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Cyclodextrin Purification with Hollow Fibers

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Abstract

Cyclodextrins are cyclic 1–4 linked oligomers of α-D-glucopyranose prepared from starch hydrolysis through enzymatic reactions. Mixtures of the three main cyclodextrins (CD), α-, β-, and γ-CDs, are always produced. A possible facile purification process is proposed. Permeation through hollow fibers made of a perfluorinated ionomer membrane, Nafion type, is shown to be an effective way to separate α-CD from β- and γ-CD. α-CD with 95% purity was obtained after permeation through a Nafion hollow fiber of an equimolar 0.02 *M* solution of the three CDs. The fiber had a 56 cm^2/cm^3 surface area per volume ratio. Kinetic studies and continuous extraction experiments with a 2-m coiled fiber showed that it is possible to obtain a 11.5 g/L α-CD solution with 92.4% purity or a 0.6 g/L α-CD solution with 97.2% purity, depending on the flow rate. The transport of CDs through the membrane could be due to moving water pools inside the ionomer. The small α-CD fits easily in such pools when the larger β- and γ-CDs are excluded by steric hindrance. Temperature raises increased the permeation rates while decreasing the selectivity. The process could be scaled-up associating hollow fibers in bundle.

INTRODUCTION

Cyclodextrins are cyclic 1–4 linked oligomers of α-D-glucopyranose with each glucopyranosyl residue being in the $^4C_1$ conformation. Schradinger, who discovered cyclodextrins (CD) in 1903 (*1*), classified the three most

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important forms as α-, β-, and γ-cyclodextrin (α-CD, β-CD, and γ-CD, respectively). Figure 1 shows the structure and carbon numbering of β-CD. Cyclodextrins are toroidal-shaped, with all of the secondary hydroxyl groups located at one end of the annulus and all of the primary hydroxyl groups located at the other end, as shown in Fig. 1 (*2*). Table 1 gives the number of glucose units, the size, the molecular weight, and the water solubility of α-, β-, and γ-CD.

CDs are prepared from starch through enzymatic reactions. The glycosyl transferase enzymes are produced by different kinds of bacteria. The en-

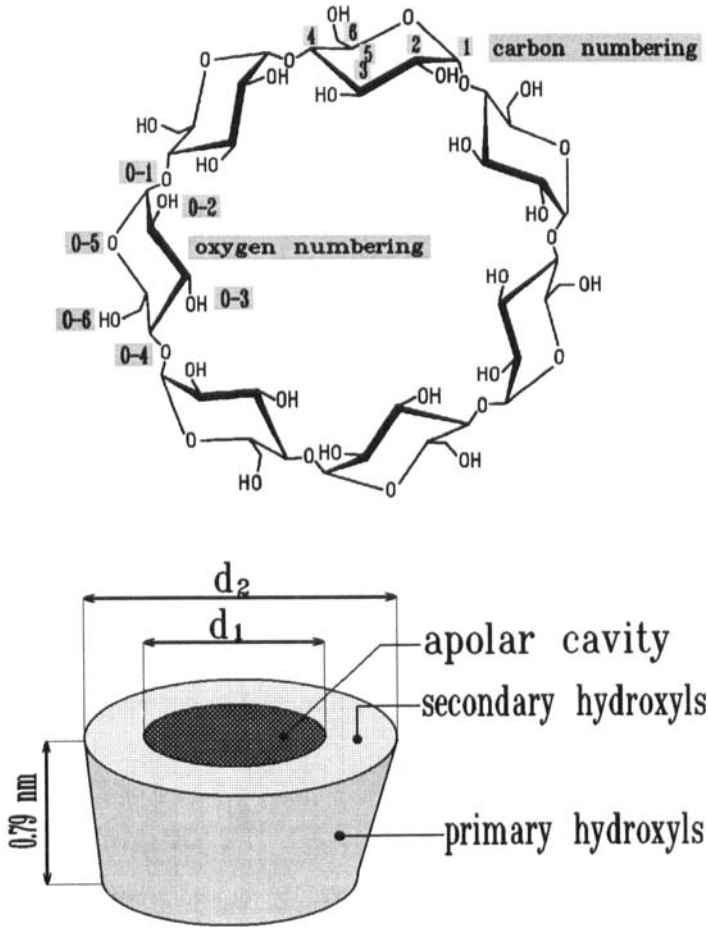


FIG. 1. Top: β-Cyclodextrin with seven glucopyranose 1–4 linked units. Bottom: Schematic shape of a cyclodextrin molecule. The cone height is 0.79 nm; the cone internal and external mean diameters are d_1 and d_2, respectively. See Table 1 for values.

