Sorption and Separation of Sugars with Adsorbents Based on Reversible Chemical Interaction

Johan A. Vente[1,2*], Hans Bosch[1], André B. de Haan[1] and Paul J.T. Bussmann[2] *(1) Separation Technology Group, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands. (2) TNO Quality of Life, P.O. Box 342, 7300 AH Apeldoorn, The Netherlands.*

(Received 12 January 2006; revised form accepted 30 January 2007)

ABSTRACT: The goal of this work was to enhance the selectivity of the separation of fructose or glucose from carbohydrate mixtures with adsorbents capable of reversible reaction. The experimental data presented demonstrate the feasibility of separating glucose from *trans*-galacto-oligosaccharides and fructose from fructo-oligosaccharides using resins as adsorbents. Comparison of the sorption properties of a resin functionalised with the bisulphite (HSO_3^-) ion with its original chloride (Cl^-) form showed that the sorption of glucose increased due to interaction with the bisulphite group. In addition, functionalisation with bisulphite resulted in selectivity towards glucose relative to fructose and lactose. Moreover, chromatographic separation of glucose from *trans*-galacto-oligosaccharides on a bisulphite-loaded resin was improved compared to the same resin in the chloride form. However, the bisulphite unfortunately oxidised.

Boronic acid-functionalised resin was selective towards fructose at pH 6.0 compared to glucose, indicating complex formation between fructose and boronic acid. Although complex formation with boronate has been assumed to be even stronger than with boronic acid, increasing the pH to a value above the pK_a of the functional group did not improve fructose sorption. Chromatographic separation of fructose from fructo-oligosaccharides was obtained on boronic acid-functionalised resin as a result of complex formation with fructose.

These results show that reversible chemical reactions can lead to an improvement in the performance of adsorbents for sugar separations.

1. INTRODUCTION

Ion-exchange resins are employed as adsorbents in current industrial carbohydrate–carbohydrate chromatographic separation processes with exclusive metal ion (usually Ca^{2+}) loading. The main cost factor in such separation processes arises from product dilution with water, which necessitates cost-intensive downstream water removal in the process (Pynnonen 1998). Hence, the use of an improved adsorbent could reduce the need for such dilution. The selectivity of currently applied adsorbents is relatively low since it is based on physical interaction between the metal ions and dipoles (Goulding 1975). In the approach chosen in the present work, the capacity and selectivity of the adsorbents was improved through reversible chemical interactions between bisulphite and boronic acid functional groups.

The bisulphite ion is known to form a more stable complex with aldehydes such as glucose [see Figure 1(A)] than with ketones such as fructose. Bisulphite-loaded anion-exchange resin

*Author to whom all correspondence should be addressed. E-mail: johan.vente@tno.nl.

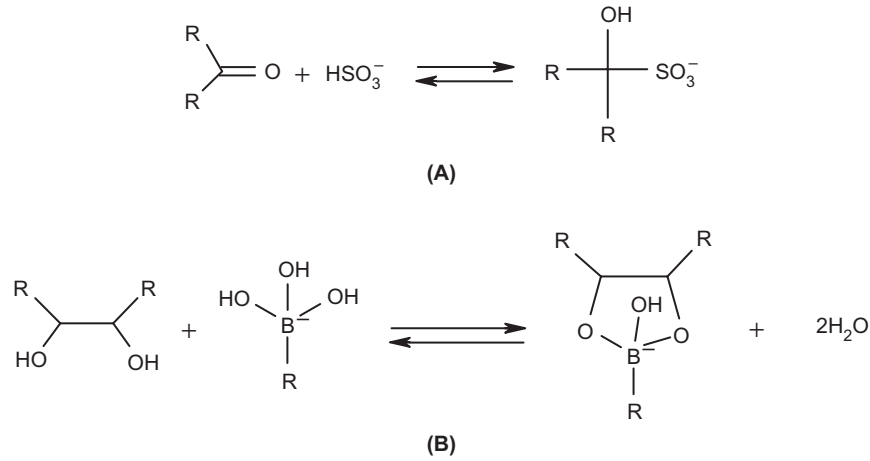

Figure 1. Schemes for (A) the reaction of a carbonyl group (e.g. as in glucose) with the bisulphite ion and (B) for the reaction of the *cis*-diol group with the boronate ion.

has been shown to separate glucose from fructose (Ganetsos and Barker 1993) and galactose from glucose (Lindberg and Slessor 1967). In contrast, bor(on)ic acid selectively interacts with fructose via the *cis*-diol group of fructose [see Figure 1(B)]. Separation of sugars on boronic acid-functionalised adsorbents using elution chromatography (Weith *et al*. 1970; Barker *et al*. 1973), cycling zone adsorption (Dore and Wankat 1976) and frontal chromatography (Dukler and Freeman 2001) has shown that fructose is retained to a greater extent than glucose and sucrose.

