CHROM. 4013

Reproducible separation of α- and β-cyclodextrin on charcoal columns

Separation of cyclodextrins by means of column chromatography has already been the subject of investigation in our laboratory and has resulted in a communication[1] describing the separation of α- and β-cyclodextrin (CD) on a cellulose column. Although analysis of cyclodextrins on a cellulose column gave good results, the maintenance of good separation characteristics was sometimes difficult in spite of protective measures. Therefore, a more reliable method has been developed using gradient elution on charcoal.

Many separations of sugar mixtures on charcoal have already been described in the literature[2–7]. However, a method of obtaining reproducible separations has not yet been published.

We found that reproducible results can be obtained by flotation of the charcoal in water until free from very small particles, before packing the column. The column can then be used many times if it is regenerated after each separation by washing with water until free of "gradient solute".

In view of the results obtained preparative column chromatography, using large columns, seems possible. Preliminary results of this method are also given.

Experimental

Materials. Charcoal: Darco G-60 batch no. 18110 (Atlas Chem. Ind., Wilmington, Del., U.S.A.). The charcoal is freed from very small particles by flotating 100 g of charcoal in demineralised water as shown in Fig. 1. After 1 or 2 days the charcoal is slurried with 1 *N* HCl and washed with water until free of chloride. Finally, it is dried at 100°.

Celite 560: Johns Manville (New York, U.S.A.).

1-Butanol: Shell Industrial Chemicals (The Hague, The Netherlands).

Cyclodextrins: These were prepared according to the method described by FRENCH *et al.*[8] and tested for purity by column chromatography on cellulose[1] and by solubility measurements[9].

Glucose and maltose: Brocades (Amsterdam, The Netherlands).

Preparation of the column. The column is prepared in an all-glass tube with an inside diameter of 3 cm, a length of 45 cm and is provided with a jacket.

Charcoal, treated in the way described, is mixed with Celite in the ratio of 4:3 and slurried with water[2]. The small column requires about 150 g charcoal–Celite mixture.

First, a wad of glass wool is placed in the bottom end of the tube, after which a little of the Celite suspension in water is poured into it until a layer of about 1 cm in height is formed. Finally, the charcoal–Celite suspension is poured into the tube applying slight suction until the bed has reached the desired height (about 43 cm). The column is now mounted as described by FRENCH *et al.*[2]. Subsequently the column is washed under pressure (0.2 atm) with 1 l water in order to compress the bed, and then with 1 l 7% 1-butanol in water to remove organic impurities which might interfere with the chromatographic separation. After the column has been washed again with water, until 1-butanol free (refractive index of effluent), it is ready for use.

