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Synthesis of Rigid Cyclodextrin-Containing Polymeric Resins for Adsorption

TINGYUE GU[1,*], GOW-JEN TSAI[2,3] and GEORGE T. TSAO[2]
[1]*Department of Chemical Engineering, Ohio University, Athens, OH, 45701, USA;* [2]*School of Chemical Engineering, Purdue University, West Lafayette, IN, 47907, USA;* [3]*Wyeth-Ayerst Research, N. Middletown Road, Building 96A, Room 216, Pearl River, NY, 10965, USA*

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Abstract

Cyclodextrins (CDs) are a family of cyclic-oligosaccharides with usually 6–8 glucopyranose units. Because of their unique donut-shaped steric structure they are able to form inclusion complexes selectively with some guest molecules. CDs are in starch-like powder form and are soluble in water to certain extents. It is desirable to produce insoluble CD-containing particles for adsorption, liquid chromatography and other applications. This work presents a novel method of synthesizing CD-containing resins. Resins synthesized with this method possess high contents of CDs and some favorable physical properties. Adsorption isotherms for three small aromatic compounds, namely phenylalanine, tryptophan and aspartame, were obtained experimentally.

Introduction

Cyclodextrins (CDs) are a family of cyclic oligosaccharide usually consisting of 6, 7, or 8 α-(1,4)-linked D-glucopyranose units, which are called α-CD, β-CD and γ-CD, respectively. Among them, β-CD is the most abundant and most widely used. The enzymatic productions of CDs and their physical properties were reviewed by several researchers [1–3].

CDs have many useful applications due to their ability to form inclusion complexes with a variety of guest molecules, such as fatty acids and esters, aromatic compounds, aliphatic alcohols, biomolecules with bulky side chains including antibiotics and proteins with aromatic amino acid residues [3]. For example, silica-based β-CD columns can be used to separate positional isomers of Suprofen that is a common anti-inflammatory medication [4]. Forty percent of all dosage-form drugs sold in 2000 were single enantiomers and they accounted for $133 billion in sales [5]. A group of Australian researchers developed a process to use CDs to remove cholesterol from egg yolks and dairy products according to Haggin [6]. Apart from their potential uses in separations, CDs are also used to enhance solubilities of some compounds in water, and to protect some compounds from oxidation. As a result, CDs are also studied for use as a drug delivery agent. Consequently, CDs have found applications in areas such as food and agricultural, pharmaceutical, and chemical industries [3]. Schneiderman and Stalcup [7] summarized various CD applications in separation science. More recently, Singh et al. [8] published a review on the applications of CDs in biotechnology.

Because CDs are soluble in water to some extents, they must be immobilized before they can be used as a stationary phase for inclusion chromatography, which is defined as a kind of chromatography that utilizes selective inclusions to provide selectivity [9, 10]. An aqueous alcohol, such as methanol, solution is often used as the mobile phase for CD columns in inclusion chromatography [11]. An updated review of the synthesis of CD-containing adsorbents was given by Crini and Morcellet [12]. There are primarily two types of synthetic CD-containing particles for such applications.

The first type consists of CD polymers with inorganic support. Silica gel has been successfully used as an inorganic support. Fujimura et al. [13] first synthesized a chemically bonded CD silica stationary phase for liquid chromatography (LC) and tested the retention behavior of several aromatic compounds. Kawaguchi et al. [14] immobilized CDs on silica gel by condensating carboxylated silica gel with CDs having amino groups, by using 1-ethyl-3-[3-(dimethyl-amino) propyl] carbodiimide. Shiraishi et al. [15] also immobilized CDs on silica gel in a simpler method that did not involve the synthesis of modified CDs. In their method, silica gel was first chlorinated with thionyl chloride or silicon tetrachloride, and then was reacted with β-CD. The commercial β-CD column by Advanced Separation Technologies

* Author for Correspondence. E-mail: gu@ohio.edu

Inc. (Whippany, NJ) is packed with 5 μm silica covalently bonded with β-CD molecules [11, 16]. The β-CD molecules are chemically bonded to silica gel via a stable spacer 6–10 atoms in length [16]. Alpha- and γ-CD bonded phase High Performance Liquid Chromatography (HPLC) columns are also available from the same company.

