Preparation of Celldextrins: An Engineering Approach

INTRODUCTION

Celldextrins are linear polymers of two to seven β-1,4 linked glucose molecules. Properties of these oligosaccharides include a decreasing water solubility with increasing molecular weight, limited solubility in nonaqueous or partially aqueous solvents, and a melting point which increases with increasing weight. These and other properties are summarized in Table 1.

Celldextrins are reaction intermediates for the formation of glucose from cellulose, which is one of the United States most abundant renewable resources. One billion tons of cellulose-containing residues are generated annually. These residues have a potential for yielding over 6 x 10^11 pounds of a valuable chemical feedstock, glucose, from which fuel alcohol and other fermentation derived chemicals can be made. At present the hydrolysis of cellulose to glucose is being intensely studied with the objective of obtaining quantitative data on this process for the engineering design of cellulose saccharification plants. For this research, relatively large quantities of individual pure components celldextrins are required. Unfortunately, except for cellobiose, celldextrins are not commercially available.

Celldextrin mixtures are readily prepared by the classic method of Miller et al. In their approach, cellulose was carefully hydrolyzed with fuming HCl to give a distribution of oligosaccharides from cellobiose to cellulose, as well as glucose. The separation of the mixture was accomplished by chromatography on a charcoal-cellulose column (90 cm long x 3 cm i.d.) using gradient elution with ethanol-water as the eluent. Separation of 2 g of a mixture of celluloses through cellobiose required two to three days of continuous elution. Once used the columns-packing material had diminished separation capability. Hence, new absorbent had to be prepared and the column repacked before each run. This method is quite satisfactory for preparing celldextrins in small quantities and is much cited in the literature. However, owing to the heavy demand for celldextrins in our laboratory for use in enzyme kinetics studies on cellulosic hydrolysis, a less time-consuming separation of celldextrins was needed. Developmental work was, therefore, carried out which resulted in several schemes for increasing productivity.

MATERIALS AND METHODS

Cellobiose Hydrolysis to Form Celldextrins

Hydrolysis of cellulose was carried out by a modified method of Miller et al. The procedure consisted of dissolving 10 g Whamian CW-11 cellulose in ice-cold fuming HCl. The clear viscous solution which formed was warmed to 25°C and kept at this temperature for 2 hr. During this time the cellulose was hydrolyzed to cellobiose. Next, the HCl was partially stripped from the hydrolysate under 20 mm Hg vacuum with an aspirator for 15 min. The mixture was then added to 500 ml ice-cold distilled water. This caused the unhydrolyzed portion of the cellulose to precipitate. The precipitate was then filtered off and the filtrate, containing the celldextrins, was subjected to a neutralization and recovery procedure.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. Wt</th>
<th>Melting point (°C)</th>
<th>Solubility (g/liter)</th>
<th>( D_2^* ) (cm(^2)/sec)</th>
<th>Molecular dimensions (Å)</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellobiose</td>
<td>342.3</td>
<td>225</td>
<td>125-147</td>
<td>insol.</td>
<td>14.6</td>
<td>6.42 hydrolisis yields 2-β-D-glucoses; insol. in ether</td>
<td>1-3</td>
</tr>
<tr>
<td>Cellotriose</td>
<td>504.43</td>
<td>200-209</td>
<td>very sol.</td>
<td>insol. slowly sol.</td>
<td>4.6-4.9</td>
<td>20.2 6.56</td>
<td>2.4 5</td>
</tr>
<tr>
<td>Celloctaose</td>
<td>666.59</td>
<td>252-253</td>
<td>78 at 25°C</td>
<td>insol. insol.</td>
<td>4.2-4.78</td>
<td>26.2 6.60</td>
<td>2.5 6</td>
</tr>
<tr>
<td>Cellopentaoose</td>
<td>828.73</td>
<td>206-268</td>
<td>40 (slowly sol. in warm water, 4.8 at 25°C)</td>
<td>insol. insol.</td>
<td>3.82</td>
<td>31.8 6.66</td>
<td>1 2 5 7</td>
</tr>
<tr>
<td>Cellohexaoose</td>
<td>990.86</td>
<td>275-278</td>
<td>10 (slowly sol. warm water)</td>
<td>–</td>
<td>3.38</td>
<td>37.6 6.68</td>
<td>1 2 5</td>
</tr>
<tr>
<td>Celloheptaoose</td>
<td>1152.90</td>
<td>283-286</td>
<td>1 (slowly sol. in warm water)</td>
<td>–</td>
<td>–</td>
<td>47.6 6.68</td>
<td>5</td>
</tr>
</tbody>
</table>

* In water at 30°C.

