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QUALITATIVE AND QUANTITATIVE DETERMINATION OF LACTOSE IN MILK AND DAIRY PRODUCTS BY CAPILLARY ELECTROPHORESIS

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Abstract

A capillary electrophoretic method was developed for carbohydrate analysis of various dairy products containing carbohydrates in the range of 1-10% using indirect UV detection. A thorough, yet simple sample preparation technique was developed to address the inherent problems of indirect detection (limited linear dynamic range, and lack of robustness). The effect of separation parameters (buffer, electric field strength, length of the capillary and capillary conditioning procedures) on the selectivity and efficiency of the separation were investigated. The applicability of the method is demonstrated by analysing different dairy products (cocoa milk, milk, yoghurt, sour milk, sour cream, etc.). A critical assessment of the developed analytical method is also provided.

Keywords: milk, lactose, capillary electrophoresis, dairy products.

Introduction

About 10 – 20 per cent of the World's population suffers from lactose intolerance (in Hungary this ratio is about 14 per cent). Therefore, it is very important to find a quick and reliable analytical method for quantitative and qualitative determination of lactose in foods. Capillary electrophoresis (CE), the instrumental approach to traditional electrophoresis, could be a suitable analytical method for solving this problem. In capillary electrophoresis the analytes are separated according to the differences in their net charge, and are detected either according to their UV absorbance or to their fluorescence emission during the separation (on capillary) [2]. Papers on capillary electrophoresis of carbohydrates published so far deal mostly with the separation theory of saccharides [3, 4]. Only one application has

been published so far in the field of carbohydrate analysis in fruit juices with CE [5].

The carbohydrate content of milk is about 5% lactose (O-β-D-galactopyranosyl-D-glucopyranose) and a small amount (less than 0.1%) of glucose, galactose, and oligosaccharides. Saccharides do not absorb in UV (except some acidic disaccharides and aminosugars), thus direct detection methods are not applicable. Detection of carbohydrates can be facilitated either via derivatisation to form UV/fluorescence active compounds or by indirect UV/fluorescence detection. In this paper a simple and fast analytical method is presented for the determination of lactose in milk and dairy products using capillary electrophoresis with indirect UV detection.

Materials and Methods

All of the chemicals used in this study were of analytical grade unless mentioned otherwise. For the preparation of the electrolytes double distilled Milli-Q water (Waters, Milford, MA, USA) was used. Sorbic acid, salicylic acid, lactose, saccharose, glucose, galactose and sodium tetraborate were purchased from Reanal (Budapest, Hungary). TRIS (tris-(hydroxymethyl)-aminomethane), NaOH and phosphoric acid were products of Acros Organics, New Jersey, USA.

When indirect detection is used, there are at least two conditions the electrolyte has to fulfil. First of all one component of the electrolyte should be UV absorbing, secondly the electrophoretic mobility of the UV active component in the electrolyte should be similar to that of the analytes in order to achieve symmetrical peak shapes [6]. Three different electrolyte systems containing UV active component were investigated in our study: sodium tetraborate (pH 9.1), TRIS (pH 7.8) and sorbate (pH 12.1). The sodium tetraborate buffer (buffer #1) was prepared by dissolving 50 mmole of sodium tetraborate in water. The pH of the buffer was adjusted with salicylic acid to 9.1, and the solution was diluted to 1 l with distilled water. TRIS buffer (buffer #2) was prepared by dissolving 50 mmole of TRIS in water. The pH of the solution was adjusted to 7.84 with salicylic acid, and the solution was diluted to 1 l with distilled water. 5.3 mmole of sorbic acid was dissolved in water to prepare the third buffer system (buffer #3) and the pH of the electrolyte was adjusted to 12.1 with 0.1 mole/l NaOH and the solution was diluted to 1 l with distilled water. Several electrophoretic conditions were investigated in order to achieve the optimum separation. The separation conditions are listed in *Table 1*.

In our experiments fused-silica capillaries (Polymicro Technologies Phonix, AZ, USA) with inner diameters of 50 and 75 μm were used. In

Table 1
List of separation conditions

Separation condition	Electrolytes	Capillaries total length L (cm)	Capillaries effective length l (cm)	Capillaries internal diameter I.D. (μm)	U Voltage (V)	E Electric field (V/cm)	Detection wavelength (nm)
1*	borax	44.5	22.5	50	15000	337	195
2	salicylic acid /TRIS	44.5	22.5	50	15000	337	298
3a		44.5	22.5	50	15000	337	256
3b		60.5	36	50	15000	248	256
3c	sorbic	70	55	50	10000	143	256
3d	acid	70	55	50	15000	214	256
3e		70	55	50	18000	257	256

* LPA coated capillary

some cases the capillaries were coated with linear polyacrylamide using a modified coating procedure of HJERTEN's [7, 8].

