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# EVALUATION OF VARIOUS PACKINGS FOR SOLID-STATE CATALYTIC REACTORS USED IN THE LIQUID CHROMATOGRAPHIC DETECTION OF NON-REDUCING CARBOHYDRATES

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## SUMMARY

The use of strongly acidic cation exchangers as catalysts in post-column packed-bed reactors has been studied. The main aim was their application in the liquid chromatographic determination of carbohydrates, *i.e.*, in the conversion of non-reducing oligosaccharides, especially sucrose, into more easily detectable products with reducing properties. Three types of polystyrene-based ion exchangers with different percentages of cross-linking (4 and 8%) and one silica-based ion exchanger were compared. Studies on the influence of flow-rate and reaction temperature on reactor performance were carried out so as to allow the determination of peak broadening in the reactor and of the rate of hydrolysis of the saccharides. In both instances the 4% cross-linked polystyrene resin gave the best results.

## INTRODUCTION

For several years, column liquid chromatography (LC) in systems using chemically bonded silica[1,2] or silica dynamically coated with amine modifiers present in the mobile phase[3,4] has been considered the best method for the separation of saccharides; however, it has been shown that these methods suffer from several disadvantages. The column lifetime, for example, is relatively short[5] and the chromatographic performance deteriorates with time[6,7]. Further, the formation of compounds analogous to Schiff bases[8] can cause serious losses of reducing sugars[9]. For the rest, carbohydrate detection based on the measurement of either UV absorbance or changes in refractive index (RI) has poor sensitivity, especially with oligosaccharides[10]. Oligosaccharides of higher molecular weight, moreover, are only sparingly soluble in the mobile phases normally utilized[11] and, finally, a high organic modifier content of the mobile phase complicates the pre-treatment of natural samples.

These disadvantages made various groups of workers turn their attention to

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the use of polystyrene-based cation exchangers for saccharide separations. These resins allow the separation of simple monosaccharide mixtures on the basis of ligand exchange[12], whereas with oligosaccharides the principal mechanism is size exclusion[13]. In addition, the use of pure aqueous phases as the eluent enhances sensitivity in RI detection[9]. Detection limits with conventional LC detectors, however, still remain in the microgram range and the lack of resolution observed for oligosaccharides with the same degree of polymerization prevents the general application of the technique to samples from the food industry. The latter problem can be partly overcome if detection systems that differentiate between reducing and non-reducing sugars are used, as most food samples contain reducing disaccharides such as maltose or lactose in addition to the non-reducing sucrose. A step in this direction was proposed in an earlier paper[14] with the use of post-column solid-state catalytic reactors for the conversion of non-reducing sugars to reducing sugars. However, the time of analysis and some system parameters did not fulfil the requirements of modern column technology and further studies and optimization with regard to band broadening and the materials used in the post-column reactors were required, and were the subject of this investigation.

## THEORETICAL

Reaction band broadening, $\sigma_r$, caused by reactions occurring in a post-column reactor packed with a suitable catalyst, has recently been studied by Nondek *et al.*[15]. Their calculations have been shown to agree satisfactorily with experimental results obtained for the anion-exchanger-catalysed hydrolysis of N-methylcarbamates[16]. In this work, the scope of our research project was extended to include the catalytic hydrolysis of sugars in post-column reactors packed with strongly acidic cation exchangers.

When making the assumption that the capacity ratio of the reaction product, $k'_p$, is larger than that of the reactant, $k'_r$, one can derive[15] for the reaction band broadening

$$\sigma_r = \sqrt{0.125 \ln 2}\,(1 - k'_p/k'_r)/k_r \qquad (1)^*$$

Obviously, eqn. 1 can in principle be used to calculate the rate constant of the reaction, $k_r$. The validity of this equation is, however, based on assumptions[15] that may not always apply to real systems. In such instances, one will have to determine the rate constant by an independent method, for example, from the change of the $\text{reactant}_{in}/\text{reactant}_{out}$ ratio, $w_{in}/w_{out}$, with time. According to Langer and Patton[17], this ratio can be written as

$$w_{in}/w_{out} = \exp(k_M t_M + k_S t_S) \qquad (2)$$

where $k_M$ and $k_S$ are the rate constants of the reaction in the mobile and stationary phase, respectively, and $t_M$ and $t_S$ are the times spent in these phases. In the case considered here, no reaction takes place in the mobile phase. Therefore, with $t_S$ =

* In ref. 15, one should read $\sqrt{0.125 \ln 2}$ instead of $0.125\sqrt{\ln 2}$.