* For extended hydrated cellobiose using Stuetz-Briegleb models.

\( D_2^* \) = Molecular Weight

\( \text{Molecular dimensions (Å)} \) = Molecular Weight

\( \text{Melting point (°C)} \) = Molecular Weight

\( \text{Solubility (g/liter)} \) = Molecular Weight

\( \text{Comments} \) = Molecular Weight

\( \text{Ref.} \) = Molecular Weight
Neutralization and Recovery of Cellulose Ions

In early experiments, the filtrate was simultaneously neutralized and deionized using a 4 x 8 column (0.9 liter) of Amberlite IRA-95 macroreticular weak-base anion exchange resin in the free base form. This resin was quite effective in desalting and neutralizing the cellulose Ions in a short time by exchanging OH⁻ for Cl⁻ ions. Unfortunately, owing to the high HC concentration (ca. 3N) in the starting cellulose filtrate, localized heating occurred in the resin bed during ion exchange. The heat, in combination with the basic environment of the resin, promoted partial degradation of the sugars as indicated in the chromatogram of Figure 1(b) obtained by low-pressure liquid chromatography (LPLC) using water as the sole eluent.*

The formation of degradation products was avoided by using a different desalting method. This approach involved using sodium bicarbonate to bring the pH of the filtrate to pH 4-5 followed by evaporation at 30-35°C in a rotary vacuum flash evaporator. After a 15- to 25-fold reduction in the volume of the filtrate must of the salt formed during the neutralization step is precipitated out and filtered off. Distilling of the remaining syrup was done using Sephadex G-15 packed in a 2.5 x 100 cm column.

Each desalting run consisted of layering 25 ml syrup onto the column and eluting.

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*Fig. 1. Liquid chromatograph of cellulose Ions having: (a) no side products and (b) some side products.
with water at a flow rate of 1 to 2 ml/min. The eluate was collected in 12 ml fractions which were analyzed using LPLC. As is evident from Figure 2, a partial separation of cellulodextrins, as well as a salt fractionation, occurred. No side products were formed by this procedure (see Fig. 1(a)). Fractions 12–23 were collected for further separation into pure components.

Separation of Cellulodextrins into Pure Components

Several alternate separation methods were initially considered. These included: partition chromatography on cellulose columns,6-9 gel chromatography on Bio-Gel P-2 and P-4,10-13 chromatography on Sephadex gels,14 ion-exchange chromatography,15 and partition chromatography on silica gel.16

Separations of cellulodextrins were initially carried out using silica gel (Bio-Sil A, 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) packed by the tap-fill method17 in a 15 × 750 mm column. The use of these silica gel columns to separate cellulodextrins was first mentioned by Streamer et al.18 However, details of his method were incomplete, and hence they had to be worked out in our laboratory by trial and error.

The Bio-Sil A columns gave satisfactory separation of cellulodextrins when a mixture of 65% isopropanol–ethyl acetate in a 1:1 volume ratio was used as the eluent.

![Diagram](image-url)

Fig. 2. Desalting of cellulodextrins of a Sephadex G-15 column (2.5 × 100 cm).
However, owing to a high pressure drop (50 to 100 psi) the flow rate through these columns was limited to 1 ml/min.

After some experience with silica gel columns we found that a commercially available, prepacked silica gel column, named the “Labar” column (EM Laboratories Inc., Elmsford, N.Y.) gave superior resolution, higher capacity, and lower pressure drops than our in-house-prepared columns. Separation of cyclodextrins, using the Labar column, was carried out by the following procedure.

