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Determination of carbohydrates by high-performance capillary electrophoresis with indirect absorbance detection[1]

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Abstract

High-performance capillary electrophoresis (HPCE) methods with indirect absorbance detection (IAD) have been developed for the determination of carbohydrates, e.g., glucose, fructose, rhamnose, ribose, maltose, lactose, sucrose and gluconic acid. The suitability and performance of six background electrolytes (BGEs), i.e., 1-naphthylacetic acid (NAA), 2-naphthalenesulfonic acid, 1,3-dihydroxynaphthalene, phenylacetic acid, *p*-cresol and sorbic acid, for the IAD method were investigated. The effects of the concentration of the BGE, pH and temperature on the CE separation of these analytes were evaluated. NAA was found to be best suited as the carrier buffer and background absorbance provider for the detection at 222 nm. The optimal CE performance was found when employing 2 m*M* NAA, pH 12.2, at 25°C. In comparison with the previous method that used sorbate as the BGE, the present method utilizing NAA shows a 3–6 fold increase in the separation efficiency and a 2–5 fold improvement in the detection limit. The calculated number of theoretical plates is in the range of $1.0–3.0 \cdot 10^5$. The precision of the present method for most sugar analytes, measured by the coefficient of variation (C.V.), typically, is less than 1% for the migration time and better than 3% for the peak height and peak area ($n=6$). The detection limit is about 0.1 m*M* for all analytes, except for ribose for which it is about 0.2 m*M*. This new method is fast, accurate and can be readily applied to real biological samples for quantitative determination of selected carbohydrates.

Keywords: Carbohydrates

1. Introduction

Carbohydrates, the most abundant organic constituents of plants, are also broadly distributed in a wide variety of tissues including micro-organisms and animals. They are also one of the richest food sources. Consequently, carbohydrate analysis is important in biochemical, pharmaceutical, agricultural and food sciences. In spite of extensive research and development effort over the past few decades, several obstacles still remain to be overcome before analysis of carbohydrates could become routine. The difficulty in carbohydrate analysis can be attributed to at least two factors: (1) presence of many structural isomers and (2) lack of an easy detection method, e.g. UV absorbance or fluorescence.

Methods currently used for the separation of carbohydrates include thin-layer chromatography (TLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) [1–3]. The TLC method, which is simple and convenient, is adequate only for qualitative analysis, e.g., determination of the composition of carbohydrates. The drawbacks are many, including long development

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time, poor separation efficiency and poor precision for quantitative purposes. The GC method has good sensitivity and separation efficiency and is also a good quantitative tool, but many carbohydrates are not volatile or are heat-labile. Furthermore, carbohydrates quite often need to be derivatized for GC analysis and stereoisomers that are formed in the process complicate the identification of the original analytes. Presently, HPLC is one of the most widely used and important separation methods for the analysis of carbohydrates. Various kinds of bonded-phase and ion-exchange columns have been developed for the HPLC method [4]. In conjunction with a pulsed amperometric detector, the HPLC method is highly sensitive and selective [5]. A major drawback of HPLC is its lack of separation efficiency, particularly for separating higher molecular mass polysaccharides. In addition, HPLC often needs longer times for column equilibration and the cost of a specialized column is substantial.

A newly developed separation tool for carbohydrate analysis is high-performance capillary electrophoresis (CE). CE has the advantages of being simple, highly efficient and selective and it is fast. It has the potential to become an important analytical separation tool for carbohydrate determination. However, two major obstacles have hindered the development of CE as a major tool in carbohydrate analysis. Firstly, most carbohydrates, except sugar acids, have rather high ionization constants (pK_a values of 12 or greater); therefore, they do not carry electrical charges at neutral pH. Secondly, simple carbohydrates do not absorb UV light above 200 nm, which is the most frequent means of detection with commercial instruments. One way to overcome both problems is to derivatize the carbohydrate with a chromophore or a fluorescent probe that also carries electrical charges. A number of derivatizing agents have been employed for carbohydrate analysis, including 2-aminopyridine, *p*-aminobenzoic acid, ethyl *p*-aminobenzoic acid, 6-aminoquinoline, 4-aminobenzonitrile, 1-phenyl-3-methyl-5-pyrazolone, 3-(4-carboxylbenzoyl)-2-quinoline carboxaldehyde and various kind of aminonaphthalene sulfonic acids [6–15]. Several drawbacks are often encountered in the derivatization method. Firstly, the reactivity of derivatizing reagent for different analytes could be very different, making the process difficult to control. Secondly, more than one adduct could form for each analyte, resulting in a complicated separation problem. Thirdly, the derivatization is often time-consuming and labor intensive.

The use of borate, which forms a complex with the hydroxyls of carbohydrate, is a simple way to introduce electrical charges and the complex can be detected via absorbance at 195 nm [16,17]. However, this method suffers from somewhat poor sensitivity, i.e., its detection limit is only in the nanomolar range. The indirect absorbance detection (IAD) method offers an alternative means of detection without the need for derivatizing the carbohydrate. A suitable background electrolyte (BGE) is added and CZE is performed at pH>12 where the hydroxyl groups of carbohydrate are ionized. Sorbic acid has been employed as a BGE and a detection limit of about a pmol of carbohydrate has been reported [18,19].

