International Journal of Pharmacy and Chemistry
2025, Vol. 11, No. 4, pp. 76-86
https://doi.org/10.11648/j.ijpc.20251104.11

Research Article

Evaluation of a Novel Ion-Exchange Resin, St-70, with a Cross-Linking Degree of 40% and Various Numbers of Methylene Groups in the Porous Shell

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Abstract

The complex relationship between the molecular structure of ion-exchange resins and carbohydrate elution presents a challenge for the development of polymer materials for high-performance liquid chromatography under a wide range of conditions. We evaluated the effect of the number of methylene groups in the functional chain of the shell on carbohydrate separation. Core-shell ion-exchange resins with a monomer weight ratio of 30:70 (denoted as St-70) were synthesized with a constant cross-linking degree of 40%. The number of methylene groups in the functional chain of the porous polymer shell was varied from two to six (denoted as St-70 (40% Me:2, 4, and 6)) to analyze the carbohydrate separation performance under strongly 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. As the number of methylene groups increased, glucose, fructose, and sucrose for St-70 (40% Me:4) at flow rates of 0.3-0.7 mL/min with 0.10 mol/L NaOH eluent showed the largest retention times. The carbohydrates for St-70 (40% Me:4) at flow rates of 0.3, 0.5, and 0.7 mL/min showed the largest theoretical plate numbers when the number of methylene groups was changed from two to six. These results suggest that St-70 core-shell ion-exchange resins are highly efficient for carbohydrate analyses. Their suitability under strongly alkaline conditions facilitates their effective use in electrochemical detection.

Keywords

High-Performance Liquid Chromatography, Core-Shell Ion-Exchange Resin, Carbohydrates, Retention Time, Theoretical Plate Number, Density-Functional Theory, Hartree-Fock

1. Introduction

Choosing an appropriate ion-exchange resin is essential for high-performance liquid chromatography (HPLC), which is a critical analytical tool. Various core-shell resins have been developed for this purpose [1, 2]. However, silica-based resins, such as octadecyl-functionalized silica resins [3-8], are not suitable for use under strongly alkaline conditions. Styrene-divinylbenzene- and acrylamide-type polymers, frequently used as base materials for organic resins [9-13], are also limited for applications in high-speed HPLC owing to their fully porous structure. To address these issues, core-shell ion-exchange resins, composed of a porous polymer shell and dense core, have been synthesized. These resins provide supe-

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Received: 3 July 2025; **Accepted:** 21 July 2025; **Published:** 27 August 2025

rior durability at high pH levels. Two commercially available core-shell ion-exchange resins prepared via precipitation polymerization around the core [14, 15] and latex-type resins using a styrene base [16-18] have been reported.

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 analysis [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 a good separation performance.

Previously, we 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 demonstrated that ion-exchange resins with a core-shell monomer weight ratio (before suspension polymerization) of 20:80 (St-80) and a cross-linking degree of 55% in the porous region had a shorter retention time in HPLC analyses of carbohydrates than that of the fully porous resin (0:100) [28-30]. We also evaluated St-80 resins with various degrees of cross-linking (10%, 40%, and 55%) in the porous shell [31], 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 shell thickness [32]. We then evaluated the carbohydrate elution behavior using St-50 and St-70 ion-exchange resins with cross-linking degrees of 10%, 40%, and 55% [33, 34], and using St-60, St-70, and St-80 resins with two, four, and six methylene groups in the functional chain (denoted as St-60 (55% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), and St-80 (55% Me:2, 4, and 6)), with the cross-linking degree held constant at 55% [35-37]. However, the effects of reducing the degree of cross-linking on carbohydrate separation have not been clearly established. We evaluated carbohydrate elution behavior using St-60 (monomer weight ratio: 40:60) with a cross-linking degree of 40% in the porous shell and two, four, or six methylene groups (denoted as St-60 (40% Me:2), St-60 (40% Me:4), and St-60 (40% Me:6), respectively) [38].

In this study, we evaluated the carbohydrate elution behavior by using St-70 (monomer weight ratio: 30:70) with a cross-linking degree of 40% in the porous shell and two, four, or six methylene groups (denoted as St-70 (40% Me:2), St-70 (40% Me:4), and St-70 (40% Me:6), respectively. This study provides insights into the factors contributing to carbohydrate elution behavior and provides a basis for optimizing resins with respect to cross-linking.

2. Materials and Methods

2.1. Materials

myo-Inositol, sucrose, and NaOH were obtained from Fujifilm Wako Chemicals Co. (Richmond, VA, USA). D(−)-Fructose and D(+)-glucose were obtained from Kanto Chemical Co. (Tokyo, Japan). 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-divinylbenzene copolymer carrier with a tertiary amine, as previously described [19].