$t_R\ k'/(k' + 1)$ and $k_{app} = (1/t_R)\ln(w_{in}/w_{out})$, where $t_R = t_M + t_S$, one can now write for the apparent rate constant

$$k_{app} = k_S k'/(k' + 1) \quad (3)$$

Hence the true reaction constant, $k_S$ (which is identical with $k_r$ in eqn. 1 and, thus, in ref. 15), has now been related to two experimentally accessible parameters, $k_{app}$ and $k'$. It is important to add that in eqns. 2 and 3, $t_M$, $t_S$, $t_R$ and $k'$ all relate to the reactant.

## EXPERIMENTAL

The set-up for the post-column catalytic hydrolysis of the oligosaccharides (Fig. 1) consisted of the following components: a Model 110 A high-pressure reciprocating pump (Altex, Berkeley, CA, U.S.A.) equipped with a Bourdon-type pulse damper, a six-port injection valve (Rheodyne, Berkeley, CA, U.S.A.) with a 20-$\mu$l loop, a 6 × 0.4 cm I.D. reactor column and a 25 × 0.6 cm I.D. stainless-steel chromatographic column, both immersed in thermostated water-baths (Thermo-Bay, Lauda, F.R.G.), and a Model LC-75 variable-wavelength UV–visible spectrophotometer (Perkin-Elmer, Norwalk, CT, U.S.A.).

Three different polystyrene-type cation-exchange resins were supplied by United Chemical and Metallurgical Works (Ústí nad Labem, Czechoslovakia). These were designated as Ostion LGKS 0800 (8% cross-linking; 10-$\mu$m particles), Ostion 0803 (8% cross-linking; 25-$\mu$m particles) and an experimental batch (4% cross-linking, 17-$\mu$m particles). All three resins are sulphonic acid-type styrene–divinylbenzene copolymers and can also be obtained with similar specifications from other manufacturers[18]. Another type of strongly acidic cation exchanger having sulphonic acid

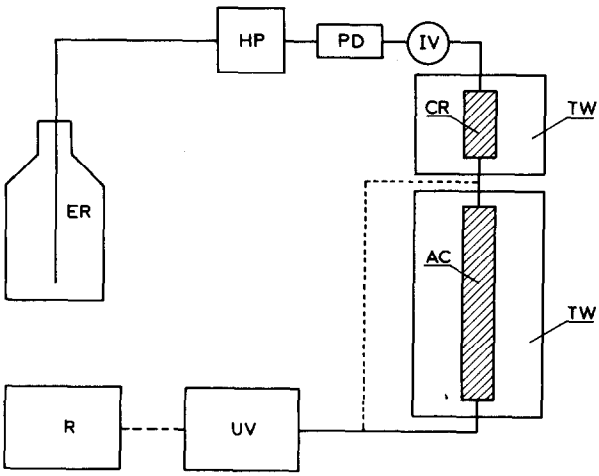


Fig. 1. Instrument set-up used for evaluation of the catalytic post-column reactor, *i.e.*, for determination of the rate of hydrolysis (full line) and band broadening of the reactor (broken line). ER, Eluent reservoir; HP, high-pressure pump; PD, pulse damper; IV, injection valve; CR, catalytic reactor; TW, thermostated water-bath; AC, analytical column; UV, UV absorbance detector; R, recorder.

groups covalently bound to a silica matrix (Ionenaustauscher CX) was obtained from Merck (Darmstadt, F.R.G.). Whereas the Merck material was supplied in the hydrogen form, the other resins had to be converted into this form by washing with 2 *M* HCl on a sintered-glass disc. Ostion LGKS 0800 was also used in its calcium form (conversion with 1 *M* $CaCl_2$) as the packing material in the analytical column.

The 8% cross-linked polystyrene cation exchangers were packed as follows: a 50% (v/v) slurry in degassed, deionized water was added to a slurry reservoir and packed downwards under constant pressure (200 bar) using water as the packing liquid. The 4% cross-linked cation exchanger was packed similarly, but using a lower pressure (25 bar).