TABLE 1
Physicochemical Properties of Cyclodextrines

Cyclodextrin CD	Glucose unit	MW (g/mol)	Size (nm)		Water solubility[b]	
			d_1[a]	d_2[a]	mol/L	g/L
α	6	972	0.57	1.37	0.149	145
β	7	1135	0.78	1.53	0.0163	18.5
γ	8	1297	0.95	1.69	0.179	232

[a]See Fig. 1.
[b]Temperature 25°C.

zymatic reactions produce mixtures of the three CDs along with other sugars, such as glucose, maltose, and maltotriose (*3*, *4*). Table 2 lists the cyclodextrin yield obtained by using glycosyl transferase from different bacteria.

To obtain a pure CD, purification of the mixture is required. β-CD, with a purity in the 80% range, is the cheapest CD because it can be easily obtained through water recrystallization. The relatively low solubility of β-CD, compared to α- and γ-CDs (Table 1), makes the process simple. The industrial approach for CD purification involves the conversion of liquefied starch in the presence of a suitable organic solvent precipitant (toluene, decanol, or cyclohexane) (*4*). Insoluble crystalline complexes of the solvent and CDs accumulate during the conversion. The solvent choice affects CD conversions. Toluene favors β-CD formation whereas decanol favors α-CD formation (*5*). In both cases the purity is in the 80% range. To date, α- and γ-CDs are not easily obtained and are expensive reagents.

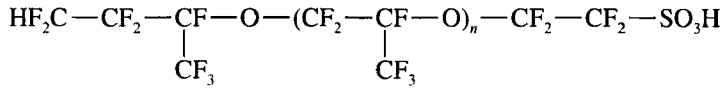
The aim of this work is to introduce a possible route for CD purification with the use of hollow fiber membranes. Such hollow fibers have a large surface area per volume. With low energy consumption, it may be possible to separate CDs and to obtain high purity CD samples.

TABLE 2
CD Formation by Bacterial Hydrolysis of Starch

Strain	CD yield (%)	α-CD (%)	β-CD (%)	γ-CD (%)
Bacillus macerans	27	44	41	15
Bacillus No. 13	58	2	76	22
Bacillus No. 17-1	60	1	74	25
Bacillus No. 38-2	74	5	73	22
Bacillus circulans	57	18	67	15

EXPERIMENTAL

The hollow fiber membranes were made of a perfluorinated anion exchanger polymer commercialized under the registered trade name Nafion by the E. I. du Pont Company (Polymer Product Department, Wilmington, Delaware). Nafion is formed by the copolymerization of tetrafluoroethylene with a perfluorovinyl ether compound. The perfluorovinyl ether is prepared through a reaction of sulfur trioxide, tetrafluoroethylene, and hexafluoropropylene oxide (*6*). The molecular structure is



The 1100 equivalent weight polymer is a mixture of units with $n = 3$, 4, and 5. The exact nature and structure of the Nafion membrane is not completely understood and still under intensive investigation.

The Nafion tubing was made from Nafion solutions. Nafion solutions are 5% solutions of 1100 equivalent weight sulfonic acid perfluorinated resin in a mixture of propyl alcohols and water (Solution Technology Inc., Mendenhall, Pennsylvania). The Nafion tubing was obtained from Perma Pure Products (Toms River, New Jersey) as 50 m coils (0.8 mm i.d., 1 mm o.d.). The surface area per volume was 56 cm^2/cm^3.

The extraction set-up was extremely simple, as shown by Fig. 2. For static experiments, 7 cm long pieces of Nafion tubing were cut. The cut tubing was set in water for at least 24 h for equilibration. Nafion tends to swell in water. Once water equilibrated, each piece of tubing was put in a 5-mL beaker containing 3 mL of an aqueous cyclodextrin mixture. 20 μL water was put inside the tube that was placed in the CD solution in such a way that the water level inside the tubing was 1–2 mm below the external solution level, as shown in Fig. 2.

