ELSEVIER

Food Chemistry 72 (2001) 385–388

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Determination of lactose in sugar-free milk powder by capillary electrophoresis with electrochemical detection

Xin Zhang, Yuhua Cao, Jiannong Ye *

Department of Chemistry, East China Normal University, Shanghai 200062, People's Republic of China

Received 26 May 2000; received in revised form 29 August 2000; accepted 29 August 2000

Abstract

Lactose in three sugar-free milk powder samples were satisfactorily determined by capillary electrophoresis with electrochemical detection using a 300 μm copper disk electrode as the working electrode in 0.1 mol/l NaOH medium. There was acceptable linearity (0.9969) between the peak current and concentration of lactose in the range from 5.0×10^{-6} to 5.0×10^{-3} g/ml with the detection limit (S/N = 3) of 1×10^{-7}g/ml. The proposed method was successfully applied to analyze the actual milk powder samples.

Keywords: Capillary electrophoresis; Electrochemical detection; Lactose; Sugar free; milk powder

1. Introduction

It is well known that lactose is a major component in milk powder (Indyk, Edwards & Woollard, 1996) and its content is closely related to the quality of the milk powder (Amarita, Fermandez & Alkorta, 1997). Therefore, lactose determination in milk powder is an important task in food analysis. The most common technique for lactose determination in milk powder has been the HPLC approach (Harvey & Aust, 1988; Indyk et al., 1996; Maureen, 1991; Pischesrieder, Gross & Schoetter, 1999). Other methods including oxidation-reduction titration method (Doi, Satoh, Kanzaki & Matsumono, 1991), spectrophotometric determination (Petrushevska-Tozi & Bauer-Petrovska, 1997) and flow-injection analysis (Naroneshingh, Stoute, Davis & Ngo, 1991) have also been used. In this work, an alternative method for lactose determination in milk powder by using capillary electrophoresis with electrochemical detection (CE-ED) approach is described, which is not only simple and convenient, but also sensitive and selective.

2. Reagents and solutions

Lactose was purchased from Shanghai Second Reagents Factory (China) and used without further purification. Three milk powder samples were Nestle soluble milk powder, Campina Birch Tree full cream milk powder and Anlene Hi-Calcium fat-free milk powder, respectively. Each milk powder sample was carefully weighed and dissolved with doubly distilled water, then filtered with 0.22-μm teflon filter paper (Shanghai Bandao Purification Material Factory, China). Prior to use, the sample solution was diluted with the electrophoresis medium (0.1 mol/l NaOH) to the required concentration.

NaOH (0.1 mol/l) solution was used as the electrophoresis medium. In this alkaline solution, copper working electrode shows excellent response to carbonhydrates including lactose (Colon, Dadoo & Zare, 1993).

3. Apparatus

The laboratory-built CE–ED system has been constructed and described previously. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided separation voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and outlet end of capillary was maintained at ground. The separation was proceeded in

Corresponding author. Tel.: +86-21-6223-2254; fax: +86-21-6257-6217

E-mail address: jnye@ecnu.edu.cn (J. Ye).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00242-9

a 75 cm length of 25-μm i.d. and 360-μm o.d. fused silica capillary (Polymicro Technologies, Phonix, AZ, USA). In order to protect the operator from the high voltage and assure the safety of the CE-ED system, the entire capillary, the buffer reservoir for CE, and all electrodes were enclosed in a Plexiglas box with a safety switch wired to turn off the power supply whenever the box was opened (Ye & Richard, 1993, Ye & Richard, 1994).

