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Determination of carbohydrates in fruit juices by capillary electrophoresis and high-performance liquid chromatography

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Abstract

A capillary zone electrophoresis (CZE) method with indirect UV detection was adapted for the routine determination of carbohydrates in a variety of fruit juices. The method was optimized with respect to the effect of buffer pH, temperature and capillary length. Potassium sorbate was chosen as the background electrolyte and chromophore for UV detection at 256 nm. Optimum separation conditions were found with a buffer of a pH of 12.2–12.3 and a subambient temperature of 15°C. The optimized CZE method was compared with a routine method for the determination of sugars in fruit juices, a high-performance anion-exchange chromatographic method with pulsed amperometric detection (HPAEC–PAD), with respect to separation efficiency, sensitivity, linearity and repeatability. The CZE method showed a 10–20-fold increase in separation efficiency compared with the HPAEC–PAD method, but the amperometric detection in the latter proved to result in detection limits of 2–3 orders of magnitude lower than those obtained by indirect UV detection. Both methods showed good linearity in the investigated concentration ranges and good repeatability for migration times and peak areas. CZE with commercial instrumentation was applied to the routine determination of carbohydrates in fruit juices such as orange, apple and grape juice. The quantitative CZE results with internal calibration showed no significant differences from those for the HPAEC–PAD reference method. It was demonstrated that capillary electrophoresis (CE) can be applied in a routine food testing laboratory. The method is simple, inexpensive and easy to implement and will further broaden the application range of CE in food analysis.

1. Introduction

In recent years, capillary electrophoresis (CE) has attracted increasing interest and its successful use in different application fields such as biochemistry, biotechnology, pharmacy and clinical chemistry has been demonstrated in a variety of studies [1–3]. However, the impact that CE can have in food science and particularly in quality control of food and food additives is today only starting to be recognized. So far, less than 100 CE applications in food science have been published. Among them, the analysis of hop bitter acids in beer [4], the differentiation and determination of milk proteins [5] and the identification of different sulfonamides in pork meat as a means to ascertain the use of animal drugs [6] are worth mentioning. CE can also be very helpful for the determination of ascorbic acid [7] and other organic acids [8] in fruit juices.

Currently, the most popular techniques for the determination of carbohydrates in food stuff are

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thin-layer chromatography (TLC), gas chromatography (GC) of volatile carbohydrate derivatives and high-performance liquid chromatography (HPLC). Although TLC is a simple and rapid method for the identification of carbohydrate compositions, the analysis times are long and the separation efficiency is often not satisfactory [9]. GC exhibits good sensitivity, but the formation of stereochemical isomers during the necessary derivatization with, for example, trimethylsilane to yield volatile sugar derivatives results in very complex chromatograms and remains a major problem [10]. Other derivatization procedures, such as the formation of alditolacetate or aldonitriloacetate derivatives, also lead to problems in the interpretation of the resulting chromatograms [11].

Another possibility for the rapid determination of simple sugars and also for polysaccharide hydrolysates is electrophoretic separation on supporting media such as paper [12] or silylated glass-fibre paper [13,14]. However, similarly to TLC, slab electrophoretic methods only provide qualitative information. Among the methods mentioned above, HPLC is certainly the most important method for carbohydrate determinations in both research applications and routine analysis. HPLC separations can be carried out on different stationary phases such as alkylated or aminoalkylated silica gels or ion-exchange resins [15]. The latter, in combination with pulsed amperometric detection (PAD) [16,17], represents a selective and sensitive system for carbohydrate determinations with the additional advantage that no derivatization step is required. A disadvantage of this HPLC method is its limited separation efficiency, especially when analysing higher oligosaccharides. In addition, HPLC can be time consuming when column equilibration procedures are necessary. Overall, the analysis costs are high owing to the expensive instrumentation, stationary phase material and solvent consumption.

CE has the potential to be an alternative method to HPLC and also to the other methods because it can bring speed, quantification and reproducibility combined with a high separation efficiency to the routine quality control of carbohydrates. At first sight, an electrophoretic method does not seem appropriate to carbohydrate determinations because the solutes lack both charge (a prerequisite for electrophoretic separation) and a suitable chromophore (necessary for on-column UV detection). To overcome these problems, several strategies can be pursued.

To attach a charge to the sugar solutes, ionization at high pH [18], complexation of their vicinal hydroxyl groups with borate, resulting in an anionic complex [19], and derivatization with a charged label [20] have been described.

On-line detection of sugars without precolumn labelling can be accomplished in the UV region at 195 nm after borate complexation, as the UV absorbance of complexed sugar molecules is increased to allow detection at the millimolar level [19]. Indirect photometric detection [18,21] represents a second possibility. Several procedures for labelling sugars with a suitable labelling reagent for sensitive UV and fluorescence detection have been described, those with 2-aminopyridine [22], 3-methyl-1-phenyl-2-pyrazolin-5-one [23] and 8-aminonaphthalenetrisulfonic acid [24] being the most popular. Other attempts to determine sugars involve different detection schemes, such as amperometric [25] and refractive index detection [26]. By freely combining these different means of charging and detecting sugars, a variety of methods for the determination of carbohydrates using CE can be explored.

Indirect photometric detection of underivatized carbohydrates represents a simple, easy and time-efficient approach. As the pK_a of monosaccharides is generally >12, this method requires a background electrolyte with a pH >12 to ensure the formation of negatively charged sugar molecules. Further, a carrier electrolyte anion with a high molar absorbance will allow adequate detection sensitivities. Although the sensitivity of indirect UV detection methods generally does not reach the levels achieved by direct UV measurements, sugar detection in the low millimolar range is possible, with sorbic acid as an absorbing additive [18].

The aim of this work was the improvement of

a CZE method for the determination of sugars in a high-pH separation system with indirect UV detection [18]. This method was then subsequently applied to the determination of sucrose, glucose and fructose in three different fruit juices. To validate the method, the quantitative CZE results were compared with results obtained for a routine control series in a government food control laboratory applying a certified HPLC method, based on anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD). Both methods were submitted to a statistical analysis to demonstrate that the CZE method with indirect UV detection is suitable for routine food testing laboratories.