The silica-based cation exchanger was suspended in isopropanol by ultrasonication and introduced into a slurry vessel, to which an empty column filled with tetrachloromethane containing 5% of methanol had been attached. Packing was conducted by pumping methanol under a maximum pressure of 400 bar.

The set-up shown in Fig. 1 was used in two different ways. For the determination of band broadening in the reactor, the effluent from the catalytic reactor column was led directly to the detector broken line). For the determination of the rate of reaction the reactant and product present in the reactor effluent were first separated using an analytical column inserted between the reaction column and the detector. In both instances, direct measurement of UV absorbance at 195 nm was used for detection.

## RESULTS

### *Band broadening in the reactor*

A solid-phase reactor that retains the analytes on its surface to a certain extent can be considered as a chromatographic column connected in series to the analytical column. In the absence of a reaction, band broadening is then solely a function of chromatographic parameters and was investigated with fructose as a non-reacting saccharide by measuring the chromatographic efficiency of the various materials of interest in terms of theoretical plate numbers. The reactor dimensions were 6 × 0.4 cm I.D.; the flow-rate was kept constant at 0.5 ml $min^{-1}$.

The influence of temperature on the various cation-exchange materials was found to be pronounced, with a five-fold increase in plate number, from 235 at 25°C to 1190 at 80°C, for the 4% cross-linked resin, and about a three-fold increase for both 8% cross-linked resins.

The influence of flow-rate on the chromatographic efficiency of the reactor columns was tested at 85°C for flow-rates of 0.2–1.0 ml $min^{-1}$. The variations were small, with an optimum in the range 0.4–0.6 ml $min^{-1}$ for the 4% cross-linked resin and no clear dependence for the others. Similar observations were made for other monosaccharides. The general consensus was that the chromatographic efficiency decreased for all the non-reacting sugars tested in the order 4% cross-linked polystyrene (17-$\mu$m particles) $\gg$ 8% cross-linked polystyrene (10- or 25-$\mu$m particles) $>$ silica-based cation exchanger (10-$\mu$m particles).

The retention characteristics of the monosaccharides and the non-hydrolysable cellobiose were found to be independent of temperature (Table I). Similar behaviour was assumed for sucrose and raffinose, although their values cannot be measured

TABLE I

ELUTION VOLUMES OF SACCHARIDES ON DIFFERENT CATION EXCHANGERS ($H^+$ FORM)

Mobile phase, distilled water; reactor dimensions, 6 × 0.4 cm I.D.

| *Compound* | *Cation exchanger** | | | |
|---|---|---|---|---|
| | *8% cross-linking, 10 μm* | *8% cross-linking, 25 μm* | *4% cross-linking, 17 μm* | *Silica matrix, 10 μm* |
| Cellobiose | – | 0.329 (b) | 0.488 | – |
| Fructose | 0.507 (b) | 0.435 (b) | 0.634 (b) | 0.824 (c) |
| Glucose | – | 0.408 (b) | 0.597 (b) | – |
| Raffinose | – | 0.317 (a) | 0.443 (a) | 0.794 (a) |
| Stachyose | – | 0.292 (a) | 0.395 (a) | – |
| Sucrose | 0.417 (a) | 0.346 (a) | 0.503 (a) | 0.814 (a) |

* (a) Values for 27°C; (b) average values for the range 27–85°C, measured at 5°C intervals; (c) average values for the range 60–100°C, measured at 5°C intervals. Relative standard deviation for (b) and (c): 1–2%.

