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Indirect detection of saccharides in reversed-phase liquid chromatography with highly alkaline mobile phases

Bing Lu, Morgan Stefansson[1], Douglas Westerlund*

Analytical Pharmaceutical Chemistry, Uppsala University Biomedical Centre, P.O. Box 574, S-751 23 Uppsala, Sweden

Abstract

An indirect UV-detection method in the separation of saccharides by reversed-phase LC was developed. Porous graphitic carbon was used as the solid phase and sorbic acid, muconic acid and furanacrylic acid were tested as UV-absorbing markers included in the alkaline sodium hydroxide–methanol mobile phase. The indirect response of the saccharides was in general found to follow earlier developed theories. The most important parameters for optimizing the response were the retention of the solutes relative to the marker, which should be close to 1, and a high loading of the marker on the solid phase. The detection sensitivity can be improved by optimizing the marker and organic solvent concentrations in the mobile phase, the pH as well as the temperature.

The stability of the saccharides was found to be adequate for the alkaline conditions used in the separation systems.

1. Introduction

Research activities on carbohydrates in the biosciences have increased considerably during recent years; especially regarding their role as components in the cell walls and membranes for molecular recognition. It is expected that many future drugs, i.e. antibiotics and diagnostics, will exert their effect by influencing the carbohydrate chemistry in the living cell. In this context it is essential to develop new analytical techniques for carbohydrate analysis regarding content, stability and purity, as well as for studies on biological materials. Gas chromatography is a well established technique in the analysis of carbohydrates, and provides in combination with mass spectrometric detection a reliable analytical technique. A drawback is, however, the necessity to produce derivatives of the analytes in order to improve their chromatographic and detection properties.

Detection is also one of the major obstacles in liquid chromatographic analysis of saccharides. As saccharides do not exhibit absorbance at wavelengths above 200 nm, direct UV detection is not applicable due to low sensitivity and high background interference. Alternatively, refractive index has been used for determination of saccharides but does not allow determination of sugars at trace levels and the use of gradient elution. Another type of detection, pulsed amperometric detection, provides sensitive detection for saccharides [1,2], but the electrode may be fouled by additives in the mobile phase.

* Corresponding author.

[1] Present address: Department of Analytical Chemistry, Institute of Chemistry, Uppsala University, P.O. Box 531, S-751 21 Uppsala, Sweden.

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However, pre- or postcolumn derivatization followed by use of photometric or fluorimetric detection are methods frequently used today and can measure saccharides at the 10 pmol range [3–5]. The time consumption and technical complications, particularly in analysis of non-reducing saccharides that require an initial hydrolysis step, are the drawbacks of such techniques.

An indirect UV-detection method, which is based on the use of a mobile phase marker with detector response, was first applied to determine sugars by Gnanasambandan and Freiser [6]. Xylose, ribose and fructose were detected by using a mobile phase containing an UV-absorbing counter ion, methylene blue. Herné et al. [7] also detected sorbose, fructose and sorbitol by adding arbutin into the mobile phase, but it seemed that sensitivity and selectivity need to be improved.

This paper describes a simple and sensitive method for analysis of saccharides in HPLC through the use of indirect UV detection with sorbic acid as the absorbing ion.

2. Theory

The principles of indirect detection of ionic compounds in reversed-phase systems have been derived earlier [8–10]: a detectable component is included in the aqueous mobile phase and is assumed to distribute to the solid phase. When a solution deviating in composition from the mobile phase is injected the established equilibria in the column are disturbed and the analytes will travel through the column in zones together with mobile phase components in concentrations differing from those of the bulk mobile phase. All mobile phase components that are interacting with an injected analyte will also create migrating zones, so-called system peaks (also called eigenpeaks). It is assumed that the most common interaction between the components is competition for the adsorption sites on the solid phase. The following relationship for the analyte response (R) is generally valid:

$$R = k\phi_s\alpha_s/(\alpha_s - 1) \quad (1)$$

where ϕ_s is the fractional loading of the marker on the solid phase; α_s is the retention of the analyte relative the system peak. In the present study the analytes are present both in charged and uncharged form involving that a correction factor (k) has to be introduced on the right hand side of the equation.