In one experiment a set of beakers (between 3 to 6) was prepared, each one containing the same CD mixture. A 7-cm piece of Nafion tubing, containing 20 μL pure water, was placed in each beaker at the same time. After 1 h, the composition of the liquid inside the the tubing of the first beaker was determined by liquid chromatography (LC). Chromatograms, like the ones shown by Fig. 3, were obtained. They allowed the calculation of permeation rates at time t for each CD. According to the results, the remaining beakers were analyzed after various time intervals. The longest permeation time has been 124 h (5 days) for the trimethylphenyl ammonium chloride (TMPAC) treated Nafion tubing. The CD bulk concentration was considered constant since the absolute amount of CD that permeated was always lower than 0.5% of the initial CD amount.

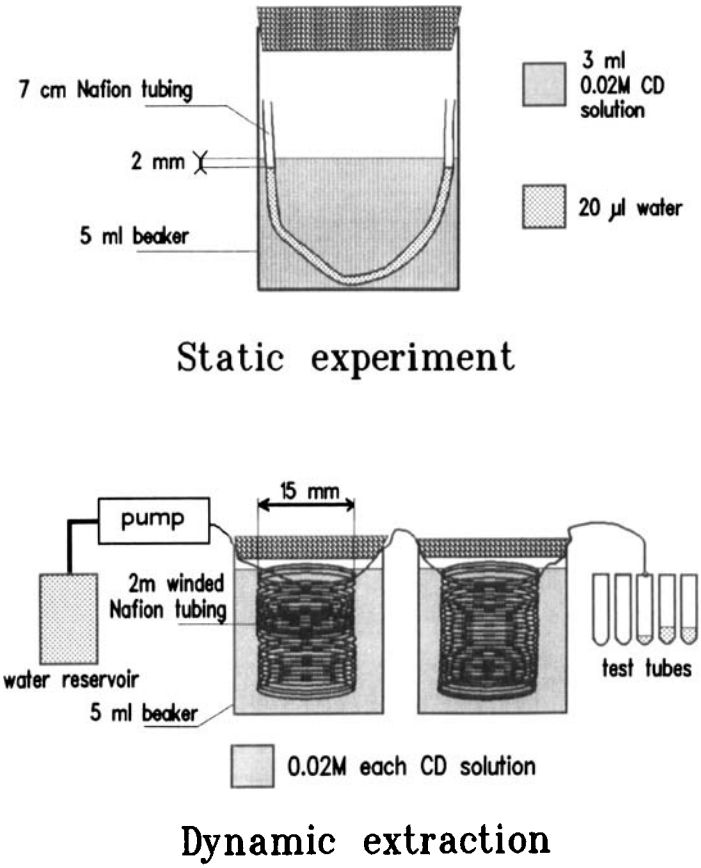


FIG. 2. Top: CD permeation through Nafion tubing in static experiment. Bottom: Set-up for dynamic CD permeation.

For dynamic extraction, 2 m of tubing were wound in two coils placed in two 5-mL beakers as shown by Fig. 2 (bottom). A Shimadzu LC6A chromatographic pump was used to circulate water inside the tubing at a flow rate of 45 µL/min (2.7 mL/h). The eluting solution was collected in test tubes and analyzed by LC.

All solutions were analyzed by LC using the procedure recently described (*7*): A Shimadzu LC chromatograph was used with a LC6A pump, a CR3A controller, a Rheodyne 7125 injector valve, an Astec Cyclobond 25 cm column (β-CD bonded silica, 5 µm particle diameter), and a Waters Model R401 differential refractometer. The mobile phase was a 50–50 v/v methanol–water solution containing 1% triethylamine and adjusted to pH 4.2

