

Elution Behavior of Carbohydrates using Core-Shell Ion-Exchange Resin St-70 with Different Numbers of Methylene Groups in the Porous Shell and a Constant Cross-Linking Degree of 55%

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ABSTRACT

Understanding the relationship between the molecular structure ion-exchange resins and the elution of carbohydrates is important for high-performance liquid chromatography (HPLC). From a novel resin development perspective, we focused on the effect of the number of methylene groups in the functional chain of the porous polymer shell. Herien, 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 55%. The number of methylene groups in the functional chain of the porous polymer shell was varied from two to six. The effect of the number of methylene groups on the carbohydrate separation performance was examined 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 for St-70 variants with different numbers of methylene groups in the porous layer. Increasing the number of methylene groups tended to increase the retention times of each carbohydrate under all conditions. The theoretical plate numbers of carbohydrates decreased and then increased as the number of methylene groups increased from two to six. The results suggest that the St-70 core-shell ion-exchange resins are highly efficient for carbohydrate analysis. Their suitability for strongly alkaline conditions allows their effective use in electrochemical detection.

Keywords

HPLC, Core-shell ion-exchange resin, Carbohydrates, Retention time, Theoretical plate number.

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], including octadecyl-functionalized silica resins [3-8]. However, silica-based resins have low adsorption ability in alkaline solutions and can exhibit solubility. Thus, they are not suitable for use under strongly alkaline conditions. Styrene-divinylbenzene- and acrylamide-type polymers are frequently

used as base materials for organic resins [9-13]. Regardless, the use of polymer resins is limited in high-speed HPLC owing to their fully porous structure. To overcome the aforementioned problems, researchers have synthesized core-shell ion-exchange resins composed of a porous polymer shell and a dense core. 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 affected by the thickness and degree of cross-linking of the shell. Because the retention time increases with the thickness of the porous shell, the shell should be as thin as possible to reduce the analysis time. Meanwhile, an appropriate

degree of cross-linking in the porous layer is necessary to achieve good separation performance. Therefore, it is important to optimize the shell thickness and degree of cross-linking in the porous layer for HPLC analysis [19].

In our previous series of studies, we investigated the effects of various factors (shell thickness, degree of cross-linking in the porous shell, concentration of 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]. First, we compared ion-exchange resins with core-shell monomer weight ratios (before suspension polymerization) of 20:80 (St-80) and 0:100 (fully porous resin), which both had a cross-linking degree of 55% in the porous region, and found that St-80 had a shorter retention time in the HPLC analysis of carbohydrates than the fully porous resin [28-30]. Next, we compared St-80 resins with cross-linking degrees of 10%, 40%, and 55% in the porous shell with regard to the carbohydrate separation performance and theoretical plate number (N) during HPLC [31]. Subsequently, we prepared ion-exchange resins with a constant cross-linking degree of 55% and altered the core-shell monomer weight ratio to 50:50, 40:60, and 30:70 (St-50, St-60, and St-70, respectively), which affected the thickness of the shell, and compared the retention time, N value, and carbohydrate separation performance during HPLC [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 St-60 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), respectively), where the cross-linking degree was constant at 55% [35], to investigate the effect of the number of methylene groups on the carbohydrate elution behavior. 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 shells 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-70 (monomer weight ratio: 30:70) with a cross-linking degree of 55% in the porous shell. The number of methylene groups was varied as two, four, and six (denoted as St-70(Me:2), St-70(Me:4), and St-70(Me:6), respectively). St-70 has a thicker porous shell than St-60 [35]; therefore, this study also provides information on the effect of the number of methylene groups at different shell thicknesses.

Materials and Methods

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 either 500 or 1000 mg/L.

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 described previously [19].

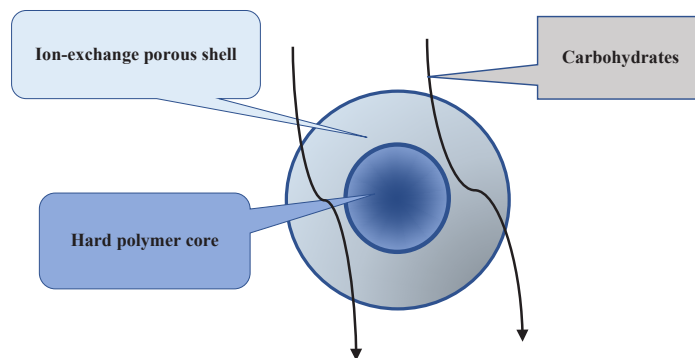


Figure 1: Structure of core-shell ion-exchange resin consisting of a dense polymer core and an ion-exchange porous polymer shell.

