CHROM. 4087

ANALYSIS OF CYCLODEXTRIN MIXTURES BY GAS CHROMATOGRAPHY OF THEIR DIMETHYLSILYL ETHERS

J. B. BEADLE
Moffett Technical Center, Corn Products Co., Box 345, Argo, Ill. 60501 (U.S.A.)

(Received March 26th, 1969)

SUMMARY

Gas chromatography provides a rapid and accurate method for analyzing mixtures of α-, β-, and γ-cyclodextrins and/or their complexes with certain organic solvents. The samples are prepared by decomposing the cyclodextrin–solvent complexes and converting the cyclodextrins to their volatile dimethylsilyl ethers.

INTRODUCTION

In order to assist in the preparation of cyclodextrins in development quantities, we have evaluated methods of cyclodextrin analysis. Our primary interest was in separation and quantitative determination of mixtures of α- and β-cyclodextrins, and on occasion γ-cyclodextrin, respectively containing 6, 7, and 8 anhydroglucose units. A typical molecular model (β-cyclodextrin) is shown in Fig. 1.

Published methods of cyclodextrin analysis were found to suffer from certain limitations. Neither thin-layer chromatography[1] nor circular paper chromatography[2] separated the compounds satisfactorily; severe streaking was observed in both cases. A column chromatographic approach has recently appeared[3] which reportedly has sufficient resolution, but requires several hours per analysis. This procedure was not studied in these laboratories.

SWEELEY *et al.*[4] reported the gas chromatographic separation of stachyose, a tetrasaccharide, as its trimethylsilyl (TMS) ether in slightly less than 1 h at the relatively moderate column temperature of 250°. While experimenting with the method developed by BROBST AND LOTT[5] for separating maltosaccharides up to DP4, we were able to elute the trimethylsilyl ether of maltotetraose in less than 10 min by changing instrument parameters. Peaks were also observed which corresponded to maltopentaose and maltohexaose, during routine syrup analysis on aged columns in which the liquid phase content had decreased from use. This, together with later work on saccharide analysis[6], suggested the use of gas chromatography for analyzing cyclodextrins of comparable molecular weight. The present communication describes the procedure developed for α- and β-cyclodextrin, the two cyclodextrins

