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Analysis of carbohydrates in beverages by capillary electrophoresis with precolumn derivatization and UV detection

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Abstract

We describe a simple, selective capillary electrophoretic method using precolumn derivatization and UV detection at 280 nm to determine carbohydrates in beverages. Three carbohydrates, glucose, maltose and maltotriose, were analysed. The labelling reagent was *p*-aminobenzoic acid (PABA). Under optimised conditions the labelling reaction took 1 h at 40 °C in the presence of 250 mM PABA solution and 20% acetic acid. A fused silica capillary column was used together with an electrophoretic medium of 20 mM borate buffer (pH 10.2). The analysis was completed in less than 12 min. The detection limits for the three analytes were in the range of 3.82–4.14 mg l^{-1}. The method could be readily applied to the analysis of carbohydrates in several beverages. Sample preparation was minimal, consisting only of degassification, dilution and/or filtration.

Keywords: Reducing carbohydrates; Capillary-zone electrophoresis; Derivatization; Beverage

1. Introduction

Carbohydrates such as glucose, maltose and maltotriose are to be found widely in many foods and beverages and are often used as food additives (Paulus & Klockow, 1996) for which the monitoring of carbohydrates in food samples is very important in the fields of nutrition, biology and food science (Molnár-Perl, 2000).

Many different analytical methods, especially those based on chromatographic techniques, in particular high-performance liquid chromatography (HPLC), using various different detectors have been assayed to this end (Glyad, 2002; Honda, 1984; Ikeguchi & Nakamura, 1999; Vonach, Lendl, & Kellner, 1998; Wilson, Cataldo, & Andersen, 1995).

As an alternative to chromatography, capillary electrophoresis (CE) is a powerful separation technique which provides high-resolution results and is becoming a standard tool for the analysis of many compounds (Baker, 1995; Cruces-Blanco, 1998; Chang & Kaplan, 2001; Cortacero-Ramírez, 2003; El Rassi & Mechref, 1996; Morales, 2002). One difference between CE and HPLC is that CE uses an open-tube capillary instead of a chromatographic column. Several CE methods have been developed with regard to carbohydrate analysis (He, Sato, Abo, Okubo, & Yamazaki, 2003; Oefner & Chiesa, 1994; Soga & Serwe, 2000). Since carbohydrates lack both charge and any strong UV chromophore, most of the methods described in the literature rely on some sort of derivatization technique (Guttman, 1997; Honda, Isawe, Makino, & Fujiwara, 1989). Whilst these methods lead to lessened sensitivity and resolution, complexity of detection is greatly increased. Some other methods do avoid these problems, such as those using in-column derivatization with 1-phenyl-3-methyl-5-pyrazole (PMP) (Honda, Suzuki, & Taga, 2003), with a combination of labelling by reductive amination using aromatic amines and reducing reagents (Hase, 1993; Jackson, 1997) or with *p*-aminobenzoic acid (PABA) (Grill, Huber, Oefner, Vorndran, & Bonn, 1993; He

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et al., 2003; Huber, Grill, Oefner, & Bobleer, 1994; Oefner, Vorndran, Grill, Huber, & Bonn, 1993).

The aim of our work here has been to evaluate the possibilities of capillary electrophoresis as an alternative to HPLC in the analysis of reducing carbohydrates by employing established precolumn derivatization techniques combined with *p*-aminobenzoic acid (PABA), the labelling reagent used for quality control in numerous food laboratories.

