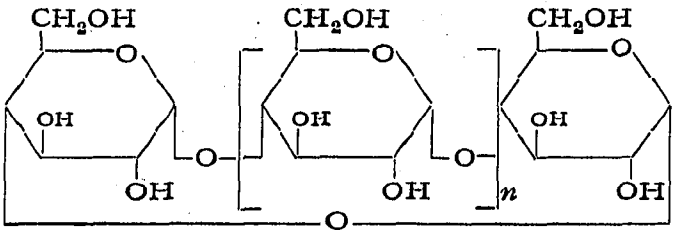
Thin-layer chromatography of cyclodextrins and some other sugars using microchromatoplates

Schardinger dextrins (cyclodextrins) are a homologous group of cyclic saccharides consisting of α 1,4 bound glucose units. They are obtained from the breakdown of starch by the action of *Bacillus macerans* amylase. Their general formula may be expressed by (I):



(I)

in which $n = 4$ for α-cyclodextrin, $n = 5$ for β-cyclodextrin, and $n = 6$ for γ-cyclodextrin.

They are important in that they can form inclusion compounds. During a study of the preparation and properties of cyclodextrins we thought it useful to develop a simple and rapid procedure to distinguish α- from β-cyclodextrin, and the cyclodextrins mentioned from some other sugars, *viz.* glucose and maltose, produced during the preparation of cyclodextrins. γ-Cyclodextrin is not included in our observations, as we have not yet succeeded in isolating it.

CRAMER[1] succeeded in separating α-, β- and γ-cyclodextrin on Schleicher & Schüll 2045b paper, using butanol-2-pyridine–water (1:1:1, v/v/v) as a developing solvent (circular chromatogram). However, we have been unable to separate α- and β-cyclodextrin in this way. On the other hand we did succeed in separating α- and β-cyclodextrin by using a developing solvent described earlier by CRAMER[2] which consisted of *n*-butanol–dimethylformamide (dmf)–water (2:1:1, v/v/v). In our opinion it is not advisable to use Schleicher & Schüll 2045b paper as described by CRAMER, as the time of separation is about 48 h. We obtained more rapid results (18 h approximately) by using Whatman No. 1 paper. In our opinion the best indicator spray is 1% alcoholic iodine solution. This results in the formation of a purple compound with α-cyclodextrin and a yellow one with β-cyclodextrin. The latter is visible only after the brown I_2 background has evaporated sufficiently; in some cases this may take 1 h.

In view of the long separation time of the method described above and of the rather poor identification procedure, we have developed a rapid chromatographic procedure to separate α- and β-cyclodextrins. For this we used the microchromatoplates as described by PEIFER[3].

Preparation of chromatoplates

1. *Silicic acid layers.* We used Kiesel-G "Merck", and followed PEIFER's[3] procedure. The plates ($7^1/_2 \times 2^1/_2$ cm) were steamed and dried to obtain less fragile coatings. The initial activity of the plates was very constant, as was shown by chromatographing the test fluid of the firm of Desaga (60, Hauptstrasse, Heidelberg, Germany)

consisting of butter yellow, sudan red and indophenol blue with benzene as a developing solvent (time of separation: 4 min).

2. *Alusil layers* (silicic acid mixed with alumina). A suspension consisting of 25 g Kieselgel-G and 25 g Aluminiumoxid-G (both "Merck") in 100 ml chloroform–methanol (2:1, v/v) was used. The plates were treated (steaming and drying) by the PEIFER[3] procedure.

Indicator spray

The indicator spray used was a mixture of 10 ml conc. sulphuric acid, 20 ml water and 3 g potassium dichromate. The spray reagent should be used as a fine cloud, and should be blown onto the coating very carefully as there is a chance of loosening the coating. Before spraying, the developing solvent is carefully vaporized by laying the chromatoplate on a hot plate. After spraying, the chromatoplate is put on the hot plate again for a short time until black dots appear. The advantages of the use of microchromatoplates are apparent here as the developing solvent is vaporized very rapidly and the oxidation takes place almost instantaneously.

Developing solvents

After having tried some thirty solvents the following two give the best results:

(1) *n*-butanol–glacial acetic acid–water–pyridine–dmf (6:3:1:2:4), and

(2) *n*-butanol–glacial acetic acid–water (6:3:1).