The home-built capillary electrophoretic system consisted of a CZE 1000 PN 30 power supply (SPELLMAN High Voltage Corporation, Plainview, NY, USA), a Spectra 100 UV/VIS detector (Thermo-Separation Products, San Jose, California, USA) and a DTK Personal Computer (Parity Ltd. Budapest, Hungary) equipped with an analogue to digital converter board (Data Translations, Framingham, MA, USA) and a data acquisition and analysis software (Caesar) (Analytical Devices Inc. Alameda, CA, USA). All the analyses were performed at ambient temperature; the capillaries were cooled by using a laboratory fan. Samples were injected into the capillary by electrokinetic injection using 10000 V for 10 seconds.

For sample and buffer preparation a 8452 A spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA) a TH 22 centrifuge (VEB MLW MEDIZINTECHNIK, Germany), a UC 450 PJ1 ultrasonic bath (TESLA, Prague, Czech Republic), a pH meter OP-211/1 (Radelkis, Budapest, Hungary) and a magnetic stirrer OP 951 (Radelkis, Budapest, Hungary) were used.

Results and Discussion

Sample Preparation (Milk and Dairy Products):

Indirect detection methods have some inherent disadvantages compared to direct detection techniques, namely a limited linear dynamic range in the detection and lack of robustness [1]. Therefore, a thorough and yet simple

sample preparation method has been developed for the analysis of milk and dairy products. The sample preparation consists of a homogenisation step (vigorous shaking for 20 seconds), a deproteinisation step (by adding 300 μl 0.1 mol/l HCl to 300 μl milk sample), centrifugation (removal of the coagulated milk protein at 6000 l/min, for 15 minutes) and dilution of supernatant with buffer (to 5 μl sample 935 μl electrolyte).

Selection of the Appropriate Background Electrolyte

Sodium tetraborate buffer (buffer#1)

Saccharides can be complexed with tetrahydroxy borate anions at basic pH [5] as it is shown in *Eq.* (1):

$$\begin{aligned} B^- + L &\Leftrightarrow BL^- + H_2O, \\ BL^- + L &\Leftrightarrow BL_2 + H_2O, \end{aligned} \tag{1}$$

where
L means: polyol ligand (non-charged carbohydrate) and
B^- means: $B[OH_4]^-$.

Upon complexation with tetraborate ions uncharged carbohydrate molecules can acquire charge and thus their charge/mass ratios will be different from each other. Moreover, they will absorb in the low UV region, and therefore direct UV detection can be applied. In order to avoid capillary wall interference in complex formation, linear polyacrylamide coated capillaries were used when this buffer system was applied (for separation conditions see *Table 1*). Using this system, the migration time of lactose was too long (over 40 min.), due to the lack of electroosmotic flow and the small charge/mass ratio of the complex, therefore this method was abandoned.

Salicylic acid (buffer#2)

Salicylic acid is strongly absorbing at 298 nm, therefore it is well suited for indirect UV detection. Using uncoated fused-silica capillaries and conditions listed in *Table 1,* poorly defined negative peaks at 3.3 minutes for lactose were obtained when lactose samples in the concentration of 0.1, 0.5, 1, 2, 3, 4, 5 mg/ml were injected into the capillary. Using this electrolyte system, a positive peak with altering size was always observed both in front of, and behind the lactose peak. Therefore the calculation of peak

Table 2
Calibration data using TRIS buffer

Lactose (mg/ml)	0.1	0.5	1	2	3	4	5
peak area (Vs)	−0.068 ±0.022	−0.140 ±0.062	−0.134 ±0.018	−0.069 ±0.027	−0.140 ±0.063	−0.155 ±0.015	−0.196 ±0.057

Table 3
Calibration data using sorbate buffer

Lactose (mg/ml)	Peak area (Vs)
1	−0.604
1.25	−0.812
1.5	−1.089
1.75	−1.355
2	−1.660