2. Materials and methods

2.1. Standards and reagents

Glucose, maltose, maltotriose, matotetraose, maltopentaose, maltohexaose and maltoheptaose were bought from Sigma Chemical Co. (St. Louis, MO, USA). Stock standard solutions of 10 mg ml^{-1} of each analyte were prepared in water doubly deionized in a Milli-Q system (Millipore, Bedford, MA, USA). *p*-Aminobenzoic acid (PABA) and sodium cyanoborohydride ($NaBH_3CN$) were also from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. Methyl alcohol (MeOH), 99.9% spectrophotometric grade, came from Aldrich Chemical Co., Inc. (Milwaukee, USA). Acetic acid (AcOH) and sodium hydroxide (NaOH) came from MERCK (Darmstadt, Germany). The running buffer was prepared by dissolving an appropriate amount of sodium borate (Sigma Chemical Co.) in doubly deionized water to obtain a final concentration of 100 mM, with its pH adjusted to 10.2.

2.2. Instrumentation

All CE experiments were made with a Beckman P/ACE^{TM} MDQ capillary electrophoresis instrument. The system included a 0–30 kV high-voltage built-in power supply, equipped with a diode array detector, and the GOLD software for system controlling and data handling. All capillaries (fused silica) had an internal diameter of 75 μm and were 57 cm in total length (Beckman Instrument Inc., Fullerton, CA, USA). The temperature was controlled using a fluorocarbon-based cooling fluid.

2.3. Electrophoretic procedure

The running buffer was 20 mM $Na_2B_4O_7$ (pH 10.2); samples were injected hydrodynamically for 8 s at 0.5 psi. Detection was carried out by on-column measurement of UV absorption at 280 nm. The separation voltage was 20 kV at a constant temperature of 25 °C. The capillary was flushed between runs with 0.1 M NaOH for 1 min followed by water for 1 min and then equilibrated with running buffer for 3 min.

2.4. Labelling reaction

The PABA reagent solution was freshly prepared before derivatization by dissolving 20 mg of $NaBH_3CN$ in 1 ml of methanolic solution containing 250 mM PABA and 20% AcOH. This solution was added to a mixture of carbohydrates at a concentration of 20 mg l^{-1} in a small test tube. The labelling reaction was facilitated by gentle vortexing for 5 min. The resulting solution was kept for 1 h at 40 °C and after cooling to room temperature diluted 1:10 with water prior to capillary electrophoretic analysis.

2.5. Analysis of beverages

For the beer analysis, the samples were degassed before being derivatized and diluted 1:20 with water and passed through a 0.22 μm membrane filter prior to injection. Peaks were identified by comparing their migration times with those of the standard spiked in the samples.

Since the soft drink to be assayed contained a large quantity of glucose, it was first diluted 1:50 with Milli-Q water, derivatized, diluted 1:20 and passed through a 0.22 μm membrane filter prior to injection.

3. Results and discussion

3.1. General

The optimum electropherogram of a standard mixture of the seven PABA-carbohydrate derivatives studied (glucose, maltose, maltotriose, maltoretraose, maltopentaose, maltohexaose and maltoheptaose), resulting from an analysis time of less than 12 min, shows very high definition between peaks (Fig. 1).

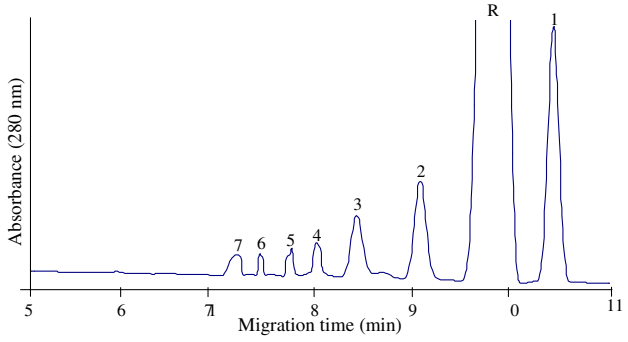

Fig. 1. Separation of a standard mixture of seven PABA-carbohydrate derivatives by CE with UV detection at 280 nm. Peak assignment: (1) glucose, (2) maltose, (3) maltotriose, (4) maltotetraose, (5) maltopentaose, (6) maltohexaose, (7) maltoheptaose and R, derivatization reagent (*p*-aminobenzoic acid). Concentration of 20 mg l^{-1}, operation conditions: 20 mM sodium tetraborate, pH 10.2, capillary $L_T = 57$ cm, $L_D = 50$ cm, I.D. = 75 μm; 20 kV, 8 s hydrodynamic injection.