No publications apparently exist in the literature in which adsorbents capable of reversible chemical interaction have been employed for the separation of monosaccharides from fructo-oligosaccharides (FOS) or *trans*-galacto-oligosaccharides (GOS). Hence, the goal of the present work was to evaluate whether it is possible to improve the capacity and selectivity of adsorbents for the separation of glucose from GOS and fructose from FOS.

The sorption behaviour of sugar was determined by measuring the individual sorption isotherms of glucose, fructose, sucrose and lactose because these sugars are constituents of GOS, FOS or both. In addition, the separation factor was calculated from the individual sugar isotherms, discussed in terms of the interactions between the adsorbent and the sugars, and then compared to the separation factors obtained for Ca^{2+} ion-loaded resin. Multi-sugar isotherms and the sorption kinetics were not determined. Instead elution chromatograms of pulse injections of two commercial oligosaccharides mixtures, i.e. GOS and FOS, respectively, were recorded and discussed to obtain an insight into how the equilibrium sorption properties influence the separation of monosaccharides from oligosaccharides.

2. MATERIALS AND METHODS

2.1. Adsorbents

The poly(styrene-*co*-divinylbenzene) (PS–DVB) trimethyl-aminated anion-exchange resin Dowex 1X-X4, mesh size 200–400 (Aldrich, Steinheim, Germany) was purchased in the Cl^- form

with an ion-exchange capacity of 3.5 mequiv/g. All water employed was prepared using a MilliQ apparatus (Millipore, Bedford, MA, U.S.A.). For isotherm measurements by frontal analysis and the recording of chromatograms (see Section 2.4), the Cl^--loaded resin was packed in a chromatographic column (Superformance: maximum length, 30.0 cm; internal diameter, 16 mm; Götec, Muehltal, Germany). The HSO_3^--loaded resin was prepared from that loaded into the column by ion exchange with a 0.2 M $NaHSO_3$ solution prepared from $Na_2S_2O_5$. As a consequence, it had the same ion-exchange capacity as the original column. The pH of the solution used for ion exchange was 2.7. Mitsuhashi *et al*. (1995) have shown that the bisulphite–sugar complex is stable over the pH range 2–6. Analysis of the solution by anion chromatography revealed that, in addition to HSO_3^-, the solution also contained some HSO_4^- ions apparently formed by oxidation (Deister *et al*. 1986). The bed porosity of both the Cl^-- and HSO_3^--loaded resin was calculated from the retention time of Dextran T2000 (Pharmacia, Sweden).

Boronate Affigel was obtained from Bio-rad (Veenendaal, The Netherlands). This is a boronic acid-functionalised poly(acrylamide) resin which is similar to Affi-Gel 601 (Bio-Rad 1995) but with a higher binding capacity (1.2–2.5 mequiv/g dry resin) (Frey 2003). Affigel was washed with water before use. Dextran eluted as multiple peaks on Affigel and could therefore not be used for the estimation of the bed porosity. In contrast, the injection of 1 kg/m^3 bovine serum albumin (Grade V: Sigma, Steinheim, Germany) resulted in a single, nearly symmetrical peak whose retention time was used to calculate the bed porosity. It was assumed that acetone is capable of entering the resin interior due to its small molecular size but does not interact with the boronic acid groups because it does not contain a *cis*-diol group. Hence, the retention time of a 10 kg/m^3 solution of acetone was used to estimate the total porosity.