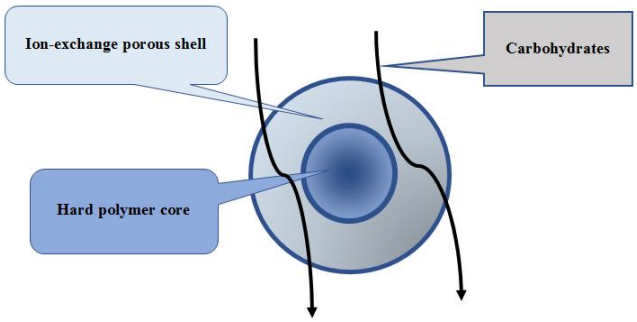


Figure 1. *Structure of the core-shell ion-exchange resin consisting of a dense polymer core and an ion exchange porous polymer shell.*

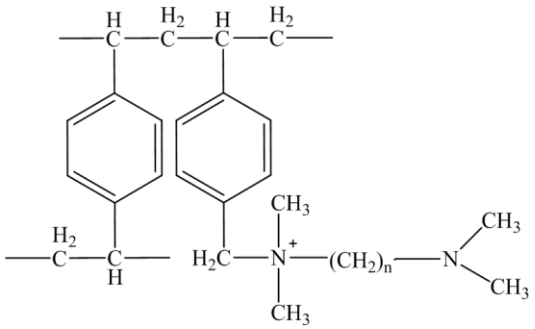


Figure 2. *Chemical structure of the porous polymer in the ion-exchange resin shell (n = 2, 4, and 6).*

The shell thickness was maintained consistently by ensuring a constant core-shell monomer weight ratio of 30:70, alongside a fixed total mass of monomers. Additionally, the degree of cross-linking within the porous layer was consistently held at 40%

by employing a styrene/divinylbenzene weight ratio of 60:40 [32]. The number of methylene groups in the functional chain of the porous layer was adjusted 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 tertiary amines to produce core-shell ion-exchange resins with two, four, and six methylene groups (denoted as St-70 (40% Me:2), St-70 (40% Me:4), and St-70 (40% Me:6), respectively). For comparison, a fully porous resin with a 40% degree of cross-linking and six methylene groups in the functional chain was prepared by reacting the chloromethylstyrene-divinylbenzene copolymer carrier (divinylbenzene weight ratio: 40%) with the *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexamethylenediamine tertiary amine (denoted as Fully (40% Me:6)). The average diameter of the prepared resins was 5 μm. We prepared 3 g of each core-shell and fully porous resin.

2.3. HPLC Analysis Conditions

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 ×150 mm I.D. stainless steel column using a conventional slurry packing method at a constant pressure of 120 kg/cm^2. The sample solution (20 μL) containing carbohydrates (inositol, glucose, fructose, and sucrose) was injected into an AS-8020 HPLC autosampler (Tosoh, Tokyo, Japan) and eluted with either a 0.10 or 0.15 mol/L NaOH eluent at room temperature (30 ℃). 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^+ atom in the functional chain and the configuration using model compounds by density functional theory using the ωB97X-D density functional and 6.31G* basis set in Spartan'20 (Figure 3).

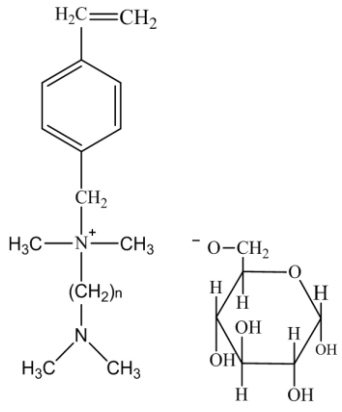


Figure 3. *Structures used for optimizing the electrostatic charge on the N^+ atoms in the functional chain and on the O^- atom in carbohydrate by using Spartan'20: (a) functional chain of the ion-exchange resin and (b) representative carbohydrate molecule.*

3. Results

3.1. Carbohydrate Separation Performance of St-70 (40% Me:2), St-70 (40% Me:4), and St-70 (40% Me:6) Ion-Exchange Resins

3.1.1. Effects of the NaOH Eluent Concentration and Flow Rate

We evaluated the retention times of glucose, fructose, and sucrose using a 0.10 mol/L NaOH eluent and St-70 (40% Me:2), St-70 (40% Me:4), and St-70 (40% Me:6) core-shell ion-exchange resins, which had a core-shell monomer weight ratio of 30:70 and two, four, and six methylene groups, respectively.

We evaluated the carbohydrate separation performance of columns packed with St-70 (40% Me:2, 4, and 6) using a 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min (Table 1). Figure 4 a-f presents the chromatograms of St-70 (40% Me:4 and 6) at flow rates of 0.3, 0.5, and 0.7 mL/min. The carbohydrate separation performance of columns packed with the St-70 (55% Me:6) resins using a 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min is shown in Table 1. Figure 5 a-c presents the retention times of glucose, fructose, and sucrose at flow rates of 0.3, 0.5, and 0.7 mL/min with 0.10 NaOH eluent.