3.2. Optimisation of the labelling reaction

The optimum parameters for the labelling reaction were established using the signals of glucose, maltose and maltotriose alone because these three carbohydrates are very representative of those commonly used in the foodstuffs industry. The labelling reaction of carbohydrates with the derivatization reagent PABA is based on the reaction of the primary amine with the reducing function of the carbohydrates, forming a Schiff's base which is subsequently reduced by $NaBH_3CN$ to produce a stable secondary amine.

Different labelling reagents were tested and PABA was chosen for obtaining the most reproducible results. One of the most important variables to be optimised in a derivatization reaction is the concentration of the labelling reagent. Thus we assayed reactions at PABA concentrations ranging from 0 to 450 mM. A minimum concentration of 150 mM was needed to complete the reaction but one of 250 mM was found ideal to guarantee the reproducibility of the peak areas for the carbohydrates selected (Fig. 2(a)) so we used this concentration for the rest of the experimental work.

Because the Schiff's base formed during the first step of the labelling reaction needs to be further reduced, a reducing agent is required, that most frequently used being $NaBH_3CN$. The effect of different concentrations of this reagent in the reaction solution of the carbohydrates was tested in the range of 0–60 mg. A minimum of 10 mg was necessary to complete the second step of the labelling reaction whilst concentrations higher than 60 mg caused a decrease in peak area (Fig. 2(b)). We chose to use 20 mg to ensure both the completion of the reaction and satisfactorily high sensitivity of detection.

Because the derivatization reagent has to be prepared in acetic acid (AcOH) we studied the influence upon the peak area of different percentages of this solvent in the labelling reaction and found it to be negligible with the analytes being studied. Thus we chose to use 20% AcOH, which provided the best level of solubility for our purposes.

Other parameters that tend to influence a derivatization reaction are heating time and temperature. The temperature was modified between 30 and 90 °C over periods of 2 h. The highest areas for the analytes were obtained after 1 h at 40 °C.

After heating for this time the reaction mixture was cooled to room temperature and then diluted 1:10 with water before analysis by CE.

3.3. Separation of carbohydrates by CE

Separation of the standard PABA-carbohydrate derivatives was performed by capillary zone electrophoresis (CZE), based on the ratio between their electric charges and molecular size. Detection was carried out at 280 nm and identified by spiking samples with standards.