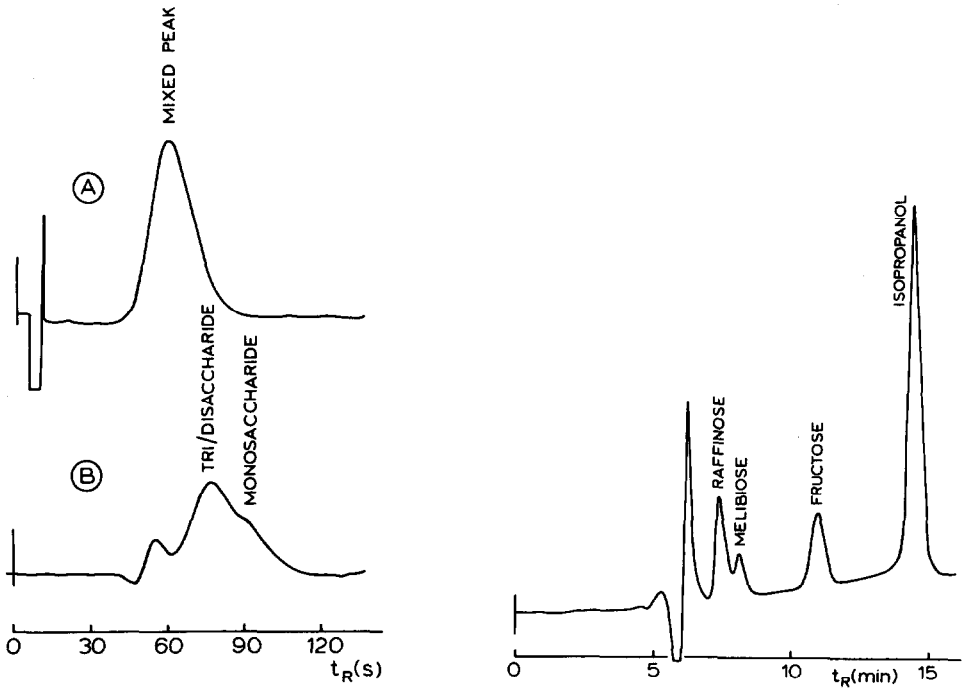


Fig. 2. Hydrolysis of raffinose recorded by using the broken-line set-up in Fig. 1; UV detection at 195 nm. Reaction conditions: 6 cm × 4 mm I.D. stainless-steel reactor packed with sulphonic acid-type ($H^+$) polystyrene cation exchanger (A, 8% cross-linked, 25-μm particles; B, 4% cross-linked, 17-μm particles); reaction temperature, 60°C; flow-rate, 0.5 ml $min^{-1}$.

Fig. 3. Hydrolysis of raffinose (10% in isopropanol) recorded by using the full-line set-up in Fig. 1; UV detection at 195 nm; catalytic reactor conditions as in Fig. 2B. Analytical system: 25 cm × 6 mm I.D. column packed with 10-μm 8% cross-linked sulphonic acid-type ($Ca^{2+}$) polystyrene cation exchanger; eluent, distilled water at 0.5 ml $min^{-1}$; temperature, 85°C.

reliably at higher temperatures as they hydrolyse significantly. As a result, the chromatographic peaks detected by UV at 195 nm are composed of reaction product(s) and remaining reactant (see Figs. 2 and 3). The apparent change in the peak width of hydrolysable oligosaccharides as a function of temperature was therefore always greater than for monosaccharides.

For sucrose, the chromatographic efficiency on 8% cross-linked material increases 2.5-fold on going from room temperature to 85°C. The plate numbers are consistently lower than those for fructose and glucose. Even at room temperature lower efficiencies are measured for sucrose on all materials tested, *i.e.*, $N = 75$–$120$ for sucrose compared with $N = 120$–$205$ for glucose and $N = 135$–$235$ for fructose. Hence molecular size also seems to play a role, as further substantiated by measurements with raffinose ($N = 65$) and the tetrasaccharide stachyose ($N = 45$) on 8% cross-linked material.

The higher efficiency of the 4% cross-linked ion exchanger is also apparent from Fig. 2, where partial hydrolysis of raffinose was monitored without a separation column (Fig. 1, broken line). Partial resolution of the reactant and products can be observed with the 4% material but only a single (mixed) peak with 8% cross-linked polystyrene. Further separation of the reaction mixture using the reactor with the analytical column (Fig. 3) reveals the presence of a UV-absorbing contaminant eluting before raffinose. Much greater amounts of UV-absorbing impurities contained in standards of some other saccharides (maltose, lactose, cellobiose) have rendered the measurement of their band broadening in the reactor difficult.