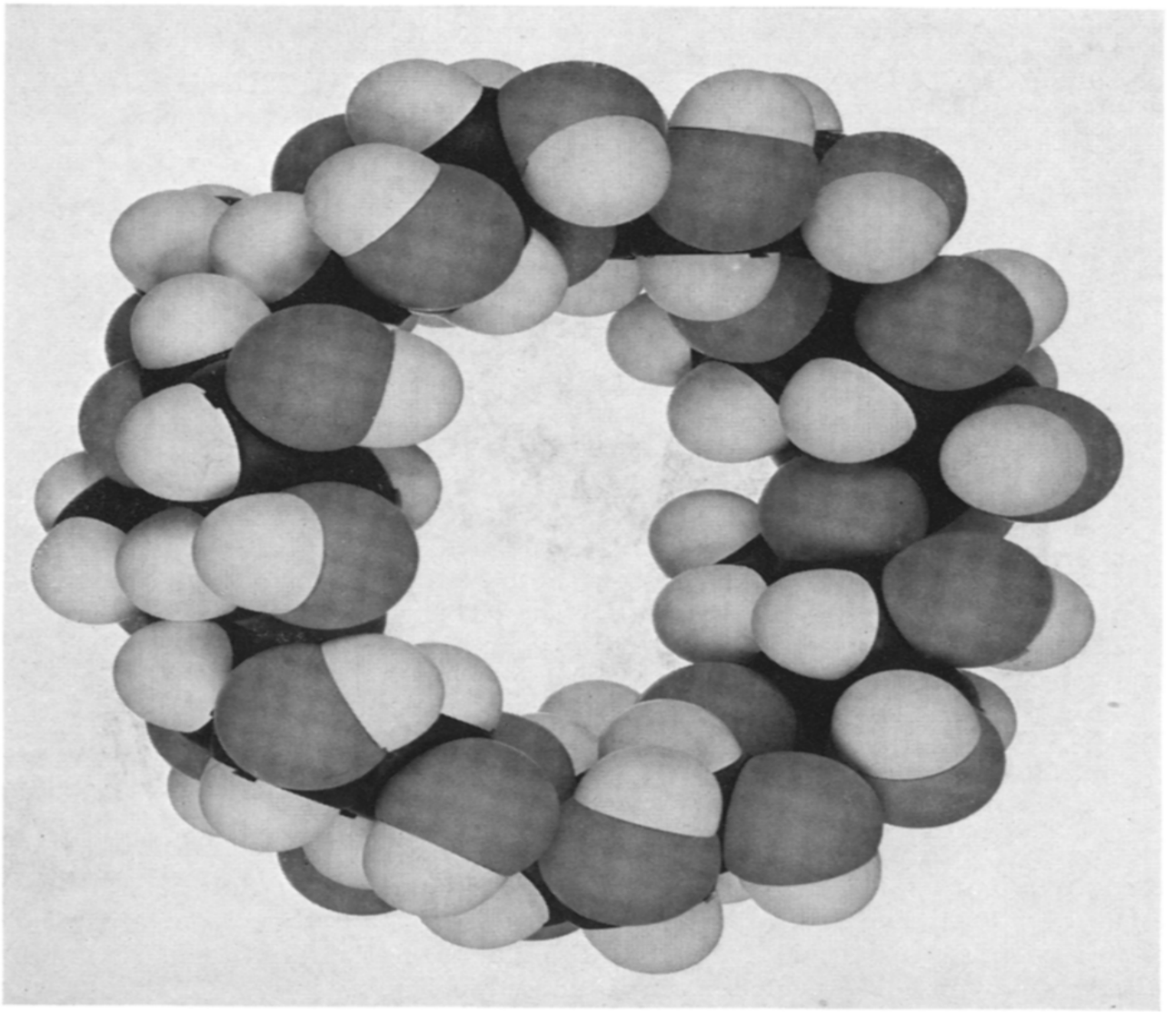

Fig. 1. Molecular model of β-cyclodextrin showing its toroidal configuration.

of present commercial interest, and the γ-cyclodextrin as well. The method is accurate, simple to perform, and rapid: an analysis rate of two samples per hour can easily be maintained.

EXPERIMENTAL

Apparatus

Our work was performed with an Aerograph Model 1520-A chromatograph equipped with a modified flame detector cell (Fig. 2); however, similar features are available on other instruments. The shortened collection grid in the modified detector eliminates the sensitivity loss caused by deposition of silica during combustion of the silylation mixture. Matched 1/8 in. by 24 in. copper columns were used, containing 3% JXR coated on 80/100 mesh Chromosorb® W (AW-DMCS). Prior to use, the columns were conditioned overnight at 350°, using a helium flow rate of 10–15 ml per

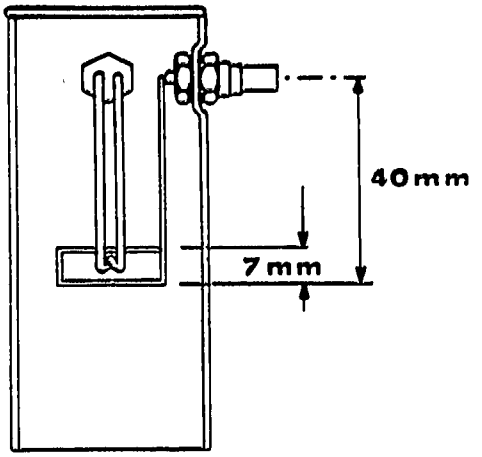

Fig. 2. Schematic diagram of modified flame detector, showing dimensions and position of the collector ring.

min. We adopted the following instrument parameters: The initial column oven temperature was 325°, programmed up at 20° per min for 4 min to a maximum of 405° which was maintained until γ-cyclodextrin eluted. After a new column is used for several days, the initial temperatures should be lowered slightly. Injector and detector temperatures were 370°. The detector temperature was unusually critical; below 350° the β- and γ-cyclodextrin peaks broaden excessively. The carrier gas used was helium at a flow rate of 45–50 ml per min.