2. Experimental

2.1. Chemicals

All chemicals and sugar standards were of analytical-reagent grade.

For the CE experiments, sugar standards of D-fructose (Fru), L-fucose (Fuc), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), D-raffinose, N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc), N-acetylneuraminic acid (NANA) and D-sucrose (Suc) were purchased from Fluka (Buchs, Switzerland), D-glucoronic acid (GlcA) from Serva (Heidelberg, Germany) and D-galacturonic acid (GalA) from Merck (Darmstadt, Germany). Buffer chemicals were supplied by either Sigma (St. Louis, MO, USA) or Fluka (Buchs, Switzerland). All buffers were prepared with water purified with a Milli-Q system (Millipore, Bedford, MA, USA) and used throughout all analyses.

The three sugar standards (Glc, Fru, Suc) used in the HPAEC analysis were purchased from Merck. Sodium hydroxide (NaOH), necessary for the preparation of the LC eluent, was supplied by Baker (Gross Gerau, Germany).

2.2. Sample pretreatment

For CE separations, the fruit juices were diluted 50–100-fold with Milli-Q-purified water. Whereas the orange juice had to be filtered through a 0.22-μm Millipore filter, the other juices could be applied without further pretreatment. For HPAEC analyses, the juices were diluted 2000–10 000-fold and filtered through a 0.45-μm filter before injection.

2.3. Procedures

The CZE background electrolyte was prepared by dissolving an appropriate amount of potassium sorbate in Milli-Q-purified water to yield a final concentration of 6 m*M*. The pH was adjusted to 11.9–12.4 by titration with 1 *M* NaOH at room temperature. The sugar standard solutions for the method development experiments contained between 0.25 and 0.4 mg ml^{-1} of each sugar. A stock standard solution of 8 mg ml^{-1} of Suc and 4 mg ml^{-1} of Glc and Fru was used for the external calibration in juice analysis. This solution was diluted to concentrations of 0.2–2.0 mg ml^{-1} for Suc and 0.1–1.0 mg ml^{-1} for the two monosaccharides.

For the determination of the response factors, samples containing 0.8 mg ml^{-1} of Suc and 0.4 mg ml^{-1} of Glc and Fru were used. Glucuronic acid was added as an internal standard, resulting in a final concentration of 0.22 mg ml^{-1} GlcA in standard samples and 0.12 mg ml^{-1} GlcA in fruit juices.

To prepare the 200 m*M* sodium hydroxide HPAEC eluent, 26 ml of 50% (w/w) sodium hydroxide solution were mixed with 1000 ml of degassed water. It was necessary to keep the eluent free of carbonate. Sugar mixtures containing 2, 10 and 50 mg l^{-1} of each sugar were used as standard solutions for external calibration.

2.4. Instrumentation

CE separations were performed on a Spectra Phoresis 1000 capillary electrophoresis system (Thermo Separation Products, Freemont, CA, USA). For data acquisition and data handling an OS/2 compatible 486 computer combined with a Spectra Phoresis software package for individual peak integration was used. Fused-silica capil-

laries of 50 μm I.D. from Polymicro Technologies (Phoenix, AZ, USA) were cut to the appropriate length of 42 or 90 cm. The detection window was placed 7 cm from the cathodic end, resulting in an effective separation distance of 35 and 83 cm, respectively. UV detection was carried out at 256 nm throughout all experiments. Injection was accomplished by applying a pressure of 105 mbar for a preset time (1 or 2 s). Unless indicated otherwise, the capillary was thermostated at 15°C. Before starting a series of analyses, the fused-silica capillaries were conditioned by flushing them with running buffer for at least 10 min, followed by an equilibration time of 15 min. Overnight, the capillaries were stored in 1 m*M* NaOH.

HPAEC analyses were accomplished on a Model 4000i ion chromatographic system (Dionex, Sunnyvale, CA, USA) with a 50-μl injection loop and a pulsed amperometric detector (PAD-1). All separations were carried out at room temperature on a Carbopac PA1 column (250 × 4 mm I.D.) combined with a Carbopac PA1 guard column (both from Dionex) at an eluent flow-rate of 1 ml min^{-1}. The detector consisted of a gold working electrode and an Ag/AgCl reference electrode. The pulse sequence used for the carbohydrate analysis consisted of three steps: sampling (50 mV, 480 ms), cleaning (600 mV, 120 ms) and reduction of the electrode surface (−600 mV, 60 ms). To ensure a carbon dioxide-free eluent during analysis, an EDM-2 degassing module (Dionex) was necessary. Data acquisition was carried out on a Maxima 820 work station (Millipore).

3. Results and discussion

3.1. CZE method development

A recently published paper by Vorndran *et al.* [18] served as the basis of the CZE method development. The reasons for choosing sorbate as the carrier electrolyte anion and chromophore for indirect photometric detection, at a concentration of 6 m*M*, are discussed therein.

Separation in CZE is based on differences in the electrophoretic mobilities (μ_{ep}) of the analytes. As the electrophoretic mobility depends mainly on the pH of the separation system, optimization of the buffer pH is important for reaching the optimum separation conditions. Because the pK_a values of sugars are in the range of 12–14 [27], the buffer pH must be >12 to ionize the sugar molecules.

In Fig. 1, the influence of pH on the experimentally determined electrophoretic mobility of four sugars in the pH range 11.9–12.4 is shown. By closely inspecting the experimental data, we could not confirm the previously published [18] linear behaviour between electrophoretic mobility and pH owing to the increased dissociation rate of the sugars with increasing pH. It should be pointed out that those monosaccharides with pK_a values within the investigated pH range (12.1–12.4) exhibit a relatively steep increase in electrophoretic mobility, indicating their higher degree of ionization. Raffinose, with a reported pK_a of 12.74 [27], shows a linear relationship.

Surprisingly, the electroosmotic flow inside the capillary decreased continuously from pH 11.9 to 12.4, resulting in longer analysis times for all solutes. This can be explained by an increase in the ionic strength of the background electrolyte, resulting in a thinner double layer and thus a lower zeta potential [28].

