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INFLUENCE OF MUTAROTATION CATALYSTS ON THE LIQUID CHROMATOGRAPHY OF MALTO-OLIGOSACCHARIDES ON A CATION-EXCHANGE RESIN

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SUMMARY

To avoid double peaks due to α- and β-anomers in complicated chromatograms of carbohydrate mixtures, mutarotation catalysts may be applied.

The effects of the addition of alkaline mutarotation catalysts to the aqueous mobile phase on the separation of malto-oligosaccharides at different separation temperatures are described. Addition of the catalyst results in a symmetrical and narrower peak for each malto-oligosaccharide tested. However, although the peak shape was improved, separation of a malto-oligosaccharide mixture at room temperature was not satisfactory, due to the peaks still being too broad. At elevated temperatures formation of isomerization products occur in the alkaline mobile phase during the separation.

The best chromatograms of malto-oligosaccharides mixtures were obtained with a cation-exchange resin in the calcium form using a neutral aqueous mobile phase and a column temperature of 90°C.

INTRODUCTION

Efficient liquid chromatographic separations of carbohydrates are often performed on a cation-exchange resin in the calcium form at elevated temperatures[1–4]. The separation mechanism is based on complex formation between the hydroxyl groups of the carbohydrates with the immobilized Ca^{2+} ions on the resin[2,5,6] and on size exclusion effects[7].

At room temperature the mutarotation of many reducing sugars is low. Consequently, the chromatographic separation of a mixture of carbohydrates at room temperature on a cation-exchange resin will result in a needlessly complicated chromatogram with double peaks and/or peaks with shoulders due to the (incomplete) separation between the α- and β-anomers of the respective sugars. Increasing the temperature increases the mutarotation by a factor of about 2.5 for every 10°C temperature rise[8]. As a result of the fast mutarotation, at elevated temperatures the α- and β-anomers of the respective carbohydrates elute together in one peak[1–4,7].

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Recently, Verhaar and Kuster[9] and Verhaar[10] reported the application of a mutarotation catalyst in the chromatographic separation of carbohydrates. Addition of 0.001 *M* triethylamine (TEA) to the mobile phase catalyses the mutarotation of the reducing sugars at room temperature during the separation on the cation-exchange column. No splitting in the α- and β-anomer peaks occurred and useful sugar chromatograms could be obtained at room temperature.

Verhaar and Kuster restricted themselves to the separation of monosaccharides such as glucose, fructose, mannose and galactose[9]. In the starch industry, separations between malto-oligosaccharides are more important. For this reason, we studied the effect of several catalysing additives to the mobile phase on the efficiency of the separation of malto-oligosaccharides on a cation-exchange resin in the Ca^{2+} form.

It is well known that the mutarotation of reducing sugars is catalysed by both acids and bases[8,11–13]. In this study, no acid catalysts were tested because acid removes the Ca^{2+} ions from the cation-exchange resin and thus deteriorates the chromatographic separation. As discussed by Verhaar and Kuster[9], the TEA-containing mobile phase also displaced gradually the Ca^{2+} ions from the column, resulting in a continuous decrease in separation efficiency. Therefore, a column regeneration procedure was required after every 2 l of eluent pumped through the column. To avoid these time-consuming column regeneration procedures when using TEA, we also used a dilute calcium hydroxide solution. In order to maintain the previously reported benefits of a calcium ethylenediaminetetraacetate (CaEDTA)-containing mobile phase[8], solutions of CaEDTA in dilute calcium hydroxide were also applied.

EXPERIMENTAL

All separations were performed on a Hewlett-Packard 1084B liquid chromatograph equipped with an automatic sample introduction system (sample volume 50 μl), column oven and Hupe Busch 1033 differential refractometer detector (maintained at 37°C). A 25 cm × 9.0 mm I.D. column was packed with the cation-exchange resin AG 50W-X4 (Bio-Rad Labs., particle size 20–30 μm) in the calcium form[7].