2.2. Measurement of sugar isotherms

The monosaccharides D-glucose and D-fructose, and the disaccharides D-sucrose and D-lactose were employed for the measurement of the individual sugar sorption isotherms. Such isotherms were measured by frontal analysis in a chromatography set-up using the packed columns with a flow rate of 10.0 cm^3/min according to a procedure described in detail elsewhere (Vente *et al*. 2005). The thermostatted column was maintained at 60°C in all measurements. The following correlation was fitted to the measured sugar sorption isotherms:

$$q = ac^2 + bc \tag{1}$$

where q is the sugar concentration in the resin (kg/m^3 wet resin), a (m^3/kg) is an equilibrium parameter correcting for the concentration dependence of the distribution coefficient at high sugar concentrations, c (kg/m^3) is the sugar concentration in the liquid phase and b (–) is the slope of the isotherm at infinite dilution. This correlation has been used previously to describe sugar sorption isotherms on zeolites (Muralidharan and Ching 1997). The separation factor of component 1 relative to component 2, $\alpha_{1,2}$, was calculated as:

$$\alpha_{1,2} = \frac{q_1/c_1}{q_2/c_2} \tag{2}$$

in which the subscripts 1 and 2 refer to the strongly and weakly adsorbing components, respectively.

2.3. Recording the chromatograms of the oligosaccharides

Commercial mixtures of fructo-oligosaccharides FOS (Raftilose® L60/75) and *trans*-galacto-oligosaccharides GOS (Elix'or® 259) were kindly donated by Orafti (Tienen, Belgium) and Borculo Domo Ingredients (Borculo, The Netherlands), respectively. HPLC analysis of FOS was performed using a Polyamine II column (250 × 4.6 mm i.d., obtained from YMC, Schermbeck, Germany) and eluted with a 60:40 acetonitrile/water mixture at a flow rate of 1.0 ml/min at 25°C with RI detection. Similarly, HPLC analysis of GOS was performed using a Rezex RSO Oligosaccharide column (200 × 10 mm, Phenomenex, Torrance, CA, U.S.A.) and eluted with water at a flow rate of 0.4 ml/min at 85°C with RI detection. Both the Raftilose and Elix'or are syrups containing ca. 75% dry substance. Raftilose consists of 6.1% m/m fructose, 32.5% sucrose, 57.9% tri- and larger sacharides together with some glucose and difructose (3.5% in total), while Elix'or consists of 20.7% glucose, 1.1% galactose, 39.5% disaccharides (including lactose) and 38.7% tri- and larger saccharides.

Chromatograms were recorded by eluting diluted samples of FOS or GOS (1.00 cm^3, 100 kg syrup/m^3) injected into the column described above, using water as the eluant in conjunction with spectrophotometric detection at 190 nm. To allow a comparison between chromatograms which differed only slightly in bed length and bed porosity, the detector signal is presented as a function of dimensionless time, τ:

$$\tau = \frac{t - t_R}{t_R} \tag{3}$$

where t is the elution time (s) and t_R the retention time (s) of dextran.

3. RESULTS AND DISCUSSION

3.1. Isotherms on bisulphite-loaded resin

The sugar sorption isotherms onto HSO_3^--loaded resin are depicted in Figure 2 where they are compared with the isotherms onto the resin in its original Cl^- form. The sorption capacities of all the sugars onto the Cl^--loaded resin were high, being higher for monosaccharides than for disaccharides, possibly due to the steric hindrance of the larger disaccharides. Sugar sorption onto the Cl^--loaded resin occurred merely as a result of partitioning (Vente 2004). What is interesting is that sorption onto the HSO_3^--loaded resin was even higher than onto the Cl^--loaded resin, particularly for glucose and lactose. Moreover, the initial slope of the glucose and lactose isotherms increased, thereby indicating the enhanced affinity of the adsorbent for these sugars. In addition, the shape of the isotherm changed from being slightly concave to convex. The selective enhancement of glucose and lactose sorption onto the HSO_3^--loaded resin indicates that the open-chain aldehyde forms of glucose and lactose must have interacted chemically with the groups in the resin. Sucrose does not exist in an open-chain form and fructose is a ketose; hence, these sugars interacted to a lesser extent or not at all with the groups.

