**Research Article** 



# Carbohydrate Separation Using the Core-Shell Ion-Exchange Resin St-80 with Different Numbers of Methylene Groups in the Porous Shell and a Constant Cross-Linking Degree

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# Abstract

Clarifying the relationship between the molecular structure of ion-exchange resins and the elution of carbohydrates is essential for analyses using high-performance liquid chromatography (HPLC). From the perspective of novel resin development, we evaluated the effect of the number of methylene groups in the functional chain of the porous polymer shell on carbohydrate separation. Core-shell ion-exchange resins with a monomer weight ratio of 20:80 (denoted as St-80) were synthesized with a constant cross-linking degree of 55%. The number of methylene groups in the functional chain of the porous polymer shell was varied from two to six for analyses of carbohydrate separation performance under strong alkaline conditions. A mixture of inositol, glucose, fructose, and sucrose was separated using a 0.10 or 0.15 mol/L NaOH eluent at flow rates of 0.3–0.7 mL/min. The retention times were compared among St-80 variants with different numbers of methylene groups in the porous layer. As the number of methylene groups increased, the retention times of each carbohydrate for St-80(Me:4) at flow rates of 0.3–0.7 mL/min with 0.10 mol/L NaOH eluent increased slightly. The theoretical plate numbers of glucose and fructose at flow rates of 0.5 and 0.7 mL/min decreased as the number of methylene groups decreased from six to two. These results suggest that St-80 core-shell ion-exchange resins are highly efficient for carbohydrate analyses. Their suitability for strongly alkaline conditions allows their effective use in electrochemical detection.

# **Keywords**

High-performance Liquid Chromatography, Core-shell Ion-exchange Resin, Carbohydrates, Retention Time, Theoretical Plate Number

# **1. Introduction**

Choosing an appropriate ion-exchange resin is essential for high-performance liquid chromatography (HPLC), a critical analytical tool. Various core-shell resins have been developed for this purpose [1, 2], including octadecyl-functionalized silica resins [3-8]. However, silica-based resins are not suitable for use under strongly alkaline conditions. Sty-rene-divinylbenzene- and acrylamide-type polymers are frequently used as base materials for organic resins [9-13].

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However, the use of polymer resins is limited in high-speed HPLC owing to their fully porous structure. To overcome these issues, core-shell ion-exchange resins composed of a porous polymer shell and a dense core have been synthesized. These resins provide superior durability at high pH. Two commercially available examples are core-shell ion-exchange resins fabricated via precipitation polymerization around the core [14, 15] and latex-type resins that use a styrene base [16-18].

The performance of these resins is mainly determined by the thickness and degree of cross-linking of the shell portion; therefore, these parameters should be optimized for HPLC analyses [19]. Because the retention time increases with the thickness of the porous shell, the shell portion should be as thin as possible to reduce the analysis time. Furthermore, an appropriate degree of cross-linking in the porous shell portion is necessary to achieve good separation performance.

We have previously investigated the effects of various factors (shell thickness, degree of cross-linking in the porous shell, concentration of the NaOH eluent, and number of methylene groups in the functional chain) on the performance of core-shell ion-exchange resins consisting of a dense polymer core and a porous polymer shell with a functional chain in the polymer structure [19-27].

We initially compared ion-exchange resins with core-shell monomer weight ratios (before suspension polymerization) of 20:80 (St-80) and 0:100 (fully porous resin) with a cross-linking degree of 55% in the porous region; St-80 had a shorter retention time in HPLC analyses of carbohydrates than that of the fully porous resin [28-30]. We also evaluated St-80 resins with various degrees of cross-linking (i.e., 10%, 40%, and 55%) in the porous shell [31] and with a constant cross-linking degree of 55% and core-shell monomer weight ratios of 50:50, 40:60, and 30:70 (St-50, St-60, and St-70, respectively), which affected the thickness of the shell [32]. We then evaluated the elution behavior of carbohydrates using St-50 and St-70 ion-exchange resins with cross-linking degrees of 10%, 40%, and 55% [33, 34]. Finally, we studied carbohydrate elution behavior using St-60 and St-70 resins with two, four, and six methylene groups in the functional chain (St-60(Me:2), St-60(Me:4), and St-60(Me:6) and (St-70(Me:2), St-70(Me:4), and St-70(Me:6), respectively), holding the cross-linking degree constant at 55% [35, 36]. However, further research is required to understand the effect of the number of methylene groups on the carbohydrate elution behavior when using core-shell ion-exchange resins with different shell thicknesses.