Although expressing band broadening in the reactor in terms of theoretical plates is illustrative, it is more important to consider the variance of peaks in the reactor[15]. In the present system it was generally impossible to determine the contribution of the reaction band broadening to the total peak variance in the reactor by the method of Nondek *et al.*[15], because (1) the variances of the reactant and product(s) are not equal, (2) sugars of interest yield two reaction products that differ as regards their column efficiency and photometric response, and also their retention volume in the reactor and (3) in most instances the reaction does not go to completion, yielding a mixed peak of reactant and products not distinguishable by direct UV photometry. One exception to this conclusion is discussed below.

Plotting the total band broadening, $\sigma_{total}$, in the system including the sample injection valve, catalytic reactor and UV detector (Fig. 1, broken line) against the volumetric flow-rate gave for a given saccharide and temperature almost identical curves for all polystyrene packings. However, when the $\sigma_{total}$ values (shown for sucrose in Fig. 4) were time-normalized, *e.g.*, plotted against the residence time of the injected saccharide in the reactor, differences between different polystyrene ion exchangers in the region of low flow velocities (high $t_R$) become clearly evident: less cross-linked resin gives less band spreading and, as expected from earlier observations, higher temperatures result in reduced band broadening. The dependence of $\sigma_{total}$ on $t_R$ was found to be fairly linear in the tested range of flow-rates and the optimum position of lines was thus determined by means of linear regression.

When eqn. 1 is used for the calculation of reaction band broadening for sucrose under conditions of full hydrolysis (85°C), taking into account only fructose as reaction product (glucose and fructose are formed in equimolar amounts, but the UV response of glucose under our experimental conditions was about six times lower

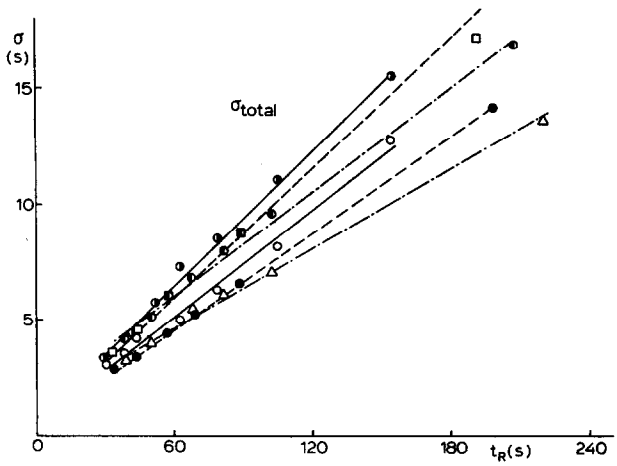


Fig. 4. Time-normalized dependence of reactor (including detector) band broadening on flow-rate for a 6 cm × 4 mm I.D. packed-bed reactor; flow-rate expressed in terms of $t_R$, the time spent by the test solute, sucrose, in the reactor. Reactor conditions (temperature, particle size, percentage cross-linking): ◑, 60°C, 25-μm, 8%; □, 60°C, 10-μm, 8%; ◐, 60°C, 17-μm, 4%; ○, 85°C, 25-μm, 8%; ●, 85°C, 10-μm, 8%; △, 85°C, 17-μm, 4%.

than that of fructose) one obtains $\sigma_r$ values of about 1–2.5 sec. These are virtually independent of flow-rate, which is in agreement with earlier results[15]. However, even if this earlier conclusion is borne out, there can be no doubt that the true $\sigma_R$ values are different from those reported here. This can be demonstrated, for example, by calculating the reaction band broadening for sucrose at 27°C, when hydrolysis is negligible. Using the above method of calculation yields a value of 1–1.3 sec, which is close to the reaction band broadening of sucrose at 85°C mentioned before. In other words, it seems likely that the true $\sigma_r$ values are substantially lower than the 1–2.5 sec reported in this paragraph.

The silica-based ion exchanger displays a markedly greater retention of sugars than do polystyrene resin (see Table I); this is probably due to the presence of residual silanol groups. On the other hand, the selectivity differences of this cation exchanger towards various sugars were negligible, which should result in virtual elimination of reaction band broadening. Unfortunately, its efficiency was so poor and the high affinity of the silica matrix for sugars resulted in such broad peaks as to render this type of material unsuitable for combination with high-efficiency columns.