A retention equation can be derived assuming that the ionized sugar (X^-) and the marker (M^-) are distributed as ion pairs with sodium to the stationary phase; sodium hydroxide may also compete for the adsorption sites. It is further assumed that the uncharged sugar (HX) also is distributed to the stationary phase and competing for the same adsorption sites:

$$k'_X = qK^0(K_{HX}a_{H^+} + K_{NaX}[Na^+]) \times [(a_{H^+} + K_{a(HX)})(1 + K_{NaM}[Na^+][M^-] + K_{NaOH}[Na^+][OH^-])]^{-1} \quad (2)$$

where k' is the capacity ratio, q is the phase ratio, K^0 is the adsorption capacity of the solid phase, $K_{a(HX)}$ is the acid dissociation constant for the sugar, and the K values are the equilibrium constants for the different adsorption equilibria. The retention of the sugar can in principle be regulated by the concentrations of sodium, the marker and hydroxide (which also determines the pH in this case), and is furthermore governed by the capacity of the solid phase, and the magnitude of the equilibrium constants including pK_a.

The retention of the marker can be described by a related equation [11]; however, it is not given here since the system peak retention was not studied in detail in this paper.

3. Experimental

3.1. Apparatus

The chromatographic system was set up with an LKB 2150 pump (Bromma, Sweden), a Rheodyne 7125 sample injector with a 20-μl loop and a Tefzel alkaline resistant rotor seal, Spectro-

Monitor III UV detector (LDC, Riviera Beach, FL, USA), and Kipp and Zonen BD 40 recorder. Two porous graphitic carbon Hypercarb columns (100 mm × 4.7 mm I.D.), containing material from the same batch Nos. 0492 and 0498 (Shandon Scientific, Runcorn, UK) were used. The column No. 0498 was probably somewhat contaminated by an unknown substance from previous studies. The test chromatogram showed that solutes were less retained on this column than on column No. 0492, but the efficiency and selectivity were similar. Determinations of molar absorbances for the markers were performed with a Spectrophotometer Model 25 (Beckman, Irvine, CA, USA).

3.2. Chemicals

Sodium hydroxide (1 *M* Titrizol), phenol and methanol were obtained from E. Merck (Darmstadt, Germany) and sorbic acid from Fluka (Buchs, Switzerland). Other chemicals were purchased from Sigma (St. Louis, MO, USA). All chemicals were of analytical-reagent grade.

3.3. Chromatographic conditions

Deionized water (Millipore, Bedford, MA, USA) was used for preparation of mobile phases. Sodium hydroxide dilutions were kept in plastic bottles under protection from carbon dioxide by soda lime tubes. The mobile phases were freshly prepared and degassed prior to use. Flow-rate was 1.0 ml/min and the eluent was monitored at 254, 258 and 294 nm when using sorbic acid (SA), muconic acid (MA) or furanacrylic acid (FA) as UV-absorbing ions, respectively. The temperature of column and injector was maintained constant (22°C), if not otherwise indicated, using a Heto type 02 pt 923 C water-bath (Birkeröd, Denmark).

3.4. Calculations

The capacity ratio, k', was calculated conventionally from: $k' = (V_R - V_m)/V_m$, where V_R is the retention volume and V_m is the interstitial volume of the mobile phase in the column. V_m was obtained from the front disturbance in the chromatogram. The molar absorbances of the markers were determined by static measurements in the relevant mobile phase in a spectrophotometer. The apparent molar absorptivity (ϵ^*) of an analyte was calculated according to the equation [12]:

$$\epsilon^* = \frac{Ysu}{mdb} \tag{3}$$

where Y is peak area, s is sensitivity setting of the detector, u is flow-rate, m is amount of analyte, d is the chart speed and b is the path length in the detector cell.

4. Results and discussion

4.1. Using sorbic acid as the UV-absorbing ion

Our previous work showed that using porous graphitic carbon (PGC) as the solid phase can give fairly high retention of di- and oligosaccharides with sodium hydroxide as the mobile phase [2]. In the same system, SA, which has a high molar absorptivity at 254 nm: log $\epsilon = 4.46$ (see Table 4), was found to have a retention similar to the disaccharides. These conditions should be applicable for using SA as an UV-absorbing ion for indirect detection of saccharides. With addition of SA to the mobile phase, di- and trisaccharides were detected with relative retention values (k'_{solute}/k'_{SA}) between 0.43 and 2.87 (Fig. 1). SA with pK_a 4.8 is negatively charged, while the saccharides, pK_a 12–14 [11], are partly charged in the highly alkaline mobile phase (pH above 12). Lactose, the retention of which was closest to that of SA, demonstrated the highest detector response (log $\epsilon^* = 5.4$), i.e. about ten times higher apparent molar absorbance than the marker. The response was lower for sugars with both lower and higher retention than the system peak [8–10], but the apparent molar absorbances were higher than ϵ for the marker in all cases. The relationship