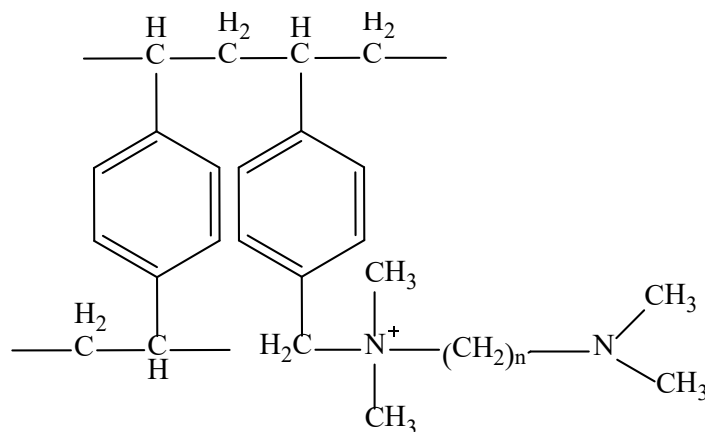


Figure 2: Chemical structure of porous polymer in the ion-exchange resin shell ($n = 2, 4, \text{ and } 6$).

The thickness of the shell was kept constant by maintaining a constant core-shell monomer weight ratio of 30:70 and a constant total mass of the 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-70(Me:2), St-70(Me:4), and St-70(Me:6), 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 chloromethylstyrene-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.

Conditions for 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/cm². 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 $^{\circ}\text{C}$). 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^+ atom in the functional chain by density functional theory using the $\omega\text{B97X-D}$ density functional and 6.31G* basis set in Spartan'20 (Figure 3).

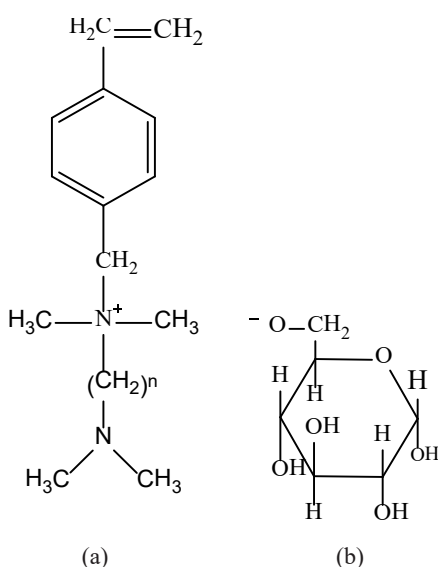


Figure 3: Structures used for optimization of the electrostatic charge on the N^+ atoms in the functional chain using Spartan'20: (a) functional chain of the ion-exchange resin and (b) representative carbohydrate molecule.

Results

Carbohydrate separation performance of St-70(Me:2), St-70(Me:4), and St-70(Me:6) ion-exchange resins

Effect of NaOH eluent concentration and flow rate

We first evaluated the carbohydrate separation performance of columns packed with the St-70(Me:2), St-70(Me:4), and St-70(Me:6) resins using a 0.10 mol/L NaOH eluent at flow rates of 0.3, 0.5, and 0.7 mL/min. Figures 4a–c present 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 results show that the retention times of each carbohydrate increased as the number of methylene groups increased, with St-70(Me:6) exhibiting the longest retention times at all flow rates. In addition, each carbohydrate demonstrated good peaks in the chromatograms, with little change in the peak shape as the number

of methylene groups increased.

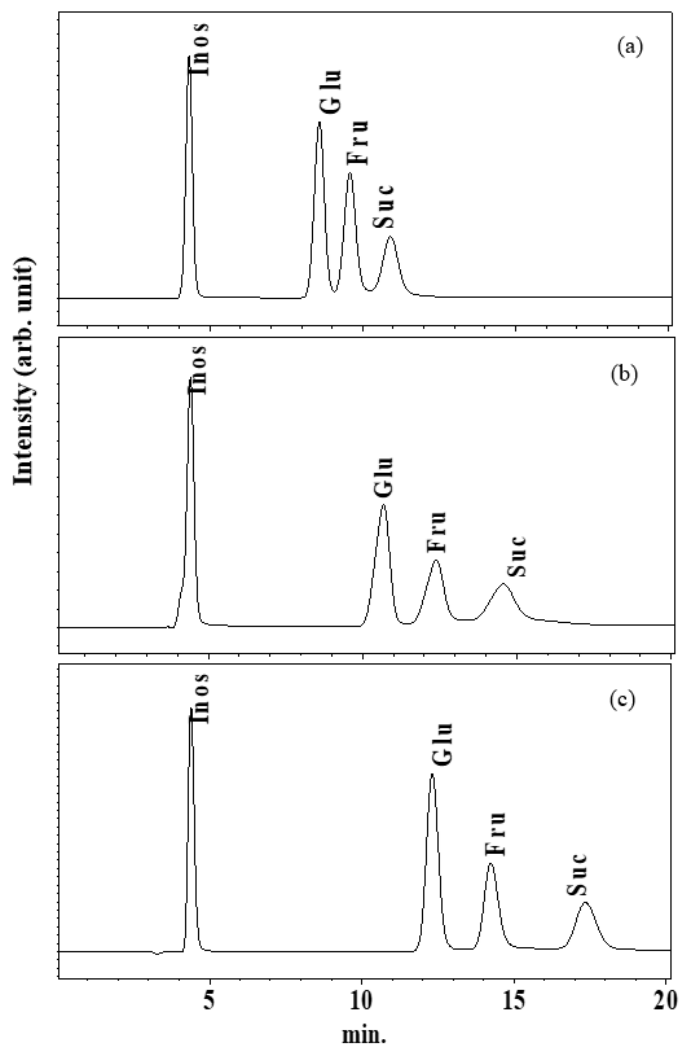


Figure 4: Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-70(Me:2), (b) St-70(Me:4), and (c) St-70(Me:6) with 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min.