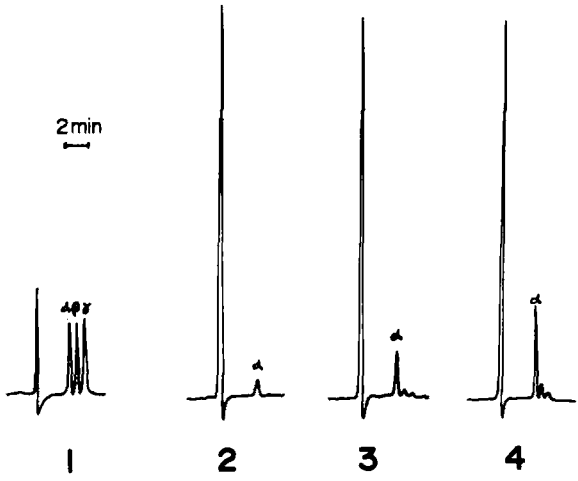


FIG. 3. Chromatograms of CD mixture used to compute the data listed in Table 3. Column: Cyclobond β-CD bonding, 5 μm particle size, 25 cm, 4.6 mm i.d. Mobile phase: methanol–water–triethylamine 49.5–49.5–1 (% v/v) adjusted to pH 4.2 with acetic acid. Flow rate: 1 mL/min. Refractive index detection. Room temperature. Injected volume: 10 μL. 1: 0.02 *M* solution of α-, β-, and γ-CD, RI attenuation 16×. 2: 90 min permeated solution, RI attenuation 4×. 3: 5 h permeated solution, RI attenuation 4×. 4: 15 h permeated solution, RI attenuation 4×.

with acetic acid. α-, β-, and γ-CD were obtained from Astec (Whippany, New Jersey).

RESULTS AND DISCUSSION

Static Experiments

Assuming the permeation of molecules in Nafion polymer can be described by a diffusion process following Fick's first law, the permeation flux N (in $mol \cdot cm^{-2} \cdot s^{-1}$) is related to the solute diffusion coefficient D (in cm^2/s) by

$$N = -D \frac{dC}{dx} \tag{1}$$

with C, the concentration, in mol/cm^3, and x, the permeation distance, in cm. Assuming the diffusion coefficients are independent of concentration, Eq. (1) can be integrated to

$$N = \frac{D}{e} \Delta C \tag{2}$$

where e is the tubing wall thickness and ΔC is the concentration difference between the inside and the outside tubing.

If the bulk concentration, C_b, is unchanged during the permeation experiment, NS moles permeate through the tubing wall per time unit, producing a concentration change, dC, in the inner liquid (*8*):

$$dC = \frac{NS}{V}\,dt \tag{3}$$

where S is the permeating surface, equal to $2\pi rL$, and V is the inner liquid volume, equal to $\pi r^2 L$. r and L are the internal radius of the tubing (= 0.4 mm) and the length of the inside contained liquid (4 cm for 20 μL), respectively. Combining Eqs. (2) and (3) yields

$$\frac{dC}{dt} = \frac{2D}{er}(C_b - c) = k(C_b - c) \tag{4}$$

which can be integrated, with the assumptions that D and C_b are constant, to give the concentration, c, inside the tube:

$$c = C_b(1 - e^{-kt}) \tag{5}$$

Table 3 lists the kinetic constant (k), diffusion coefficient (D), temperature of experiments, and initial composition of the permeation experiments done with water-equilibrated Nafion tubing. Three repeated

TABLE 3
Cyclodextrin Permeation Rate through Nafion Tubing

Bulk composition (mol/L)			Temperature	Permeation rate k^a (10^7 s^{-1})			Diffusion coefficient^a (10^9 cm^2/s)		
α	β	γ	(°C)	α	β	γ	α	β	γ
0.02	0.02	0.02	40	110	4.2	1.1	2.8	0.11	0.028
0.02	0.02	—	23	100	1.9	—	2.4	0.047	—
0.02	0.02	—	40	190	18	—	4.9	0.45	—
0.02	0.02	—	50	600	36	—	15	0.90	—
0.02	0.02	—	60	370	60	—	9.3	1.5	—
Tubing Treated with Trimethylphenyl Ammonium Chloride (TMPAC)									
0.02	0.02	0.02	40	8.5	4	0.53	0.22	0.10	0.013
0.02	—	0.02	40	7.1	—	0.37	0.18	—	0.009
0.02	—	0.02	50	25	—	1.1	0.61	—	0.027
0.02	—	0.02	60	28	—	2.0	0.69	—	0.050

^{a}Average value of three experiments, uncertainty: 40%.

experiments produced permeability and diffusion coefficients in agreement within a ±40% range. Given the duration of the experiments (between 6 and 124 h), it was not possible to obtain enough data to decrease statistically the uncertainty below 40%. Within the indicated variability, the CD diffusion in Nafion tubing can be represented by Eq. (5).

By comparing Tables 3 and 1, it is apparent that the permeation rates through Nafion tubing are not directly related to the CD water solubility. Rather, the values seem related to the CD size.