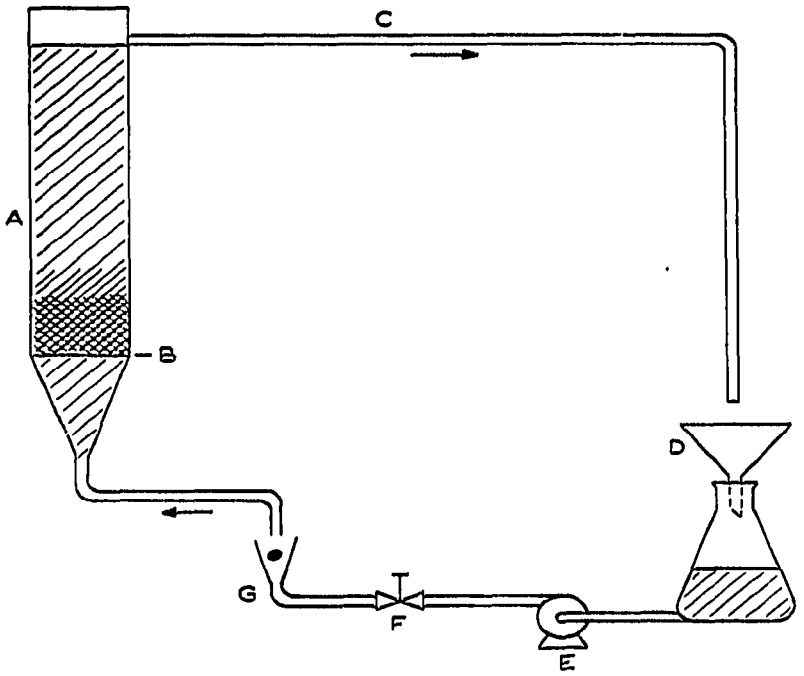

Fig. 1. Continuous recirculating flotation unit. A = Flotation column (340/90 mm); B = sintered glass plate; C = overflow carrying with it very small particles; D = paper filter; E = pump; F = regulating valve; G = flowmeter, liquid velocity 85 ml/min.

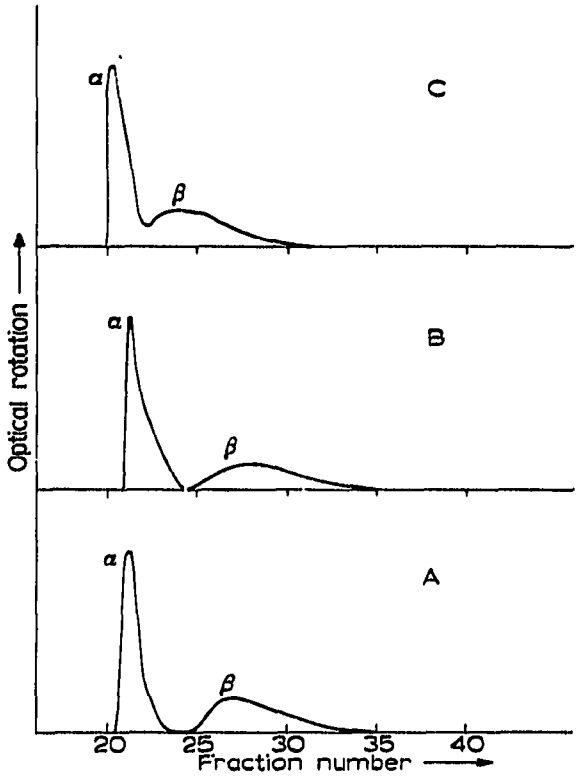

Fig. 2. Separation of α- and β-CD on unflotated charcoal. Gradient: 7% 1-butanol → 1.5 l 3% 1-butanol; sample: 5 ml 3% α-, 3% β-CD. A = Fresh column, flow velocity 40 ml/h; B = column used once, flow velocity 42 ml/h; C = column used twice, flow velocity 47 ml/h.

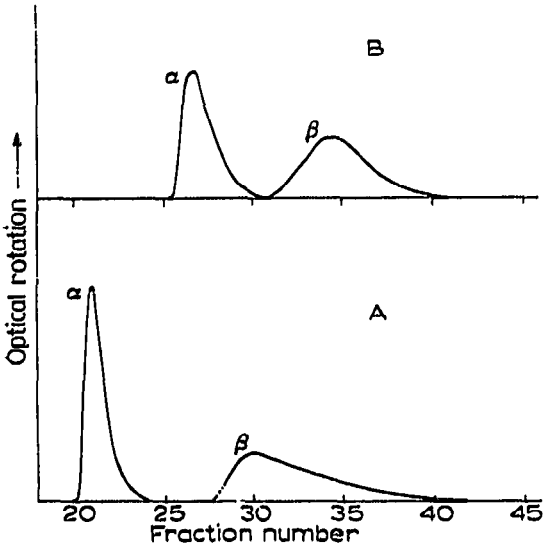

Fig. 3. Separation of α- and β-CD on flotated charcoal. (A) Gradient: 7% *n*-butanol → 1.5 l 3% *n*-butanol; sample: 5 ml 3 % α-, 3% β-CD; flow velocity 151 ml/h. (B) Gradient: 7% *n*-butanol→ 500 ml water; sample: 4 ml 3 % α-, 3 % β-CD; flow velocity 130 ml/h.