3.1.2. Comparison Among St-70 (40% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), and Fully (40% and 55% Me:6)

Each carbohydrate for St-70 (40% Me:2, 4, and 6) showed longer retention times than those of St-70 (55% Me:2, 4, and 6) at flow rates of 0.3, 0.5, and 0.7 mL/min with 0.10 mol/L NaOH eluent. A similar trend was observed for Fully (40% Me:6) at the flow rates of 0.3, 0.5, and 0.7 mL/min. The chromatograms of St-70 (55% Me:6) showed clean peaks, as shown in Figure 5 a-c [35].

Next, the eluent concentration was increased to 0.15 mol/L NaOH. The retention times of glucose, fructose, and sucrose for St-70 (40% Me: 2, 4, and 6) and St-70 (55% Me:2, 4, and 6) at flow rates of 0.3, 0.5, and 0.7 mL/min are listed in Table 2. Glucose, fructose, and sucrose for St-70 (40% Me:4) at flow rates 0.3, 0.5, and 0.7 mL/min with 0.15 mol/L NaOH eluent showed longer retention times than those of St-70 (55% Me:4). Glucose, fructose, and sucrose for St-70 (40% Me:2 and 6) and St-70 (55% Me:2 and 6) showed similar retention times at a flow rate of 0.5. and 0.7 mL/min.

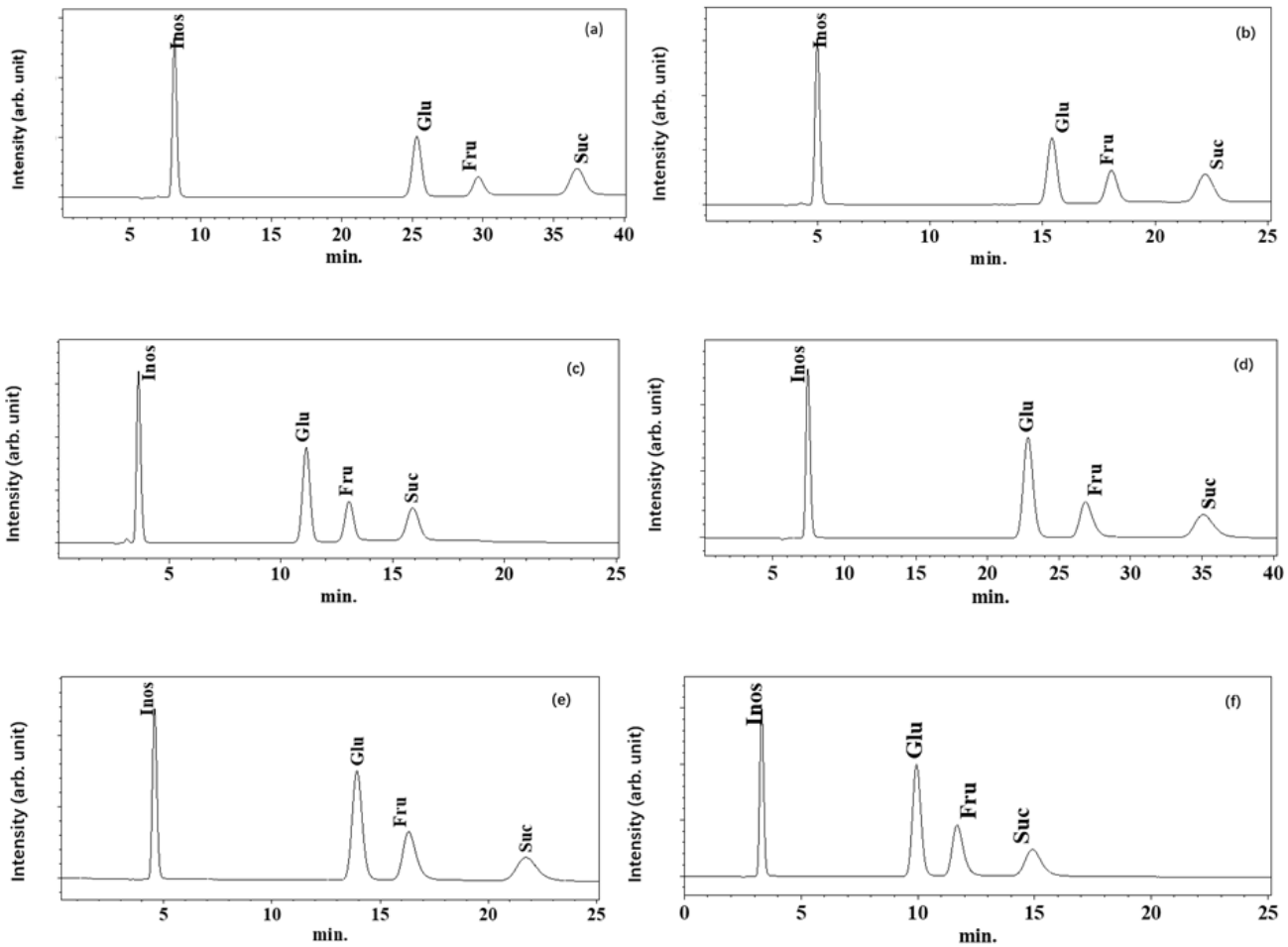


Figure 4. *Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using St-70 (40% Me:4) with a 0.1 mol/L NaOH (a) 0.3 ml/min, (b) 0.5 ml/min, (c) 0.7 ml/min, and St-70 (40% Me:6) (d) 0.3 ml/min, (e) 0.5 ml/min, (f) 0.7 ml/min.*