Separation of α- and β-cyclodextrins

1. *On silicic acid layers.* The difficulty was to find a suitable developing solvent as the solvents used for "common sugars"[4] were found unsuitable for our purpose; however, solvent (1) was found to be most suitable. The R_F of α-cyclodextrin is 0.0 and that of β-cyclodextrin is about 0.5. After evaporating solvent (1) from the plate with a hair-dryer, the plate can be put in, for example, dmf for a short time, in such a way that the dmf front travels $^1/_3$ of the distance of the solvent (1) front ("Stufentechnik"); the R_F of α- and β-cyclodextrin in dmf is about 0.9. It is also possible to make a two-dimensional chromatogram using solvent (1) and dmf; however, the "Stufentechnik" referred to gave the best results.

2. *On alusil layers.* α- and β-cyclodextrins could also be separated on layers of alusil with solvent (1). The R_F of α-cyclodextrin is again 0.0 and that of β-cyclodextrin about 0.5 but the spots were less sharp than when silicic acid layers were used.

Investigations on solvent (1) showed that:

(a) omission of water resulted in tailing of the α-cyclodextrin,

(b) omission of pyridine resulted in tailing of the β-cyclodextrin,

(c) omission of glacial acetic acid resulted in tailing of the α-cyclodextrin,

(d) omission of dmf resulted in an $R_F = 0.0$ for β-cyclodextrin.

It was also observed that cyclodextrins are very soluble in dmf. β-Cyclodextrin has a solubility of 20.0 ± 0.5 g/100 g dmf at 24°, compared with its solubility in water of only 1.5 ± 0.3 g/100 g water.

It is advisable not to put more than 5 μg of each cyclodextrin on the plate.

Separation of α- and β-cyclodextrin, glucose and maltose

The best results were obtained on silicic acid plates (even when separating glucose

and maltose, this in contradiction to STAHL's[4] observations on "macro" plates) using the "Stufentechnik". Development was first with solvent (2), followed by solvent (1). It is advisable to allow solvent (2) to reach the end of the adsorbent and then leave the chromatoplate in this solvent for another 4 min. After this the solvent is vaporised with a hairdryer and the plate is put in solvent (1) (the R_F of α-cyclodextrin in solvent (2) is slightly greater than 0.0). Fig. 1b shows the result. For pur-

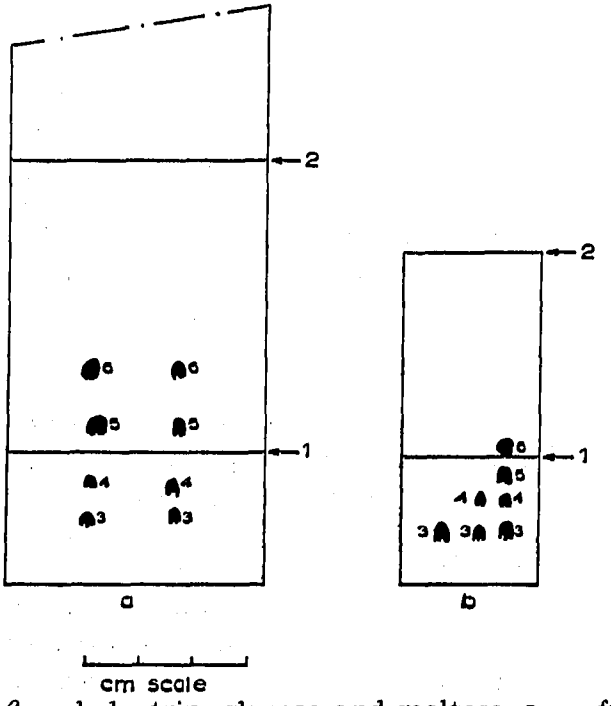


Fig. 1. Separation of α- and β-cyclodextrin, glucose and maltose. 1 = front of solvent (1); 2 = front of solvent (2); 3 = α-cyclodextrin; 4 = β-cyclodextrin; 5 = maltose; 6 = glucose.

pose of comparison (Fig. 1a) we made a chromatogram on a plate prepared by the usual spreading technique with the aid of the Desaga applicator (thickness of the layer 250 μg). When alusil plates were used the spots were not as sharp.

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