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Separation of cyclodextrins and their derivatives by thin-layer and preparative column chromatography

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Abstract

Cyclodextrins, their derivatives and products of their partial hydrolysis are clearly separated by chromatography on silica gel by mobile phases containing aqueous ammonia and organic solvent (acetonitrile or 1-propanol). This system allows separation of compounds with different numbers of substituents on cyclodextrin, but does not allow the separation of isomers; the system can be used both for thin-layer chromatography and for preparative column chromatography. The isomers in the fraction thus obtained can be in some cases separated, after peracetylation, by chromatography on silica gel using a mobile phase consisting of methanol and dichloromethane. This chromatographic sequence potentially yields derivatives of cyclodextrins in quantities which enable evaluation of their solubilization and biological properties.

Keywords: Cyclodextrins; Thin-layer chromatography; Preparative column chromatography

1. Introduction

In some applications, organic solvents have effects adverse to health. Cyclodextrins and their derivatives form water-soluble inclusion complexes with nonpolar compounds and have the potential to replace organic solvents not only in some analytical and laboratory scale preparative procedures, but even in some large scale technical operations; these applications have been reviewed [1-3]. Cyclodextrins and their derivatives are also being used as ligands in various affinity chromatography methods and as components of pharmaceutical formulations, where they assist in absorption of drugs by

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the organism [1–3]. Underivatized α-, β-, and γ-cyclodextrins (cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively) are less well suited for these uses than are the mixtures of their derivatives. Mixtures of hydroxypropyl or methyl ethers of β- and γ-cyclodextrins already are commercially available, and other mixtures

