Journal of Chromatography, 189 (1980) 98–100

CHROM. 12,420

Note

Analysis of cyclodextrins by high-performance liquid chromatography

H. HOKSE

Experiment Station for the Utilization of Potatoes, Rouaanstraat 27, 9723 CC Groningen (The Netherlands)

(Received October 1st, 1979)

The chromatographic separation of cyclodextrins has already been described[1–5] and a high-performance method for the rapid chromatographic analysis of mixtures of α-, β- and γ-cyclodextrins (α-CD, β-CD and γ-CD, consisting of six, seven and eight α-1,4-linked glucose units, respectively) has been published recently[6].

A method using a column packed with a silica derivative containing amino groups, with acetonitrile–water mixtures as the eluent, has been described for the analysis of mixtures of mono- and disaccharides[7,8]. However, this method does not seem very suitable for the direct analysis of the formation of cyclodextrins from starch, as the low solubility of starch in the eluent may cause problems during the chromatographic run.

The chromatographic analysis of linear oligosaccharides, produced from starch by enzymatic or chemical degradation, is mostly carried out on cation-exchange resins at elevated temperature with water as the eluent[9]. We have found this method to be suitable for the separation and analysis of cyclodextrins.

EXPERIMENTAL

The cyclodextrins (α-CD, β-CD and γ-CD) were obtained from Sigma (St. Louis, Mo., U.S.A.).

Standard solutions of cyclodextrins or linear oligosaccharides at concentrations up to 20 mg/ml were prepared.

A Hewlett-Packard Model 1084B liquid chromatograph, equipped with an automatic sampling system and a Model HP 1031 differential refractometer as a detector, was used. The column system consisted of two columns (25 × 0.62 cm I.D.), connected by unions of low dead volume. Both columns were packed with Aminex 50W-X4 (Ca^{2+}) (20–30 μm). The temperatures of the columns and the detection system were 90° and 37°, respectively. Only water, filtered through 0.2-μm filters and deaerated at 90°, was used for elution.

RESULTS

Using the standard conditions for the analysis of linear oligosaccharides, it was found that α-CD, β-CD and γ-CD were much more retarded on the column

system than their linear analogues. Some results are shown in Fig. 1. Clearly the interactions of the cyclodextrins with the cation-exchange resin are different from those of the linear analogues, which are assumed to be based on complex formation between the calcium ions of the resin and the hydroxyl groups of the glucose molecules[10,11].

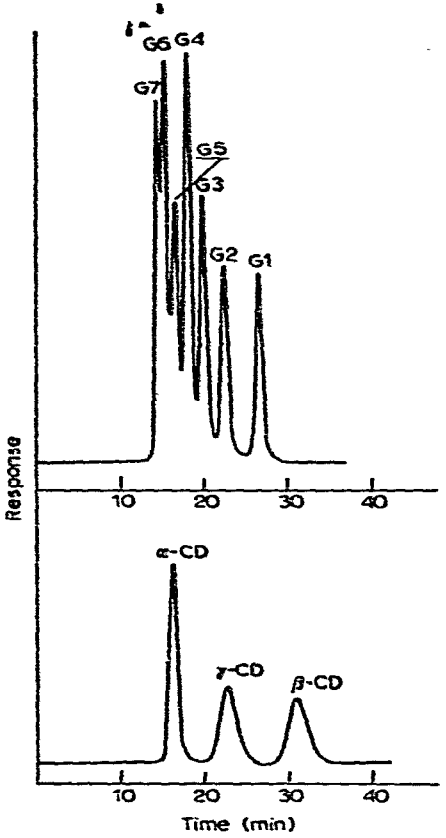

Fig. 1. Chromatographic separation of linear gluco-oligosaccharides (G_1–G_7) and cyclodextrins on a cation-exchange resin. Columns (two in series), 25 × 0.62 cm I.D., 90°; eluent, water, 90°; flow-rate, 0.3 ml/min; resin, Aminex 50W-X4 (Ca^{2+}) (20–30 μm); detection, differential refraction at 37°; sample size, 50 μl; recorder chart speed, 0.2 cm/min.