Reagents

Standard α- and β-cyclodextrins were prepared by repeated recrystallization from water. γ-Cyclodextrin was available only as the anthracene complex, and was used without purification merely to obtain retention data. Tetramethyldisilazane was obtained from Pierce Chemical Company, Box 117, Rockford, Ill. Eastman trifluoroacetic acid (No. 6287) was used, and anhydrous reagent grade pyridine from any source was found satisfactory.

Standardization

Standard cyclodextrin mixtures were prepared in proportions approximating those anticipated in the samples. The desired amount of each pure cyclodextrin was weighed into a 25 ml volumetric flask, the total not exceeding 110 mg. Reagents were added in the following order: 4.0 ml of pyridine, 4.0 ml of tetramethyldisilazane, and 0.4 ml of trifluoroacetic acid. The flask was left unstoppered. After the initial reaction subsided, the contents of the flask were mixed by swirling; two 4 mm glass beads were added, and the flask was placed on a hot plate operating at the lowest temperature which maintained boiling. After 15 minutes' boiling, the flask was cooled, and the solution was diluted to volume with pyridine; the negligible volume of the beads was ignored. A 3.0 μl aliquot was chromatographed, and peak area per mg in the standard solution was calculated for each cyclodextrin.

Sample analysis

Because many samples were expected to contain residual complexant, the

following procedure was applied to remove the complexant prior to derivatization. About 5 ml of distilled water was added to 100–110 mg (dry basis) of sample contained in a 25 ml volumetric flask. The sample was placed on the steam bath with an air stream directed at the liquid suface to speed evaporation. When completely dry, the sample was derivatized as described for the standards. Area under each peak was compared with the corresponding standard value to calculate component concentration. The evaporation step was omitted when complexants were known to be absent.

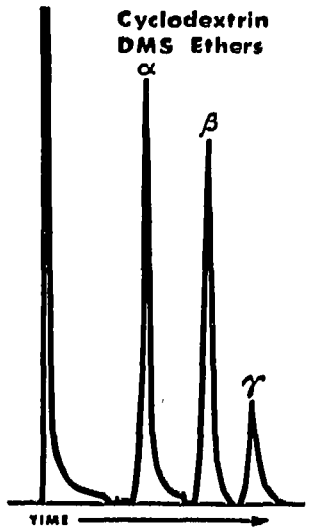

Fig. 3. Chromatogram of a mixture of α-, β- and γ-cyclodextrins. The components elute in order of increasing molecular weight.

RESULTS AND DISCUSSION

We originally planned to utilize the cyclodextrin TMS ethers, but these derivatives showed numerous peaks for each cyclodextrin. One possible explanation is that steric hindrance prevented complete derivatization, although other equally probable causes can be offered. The dimethylsilyl (DMS) ethers form under the same conditions normally used to prepare the TMS ethers, so that the only change required in the procedure was to substitute tetramethyldisilazane as the derivatizing agent[7]. As a result we obtained single peaks for pure compounds. A typical chromatogram is shown in Fig. 3.

Day-to-day reproducibility of the procedure was evaluated by analyzing known "sample" mixtures of pure α- and β-cyclodextrin, over a wide range of proportions, using similar mixtures as standards for calculation. Typical results appear in Table I. Standard deviations, which include errors in both "sample" and "standard" values, were 0.7% and 0.8% absolute for α- and β-cyclodextrin, respectively.

While these samples were analogous to finished products, a majority of the samples of interest were process materials still containing varying amounts and types of complexants. Although some cyclodextrin complexes can be derivatized directly, others (*e.g.*, the cyclohexane-α-cyclodextrin complex) must be broken down by steam distillation prior to derivatization. This can be rapidly performed on an analytical

TABLE I

ANALYSIS OF PURE α- AND β-CYCLODEXTRIN MIXTURES

α-Cyclodextrin		*β-Cyclodextrin*		*Recovery (%)*
Added (%)	*Found (%)*	*Added (%)*	*Found (%)*	
4.1	4.4	95.9	94.5	98.9
30.9	32.7	69.1	70.2	103.0
34.4	34.5	65.6	66.3	100.8
44.8	45.2	55.2	56.4	101.6
46.9	45.7	53.1	51.9	97.6
			Mean	100.4