Silica-based CD-containing beads are excellent for HPLC packing because of the known advantages of spherical silica beads used as a rigid support. Some researchers have used these analytical columns to separate substituted phenolic compounds in reversed phase HPLC [11] and to separate diastereomers and structural isomers [4, 16, 17]. Unfortunately, such silica-based beads are not suitable for preparative- or large-scale separations, because the use of silica beads as the backbone support inherently limits the content of CD on silica gel to only a few weight percent, typically 3%. It is necessary to have a much larger content of CD since CD has a relatively large molecular weight and one such big molecule usually can only include one guest molecule at most.

The second type consists of CD-containing copolymers with organic supports. One of the first reported CD stationary phases is a polymeric CD-epichlorohydrin resin [18]. It has been used to separate various natural products (vitamins, perfumes, aromatic amino acids, *o*- or *p*-nitrophenol substituted chlorobenzoic acids derivatives and the diastereomers of $Co(NH_3)_4$-glucose-6-phosphate ATP. The gel-like β-CD-epichlorohydrin polymer suffers from a water-swelling rate of up to 500%. It has a quite different swelling rate in alcohols. This kind of large and non-uniform swelling rate is undesirable in large-scale uses. CD polymer gels, obtained from their polymerization in solution with poly (vinyl alcohol) using ethylene glycol-bis(epoxypyl) ether as cross-linking agent have also been employed as stationary phases for inclusion chromatography [18]. Tanaka et al. [19] prepared two chemically bonded β-CD gels, β-en-Bio-Gel and β-en-agarose, by coupling mono-(6-β-aminoethylamino-6-deoxy)-β-CD with either succinyl hydrazide Bio-Gel P-2 or 1,4-butanediol diglycidyl ether. Several α- and β-CD-containing polyurethane resins cross-linked with different diisocyanates have been used with some successes in both LC and Gas Chromatography (GC) [20–22]. Mizobuchi et al. [22] reported that β-CD polyurethane resins contained high contents of β-CD (ca. 65 wt%) and are stable in organic solvents. However they become unstable in acidic solutions. The α-CD polyurethane resin also contains a high α-CD content, but it is unstable in organic solvents.

Unlike synthesizing silica-based beads, it is possible to synthesize CD-containing polymeric resins that have a large weight percentage of CD. This increases their loading capacities in preparative- and large-scale applications. Other physical properties, such as mechanical strength, swelling rate, insolubility and wettability are also very important in the practical use of CD-containing resins. When the CD content in a resin is high, the resin tends to be less insoluble, and weak in strength since CDs are starch-like substances with little physical strength.

There are two intrinsic drawbacks in most of the reported methods for the synthesis of CD polymers. First, researchers tend to choose a support polymer as backbone and then try to attach CD molecules to the support. This leads to the low CD content since the majority of the product will be the support polymer. Second, some researchers did use a small polyfunctional molecule as the cross-linking agent in order to improve the CD content in the product, as in the case of CD-epichlorohydrin resin. Unfortunately the cross-linking agent is weak in its structure. Thus the polymer has a gel-like appearance with a weak mechanical strength even though the degree of cross-linking is high [12].

A new method for the synthesis of CD-containing polymers is presented in this work. The basic idea is to select a polyfunctional cross-linking agent that is small in molecular size and yet strong in structure. 1,2,4,5-benzenetetracarboxylic anhydride (BA) seems to be an excellent candidate. It is a small molecule that has four functional groups and a very strong aromatic ring as its backbone. It can form a highly cross-linked copolymer with CDs through esterification with the abundant hydroxyl groups of CDs. These important factors ensure that the product polymers contain high contents of CDs and possess excellent physical properties due to a high degree of cross-linking. Experimental results indicated that polymers with up to 70 wt% CD can be synthesized.

Materials and experimental methods

Alpha-, β-CDs were purchased from the Ensuiko Sugar Company, Japan. Prior to polymer synthesis, CDs were purified to remove water trapped in CD cavities. This was done with acetone wash and cyclohexane wash, respectively followed by vacuum oven drying at 93.3 °C (200°F) overnight in a Model 289 vacuum oven from Fisher Scientific (Pittsburgh, PA). BA was purchased from Sigma-Aldrich (St. Louis, MO). N,N-Dimethyl Formamide (DMF) (Certified ACS grade) was purchased from Fisher Scientific. It was mixed with CaO and refluxed overnight and then distillated to remove trace amount of water before use. 1,3-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, L-phenylalanine and L-tryptophan were all purchased from Sigma-Aldrich.