The temperature of the buffer system is also an important parameter that has a pronounced influence on the separation of low-molecular-mass carbohydrates. According to the literature, an increase in temperature of 1°C should increase the electrophoretic mobility by approximately 2% [29]. However, in our experiments, an increase in the electrophoretic mobilities with decreasing temperature was observed. This behaviour is due to a change in pH rather than to a temperature effect alone. At lower temperature, the dissociation of water molecules is dictated by a smaller ion-product value, resulting in a lower proton concentration and thus in a higher pH.

Table 1 demonstrates the effects that pH and temperature changes have on the resolution of various sugar pairs. From these values it can be concluded that, in order to increase the dissocia-

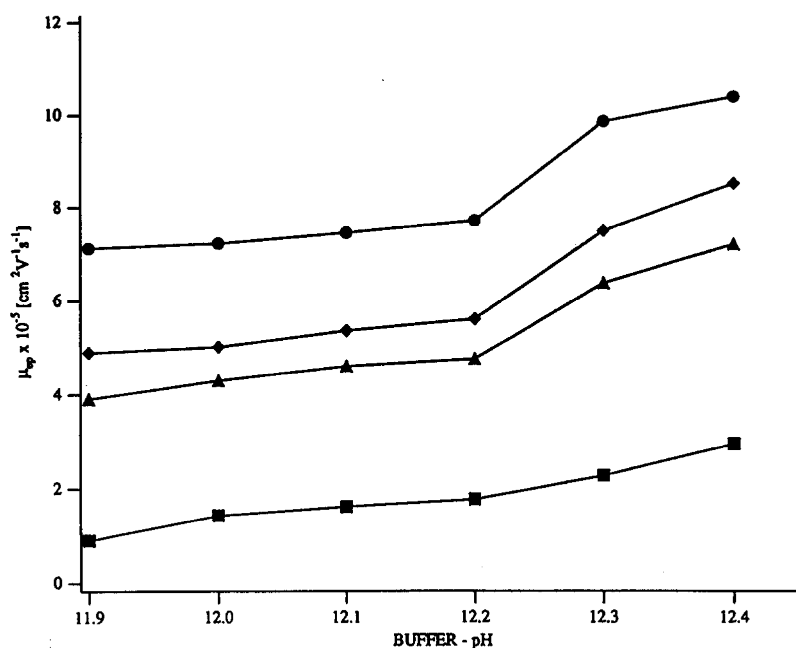

Fig. 1. Experimentally determined electrophoretic mobilities of carbohydrates as a function of pH. Running conditions: buffer 6 m*M* sorbate adjusted to different pH; capillary, 42 cm (35 cm effective length) × 50 μm I.D.; 230 V cm^{-1}; 15°C; indirect UV detection at 256 nm; 2-s injection. Solutes: ● = mannose; ◆ = glucose; ▲ = galactose; ■ = raffinose.

tion and improve the resolution of the sugars, it is best to choose a temperature as low and a pH as high as possible for the separation. However, there are instrumental limitations. Above pH 12.3, fluctuations in the UV baseline were observed, rendering an analysis impossible. Consequently, for practical purposes a buffer pH of 12.2–12.3 and a temperature of 15°C were chosen. All subsequent experiments were carried out under these conditions.

Table 1
Resolution as a function of pH and temperature

	Resolution			
	μ_{eo}–Raf	Raf–Gal	Gal–Glc	Glc–Man
pH 11.9	0.41	1.49	0.44	1.69
pH 12.0	0.77	1.75	0.54	1.77
pH 12.1	1.00	2.10	0.59	1.82
pH 12.2	1.31	2.43	0.71	1.76
pH 12.3	1.49	3.10	1.10	2.18
pH 12.4	1.90	3.73	1.15	2.12
40°C	0.47	0.86	0	0.84
30°C	0.60	1.27	0.28	1.07
25°C	0.79	1.48	0.46	1.29
20°C	0.93	1.97	0.64	1.47
15°C	1.19	2.55	0.85	1.81

Theory predicts that the separation efficiency in CZE is proportional to capillary length when the electric field is kept constant [30]. In addition, a contribution from injection to band broadening can be observed, which has an impact on the separation efficiency and resolution. This influence of capillary length on the separation is demonstrated in Fig. 2. Fig. 2a shows the separation of the eight monosaccharides expected to occur in glycoproteins. It can be calculated that a 1-s injection at 105 mbar into a 42-cm capillary will result in an injection plug of

