potential waveforms were required for acceptable long-term usage of these electrode materials. In the second approach, transition metals such as nickel (Casella, Cataldi, Salvi, & Desimoni, 1993) or copper (Štulík & Pacáková, 1988), have been developed and characterized. The utilities of them were demonstrated with high sensitivity, but continuous anodization of the electrode surface destabilized background signal. The benefits of using chemically modified electrodes (CMEs) in these systems include acceleration of electron transfer reactions, permselective transport, reduction in fouling, and preferential accumulation of analyte. A lot of work has illustrated the unique reactivity and the unusual stability associated with the use of metal oxide modified electrodes (Luo, Prabhu, & Baldwin, 1990; Prabhu & Baldwin, 1989; Wang & Taha, 1990; Xie & Huber, 1991).

Recently, CE has emerged as a highly promising technique consuming an extremely small amount of sample and capable of the rapid, high-resolution separation, characterization, and quantitation of analytes. There are many reports on determination of carbohydrate compounds by capillary electrophoresis with electrochemical detection (CE-EC) at a copper electrode (Cao, Wang, Chen, & Ye, 2004; Chen, Zhang, & Zhu, 2006; Wang, Yu, Zong, He, & Fang, 2003). Despite of many advantages of CMEs, few reports of their application in CE have appeared.

In this paper, we demonstrated the first application of nano-NiO modified carbon paste electrode in capillary zone electrophoresis with amperometric detection (CZE-AD) to detect carbohydrates, which exhibited a highly stable electrocatalytic oxidation and low detection limits (S/N = 3) ranging from 3.0×10^{-7} to 6.0×10^{-7} mol L^{-1}. To evaluate the performance of the system for the analysis of a real sample, the determination of carbohydrates in honey, which mainly contains fructose, glucose and sucrose, was investigated. The results were also satisfying.

2. Materials and methods

2.1. Apparatus

CZE-AD system was laboratory-built (Fu, Song, & Fang, 1998; Wang et al., 2003). Electrophoresis was driven by a high-voltage supplier (±30 kV, Shanghai Institute of Nuclear Research, China). Separations were performed in a fused silica capillary (Hebei Yongnian Laser-fiber Factory, China) with 25 μm i.d., 360 μm o.d. and 57 cm long. Potential control and current output were employed by a CH-2 amperometric detector (Jiangsu Electrochemical Analytical Instrumental Factory, China). Electropherograms were recorded by a chart recorder (Shanghai Dahua Instrument Factory, China). Other electrochemical experiments were carried out by a CHI 830b electrochemical analyzer (CH Instruments, USA). A three-electrode system, which consisted of a nano-nickel oxide modified carbon paste electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode, was used in both electrochemistry and detection experiments.

2.2. Chemicals

All reagents were analytical-reagent grade and used as received unless otherwise mentioned. All solutions were prepared with double distilled water. Glucose, sucrose and fructose were purchased from Shanghai Sinopharm Chemical Reagent Company. Stock solutions were prepared fresh daily, stored at 4 °C, and diluted with running buffer to needed concentrations in CE experiments. Nano-NiO was obtained from inorganic chemistry laboratory (East China Normal University, China) (Li et al., 2007). Before CZE experiment, all used solutions were filtered through 0.22 μm polypropylene acrodisc syringe filter and sonicated for 5 min to remove bubbles.

2.3. Procedure

2.3.1. Preparation of nano-NiO modified carbon paste electrode

Nano-NiO modified carbon paste was prepared by mixing weighed amounts of specpure carbon powder, paraffin oil and nano-NiO (45%:35%:20% W/W) thoroughly in an agate mortar until perfect homogenization was attained. A portion of the ready-prepared paste was then packed firmly into the cavity of a Teflon tube (0.5 mm i.d., 3 mm o.d.). Electrical contact was established via a copper wire, which was threaded into the opposite end of the electrolyte body and embedded into the carbon paste. Then the electrode was left unused for a certain time (12–24 h) to allow their final homogenization to proceed (Švancara & Schachl, 1999). Prior to use, the surface of the modified carbon paste electrode was smoothed by polishing on weighing paper. After a day's experiment the surface was renewed by removing the top of the used paste. This type of electrode could be used at least for one week.