*Hydrolytic activity of the reactor*

It has been demonstrated earlier[14] that the hydrolytic activity of a catalytic reactor packed with a polymer-type ion-exchange resin depends on the degree of polymerization of the saccharide and on the type of its glycoside bands. As the main purpose of the reaction column is to allow for the determination of sucrose and possibly of structurally similar non-reducing higher oligosaccharides such as raffinose and stachyose, most attention was given to the hydrolysis of sucrose and a limited number of experiments were performed with raffinose. For these two oligosaccharides, an acceptable resolution of reactant and reaction products was obtained by using an analytical column in sequence after the reaction column. This order of

columns is, of course, different from that used in an operational analytical system for the determination of saccharides, in which separation precedes conversion in the catalytic reactor and final detection (see below).

The dependence of sucrose (saccharose) hydrolysis on temperature in reactors filled with various types of polystyrene-based cation-exchange resins ($H^+$ form) is shown in Fig. 5. For a given flow-rate (0.5 ml $min^{-1}$) 100% conversion was reached at 75°C using the 4% cross-linked resin, whereas both types of 8% cross-linked resin required a further 10°C increase in temperature to obtain complete hydrolysis.

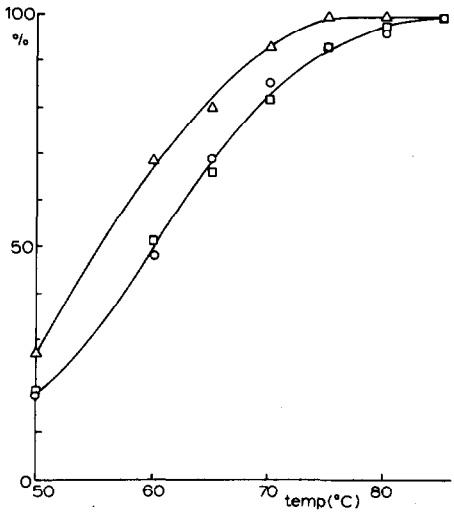


Fig. 5. Dependence of efficiency of sucrose hydrolysis on temperature in a 6 cm × 4 mm I.D. reactor packed with polystyrene-based cation exchangers; flow-rate, 0.5 ml $min^{-1}$. △, 17-μm, 4% cross-linking; ○, 10-μm, 8% cross-linking; □, 25-μm, 8% cross-linking.

In order to study hydrolysis as a function of flow-rate, plots of $\ln(\text{reactant}_{in}/\text{reactant}_{out})$ *versus* the residence time of the chosen saccharide in the reactor were constructed (Fig. 6). As in Fig. 4, these $t_R$ values were assumed to be essentially the same as those recorded at 27°C (see Table I). Plotting the results as in Fig. 6 has the following advantages: (1) with first-order kinetics straight lines should be obtained; (2) apparent rate constants can be calculated from their slopes; and (3) the results are time-normalized for all ion exchangers, thus eliminating the influence of small variations in flow-rate.

Besides verifying the first-order kinetics nature of the reaction, the results in Fig. 6 show that the rate of hydrolysis using a less cross-linked resin is really more rapid (see Fig. 5) and that the differences in the degree of sucrose decomposition cannot be attributed primarily to differences in retention. Again, no difference between the two particle sizes of 8% cross-linked resin was observed. Comparative data for the hydrolysis of raffinose shown in Fig. 6 demonstrate that the decomposition of this trisaccharide proceeds at a lower rate than the hydrolysis of sucrose even when differences in retention are compensated for.

Apparent rate constants, $k_{app}$, calculated for the hydrolysis of sucrose at 60°C

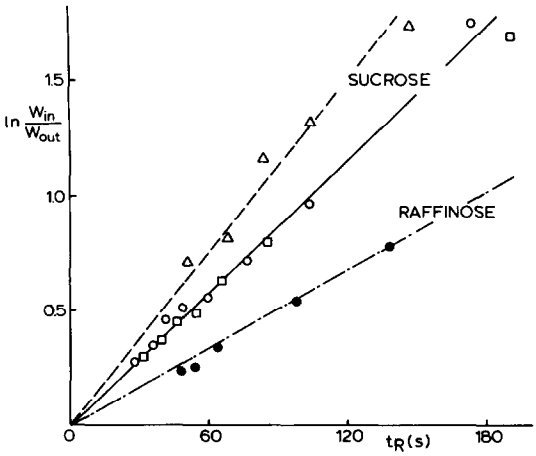


Fig. 6. Influence of flow-rate (residence time) on the hydrolysis and verification of the first-order reaction rate for the hydrolysis of oligosaccharides catalysed by polystyrene-based cation exchangers. △, Sucrose, 17-μm 4% cross-linked particles; ○, sucrose, 10-μm 8% cross-linked particles; □, sucrose, 25-μm 8% cross-linked particles; ●, raffinose, 17-μm 4% cross-linked particles. Temperature, 60°C.