Table 1 lists the separation factors for the different combinations of sugars studied. The separation factors for the Cl^--loaded resin were all low ($\leq$ 1.2) and only some selectivity was observed for monosaccharides relative to disaccharides, indicating that steric effects play a role. The separation factor for glucose/fructose on the HSO_3^--loaded resin was 1.1 at the high

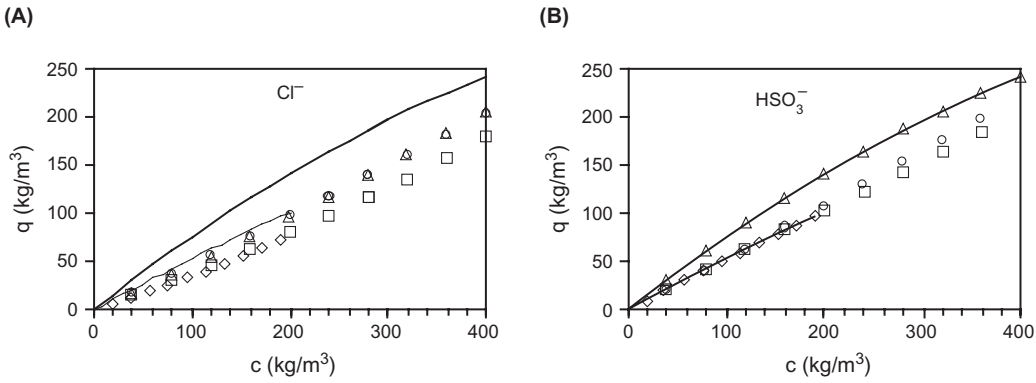

Figure 2. Sugar sorption isotherms for (A) Cl^--loaded and (B) HSO_3^--loaded Dowex 1X-X4 resin at 60°C. Data symbols refer to the following: △, glucose; ○, fructose; □, sucrose; and ◇, lactose. The lines correspond to the best fits obtained from an application of equation (1) to the data for the sorption of glucose (—) and lactose (——) onto the HSO_3^--loaded resin.

TABLE 1. Separation Factors, $\alpha_{1,2}$, for Ion-loaded Resins

Sugars	c_1	c_2	Separation factors, $\alpha_{1,2}$		
	(kg/m³)	(kg/m³)	Ca^{2+} [a]	Cl^-	HSO_3^-
Glucose/fructose	400	300	0.7	1.0	1.1 [b]
Glucose/lactose	130	250	1.2	1.2	1.5
Fructose/sucrose	50	270	2.0	1.1	1.1

[a] Values taken from Vente (2004). [b] Up to 1.5 for low concentrations.

concentrations encountered in industrial processes and thus very low. However it increased to 1.5 for dilute solutions of glucose and fructose. Moreover, the selectivity of glucose/fructose on the HSO_3^--loaded resin was the inverse of that observed on the Ca^{2+}-loaded resin. Table 1 also lists the separation factors for glucose/lactose and fructose/sucrose which are indicative for the separation of glucose from GOS and fructose from FOS, respectively. The separation factor for glucose/lactose depended slightly on concentration and was rather high, i.e. 1.5, at concentrations relevant to the separation of glucose from GOS. Moreover, it was higher than that for cation-loaded resins (Vente 2004). The separation factor for fructose/sucrose was even lower than obtained with the Cl^--loaded resin, indicating that steric effects played a less significant role in the HSO_3^--loaded resin than in the Cl^--loaded resin. Indeed, the fact that complex formation with improved the sorption capacity and selectivity suggests that this procedure has the potential for the preparation of functionalised adsorbents for the efficient removal of glucose from GOS.

3.2. Chromatograms of GOS on anion-exchange resin in the bisulphite form

Figure 3 shows the results for the elution of GOS from columns packed with Cl^-- and HSO_3^--loaded resin as represented by a non-calibrated, non-linear UV signal. The triangles on the x-axis of both parts represent the elution times for lactose (▲) and glucose (△), respectively, as calculated