2.3.2. CZE experiments

Before CZE experiments, the three-electrode system was fixed in the corresponding holes of the electrochemical cell and the modified carbon paste electrode was positioned straightly opposite the capillary outlet as close as possible by a three dimension positioner. Before each run in CE experiments, the capillary was sequentially rinsed with 0.1 mol L^{-1} hydrochloric acid, doubly distilled water, 0.1 mol L^{-1} sodium hydroxide, 3 min for each and with running buffer till the inside current of the capillary reached stability. This was important to get a reproducible electro-osmotic flow. CZE was performed at the separation voltage of 10 kV with 50 mmol L^{-1} NaOH solution used as running buffer. The potential applied to the working electrode was 0.55 V (vs. SCE). Samples were electrokinetically injected at 10 kV for 10 s.

2.3.3. Samples

The proposed method was applied to eight honey samples, which were purchased at a local supermarket, including acacia honey, astragalus honey, and so on. Among them, four honey samples were from Shanghai (China), one from Zhejiang province (China), one from Anhui province (China), one from Jiangsu province (China), and the other one came from Beijing (China). Honey was diluted 10,000 times with running buffer, filtered through 0.22 μm polypropylene acrodisc syringe filter, and then analyzed directly without any other sample treatment.

3. Results and discussion

3.1. Selection of detection potential

Carbohydrates are unusually electroactive at carbon electrodes, the most commonly used working electrode in AD, but can be detected by nano-NiO modified carbon paste electrodes at a constant applied potential in strongly alkaline media based on electrocatalytic oxidation. A TEM image of the NiO nanoparticles is shown in Fig. 1. Fig. 2 are the cyclic voltammograms of sucrose, glucose and fructose in 50 mmol L^{-1} NaOH solution, in which all analytes exhibited obvious anodic and cathodic peaks. The peak currents increased gradually until steady state was reached. It is believed that nano-NiO particles were transformed to $Ni(OH)_2$ by hydration upon exposure of the electrode surface to solution. Then the modifier was oxidized from $Ni(OH)_2$ to NiOOH back and forth by potential cycling (Luo et al., 1990; Prabhu & Baldwin, 1989; Štulík & Pacáková, 1988; Wang & Taha, 1990).

The potential applied to the working electrode directly affects the sensitivity of this method. According to the cyclic voltammograms, it was found that the signal-to-noise ratio was the best for detecting all analytes simultaneously, when potential equating to 0.55 V. Therefore, 0.55 V was selected as the most suitable detection potential in this experiment. The signal-to-noise ratio and the reproducibility were high at the optimum potential.

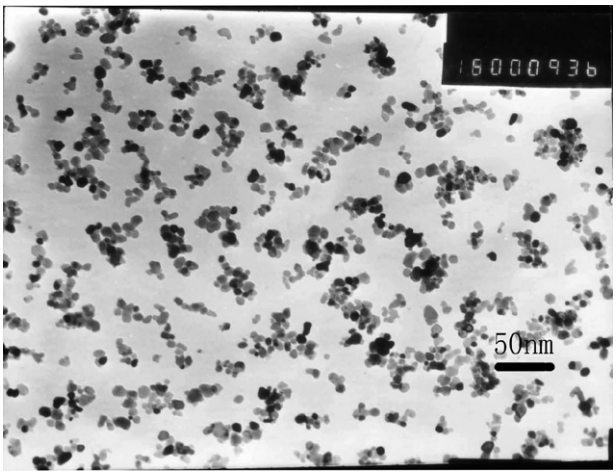

Fig. 1. TEM image of the prepared NiO nanoparticles.