In this research, we have studied the CE separation of glucose, fructose, rhamnose, ribose, maltose, lactose, sucrose and gluconic acid and have investigated the suitability of several BGEs for the IAD method. The performance of six BGEs, including 1-naphthylacetic acid (NAA), 2-naphthalenesulfonic acid, 1,3-dihydroxynaphthalene, phenylacetic acid, *p*-cresol and sorbic acid was evaluated and compared. The effects of the concentration of BGE, pH and temperature on the CE separation of the analytes were also studied.

2. Experimental

2.1. Chemicals

D-Glucose, D-galactose, D-mannose, D-fructose, D-arabinose, D-ribose, D-xylose, D-lyxose, D-melibiose, D-cellobiose, D-turanose, L-rhamnose, L-sorbose, lactulose, lactose, maltose, sucrose, D-raffinose, glucuronic and D-gluconic acids, of the highest purity available (most have a purity of greater than 99%, except for some disaccharides), were purchased from Sigma (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA) and Janssen (Geel, Belgium). 1-Naphthylacetic acid (NAA), 2-naphthalene-sulfonic acid (NSA), 1,3-dihydroxynaphthalene (DHN), dimethylsulfoxide (DMSO), phenylacetic acid (PAA),

p-cresol and sorbic acid, of analytical or reagent grade, were obtained from Sigma, Janssen and Riedel-de-Haen (Seelez, Germany). Various brands of beverage and food items were purchased from local market stores. Doubly deionized water prepared from a Milli-Q system (Millipore Corp, Bedford, USA) or doubly deionized–distilled water was used exclusively for all solutions.

2.2. Samples, buffers and pH adjustment

Various sugars and gluconic acid were prepared either as 0.01 *M* or 0.1 *M* stock solution and kept at 4°C. These solutions were mixed in proportions, diluted and used as the standard solutions when required. Real samples of beverages and drinks were diluted 50- to 100-fold with deionized water. Serum samples were diluted 10-fold with water. After dilution, most samples could be directly injected for CE analysis except for serum and dairy samples which must be filtered through 0.2 μm membranes. BGE buffer solutions were prepared as 2–10 m*M* stock solutions and their pH values were adjusted with 1 *M* or 0.1 *M* NaOH to 11.8–12.5 Additives were added directly to the buffer solution to the desired concentration and the pH was adjusted afterwards. The pH of the buffer was checked periodically and readjusted when necessary. All buffer solutions were filtered through 0.2-μm membranes and degassed under vacuum for 10 min.

2.3. Electrophoretic procedures

CE experiments were carried out in a fully automated Spectra Phoresis Model 1000 instrument (Thermo Separation Products, Fremont, CA, USA), as described previously [20]. In most experiments, except in the electrophoretic mobility determination of BGEs, the detector wavelength was fixed at the optimal wavelength depending on the BGE used, as specified in Table 1. In the IAD method, peaks in the electropherogram appear originally as negative peaks but are inverted to positive peaks by using the vendor's software. The separation capillaries (bare fused-silica) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Their length and diameter varied as specified in the figure legends.

Procedures for the pretreatment of new capillaries and for pre-run washes were similar to those reported previously [20]. Post-wash treatment after each real sample run was carried out using 1 *M* NaOH for 3 min, deionized water for 3 min and then the running buffer for 5 min. This special precaution was taken to ensure that the complex matrices of real samples would not affect the intricate electrical behavior of the capillary wall. After the background signal of running buffer solution became stable, sample injection was effected in the hydrodynamic (HD) mode. The separation run was carried out at +25 kV constant voltage, at various temperatures (15–60°C depending on the experiment) and with a current of about 10–20 μA. Between runs, the capillary was post-washed with deionized water for 5 min. Peak identification for each analyte was carried out by spiking with the known standard, and the peak with increased height was identified.

Table 1
Molar absorptivities, pK_a values and electrophoretic mobilities of various BGEs

BGE	Molar absorptivity		pK_a [a]	Mobility (10^{-5} cm^2 V^{-1} s^{-1})
	λ_{max}	ϵ_{max}		
NAA	222	81 100	4.24	−26.3
p-Cresol	236	8 320	10.26	−28.5
DHN	256	14 680	9.37,10.93	−29.3
Sorbic acid	254	24 120	4.77	−29.6
PAA	209	7 600	4.31	−30.0
NSA	225	86 000	2.60	−30.2

NAA: 1-naphthylacetic acid. DHN: 1,3-dihydroxynaphthalene. PAA: phenylacetic acid. NSA: 2-naphthalene sulfonic acid.
[a] From Refs. [29,30].

2.4. Electrophoretic mobility determination

The mobilities of various BGEs under the specified CE conditions were determined in a buffer containing 20 m*M* sodium phosphate, at pH 12.1. The standard solution of mixtures of all BGEs (0.1 m*M* each) in deionized water, containing 0.05% DMSO (as the neutral marker), was injected in the HD mode for 1 s. The CE voltage applied was +20 kV. Detection was effected by rapid scanning of absorbance from 200 to 350 nm, which allowed a positive identification of the background absorbance

provider. Electroosmotic mobility, μ_{eo}, and the electrophoretic mobility of the BGE, μ_e, were calculated from the observed mobility, μ_{obs}, using the equations described previously [21]. UV–VIS absorption spectra, wavelength maxima and molar absorptivities for the various BGEs were determined by using a Model U-2000, double-beam scanning spectrophotometer (Hitachi, Tokyo, Japan).