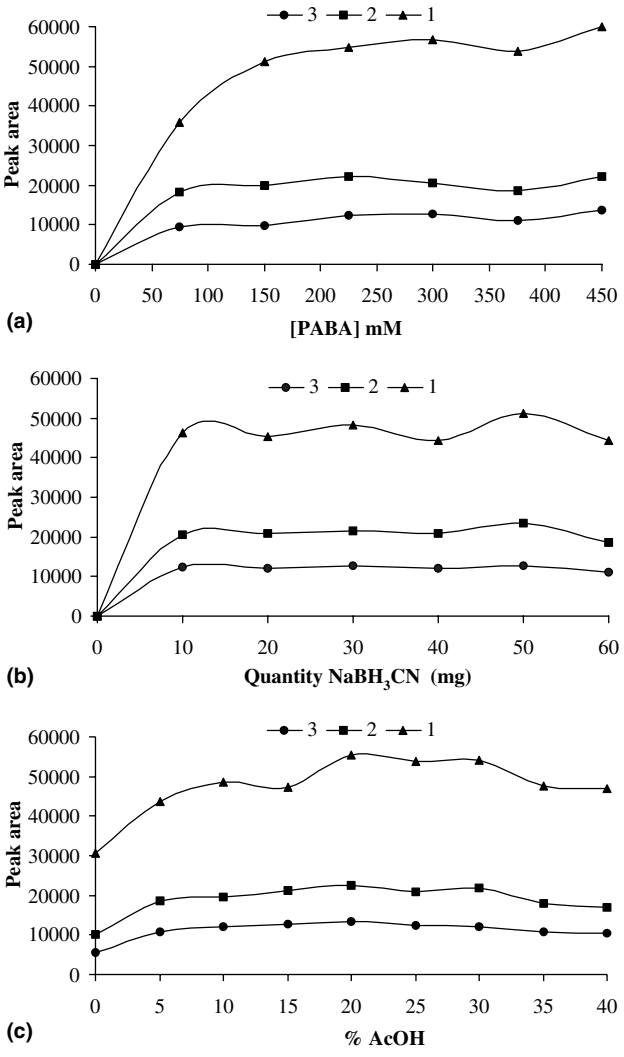

Fig. 2. Optimisation of the experimental conditions of the labelling reaction: (a) PABA concentration; (b) $NaBH_3CN$ quantity; (c) AcOH percentage. Reaction temperature: 40 °C, time 60 min. Electrophoretic conditions as in Fig. 1 (1) glucose, (2) maltose, (3) maltotriose.

To guarantee the ionization of the analytes we made a detailed study of the pH of the separation media over a range between pH 8 and 11. The best resolution versus the lowest migration times was obtained at pH 10.2, at which value the PABA-carbohydrate derivatives are negatively charged.

The buffer concentration must also be optimised because of its influence on both electro-osmotic flow and electrophoretic mobility in CE. It also affects the symmetry of the peaks. If the concentration of the analyte ions is higher than that of the buffer ions, the electric field in the capillary may become distorted, leading to irregular peak shapes (Frazier, Ames, & Nursten, 2000). The effect of buffer concentration on the mobility and

resolution of the selected carbohydrates was investigated by using different concentrations of sodium-tetraborate carbohydrate buffer solution at pH 10.2. Although resolution can be improved by increasing the concentration there are limitations to high buffer concentrations because of Joule heating (Knox & McCormack, 1994). Taking all effects into account, we decided on a concentration of 20 mM for our further studies.

Among the instrumental parameters, the effect of different voltages from 5 to 30 kV upon capillary electrophoretic separation was tested. As expected, the mobility of the PABA-carbohydrate derivatives de-

Table 1
Performance parameters

Parameter	($mg\,l^{-1}$)	Glucose	Maltose	Maltotriose
Analytical sensitivity ($mg\,l^{-1}$)		1.38	1.32	1.31
Detection limit ($mg\,l^{-1}$)		4.01	4.14	3.82
Quantitation limit ($mg\,l^{-1}$)		13.37	13.81	12.73
Linear range ($mg\,l^{-1}$)		4.01–60	4.14–60	3.82–60
Precision (RSD) (%)	10	9.41	9.89	9.25
	20	4.58	4.39	4.51
	30	2.97	2.78	2.74
	40	2.16	2.03	2.01
	50	1.78	1.72	1.72
	60	1.65	1.59	1.57