BA was chosen as the cross-linking agent and DMF was used as the solvent for polymerization. A direct polymerization method was used to copolymerize β-CD with BA. The same method was also applied to the synthesis of α- and γ-CD polymers. In this method, no chemical modification of CD was needed before polymerization. Below is the polymerization procedure.

In a 1000 ml round bottom flask, 23.3 g of purified β-CD is dissolved in 600 ml dried DMF. About 10.0 g of BA is then added and dissolved in the solvent by vigorous stirring. After this, 18.8 g 1,3-dicyclohexylcar-

bodiimide and 1.5 g 4-dimethylaminopyridine are added as an esterification catalyst system. The flask is then sealed and the solution is stirred rigorously. The reaction liquid becomes thicker and thicker during the course of the reaction and after half-an-hour the liquid becomes a sticky opaque gel indicating the formation of a highly cross-linked polymer

The post-treatment of the reaction product begins with the evaporation of DMF in the gel by using a rotary vacuum evaporator (Fisher Scientific) with its water bath temperature set at 85 °C. After this, the product is vacuum dried at 93.3 °C to remove the residual DMF from the product. The dried product is further treated with methanol in a Soxhlet extraction apparatus (ACE Glass, Vineland, NJ) for a couple of days to wash away the remaining catalysts. Finally, the product is dried in vacuum again to yield the final product that has the appearance of lumps of particles with a light-yellowish color.

The CD resin was ground and sieved and the fraction in the 120–170 mesh range was collected. The batch adsorption method was used to measure the adsorption capacities of the resins corresponding to different equilibrium concentrations in the bulk liquid. The capacities were measured in grams of solute taken up by each gram of dry resin at a fixed equilibrium temperature. Twenty-four hours was allowed to assure equilibrium. The bulk liquid phase concentration was analyzed using liquid chromatography to obtain the solute concentration. Two common aromatic amino acids, phenylalanine and tryptophan were used as solutes to test adsorption capacities of the synthesized polymeric CD resins. Aspartame (L-aspartyl-L-phenylalanine) that is a small peptide was also used.

Results and discussion

The analysis of the steep water used to wash the product after copolymerization of β-CD and BA prior to the methanol treatment gave no detectable amount of dissolved β-CD. This indicated that all β-CD was copolymerized during the reaction. The amount of BA reacted was easily determined by the common acid–base titration of the steep water and was found to be very close to 100%. Each batch of the end product before grinding typically weighed 1 g or 2 below the combined weight of β-CD and BA in the feed at the beginning of the polymerization reaction. It was possible that some polymers were washed away during the extraction with methanol in the post-treatment stage because their degree of cross-linking was not high enough to make them absolutely insoluble in methanol.

β-CD-containing resins with different β-CD contents can be obtained by changing the β-CD to BA feed ratio. The β-CD content can easily reach up to 70 wt%. The physical properties of such resins are also quite good in terms of physical strength, insolubility in water and organic solvents. Dried resin particles were placed in a graduated cylinder and mixed with water or ethanol to test swelling. It was found that the resin level inside the cylinder increased only slightly and the water and ethanol gave similar results. The density of such resins is around 1.3 g/cm^3. It was found that the resins are acid resistant, but they decompose in alkaline solutions. Figure 1 shows the IR spectrum of a β-CD-containing resin synthesized using the method above. It has a large negative peak corresponding to the OH stretching at the 3391 cm^{-1} position that was reported by Bongiorno et al. [23] for pure β-CD crystals. This indicates that the resin contains a large amount of OH groups contributed by β-CD.

β-CD-containing resins with a higher (than 70 wt%) β-CD content can also be synthesized using the method, but such resins have less desirable physical properties because when the CD content is too high there is not sufficient structural support from the cross-linking agent. The physical properties of a CD-containing resin largely depend on the degree of cross-linking. The higher