3. Results and discussion

3.1. Selection of a suitable BGE

As discussed in our previous paper [22] and in the literature [23], several factors should be considered when selecting a BGE suitable for CE. The mobility of the BGE should match closely with those of the majority of the analytes, which would result in a better separation and resolution. The molar absorptivity of the BGE should be high to provide better sensitivity. Furthermore, for carbohydrates to carry charges, CE must be performed at high pH and the BGE must be stable at such a pH. Therefore, NAA, DHN, NSA, PAA, *p*-cresol and sorbic acid were first investigated for their suitability as the BGE for CE analysis of carbohydrates. The molar absorptivities, pK_a values and electrophoretic mobilities for these background absorbance providers are listed in Table 1. Among these six compounds, *p*-cresol and DHN are very weak acids, having a $pK_a > 9$, while the other four acids have pK_a values of around 2.6–4.8. The mobilities (expressed as negative values for the anion) of these compounds are in the order of NAA<*p*-cresol<sorbate<NSA. NAA and NSA have considerably higher molar absorptivities than the other four chromophores. The pK_a values for the hydroxyl group of sugars are typically about 12 or greater, therefore, in order to ionize the analytes, CE must be performed at pH>12. The electropherogram of five BGEs (0.1 m*M* each) and of the neutral marker, DMSO (0.05%), in 20 m*M* phosphate buffer, pH 12.1, are shown in Fig. 1. Since the molar absorptivity of NAA is three times higher than that of sorbic acid, which was used in the previous study [19], it resulted in a better sensitivity as shown in Fig. 1 and therefore, NAA was chosen for further evaluation.

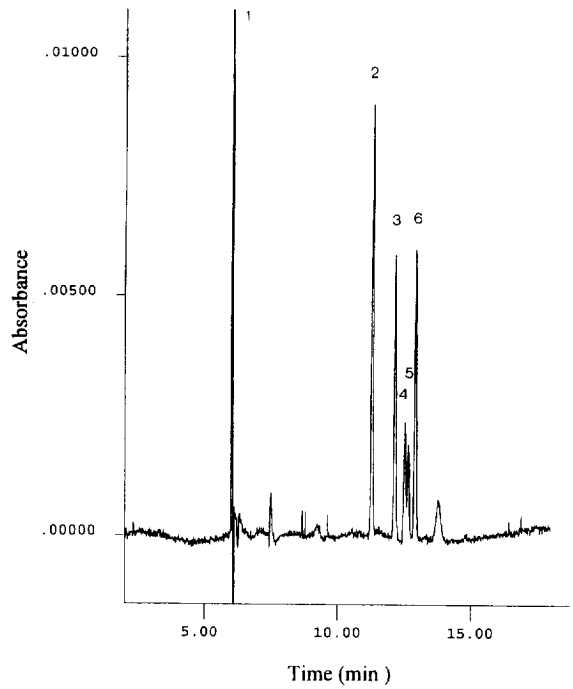

Fig. 1. Electropherogram of a mixture of five BGEs (0.1 m*M* each) and DMSO (0.05%, as the neutral marker) in 20 m*M* phosphate buffer, pH 12.1. CE conditions: capillary, 70 cm (63 cm to the detector) × 75 μm I.D.; voltage, +20 kV; temperature, 25°C; rapid scanning detection, from 200 to 350 nm; injection, 1 s HD. Peaks: 1 = DMSO; 2 = NAA; 3 = *p*-cresol; 4 = DHN; 5 = sorbate; 6 = NSA.

3.2. CE separation of the selected carbohydrates

The electropherogram of mixtures of mono- and disaccharides, and of gluconic acid (1 m*M* each) in a 2 m*M* NAA, pH 12.2 solution is displayed in Fig. 2. The migration times for these analytes increased in the following order: sucrose<lactose<maltose<glucose<rhamnose<fructose<ribose<gluconic acid. The separation of these analytes was based primarily on their degrees of ionization (i.e., difference in pK_a) and secondly on differences in their charge/mass ratios. The molecular masses, pK_a values and mobilities of the eight analytes are compared with various other carbohydrates in Table 2. The disaccharides have higher molecular masses and thus move slower towards the anode (opposite to the detector side). Glucose and fructose have the same mass which is greater than that of rhamnose; however, fructose has a higher mobility, probably be-