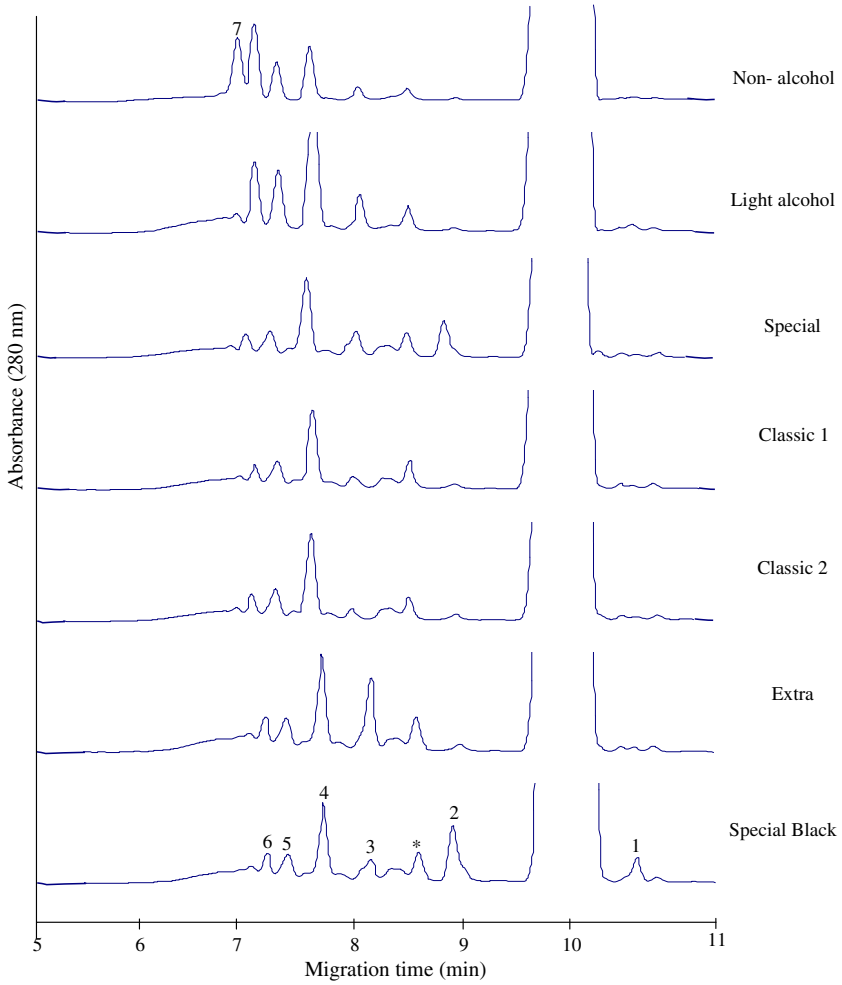

Fig. 3. Electropherograms of PABA from different beer samples. (*) unknown peak, CE conditions and peak assignment as in Fig. 1.

creased concomitantly with a decrease in voltage. A voltage of 20 kV was employed.

Injection time is another important instrumental parameter in capillary electrophoretic separations. We tried times of between 4 and 16 s and obtained the best results at 8 s.

3.4. Linearity and sensitivity

The linearity and sensitivity of the method were tested against glucose, maltose and maltotriose, the most common carbohydrates found in foods and beverages. All three calibration curves showed good linearity from 4 to 60 mg l^{-1}. Each point of the calibration plot was repeated three times in an independent solution prepared in the same way. The calibration plots indicate good correlation between peak areas and PABA-carbohydrate derivative concentrations; regression coefficients were 0.997 for the three compounds. The precision of the measurements was checked for three injections for all points of the calibration plots.

The detection and quantitation limits for the carbohydrates were calculated using the method described by Cuadros-Rodriguez, García-Campaña, Jiménez-Linares, and Román Ceba (1993). All the results obtained for the three PABA-carbohydrate derivatives are summarised in Table 1.

3.5. Application to real samples

Our aim in this work has been to demonstrate the versatility and confirm the potential resolution of the CE separation technique using UV detection to determine carbohydrates in different types of beers (alcoholic and non-alcoholic) and non-alcoholic beverages such as dietetic fruit juices and a soft drink.

The three analytes quantified (glucose, maltose and maltotriose) are fermentable carbohydrates which may remain in beer depending on the type of yeast used (Pollock, 1981). The contribution of the most important carbohydrates to sweetness can be readily gauged (the range found in beers is 0.04–1.1 g l^{-1} for glucose, 0.7 – 3.0 g l^{-1} for maltose and 0.4–3.4 g l^{-1} for maltotriose). If the carbohydrates consist of more than four glycosil units the beverage in question is hardly sweet (Hughes & Baxter, 2001). As can be seen in Fig. 3, the maltotriose peak appears in all the types of beer analysed, whilst maltose is present in two of them, the "*Special Beer*" and the *"Special Black Beer"*. Glucose was only detected in the "*Special Black Beer*" but this is due to the final special treatment given to this kind of beer.