The following solutions were used as mobile phases: pure water, an 50 ppm aqueous solution of CaEDTA (pH 6.2), a 250 ppm aqueous solution of CaEDTA (pH 6.4), a dilute aqueous solution of calcium hydroxide (pH 10.6), a 50 ppm solution of CaEDTA in diluted calcium hydroxide solution (pH 10.6) and a 0.001 *M* aqueous solution of TEA (pH 10.2).

The test samples were glucose, maltose, maltotetraose and an acid-hydrolysed potato starch syrup (5%, w/v).

Separations have been carried out at different temperatures between 30 and 90°C. However, for mobile phases containing TEA, a maximum column temperature of 70°C was applied because of the low boiling point of TEA.

RESULTS AND DISCUSSION

Glucose

The effects of both the composition of the mobile phase and the column temperature on the glucose chromatograms were studied.

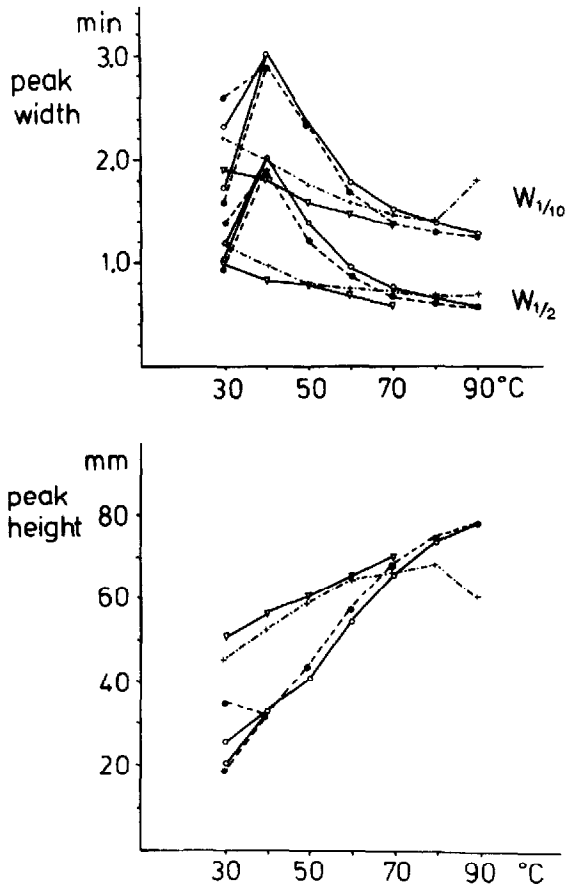

Fig. 1. Effect of the column temperature and the composition of the mobile phase on the peak height and the peak width at 50% and 10% of the peak height ($W_{1/2}$, $W_{1/10}$) in the glucose chromatograms. Composition of mobile phase: ●----●, water; ○——○, 50 ppm CaEDTA; ▽——▽, 0.001 *M* TEA (pH 10.2); + .–. +, 50 ppm CaEDTA in $Ca(OH)_2$ (pH 10.6).

Fig. 1 shows the influence of column temperature on peak height and peak width for the different mobile phases and Fig. 2 shows the effect of the composition of the mobile phase on the chromatograms using a column temperature of 30°C. In Fig. 3 the effect of column temperature on the shape of the glucose peak, using a neutral or alkaline mobile phase, is demonstrated.

The catalytic effect of alkali on the mutarotation at room temperature is evident. However, at elevated temperatures the glucose peak in the chromatograms obtained with the alkaline mobile phases becomes distorted (Figs. 1 and 3). It is known that in aqueous alkaline solution transformation and degradation reactions with reducing sugars occur. The Lobry de Bruin-Alberda van Ekenstein transformation of glucose results in numerous products, including fructose, mannose and psicose[8,12–15]. As shown by Kainuma and Suzuki[16], higher temperatures accelerate the alkaline isomerization considerably. It is possible to isomerize about 24% of the glucose originally present in 20 min at 71°C. Taking into account that it takes18–19

