Separation of Sulfuric Acid and Monosaccharides with a Strong-Base Anion Exchange Resin Containing Polystyrenesulfonate as Polymeric Counter Ion to Reduce Tailing of Sulfuric Acid

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Abstract
This paper describes the separation of sulfuric acid and monosaccharides with an amphoteric ion exchange resin to reduce the tailing of sulfuric acid. The amphoteric ion exchange resin of snake cage type was prepared by polymerizing p-styrenesulfonate ions contained in a strong-base anion exchange resin (Dowex 1X4) as counter ion. First, chloride form Dowex 1X4 (100-200 mesh) was changed into p-styrenesulfonate ion form. Then, p-styrenesulfonate ions in the resin were polymerized for 3 h at 72.5±2.5°C and for more 3 h at 82.5±2.5°C using a water soluble radical initiator. The resulting resin named Dowex 1X4-SS was treated with 1 M (M = mol dm$^{-3}$) NaCl and finally washed with water in a column-mode. FT-IR spectra and elemental analyses of Dowex 1X4-SS suggested its amphoteric structure of snake cage type. The separation of glucose and sulfuric acid was tested by using a column (i.d. 1.5 cm, resin bed length 29-30 cm) at 50°C. The tailing of sulfuric acid was markedly reduced in case of Dowex 1X4-SS, compared to that of the parent resin Dowex 1X4. In addition, the newly developed Dowex 1X4-SS successfully applied to the continuous separation of monosaccharides and sulfuric acid in concentrated sulfuric acid hydrolyzates of bamboo by means of a simulated moving bed chromatography.

Keywords: Amphoteric resin, Acid sugar separation, Simulated moving bed chromatography

1. Introduction
Recently, Sun et al. reported a possible process for the ethanol production from bamboo$^{11}$. This process consisted of (i) hydrolysis of bamboo with concentrated sulfuric acid, (ii) removal of colored compounds in the acid hydrolyzates with activated charcoal, (iii) separation of sulfuric acid and monosaccharides (sugars) in the decolorized acid hydrolyzates by a simulated moving bed chromatography (SMBC), (iv) hydrolysis of oligosaccharides remained in the sugar fraction, and (v) continuous ethanol fermentation. In this system, the step (iii) is the most important because sulfuric acid must be repeatedly used via the separation of sulfuric acid from sugars by SMBC and the followed concentration of sulfuric acid by distillation under reduced pressure. Sun et al. used a SMBC with six columns packed with a strong-base anion exchange resin Diaion MA03SS. However, the marked tailing of sulfuric acid was observed, although sulfuric acid was beautifully separated from sugars$^{11}$. The marked tailing of sulfuric acid lowered the concentration of the eluted sulfuric acid and results in increase of energy cost in the concentrating operation of the eluted sulfuric acid. In case of the separation of sulfuric acid and sugars with a strong-acid cation exchange resin, on the

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contrary, no tailing of sulfuric acid was observed although the separation efficiency of sulfuric acid and sugars was not so high compared with that of the strong-base anion exchange resin\(^2\). These two works\(^1,2\) led us to a study on behavior of an amphoter ion exchange resin containing both quaternary ammonium and sulfonate groups because it was expected that the quaternary ammonium groups give high efficiency of the separation of sulfuric acid and sugars, and sulfonate groups prevent the marked tailing of sulfuric acid. Unfortunately, we were not able to find such kind of commercially available amphoteric resin and then prepared an amphoteric resin of snake cage type\(^3\) (hereafter abbreviated as Dowex 1X4-SS), in which a strong-base anion exchange resin Dowex 1X4 was used as parent resin and polystyrenesulfonate ions were contained as polymeric counter ion (Scheme 1).

![Scheme 1 Structures of resins](image)

This paper reports the preparation and characterization of Dowex 1X4-SS and its performances in the chromatographic separation of sulfuric acid and glucose. In addition, it was applied to the SMBC separation of sulfuric acid and monosaccharides in a sulfuric acid hydrolyzate of bamboo.