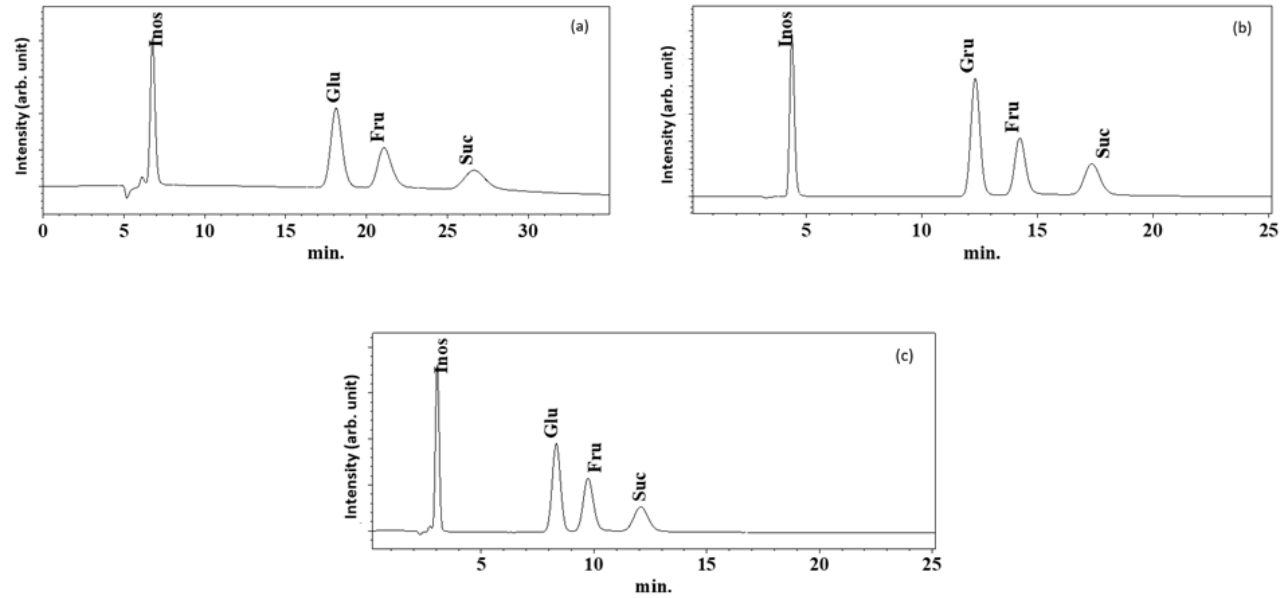


Figure 5. *Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using St-70 (55% Me:6) with a 0.1 mol/L NaOH (a) 0.3 ml/min, (b) 0.5 ml/min, (c) 0.7 ml/min.*

Table 1. *Retention times (min) of glucose, fructose, and sucrose using St-70 (40% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), Fully (40% Me:6), and Fully (55% Me:6) with 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min.*

Flow rate	The Number of CH_2 / Cross-linking	Glu		Fru		Suc	
		40%	55%	40%	55%	40%	55%
0.3 mL/min	2	19.5	14.1	22.4	15.7	28.2	18.0
	4	25.3	17.6	29.7	20.4	36.6	24.1
	6	22.8	18.1	26.9	21	35.1	26.6
	Fully porous 6	34.9	26.9	43.5	32.5	58.6	44.2
0.5 mL/min	2	11.9	8.6	13.6	9.6	16.9	10.9
	4	15.4	10.7	18.1	12.4	22.2	14.6
	6	13.9	12.3	16.3	14.2	21.7	17.3
	Fully porous 6	21.1	16.4	26.6	19.6	35.5	27.2
0.7 mL/min	2	8.6	6.1	9.9	6.9	12.2	7.9
	4	11.1	7.7	13.1	8.9	15.9	10.5
	6	9.9	8.3	11.7	9.7	14.9	12.1
	Fully porous 6	15.3	11.8	19.2	14.2	25.7	19.9

This data of St-70 (55% Me:2, 4, and 6) is shown in Ref 36.