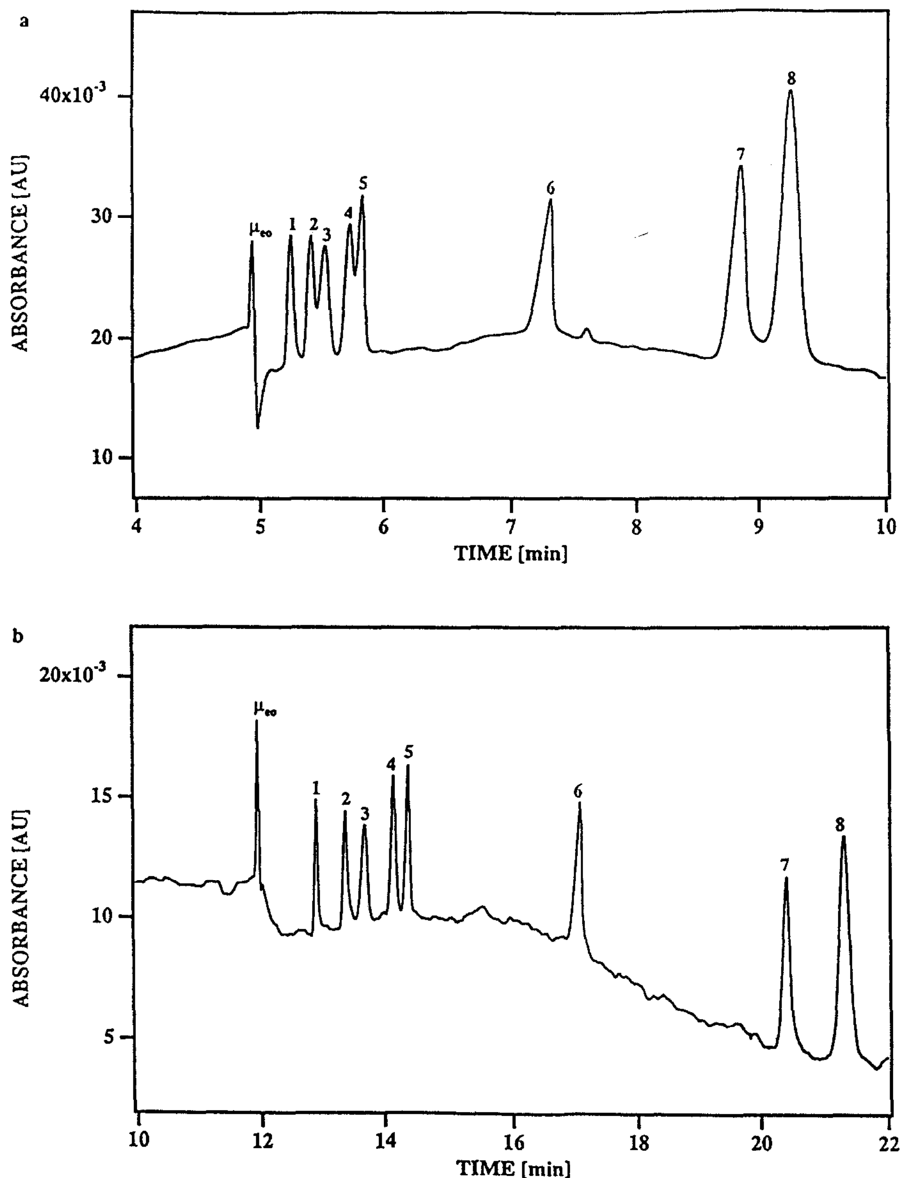

Fig. 2. Capillary zone electrophoresis of carbohydrates. Running conditions: buffer 6 mM sorbate adjusted to pH 12.2; 230 V cm^{-1}; 15°C; indirect UV detection at 256 nm; 1-s injection. Sugars, 0.97–1.55 mM; 1 = fucose; 2 = galactose; 3 = glucose; 4 = N-acetylgalactosamine; 5 = N-acetylglucosamine; 6 = N-acetylneuraminic acid; 7 = galacturonic acid; 8 = glucuronic acid. (a) Capillary 42 cm (35 cm effective length) × 50 μm I.D.; (b) capillary 90 cm (83 cm effective length) × 50 μm I.D.

1.95 mm. This leads to an incomplete resolution of the sugar pairs Gal–Glc and GalNAc–GlcNAc, because more of the capillary volume was occupied by the injection plug compared with a longer capillary, resulting in a shorter effective separation length. If the same experiment was performed in a 90-cm capillary, as shown in Fig. 2b, the separation efficiency more than doubled, resulting in baseline resolution of these sugar pairs, but at the cost of doubling the analysis time. The better resolution can be attributed to two effects: the smaller contribution of the shorter injection plug of 0.9 mm, which results from a higher pressure drop at the longer capillary, and the longer separation distance with the same electric field strength.

As the resolution of the three sugars Glc, Fru and Suc, occurring in fruit juices, proved to be sufficient with the shorter capillary, the 42-cm capillary was chosen for further separations.

3.2. Comparison of CZE with HPAEC

In order to evaluate the usefulness of the above-described CZE method, the results of the CZE experiments were compared with those obtained with the state-of-the-art method for carbohydrate analysis in routine work, HPAEC–PAD. The comparison was made with respect to separation efficiency, sensitivity, linearity and repeatability.

Separation efficiency

The separation power of two analytical systems can be evaluated by the separation efficiency, expressed by the number of theoretical plates (N). N describes the band broadening in a given analysis system and can be calculated by the expression

$$N = 5.54(t_m / w_h)^2$$

where t_m is the migration time and w_h the peak width at half-height [31]. As shown in Table 2, typical plate numbers for the sugar solutes in the HPAEC system are in the range 3000–4000. In CZE, the separation efficiency proved to be 10–20 times higher with theoretical plate numbers of 30 000–70 000 (Table 2). This is important, because a high separation efficiency has a positive effect on the resolution of the analytes.

Although the number of theoretical plates in CZE is more than one order of magnitude higher than in HPAEC, even higher plate numbers, up to 400 000, would be expected with indirect UV detection [32]. However, this requires an effective mobility of the analyte ions close to that of the background electrolyte co-ion, in order to avoid a concentration overload [33]. In the separation system discussed here, the electrophoretic mobility of the sorbate anion was measured at pH 12.2 to be $42.8 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1} whereas the sugars showed the following, much lower mobilities: Suc, $2.24 \cdot 10^{-5}$; Glc, $7.12 \cdot 10^{-5}$; and Fru, $8.89 \cdot 10^{-5}$ cm^2 V^{-1} s^{-1}. Therefore, the separation efficiency was relatively low compared with other CE separations, but still compares favourably with the HPAEC results.

Limit of detection (LOD)

The LOD has an impact on the degree of sample dilution and the reproducibility of the

Table 2
Comparison of separation efficiency and limits of detection in CZE and HPAEC

Sugar	Separation efficiency (N)		Limit of detection (LOD)	
	CZE	HPAEC	CZE (mM)	HPAEC (μM)
Sucrose	49 700	4 350	0.29	0.58
Glucose	29 300	3 400	0.23	1.11
Fructose	69 600	3 100	0.24	1.11

measurements because of the decreased matrix effects at higher dilutions. The LOD was calculated as the concentration that gave a signal three times greater than the baseline noise. As shown in Table 2, indirect UV detection with sorbate resulted in LODs of 0.23–0.29 m*M*, whereas PAD was able to detect as little as 0.5–1 μM concentrations of sugars. Thus PAD resulted in detection limits of 2–3 orders of magnitude better than those with indirect UV detection.