2. Experimental

2.1 Materials

Dowex 1X4 (chloride ion form, particle size: 0.11-0.25 mm, total anion exchange capacity: 1.0 meq/mL) was purchased from Muromachi Technos Co. Ltd., Japan. Diaion MA03SS (chloride ion form, particle size: 0.15-0.25 mm, total anion exchange capacity: 1.6 meq/mL) was kindly provided by Mitsubshi Chemical Co., Japan, and this is a strong-base anion exchange resin developed for separation of sulfuric acid and sugars by means of SMBC. Sodium p-styrenesulfonate and 2, 2’-azobis[N-(2-carboxyethyl)-2-methylpropionamidine]hydrate were obtained from Wako Pure Chemical Industries, Co. Ltd., Japan. Unless otherwise noted, usual reagents were special grade chemicals.

2.2 Synthesis of amphoter ion exchange resin of snake cage type\(^3\)

Dowex 1X4 was conditioned in a column-mode. To the Dowex 1X4 (500 mL or 1 L) packed column, electrolyte solutions and water were supplied in the order of 1 M NaCl, water, 1 M NaOH, water, 1 M HCl, and water. Volume of each electrolyte solution was 5 L or 10 L. Water was supplied until column effluents became electrolyte free. After conditioning, 1 M sodium p-styrenesulfonate solution (2.5 L) was supplied to the chloride ion form Dowex 1X4 (500 mL) packed column to obtain the p-styrenesulfonate ion form Dowex 1X4. Then, the column was washed with water until no p-styrenesulfonate ion was detected in column effluents. The p-styrenesulfonate ion form Dowex 1X4 (500 mL) was transferred into a 2 L round-bottom reaction flask with 1 L of water. Then, a water soluble radical initiator (2,2’-azobis[N-(2-carboxyethyl)-2-methylpropionamidine]hydrate, 30 g) was added to the reaction flask. After the initiator was dissolved, the reaction flask was stood for 2 days at room temperature under occasional stirring to diffuse the initiator into the resin. Then the mixture in the flask was heated under stirring for 3 h at 72.5±2.5°C and then for more 3 h at 82.5±2.5°C to polymerize p-styrenesulfonate ions in the resin. After cooling, the resulting resin Dowex 1X4-SS was washed with water. Then the resin was treated with 1 M NaCl in a column-mode to exchange unreacted p-styrenesulfonate ion in the resin with chloride ion. After feeding 1 M NaCl (5 L), no p-styrenesulfonate ion was detected in column effluents by means of UV-visible spectrometry.

Syntheses of Dowex 1X4-SS were repeated 7 times. The resulting resin in each run was characterized by means of FT-IR spectrum measurement and CHN analysis. The sulfur content was also determined by means of ICP-AES after the acid digestion of resin samples.

2.3 Chromatographic separation of glucose and sulfuric acid by a conventional column chromatography

Dowex 1X4-SS was packed into a glass column (i.d. 1.5 cm, length 30 cm) with a jacket for water circulation. The volume of the resin bed was 50.9-53.4 mL. The resin packed column was conditioned by feeding 3.73 M (ca. 30 wt%) sulfuric acid and then was washed with water until effluent became acid free. The temperature of the column was kept at 50°C by circulating water from a water bath. The sample solution was an aqueous solution containing 326 g/L (3.32 M) sulfuric acid and 150 g/L (0.832 M) glucose. After the sample solution (15 mL) was loaded on the column, water as eluent was immediately supplied to the column. A flow rate of liquids in both loading and elution operation was 2 bed volumes (BV) per hour. All effluents from the column were collected on a fraction collector. The fraction volume was 5 mL (ca. 0.1 BV). Concentrations of sulfuric acid and glucose in each fraction were determined by means of an acid-base titration and a sugar analyzer SU-300, TOA DKK, Japan, respectively. For comparison, Dowex 1X4 and Diaion MA03SS were also tested.

2.4 Effect of the sample size on the separation of glucose and sulfuric acid by means of a conventional chromatography

The method and the apparatus were the same as those described in the section 2.3 except for concentrations of glucose and sulfuric acid. The loaded volume of sample solutions was
fixed at 15.0 mL. Compositions of sample solutions are given with the results in the later section.