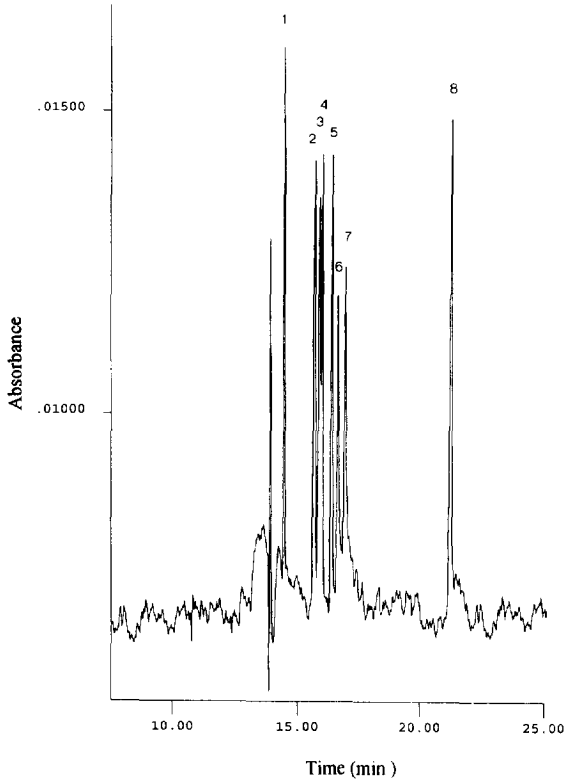

Fig. 2. Electropherogram of a mixture of eight selected monosaccharides (1 m*M* each) in 2 m*M* NAA, pH 12.2. CE conditions: capillary, 120 cm (113 cm to the detector) × 50 μm I.D.; voltage, +25 kV; temperature, 25°C; detection, IAD at 222 nm; injection, HD, 3 s; Peaks: 1 = sucrose; 2 = lactose; 3 = maltose; 4 = glucose; 5 = rhamnose; 6 = fructose; 7 = ribose; 8 = gluconic acid.

cause it has a lower pK_a. Ribose has a lower mass than the other three monosaccharides, thus it has higher mobility. Gluconic acid is fully ionized and thus has the highest mobility (longest migration time). Since CE was performed with a positive voltage applied and at a high pH, the electroosmotic flow (EOF) is considerably larger than the electrophoretic mobilities of the anions, the species that has the highest mobility (gluconic acid) would migrate last past the detector. Thus the observed migration order of these eight analytes agrees well with the expected order. Also, it is clear from Table 2 that many carbohydrates (e.g., lyxose, xylose, sorbose and mannose) have very close mobilities (determined individually) and the differences in their pK_a values are too small for CE separations of these sugars to be practical, under the present conditions. However, it is interesting to note that the CE separation between the two disaccharides, lactose and maltose, was better than that between glucose and maltose. Apparently, this could be attributed to maltose having a lower pK_a value (thus having a higher degree of ionization).

Table 2
First ionization constants, molecular masses and mean electrophoretic mobilities of various carbohydrates

Compound	pK_a [a]	M_r	Mobility [b]
Raffinose	12.74	504.5	−2.36
Sucrose	12.51	342.3	−2.41
D-Galactose	12.35	180.2	−6.86
Lactose	11.98	342.3	−6.94
Melibiose	NA[c]	342.3	−7.12
Cellobiose	NA	324.3	−7.76
D-Arabinose	12.43	150.1	−7.85
Maltose	11.94	342.3	−7.90
D-Glucose	12.35	180.2	−8.04
Lactulose	NA	342.3	−8.16
L-Rhamnose	NA	164.2	−9.37
Turanose	NA	342.3	−9.52
D-Lyxose	12.11	150.1	−10.03
D-Xylose	12.29	150.1	−10.06
D-Fructose	12.03	180.2	−10.21
D-Mannose	12.08	180.2	−10.25
L-Sorbose	11.5	180.2	−10.42
D-Ribose	12.21	150.1	−10.65
D-Gluconic acid	3.86	196.2	−22.80
D-Glucuronic acid	NA	194.1	−25.20

[a] From [31].
[b] 10^{-5} cm^2 V^{-1} s^{-1} CE condition: buffer, 2 m*M* NAA, pH 12.2; separation voltage, 25 kV; temperature, 25°C; capillary, 120 cm (113 cm to the detector) × 50 μm I.D.
[c] Not available.

The mobilities of these carbohydrates (except the sugar acids) are in the range from −2.36 to -10.65×10^{-5} cm^2 V^{-1} s^{-1} (see Table 2), whereas the mobilities for NAA and sorbate are −26.3 and -29.6×10^{-5} cm^2 V^{-1} s^{-1} (see Table 1), respectively, at the same pH. Although none of these BGEs have mobilites that match those of the analytes, NAA has a closer match than sorbate as the BGE for the CE analysis of carbohydrates. The separation efficiency, as measured by the number of theoretical plates (N) is higher in NAA (100 000–300 000), which is about three to six times higher than the corresponding values when employing sorbate as the BGE [19].

In comparison to the HPLC method [24], the CE–IAD method, using NAA as the BGE, has N values that are about 50–100 higher. CE separations between the galactose–lactose pair and between the glucose–maltose pair were very difficult because the electrophoretic mobilities of the two analytes in each pair were very close. In contrast, separation of the analytes in each pair using the HPLC method yielded a more satisfactory result. However, the separation in the galactose–mannose pair which was difficult in the HPLC method could be easily resolved in the CE method. Thus, CE and HPLC methods should be used in a complementary manner for the analysis of carbohydrates.