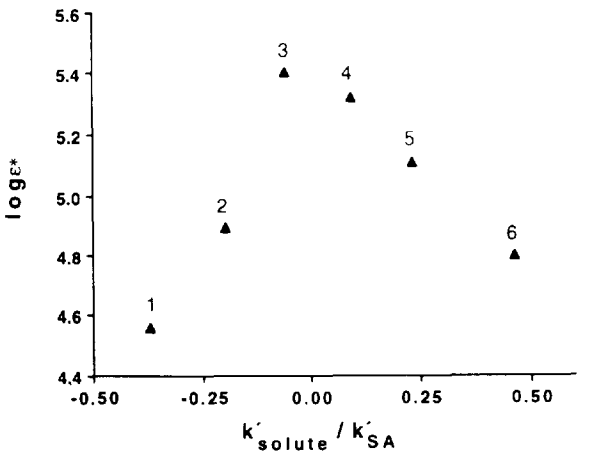

Fig. 1. Response of saccharides with sorbic acid (SA) as UV-absorbing ion. Mobile phase: $2 \cdot 10^{-5}$ *M* SA in 0.1 *M* NaOH and 10% (v/v) methanol. Solid phase: PGC No. 0492. 1 = Melibiose; 2 = sucrose; 3 = lactose; 4 = melezitose; 5 = gentiobiose; 6 = cellobiose.

between detection sensitivities and relative retentions (k'_{solute}/k'_{SA}) for individual sugars were in agreement with earlier developed theories; a graphical illustration of Eq. 1 is given in Fig. 2; a negative response is represented by a negative value of ϵ^*.

4.2. Influence of SA concentration on detector response

The detector responses of four disaccharides were determined using mobile phases that contained different concentrations of SA ($5 \cdot 10^{-6}$–$3 \cdot 10^{-5}$ *M*). The detection sensitivity increased with increasing concentrations of SA, and the logarithm of the apparent molar absorptivity (ϵ^*) of solutes versus the logarithm of the concentration of SA demonstrated linear relationships in this concentration range (Fig. 3). However, the system became less stable as the background absorbance approached the limit of the detector; consequently a SA concentration higher than $3 \cdot 10^{-5}$ *M* was not tested. Generally, a higher concentration of UV-absorbing ion in a mobile phase would give a higher loading (Φ) of the UV-absorbing ion on solid phase, therefore the detection sensitivity would improve according to Eq. 1. However, an increase in marker concentration will also increase noise, and an optimal signal-to-noise ratio will be obtained at an intermediate value.

The retentions of the saccharides were not

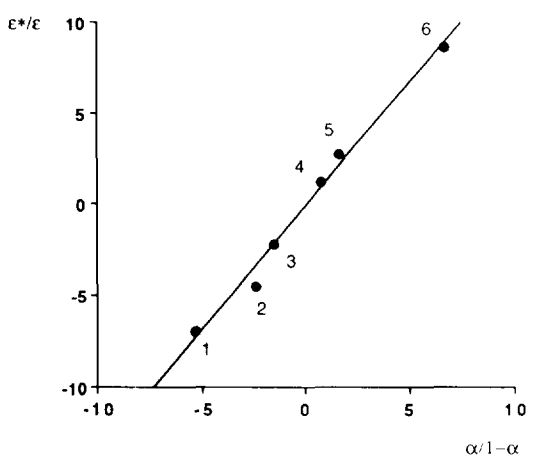

Fig. 2. Graphical computation of the response function (Eq. 1). Chromatographic conditions as in Fig. 1. 1 = Melezitose; 2 = gentiobiose; 3 = cellobiose; 4 = melibiose; 5 = sucrose; 6 = lactose.

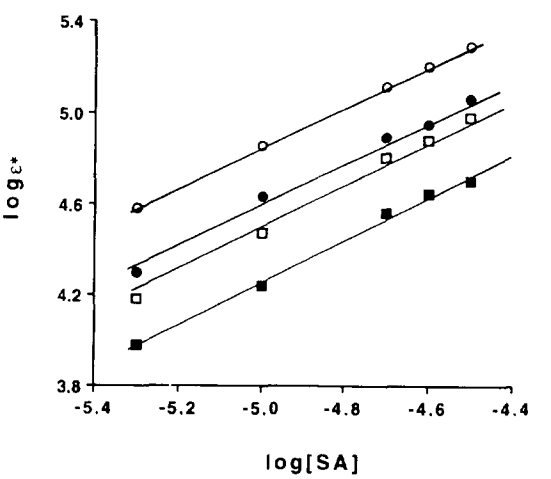

Fig. 3. Influence of sorbic acid concentration on response. Mobile phases: $5 \cdot 10^{-6}$–$3 \cdot 10^{-5}$ *M* SA in 0.1 *M* NaOH and 10% (v/v) methanol. Solid phase: PGC No. 0492. ○ = Gentiobiose; ● = sucrose; □ = cellobiose; ■ = melibiose.

influenced by a variation of the sorbate concentration in the range tested; this means that the term $K_{NaM}[Na+][M-]$ in Eq. 2 is negligible in the concentration range studied. The analyte retentions are then governed mainly by pH, the sodium and hydroxide concentrations.