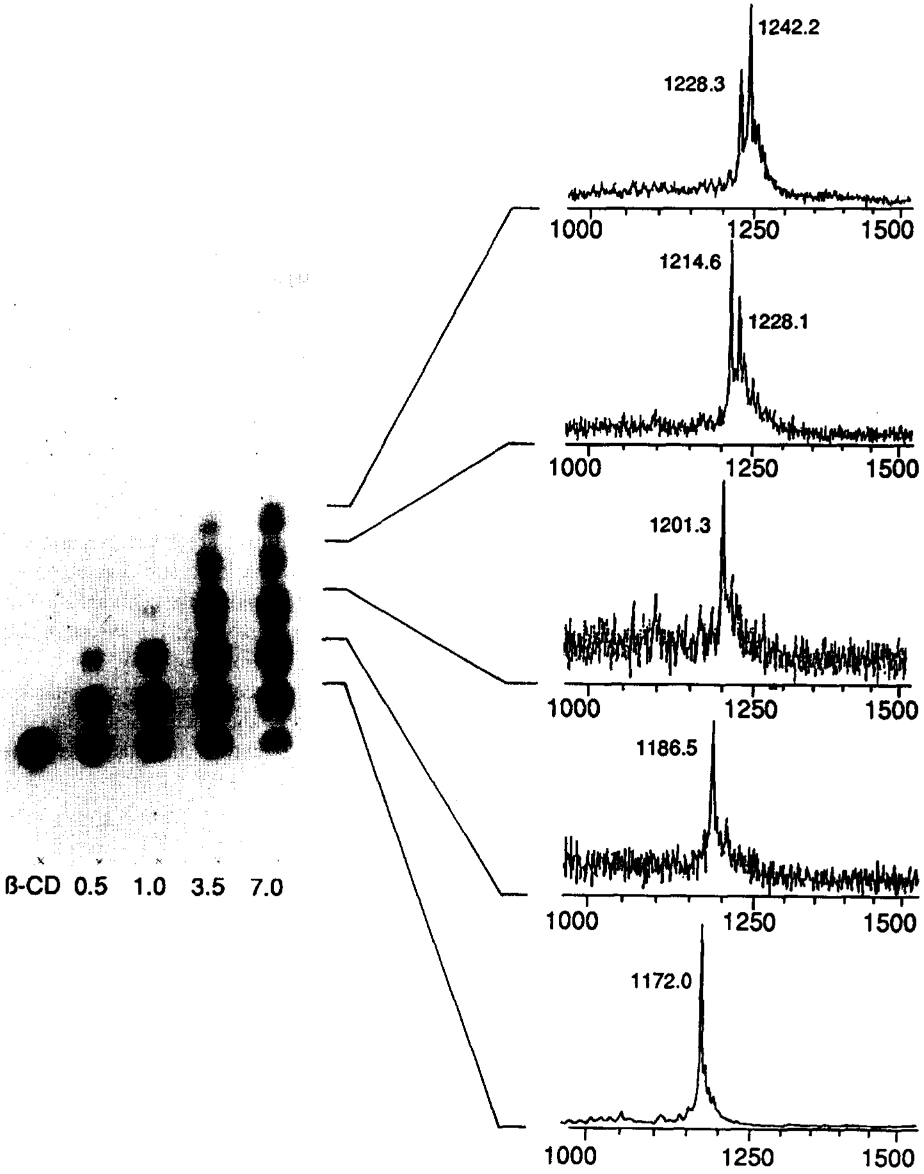

Fig. 1. Thin-layer chromatogram (developed with 1-propanol–ethyl acetate–water–concd aq ammonia, 6:1:3:1) of β-cyclodextrin (β-CD) and of the mixtures obtained by its reaction with 0.5, 1.0, 3.5, and 7 mole-equiv of dimethyl sulfate (denoted by numbers). To the right of the plate are ^{252}Cf MS spectra of the eluted spots.

are continuously being developed and tested. Because these mixtures are obtained by a partial substitution of the 18, 21, or 24 hydroxyls of α-, β-, or γ-cyclodextrins, respectively, the number of components of these mixtures is in the thousands. Even the most sophisticated methods cannot be expected to separate and identify all components. Even if such information were available, it would have only limited impact on the advance in preparation and use of such mixtures. Furthermore, sophisticated separation methods become tedious when gram quantities of material are needed, as invariably happens in tests of solubilization potency and tests of toxicity in animals. Here we describe low-technology practical systems to be used in such projects. These systems separate mixtures of cyclodextrins principally on the basis of the number of substituents per molecule without separation of isomers within these groups; the latter may subsequently be separated by additional chromatography performed after their derivatization. These fractionation techniques can assist in an approximate characterization and optimization of composition of mixtures of cyclodextrin derivatives, and in special situations may help in the preparation of chemically pure compounds.

2. Results and discussion

For thin-layer chromatography of cyclodextrins and their derivatives on silica gel, two ammonia-containing developing solvents were found suitable. The preferred solvent consists of acetonitrile–water–concd aq ammonia (6:3:1); development is fast, and the developing solvent is stable upon storage. The second developing system contains 1-propanol–ethyl acetate–water–concd aq ammonia (6:1:3:1). Development times with this solvent are three to four times slower than with the acetonitrile system; upon storage

Table 1
Resolutions of cyclodextrins, glucosyl derivatives of cyclodextrins, and linear oligosaccharides by thin-layer chromatography

Compound	R_f (acetonitrile system [a])	R_f (propanol system [a])
α-Cyclodextrin	0.18	0.21
β-Cyclodextrin	0.14	0.18
γ-Cyclodextrin	0.11	0.14
Glucosyl-β-cyclodextrin [b]	0.08	0.11
Maltosyl-β-cyclodextrin [b]	0.06	0.09
Dimaltosyl-β-cyclodextrin [b]	0.03	0.05
Glucose	0.23	0.31
Maltose	0.19	0.26
Maltotriose	0.15	0.24
Maltotetraose	0.12	0.19
Maltopentaose	0.09	0.15
Maltohexaose	0.07	0.12
Maltoheptaose	0.06	0.08

[a] Systems: acetonitrile–water–concd aq ammonia (6:3:1), 1-propanol–ethyl acetate–water–concd aq ammonia (6:1:3:1).

[b] Product of Ensuiko Sugar Refining Co., Yokohama.

Table 2

R_f values of 2-*O* and 6-*O* alkyl derivatives of β-cyclodextrin

Substituent on β-cyclodextrin	R_f (acetonitrile system)	R_f (propanol system)
None	0.15	0.18
2-*O*-Methyl [a]	0.20	0.25
2-*O*-Ethyl [a]	0.23	0.30
2-*O*-Propyl [a]	0.27	0.36
2-*O*-Allyl [a]	0.26	0.34
2-*O*-(*S*)-2-Hydroxypropyl [b]	0.20	0.27
2-*O*-(*R*)-2-Hydroxypropyl [b]	0.20	0.27
6-*O*-(*S*)-2-Hydroxypropyl [b]	—	0.24
6-*O*-(*R*)-2-Hydroxypropyl [b]	—	0.24
2-*O*-(*S*)-2,3-Dihydroxypropyl [c]	0.12	0.18
2-*O*-(*R*)-2,3-Dihydroxypropyl [c]	0.12	0.18
2-*O*-2,2-Hydroxymethylpropyl [c]	—	0.31

[a] Ref. [6].
[b] Ref. [8].
[c] Ref. [9].

the solvent decomposes slowly, with formation of ammonium acetate and acetamide. The advantage of the latter system is that the R_f values of many compounds are known because this system has been used for about 10 years [4,5].