A three-electrode cell system consisting of a 300-μm copper disc working electrode, whose side area was covered with a nonconductive coating, a platinum auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. Before use, the copper disc electrode was successively polished with emery paper, sonicated in doubly distilled water and finally positioned carefully opposite the outlet of the capillary with the aid of a Oriel Corp (Stratford, CT, USA) Model 14901 micropositioner and arranged in a wall-jet configuration. The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument Factory). The electrophoresis medium was 0.1 mol/l NaOH and sample injection was made by electromigration for 10 s at 20kV. In order to minimize the effect of CO_2 pickup from the atmosphere, NaOH solution was replaced daily. The potential applied to the working electrode was 0.62 V (vs. SCE). Samples were separated at 20 kV.

4. Hydrodynamic voltammogram

In amperometric detection, the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Therefore, the effect of working electrode potential on the peak current (calculated by measuring the peak height) of the analyte was investigated to obtain optimum detection. Fig. 1 illustrates the hydrodynamic voltammogram of lactose. When the applied potential reaches +0.3 v (vs. SCE), the peak current increases rapidly. However, when the potential exceeds 0.60~0.65 v (vs. SCE), the current levels off. Although applied potential greater than +0.65 v (vs. SCE) results in larger peak current, solvent oxidation becomes pronounced, both the baseline noise and the background current increase, resulting in an unstable baseline, which is a disadvantage for sensitive and stable detection. Therefore the applied potential to the working electrode was maintained at +0.62 v (vs. SCE) where the background current is not too high and the S/N ratio is the highest. Moreover, the working electrode showed good stability and high reproducibility at this optimum potential.

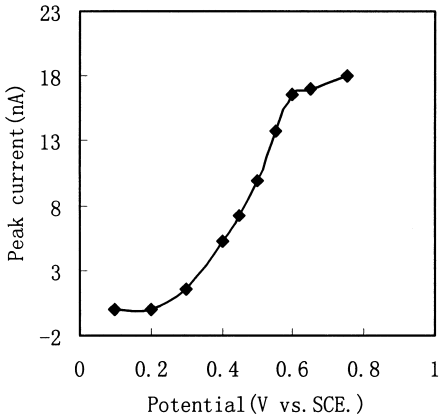

Fig. 1. Hydrodynamic voltammogram (HDV) for lactose. Fused-silica capillary: 25 μm i.d.×75 cm; working electrode: 300 μm diameter copper disk electrode; electrophoresis medium: 0.10 mol/l NaOH; separation voltage: 20 kV; injection: 20 kV, 10 s; lactose concentration: 5×10^{-5}g/ml.

4.1. Effect of injection time

Injection time determining the amount of sampling affects both the peak current and peak shape. The effect of injection time on CE separation was investigated by changing the sampling time from 2 to 16 s at injection voltage of 20 kV. As shown in Fig. 2, peak current increases with increasing sampling time, and the peak width of the analyte increases simultaneously. When the injection time is longer than 10 s, the peak current levels off and the peak broadening becomes more severe. In this experiment, injection time of 10 s at 20 kV is selected. At the optimum conditions, the typical electropherogram for lactose standard is shown in Fig. 3B, and the lactose peak appears at 20 min.

5. Reproducibility, linearity, detection limit and recovery

The repeatability of the peak current and the migration time in this experiment was determined by repeatedly ($n=7$) injecting the lactose standard (5.0×10^{-5}g/ml)

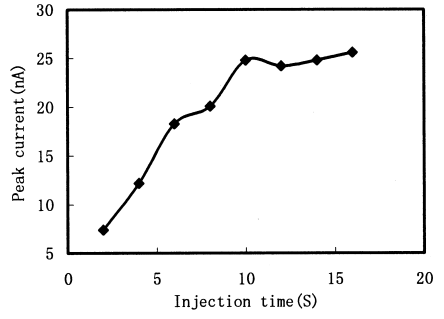

Fig. 2. Effect of the injection time on lactose peak current. Working electrode potential is 0.62 v (vs. SCE). Other conditions are the same as in Fig. 1.

into the system under the optimum conditions. The relative standard deviation (RSD) was found to be 3.6% for peak current, and 1.3% for migration time. A series of lactose standard solutions with the concentration range of 1.0×10^{-7}~1.0×10^{-2} g/ml was tested to determine the linearity of this method. The calibration curve exhibits excellent linear behavior over the concentration range from 5.0×10^{-6} to 5.0×10^{-3} g/ml. The correlation coefficient was 0.9969, as shown in Fig. 4 and the detection limit was 1.0×10^{-7} g/ml. The recovery experiment of lactose in milk powder sample was also performed, and the results are listed in Table 1.