Enrichment Factor

Starting with an identical concentration for each CD and defining the enrichment factor, ϕ, as the ratio of two CD concentrations inside the tubing with $c_1 > c_2$ at time t, Eq. (5) gives

$$\phi = \frac{c_1}{c_2} = \frac{1 - e^{-k_1 t}}{1 - e^{-k_2 t}} \tag{6}$$

The maximum ϕ value occurs at time $t = 0$:

$$\lim_{t \to 0} \phi = k_1/k_2 \tag{7}$$

and the minimum ϕ value is

$$\lim_{t \to \infty} \phi = 1 \tag{8}$$

Figure 4 shows the enrichment factor, ϕ, versus time for the 40° C experiment with the three CD mixture. Figure 4 shows that ϕ decreased rapidly when the two permeation rates were very different. The ratio $\phi_{\alpha/\gamma}$ was 99 at time $t = 30$ min, and only 70 at time $t = 20$ h. However, the α, β, and γ concentrations were 3.9×10^{-4}, 1.5×10^{-5}, and 4×10^{-6} mol/L, respectively, at 30 min. This eluent would allow the production of 400 mg CDs per liter, with a purity of α-CD equal to 95%. At time $t =$ 20 h, the α, β, and γ concentrations were 0.011, 6×10^{-4}, and 1.6×10^{-4} mol/L, respectively, which would allow the production of 11.5 g CDs per liter, with a purity of α-CD = 92.4%. Obviously, there is a trade-off between increased yield and decreased purity. However, the 30-fold increase in yield may far outweight the 2.6% decrease in purity.

Dynamic Extraction

To optimize the CD extraction and purification process, a dynamic extraction set-up was designed as described in the Experimental section and illustrated by Fig. 2. A flow rate of 45 μL/min was chosen so that the

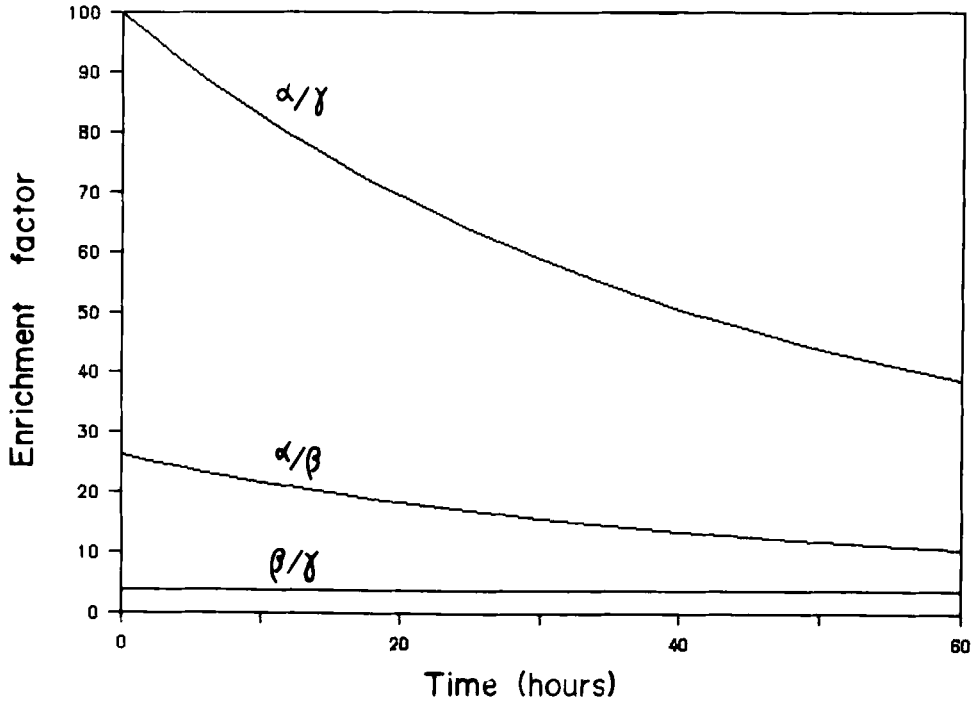


FIG. 4. Enrichment factor, ϕ, versus time in hours obtained using Eq. (6). Temperature: 40°C. The enrichment factor is obviously dependent on the initial bulk concentrations which are identical in this case.