In this study, we focused on the effect of the number of methylene groups in the functional chain on the carbohydrate elution behavior using St-80 (monomer weight ratio: 20:80) with a cross-linking degree of 55% in the porous shell. Two, four, and six methylene groups were evaluated (denoted as St-80(Me:2), St-80(Me:4), and St-80(Me:6), respectively). St-80 has a thicker porous shell than those of St-60 [35] and St-70 [36] and, therefore, this study also provides insight into

the effect of the number of methylene groups at different shell thicknesses.

## 2. Materials and Methods

#### **2.1. Materials**

*myo*-Inositol, sucrose, and NaOH were obtained from Fujifilm Wako Chemicals Co. D(-)-fructose and D(+)-glucose were obtained from Kanto Chemical Co. Ultrapure water (ELGA) was used to prepare the eluent and sample solutions. Sample solutions were prepared by sequentially mixing and diluting the stock solutions to concentrations of 500 or 1000 mg/L.

#### 2.2. Preparation of Core-shell ion-exchange Resins

The core-shell ion-exchange resin consisted of a hard polymer core and a porous shell containing functional chains, as shown in Figures 1 and 2 [31]. The porous shell was synthesized by reacting a chloromethylstyrene-divinylbenze copolymer carrier with a tertiary amine, as described previously [19]. The thickness of the shell was kept constant by maintaining a constant core-shell monomer weight ratio of 20:80 and a constant total mass of monomers. The degree of cross-linking in the porous layer was also kept constant at 55% by employing a styrene/divinylbenzene weight ratio of 45:55 [32]. The number of methylene groups in the functional chain of the porous layer was adjusted by using N, N, N', N'-tetramethyl ethylenediamine, N,N,N',N'-tetramethyl-1,4-butanediamine, and N,N,N',N'-tetramethyl-1,6-hexamethylenediamine as the tertiary amines to produce core-shell ion-exchange resins with two, four, and six methylene groups (denoted as St-80(Me:6), St-80(Me:4), and St-80(Me:2), respectively). For comparison, a fully porous resin (i.e., with no core) with a degree of cross-linking of 55% and six methylene groups in the functional chain was prepared by reacting the chloromethylstyren-divinylbenzene copolymer carrier (divinylbenzene weight ratio: 55%) with the *N*,*N*,*N*',*N*'-tetramethyl-1,6-hexamethylenediamine tertiary amine. The prepared resins had an average diameter of 5 µm. We prepared 3 g of each core-shell and fully porous resin.



*Figure 1.* Structure of the core-shell ion-exchange resin consisting of a dense polymer core and an ion-exchange porous polymer shell.



*Figure 2.* Chemical structure of the porous polymer in the ion-exchange resin shell (n = 2, 4, and 6).

#### 2.3. Conditions for the HPLC Analysis

HPLC was performed using a DKK-TOA SU-300 instrument equipped with an electrochemical detector and a gold electrode. The resins were mixed with 10 mL of a 0.10 mol/L NaOH eluent and packed into a 4.6 mm  $\times$  150 mm I. D. stainless steel column using a conventional slurry packing method at a constant pressure of 120 kg/cm<sup>2</sup>. The sample solution (20 µL) containing carbohydrates (inositol, glucose, fructose, and sucrose) was injected into an AS-8020 HPLC autosampler (Tosoh) and eluted with either a 0.10 or 0.15 mol/L NaOH eluent at room temperature (30 °C). Flow rates of 0.3, 0.5, and 0.7 mL/min were used. The theoretical plate number (N) of each carbohydrate in the standard solution was determined using a built-in data-processing program. We calculated the electrostatic charge on the N<sup>+</sup> atom in the functional chain by density functional theory using the ωB97X-D density functional and 6.31G\* basis set in Spartan'20 (Figure 3).