4.3. Influence of pH on retention and response

Since the pK_a values of the saccharides are ca. 12 or higher a high pH is needed to dissociate the solutes in the mobile phase. When the sodium hydroxide concentration was changed from 0.1 to 0.02 *M*, the retentions of all solutes increased, whereas the SA retention was constant (Fig. 4). The retention behaviour shows that the distribution of the uncharged form of the sugar dominates in the studied pH interval (see Eq. 2). The distribution of the charged form as ion pair with sodium also plays a role, since otherwise there would be a linear relationship between log k' and pH, which is not the case. The change in elution order for melibiose and sucrose with $pK_a = 12.6$ [13] is probably due to a difference in their pK_a values. Sorbic acid ($pK_a = 4.8$) is anionic during the whole pH interval, and its unchanged retention shows that sodium hydroxide, in this concentration interval, does not compete significantly for the adsorption sites with the marker.

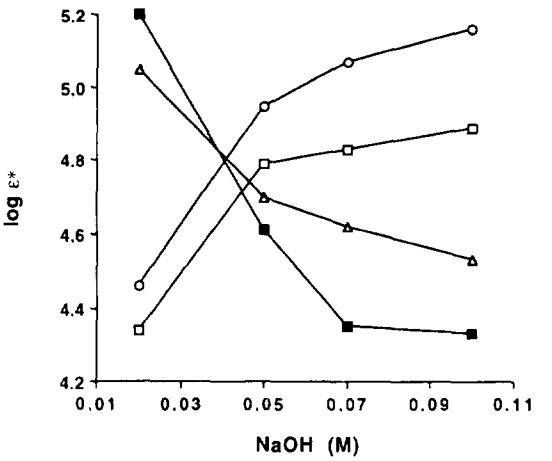

Fig. 5. Influence of pH on response. Chromatographic conditions and symbols as in Fig. 4.

The detector responses increased for melibiose and sucrose, while they decreased for gentiobiose and cellobiose with decreasing pH (Fig. 5). The response for sucrose was smaller than that of melibiose when sucrose eluted earlier than melibiose. These results were caused by the change of relative retentions of solutes to the

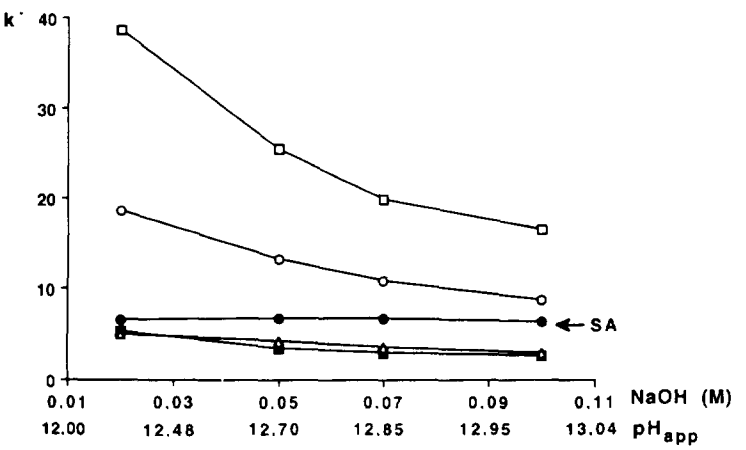

Fig. 4. influence of pH on retention of saccharides. Mobile phases: $2 \cdot 10^{-5}$ *M* SA in $2 \cdot 10^{-2}$–$1 \cdot 10^{-1}$ *M* NaOH, and 8% (v/v) methanol. Solid phase: PGC No. 0498. □ = Cellobiose; ○ = gentiobiose; ● = SA; △ = sucrose; ■ = melibiose.

system peak, Eq. 1 (see Fig. 4), again illustrating that solutes eluting closer to the UV-absorbing compound will have lower detection limits. Since the indirect detection mechanism involves a mutual interaction between the marker and the analyte, it is interesting that the main factor determining the magnitude of the response is the closeness of the analyte and system peaks. As discussed above the change in retention order between sucrose and melibiose is probably due to differences in the pK_a values, and this means that the fraction of uncharged melibiose is larger than that of sucrose at the lowest pH studied. This indicates that the charged marker competes with both the uncharged and charged forms of the sugars for the adsorption sites, which also is an assumption made in the derivation of the retention equation.