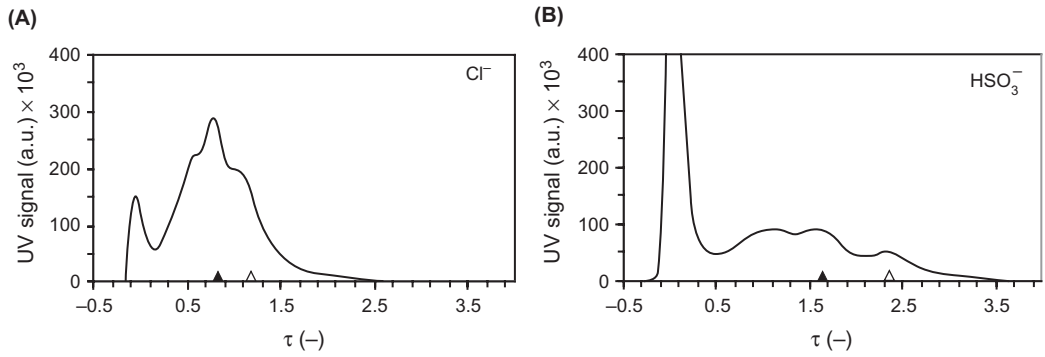

Figure 3. Chromatograms of GOS on (A) Cl^--loaded and (B) HSO_3^--loaded resins at 60°C. Experimental conditions: GOS conc. = 100 kg syrup/m^3; flow rate = 0.545 cm^3/min. The data points on the x-axis of both parts depict the elution times for lactose (▲) and glucose (△), respectively.

from the initial slope of the isotherms (Vente 2004). These elution times correspond to peaks on the chromatograms indicating the elution of that particular sugar. Apparently the elution behaviour of sugars in the multi-sugar mixture was well predicted by the isotherm data for the individual sugars.

Furthermore, the retention time increased when the resin was converted from the Cl^- form into the HSO_3^- form. In addition, the separation resolution improved as expected from the discussion of the separation factors in Section 3.1 above. However, no complete peak separation was obtained between glucose and other saccharides. This indicates that the sorption kinetics were rather slow. A further increase in temperature might improve the sorption rate but this is hampered by the limited stability of anion-exchange resins above 60°C.

Because of its superior capacity and selectivity, the HSO_3^--functionalised resin is capable of competing with metal ion-loaded resins. However, before a feasible system may be obtained, several aspects such as the limited stability of HSO_3^--functionalised and anion-exchange resins need to be addressed. Indeed, we have found that a 39% $NaHSO_3$ solution prepared by dissolving SO_2 into an NaOH solution provided a more stable source of ions. This solution had a higher pH value (3.7 compared to 2.7) and anion-exchange chromatography indicated that the solution contained no HSO_3^- ions. Furthermore, deoxygenating the feed solution stabilises the HSO_3^- ion (Deister *et al*. 1986). The thermal stability of anion-exchange resins should be considered relative to those conditions where the lifetime of the resin is sufficient and yet the sorption kinetics are fast.

3.3. Isotherms of sugars on the boronate-functionalised resin

Figure 4 shows the isotherms of glucose and fructose onto Affigel Boronate. Two quantities were used to express the adsorbent volume. The first was determined from the retention time of acetone and the second from that of albumin. Assuming that both tracers did not interact with the functional groups of the resin and that the albumin molecule was too large to penetrate the interstices of the resin, the adsorbent volume determined with acetone represents the polymer volume of the adsorbent only whereas that with albumin represents the volume of swollen adsorbent including solvent in the interstices of the polymer. The isotherm calculated on the basis