with reactors filled with polystyrene cation exchangers were $1.27 \cdot 10^{-2}$ (4% cross-linking) and $9.5 \cdot 10^{-3}$ (combined data for 10- and 25-μm 8% cross-linked resin). The rate constant for the hydrolysis of raffinose to fructose and melibiose was $5.6 \cdot 10^{-3}$ for the 4% cross-linked resin. Hydrolysis using a silica-based ion exchanger proceeds at a slower rate. For sucrose hydrolysis the results for 95°C are close to those obtained for the 8% cross-linked resins at 60°C and an apparent rate constant of about $8.7 \cdot 10^{-3}$ was calculated from the slope of the sucrose curve. The results for raffinose, on the other hand, are similar to those for the 4% cross-linked resin ($k_{app} = 6.8 \cdot 10^{-3}$), indicating that the silica matrix eliminates to a certain extent the differences among saccharides caused by the different molecular size. Unfortunately, complete hydrolysis of sucrose or raffinose to two reducing subunits was never achieved on a silica-based ion exchanger over the tested temperature interval (60–100°C). The Arrhenius plots (Fig. 7) constructed for polystyrene resins are slightly curved which, in earlier work[15], has been attributed to the restrictions of mass transfer in the stationary phase. From a practical point of view, it can be concluded from these data that increasing the reaction temperature above 85°C offers only limited advantages as regards the speed of hydrolysis.

*Analytical systems*

The instrumental set-up used for testing the heterogeneous catalytic post-column reactors (Fig. 1) can be converted to an analytical system for carbohydrate determination by reversing the sequence of columns. The sample is introduced and separated on the analytical column, then enters the catalytic reactor and is finally detected via a photometric or fluorigenic reaction for reducing saccharides. When used in this mode, the present column–reactor combination, with 17-μm 4% cross-linked polystyrene cation exchanger as the reactor packing, allows at least a two-fold reduction in the analysis time compared with previous results[14], together with a

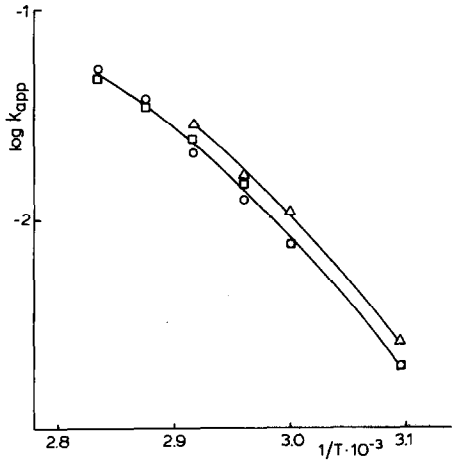


Fig. 7. Arrhenius plots for the hydrolysis of sucrose on 17-μm 4% cross-linked (△), 10-μm 8% cross-linked (○) and 25-μm 8% cross-linked (□) polystyrene-based cation exchangers. Flow-rate, 0.5 ml $min^{-1}$.

substantial improvement in the raffinose–sucrose separation. A typical chromatogram obtained by this approach is shown in Fig. 8. In this instance, reaction with the cyanoacetamide reagent in 0.3 *M* borate buffer[19] followed by UV photometry at 276 nm was used for detection step. Other derivatization procedures are presently