4.4. Influence of methanol concentration on detector response

In order to shorten the chromatographic run times and to improve system selectivity, an organic modifier, methanol, was added to the mobile phase. As expected, the capacity ratios of both the system and sample peaks were reduced with increasing concentrations of methanol from 4 to 12%. However, the effect was more pronounced on the analytes, particularly on the highly retained saccharides, than on the marker (SA) (see Table 1). Consequently, gentiobiose eluted closer to the marker, while the peaks of melibiose and sucrose generally became more distant from the system peak with increasing methanol content.

The detection sensitivity varied with increasing concentration of methanol (Table 1). The apparent molar absorbance of melibiose and sucrose decreased significantly, whereas that of gentiobiose showed a considerable increase. In general, addition of an organic modifier to the mobile phase will lead to a decrease in detection sensitivity, as a result of a reduced loading of the UV-absorbing component on the solid phase [14–16]. Furthermore, a change in relative retention (k'_{solute}/k'_{SA}) will also cause an alteration in the detection sensitivity. For melibiose and sucrose, the large decrease in detector response was likely due to the sample peaks becoming more distant from the system peak and a reduction in loading of SA on the solid phase at higher concentration of methanol. However, the relative retention (k'_{solute}/k'_{SA}) of gentiobiose became closer to 1. Consequently, the effect of the decrease in loading of SA on the solid phase was counteracted by an improvement in relative retention.

A typical chromatogram is given in Fig. 6 which shows the separation of some di- and trisaccharides. It clearly demonstrates that sugars eluting close to the system peak have lower detection limits than those eluting more distant to this peak. However, the peak shape of compounds that are eluted close to the system peak may be distorted [e.g. lactose (peak 3 in Fig. 6)],

Table 1
Influence of methanol concentration on the retention and indirect detector responses of sugars

Sugars	Concentration of methanol (%, v/v)							
	4.0		8.0		10.0		12.0	
	α_s	Log ϵ^*	α_s	Log ϵ^*	α_s	Log ϵ^*	α_s	Log ϵ^*
Melibiose	0.76	5.20	0.49	4.79	0.41	4.54	0.37	4.30
Sucrose	0.82	5.48	0.75	5.16	0.59	4.89	0.56	4.68
Gentiobiose	3.36	4.87	2.03	5.17	1.62	5.14	1.41	5.26

Mobile phase: $2 \cdot 10^{-5}$ *M* sorbic acid and methanol in 0.1 *M* NaOH. Solid phase: PGC No. 0492. $\alpha_s = k'_{solute}/k'_{SA}$; ϵ^* = apparent molar absorptivity.

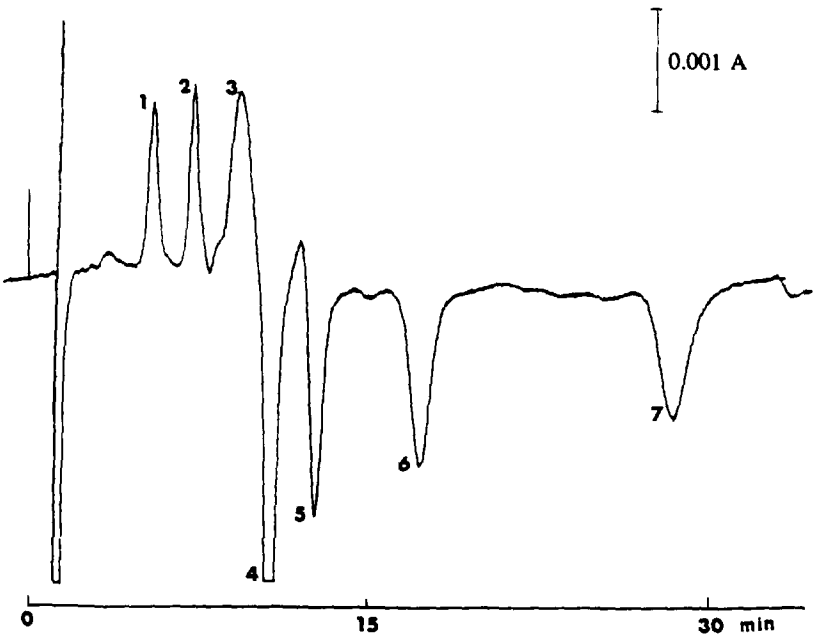

Fig. 6. Separation of di- and trisaccharides. Mobile phase: $2.5 \cdot 10^{-5}$ *M* SA in 0.1 *M* NaOH and 10% (v/v) methanol. Solid phase: PGC No. 0492. Temperature: 22°C. Peaks: 1 = melibiose (1.36 μg); 2 = sucrose (0.68 μg); 3 = lactose (0.34 μg); 4 = system peak (sorbic acid); 5 = melezitose (0.50 μg); 6 = gentiobiose (0.68 μg); 7 = cellobiose (1.36 μg).