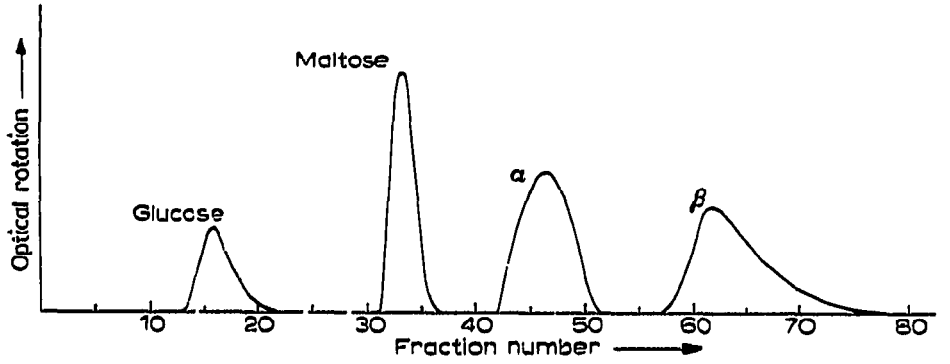

Fig. 4. Separation of glucose, maltose, and α- and β-CD. Gradient: 7 % *n*-butanol → 1.5 l water; sample: 5 ml 3 % of each sugar; flow velocity 132 ml/h.

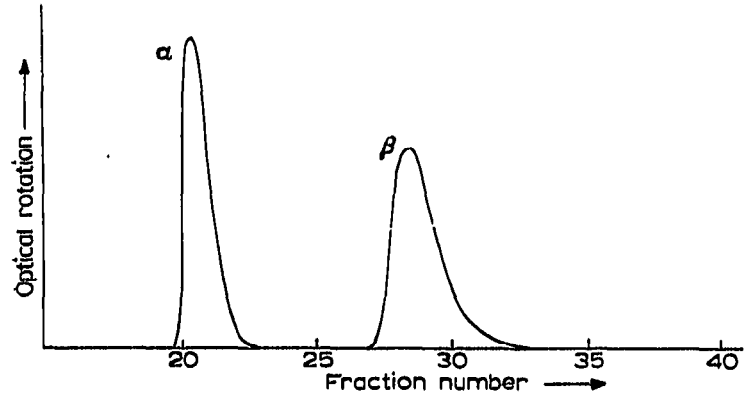

Fig. 5. Separation of α- and β-CD. The gradient is steepened at fraction 15. Original gradient: 7 % *n*-butanol → 1.5 l 3 % *n*-butanol. At fraction 15 1250 ml liquid is removed from the mixing chamber; gradient: 7 % *n*-butanol → 250 ml 3.9 % *n*-butanol (calculated according to ALM *et al.*[10]). Flow velocity: 165 ml/h; sample: 5 ml 3 % α-, 3 % β-CD.

It can be regenerated by washing with water until butanol free, which takes about 10 h; the column must be prevented from running dry.

Operation of the column. All chromatographic experiments are carried out at 25° by thermostating the column.

1-Butanol is chosen as the gradient eluant because only a small concentration is required to elute the sugars from the column[2]. A 7 % aqueous solution of 1-butanol in the solvent reservoir is led into the mixing chamber containing initially 1.5 l 3 % 1-butanol in water. In some experiments the gradient has been increased in order to elute the β-CD faster.