Table 2. *Retention times (min) of glucose, fructose, and sucrose using St-70 (40% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), Fully (40% Me:6), and Fully (55% Me:6) with 0.15 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min.*

Flow rate	The Number of CH_2 / Cross-linking	Glu		Fru		Suc	
		40%	55%	40%	55%	40%	55%
0.3 mL/min	2	19.5	14.1	22.4	15.7	28.2	18.0
	4	25.3	17.6	29.7	20.4	36.6	24.1
	6	22.8	18.1	26.9	21	35.1	26.6
	Fully porous 6	34.9	26.9	43.5	32.5	58.6	44.2
0.5 mL/min	2	11.9	8.6	13.6	9.6	16.9	10.9
	4	15.4	10.7	18.1	12.4	22.2	14.6
	6	13.9	12.3	16.3	14.2	21.7	17.3
	Fully porous 6	21.1	16.4	26.6	19.6	35.5	27.2
0.7 mL/min	2	8.6	6.1	9.9	6.9	12.2	7.9
	4	11.1	7.7	13.1	8.9	15.9	10.5
	6	9.9	8.3	11.7	9.7	14.9	12.1
	Fully porous 6	15.3	11.8	19.2	14.2	25.7	19.9

This data of St-70 (55% Me:2, 4, and 6) is shown in Ref 36.

Glucose, fructose, and sucrose for Fully (40% Me:6) showed longer retention times than those of Fully (55% Me:6) at flow rates of 0.3, 0.5, and 0.7 mL/min with 0.10 mol/L NaOH eluent (Table 1). Glucose, fructose, and sucrose for

Fully (40% Me:6) also showed longer retention times than those of Fully (55% Me:6) at all flow rates with the 0.15 mol/L NaOH eluent (Table 2).

3.2. Resolution Between Glucose and Fructose Peaks

To further investigate the carbohydrate-separation performance of the resins, we evaluated the resolution of the glucose and fructose peaks (Table 3), which were adjacent to the chromatograms. When using the 0.10 mol/L NaOH eluent, resolutions of ≥1.5 were achieved for the St-70 (40% 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. The resolutions for St-70 (40% Me:4) were 2.8, 2.6, and 2.4 at flow rates of 0.3, 0.5, and 0.7 mL/min, respectively. When using the 0.15 mol/L NaOH eluent, St-70 (40% Me:4) core-shell resins showed a higher resolution than that of St-70 (55% Me:4) at all flow rates. St-70 (40% Me:6) core-shell resins showed the opposite trend [39].

Table 3. *The resolution between glucose and fructose using St-70 (40% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), Fully porous resin (40% Me:6), and Fully porous resin (55% Me:6) with 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min.*

Flow rate	CH_2-2		CH_2-4		CH_2-6		Fully CH_2-6	
(mL/min)	40%	55%	40%	55%	40%	55%	40%	55%
0.3	2.0	1.4	2.8	1.6	2.4	2.2	3.4	3.0
0.5	1.9	1.3	2.6	1.5	2.1	1.9	3.2	2.6
0.7	1.7	1.2	2.4	1.4	1.9	1.6	3.0	2.3

When using Fully (40% Me:6) with 0.10 mol/L NaOH eluent, the resolutions between the glucose and fructose peaks were 3.4, 3.0, and 3.0, respectively, at flow rates of 0.3, 0.5, and 0.7 mL/min, respectively, indicative of good separation performance. When using Fully (40% Me:6) with the 0.15 mol/L NaOH eluent, the resolutions between the glucose and fructose also showed good results, like the results for Fully (40% Me:6) with the 0.10 mol NaOH eluent.

3.3. Theoretical Plate Numbers (*N*) Using St-70 (40% Me:2), St-70 (40% Me:4), and St-70 (40% Me:6) Core-Shell Ion-Exchange Resins

Resins with different cross-linking degrees in the porous shell (40% and 55%) were further compared in terms of the *N* values of glucose, fructose, and sucrose when using the 0.10 and 0.15 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min (Figure 6 a, b).

Glucose, fructose, and sucrose for St-70 (40% Me:2 and 4) showed larger theoretical plate numbers than those of St-70 (55% Me:2 and 4) at all flow rates with 0.10 mol/L NaOH eluent. Glucose, fructose, and sucrose for St-70 (40% Me:6) showed smaller theoretical plates than for St-70 (55% Me:6) at all flow rates at the same concentration. As the number of methylene groups in the porous shell increased from two to six, the *N* values of glucose, fructose, and sucrose for St-70 (40% Me:2, 4, and 6) increased and then decreased at flow rates of 0.3, 0.5 and 0.7 mL/min with 0.10 mol/L NaOH eluent. As the number of methylene groups increased, the *N* values of glucose, fructose, and sucrose for St-70 (40% Me:2, 4, and 6) increased and then decreased at flow rates of 0.3, 0.5, and 0.7 mL/min with 0.15 mol/L NaOH eluent. When four methylene groups were present, all carbohydrates showed the largest theoretical plate number at all flow rates.