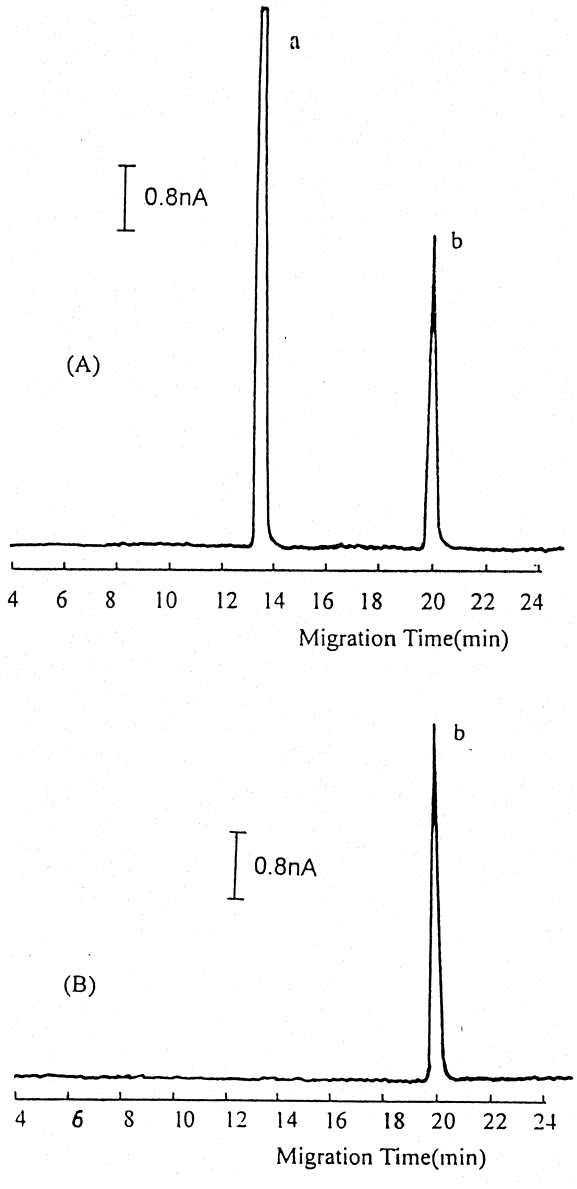

Fig. 3. The electropherogram of (A) lactose standard solution (5×10^{-5}g/ml) and (B) milk powder sample solution (9.0×10^{-5}g/ml). Experiment conditions are the same as in Fig. 2.

5.1. Sample analysis

Under optimum conditions, the determination of lactose in the three milk power samples was carried out according to the procedures described earlier. Typical electrophorogram of milk powder sample is shown in Fig. 3A. As we can see from Fig. 3A, the electrophorogram consists of only two peaks, *peak b* is proved to be the lactose peak either by comparing its migration time with that of Fig. 3B, or by the standard-addition method. *Peak a* in Fig. 3A is the system peak caused by some high molecular compounds including hydrocarbons and proteins, which exist in milk powder samples. It is found that upon hydrolysis the height of *peak a* reduces significantly, and some new peaks at longer migration time appear. The assay results of three milk powder samples are summarized in table 2,which indicate that the assay