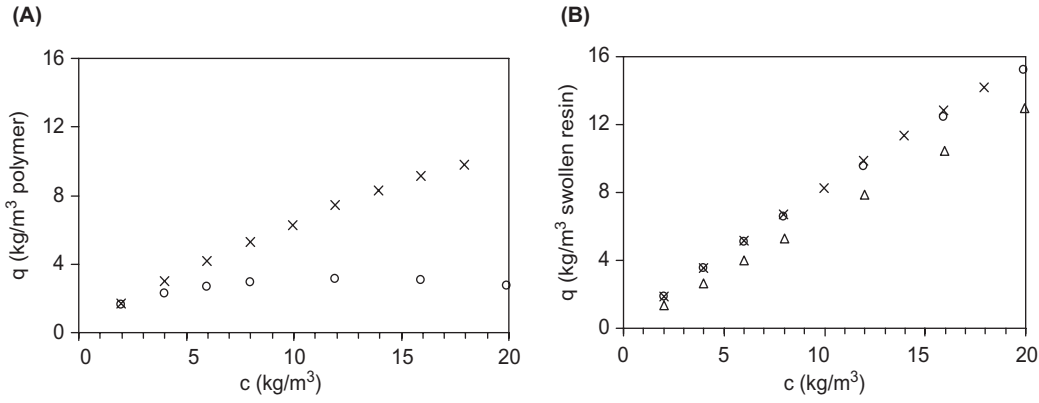

Figure 4. (A) Adsorption (acetone as a tracer) and (B) sorption (albumin as a tracer) isotherms for sugars onto Affigel Boronate at 60°C. Data points correspond to the following: (×) fructose at pH 9.0; (○) fructose at pH 6.0; and (△) glucose at pH 6.0.

of acetone as a tracer can be interpreted as the *ad*sorption isotherm (accumulation of sugar at the polymer/liquid interface), whereas the isotherm based on albumin represents the sorption isotherm (uptake of solute due to partitioning and adsorption). For both quantities, the isotherms depicted in Figure 4 show that fructose was better adsorbed than glucose. Indeed, a larger difference in sorption between glucose and fructose was observed for the *ad*sorption isotherms than for the sorption isotherms. These isotherms illustrate the difference in affinity of boronic acid-functionalised polymer for the sugars. Because of the large amount of sorption resulting from non-selective partitioning, much less difference was observed between glucose and fructose sorption when albumin was employed as the tracer. The separation factor calculated from the sorption isotherms decreased from 1.4 at concentrations close to zero to 1.2 at a concentration of 20 kg/m^3.

Complex formation with boronic acid may proceed via the neutral boronic acid, R–B(OH)$_2$, or via the boronate anion, R–B(OH)$_3^-$. Complex formation via neutral boronic acid may allow desorption by concentration swing, whereas complex formation via the boronate anion may require a decrease in pH for desorption to occur. It would appear that the route via the boronate anion prevails in aqueous solution at high pH values (Springsteen and Wang 2002). Since the pK_a value of 3-aminophenylboronic acid, the functional group of the adsorbent, is 8.75 (Singhal *et al*. 1991), the boronic acid groups in the adsorbent are mainly in the neutral form at a pH value of 6.0. Figure 4 shows that even at this pH value there is still interaction between fructose and the adsorbent, thereby indicating that although chemical interaction may not necessarily occur with the boronate anion it can take place with boronic acid.

To investigate such complex formation further, the boronic acid-functionalised resin was treated with excess 0.01 M NaOH and subsequently washed with water whose pH value had been adjusted to 9.0 with NaOH. This led to the conversion of boronic acid into the boronate form, with the uptake of OH^- ions being compensated by the uptake of Na^+ ions to ensure electrical neutrality. This caused the adsorbent to function as an ion-exchanger and become much more hydrophilic. As a consequence, the swollen adsorbent expanded, the resin volume as determined using albumin as a tracer increasing by as much as 2.57-times. Such expansion diluted the concentration of boronic acid groups. Figure 4(B) shows the isotherm of fructose determined with solutions whose pH values had been adjusted to 9.0 by the addition of NaOH. Under these circumstances, the

sorption of fructose was little changed compared to the situation at pH 6.0. Furthermore, the pH value of the column effluent was ca. 6.0, i.e. considerably lower than the pH of the feed. Nagai *et al*. (1993) observed previously that complex formation decreases as the pH value decreases. Glucose adsorption onto Affi-Gel 601, a boronic acid-functionalised resin similar to the Affigel Boronate used for this work, indicated that glucose adsorption increased only at pH values above 10 (Matsumoto *et al*. 2002). Due to dilution of the boronic acid groups and to acidification resulting from fructose interaction with boronate, fructose sorption also only increased marginally compared to the situation with the adsorbent that was not treated with NaOH. Furthermore, the volume of the adsorbent decreased during the experiments, thereby indicating that the boronate converted slowly into boronic acid, in agreement with the low pH of the column effluent.

3.4. Chromatograms of sugars on the boronate-functionalised resin

Figure 5 shows the chromatograms of glucose, fructose and a mixture of these sugars eluted on the boronate-functionalised resin. As expected from the isotherms, glucose and fructose eluted at different times with glucose being eluted first. Furthermore, the elution of a mixture of glucose and fructose resulted in two partly separated peaks, with the elution of glucose in the mixture being slightly later and that of fructose slightly earlier than that of the individually injected sugars. It is not clear why the glucose in the mixture eluted later. The earlier fructose elution time may perhaps be the result of competition with glucose for the adsorption sites on the resin surface.