The theoretical plate numbers of glucose, fructose, and sucrose for Fully (40% Me:6) and Fully (55% Me:6) with 0.10 mol/L NaOH eluent are shown in Figure 6 a. When comparing Fully (40% Me:6) and Fully (55% Me:6), glucose for the former showed a smaller theoretical plate number than that of the latter at all flow rates. Fructose for Fully (40% Me:6) showed larger theoretical plate numbers than those of Fully (55% Me:6) at all flow rates. The theoretical plate numbers of glucose, fructose, and sucrose for Fully (40% Me:6) and Fully (55% Me:6) with 0.15 mol/L NaOH eluent are shown in Figure 6 b. Glucose for Fully (40% Me:6) showed smaller theoretical plate numbers than those of Fully (55% Me:6) at all flow rates with 0.15 mol/L NaOH eluent. *N*-values for fucose exhibited an opposite trend to that for glucose.

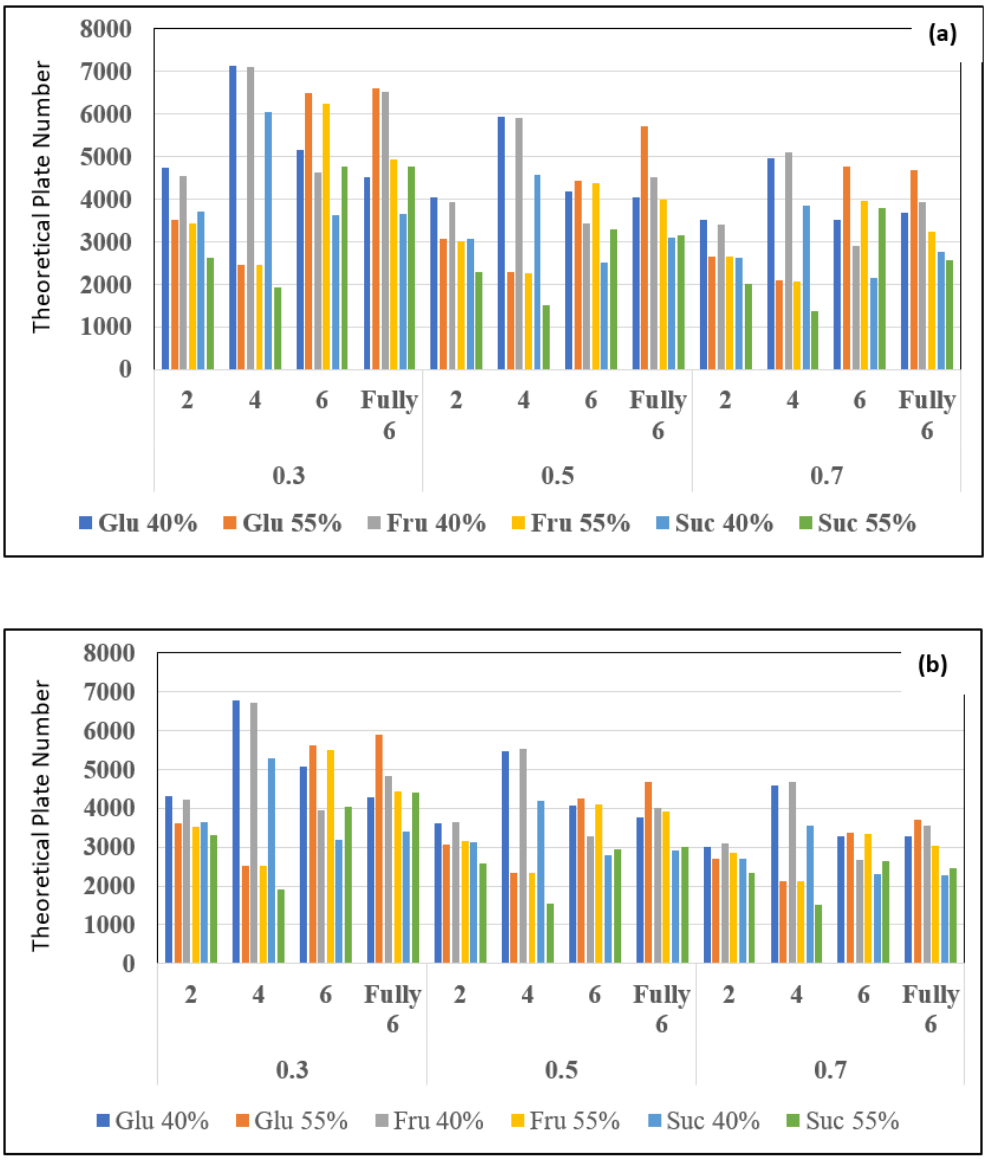


Figure 6. *Theoretical plate numbers N of glucose, fructose, and sucrose using St-70 (40% Me:2), St-70 (40% Me:4), St-70 (40% Me:6), and Fully (40% Me:6) with (a) 0.10 mol/L and (b) 0.15 mol/L NaOH eluents at flow rates of 0.3, 0.5, and 0.7 mL/min.*

3.4. Mechanism Underlying Retention Time Variation

Table 5 summarizes the glucose retention times and *N* values with the 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min, along with the electrostatic charge on the N^+ and O^- atoms, and the ion-exchange capacity of the core-shell resins. As the number of methylene groups increases from two to six, the electrostatic charge on N^+ atom initially decreases and then increases, whereas the ion-exchange capacity initially increases and then decreases. Specifically, St-70 (40% Me:2) had the largest electrostatic charge on N^+ atom and smallest ion-exchange capacity. The electrostatic charge on the O^- atom showed a different tendency from that of the N^+ atom. When the number of methylene groups increased, the ion-exchange capacity increased and then decreased. The retention time of glucose for St-70 (40% Me:2, 4, and 6) increased and then decreased as the number of methylene groups increased. The retention time of glucose and ion-exchange capacity showed the similar trend when the number of methyl groups increases from 2 to 6.