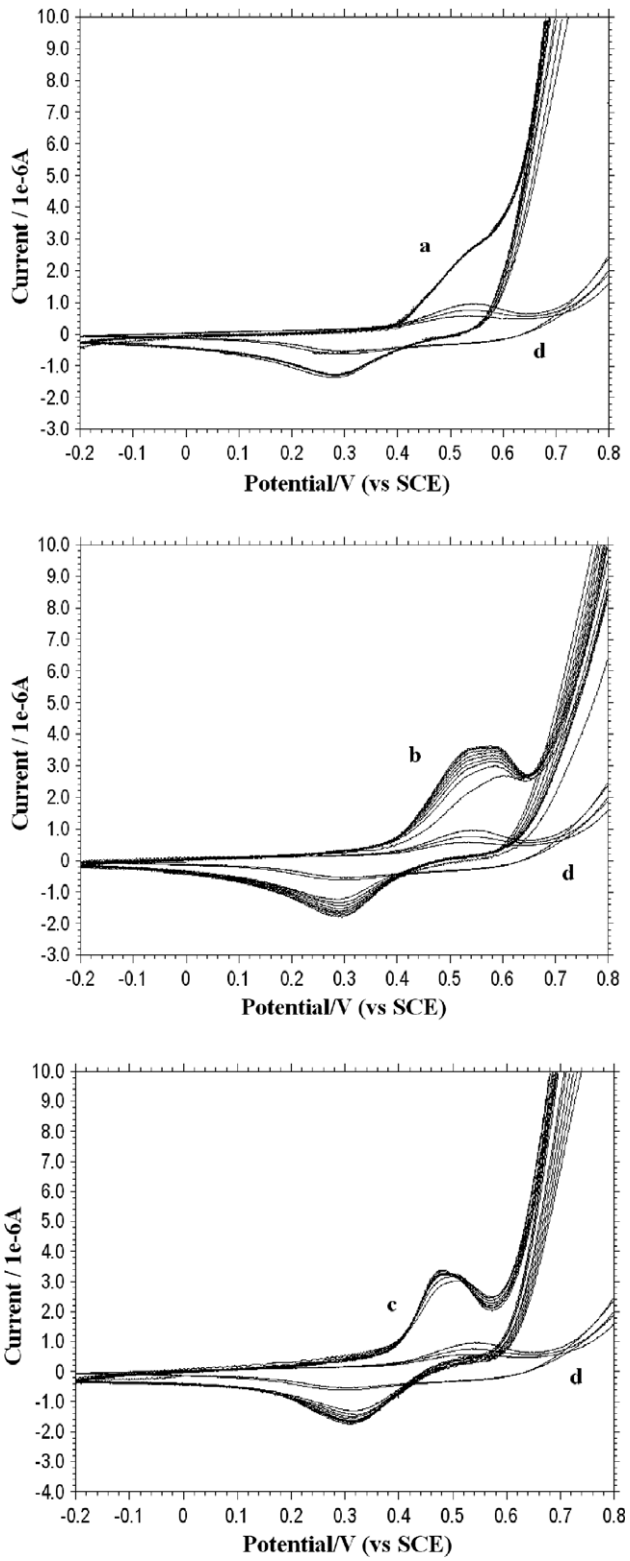

Fig. 2. Cyclic voltammograms showing catalytic oxidation of three carbohydrates with a concentration of 1.0×10^{-3} mol L^{-1}at nano-NiO modified carbon paste electrode: (a) sucrose; (b) glucose; (c) fructose; (d) 50 mmol L^{-1} NaOH blank solution; scan rate 100 mV/s.

3.2. Effects of the NaOH concentration

The NaOH concentration affects the zeta-potential (ζ) of the inner wall of the capillary, the viscosity coefficient of the solution, the electro-osmotic flow (EOF) as well as the overall charge of the analytes, which determine the migration time, peak height, and the separation resolution of the analytes. The effects of the running buffer concentration were studied within the concentration range from 25 to 75 mmol L^{-1}. It was found that when NaOH concentration reached 50 mmol L^{-1}, the base-line separation for the three carbohydrates could be achieved. With further increase of the concentration, the migration time of the three analytes prolonged obviously. It was because that the increase of ionic strength of NaOH solution resulted in the decrease of electro-osmotic flow in the capillary. Meanwhile, the peak current was low and the peak shape became poor, because the electric current in the capillary also increased resulting in Joule heating and peak broadening. Therefore, the optimum running buffer concentration is 50 mmol L^{-1}, and the three carbohydrates can be well-separated within a relatively short time.