#### **3. Results**

# 3.1. Carbohydrate Separation Performance of St-80 (Me:2), St-80 (Me:4), and St-80 (Me:6) Ion-exchange Resins

#### 3.1.1. Effects of the NaOH Eluent Concentration and Flow Rate

We first evaluated the carbohydrate separation performance of columns packed with the St-80(Me:2), St-80(Me:4), and St-80(Me:6) resins using a 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min. Figure 4a–c. presents the chromatograms at a flow rate of 0.5 mL/min, and Table 1 presents the retention times of glucose, fructose, and sucrose at each flow rate. The retention times of each carbohydrate did not change substantially when the number of methylene groups increased and St-80(Me:4) exhibited slightly longer retention

times than those of other resins at all flow rates. In addition, each carbohydrate demonstrated clear peaks in the chroma-

tograms, and the peak shapes changed only slightly as the number of methylene groups increased.



**Figure 3.** Structures used for optimization of the electrostatic charge on the  $N^+$  atoms in the functional chain using Spartan'20: (a) functional chain of the ion-exchange resin and (b) representative carbohydrate molecule.



*Figure 4.* Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-80(Me:2), (b) St-80(Me:4), and (c) St-80(Me:6) with 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min.

Next, the eluent concentration was increased to 0.15 mol/L NaOH. The chromatograms for a flow rate of 0.5 mL/min are

shown in Figures 5a–c, and the retention times of glucose, fructose, and sucrose at flow rates of 0.3, 0.5, and 0.7 mL/min are listed in Table 2. Similar to the results obtained using the 0.10 mol/L NaOH eluent, St-80 (Me:4) had the longest retention times for each carbohydrate, and similar peak shapes were observed at flow rates of 0.3 and 0.7 mL/min.

#### 3.1.2. Comparison Among St-60, St-70, and Fully Porous Resins

We also evaluated the retention times of sucrose using the 0.10 mol/L NaOH eluent and St-60(Me:2), St-60(Me:4), and St-60(Me:6) core-shell ion-exchange resins, which had a core-shell monomer weight ratio of 40:60 and two, four, and six methylene groups, respectively. Good chromatograms were obtained using St-60(Me:2, 4, 6) at all flow rates with 0.10 mol/L NaOH eluent (Table 3) [35].



**Figure 5.** Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-80(Me:2), (b) St-80(Me:4), and (c) St-80(Me:6) with 0.15 mol/L NaOH eluent at a flow rate of 0.5 mL/min.

Good chromatograms were also obtained using St-70 (Me:4 and 6) at a flow rates of 0.3 and 0.5 mL/min with 0.10 mol/L NaOH eluent (Table 3) [36].

A fully porous resin (i.e., with no dense core) with six

methylene groups (Me:6) and the same cross-linking degree (55%) used for the porous shells of the St-80(Me:2, 4, and 6), St-70(Me:2, 4, and 6), and St-60(Me:2, 4, and 6) core-shell resins was evaluated for comparison. Notably, the core-shell resins all exhibited shorter retention times for sucrose than that of the fully porous resin (Me:6) at all flow rates and NaOH eluent concentrations (Table 3). Because the core-shell resins only contain a thin shell of porous polymer, the interaction time between the carbohydrates and porous region is shorter than that for the fully porous resin (Me:6), resulting in significantly shorter retention times [32].