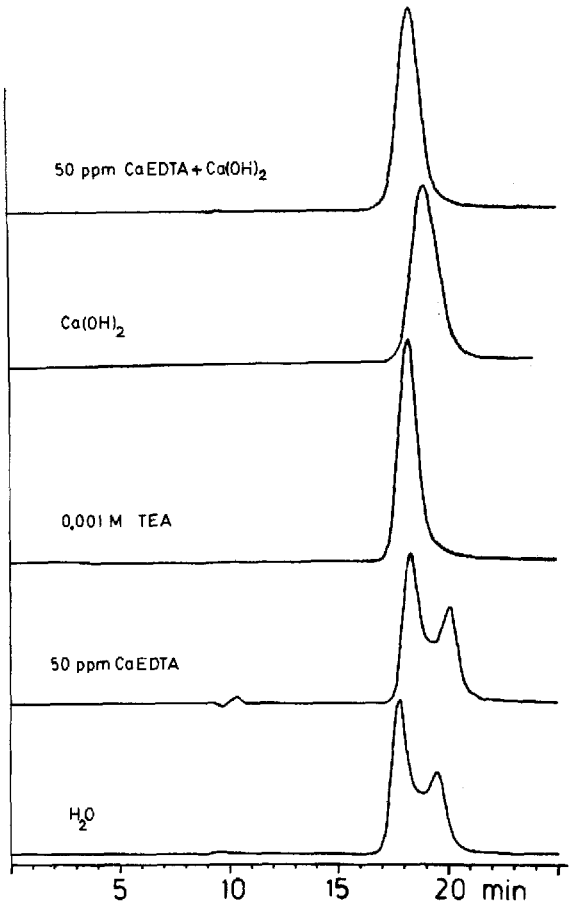

Fig. 2. Effect of the composition of the mobile phase on the glucose chromatogram at 30°C. Samples: 50 μl of 1 % glucose solution.

min to elute glucose from the chromatographic column (Fig. 3), it is obvious that the above-mentioned isomerization reactions in the alkaline mobile phase at elevated temperatures cannot be neglected.

Isomerization of glucose during the separation caused peak tailing, probably because of fructose formation (increase in $W_{1/10}$) and subsequently a decrease in the glucose peak height (Fig. 1). Fig. 3 shows the distorted glucose peaks that were obtained by using alkaline CaEDTA solution as the mobile phase. On the other hand, high temperatures increase the mutarotation considerably and, at the same time, decreases the viscosity of the aqueous mobile phase significantly. According to the Wilke–Chang equation[17], both a temperature rise and a decrease in viscosity result in higher diffusion coefficients of the compounds to be separated. Hence both the increased mutarotation and the decreased viscosity give rise to narrower and thus higher peaks. Therefore, applying a neutral mobile phase in combination with a column temperature of 80–90°C results in narrower and higher glucose peaks than applying a mobile phase with a mutarotation catalyst at room temperature (Fig. 1).

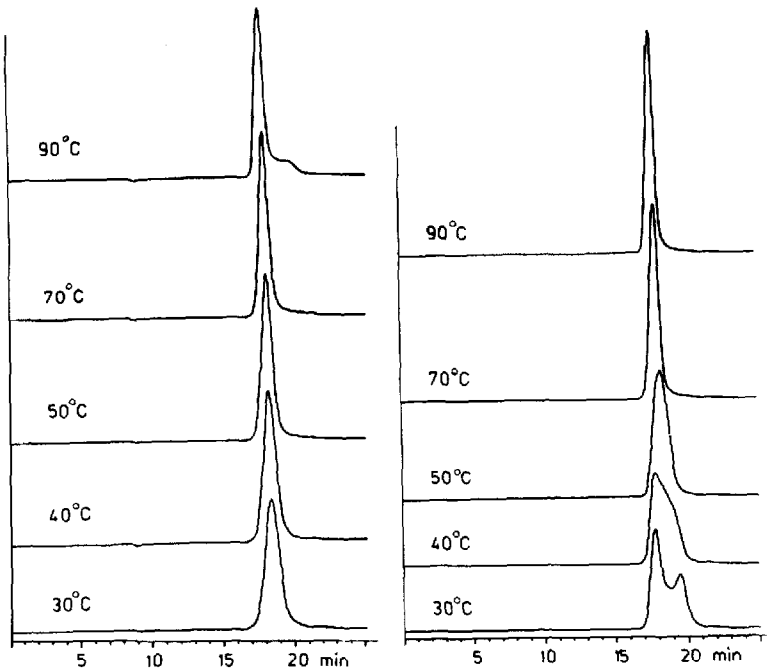

Fig. 3. Effect of the column temperature on the glucose chromatograms. Test sample: 50 µl of 1 % glucose solution. Right: pure water as mobile phase. Left: 50 ppm solution of CaEDTA in $Ca(OH)_2$ (pH 10.6).