since the relative response for the part of the peak coming closer to the system peak will be higher than more distant parts.

4.5. Effect of temperature on detector response

The influence of temperature on the detection sensitivity showed that the detector responses were significantly reduced, when the temperature was raised from 12 to 42°C (Fig. 7). The retentions of sugars decreased at a similar rate to that of SA (see Fig. 8, which is discussed below), hence the retentions of the solutes relative to SA essentially remained constant at different temperatures. The main reason to the decreasing response, in accordance with theory (see Eq. 1), was due to decreased loading of the marker on the solid phase with increasing temperature (Table 2). The decrease in loading from 12 to 42°C was about 2.6 times and the decreases in response for the different analytes were of the same magnitudes. The Van 't Hoff plots (Fig. 8) were linear in the studied temperature interval indicating an unchanged retention mechanism for the saccharides. The thermodynamic parameters calculated from the relationships (Table 3)

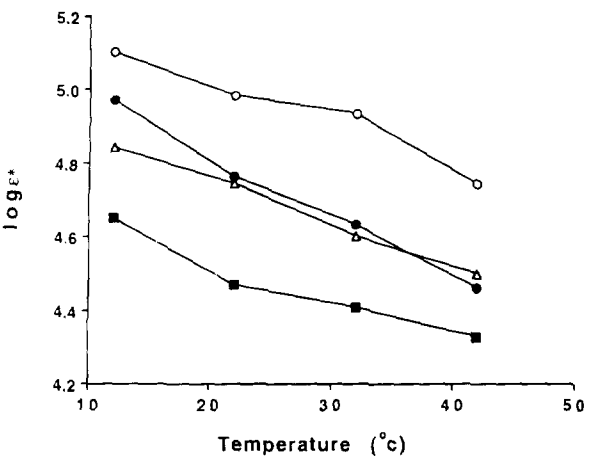

Fig. 7. Effect of temperature on the detector response. Mobile phase: $2 \cdot 10^{-5}$ *M* SA in 0.1 *M* NaOH and 8% (v/v) methanol. Solid phase: PGC. No. 0498. ○ = Gentiobiose; ● = cellobiose; △ = sucrose; ■ = melibiose.

Table 2
Influence of temperature on the adsorption of sorbic acid on the solid phase

Temperature (°C)	Amount adsorbed (μg)[a]
12	29.6
22	21.9
32	14.4
42	11.2

Mobile phase: $2 \cdot 10^{-5}$ *M* SA and 8% (v/v) methanol in 0.1 *M* NaOH. Solid phase: PGC No. 0498.
[a] Measured by breakthrough techniques.

indicate that the retention of the carbohydrates is due to the enthalpic factors (bonding to the solid phase), while the bonding results in increasing order in the system (negative entropy values). The magnitude (but not the sign) of the entropy data are uncertain, however, due to the non-accessibility of the phase ratio, which was roughly calculated according to $(V_t - V_m)/V_m$, where V_t = total volume of the column. The results seem to confirm that the retention mechanism of the marker, SA, is the same as for the analytes, which was assumed in deriving the retention equation, Eq. 1.

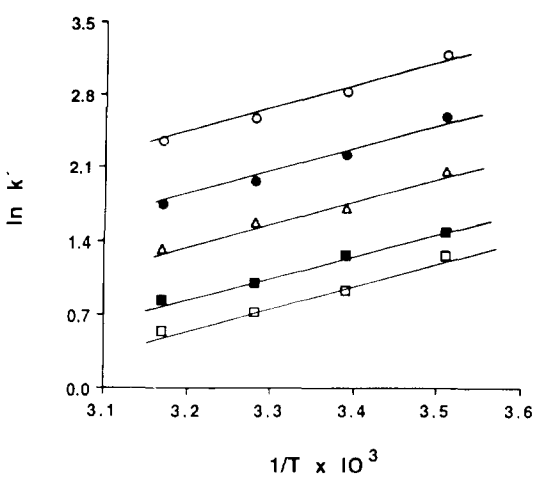

Fig. 8. Indirect detection of sugars in HPLC Van 't Hoff plots. Mobile phase: $2 \cdot 10^{-5}$ *M* sorbic acid in 0.1 *M* NaOH and 8% (v/v) methanol. Solid phase: PGC No. 0498. Solutes: ○ = cellobiose; ● = gentiobiose; ■ = sucrose; □ = melibiose; △ = sorbic acid (marker).