Table 4. *The resolution between glucose and fructose using St-70 (40% Me:2, 4, and 6), St-70 (55% Me:2, 4, and 6), Fully porous resin (40% Me:6), and Fully porous resin (55% Me:6) with 0.15 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min.*

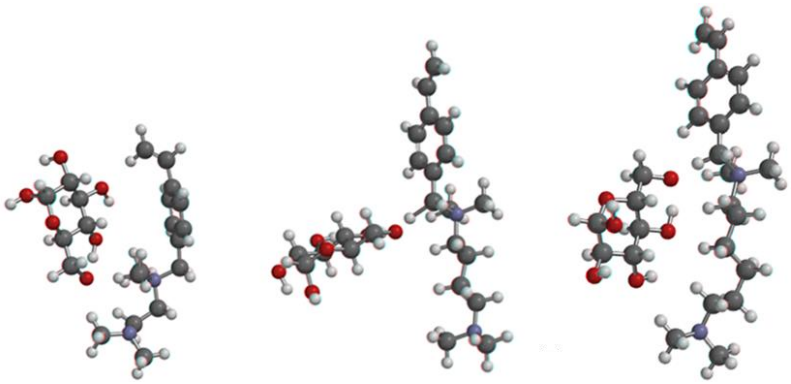
Flow rate	CH_2-2		CH_2-4		CH_2-6		Fully CH_2-6	
(mL/min)	40%	55%	40%	55%	40%	55%	40%	55%
0.3	1.3	1.3	2.6	1.4	1.6	1.9	3.1	2.3
0.5	1.2	1.1	2.3	1.4	1.4	1.7	2.8	1.9
0.7	1.1	1.0	2.1	1.3	1.3	1.5	2.6	2.0

Table 5. *Retention times and theoretical plate numbers (N) of glucose, electrostatic charges on N^+ and O^-, and ion-exchange capacities of St-70 resins with (Me:2), (Me:4), and (Me:6) (All resins had 40% crosslinking and were evaluated using 0.10 mol/L NaOH as the eluent at a flow rate of 0.5 mL/min.).*

Ion-exchange resin	Glu Retention Time (min)		Theoretical plate number (*N*)		Electrostatic charge N^+	Electrostatic charge O^-	Ion-exchange capacity (mEq/mL)	
Cross-linking	40%	55%	40%	55%	(a) in Figure 3	(b) in Figure 3	40%	55%
St-70(Me:2)	11.9	8.6	4050	3050	+0.720	−0.651	0.346	0.224
St-70(Me:4)	15.4	10.7	5920	2300	+0.637	−0.648	0.386	0.308
St-70(Me:6)	13.9	12.3	4170	4440	+0.668	−0.788	0.356	0.232

In our previous study, the ion-exchange capacities of St-70 (55% Me:2, 4, and 6) with 0.10 mol/L NaOH eluent increased and then decreased. Meanwhile, the electrostatic charge on the N^+ atom decreased and then increased as the number of methylene groups increased from two to six. However, the retention time of glucose steadily increases [36].

We hypothesized that the observed retention time results could be attributed to two opposing factors. For St-70 (40% Me:2, 4, and 6), the positive charge of the N^+ atom within the functional chain initially decreased and subsequently increased, while the ion-exchange capacity initially increased and then decreased as the number of methylene groups increased. However, the ion-exchange capacity of St-70 (40% Me:2, 4, and 6) differed from that of St-70 (55% Me:2, 4, and 6) and glucose for St- St-70 (40% Me:4) showed the largest retention time. when comparing the retention time of glucose for two ion-exchange resins.



(a) (b) (c)

Figure 7. *The most stable configuration of model complexes formed between the monovalent negative carbohydrate ion and positive N^+ group in the porous shell region for (a) Me:2, (b) Me:4, and (c) Me:6 (calculated using HF 3.21G Spartan' 20).*

The stable configuration of these molecules between the monovalent anion of a carbohydrate and the cation of an ion-exchange model compound (in Figure 3a, b) was investigated using Spartan'20 (Figure 7 a-c). The distances between N^+ and O^- in the complexes were 3.938, 4.206, and 3.187 Å for the model complexes (Figure 7), respectively. These results indicate that it is necessary to comprehensively consider several factors to explain the differences in carbohydrate retention times.