3.3. Effect of pH on BGEs

Because the pH of the running BGE solution was fairly high, the concentration of the hydroxyl ion in the solution could affect the transfer ratio, TR_{tot}. TR_{tot} is a measure of the efficiency of the non-absorbing analyte to replace the co-migrating BGE, as the former migrates past the detector. The higher the TR_{tot}, the larger the absorbance signal (negative peak). In the present case, TR_{tot} can be expressed by the following equation:

$$TR_{tot} = \alpha[\text{sugar}]/\{[C] + [OH^-]\} \quad (1)$$

where α[sugar], [C], and [OH^-] are concentrations of the ionized sugar, of the BGE and of hydroxyl ion, respectively. At given concentrations of analyte and BGE, the α value and [OH^-] are two competing functions of pH. Thus, when plotting TR_{tot} as a function of pH, TR_{tot} will reach a maximum value then decline, as will the sensitivity of the IAD method.

The effects of pH on the electroosmotic flow and on the electrical current of the BGE solution are shown in Fig. 3. As the pH was raised from 11.8 to 12.4, the current increased while the EOF decreased. This resulted in increased analysis time. The decrease in the EOF was due mainly to the increase in the ionic strength of the BGE which reduced the thickness of the electric double layer, thus the zeta potential [25]. For example, in the case of ribose, changing the pH from 11.8 to 12.4 resulted in an increase in the migration time from 14.5 to 18.0 min

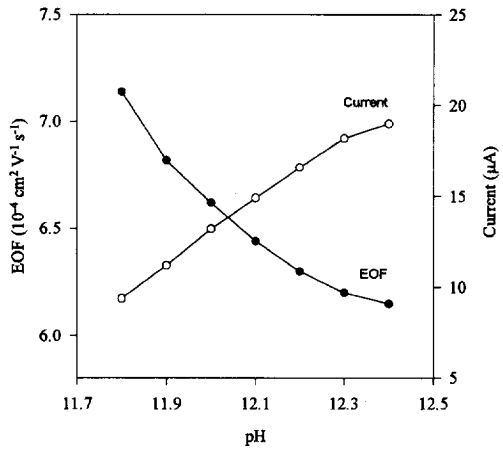

Fig. 3. Effects of pH on the EOF and electric current of the CE buffer. Other conditions as in Fig. 2.

(data not shown). In addition, as the pH became higher, the increase of electric current, presumably, also caused higher background noise. The overall effect was a reduction of detection sensitivity.

Fig. 4. shows the effect of pHs from 11.8 to 12.4 on the electrophoretic mobilities of eight selected sugars. With the exception of gluconic acid, the mobilities of these sugars generally increased (became more negative) as the pH was increased, because the analytes were more ionized at higher pH. However, above pH>12.3, the pH effect on the

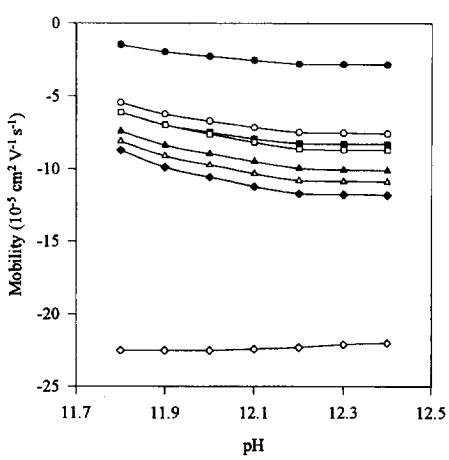

Fig. 4. Effects of pH on the electrophoretic mobilities of eight selected sugars. Other conditions are the same as in Fig. 2. The symbols from top to bottom: ● = sucrose; ○ = lactose; ■ = maltose; □ = glucose; ▲ = rhamnose; △ = fructose; ◇ = ribose; ◇ = gluconic acid.

mobility began to level off; presumably, the zeta potential began to decline which negates the ionization effect. It is interesting to note that at pH<12.0, maltose (curve ■) and glucose (curve □) are almost indistinguishable, because the pK_a of these two analytes are very close (11.94 and 12.35, respectively. See Table 2). As the pH was raised, the difference in electrical charges carried by the two analytes also increased; thus, at pH>12.2 a small improvement in the resolution of these two compounds was observed.

3.4. Influence of temperature

For separating small carbohydrates (i.e. low molecular masses), the running temperature of CE also plays a determinant role. Increasing the temperature would decrease the viscosity of the running BGE solution, thus increasing the electrophoretic mobility. It has been estimated that for every 1°C rise in temperature, the mobility increases by about 2% [26]. However, in the case of sugars, an opposite effect was observed, i.e., increasing the temperature actually lowered their mobility (less negative). This anomaly could be attributed to the temperature effect on the ionization constant of water, K_w. At lower temperatures, K_w is lower and therefore the pH is higher. The higher pH causes carbohydrates to be ionized more, resulting in higher mobility. As can be seen in Fig. 5, increasing the temperature from 15 to 40°C caused both the EOF and the current to increase almost linearly. This phenomenon could be attributed to the reduction of the viscosity of the running buffer. As a result, the EOF is increased and the analysis time is shortened. For example, the migration time for ribose decreased from 21.4 min at 15°C to 9.5 min at 50°C (data not shown), a considerable time saving. The effects of temperature on the electrophoretic mobilities of seven sugars and gluconic acid are depicted in Fig. 6a and b, respectively.