TABLE II

ANALYSIS OF MIXTURES OF CYCLODEXTRINS AND COMPLEXES

α-Cyclodextrin		*β-Cyclodextrin*		*Recovery (%)*
Added (%)	*Found (%)*	*Added (%)*	*Found (%)*	
α-Cyclodextrin + β-cyclodextrin added as the toluene complex				
28.7	27.3	71.3	67.8	95.1
32.6	32.4	67.4	65.4	97.9
58.7	60.3	41.3	40.5	100.8
84.5	86.5	15.5	16.9	103.4
β-Cyclodextrin + α-cyclodextrin added as the cyclohexane complex				
10.9	10.3	89.1	92.3	102.6
23.8	23.2	76.2	76.8	100.0
32.7	31.8	67.3	67.4	99.3
61.4	64.8	38.6	39.0	103.8
90.8	90.3	9.2	6.5	96.8
			Mean	100.0

TABLE III

MATERIAL BALANCE OF CYCLODEXTRIN PROCESS SAMPLES

Indicated component (%)				*Total (%)*
α-Cyclodextrin	*β-Cyclodextrin*	*Complexant*	*Water*	
66.2	21.5	8.0	1.3	97.0
70.9	15.4	10.4	1.4	98.1
86.2	1.5	8.2	1.0	96.9
86.4	0.0	8.1	1.2	95.7
87.6	0.0	8.7	1.1	97.4
89.6	0.0	8.2	1.1	98.9
87.9	0.0	8.9	1.3	98.1
91.3	0.0	8.2	1.3	100.8
			Mean	97.9

scale by adding water to the weighed sample and evaporating to dryness on a steam bath under an air stream. To demonstrate efficacy of this procedure, several grams of cyclohexane-α-cyclodextrin and toluene-β-cyclodextrin complexes were dried in a vacuum oven at 85° for 16 h, thus essentially eliminating water and partially removing the complexant. The amount of residual complexant was determined in each of these materials by pyrolysis–gas chromatography. Then, known mixtures of each complex with the opposite pure cyclodextrin were analyzed as described above, using similar mixtures of the two pure cyclodextrins as standards. Table II shows that recoveries were generally satisfactory.

As a further test, we analyzed a series of authentic process samples, containing various proportions of α- and β-cyclodextrin and complexant. Water was also determined, by the Karl Fischer method, to provide a material balance. The results (Table III) show that the maximum total error of all analyses was 4.3% relative; the average recovery was 97.9%.

The need for a standard approximating the sample in proportions of the cyclodextrins present stems from a small loss of β-cyclodextrin (and probably γ-cyclodextrin as well) on the column, assumed to result from adsorption. For this reason, where wide variation in sample composition is encountered, analysis of sample first, followed by selection of an appropriate sample mixture, minimizes repeat analyses.

Problems sometimes occurred in derivatizing samples carried through the evaporation step, because they tended to form a glass. When the reagents were added, the outer surface of the sample reacted, but this layer could not be penetrated by the reagents at room temperature. Introduction of the reflux step increased solution rate of the derivative and permitted the reaction to go to completion.

ACKNOWLEDGEMENT

We thank J. H. SMITH of Moffett Technical Center for preparing the standard cyclodextrins used in this investigation.

REFERENCES

1 H. WIEDENHOF, *J. Chromatog.*, 15 (1964) 100.
2 F. CRAMER AND D. STEINLE, *Ann. Chem.*, 595 (1955) 81.
3 J. N. J. J. LAMMERS, *Stärke*, 19, No. 3 (1967) 70.
4 C. C. SWEELEY, R. BENTLEY, M. MAKITA AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497.
5 K. M. BROBST AND C. E. LOTT, *Cereal Chem.*, 43 (1966) 35.
6 J. B. BEADLE, *J. Agr. Food Chem.*, in press.
7 W. WELLS, C. C. SWEELEY AND R. BENTLEY, in H. SZYMANSKI (Editor), *Biomedical Applications of Gas Chromatography*, Plenum Press, New York, 1964, p. 199.