Table 3
Thermodynamic parameters for retention of saccharides on porous graphitic carbon

Solutes	r^{2} [a]	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol · K)	$T\Delta S^{0}$ [b] (kcal/mol)
Melibiose	0.987	−4.23	−10.7	−3.2
Sucrose	0.993	−3.96	−9.3	−2.7
Gentiobiose	0.989	−5.08	−11.0	−3.3
Cellobiose	0.990	−4.96	−9.4	−2.8
Sorbic acid[c]	0.978	−4.27	−9.3	−2.7

Mobile phase: $2 \cdot 10^{-5}$ *M* SA and 8% (v/v) methanol in 0.1 *M* NaOH. 1 cal = 4.184 J.
[a] Correlation coeffient for the linear fit of the Van 't Hoff plot.
[b] At 295 K.
[c] Marker.

Lower column temperature would give rise to higher response, but a disadvantage is an increased zone broadening, on average an almost 3-fold decrease in the number of theoretical plates was obtained in going from 42 to 12°C.

4.6. Responses with different UV-absorbing ions

In addition to SA, MA and FA were tested as potential markers under the same chromatographic conditions. As shown in Table 4, MA, SA and FA exhibited approximately the same molar absorptivities at 258, 254 and 294 nm, respectively. The loading of the individual ions on the solid phase varied depending on differences in their hydrophobic properties. In order to obtain comparable background absorbances, each UV-absorbing ion was added in the same

Table 4
Properties of markers

Marker	ϵ [a]	Amount adsorbed on the solid phase (μg)[b]
MA	$2.55 \cdot 10^4$ (258 nm)	4.5
SA	$2.88 \cdot 10^4$ (254 nm)	21.9
FA	$2.25 \cdot 10^4$ (294 nm)	134.1

[a] Molar absorptivity in the solvent: 8% methanol in 0.1 *M* NaOH.
[b] Measured by breakthrough techniques; chromatographic conditions were the same as in Table 5.

concentration to the mobile phases. The detector was set at the wavelength of maximum absorption of respective marker. Responses and relative retentions for sugars in these systems are summarized in Table 5. The detection sensitivity was shown to be dependent on the retentions of both solutes and UV-absorbing ions. MA, which is a divalent acid, was less retained than the solutes and gave rise to lower and approximately the same response for all actual sugars. This was unexpected and not in accordance with the general theory; a constant response would not be obtained until the relative retentions were >20. It would be of interest to study di- and polyvalent acids further in order to find out whether this is a general phenomenon. Markers of that type would then make the application of indirect detection principles more convenient. In the case of FA, all solutes were eluted earlier than the marker. The loading of FA on the solid phase was considerably higher (see Table 4); the detector responses of individual sugars increased with increasing retention. A very high response was noted for the most retained sugar, cellobiose —an almost 15-fold higher apparent molar absorbance compared to the ϵ of FA. It indicates that FA might be very useful for more retained saccharides, i.e. higher oligo- and polysaccharides. However, as shown in Table 5, using SA, which had about the same retention as that of the solutes, as the marker gave generally the highest detection sensitivity in spite of the fact that the amount of SA adsorbed on the solid phase was 6-fold lower than that of FA in the system. This demonstrates again the importance of choosing a marker that gives retentions relative the solutes close to 1 in order to obtain high detection sensitivities in indirect detection systems.

These systems were not suitable for detection of monosaccharides and amino monosaccharides due to their very low retention; using sorbic or muconic acid as markers the small sugars were unretained on the PGC column. By using the more polar marker phenol as the UV-absorbing ion, three monosaccharides and amino monosaccharides could be separated and detected as shown in Fig. 9.