*Table 1.* Retention times (min) of glucose, fructose, and sucrose using St-80(Me:2), St-80(Me:4), St-80(Me:6), and fully porous (Me:6) resins with 0.10 mol/L NaOH eluent at a flow rates of 0.3–0.7 mL/min.

| Flow rate     | Number of CH <sub>2</sub> | Glu  | Fru  | Suc  |
|---------------|---------------------------|------|------|------|
|               | 2                         | 20.5 | 24.6 | 27.3 |
| 0.3           | 4                         | 21.8 | 25.9 | 31.5 |
| mL/min        | 6                         | 19.3 | 22.1 | 26.7 |
|               | Fully porous, 6           | 26.9 | 32.5 | 44.2 |
|               | 2                         | 12.4 | 14.8 | 16.9 |
| 0.5           | 4                         | 13.1 | 15.6 | 18.9 |
| mL/min        | 6                         | 12.9 | 15.0 | 18.6 |
|               | Fully porous, 6           | 16.4 | 19.6 | 27.2 |
| 0.7<br>mL/min | 2                         | 8.9  | 10.6 | 12.2 |
|               | 4                         | 9.5  | 11.2 | 13.6 |
|               | 6                         | 9.2  | 10.7 | 13.2 |
|               | Fully porous, 6           | 11.8 | 14.2 | 19.9 |

#### 3.2. Resolution Between Glucose and Fructose Peaks

To further investigate the carbohydrate separation performance of the resins, we evaluated the resolution between the glucose and fructose peaks (Table 3), which were adjacent in the chromatograms. When using the 0.10 and 0.15 mol/L NaOH eluent, resolutions of  $\geq$ 1.5 were achieved for the St-80(Me:2, 4, and 6) core-shell resins at flow rates of 0.3, 0.5, and 0.7 mL/min, indicating that they had good separation performance [37].

Only St-70(Me:4 and 6) exhibited a resolution of  $\geq$ 1.5 at flow rates of 0.3 and 0.5 mL/min with 0.10 mol/L NaOH eluent, whereas those of St-70(Me:2) and St-70(Me:4) were 1.2 and 1.4 at a flow rate of 0.7 mL/min, respectively [36]. St-60(Me:2, 4, and 6) exhibited good separation performance with the 0.10 mol/L NaOH eluent at all flow rates.

*Table 2.* Retention times (min) of glucose, fructose, and sucrose using St-80(Me:2), St-80(Me:4), St-80(Me:6), and fully porous (Me:6) resins with 0.15 mol/L NaOH eluent at flow rates of 0.3–0.7 mL/min.

| Flow rate     | Number of CH <sub>2</sub> | Glu  | Fru  | Suc  |
|---------------|---------------------------|------|------|------|
| 0.3<br>mL/min | 2                         | 15.7 | 18.3 | 21.3 |
|               | 4                         | 17.1 | 19.8 | 24.9 |
|               | 6                         | 16.3 | 18.6 | 23.7 |
|               | Fully porous, 6           | 20.0 | 23.3 | 33.6 |
| 0.5<br>mL/min | 2                         | 9.6  | 11.1 | 13.2 |
|               | 4                         | 9.4  | 10.8 | 13.9 |

| Flow rate | Number of CH <sub>2</sub> | Glu  | Fru  | Suc  |
|-----------|---------------------------|------|------|------|
|           | 6                         | 10.1 | 11.6 | 14.5 |
|           | Fully porous, 6           | 11.9 | 13.8 | 19.2 |
|           | 2                         | 6.9  | 8.0  | 9.5  |
| 0.7       | 4                         | 7.4  | 8.6  | 11.0 |
| mL/min    | 6                         | 7.4  | 8.5  | 10.9 |
|           | Fully porous, 6           | 8.9  | 10.5 | 14.6 |

Table 3. Resolution between glucose and fructose and retention times of sucrose using St-80(Me:6), St-70 (Me:6), St-60(Me:6), and fully porous (Me:6) resins with 0.10 and 0.15 mol/L NaOH eluents at flow rates of 0.3–0.7 mL/min.