Concentration detection limits in the 10^{-4} *M* range for carbohydrates are not very impressive. Assuming a 50-μm capillary and molar absorptivities (ε) of 10 000–100 000 l mol^{-1} cm^{-1}, concentration detection limits in the 10^{-5}–10^{-7} *M* range can be expected according to the Lambert–Beer law. If, in a separation method, labelling of the carbohydrates should be avoided, indirect detection is the only alternative. In that event, the spectroscopic characteristics of the indirect UV absorber is responsible for the detection limits. The chosen compound should match the mobilities of the solutes and be compatible with the separation buffer, e.g., between pH 12 and 13. Up to now, only sorbate has been reported to come close to these requirements. With $\varepsilon = 26\,000$ l mol^{-1} cm^{-1}, measured at pH 12.3 and 256 nm, low micromolar detection concentrations seem possible. Joule heat effects resulting in high baseline noise and non-ideal peak shapes originating from a mismatch of the electrophoretic mobilities of solutes and the indirect UV absorber are the reasons why the theoretical limits could not be realized.

In terms of mass detection, the comparison is in favour of CE. Assuming a 50-μl injection for HPAEC and a ca. 4-nl injection for CE, the absolute amounts detectable are 25–50 pmol for HPAEC and 0.9–1.1 pmol for CE.

Linearity

The linearity describes the molar range in which the detector signal depends linearly on the analyte concentration. The correlation coefficients for the HPAEC calibration graphs were 0.9999 for all three standard sugars in the concentration range 1–50 mg l^{-1}. The calculated correlation coefficients for the extremal CE calibration are given in Table 3. For Suc, calibration was carried out in the concentration range 0.2–2.0 g l^{-1} and for Glc and Fru in the range 0.1–1.0 g l^{-1}. In summary, both methods resulted in good linearity in the investigated concentration ranges.

Repeatability

To determine the repeatability, a sugar mixture, as given in Table 4, was injected several times into the CE and the HPAEC systems. In CZE experiments, the peak-area repeatability was found to be between 2.0 and 2.4% and the migration time repeatability was <0.3% (Table 4). HPAEC showed a better peak-area repeatability, mainly owing to lower noise and automated peak integration and almost the same repeatability for migration time as CE. It can therefore be concluded that both methods are suitable for routine application.

Although the repeatability of the CZE separations on a single day is satisfactory, the day-to-day analysis resulted in scattered values, leading to relative standard deviations (R.S.D.s) of 10–

Table 3
Linearity of carbohydrate determination with CZE and HPAEC

Sugar	Method	Concentration range	Correlation coefficient
Sucrose	CZE	0.2–2.0 g l^{-1}	0.9993
	HPAEC	1–50 mg l^{-1}	0.9999
Glucose	CZE	0.1–1.0 g l^{-1}	0.9977
	HPAEC	1–50 mg l^{-1}	0.9999
Fructose	CZE	0.1–1.0 g l^{-1}	0.9934
	HPAEC	1–50 mg l^{-1}	0.9999

Table 4
Repeatability in CZE and HPAEC [R.S.D. (%)]

Sugar	CZE concentration (mM)	HPAEC concentration (mM)	CZE (area) ($n = 7$)	HPAEC (area) ($n = 10$)	CZE (t_m)[a] ($n = 7$)	HPEAC (t_R)[b] ($n = 10$)
Sucrose	2.2	0.3	2.0%	1.5%	0.2%	0.9%
Glucose	2.2	0.6	2.2%	0.6%	0.3%	0.5%
Fructose	2.2	0.6	2.4%	0.5%	0.3%	0.5%

[a] t_m = Migration time.
[b] t_R = Retention time.

18% for peak areas. The main reason lies in the characteristics of the inner capillary wall, which are very difficult to keep constant in the high pH range over several days and which have a considerable effect on the electroosmotic flow and therefore on the overall mobilities of the analytes.

In addition, a background electrolyte with buffering capacity is necessary to keep the degree of dissociation of the sugars constant. However, at pH > 12, sorbic acid with a pK_a of 4.8 [34] has no buffering properties at all, resulting in pH shifts due to ion depletion. As demonstrated in Fig. 1, even a small change in pH of 0.1 unit has a strong effect on the electrophoretic mobility of the sugars. Because the peak area of any analyte in CZE depends on its electrophoretic mobility, a shift in the pH of the background electrolyte results in a change in the peak-area measurements [32].

3.3. *Comparison of CZE fruit juice analysis with HPAEC*

As shown above, the described CZE method allows a rapid separation of sugars with sufficient selectivity, within a linear range from 0.1 to 1.0 g l^{-1}. As it can be generally expected that sugar contents in fruit juices will be in the range 10–100 g l^{-1} [35], a dilution of 1:50 to 1:100 should allow a CE analysis.

To show that CE can be applied in routine analysis, three fruit juices, apple, orange and grape juice, were analysed with respect to their sugar compositions and contents. The applicability of the CZE method was demonstrated by its comparison with an HPLC method for carbohydrate determination, the HPAEC–PAD method. The HPLC analyses were carried out in the Kantonales Laboratorium, Basle, a government food control laboratory, within the scope of its daily routine work on fruit juices control.

Examples of CZE and HPAEC separations are shown in Figs. 3 and 4. Three main sugars, Suc, Glc and Fru, are expected to be present in fruit juices. The CZE separation of a standard mixture of these sugars under optimum conditions is shown in Fig. 3a. Fig. 3b shows the analysis of an apple juice, which was diluted 1:50 with water prior to injection. The analysis of the orange and grape juices yielded very similar electropherograms. Therefore, only the quantitative results are summarized in Table 5. It is important to note that in Fig. 3b no matrix peaks interfere with the sugar separation. The same is true for diluted orange and grape juices. For comparison, Fig. 4 displays a typical HPAEC trace from an orange juice analysis.