3.3. Effects of separation voltage and injection time

The separation efficiency of CZE was investigated within the separation voltage range from 5 to 12 kV. The migration time of the carbohydrates were significantly shortened when the separation voltage was increased. However, if the separation voltage was above 10 kV, the peaks of fructose and glucose would overlap. Therefore, 10 kV was selected as the optimum separation voltage in this experiment.

Electrokinetic injection was used in the CZE experiment. The effects of injection time were investigated by selecting different injection time (4, 6, 8, 10, 12 s at a voltage of 10 kV). It was found that when the injection time was prolonged, the peak currents increased correspondingly. However, the current response peaks of the carbohydrates were obviously broadened if the injection time was more than 12 s. So, 10 s was selected as the injection time in this experiment and satisfactory results were obtained under this condition.

Through the experiments above, the optimum conditions of CZE-AD for determining the carbohydrates were detection potential 0.55 V (vs. SCE), separation voltage 10 kV, electrokinetic injection time 10 s (10 kV) and 50 mmol L^{-1} NaOH solution. The typical electropherograms for a standard sucrose, glucose and fructose mixture solution are shown in Fig. 3. A base-lined separation for the three analytes could be achieved within 20 min.

3.4. Reproducibility, stability, linearity and detection limits

A standard mixture solution of 5.0×10^{-5} mol L^{-1} sucrose, glucose and fructose was used to determine the reproducibility of the current response and migration time

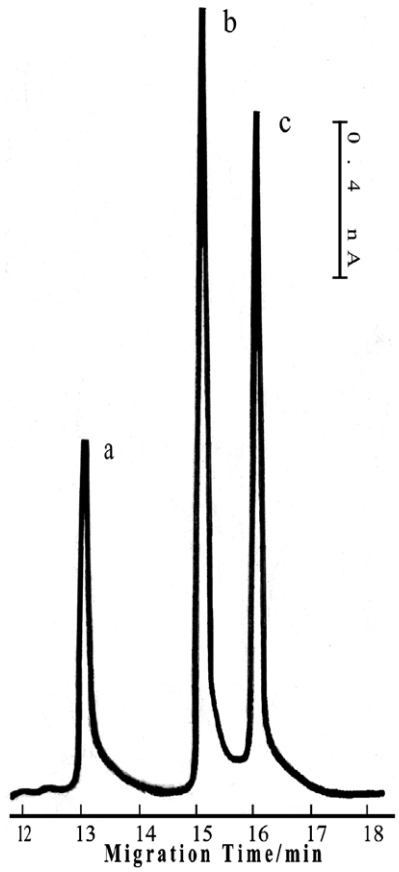

Fig. 3. Electropherograms of a standard solution containing sucrose, glucose and fructose with a concentration of 5.0×10^{-5} mol L^{-1}, under the optimal CZE-AD conditions: (a) sucrose; (b) glucose; (c) fructose.

under the optimum conditions in this experiment. The relative standard deviations (R.S.D.) of peak current and migration time were 1.2% and 4.4% for sucrose, 0.8% and 3.7% for glucose, 1.1% and 3.6% for fructose, respectively, when the analysis was repeated for five times under the same conditions, which demonstrated that this method was of good repeatability. The long-term stability was also measured over a consecutive 7-day period of constant operation, and no obvious deterioration was apparent, which demonstrated that this method was of good stability.

To determine the linearity for the three analytes by this method, a series of mixed standard solutions with different concentrations were tested. The linear ranges, regression equations, correlation coefficients and detection limits are listed in Table 1. The linearity ranges of glucose and fructose were from 1.0×10^{-6} to 1.0×10^{-3} mol L^{-1}, and that of sucrose was from 2.0×10^{-6} to 1.0×10^{-3} mol L^{-1}. Their detection limits were 3.0×10^{-7}, 3.0×10^{-7} and 6.0×10^{-7} mol L^{-1}, respectively (S/N = 3). Above results showed that this method was very sensitive.