Cyclodextrins and linear oligosaccharides were well resolved in both systems (Table 1). An increase in the number of monosaccharide residues led to a decrease in the R_f value, and cyclic oligosaccharides had higher R_f values than linear oligosaccharides having the same number of residues.

The R_f values of methyl, ethyl, propyl, hydroxypropyl, and allyl derivatives are given in Table 2. R_f values increase with the size and decrease with the polarity of the substituents. Resolution between isomeric compounds (2-*O* and 6-*O* derivatives) or between pairs of diastereomers, which differed by chirality in the substituent, was not large enough to be useful.

The resolution of mixtures into fractions containing the same number of substituents per cyclodextrin was demonstrated on a product obtained by nonregiospecific reaction of β-cyclodextrin with increasing amounts of dimethyl sulfate in aq sodium hydroxide. Individual spots of the thin-layer chromatogram (Fig. 1) were extracted and mass spectrometry indicated that they represent a series in which the number of methyl groups increases from one to six per β-cyclodextrin. Thus compounds containing different numbers of methyl groups per cyclodextrin were resolved, and the R_f value increased with the total number of substituents; isomers were not resolved. A similar resolution was obtained for a series containing from one to three 2-hydroxypropyl groups per β-cyclodextrin (results not shown) and for a series containing from one to three allyl groups (cf. Table 3).

Preparative separation of several mono-alkyl derivatives [6] of β-cyclodextrin on a silica gel column was accomplished successfully with the propanol solvent system. The main problem with this system is the dissolution of carbohydrates in a minimal volume of the relatively non-polar elution solvent; a process required when the sample is applied

Table 3
Column chromatography [a] of *O*-allyl-β-cyclodextrin derivatives

Compound/mixture of isomers	R_f [a] (TLC)	V (mL) [b]	MS (m/z) [c]			
			β-CD 1135	mono 1175	di 1215	tri 1255
Tri-*O*-allyl-β-cyclodextrins	0.58	540–600	—	—	30	100
Di-*O*-allyl-β-cyclodextrins	0.49	660–900	—	8	100	—
Mono-*O*-allyl-β-cyclodextrins	0.33	1000–1440	8	100	—	—
β-Cyclodextrin	0.17	1540–2020	100	—	—	—

[a] Solvent: 1-propanol–toluene–water–concd aq ammonia (6:1:3:1).
[b] Column elution volumes.
[c] Relative intensities of peaks of isolated fractions obtained by FAB-MS.

to the column. This problem was circumvented by dissolving the carbohydrate sample in water and evaporating it onto a small amount of silica gel, which was then applied to the top of the prepared column. The use of an elution solvent containing ethyl acetate and ammonia for column chromatography invariably resulted in contamination of the eluted fractions with nitrogen-containing solids (ammonium acetate, acetamide). Therefore, in the case of preparative chromatography, ethyl acetate was not added to the elution mixture or was replaced by toluene if a decrease in R_f values was desirable. An example of this type of separation is given in Table 3. Chromatography separated cleanly β-cyclodextrin and its mono-, di- and tri-*O*-allyl derivatives. FAB mass spectral analysis indicated a presence of cyclodextrin derivatives with fewer allyl groups in these fractions; loss of allyl groups, which form relatively stable cations, apparently occurred during the ionization in the spectrometer.

Results show that the resolution of compounds in acetonitrile- and propanol-containing systems is about the same. The acetonitrile system, in addition to its chemical stability, has the advantage of easy manipulation of R_f values through changes in the composition of the solvent. An increase in acetonitrile concentration decreases R_f values, and because such manipulation is not restricted by immiscibility problems, solvent composition can be optimized easily for maximal resolution. The mobility of compounds in the propanol-containing system can also be changed by manipulation of the ethyl acetate concentration (an increase leads to a decrease of R_f values) but the range of such changes is more limited in this case. The mobilities are also strongly affected by ammonia concentration; decreasing its concentration increases mobility.

The mobilities of the oligosaccharides in the ammonia-containing systems depended primarily on (a) whether they were linear or cyclic (the latter have larger R_f values) and (b) on the total number of hydrophilic (e.g., glucosyl) or lipophilic groups (e.g., methyl or 2-hydroxypropyl [5]) (the former decrease, and the latter increase the R_f values). This type of dependency may be expected when mobilities of compounds are affected primarily by their adsorption to polar surfaces of the stationary phase.