Most striking is the difference in the elution and migration order of the sugars in the two separation systems. From the basic principles of both methods, a separation according to the differences in pK_a of the sugars is expected. Therefore, the sugar molecules should be eluted in the order of their pK_a values, viz., Fru > Glu > Suc [27]. In CZE, the expected migration order is reversed owing to the strong electroosmotic flow (EOF) inside the capillary at pH 12.2. The EOF is generated at high pHs by the negatively charged silica surface of the inner capillary wall and causes a bulk flow inside the capillary towards the cathode. Because the EOF

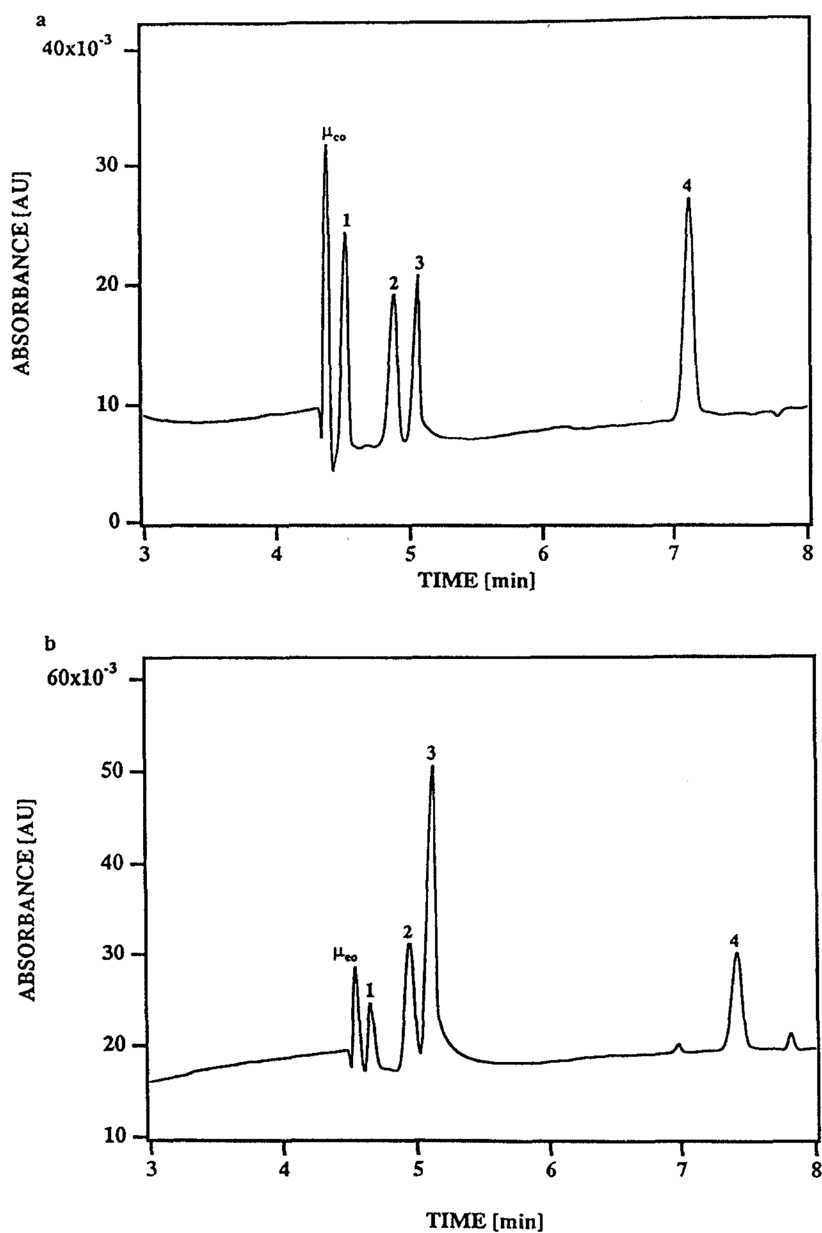

Fig. 3. Capillary zone electrophoresis of fruit juices: (a) standard mixture (sugars, 2.22–2.42 m*M*) and internal standard (GlcAc, 1.15 m*M*); (b) apple juice (diluted 1:50). Running conditions as in Fig. 1; 1-s injection. 1 = Sucrose; 2 = glucose; 3 = fructose; 4 = glucuronic acid.

is stronger than the electrophoretic mobility of the negatively charged sugars, the latter are also directed to the cathode. As a consequence, Suc, less dissociated at the separation pH, elutes first because it is less able to migrate against the direction of the EOF towards the anode, whereas the higher dissociation of Glc and Fru causes them to elute later.

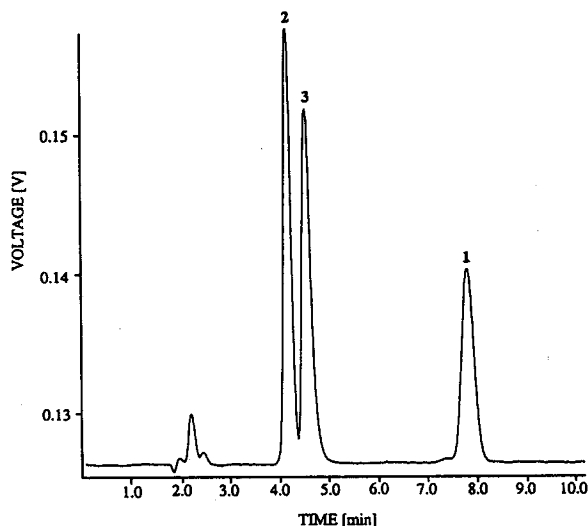

Fig. 4. HPAEC analysis of an orange juice (diluted 1:2000) on a Carbopac PA1 column with 200 m*M* NaOH as eluent at a flow-rate of 1 ml min^{-1}. Other separation conditions are described under 'Experimental'. Peak assignment as in Fig. 3.

The HPAEC separation is also based on the pK_a differences of the sugars, which influence the interaction of the analytes with the stationary phase, in this instance an anion–exchange material. This interaction depends on a dissociation equilibrium between the ionized and the non-ionized forms of the sugar molecules, as only the former is able to interact with the stationary phase. Therefore, an analyte with a lower pK_a is more strongly retained on the stationary phase than an analyte with a higher pK_a. Consequently, Glc is eluted before Fru, as shown in Fig. 4. However, Suc does not follow this separation principle. Although its pK_a is higher than that of Glc, it elutes later. The reason for that is a greater anion-exchange affinity of disaccharides compared with monosaccharides and therefore a higher retention [36].

Table 5 summarizes the results of the sugar determinations in apple, orange and grape juice. Each value represents the mean of at least three injections. The HPAEC data were evaluated only with external calibration. This proved to be satisfactory, as the injection volume of 50 μl with the appropriate injection loop was expected to be reproducible within less than 1% R.S.D. Owing to the strong dilution of at least 1:2000, no matrix effects were observed.