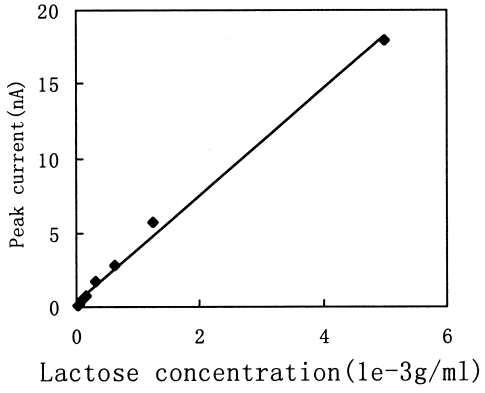

Fig. 4. Lactose calibration curve (5×10^{-6}g/ml ~5×10^{-3}g/ml). Experiment conditions are the same as in Fig. 2.

Table 1
Recovery rate of lactose in Anlene Hi-Calcium fat-free milk powder

Lactose content (g/100 g,%)	Lactose added (g/100 g,%)	Lactose increasement (g/100 g, %)	Recovery
37.8	35.3	33.6	95.3

Table 2
Results of lactose determination in three sugar-free milk powder samples

Samples	Target analyte	Label claim (g/100 g)	Lactose found (g/100 g of sample)	RSD (%) ($n=3$)
Sample 1[a]	Lactose	30	30.7	1.7
Sample 2[b]	Lactose	36	32.5	3.2
Sample 3[c]	Lactose	40	37.8	2.3

[a] Nestle soluble milk powder.
[b] Campina Birch Tree full cream milk powder.
[c] Anelene Hi-Calcium fat free milk powder.

results of lactose in milk powder samples are consistent with the labeled amount of the manufactures.

Acknowledgements

This work was supported by the Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Academia Sinica.

References

Amarita, F., Fermandez, C. R., & Alkorta, F. (1997). Hybrid biosensors to estimate lactose in milk. *Anal. Chem. Acta, 349*, 153–158.

Colon, L. A., Dadoo, R., & Zare, R. N. (1993). Determination of carbohydrates by capillary zone electrophoresis with anperometric detection at a copper microelectrode. *Analytical Chemistry, 65*, 476–481.

Doi, T., Satoh, K., Kanzaki, M., & Matsumono, K. (1991). Determination of lactose by oxidation-reduction reaction and its application to some kinds of milk and milk products. *Nippon Shokuhin Kogyo Gakkaishi, 38*, 575–580.

Harvey, J., & Aust, J. (1988). High performance liquid chromatographic method for lactose determination in Milk. *Dairy Technology, 43*, 19–20.

Indyk, H. E., Edwards, M. J., & Woollard, D. C. (1996). High performance liquid chromatographic analysis of lactose hydrolysed milk. *Food. Chemistry, 57*, 575–580.

Maureen, J. (1991). Sugars and lactose in Milk. *Lab, 2000*, 542-43.

Naroneshingh, D., Stoute, V. A., Davis, G., & Ngo, T. T. (1991). Flow-injection analysis of lactose using covalently immobilized β-galactosidase, mutarotase and glucose oxidase-peroxidase on a 2-fluo-1-ethylpyridinium salt-activated fractogel support. *Analytical Biochemistry, 194*, 16–24.

Petrushevska-Tozi, L., & Bauer-Petrovska, B. J. (1997). Spectrophotometric determination of lactose in milk with palladium chloride. *Agricultural Food Chemistry, 45*, 2112–2114.

Pischesrieder, M., Gross, V., & Schoetter, C. (1999). Detection of maillard products of lactose in heated or processed milk by high liquid chromatographic with diode-array detection (DAD) Z. *Lebensm-Unters.-Forsch, 208*, 172–177.

Ye, J., & Baldwin, R. P. (1993). Amperometric detection in capillary electrophoresis with normal Size electrodes. *Analytical Chemistry, 65*, 3525–3527.

Ye, J., & Baldwin, R. P. (1994). Determination of amino acids and peptides by capillary electrophoresis and electrochemical detection at a copper electrode. *Analytical Chemistry, 66*, 2669–2674.