| Flow rate     | Number of CH2   | 0.10 mol/L NaOH |       | 0.15 mol/L NaOH |       | RT (Suc) |       |       |       |       |
|---------------|-----------------|-----------------|-------|-----------------|-------|----------|-------|-------|-------|-------|
|               |                 | St-80           | St-70 | St-60           | St-80 | St-70    | St-60 | St-80 | St-70 | St-60 |
|               | 2               | 3.2             | 1.4   | 1.8             | 2.6   | 1.3      | 1.5   | 27.3  | 18.0  | 22.3  |
| 0.3           | 4               | 2.7             | 1.6   | 1.9             | 2.3   | 1.4      | 1.5   | 31.5  | 24.1  | 23.1  |
| mL/min        | 6               | 2.2             | 2.2   | 2.6             | 2.0   | 1.9      | 2.1   | 26.7  | 26.6  | 23.8  |
|               | Fully porous, 6 | 3.0             | 3.0   | 2.9             | 2.3   | 2.3      | 2.3   | 44.2  | 44.2  | 44.2  |
|               | 2               | 2.7             | 1.3   | 1.6             | 2.2   | 1.1      | 1.3   | 16.9  | 10.9  | 13.5  |
| 0.5           | 4               | 2.5             | 1.5   | 1.7             | 2.1   | 1.4      | 1.3   | 18.9  | 14.6  | 13.8  |
| mL/min        | 6               | 2.2             | 1.9   | 2.2             | 1.9   | 1.7      | 1.8   | 18.6  | 17.3  | 14.1  |
|               | Fully porous, 6 | 2.6             | 2.6   | 2.6             | 1.9   | 1.9      | 1.9   | 27.2  | 27.2  | 27.2  |
|               | 2               | 2.4             | 1.2   | 1.5             | 1.9   | 1.0      | 1.2   | 12.2  | 7.9   | 9.8   |
| 0.7<br>mL/min | 4               | 2.3             | 1.4   | 1.5             | 2.0   | 1.3      | 1.2   | 13.6  | 10.5  | 10.2  |
|               | 6               | 1.9             | 1.6   | 2.0             | 1.7   | 1.5      | 1.6   | 13.2  | 12.1  | 9.6   |
|               | Fully porous, 6 | 2.3             | 2.3   | 2.3             | 2.0   | 2.0      | 2.0   | 19.9  | 19.9  | 19.9  |

\*St-60 data are reported in Ref 35. \*St-70 data are submitted in Ref 36 (under review).

Resolutions of  $\geq 1.5$  were achieved for the St-60 (Me:2, 4, and 6) core-shell resins at all flow rates with 0.10 mol/L NaOH eluent.

When the eluent concentration was increased to 0.15 mol/L NaOH, the resolution was  $\geq 1.5$  for St-80(Me:2, 4, and 6) at all flow rates, demonstrating that they had good separation performance.

When using the fully porous resin (Me:6) with the 0.10 mol/L NaOH eluent, the resolutions between the glucose and fructose peaks were 3.0, 2.6, and 2.3 at flow rates of 0.3, 0.5, and 0.7 mL/min, respectively, indicative of good separation

performance (Table 3).

# 3.3. Electrostatic Charge and Ion-exchange Capacity

The ion-exchange capacities of St-80(Me:2), St-80(Me:4), and St-80(Me:6) are shown in Table 4, along with the electrostatic charges of the  $N^+$  atoms in their functional chains (Figure 3), as calculated using Spartan'20. The carbohydrate retention times increased as the number of methylene groups in the functional chain increased (Table 4). St-80(Me:2), which had the fewest methylene groups, had the highest positive charge on the nitrogen ion, lowest ion-exchange capacity, and shortest retention times among the core-shell ion-exchange resins.

We hypothesized that the change in retention times may be related to the electrostatic charge on the  $N^+$  atom in the functional chain as well as the ion-exchange capacity.

However, while the retention time increased as the number of methylene groups increased from two to six, the electrostatic charge decreased and then increased, and the ion-exchange capacity increased. Thus, it is possible to explain the constant increase in retention time based on the trends in ion-exchange capacity.

# 3.4. Theoretical Plate Numbers (N) Using St-80 (Me:2), St-80 (Me:4), and St-80 (Me:6) Core-shell Ion-exchange Resins

The resins were further compared in terms of the N values

of glucose, fructose, and sucrose when using the 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min (Figure 6a). As the number of methylene groups in the porous shell increased from two to six, the N values of glucose and fructose decreased at flow rates of 0.5 and 0.7 mL/min. As the number of methylene groups in the porous shell increased from two to six, the N values of sucrose decreased at the number of six, the N values of sucrose decreased at the number of sucrose decreased and then increased.