The dependence of electrophoretic mobility on temperature varied substantially among these analytes. While all seven sugars have positive slopes on the temperature-dependent curves, gluconic acid has a negative slope. At pH 12.0, gluconic acid is completely ionized, increasing the temperature lowers the viscosity of the solution and thus increases its

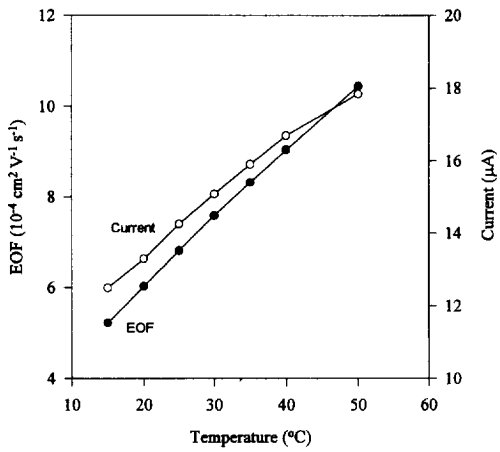

Fig. 5. Effects of temperature on the electroosmotic mobility and electric current of the CE buffer. Other conditions are the same as in Fig. 2.

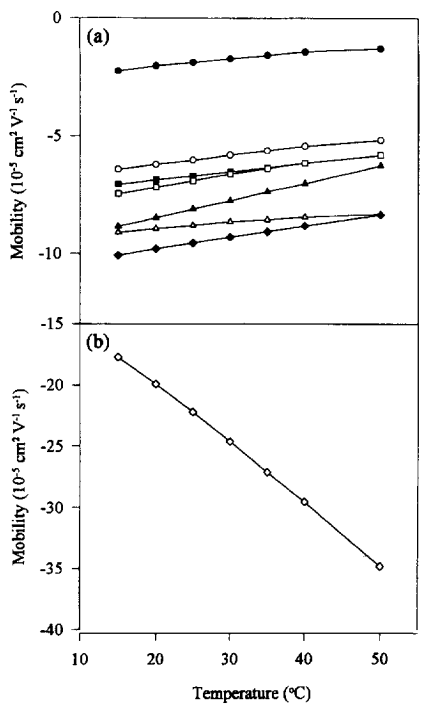

Fig. 6. Effects of temperature on the electrophoretic mobility of (a) selected sugars and (b) gluconic acid. The symbols from top to bottom are: ● = sucrose; ○ = lactose; ■ = maltose; □ = glucose; ▲ = rhamnose; △ = fructose; ◇ = ribose; ◇ = gluconic acid. Other conditions are the same as in Fig. 2.

mobility (more negative) as expected. Ribose (curve ▲) and rhamnose (curve ◇) have steeper slopes than the other five sugars, whose slopes are quite flat. The influence of temperature on their mobilities is largely dependent on the pH, which greatly affects the degree of ionization of the analyte. Knowing the water ionization product constants at various temperatures, it could be estimated that lowering the temperature from 25°C to 15°C resulted in pH changes from 12.5 to 12.2. Conversely, lowering the temperature increases the pK_a of the analyte, which decreases its ionization. Therefore, the effects of temperature on the pH and pK_a counteract each other's influence on the mobilities of the sugars. The overall influence on the mobility of analyte was dominated by the effect of the pH change. From Fig. 6, it is obvious that in order to obtain good CE resolutions for these seven sugars, the choice of the optimal running temperature is important. The optimal temperature for these eight sugars to be well resolved is in a rather narrow range, about 20 ± 2°C.

3.5. Precision, linearity, and detection limit

The precision data for the various analytes are summarized in Table 3. The precision for the eight analytes is excellent for the migration time (C.V.< 0.9%) and fairly good for the peak area or height (C.V. = 1.9–3.3%). The above data are for the within-day precisions, however, for the day-to-day run, the reproducibility suffered considerably. This was due mainly to the high pH employed for the CE run. Firstly, the surface electrical property of the capillary wall was not invariant; even a small change in pH could affect the EOF and therefore the mobilities of analytes. Secondly, at pH>12, the BGE (NAA in this case) has no buffer capability; over a time period, continuing dissolution of carbon dioxide (from the air) could affect the pH of the BGE solution. Therefore, it is imperative to wash the capillary after each run and to periodically readjust the pH of the BGE solution. To determine the four commonly found sugars, i.e., glucose, fructose, sucrose and lactose in foodstuffs, the calibration curves (Fig. 7) for these analytes were established. Over a concentration range of 0.3–10 m*M*, all analytes exhibit good linearity, as judged by the correlation coefficient, r^2, which was better than 0.999 (Table 3). The detection limits (at a signal-to-noise ratio>3) for these analytes were also determined. They were about 0.1 m*M* for most analytes, except for gluconic acid (0.01 m*M*) and for ribose (0.2 m*M*). The CE–IAD method is about 100 times less sensitive in comparison to the HPLC method employing pulsed amperometric detection [27]. The present method for the carbohydrate analysis is also about 10–100 times less sensitive compared to the comparable CE–IAD methods for other analytes, e.g. metal cations or organic acids [21,28]. The poorer detection limit observed here is due partly to the less

Table 3
Precision and linearity

Sugars	Precision (C.V., %)[a]			Linearity[b]		
	MT[c]	Area	Height	Slope $\times 10^{-4}$	Intercept $\times 10^{-4}$	r^2
Sucrose	0.35	2.4	2.7	5.3	−1.2	0.9996
Lactose	0.32	1.9	2.1	6.6	−7.2	0.9996
Maltose	0.33	2.3	2.2	6.0	−1.1	0.9995
Glucose	0.30	3.1	2.2	5.3	−2.9	0.9996
Rhamnose	0.36	2.4	2.4	5.0	−6.8	0.9994
Fructose	0.26	3.1	2.7	4.2	−7.6	0.9996
Ribose	0.23	3.1	2.9	4.1	−1.5	0.9991
Gluconic	0.85	2.6	3.3	13.8	−1.1	0.9998

[a]CE conditions are the same as in Fig. 5.2. C.V. = coefficient of variation (n = 6).
[b]Concentration range: 0.3–10 m*M* except for gluconic acid (0.1–0.5 m*M*).
[c]Migration time.