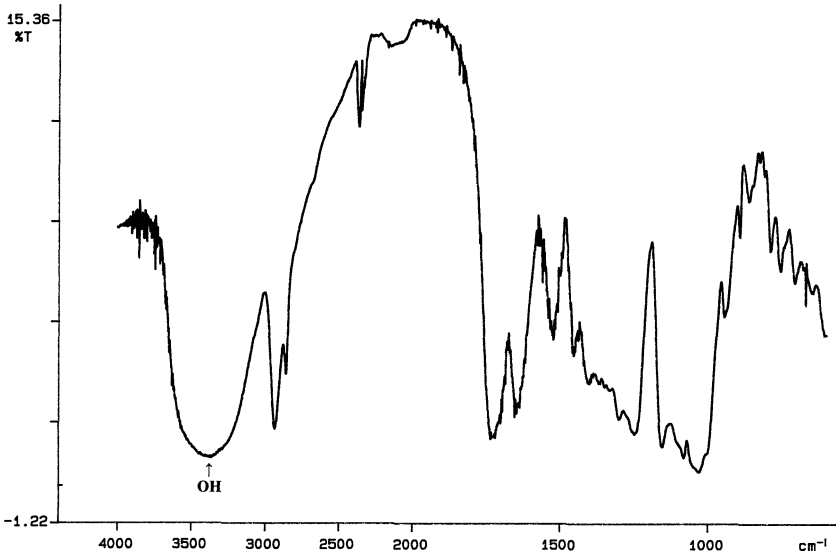

Figure 1. IR spectrum of a β-CD resin.

the degree of cross-linking, the harder and more insoluble the resin becomes.

The polymerization method was also used to synthesize α-CD-containing polymeric resins. Similar results were obtained. Figure 2 is the IR spectrum of a α-CD resin containing 63 wt% of α-CD. The α-CD resins produced using the method described above are insoluble in organic solvents, unlike α-CD polyurethane resins that are unstable in organic solvents [22].

Figure 3 shows the adsorption isotherms of a resin containing 63 wt% β-CD for phenylalanine, tryptophan and aspartame, respectively. In Figure 3, the liquid-phase equilibrium concentration ranges for tryptophan and aspartame are small because their solubilities in water are quite limited without pH adjustments. The isotherms in Figure 3 resemble the popular Langmuir isotherm that exhibits a straight-line in a double reciprocal plot. To verify this, isotherms in Figure 3 were converted to double reciprocal plots as shown in Figure 4 and excellent linearities were obtained. Figure 5 is for the adsorption isotherm of phenylalanine on a resin containing 63 wt% α-CD.

Table 1 shows that the adsorption capacities for phenylalanine and tryptophan are quite high because at the highest liquid-phase equilibrium concentration tested in this work, nearly half of the CD cavities are occupied. The data in Table 1 were calculated using the solute and CD molecular weights and CD percentages in the resins together with the highest isotherm uptake data in Figures 3 and 5. Non-specific binding was neglected in the calculations. It should be noted higher uptakes are possible than in Figures 3 and 5 by using higher equilibrium concentrations in the experiments until solubilities of the solutes become a problem even after pH adjustment.

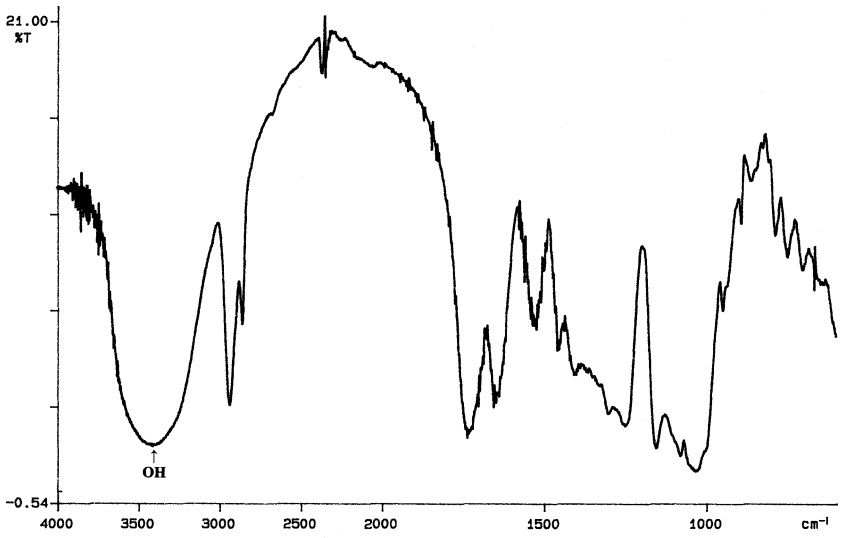

Figure 2. IR spectrum of an α-CD resin.