A remarkable effect was that at neutral pH and temperatures lower than 40°C, addition of CaEDTA to the mobile phase improved the separation between the α- and β-glucose anomers (Fig. 2), especially at higher CaEDTA concentrations (*e.g.*, 250 ppm).

Maltose

The effects of the composition of the mobile phase and the column temperature on the maltose chromatograms were similar to the effects found with glucose, although less pronounced. This is not unexpected, because the maltose anomers differ only in the geometric configuration of one hydroxyl group in one of the two glucose units. For a similar reason there is also less chromatographic difference between maltose (4-O-α-D-glucopyranosyl-D-glucose) and its isomerization product maltulose (4-O-α-D-glucopyranosyl-D-fructose) than between glucose and fructose. Under the chromatographic conditions usually applied[7], glucose and fructose are separated completely, but maltose and maltulose are not. Therefore, the isomerized maltose peak in the chromatograms seems less distorted than the isomerized glucose peak using a warm alkaline mobile phase.

A neutral mobile phase and a column temperature of less than 40°C result in the elution of a double peak or a peak with a shoulder. The catalytic effect of alkaline mobile phases on the mutarotation was demonstrated by the elution of nearly Gauss-shaped chromatographic peaks of maltose at room temperature. The peak appeared to be narrower and thus higher than those obtained by using a neutral mobile phase.

Almost the same plot as shown for glucose in Fig. 1 could be obtained for maltose. As for glucose, the best chromatograms for maltose were obtained by applying a neutral mobile phase in combination with a column temperature of 90°C.

Maltotetraose

The anomer forms of maltotetraose resemble each other even more closely than the maltose anomers. Therefore, the results were essentially the same as for maltose, but again less pronounced.

Hardly any peak deformation due to isomerization reactions at elevated temperatures in the alkaline mobile phases could be observed. This does not necessarily indicate that no isomerization of the maltotetraose occurs. One of the main isomerization products in aqueous alkaline solution will be maltotetraulose, in which the end-stranding glucose unit is transformed into a fructose unit. This transformation does not change the molecular weight of the molecule and thus the effect on the size-exclusion separation mechanism on the chromatographic column is negligible. Although the complexability of a fructose unit with the Ca^{2+} ions of the resin is stronger than that of a glucose unit, it is believed that for maltotetraose and maltotetraulose this is of minor importance. Hence it can be concluded that a transformation reaction of maltotetraose into maltotetraulose on the chromatographic column will not distort the peak shape.

The chromatograms of maltotetraose recorded at 30°C with a neutral mobile phase show a peak with a shoulder. This shoulder was again enhanced by the addition of CaEDTA to the mobile phase. The catalytic effect of the alkaline mobile phases at relative low column temperatures is obvious: the peak width is reduced and the peak height is increased compared with the use of a neutral mobile phase at the same column temperature. However, the highest and narrowest maltotetraose peak is obtained again by simply applying a column temperature of 90°C and a neutral (CaEDTA-containing) mobile phase.

Acid-hydrolysed potato starch

As shown above, addition of an alkaline mutarotation catalyst as TEA to the mobile phase results in the elution of a single Gaussian-shaped peak at room temperature for each malto-oligosaccharide tested. However, the eluted peaks were relatively broad compared with those obtained by applying a neutral mobile phase at 80 or 90°C. Therefore, it was not possible to obtain at room temperature a well developed chromatogram of an acid-hydrolysed potato starch solution containing a homologous series of malto-oligosaccharides (Fig. 4). If, however, a comparison is made between the use of TEA-containing and a neutral mobile phase at the same low temperature then, owing to the catalytic effect of TEA on the mutarotation, the chromatograms of a TEA-containing mobile phase were significantly better than those obtained with a neutral mobile phase. In the latter instance the α- and β-glucose anomers partly coincide with the maltose peak. At a column temperature of 50°C a TEA-containing mobile phase still results in a superior separation compared with a neutral mobile phase at 50°C. However, the best chromatograms of mixtures of malto-oligosaccharides were recorded by using a neutral (50 ppm CaEDTA-containing) mobile phase at 90°C, as described before[7]. Almost the same chromatographic resolution was achieved by applying the TEA mobile phase at 70°C, but owing to the