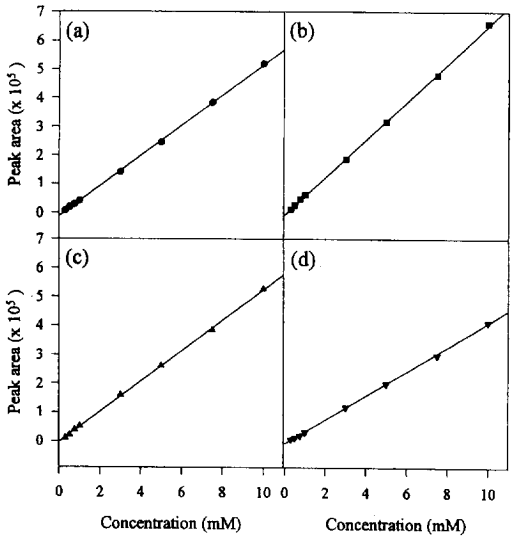

Fig. 7. Linearity of the standard calibration curves for (a) sucrose, (b) lactose, (c) glucose and (d) fructose. Concentration range, 0.3–10 m*M*. CE conditions as in Fig. 2.

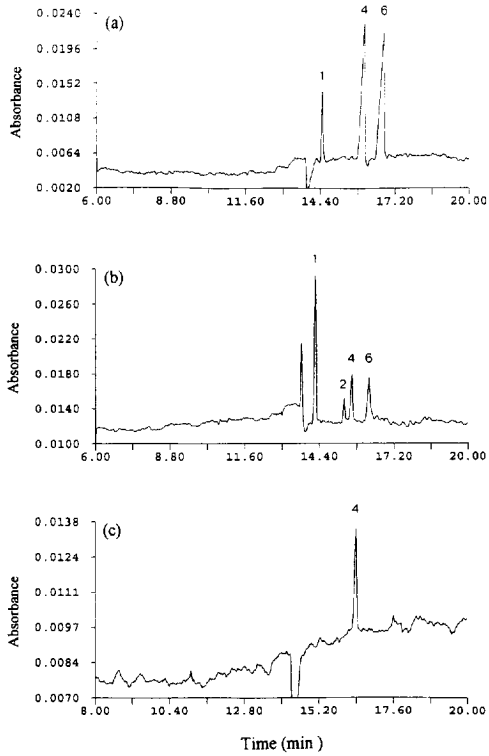

Fig. 8. Electropherograms of some sugars present in the representative real samples: (a) fruit juice, (b) dairy product (items 14 and 20 in Table 4) and (c) a typical normal serum. CE conditions as in Fig. 2 except in (c) which used a 92 cm capillary and had a HD injection of 5 s.

efficient transfer ratio at high pH and partly to the rather large mobility differences between the BGE and the analytes.

3.6. Analysis of real samples

To demonstrate the use of the present CE method for practical applications, the contents of carbohydrates in various samples were analyzed. These samples fall into five categories: (1) sport-drinks, (2) nutrient-added drinks, (3) natural or artificial flavor fruit juices, (4) dairy products and (5) serum samples. Typical electropherograms for (a) fruit juice, (b) dairy product and (c) serum sample are shown in Fig. 8. The composition of sucrose, glucose, fructose and lactose in 22 real food samples was analyzed and their concentrations were determined using the calibration curves shown in Fig. 7. The composition of selected sugars in representative real samples is summarized in Table 4. Since all these samples had fairly high concentrations of sugars (10–100 g/l), 50–100-fold dilutions were required usually in order to bring the concentrations of analytes in the diluted sample into the linear range of the calibration curves.

Although the concentration of each type of sugar varies widely from sample to sample, with only a few exceptions, the total sugar content in each sample was more or less the same. In sport-drinks (items 1–4) and natural fruit juices (items 11–14), sucrose, glucose and fructose were the major ingredients. In artificially flavored juices (items 15–18) and in some nutrient-added drinks (items 5, 6 and 8) only the monosaccharides, glucose and fructose, were present. In milk samples, only lactose was found. A random selection of ten hospital serum samples was also analyzed. Glucose was the only carbohydrate found in these samples and was determined by the CE method. Nine samples appeared to have serum glucose in the normal, or close to normal, range (80 to 120 mg/dl). Only one sample gave a high glucose

Table 4
Compositions of selected sugars in representative real samples

Item[a]	Sucrose	Glucose	Fructose	Lactose	Total
1	56	19	21		96
2	20	31	43		94
3	13	33	49		96
4	39	24	35		98
5		78	40		119
6		87	103		190
7	16	61	87		164
8		54	75		129
9		39	82	11	133
10		42	57	15	114
11	20	57	67		144
12	15	29	68		112
13	20	61	60		141
14	32	51	71		154
15		47	84		131
16		44	64		108
17		37	52		89
18		37	47		84
19				113	113
20	166	30	53	37	270
21	68			36	104
22	199	3		36	238

[a]Items 1–4, sport-drinks; items 5–10, nutrient-added drinks; items 11–18, natural or artificially flavored fruit juices; item 19–22, fresh milk or dairy products.

value of 240 mg/dl, presumably, from a diabetic patient.