As no quantitative data on the determination of sugars in fruit juices with CE and indirect UV detection are available, the CZE method was validated with both external and internal calibration, with GlcA as the internal standard.

Usually, R.S.D.s in liquid chromatographic determinations are expected to be up to 3%. This holds true for the determination of sugars in apple and grape juice, although their absolute sugar contents differ by a factor of 3–4 (Table 5). An exception is the orange juice analysis, which shows R.S.D.s up to 6%. This could possibly be explained by the temperature sensitivity of the HPAEC system. In three consecutive runs, increasing peak areas for the individual sugars were observed. This could partly be a consequence of an increase in room temperature due to malfunctioning of the air conditioning system.

In CE, exact control of the injection volume, which is in the range 5–10 nl, is difficult to achieve. Nevertheless, typical R.S.D.s for both external and internal calibration are between 1% and 3%, except for the determination of Fru in orange juice and the determination of Suc in apple juice.

From the data in Table 5, it is obvious that all measured values were in the expected range. However, values obtained with CZE by external calibration differed by up to 15% from the corresponding HPAEC data. Internal calibration results in sugar concentrations much closer to those measured by HPAEC. The main reason for the larger deviations of the external CZE calibration results might be the poor reproducibility of the nanolitre injection volumes in CE. It is also possible that the silica surface of the inner capillary wall changed during a series of experiments, thus slightly changing the separation conditions from the first to the last injection.

The results of the CZE and HPAEC experiments were evaluated statistically for a correlation by the *t*-test [37]. The *t*-test is a means of proving that the mean values of two independent series of data originate from the same normal

Table 5
Sucrose, glucose and fructose contents in fruit juices

Sample	Sugar	Method	Calibration	Concentration found (g l^{-1})	R.S.D. (%)	Expected range[a] (g l^{-1})
Apple juice	Sucrose	CZE	External	16.6 ± 0.42	2.5	12.0–23.0
			Internal	17.1 ± 0.83	4.9	
		HPAEC	External	19.2 ± 0.50	2.6	
	Glucose	CZE	External	23.3 ± 0.50	2.2	17.0–30.0
			Internal	22.7 ± 0.74	3.3	
		HPAEC	External	24.3 ± 0.23	1.0	
	Fructose	CZE	External	56.5 ± 0.35	0.6	51.0–77.0
			Internal	62.8 ± 2.04	3.3	
		HPAEC	External	59.8 ± 1.29	2.2	
Orange juice	Sucrose	CZE	External	31.9 ± 1.03	3.2	27.0–48.0
			Internal	34.6 ± 0.70	2.0	
		HPAEC	External	36.6 ± 1.49	4.1	
	Glucose	CZE	External	21.5 ± 0.50	2.3	23.0–29.0
			Internal	22.0 ± 0.10	0.5	
		HPAEC	External	24.3 ± 1.42	5.8	
	Fructose	CZE	External	19.5 ± 1.10	5.6	27.0–48.0
			Internal	25.6 ± 0.90	3.5	
		HPAEC	External	26.6 ± 1.64	6.2	
Grape juice	Glucose	CZE	External	87.1 ± 0.76	0.9	81.0
			Internal	73.5 ± 1.01	1.4	
		HPAEC	External	74.3 ± 1.06	1.4	
	Fructose	CZE	External	86.5 ± 1.79	2.1	83.0
			Internal	81.2 ± 1.57	1.9	
		HPAEC	External	79.1 ± 2.93	3.7	

[a] Values from Ref. [35].

distribution with the same mean value. If this is the case, the difference in the mean values of the two series should not be significant. However, in order to apply the t-test to two sets of data, the standard deviations of both sets of data must be comparable, indicated by the F-value [37]. As can be seen from the t-values in Table 6, the differences between the HPAEC results and the results of the CZE analyses obtained by internal calibration are not significant for all data sets at the 99% confidence level. In contrast, the data for the external CZE calibration differed significantly in all except two instances from those obtained by HPAEC, indicating that external calibration of CZE did not yield the same values as HPAEC and is therefore an invalid method. This again confirms the previous statement, that the results from the internal CE calibration are much closer to the HPLC results than those from the external CE calibration. Therefore, the internal calibration method is to be preferred when working with CE.

In summary, good agreement between the sugar contents declared on the fruit juice packaging and the sugar contents determined by CZE and the HPAEC method was found (Table 7). On inspecting the CZE and HPAEC results closely, it is striking that only the data for the orange juice are lower than the declared values. This could be due to partial adsorption of soluble sugars on haze particles, such as polyphenols, polysaccharides and proteins, which are removed by filtration, resulting in an underestimation of the sugar content in this juice.

It is evident from these results that the described CZE method is suitable as a routine method for the determination of soluble carbohydrates in fruit juices.

Table 6
Statistical evaluation of the results obtained by CZE and HPAEC

Juice	Sugar	Method[a]	*F* value	*t* value	Difference CZE-HPAEC
Apple	Sucrose	CZE ext. cal.	1.42	7.88	Significant
		CZE int. cal.	2.76	4.34	Not significant
	Glucose	CZE ext. cal.	4.73	3.57	Not significant
		CZE int. cal.	3.22	4.10	Not significant
	Fructose	CZE ext. cal.	13.58	4.93	Significant
		CZE int. cal.	2.50	2.03	Not significant
Orange	Sucrose	CZE ext. cal.	2.09	5.16	Significant
		CZE int. cal.	4.53	2.44	Not significant
	Glucose	CZE ext. cal.	8.07	3.73	Not significant
		CZE int. cal.	201.60	3.42	Not significant
	Fructose	CZE ext. cal.	2.22	7.17	Significant
		CZE int. cal.	3.32	1.06	Not significant
Grape	Glucose	CZE ext. cal.	1.95	19.69	Significant
		CZE int. cal.	1.10	1.10	Not significant
	Fructose	CZE ext. cal.	2.68	4.30	Not significant
		CZE int. cal.	3.48	1.27	Not significant

Test conditions: for F ($P = 0.95$; $f_1 = f_2 = 2$) $\leq 19.00 \Rightarrow t(P = 0.99; f = 4) \leq 4.60$; for F ($P = 0.95$; $f_1 = f_2 = 2$) $> 19.00 \Rightarrow t(P = 0.99; f = 2) \leq 9.92$. P = confidence level; f = degrees of freedom.
[a] Ext. cal. = external calibration; int. cal. = internal calibration.