internal tubing volume (1 mL) was flushed in 22 min. At 40°C, after 1 h of equilibration time, the aqueous eluent was collected in test tubes and analyzed by LC. The CD concentrations were found constant, at any time, and were 6×10^{-4} and 1.1×10^{-5} mol/L for α-CD and β-CD, respectively. The γ-CD concentration was lower than 5×10^{-6} mol/L, which was the limit of detection of the LC method with refractive index detection. The set-up shown in Fig. 2 allows the purification of a CD mixture of α-, β-, and γ-CDs producing a 600 mg/L solution of 97.2% pure α-CD. The water solubility difference allows the separation of β- and γ-CDs by precipitation (Table 1). While 15 days are required to produce 1 L of α-CD solution with a single fiber, typical hollow fiber modules contain hundreds or thousands of fibers in parallel. Hence, a very high throughput is possible.

Transport Mechanism

The permeation process through a polymeric membrane involves three steps: 1) adsorption or solution of the permeating CD at the upstream side

of the membrane, 2) diffusion of the CD through the membrane, and 3) desorption at the downstream side of the membrane (*8*). The diffusion of molecules in the membrane takes place through amorphous parts of the polymer. In the case of hollow fibers, the membrane resistance was defined as the ratio of the diffusion coefficient inside the membrane over the membrane thickness (*9*, *10*):

$$k = D/\delta \tag{9}$$

In our case the membrane resistance factor was within the range 10^{-9}–1.5×10^{-6} cm/s, depending on the CD and temperature.

Nafion was thought to be able to form moving water pools inside the amorphous parts of the polymer (*6*). A water pool is stabilized by the sulfonic acid groups connected to the perfluorinated hydrophobe polymer. The pool size has about 3 to 4 nm diameter (*6*). Figure 5 illustrates how this Nafion property could explain the CD transportation by size rather than by water solubility.

To check the aforementioned hypothesis, the Nafion tubing was modified somewhat. A large cation was substituted for the hydrogen ion of the $-SO_3H$ groups to reduce the room inside the pools (Fig. 5). Trimethylphenyl ammonium chloride (TMPAC) was used for this purpose. Nafion tubings were boiled for 3 h in a 0.1-*M* TMPAC solution and equilibrated overnight in the same solution. After rinsing in distilled water for 3 h, the TMPAC-treated tubings gave the results listed in Table 3 (bottom). The permeation rate of α-CD was 15 times lower, and those for β- and γ-CD were not significantly modified. It seems that α-CD is too big to penetrate easily into the TMPAC sterically hindered Nafion pools.

Temperature Effect

Raising the temperature produced permeation rate increases. Within the 23–60°C range studied, drastic changes with temperature in the CD permeation coefficient values were not observed. This seems to indicate that the overall viscosity of the amorphous parts of the ionomer decreased with temperatures increases. However, the moving water pool size was not modified by temperature changes. The CD diffusion coefficients inside the Nafion membrane increased when the temperature was raised, due to permeation rate increases (Table 3). Assuming the permeation rate has an Arrhenius form:

$$k = k_0 e^{-E/RT} \tag{10}$$

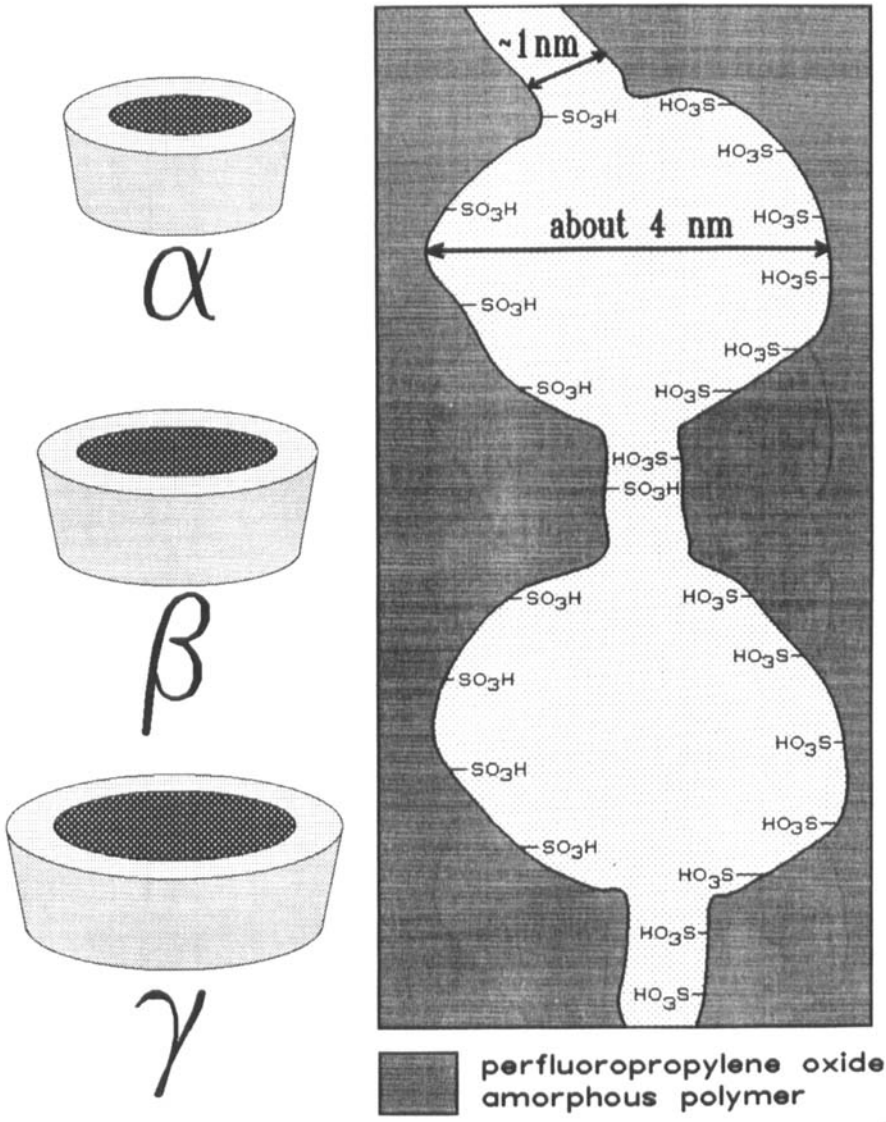


FIG. 5. Oversimplified view of the moving water pools inside the Nafion membrane. The β- and γ-CD are too big to fit inside the pools. TMPAC treatment reduced the room inside the pools so that α-CD could not fit inside anymore; see text.

TABLE 4
Activation Energy for Permeation (Eq. 10)

CD	Tubing treatment	k_0 (s^{-1})	Permeation energy		Regression coefficient
			kJ/mol	kcal/mol	
α	Water equilibrated	10^{-2}	−15.8	− 3.8	0.72
β	Water equilibrated	2.2×10^{7}	−80	−19	0.93
α	TMPAC treated	1.8×10^{4}	−62	−15	0.91
γ	TMPAC treated	10^{5}	−75	−18	0.99

where k_0 is a constant (s^{-1}), E is the activation energy for permeation (kJ/mol), and T is the absolute temperature (K). An Arrhenius plot, ln k versus $1/T$, can be used to obtain an estimated value of E. Table 4 lists the values of k_0, E, and the corresponding regression coefficient corresponding to the data of Table 3. The activation energy was five times lower for the α-CD than for the β-CD. The TMPAC treatment induced a fourfold increase of the activation energy of α-CD that reached a value of −62 kJ/mol (−15 kcal/mol), which is of the same order of magnitude as the activation energy of the two other CDs with or without TMPAC treatment. These results seem to corroborate the Nafion water pool theory (Fig. 5). The low regression coefficients were due to the high uncertainty of the permeation coefficients. The activation energies, listed in Table 4, have the same uncertainty. The k_0 values have so high an uncertainty that they must be considered as indicative only.

CONCLUSION

Nafion tubing was able to extract α-CD from a mixture of α-, β-, and γ-CDs. Used in continuous extraction from a 0.02-*M* solution of α-, β-, and γ-CDs, one 2-m fiber could produce 40 mg of α-CD with a 97.2% purity per day. To scale-up the process, Nafion tubing could be associated in bundles to obtain a high area/volume ratio. If two hundred 2-m fibers were associated in a hollow fiber module, as recently described (*11*), gram-scale and larger amounts of 97.2% pure α-CD could be produced daily with minimum energy consumption.

Acknowledgments

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