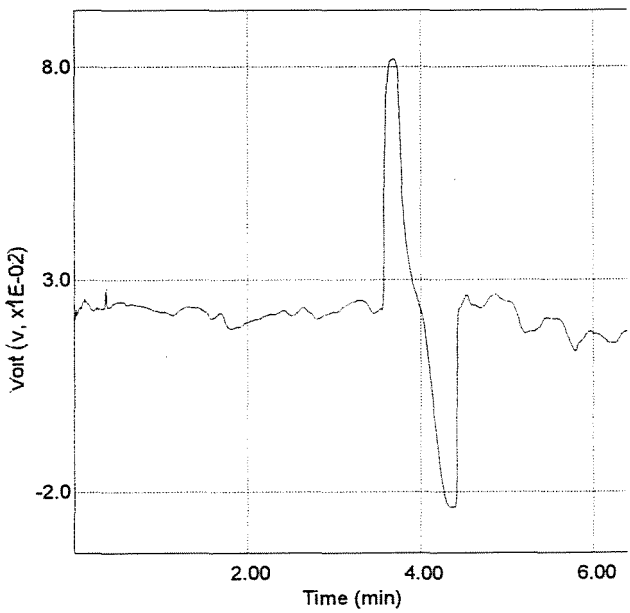

Fig. 1. Electropherogram of a milk sample. For separation conditions see text.
1. lactose

area was not reliable, and thus the buffer system was abandoned. The above phenomena are demonstrated by data in *Table 2.*

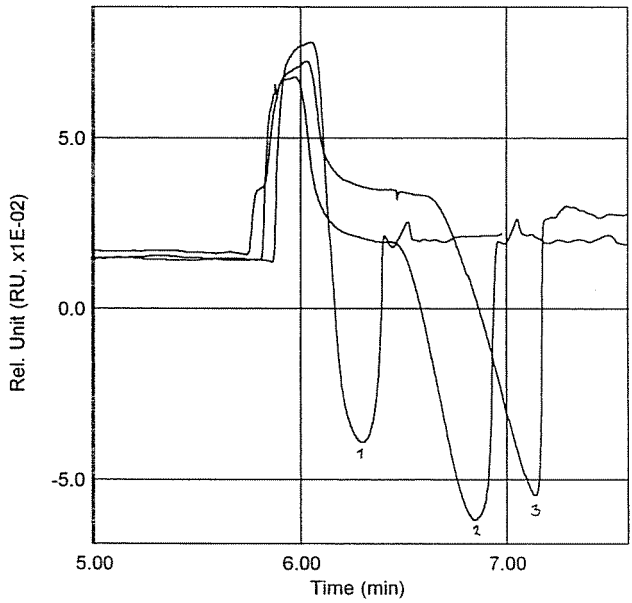

Fig. 2. Electropherograms of lactose, galactose, glucose. For separation conditions see text. 1. lactose, 2. galactose, 3. glucose

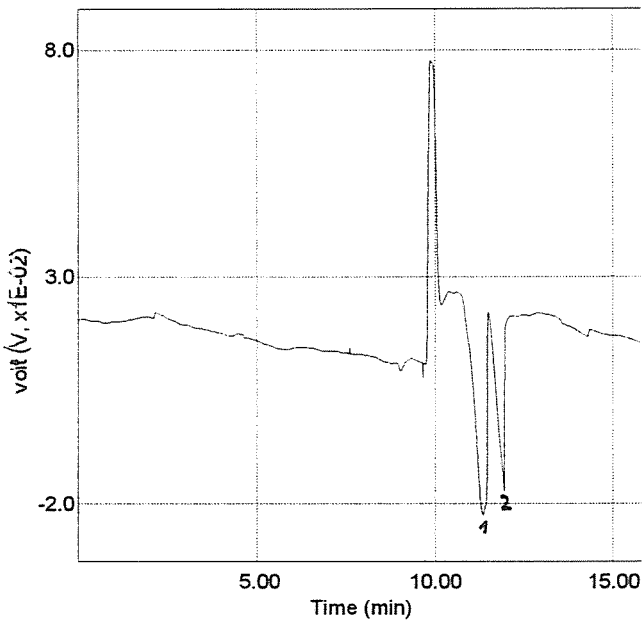

Fig. 3. Electropherogram of a chocolate milk sample. For separation conditions see text. 1. lactose, 2. saccharose