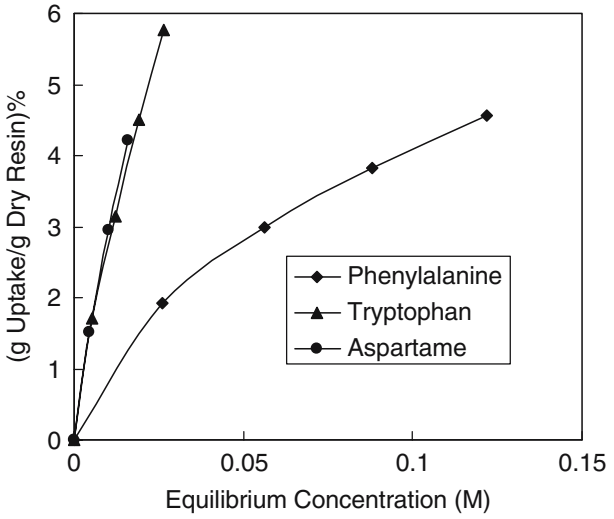

Figure 3. Adsorption isotherms of phenylalanine, tryptophan at 24 °C and aspartame at 21.5 °C on a β-CD resin.

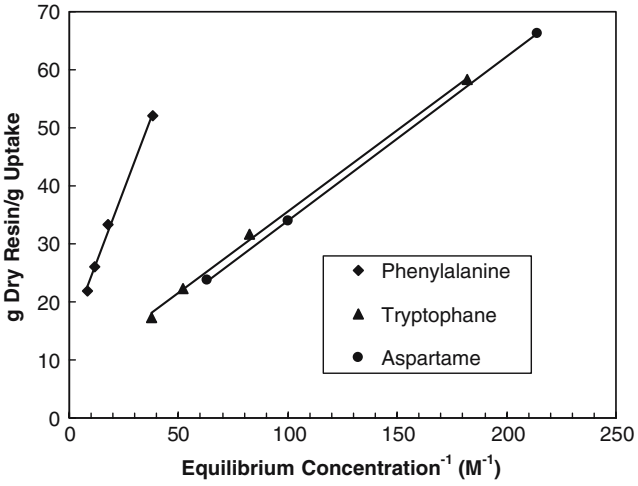

Figure 4. Double reciprocal plot of Figure 3.

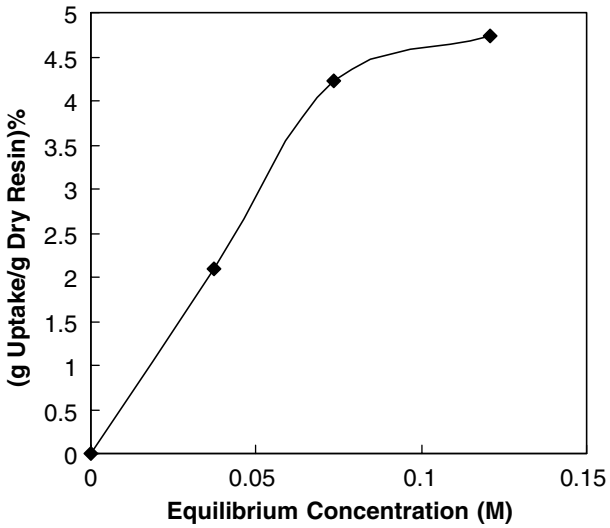

Figure 5. Adsorption isotherm of phenylalanine on an α-CD resin at 21.5 °C.

Table 1. Percentages of CD cavities occupied by solutes corresponding to the equilibrium solute molar concentrations in the liquid phase (shown in brackets) for the two resins mentioned in Figures 3 and 5

Solute	α-CD resin	β-CD resin
Phenylalanine	44.2% (at 0.121 M)	49.8% (at 0.122 M)
Tryptophan		50.8% (at 0.0265 M)
Aspartame		25.8% (at 0.0159 M)

Conclusions

A novel method was developed for the synthesis of rigid CD-containing polymeric resins. This method uses a strong cross-linking agent to copolymerize directly with CD molecules. The resins synthesized with this method contain large weight percentages of CDs, and possess quite good physical properties in terms of insolubility and physical strength. The β-CD resin synthesized with this method has a density of about 1.3 g/cm^3 and a very small swelling rate in water and organic solvent. Adsorption capacities of phenylalanine, tryptophan and aspartame were found to be quite high. Apart from batch adsorption, the insoluble resins may find many other applications in which immobilized CDs are desired. They include fix-bed adsorption and catalysis.

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