Figure 6 shows the chromatograms of FOS eluted with water on the boronate-functionalised resin. In this case, the saccharides were only partly separated with the large peak in the chromatogram being that for fructose. Analysis of the feed showed that the FOS contained much more fructose than usual, suggested that the FOS had been partly hydrolysed. Furthermore, the other peak in the chromatogram eluting at $\tau = 1.15$ coincided with the elution of pure sucrose. In this case, good separation of fructose and sucrose is observed and hence the application of boronic acid-functionalised resins may be of interest in separation processes. However, the poly(acrylamide) polymer was not a very attractive adsorbent for process applications since it was

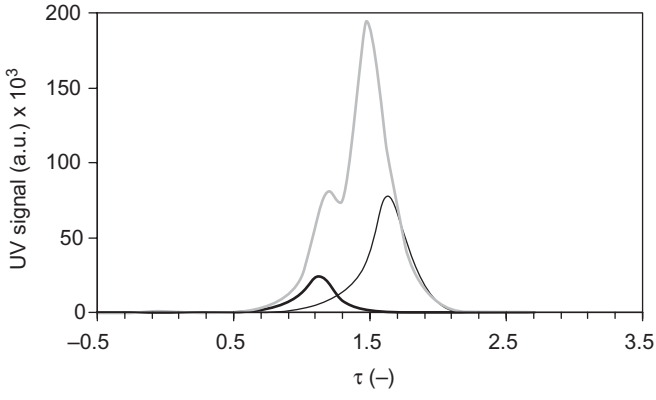

Figure 5. Chromatograms of sugars eluted with water on an Affigel Boronate packed column at 60°C and pH 6.0. Experimental conditions: flow rate = 1.00 cm^3/min. The curves depicted correspond to (——) glucose (10 kg/m^3), (——) fructose (10 kg/m^3) and (——) a mixture of glucose and fructose (each at 5 kg/m^3).

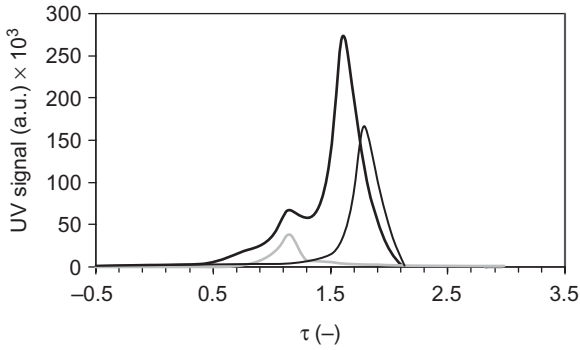

Figure 6. Chromatograms of FOS eluted with water on an Affigel Boronate packed column at 60ºC and pH 6.0. Experimental conditions: flow rate = 0.20 cm^3/min. The curves depicted correspond to (——) FOS (100 kg syrup/m^3), (——) fructose (10 kg/m^3) and (——) sucrose (10 kg/m^3).

highly elastic. This would undoubtedly result in practical problems if it were used in the operation of large columns. A more practical polymer which swells and shrinks less would be poly(styrene-*co*-divinylbenzene). It is known that this polymer can be functionalised with boronic acid (Frechet *et al*. 1979).

4. CONCLUSIONS

Resin loaded with bisulphite groups exhibited selective sorption towards glucose and lactose relative to fructose and sucrose. Furthermore, separation of glucose from GOS on an HSO_3^--functionalised anion-exchange resin showed improved behaviour relative to the same resin in the Cl^- form. Resin functionalised with boronic acid showed selective sorption for fructose relative to glucose. Indeed, even at a pH value of 6.0 and using water as the eluent, separation was obtained between fructose and glucose and between fructose and FOS. The work described demonstrates that the selectivity of sugar sorption may be enhanced selectively using adsorbents functionalised with groups capable of interacting via reversible chemical reactions. Thus, functionalisation provides a means of developing more efficient adsorbents for the separation of monosaccharides from oligosaccharides.

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