The *N* values of glucose, fructose, and sucrose were also evaluated when using the 0.15 mol/L NaOH eluent (Figure 6b). As the number of methylene groups in the porous shell increased from two to six, the *N* values of glucose, fructose, and sucrose decreased at flow rates of 0.5 and 0.7 mL/min.

Subsequently, we compared the *N* values with those for the fully porous resin(Me:6). With the 0.1 mol/L NaOH eluent, the *N* values for St-80(Me:2) were larger than those for the fully porous resin at all flow rates (Figure 6a). Similarly, with the 0.15 mol/L NaOH eluent, the *N* values of glucose, fructose, and sucrose for St-80(Me:2) were larger than those for the fully porous resin at all flow rates (Figure 6b).

*Table 4.* Retention time and theoretical plate number N of glucose, electrostatic charges on  $N^+$ , and ion-exchange capacity of St-80(Me:2), St-80(Me:4), and St-80(Me:6) (Eluent: 0.10 mol/L NaOH; flow rate: 0.5 mL/min).

| Ion-exchange resin | Glu retention time (min) | Theoretical plate num-<br>ber | Electrostatic charge on $N^+$ | Ion-exchange capacity<br>(meq/mL) |
|--------------------|--------------------------|-------------------------------|-------------------------------|-----------------------------------|
| St-80(Me:2)        | 16.9                     | 5723                          | +0.720                        | 0.172                             |
| St-80(Me:4)        | 18.9                     | 4821                          | +0.637                        | 0.346                             |
| St-80(Me:6)        | 18.6                     | 4661                          | +0.668                        | 0.399                             |







*Figure 6.* Theoretical plate numbers N of glucose, fructose, and sucrose using St-80(Me:2), St-80(Me:4), St-80(Me:6), and fully porous (Me:6) resin with (a) 0.10 mol/L and (b) 0.15 mol/L NaOH eluents at flow rates of 0.3, 0.5, and 0.7 mL/min.

#### 3.5. Mechanism of Retention Time Variation

Table 4 summarizes the glucose retention times and N values for St-80(Me:2), St-80(Me:4), and St-80(Me:6) with the 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min as well as the electrostatic charge on the N<sup>+</sup> atom and the ion-exchange capacity of the core-shell resins. As the number of methylene groups increased from two to six, electrostatic charge decreased, whereas the ion-exchange capacity showed an increasing trend.

Specifically, St-80(Me:6) had the smallest *N* value and a small electrostatic charge but the highest ion-exchange capacity. There was not a clear relationship between the number of methylene groups and electrostatic charges on N<sup>+</sup> atoms. The retention times of sucrose for St-80(Me:4) and St-80(Me:6) increased steadily (18.9 and 18.6 min) as the number of methylene groups increased.

In our previous study, the ion-exchange capacities of St-60 under the same conditions increased as the number of methylene groups increased from two to six. However, the retention times were approximately the same [35]. We hypothesized that this stability in the retention times of the St-60 resins was caused by two opposing factors: the decrease in the positive charge of the  $N^+$  atom in the functional chain and the increase in the ion-exchange capacity.

For St-80(Me: 2, 4, and 6), the ion-exchange capacity increased as the number of methylene groups increased. These findings indicate that the ion-exchange capacity is a determinant of the retention time. However, the positive charge of the  $N^+$  atom in the functional chain had no clear effect. Other factors need to be considered in addition to those included in

the analysis.

#### **4.** Discussion

In this study, we evaluated the performance of a core-shell ion-exchange resin, St-80, with different numbers of methylene groups (two, four, and six) in the functional chains of the polymer in the porous shell. The cross-linking degree was constant at 55%. The quantitative determination of carbohydrates can provide valuable information on the properties of foods. Thus, the carbohydrate separation behavior of a standard solution of inositol, glucose, fructose, and sucrose was used to evaluate the HPLC performance of the core-shell ion-exchange resins. Importantly, the use of an electrochemical detector meant that the solution did not require pretreatment for the carbohydrate analysis.