3.5. Sample analysis and recovery

Under the optimum conditions, CZE-AD was applied for the determination of glucose, sucrose and fructose in

Table 1
Regression equations and the detection limits of sucrose, glucose and fructose

Analytes	Regression equation C (mol L^{-1}); I (nA)	Correlation coefficient	Linear range (mol L^{-1})	Detection limit[a] (mol L^{-1})
Sucrose	$I = 1.70 \times 10^4$ C + 0.062	0.9980	2.0×10^{-6} –1.0×10^{-3}	6.0×10^{-7}
Glucose	$I = 3.75 \times 10^4$ C + 0.176	0.9992	1.0×10^{-6} –1.0×10^{-3}	3.0×10^{-7}
Fructose	$I = 3.42 \times 10^4$ C + 0.160	0.9985	1.0×10^{-6} –1.0×10^{-3}	3.0×10^{-7}

[a] Detection limit set at signal:noise ratio of 3.

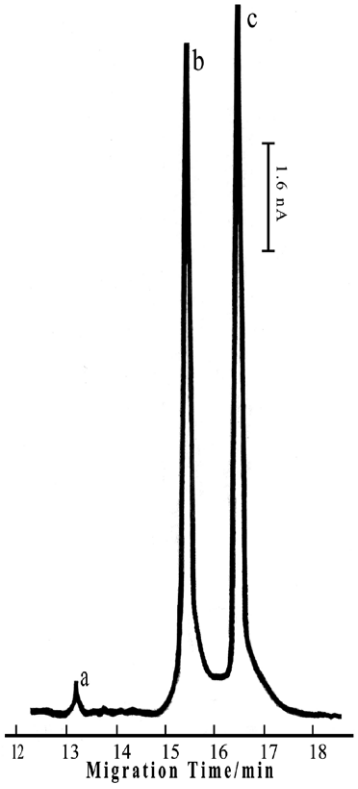

Fig. 4. Electropherograms of the honey (sample 1) being diluting 10,000 times under the optimal CZE-AD conditions: (a) sucrose; (b) glucose; (c) fructose.

Table 2
Assay results (g/100 g) of the analytes in the honey ($n = 5$)

Samples	Sucrose	Glucose	Fructose
Multifloral honey			
Sample 1	4.8 (4.8)	37.2 (4.6)	39.0 (3.7)
Sample 2	5.2 (5.6)	36.0 (4.4)	38.0 (5.0)
Eucalyptus honey			
Sample 3	7.4 (4.5)	28.0 (3.0)	33.5 (3.5)
Acacia honey			
Sample 4	2.4 (3.6)	30.2 (4.4)	37.0 (3.6)
Sample 5	3.0 (3.9)	37.5 (4.7)	39.2 (4.0)
Orange honey			
Sample 6	5.4 (3.9)	33.0 (2.9)	35.5 (4.4)
Sample 7	4.6 (4.7)	32.8 (5.2)	36.5 (4.0)
Astragalus honey			
Sample 8	2.8 (3.4)	38.5 (4.6)	41.2 (3.5)

The data in the brackets are the R.S.D.s (%).

eight honey samples. The determinations were carried out according to the procedures described earlier. The typical electropherograms for sample 1 are shown in Fig. 4, which are similar to others. Peak identification was performed by the standard-addition method, and the assay results are summarized in Table 2.

Recovery test was performed in this experiment too. The average recoveries and the corresponding R.S.D.s were 96.7% and 3.5% for sucrose, 98.1% and 2.8% for glucose, 104.0% and 3.7% for fructose, respectively ($n = 5$). The results demonstrated that this method was accurate and practical for the analytes tested.

4. Conclusion

Determination of carbohydrates by CZE-AD at a nano-NiO modified carbon paste electrode was performed in this experiment and the results showed that this method was of high separation efficiency, short analysis time, convenience of analysis. Under the optimum conditions, the three carbohydrate compounds were separated completely within 20 min, with good linearity, reproducibility and detection limits were obtained, too.

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