At lower column temperatures the retention times of β-CD and γ-CD increased (Fig. 2) and the peaks broadened considerably. With the linear glucose oligomers no such change in retention times was observed. With α-CD only a small but consistent increase in retention time was found.

Cyclodextrins form inclusion complexes with many low-molecular-weight organic and inorganic compounds[12], and recently, it has been reported that they also show interactions with polymers in aqueous solution, *e.g.*, a polystyrenesulphonic acid solution[13]. The long retention times of the cyclodextrins might reasonably be explained by such interactions with the polystyrene backbone of the resin.

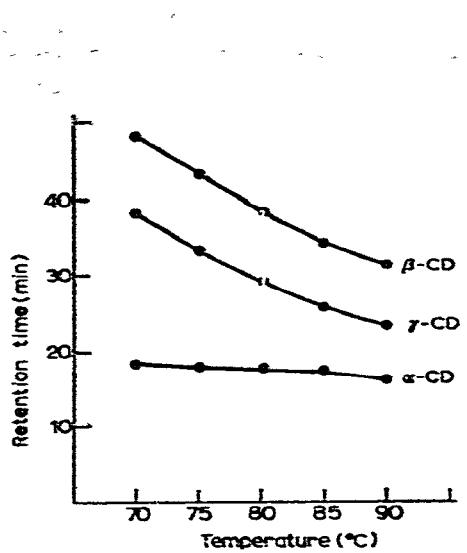

Fig. 2. Retention times of cyclodextrins at different column temperatures. Other conditions as in Fig. 1.

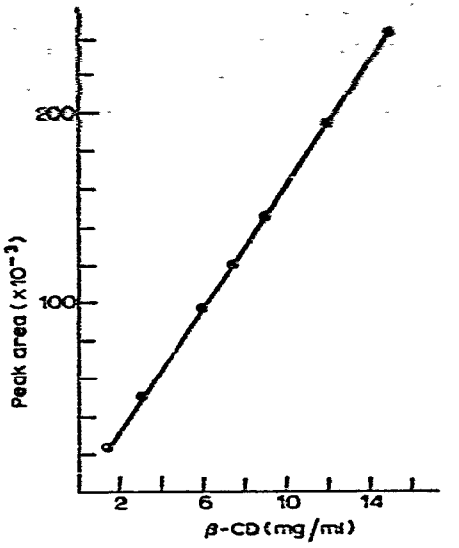

Fig. 3. Relationship between peak area and β-CD concentration. Conditions as in Fig. 1; detector attenuation, 4.

The chromatographic system described here provides a method for the analysis of cyclodextrins in the presence of many other gluco-oligosaccharides. The considerable temperature dependence of the retention times of β-CD and γ-CD makes the system flexible. With appropriate calibration the method can be used quantitatively, as shown in Fig. 3 for β-CD by the relationship between peak area and cyclodextrin concentration.

REFERENCES

1 J. N. J. J. Lammers, *Stärke*, 19 (1967) 70.
2 J. N. J. J. Lammers, *J. Chromatogr.*, 41 (1969) 462.
3 J. H. Carter and E. Y. Lee, *Anal. Biochem.*, 39 (1971) 521.
4 K. Kainuma, A. Nogami and C. Mercier, *J. Chromatogr.*, 121 (1976) 361.
5 B. Zsadon, M. Szilasi, J. Szejtli, G. Seres and F. Tüdös, *Stärke*, 30 (1978) 276.
6 B. Zsadon, K. H. Otta, F. Tüdös and J. Szejtli, *J. Chromatogr.*, 172 (1979) 490.
7 J. L. Linden and C. L. Lawhead, *J. Chromatogr.*, 105 (1975) 125.
8 D. L. Dunmire and S. E. Otto, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 176.
9 M. R. Ladisch and G. T. Tsao, *J. Chromatogr.*, 166 (1978) 85.
10 S. J. Angyal, G. S. Bethell and R. J. Beveridge, *Carbohyd. Res.*, 73 (1979) 9.
11 R. W. Goulding, *J. Chromatogr.*, 103 (1975) 229.
12 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, Heidelberg, New York, 1978.
13 T. Moro, S. Kobayashi, K. Kainuma, T. Uemura, T. Harada, J. Komiyama and T. Iijima, *Carbohyd. Res.*, 75 (1979) 345.