4.7. Stability of the sugars at alkaline conditions

Since highly alkaline sodium hydroxide solutions were used as the mobile phase, the stability of the sugars under such conditions must be adequately high in order to achieve reliable separations. This was investigated by dissolving each sugar in 0.1 *M* sodium hydroxide solution and analyzing the solutions stored at ambient temperature, about 20°C, by the indirect detection technique developed, at increasing time intervals (see Table 6). The non-reducing sugar, melezitose, was stable during the whole period studied, 2 h. The reducing sugars were degraded slowly; gentiobiose and melibiose being the most sensitive, a slight degradation could be observed after 15 min. Sucrose, cellobiose and lactose

Table 5
Relative retentions and indirect detector responses of sugars with different markers

Sugars	k'_{solute}/k'_{system}			log ϵ*		
	MA	SA	FA	MA	SA	FA
Melibiose	2.2	0.4	0.1	3.93	4.33	4.16
Sucrose	2.8	0.6	0.1	4.10	4.53	4.33
Lactose	3.9	0.8	0.2	4.23	5.06	4.56
Melezitose	4.6	0.9	0.2	3.81	5.24	4.57
Gentiobiose	7.8	1.6	0.3	4.00	5.16	4.81
Cellobiose	13.4	2.8	0.6	3.74	4.83	5.52

Mobile phase: $2 \cdot 10^{-5}$ *M* marker and 8% (v/v) methanol in 0.1 *M* NaOH. Solid phase: PGC No. 0498. UV detection: 258, 254 and 294 nm for MA, SA and FA, respectively.

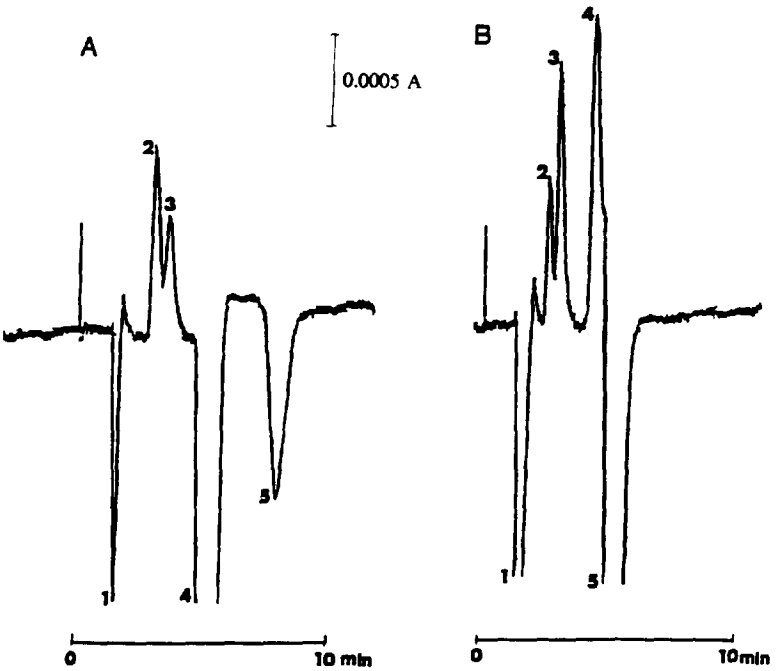

Fig. 9. Separation of amino sugars and monosaccharides with phenol as the UV-absorbing ion. Mobile phase: 0.1 *M* NaOH and $1.5 \cdot 10^{-4}$ *M* phenol. Solid phase: PGC No. 0492. UV detection at 287 nm. (A) 1 = System peak; 2 = glucosamine (0.72 μg); 3 = galactosamine (0.18 μg); 4 = system peak; 5 = N-acetylgalactosamine (0.22 μg). (B) 1 = System peak; 2 = glucose (0.72 μg); 3 = galactose (0.72 μg); 4 = fucose (0.33 μg); 5 = system peak.

were, however, more stable. It is obvious that for some reducing sugars the chromatographic run time should be kept adequately short in order to avoid degradation and to achieve reliable results in quantitative determinations. An advantageous alternative might be to use a lower pH for sensitive sugars, for example gentiobiose. The retention would then be longer (see Fig. 4) and the response somewhat lower (see Fig. 5) using the standard conditions. It is probable, however, that it would be possible to adapt the mobile phase conditions (amount of organic modifier, concentration of marker, temperature) in order to achieve suitable conditions for the analysis also of a pH-sensitive carbohydrate of interest.

Table 6
Stability of saccharides in alkaline solution

Sugars	Relative peak area (%)				
	0 min	15 min	30 min	60 min	120 min
Melezitose	100	100	100	100	100
Sucrose	100	100	100	98.4	92.3
Cellobiose	100	100	100	96.2	93.6
Lactose	100	100	97.8	91.1	84.4
Gentiobiose	100	98.3	94.5	91.6	87.3
Melibiose	100	97.4	93.6	89.4	86.2

Chromatographic conditions as in Fig. 7. Solutes were dissolved in 0.1 *M* NaOH and stored at ambient temperature.

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