2.5 Chromatographic separation of glucose and sulfuric acid by means of a SMBC system

In this work, a divided improved simulated moving bed chromatography (DISMBC) system was used and this system was purchased from Nippon Rensui Co. Ltd., Japan. Figure 1 shows the schematic configuration of the DISMBC system, which consists of 6 columns and is divided into 4 zones. Dowex 1X4-SS was packed into each column (i.d. 3.2 cm, length 50.0 cm, resin bed volume 402 mL) and the total volume of the resin was 2412 mL.

In Zones a, b, and d, one column was allocated, and remaining 3 columns were in Zone c. All 6 columns were submerged in a thermostated water tank at 50°C. One cycle of the separation operation consisted of 6 steps and each step was divided into 4 sub-steps (A, B, C, and D) as shown in Fig. 1. In sub-step A, the saccharified solution F was introduced and the R1 fraction containing mainly sugars was simultaneously extracted. In sub-step B, water W as eluent was introduced and the P fraction containing mainly sulfuric acid was simultaneously extracted. In sub-step C, water W as eluent was introduced and the R2 fraction containing mainly sugars was simultaneously extracted. In sub-step D, the liquid in all columns was circulated through the 6 columns. Then, the location of saccharide liquid and water inlets (F and W) and extracting outlets (P, R1, and R2) were moved to the next column, and the second step was started and repeated by 6 times. After finishing the first cycle, the next cycle was started in the same way. Flow rate was 29.54 mL/min. Times during sub-steps A, B, C, and D were 174, 316, 26, and 413 sec, respectively.

3. Results and Discussion
3.1 Characterization of Dowex 1X4-SS

It is expected that an amphoteric ion exchange resin containing both quaternary ammonium and sulfonate groups provides excellent performances for chromatographic separation of sulfuric acid and sugars in terms of high separation efficiency and suppression of tailing of sulfuric acid. As far as we searched, such kind of amphoteric ion exchange resin was not commercially available. Thus, we prepared an amphoteric resin of snake cage type by polymerizing p-styrenesulfonate ions contained in the strong-base anion exchange resin as counter ion. A strong-base anion exchange resin Diaion MA03SS is provided by the ordering production, and then it is difficult to use it as parent resin for the synthesis of more than 2.4 L of the amphoteric resin for the SMBC study. In the present study, Dowex 1X4 was selected as parent resin because its particle size distribution is close to that of Diaion MA03SS and its degree of crosslinking is clearly disclosed.

FT-IR spectra of the resulting resins showed the strong adsorption band of sulfonate ions at 1180 cm⁻¹, where the parent resin Dowex 1X4 did not have the strong absorption. Table 1 summarizes nitrogen and sulfur contents of the resulting Dowex 1X4-SS. As shown in Table 1, ca. 70% of chloride ions (35.45 mmol/g) were exchanged by polystyrenesulfonate ions (molecular weight of monomer 183.2 g/mol). Therefore nitrogen contents in mmol/g of Dowex 1X4-SS decreased down to 2.7±0.1 mmol/g from that of the parent Dowex 1X4 (3.5 mmol/g).

![Fig. 2 Wet volumes of Dowex 1X4 (chloride ion form) and Dowex 1X4-SS. Each dried resin (1 g) was equilibrated with water.](image)
Relative standard deviations of nitrogen and sulfur contents were 5% and 11%, respectively, and that of sulfur to nitrogen molar ratio (S/N) was 15%. Thus, the precision of nitrogen analysis was higher than that of sulfur analysis. Despite of the somewhat lower precision of sulfur analysis, it can be conceded that ca. 70% of p-styrenesulfonate ions were polymerized in the resin phase resulting in polymeric counter ions.