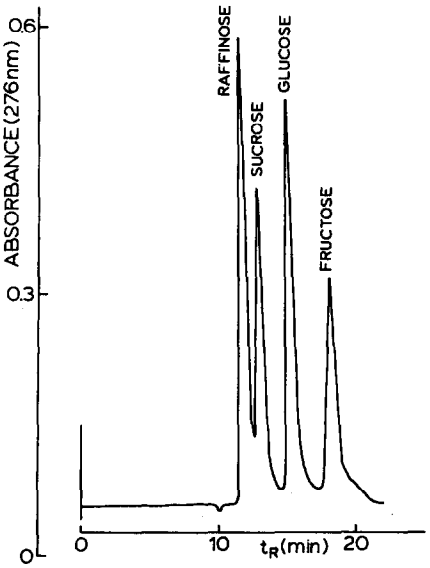


Fig. 8. HPLC of sugar mixture using post-column catalytic hydrolysis and cyanoacetamide reaction detection. Amounts injected: raffinose, 37 μg; sucrose, 18 μg; glucose, 16 μg; fructose, 365 μg. Conditions: 25 cm × 6 mm I.D. analytical column packed with Ostion LGKS 0800 ($Ca^{2+}$) and 6 cm × 4 mm I.D. catalytic reactor column packed with 4% cross-linked Ostion LGKS ($H^+$); eluent, distilled water at 0.3 ml $min^{-1}$; temperature of both columns, 85°C. Detection: 0.2 ml $min^{-1}$, addition of 0.5% cyanoacetamide in 0.3 *M* borate buffer (pH 8.0); 2-min residence time in reaction coil at 100°C; detection at 276 nm.

being investigated in order to obtain a higher sensitivity and an improved response for fructose[20].

## DISCUSSION AND CONCLUSIONS

Our results demonstrate that the 4% cross-linked polystyrene resin has the best mass transfer characteristics and catalytic activity of all the materials tested. Here, one should also consider the observation[21] that less cross-linked resins form more tightly packed beds than more cross-linked resins, which can also result in improved column efficiency.

Because of the nature of the reaction investigated, *i.e.*, the relative retention and the number of products formed, it was not possible to calculate the reaction band broadening using the method of Nondek *et al.*[15]. Recently, other workers[22] suggested a simple approach for distinguishing a single exponential modifier (for example, the contribution of the reaction band broadening) of a Gaussian peak. Unfortunately, in the resin-catalyzed post-column hydrolysis of sugars the non-Gaussian modifiers of the peak shape are of two different types. The first is the usual chromatographic tailing, which affects the tail end of all peaks. The reaction band broadening, on the other hand, affects the leading edge of the peaks, as the reactant has a lower capacity factor than has the reaction product, thereby improving the symmetry of the peaks.

The differences in the rate of hydrolysis between resins having different degrees of cross-linking cannot easily be explained. In addition to the substantial differences in activity coefficients in the gel phase among resins having different degrees of cross-linking and the possible steric hindrance as regards the orientation of saccharide molecules towards active sites in the more cross-linked exchanger, one could propose a speculative explanation, *viz.*, that the ion-exchange groups act simultaneously as catalyzing groups and as active sites exhibiting a chromatographic effect. The greater difference in the retention of the reactants and products in the less cross-linked resin may thus allow a more effective separation of reaction products from the moving reaction zone, which could otherwise be partially passivated by binding the reaction product.

The data in Fig. 7 indicate a possible role of the resistance to mass transfer in the stationary phase. However, they should be evaluated with care because of the notorious low precision of a rate constant determination based on measurements at a single reaction time[21]. The quantitative criterion for testing the role of the mass transfer resistance proposed by Weisz and Prater[23] cannot be applied because of the lack of reliable data on the diffusion of large saccharide molecules into the swollen resin. Data obtained for amino acids on an 8% cross-linked resin[24] indicate a strong dependence of diffusion coefficients on small changes in the molecular size.

The applicability of the relatively soft 4% cross-linked polystyrene cation exchanger for reactors used in post-column reaction detectors is limited by its pressure stability of 20–25 bar. The back-pressure of all detection system parts situated behind the catalytic reactor (reaction coil, detector cell and, possibly, the back-pressure device) must not exceed this value.

Finally, it can be concluded that the catalytic hydrolysis principle permits an efficient simultaneous analysis of non-reducing and reducing sugars, the final per-

formance being dependent on the proper coupling of the catalytic solid-state reactor with a suitable chromophore- or fluorophore-producing homogeneous detection system. These aspects have been examined in detail elsewhere[20].

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