4. Conclusion

NAA was found to be best suited as the carrier buffer and background absorbance provider for detection at 222 nm. The optimal CE performance was found when employing 2 m*M* NAA, pH 12.2, at 25°C. In comparison with the previous method that used sorbate as the BGE, the present method (using NAA) shows a three to six fold increase in separation efficiency and a two to five fold improvement in the detection limit. The calculated number of theoretical plates is in the range $1.0-3.0\times10^5$. The C.V. of the present method for most sugar analytes is typically less than 1% for the migration time and better than 3% for the peak height and peak area ($n = 6$). The detection limit is about 0.1 m*M* for all analytes, except for ribose for which it is about 0.2 m*M*. The present method could be readily applied to a wide variety of biological samples. Although in this work, only samples from foodstuffs and serum from patients have been analysed, it is conceivable that the method developed here could also be applied to other types of biological samples.

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References

[1] S.C. Churms, J. Chromatogr., 500 (1990) 555.
[2] R. Macrae, HPLC in Food Analysis, Academic Press, London, 2nd ed., 1988.
[3] M.H. Gordon, Principle and Application of GC in Food Analysis, Ellis Hoorwood, Chichester, 1990.
[4] S. Honda, Anal. Biochem., 140 (1984) 1.
[5] C. Corradini, A. Cristalli and D. Corradini, J. Liq. Chromatogr., 16 (1993) 3471.

[6] S. Honda, S. Iwase, A. Makino and S. Fujiwara, Anal. Biochem., 176 (1989) 72.
[7] P.J. Oefner, A.E. Vorndran, E. Grill, C. Huber and G.K. Bonn, Chromatographia, 34 (1992) 109.
[8] E. Grill, C. Huber, P.J. Oefner, A.E. Vorndran and G.K. Bonn, Electrophoresis, 14 (1993) 1004.
[9] W. Nashabeh and Z. El Rassi, J. Chromatogr., 600 (1992) 279.
[10] T. Saitoh, H. Hoshino and T. Yotsuyanagi, J. Chromatogr., 469 (1989) 175.
[11] S. Honda, K. Yamamoto, S. Suzuki, M. Ueda and K. Kakehi, J. Chromatogr., 588 (1991) 327.
[12] J. Liu, O. Shirota and M. Novotny, Anal. Chem., 64 (1992) 973.
[13] Y. Mechref and Z. El Rassi, Electrophoresis, 15 (1994) 627.
[14] C. Chiesa and R.A. O'Neill, Electrophoresis, 15 (1994) 1132.
[15] A. Guttman, N. Cooke and C.M. Starr, Electrophoresis, 15 (1994) 1518.
[16] S. Hoffstetter-Kuhn, A. Paulus, E. Gassmann and H.M. Widmer, Anal. Chem., 63 (1991) 1541.
[17] A. Marie, S. Michaelsen and H. Sorensen, J. Chromatogr. A, 652 (1993) 517.
[18] A.E. Vorndran, P.L. Oefner, H. Scherz and G.K. Bonn, Chromatographia, 33 (1992) 163.
[19] A. Klockow, A. Paulus, V. Figueiredo, R. Amado and H.M. Widmer, J. Chromatogr. A, 680 (1994) 187.
[20] T.-I. Lin, Y.-H. Lee and Y.-C. Chen, J. Chromatogr. A, 654 (1993) 167.
[21] Y.-H. Lee and T.-I. Lin, J. Chromatogr. A, 675 (1994) 227.
[22] Y.-H. Lee and T.-I. Lin, J. Chromatogr. A, 680 (1994) 287.
[23] W. Buchberger, S.M. Cousins and P.R. Haddad, Trends Anal. Chem., 13 (1994) 313.
[24] R. Pecina, G. Bonn, E. Burtscher and O. Bobleter, J. Chromatogr., 287 (1984) 245.
[25] K. Salomon, D.S. Burgi and J.C. Helmer, J. Chromatogr., 559 (1991) 69.
[26] H.H. Lauer and J.B. Ooms, Anal. Chim. Acta, 250 (1991) 45.
[27] D.A. Martens and W.T. Frankenberger, Jr., Chromatographia, 29 (1990) 7.
[28] C.H. Wu, Y.S. Lo, Y.-H. Lee and T.-I. Lin, J. Chromatogr. A, 716 (1995) 291.
[29] D.R. Lide and H.P.R. Frederikse (Editors), Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 75th ed., pp. 1994–1995.
[30] J.A. Dean (Editor), Lange's Handbook of Chemistry, McGraw-Hill, New York, 13th ed., 1985.
[31] R.F. Gould (Editor) Carbohydrate in Solution. American Chemical Society, Washington, DC, 1973.