From various attempts to separate the above fractions further, only one merits a description. Column chromatography of the peracetylated, monosubstituted allyl derivatives of β-cyclodextrin with a mobile phase of methanol–chloroform separated the expected three regioisomers easily. The same process, when used on a mixture of monomethyl derivatives of β-cyclodextrin, gave only a partial separation and a crystallization had to be used to obtain pure compounds [6]. It also may be noted that column chromatography of perbenzoylated mixtures was previously used successfully to obtain pure heptakis-2,6-di-*O*-methyl-β-cyclodextrin [7]. Since peracetylation and deacetylation give high yields and are easily scaled up, the above sequence is practical when applicable.

3. Experimental section

Materials.—TLC plates (Kieselgel 60 F_{254}, 5 × 10 cm, 0.25 mm layer thickness) were obtained from E. Merck, Darmstadt. Solvents and chemicals were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Silica gel for column chromatography was obtained from ACE Scientific Supply Co., Inc., East Brunswick, NJ. Other compounds were purchased from Sigma Chemical Co., St. Louis, MO, or their origin is denoted in footnotes to Tables 1 and 2. The sample of β-cyclodextrin substituted with one to six methyl groups was obtained by the reaction of β-cyclodextrin (3.2 mmol) with dimethyl sulfate (21 mmol) performed in aqueous 0.4 M sodium hydroxide (140 mL).

Mass spectra were recorded with a ^{252}Cf mass spectrometer constructed for National Heart, Blood and Lung Institute (NHBLI) of National Institutes of Health by Dr R.D. Macfarlane, and with a FAB VG 7070E-HF instrument (glycerol:TFA matrix) located at the University of Minnesota.

Thin-layer chromatography (ammonia systems).—The analyzed mixture was dissolved in water in a 1% concentration and 0.2 μL was spotted on the TLC plate. 1-Propanol–ethyl acetate–water–concd aq ammonia (6:1:3:1) or acetonitrile–water–concd aq ammonia (6:3:1) was used as an eluent. Development times were typically 20 and 5 min, respectively. Detection was performed by brief immersion of the plate into Vaugh's reagent [1 g $Ce(SO_4)_2$, 24 g $(NH_4)_2MoO_4$, 50 mL concd H_2SO_4, 450 mL H_2O] and placing the TLC plate on a hot plate until blue spots developed.

Preparative column chromatography (ammonia systems).—In a typical experiment, 6 g of a mixture of cyclodextrin *O*-alkyl-derivatives containing mainly mono-alkyl-derivatives [6] was dissolved in water (100 mL) and 30 g of silica gel were added. The suspension was evaporated to dryness (weight 36 g). The powdered solid thus obtained was added to the top of a silica gel (300 g) column (i.d. 5 cm), which was poured using the eluent mixture 1-propanol–toluene–water–concd aq ammonia (6:1:3:1) or 1-propanol–water–concd aq ammonia (6:3:1). The column was eluted with 3 L of eluent mixture, using pressurized air (70 kPa) at the rate of 20 mL/min; 20 mL fractions were collected. Separation was followed by TLC of fractions.

Chromatography of peracetylated cyclodextrins.—In a typical experiment the fraction from column chromatography (ammonia systems) (1 g) was dissolved in pyridine (10 mL), acetic anhydride (5 mL) was added, and the mixture was heated for 24 h to 80°C. The mixture was then evaporated to dryness, toluene (50 mL) was added and the mixture again evaporated to dryness. The resulting solid was chromatographed on a silica gel column (200 g) using CH_2Cl_2–MeOH (100:1). Collected fractions were assayed by thin-layer chromatography on silica gel plates developed with $CHCl_3$–MeOH (20:1); spots were developed with Vaugh's reagent. For deacetylation, the combined fraction (200 mg) was dissolved in 4 mL of a ~ 0.1 M solution of sodium methoxide in anhyd MeOH. After 5 h at room temperature a small amount of Dowex 50 (acid form) and water (4 mL) were added and the mixture stirred for 10 min, filtered and the filtrate evaporated to dryness in vacuo. This procedure applied to a fraction of mono-*O*-allyl-β-cyclodextrins yielded two pure compounds. In addition to the known 2-allyl-β-cyclodextrin, whose structure was previously determined by X-ray crystallography [6], a solid (presumably 3-*O*-allyl-β-cyclodextrin) was obtained in an amount about one-half that of the 2-*O* derivative; its structure is presently being investigated by X-ray crystallography. The third isomer (with the R_f of peracetate between the other two isomers) was not isolated due to its low and variable content in the peracetylated mixture. Isolated substances had correct and identical molecular weights by mass spectroscopy.

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