Cyclodextrins (500 to 800 mg) were dissolved in 10 ml water and injected into the column. Eluent, consisting of ethyl acetate-45% isopropanol in a 1:1 volume ratio, was pumped through the column at 2 to 3 ml/min. The material eluting from the column was collected in 15 ml fractions and assayed for sugar using the phenolsulfonic acid assay procedure. After cellulotriose (G₃) had eluted, a step change in the eluent composition was made to 1:1.5 ethyl acetate-45% isopropanol and elution continued until G₄ and G₅ came out. The fractions containing the various pure cyclodextrin components (see Fig. 3) were then pooled. These were then dried in a rotary flash evaporator at 50–55°C and then washed with ethanol to remove impurities remaining from the solvent. Liquid chromatography of the various components obtained from this process showed them to be relatively pure (Fig. 4).

Owing to the insolubility of higher-molecular-weight cyclodextrins in partially aqueous solutions, the higher cyclodextrins, especially G₅, G₆, and G₇, tended to precipitate out at the top of the column after eluent flow started. To avoid this problem the cyclodextrin mixture was dissolved in the same solvent used for eluent. The cyclodextrin which did not dissolve was spun down by centrifugation. The clear supernatant was injected into the column.

Using the Labar column, one run took approximately 30–35 hr. After the column was re-equilibrated with solvent of the appropriate composition it was ready for another run. Although this column had the advantages of speed of separation and good resolution, the partially aqueous eluent limited the amount of higher-molecular-weight cyclodextrins that could be obtained owing to the insolubility properties of the cyclodextrins. Thus other partition chromatography methods were not considered.

Fig. 3. Chromatograph of elution profile from Labar column.
Instead an all-aqueous system was developed where desalting and separation of cellodextrins were attained in less than 6 hr using water as the eluent.

One-Step Cellodextrin Desalting and Separation

Separation of oligosaccharides by gel permeation using water as the eluent has received much attention with respect to maltooligosaccharides. Although these methods gave good separation, the capacities appeared to be low while elution times were rather long owing to the mechanical instability of the packing materials. In comparison, separation of cellodextrins on ion-exchange resins seemed more promising. For this and other reasons, mentioned below, ion-exchange chromatography was tried.

Concurrent with efforts to develop preparative-scale separation techniques for cellodextrins, an analytical-scale separation of cellodextrins by liquid chromatography (LC) using low-pressure columns was developed. Good separation of cellodextrins was obtained on columns of cation-exchange resin using water as the eluent.
The experience gained in packing and operating the analytical-scale columns was applied to designing and building a preparative-scale column. The results are described below.

The preparative-scale jacketed column had dimensions of 2.5 i.d. x 120 cm. It was packed with AG-50W X4 cation-exchange resin (400 mesh, BioRad Laboratories, Richmond, Calif.) converted to the calcium form as described elsewhere.23 After heating the column to 85°C, 10 ml desalted cello dextrans were injected and eluted with water. Since initial runs with this column went well, the Sephadex G-15 column was connected in series with the resin column with the Sephadex column preceding the resin column.

Runs with the combined column system consisted of injecting ca. 10 ml cello dextrans-salt concentrate into the Sephadex column and then eluting with water at a

Fig. 5. Chromatograms showing preparative-scale separation of cello dextrans and resulting pure-component cello dextrans.
flow rate of about 1–2 ml/min. At first the cellodextrin-salt mixture passed through
the Sephadex column where fractionation of the glucose and salt from the rest of
the cellodextrins occurred. Once the cellodextrins, G, G, and some G, had passed into
the ion-exchange column, the Sephadex column was disconnected while elution of
the ion-exchange column continued. The salts and glucose were then washed from
the Sephadex column by gravity elution using water. The cellodextrins were sepa-
rated on the ion-exchange column and were collected in 5 ml fractions. In a typical
run, 300–700 mg cellodextrins were separated in about 6 h time. Pooled fractions
gave pure-component cellodextrins in the quantities indicated in Figure 5. Since the
cellodextrins were eluted in water, they could be used 'as is' for kinetic studies.
Drying of these components was not required. This resulted in further time savings.

SUMMARY

The combination of Sephadex G-15 and ion-exchange resin columns allows one-
step desalting and separation of cellodextrins using water as the sole eluent. The
column apparatus described in this paper has the potential of producing up to 3 g
cellohextrins in one day. In addition, the columns described are stable and do not
require repacking or regeneration after each run. Hence the potential exists for
scaling up this system for even greater production of cellohextrins if need be.

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