Figure 2 compares swelling volumes of Dowex 1X4 and Dowex 1X4-SS in water. Here, each resin (1.00 g in dried state) was equilibrated in water at room temperature. Volumes of wet resins were 2.8 and 1.9 mL/g-dry resin, respectively. The swelling volume of Dowex 1X4-SS was two thirds that of the parent Dowex 1X4. Seemingly this indicates strong electrostatic binding of polystyrenesulfonate anions with polymeric cations fixed on the crosslinked polymer matrix.

3.2 Separation of glucose and sulfuric acid by means of a conventional chromatography

Dowex 1X4-SS was prepared for the suppression of tailing of sulfuric acid. Then, performances of Dowex 1X4-SS in the chromatographic separation of sulfuric acid and glucose were examined and compared with those of strong-base anion exchange resins Dowex 1X4 and Diaion MA03SS. In this work, the resin synthesis was repeated 7 times to secure about 3 L of Dowex 1X4-SS for the SMBC study. Then, we investigated chromatographic performances of the resin Dowex 1X4-SS obtained by all synthetic runs.

Figure 3 (a, b, c) compares results of the chromatographic separation of glucose and sulfuric acid by using three respective columns, in which Dowex 1X4-SS, Dowex 1X4 and Diaion MA03SS were packed, and the numerical results are given in Table 2.

As judged from Fig. 3 (a, b, c), these resins prefer sulfuric acid to glucose; first glucose was eluted and then sulfuric acid. For glucose, each resin gave the elution profile close to a Gaussian distribution curve. On the other hand, the elution profile of sulfuric acid was significantly different between Dowex 1X4-SS and two strong-base anion exchange resins (Dowex 1X4 and Diaion MA03SS). First, the maximum concentration of sulfuric acid was about 252±8 g/L in case of Dowex 1X4-SS but not so high in cases of the strong-base anion exchange resins (Table 2). Second, the elution profile of sulfuric acid was classified into two parts; one is the region (1) where the concentration of sulfuric acid was higher than ca. 0.4 M and the other is the region (2) where the concentration of the acid was less than ca. 0.4 M because the dissociations of sulfuric acid are expressed as follows:

\[
\begin{align*}
H_2SO_4 & \rightleftharpoons H^+ + HSO_4^- , \quad \text{strong acid} \\
HSO_4^- & \rightleftharpoons H^+ + SO_4^{2-} , \quad K_{a2} = 10^{-1.9}.
\end{align*}
\]

Above ca. 0.4 M, sulfuric acid exists as the species H^+ and HSO_4^- and then the elution profile of the acid was nearly symmetric because the univalent anion is not so strongly partitioned into the resin phase. Below ca. 0.4 M, the lower the...
concentration of the acid, the greater the molar fraction of divalent \( \text{SO}_4^{2-} \) species. Then, the tailing of the sulfuric acid was extremely prolonged with decreasing the acid concentration owing to the electroselectivity phenomenon proposed by Hellferich\(^5\).

As mentioned above, both strong-anion exchange resins showed the prolonged tailing of sulfuric acid in its concentration region less than 30-40 g/L (0.3-0.4 M), where the second step dissociation of sulfuric acid becomes marked with decreasing the sulfuric acid concentration. In case of the amphoteric resin Dowex 1X4-SS containing both quaternary ammonium and polymeric sulfonate sites, the latter sites more strongly repel the divalent anion species \( \text{SO}_4^{2-} \) than does the univalent species \( \text{HSO}_4^- \), therefore the strong tailing was significantly reduced as shown in Fig. 3.

Next, we refer to the numeric data given in Table 2; here, No. for Dowex 1X4-SS in the second column corresponds to the run No. in Table 1. In case of Dowex 1X4-SS, retention volumes of glucose and sulfuric acid were 0.807 ± 0.056 and 1.08 ± 0.06 BV, respectively. Coefficient of variation (relative standard deviation) on the retention volumes of the sugar and acid were 6.9% and 5.6%, respectively. These data mean that the precision of RV and HBW for each resin were average of duplicated runs.