4. Discussion

The quantitative analysis of carbohydrates offers significant insights in the field of food chemistry. In this study, we assessed the efficacy of a core-shell ion-exchange resin, St-70 (40% Me:2, 4, and 6), which incorporates varying numbers of methylene groups (two, four, and six) within the functional chains of the polymer in the porous The cross-linking degree was constant at 40%. The carbohydrate separation behavior of a standard solution of inositol, glucose, fructose, and sucrose was used to investigate the HPLC performance of the core-shell ion-exchange resins. Importantly, a sample solution containing carbohydrates can be analyzed using an electrochemical detector without any special pretreatment.

St-70 (40% Me:2, 4, and 6) displayed high resolutions (≥1.5) at flow rates of 0.3-0.7 mL/min with 0.10 mol/L NaOH eluent, demonstrating good carbohydrate separation performance. St-70 (40% Me:4) displayed high resolution (≥1.5) at flow rates of 0.3-0.7 mL/min with 0.15 mol/L NaOH eluent. Good chromatograms were obtained for glucose, fructose, and sucrose, regardless of the number of methylene groups, demonstrating a good carbohydrate separation performance. When increasing the number of methylene groups in the functional chain, glucose, fructose, and sucrose for St-70 (40% Me:4) with 0.10 and 0.15 mol/L NaOH eluent showed the largest retention time.

St-70 (40% Me 2, 4, and 6) at all flow rates with 0.10 and 0.15 mol/L NaOH eluent showed shorter carbohydrate retention times than those for a fully porous resin (40% Me:6) without a dense core. At a high pH, carbohydrates become more highly ionized, and their interaction with the porous layer increases. Thus, the elution sequence of carbohydrates (glucose followed by fructose) is consistent with the pK_a sequence [23]. Various factors contribute to the separation properties of the core-shell ion-exchange resins. First, the core suppressed solute diffusion along the column axis. Because the porous layer is thin, the solute moves a shorter distance within the shell. The resins evaluated in this study were 5 μm in size, which was expected to allow effective separation. Second, the concentration of the NaOH eluent played a critical role in the separation of carbohydrates. Accordingly, the optimization of this parameter is expected to allow for more effective separation. Finally, St-70 (40% Me:4) with 0.10 and 0.15 mol/L NaOH eluents at all flow rates showed longer retention times for carbohydrates than those of St-70 (55% Me:4 and 6).

As the number of methylene groups increased from two to six, the ion-exchange capacity increased and then decreased. A comparable pattern was also observed in the retention time of glucose. Based on this result, a correlation between the ion-exchange capacity and retention time is anticipated. Furthermore, other factors should be considered to explain the differences in carbohydrate retention times during the analysis of carbohydrates in food. Although the data are not complete, the following can be said. From the data collected to date, it is necessary to determine the best separation 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 ≥1.5 under 0.10 ml/L NaOH eluent were as follows: St-70 (40% Me:2) at flow rate of 0.5 mL/min and St-70 (40% Me:2, 4, and 6) at flow rate of 0.7 mL/min. The resins with a short sucrose retention time of 17 min or less and resolution of ≥1.5 under conditions of 0.15 mol/L were as follows: St-70 (40% Me:4) at a flow rates of 0.5 and 0.7 mL/min.

The performance of the resins could not be fully explained by the four factors evaluated in this study (concentration of NaOH eluent, thickness of the shell portion, electrostatic charges on the N^+ and O^- atoms, and ion-exchange capacity). The following new perspective emerges: the stabilization energy between the O^- atom in carbohydrate and N^+ of the functional groups may play a crucial role in governing molecular interactions. This perspective should be considered when designing future experiments. Our data on this complex obtained through Spartan may serve as the basis for future research. Upon examining the configuration of these complexes between the model compounds ((a) and (b) in Figure 3), the complex with four methyl groups exhibited a different configuration from the other two. This result regarding the unique data for CH_2-4 (Figure 7 b) may be attributed to its lower steric hindrance or to differences in its surrounding electronic and steric environment.

5. Conclusions

Analyses of the retention time resolution and theoretical plate number under various numbers of methyl groups suggested that St-70 (40% Me:2, 4, and 6) core-shell ion-exchange resins are highly efficient for carbohydrate analyses, for example, with respect to the retention time and resolution between glucose and fructose. Their suitability under strongly alkaline conditions allows their effective use in electrochemical detection without any special pretreatment. These resins also possess outstanding durability, owing to their polymeric cores and shells.

Abbreviations

St-70 (40% Me:2, Me:4, and Me:6)	Constant Core-Shell Monomer Weight Ratio of 30:70 and Degree of Cross-Linking of 40%, with Two, Four, and Six Methylene Groups in the Porous Layer, Respectively
Rt	Retention Time
N	Theoretical Plate Number

Acknowledgments

We thank Dr. Nobuharu Takai and Dr. Toshiki Mutai for valuable discussions.

Conflicts of Interest

The authors declare no conflicts of interest.

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