Good chromatograms were achieved for glucose, fructose, and sucrose, regardless of the number of methylene groups. St-80(Me:2, 4, and 6) displayed high resolutions ( $\geq$ 1.5) at flow rates of 0.3–0.7 mL/min and eluent concentrations of 0.10 and 0.15 mol/L NaOH, demonstrating good carbohydrate separation performance.

When increasing the number of methylene groups in the functional chain, the retention times for St-80 (Me:2, 4, and 6) were nearly identical. The retention time of sucrose for St-80(Me 4) at a flow rate of 0.3 mL/min was slightly longer than those of St-80(Me:2 and 6). However, all of the core-shell ion-exchange resins demonstrated shorter carbohydrate retention times than that of a fully porous resin (Me:6) without a dense core. At high pH, carbohydrates become more highly ionized, and their interaction with the porous layer increases. Thus, the elution sequence of the carbohydrate sequen

drates (glucose followed by fructose) was consistent with the  $pK_a$  sequence [23].

Various properties are important for understanding the separation properties of the core-shell ion-exchange resins. First, the core suppresses solute diffusion along the column axis. Because the porous layer is thin, the solute moves across a shorter distance within the shell. Second, the concentration of the NaOH eluent plays a critical role in the separation of these carbohydrates. Finally, when the number of methylene groups in the porous shell increased from two to six, the carbohydrate retention times were almost the same.

The trend in retention times, which were nearly identical, as the number of methylene groups increased, could not be explained by the electrostatic charge on the  $N^+$  atom in the functional chain, which decreased as the number of methylene groups increased, or the ion-exchange capacity, which increased. Thus, other factors should be considered to explain the change in carbohydrate retention times.

At all flow rates and eluent concentrations, the *N* values for glucose, fructose, and sucrose were highest when using the St-80(Me:2) resin. In addition, as the number of methylene groups for St-80(Me:2, 4, and 6) increased, the *N* values of glucose and fructose decreased at flow rates of 0.5 and 0.7 mL/min with 0.10 mol/L NaOH eluent. The *N* values of St-80(Me:2) were larger than those of the fully porous resin. The three types of St-80 reported here were compared with St-70(Me:2, 4, and 6) and St-60(Me:2, 4, and 6) with respect to the retention times and resolution between glucose and

fructose (Table 3).

It is necessary to investigate the conditions for ion-exchange resins that provide the shortest retention time for sucrose and high resolution. The resins with a short sucrose retention time of 17 min or less and resolution of  $\geq$ 1.5 were as follows: St-80 (Me:2) at a flow rate of 0.5 mL/min and St-80 (Me:2, 4, and 6) at a flow rate of 0.7 mL/min. S-60(Me:2, 4, and 6) had a greater number of resins that met this condition than those for St-70(Me:2, 4, and 6). St-80(Me:2, 4, and 6) were the most suitable resins for separating carbohydrates based on the retention time, resolution, and theoretical plate number results.

The performance of resins could not be fully explained by the four factors (concentration of NaOH eluent, thickness of the shell portion, electrostatic charge on the  $N^+$  atom, and ion-exchange capacity) evaluated in this study. Thus, further investigations covering a wider range of parameters are required.

#### **5.** Conclusions

Analyses of retention time resolution and theoretical plate number under various numbers of methyl groups suggested that St-80 core-shell ion-exchange resins are highly efficient for carbohydrate analyses. Their suitability for strongly alkaline conditions allows their effective use in electrochemical detection. These resins also possess outstanding durability owing to their polymeric core and shell.

#### Abbreviations

| St-80(Me:2, Me:4, and Me:6) | A Constant Core-shell Monomer Weight Ratio of 20:80, The Degree of Cross-linking of |
|-----------------------------|---|
|                             | 55% with Two, Four, and Six Methylene Groups in Porous Layer, Respectively          |
| Rt                          | Retention Time  |
| Ν                           | Theoretical Plate Number  |
|                             |   |

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## **Conflicts of Interest**

The authors declare no conflicts of interest.

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