Table 2 Numerical data on the separation of glucose and sulfuric acid by means of conventional column chromatography

<table>
<thead>
<tr>
<th>Resin</th>
<th>Glucose</th>
<th>Sulfuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV (^a)</td>
<td>HBW (^b)</td>
</tr>
<tr>
<td>1</td>
<td>0.82</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>0.81</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>0.92</td>
<td>0.30</td>
</tr>
<tr>
<td>4</td>
<td>0.79</td>
<td>0.34</td>
</tr>
<tr>
<td>Dowex 1X4-SS</td>
<td>0.76</td>
<td>0.28</td>
</tr>
<tr>
<td>5</td>
<td>0.80</td>
<td>0.35</td>
</tr>
<tr>
<td>6</td>
<td>0.75</td>
<td>0.28</td>
</tr>
<tr>
<td>7</td>
<td>0.80</td>
<td>0.32</td>
</tr>
<tr>
<td>Dowex 1X4</td>
<td>1.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Diaion MA03SS</td>
<td>0.86</td>
<td>0.32</td>
</tr>
</tbody>
</table>

a) Retention volume, b) Bed volume, c) Half band width, d) Maximum concentration, e) Average, f) Standard deviation, g) Coefficient of variation (Relative standard deviation).

As quantitative measures of the peak tailing, peak asymmetry factor and U.S. Pharmacopeia tailing factor were proposed\(^6\). In the present work, however, the chromatographic profile of sulfuric acid was clearly classified into the two regions as already discussed. Taking into account this specific situation, we introduced an index named \( DT \) to express the degree of the tailing of sulfuric acid as follows:

\[
DT = B/(A+B)
\]

here, \( A \) and \( B \) are the amount of sulfuric acid eluted within 2 BV of the effluent and that from 2 to 8 BV of the effluent, respectively. The boundary of 2 BV was selected because the majority of both solutes were eluted up to 2 BV of the effluent in case of Dowex 1X4-SS. The smaller the \( DT \), the lower the tailing. Table 3 shows numeric data for \( A, B, \) and \( DT \). In cases of the strong-base resins, Dowex 1X4 and Diaion MA03SS, \( DT \) values were 0.435 and 0.357, respectively. On the other hand,

Table 3 Numeric data for the tailing of sulfuric acid

<table>
<thead>
<tr>
<th>Resin</th>
<th>Eluted H(_2)SO(_4) (g)</th>
<th>( DT )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2 BV</td>
<td>2-8 BV</td>
</tr>
<tr>
<td>Dowex 1X4-SS</td>
<td>4.54</td>
<td>0.42</td>
</tr>
<tr>
<td>Dowex 1X4</td>
<td>2.58</td>
<td>1.99</td>
</tr>
<tr>
<td>Diaion MA03SS</td>
<td>3.04</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Note: Values of RV and HBW for each resin were average of duplicated runs.

\( DT \) value was only 0.085 for Dowex 1X4-SS; thus, the marked tailing of sulfuric acid for the parent resin Dowex 1X4 was significantly reduced by its chemical modification proposed by the present study. The significant improvement of the tailing of sulfuric acid brings some practical benefits, such as the recovery of sulfuric acid at high concentration and marked decrease in both time and eluent volume required in the separation operation. Therefore, the further study was limited to Dowex 1X4-SS.
3.3 Effect of the sample size on the separation of glucose and sulfuric acid by means of a conventional chromatography

The resolution of two solutes in the chromatographic separation depends on loaded amounts of both solutes (sample size). Then, the effect of sample size was examined by using four kinds of sample solutions. Figure 4 (a, b, c, d) shows results. The composition of the sample solution (a) was 326 g/L (3.32 M) sulfuric acid and 150.0 g/L (0.832 M) glucose. Sample solutions (b), (c), and (d) were prepared by diluting the sample solution (a) with water, as written in caption of Fig. 4. Then, the sulfuric acid to glucose molar ratio was constant (3.99) in all sample solutions. As judged from Fig. 4 (a, b, c, d), overlapping area of two peaks decreased with decreasing the sample size.