Other non-fermentable carbohydrates such as maltotetraose, maltopentaose, maltohexaose and maltoheptaose also appeared in the samples analysed, as can be seen in the electropherograms, buy they have not been quantified.

We also analysed a plum juice, an orange juice and a soft drink. The total of carbohydrate content declared in the plum and orange juices is 4.5%. The electropherograms of the corresponding analysis carried out for the two juices and the soft drink are presented in Fig. 4. As it can be seen in these electropherograms, the only carbohydrate we detected and analysed in all the drinks was glucose (Table 2). In all cases we checked our results against a standard-addition method of calibration, obtaining 1.50% and 1.98% glucose for the plum and orange juices, which differ from the contents declared by

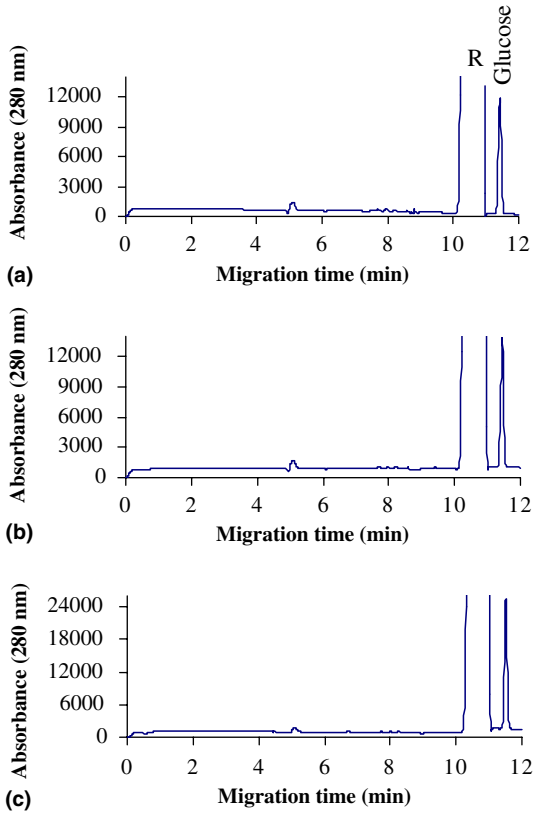

Fig. 4. Electropherograms of PABA-carbohydrate derivatives in: (a) plum juice; (b) orange juice; (c) soft drink. CE conditions and peak assignment as in Fig. 1.

Table 2
Analysis of carbohydrates in beverages

Sample	Glucose	Maltose	Maltotriose
Non alcohol beer (g l^{-1})	–	–	0.26
Light alcohol beer (g l^{-1})			0.98
Special beer (g l^{-1})	–	0.79	0.61
Classic 1 beer (g l^{-1})	–	–	0.23
Classic 2 beer (g l^{-1})		–	0.22
Extra beer (g l^{-1})	–	–	1.93
Special black beer (g l^{-1})	0.14	1.08	0.59
Plum juice (%)	1.50	–	–
Orange juice (%)	1.98	–	–
Soft drink (g l^{-1})	7.43	–	–

the manufacturer (4.5%) because this data corresponds to the total carbohydrate content.

4. Conclusion

The PABA method described here can be used for derivating any kind of reducing carbohydrates. The derivatives can then be efficiently separated by CE. The strong absorption in the UV region ensures a wide application in routine quality control which calls for rapid, multi-sample analyses. Separation by CE proves to be a viable alternative to HPLC and will provide more highly efficient separations in food analysis in the future.

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