Sorbic acid

In a previous study Vorndran and co-workers [1] evaluated the use of sorbic acid for the indirect detection of saccharides. At a high pH (pH around 11) the OH-groups of saccharides are dissociated, therefore they are negatively charged. In a systematic study CHIESA – HORVATH [4] found 6 mmol/l of sorbic acid to be the optimum concentration for indirect UV detection considering the absorbance of the background electrolyte and the dissociation ratio of sample. Our preliminary results were similar to their findings, therefore 6 mmol/l sorbic acid was applied during subsequent investigations (for separation conditions see *Table 1*). The use of this buffer system resulted in a well defined negative peak for lactose with an acceptable peak shape. The signal-to-noise ratio was sufficiently high (*Fig. 1*). The shape of the lactose peak was not symmetric, as the electrophoretic mobilities of lactose and that of the sorbic acid were different – this is a frequently occurring phenomenon when indirect detection is used [9]. The biggest problem in this system arose from the signal of water, as its mobility at this pH was almost the same as that of lactose. Since 90 per cent of milk is water, the interference of the water peak could not be neglected. Increasing the effective length of the capillary and using the same electric field strength as before, the signals of lactose and water are separable. These conditions are suitable for the separation of different types of saccharides from lactose as well (*Fig. 2*). In conclusion, the optimum separation conditions for lactose determination were as follows: using fused silica capillary (ID=50 μm, $l = 55$ cm, $L = 70$ cm); background electrolyte: 5.3 mmol/l sorbic acid in TRIS-buffer (pH= 12.1); 257 V/cm electric field.

The migration time of the sample compounds proved to be reproducible (rsd= 2.9%, $n = 5$).

The calibration curve for quantitative lactose analysis was determined by injecting lactose samples of concentration of 1, 1.25, 1.5, 1.75, 2 mg/ml into the capillary (for injection conditions see Methods). A linear correlation was found in the concentration range of 1 – 2 mg/ml, with a regression coefficient of −1.062.

Applications

Using the calibration curve this method is suitable for quantitative determination of lactose in dairy products with or without supplementary sweeteners. This is shown in *Fig. 2,* where the electropherogram of chocolate milk is presented. (For separation conditions see Figure captions). In the electropherogram of chocolate milk two characteristic negative peaks can be seen; the first negative peak is saccharose, the second one is lac-

tose. Similarly the method is suitable to analyse other dairy products (for example fruit yoghurts, Bulgarian kefir and sour cream) as well (data not shown). The limitation of this method is, that it is not suitable for the analysis of dairy products containing less than 0.1% lactose. Samples with very different consistence from that of milk (for example dry milk powder, cheese, etc.) might need a different sample preparation method than discussed in this paper.

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References

1. VORNDRAN, A. E. – OEFNER, P. J. – SCHERZ, H. – BONN, G. K. (1992): Indirect UV Detection of Carbohydrates in Capillary Zone Electrophoresis. *Chromatographia,* Vol. 33, p. 163.
2. JORGENSON, J. W. – LUKACS, A. D. (1981): Zone Electrophoresis in Open-tubular Glass Capillaries. *Analytical Chemistry,* Vol. 53 pp. 1298–1302.
3. LIU, J. – SHIROTA, O. – NOVOTNY, M. (1992): Sensitive, Laser Assisted Determination of Complex Oligosaccharide Mixtures Separated by Capillary Gel Electrophoresis at High Resolution. *Anal. Chem.,* Vol. 64, p. 973.
4. CHIESA, C. – HORVÁTH, G. Cs. – OEFNER, J. P. – O'NEILL, A. R. (1996): Analysis of Glycoproteins, Oligo- and Monosaccharides. *Analytical Biotechnology,* CRC Press.
5. HOFFSTETTER-KUHN, S. – PAULUS, A. – GASSMANN, E. C. – WIDMER, H. M. (1991): Influence of Borate Complexation on the Electrophoretic Behaviour of Carbohydrates in Capillary Electrophoresis. *Anal. Chem.,* Vol. 63, p. 1541.
6. HEIGER, D. N. (1992): High Performance Capillary Electrophoresis – an Introduction. Hewlett-Packard.
7. HJERTEN, S. (1985): High Performance Electrophoresis Elimination of Electroendosmosis and Solute Adsorption. *J. Chromatogr.,* Vol. 347, p. 191.
8. GANZLER, K., – GREVE, K. S. – COHEN, A. S. – KARGER, B. L. – GUTTMAN, A. – COOKE, N. C. (1992): Separation of SDS-Protein Complexes by Capillary Electrophoresis Using UV Transparent Polymer Networks. *Anal. Chem.,* Vol. 64., pp. 2665–2671.
9. CIKALO, M. G., – GOODALL, D. M. – SANCHEZ-FELIX, M. – BLAGBROUGH, T. – REILLY J.: Investigation of Indirect Detection for Capillary Zone Electrophoresis. Poster, presented at *HPCE'95* Würzburg.