Table 4 lists retention volumes and half band widths of two solutes. Retention volumes of both solutes were independent of the sample size, because averaged (n = 4) retention volume of glucose was 0.798±0.017 BV (coefficient of variation = 2.1%), and that of sulfuric acid was 1.105±0.017 BV (coefficient of variation = 1.5%). On the other hand, half band widths of both solutes decreased with decreasing the sample size.

The degree of the separation of two solutes is quantitatively expressed by the parameter called resolution (Rs). Recently, Dolan6) recommend to use the following equation in calculation of Rs:

\[ R_s = 1.18(R_2-R_1)/(H_1+H_2) \]  (2)

where \( R_2 \) and \( R_1 \) are respective retention volumes of second and first peaks, and where \( H_1 \) and \( H_2 \) are half band widths of first and second peaks, respectively. This equation is significantly useful when it is difficult to determine peak widths measured at the baseline between tangents drawn to the peak sides because of the marked peak tailing.

In addition to \( R_s \), percentages of each solute in overlapped area of two peaks (\( O_p \)) were calculated by the following equation.

\[ O_p = 100(\Sigma (C_m)V_j)/W_{sum} \]  (3)
where \((C_m)_j\) is the concentration of a minor solute in the \(j\)-th fraction in the overlapped area of two peaks and \(V_j\) is volume of the \(j\)-th fraction, and where \(W_{\text{sum}}\) is a total amount of each solute eluted.

Table 5 lists \(R_s\) and \(O_p\). With a decrease in the sample size, \(R_s\) increased resulting in the enhancement of the separation efficiency. Indeed, values of \(O_p\) on both solutes decreased with decreasing the sample size. The value of \(O_p\) for glucose was greater than that for sulfuric acid, because the concentration of sulfuric acid in sample solutions was about twice that of glucose. As shown in Table 5, it is possible to control \(R_s\) and \(O_p\) by changing sample size. However, the high resolution is incompatible with the high concentration of the eluted solute. Then, it is important to find the optimum conditions for the contradiction of these parameters from the practical standpoints.

### Table 5 Effect of the sample size on resolution and percentages of each solute in the overlapped area of two peaks

<table>
<thead>
<tr>
<th>Symbol for sample solution in Fig. 4</th>
<th>(R_s)</th>
<th>Glucose</th>
<th>Sulfuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>0.49</td>
<td>46</td>
<td>20</td>
</tr>
<tr>
<td>(b)</td>
<td>0.56</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>(c)</td>
<td>0.61</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>(d)</td>
<td>0.72</td>
<td>5.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Here, the results of Run 2 in Fig. 4 were analyzed.

### 3.4 Chromatographic separation of glucose and sulfuric acid by DISMBC system

Strictly speaking, Dowex 1X4-SS resins prepared 7 times exhibit the slightly different performances in the chromatographic separation of glucose and sulfuric acid, as shown in Table 2. Since the present SMB system needed 6 columns, all Dowex 1X4-SS resins prepared in each run were mixed well by stirring for several hours. Then, the mixed resin was packed into each column to even up the chromatographic performances of 6 columns.

First, a model sample solution containing 332 g/L of sulfuric acid and 92.1 g/L of glucose was loaded into the DISMBC system. Both sulfuric acid and glucose were recovered with high efficiency; recovery efficiency of sulfuric acid in the P fraction was 97.1% and that of glucose in the R fraction was 98.0%. Concentration of sulfuric acid in the P fraction was 163 g/L.

Next, the separation of monosaccharides and sulfuric acid in the decolored sulfuric acid hydrolyzate of bamboo was conducted. This saccharified liquid contained 388 g/L of sulfuric acid, 71.9 g/L of glucose, and 36.6 g/L of xylose. High recovery efficiencies of sulfuric acid and sugars were also observed; the recovery efficiency of sulfuric acid in the P fraction was 97.5% and that of sugars (glucose and xylose) in the R fraction was 96.9%. Concentration of sulfuric acid in the P fraction was 193 g/L. It was proved that Dowex 1X4-SS was successfully applied to the separation of sulfuric acid and monosaccharides in a sulfuric acid hydrolyzate of bamboo by means of the DISMBC method.

### Acknowledgements

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### References