4. Conclusions

A simple, reproducible and inexpensive CZE method for the separation of soluble, low-molecular-mass carbohydrates with indirect UV detection was adapted to fruit juice analysis. The sensitivity proved to be sufficient to measure levels of sugars in common fruit juices. In principle, other foodstuffs with similar carbohydrate concentrations such as jellies, honey, candies or soft drinks could also be within the scope of this method. The CZE method was compared with the routine HPLC method, yielding similar results in terms of resolution, reproducibility and recovery. A statistical comparison (*t*-test) showed that there is no significant difference in the results obtained by the two independent methods, provided the internal calibration method was used for CZE.

Therefore, it can be concluded that CE with the additional advantages of a short analysis time, high separation efficiency and low running costs is attractive for routine work. Additionally, CE provides the possibility of validating HPLC results or results from any other carbohydrate analysis with a second independent analytical method.

Although CE is still a relatively young analytical technique, it certainly will continue to grow in the future and find more and more applications in the area of food science. Eventually, validated CE methods should provide fast, automated and high-resolution assays in food control laboratories.

Table 7
Total carbohydrate content

Juice	Concentration ($g\ l^{-1}$)		
	Declared	Found	
		CZE[a]	HPAEC
Apple	110.0	102.6	102.4
Orange	100.0	82.2	86.8
Grape	150.0	154.7	152.6

[a] Results from internal calibration.

References

[1] B.L. Karger, A.S. Cohen and A. Guttmann, *J. Chromatogr.*, 492 (1989) 585.
[2] H. Nishi and S. Terabe, *Electrophoresis*, 11 (1990) 691.
[3] Y. Xu, *Anal. Chem.*, 65 (1993) 425R.
[4] J. Vindevogel and P. Sandra, *J. High Resolut. Chromatogr.*, 14 (1991) 795.
[5] N. de Jong, S. Visser and C. Olieman, *J. Chromatogr. A*, 652 (1993) 207.
[6] M.T. Ackermans, J.L. Beckers, F.M. Everaerts, H. Hoogland and M.J.H. Tomassen, *J. Chromatogr.*, 596 (1992) 101.
[7] B. Lin Ling, W.R.G. Baeyens, P. van Acker and C. Dewaele, *J. Pharm. Biomed. Anal.*, 10 (1992) 717.
[8] B. Kenney, *J. Chromatogr.*, 546 (1991) 423.
[9] R. Macrae, *HPLC in Food Analysis*, Academic Press, London, 2nd ed., 1988.
[10] M.H. Gordon, *Principles and Applications of GC in Food Analysis*, Ellis Hoorwood, Chichester, 1990.
[11] I.B. Niederer, *Diss. ETH*, No. 10070, ETH, Zürich, 1993.
[12] H. Weigel, *Adv. Carbohydr. Chem.*, 18 (1963) 61.
[13] B. Bettler, R. Amadò and H. Neukom, *J. Chromatogr.*, 498 (1990) 213.
[14] H. Schäfer and H. Scherz, *Z. Lebensm.-Unters.-Forsch.*, 177 (1983) 193.
[15] S. Honda, *Anal. Biochem.*, 140 (1984) 1.
[16] *Technical Note 20*, Dionex, Sunnyvale, CA, 1989.
[17] C. Corradini, A. Cristalli and D. Corradini, *J. Liq. Chromatogr.*, 16 (1993) 3471.
[18] A.E. Vorndran, P.J. Oefner, H. Scherz and G.K. Bonn, *Chromatographia*, 33 (1992) 163.
[19] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, *Anal. Chem.*, 63 (1991) 1541.
[20] W. Nashabeh and Z. El Rassi, *J. Chromatogr.*, 514 (1990) 57.
[21] T.W. Garner and E.S. Yeung, *J. Chromatogr.*, 515 (1990) 639.
[22] S. Honda, S. Iwase, A. Makino and S. Fujiwara, *Anal. Biochem.*, 176 (1989) 72.
[23] S. Honda, S. Suzuki, A. Nose, K. Yammamoto and K. Kakehi, *Carbohydr. Res.*, 215 (1991) 193.
[24] C. Chiesa and Cs. Horváth, *J. Chromatogr.*, 645 (1993) 337.
[25] L.A. Colon, R. Dadoo and R.N. Zare, *Anal. Chem.*, 65 (1993) 476.
[26] B. Krattiger, G.J.M. Bruin and A.E. Bruno, *Anal. Chem.*, 66 (1994) 1.
[27] J.A. Rendleman, Jr., *Adv. Chem. Sci.*, 117 (1972) 51.
[28] J.H. Knox and I.H. Grant, *Chromatographia*, 24 (1987) 135.
[29] H.H. Lauer and J.B. Ooms, *Anal. Chim. Acta*, 250 (1991) 45.
[30] J.W. Jorgenson and K. Lukacs, *Science*, 222 (1983) 266.
[31] Cs. Horváth and W.R. Melander, in E. Heftmann (Editor), *Chromatography —Fundamentals and Applications of Chromatographic and Electrophoretic Methods, Part A: Fundamentals and Techniques* (*Journal of Chromatography Library*, Vol. 22A), Elsevier, Amsterdam, 1983, p. A44.
[32] M.W.F. Nielen, *J. Chromatogr.*, 588 (1991) 321.
[33] F. Foret, S. Fanali and L. Ossiani, *J. Chromatogr.*, 470 (1989) 299.
[34] A. Bergholdt, J. Overgaard, A. Colding and R.B. Frederikson, *J. Chromatogr.*, 644 (1993) 412.
[35] S.W. Souci, W. Fachmann and H. Kraut, *Die Zusammensetzung der Lebensmittel, Nährwerttabellen 1989/90*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 4th ed., 1989.
[36] R.D. Rocklin and C.A. Pohl, *J. Liq. Chromatogr.*, 6 (1983) 1577.
[37] K. Doerfel, *Statistik in der Analytischen Chemie*, Deutscher Verlag für Grundstoffindustrie, Leipzig, 5th ed., 1990.