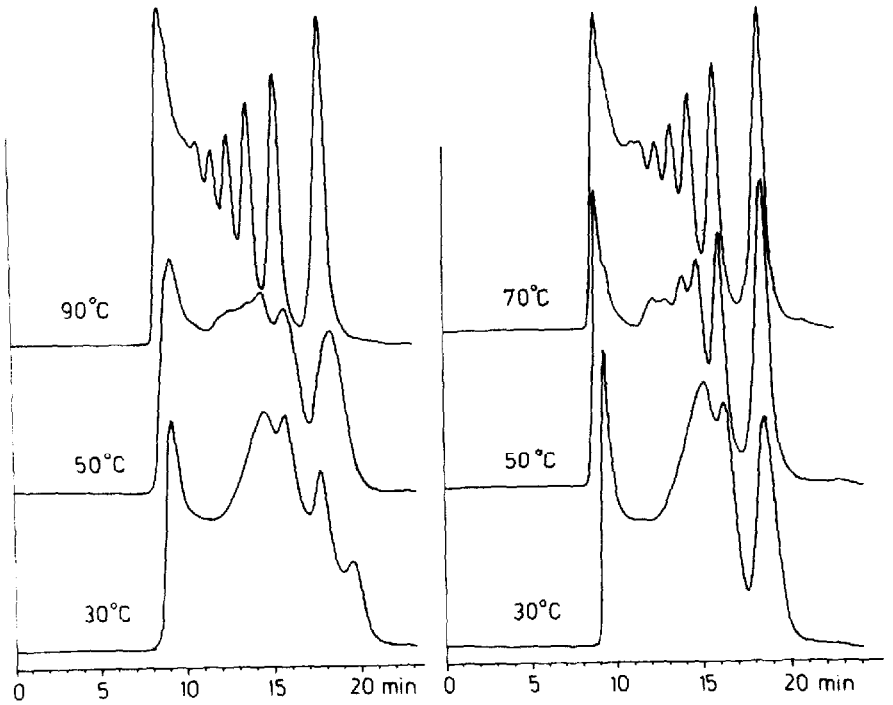

Fig. 4. Chromatograms of acid-hydrolysed potato starch solutions (5%, w/v) at different temperatures using pure water (left) or 0.001 *M* TEA solution (pH 10.2) (right) as the mobile phase.

formation of isomerization products (*e.g.*, at the foot of the glucose peak, Fig. 4) the chromatograms no longer represent the original composition of the samples being analysed.

CONCLUSIONS

Contrary to the experiences of Verhaar and Kuster, dealing with the separation of different monosaccharides[14], addition of an alkaline mutarotation catalyst to the mobile phase does not improve the chromatography of malto-oligosaccharides in such a way that separation can be performed at room temperature instead of 90°C.

Moreover, at elevated temperatures the formation of isomerization products occurs when an alkaline mobile phase is used and the TEA-containing mobile phase will gradually displace Ca^{2+} from the cation-exchange resin, which results in a continuous decrease in separation efficiency[14]. Therefore, a regular column regeneration procedure is required.

The best chromatograms of mixtures of malto-oligosaccharides were obtained by applying a neutral 50 ppm CaEDTA solution as the mobile phase and a column temperature of about 90°C, as described before[7]. Apparently, at neutral pH heat is as good a mutarotation catalyst without causing isomerization and/or degradation reactions. Moreover, owing to the elevated temperatures the viscosity of the mobile phase decreases significantly and thus the diffusion coefficients of the compounds

separated increase (Wilke–Chang equation), resulting in narrower and therefore higher peaks.

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