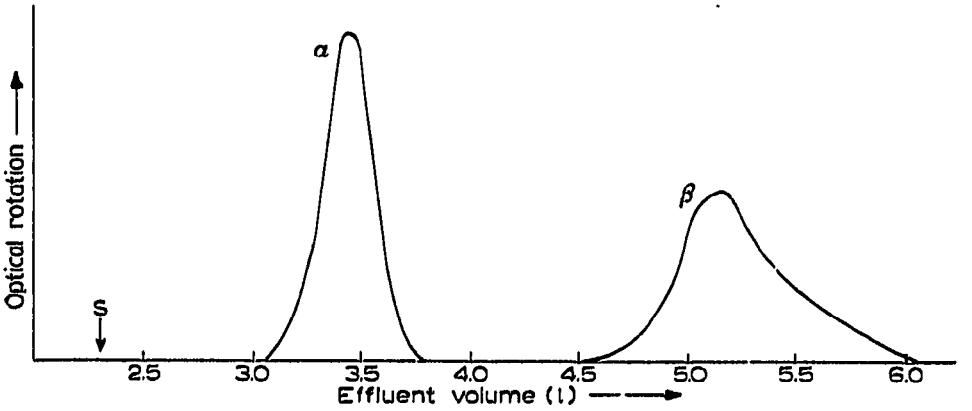

Fig. 6. Separation of α- and β-CD on the large column. Gradient: 6 % 1-butanol → 7.5 l 3 % 1-butanol; flow velocity: 280 ml/h. The gradient is steepened at point S by removing 6 l liquid from the mixing chamber; flow velocity reduces then to 200 ml/h; sample: 50 ml 2 % α-, 2 % β-CD.

The sample consists of an aqueous solution of α- and β-CD (mostly 5 ml of 3 % α- and 3 % β-CD; supersaturated with respect to β-CD). It is applied to the column in the usual way[1]. After the sample is adsorbed on to the column, elution is started.

The flow rate is about 130–170 ml/h. This rate is kept almost constant by an air pressure of 0.2 atm and an additional hydrostatic head of about 30 cm. Separation of α- and β-CD is accomplished within 12 h.

The effluent is analysed by means of an automatic polarimeter[1]; fractions of 25 ml are automatically marked on the chromatogram[1]. Impurities of α- in β-CD and *vice versa* can be determined by means of the "paper test"[1].

The preparative column. The mounting of the column is essentially the same as that of FRENCH *et al.*[2]. The solvent reservoir has a volume of 20 l, the mixing chamber of 10 l and the all-glass column tube has an inside diameter of 7 cm and a length of 55 cm. It has a sintered glass disc at the bottom of the tube and a jacket provided with expansion bellows. The tube is filled to a height of 51 cm in the same way as the small tube and needs about 900 g charcoal–Celite mixture.

Further operations are effected in a similar manner to those applied to the small column. The mixing chamber contains initially 7.5 l 3 % 1-butanol. Fractions of effluent are collected on a time base (each fraction 40 min) and have a size of about 200 ml. Hence the flow rate is about 300 ml/h and one separation is performed within 20 h.

Results and discussion

Small column. Initial experiments were carried out with unflotated charcoal. The results were not reproducible when the experiment was repeated with the same column (after regenerating each time with water until butanol free). Separation gradually became worse and the flow velocity increased although the pressure was kept constant (see Fig. 2). The bad reproducibility is perhaps caused by channelling, which gradually develops when small particles are carried along with the mobile phase.

When flotated charcoal is used the flow velocity is almost constant and much larger than when using unflotated charcoal. Reproducible elutions can now be accomplished. Fig. 3 shows two examples of the separation of α- and β-CD and Fig. 4 the separation of glucose, maltose, α- and β-CD.

Obviously correct regeneration of the column is important because if 1-butanol is still present, complete adsorption of CD will not be attained, so that part of the CD is eluted too fast.

In order to reduce the tail of the β-CD peak, so that faster separations can be accomplished, the gradient is steepened at a given moment during a separation run as can be seen in Fig. 5.

We proved, that all α- and β-CD adsorbed on the column is eluted by weighing the CD input and the dried residue of the appropriate effluent fractions, which is in accordance with other results[7]. Thus this method can be used as a purity criterion for α- and β-CD with respect to each other in a similar way to that described previously[1].

The chromatographic method described can also be used, during the production of cyclodextrins, to determine the composition of mixtures of α- and β-CD (in order to be able to choose an efficient precipitating agent to separate α- and β-CD[8]).

Large column. An example is shown of the separation of α- and β-CD in Fig. 6

We found that regeneration of the column could be accomplished somewhat faster at 50° than at 25° (8 l water instead of 11 l). It is hoped to use this column for the preparation of very pure α-, β- and perhaps γ-CD starting from raw mixtures.

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