Table 1: Retention times (min) of glucose, fructose, and sucrose using St-70(Me:2), St-70(Me:4), St-70(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	Ion-exchange resin	Glu	Fru	Suc
0.3 mL/min	St-70(Me:2)	14.1	15.7	18.0
	St-70(Me:4)	17.6	20.4	24.1
	St-70(Me:6)	18.1	21.0	26.6
	Fully porous (Me:6)	26.9	32.5	44.2
0.5 mL/min	St-70(Me:2)	8.6	9.6	10.9
	St-70(Me:4)	10.7	12.4	14.6
	St-70(Me:6)	12.3	14.2	17.3
	Fully porous (Me:6)	16.4	19.6	27.2
0.7 mL/min	St-70(Me:2)	6.1	6.9	7.9
	St-70(Me:4)	7.7	8.9	10.5
	St-70(Me:6)	8.3	9.7	12.1
	Fully porous (Me:6)	11.8	14.2	19.9

Next, the eluent concentration was increased to 0.15 mol/L NaOH.

The chromatograms at 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. Similarly, to the results using the 0.10 mol/L NaOH eluent, St-70(Me:6) had the longest retention times for each carbohydrate, and the peak shape was consistent at all flow rates.

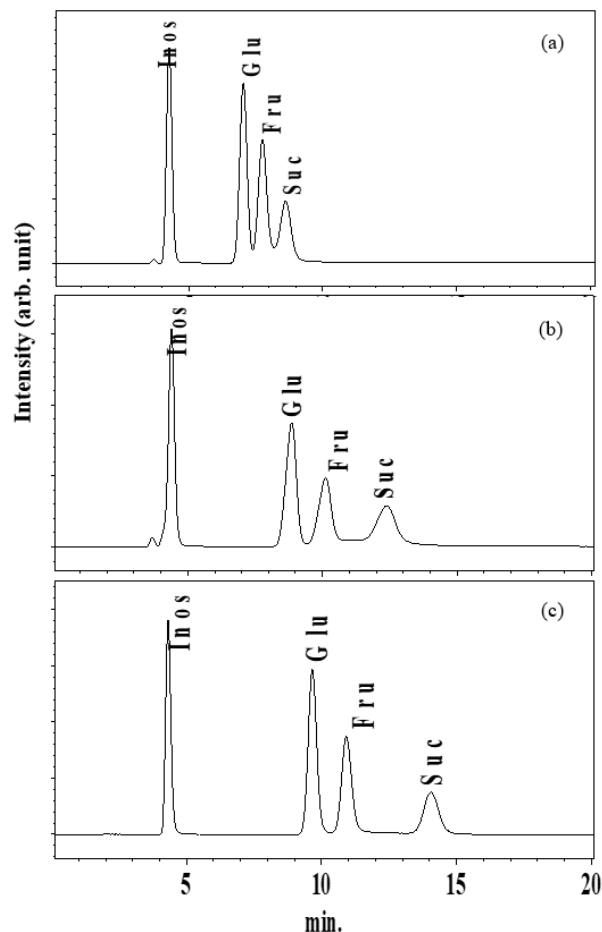


Figure 5: Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-70(Me:2), (b) St-70(Me:4), and (c) St-70(Me:6) with 0.15 mol/L NaOH eluent at a flow rate of 0.5 mL/min.

Table 2: Retention times (min) of glucose, fructose, and sucrose using St-70(Me:2), St-70(Me:4), St-70(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	Ion-exchange resin	Glu	Fru	Suc
0.3 mL/min	St-70(Me:2)	11.5	12.7	14.1
	St-70(Me:4)	14.6	16.6	20.4
	St-70(Me:6)	15.7	17.8	22.8
	Fully porous (Me:6)	20.0	23.3	33.7
0.5 mL/min	St-70(Me:2)	7.0	7.8	8.6
	St-70(Me:4)	8.9	10.1	12.4
	St-70(Me:6)	9.7	10.9	14.0
	Fully porous (Me:6)	11.9	13.8	19.2
0.7 mL/min	St-70(Me:2)	5.1	5.6	6.2
	St-70(Me:4)	6.4	7.3	8.9
	St-70(Me:6)	7.0	8.0	10.3
	Fully porous (Me:6)	8.9	10.5	14.6

Comparison with St-50 and fully porous resins

We also evaluated the carbohydrate retention times with the 0.10 mol/L NaOH eluent using St-50(Me:2), St-50(Me:4), and St-50(Me:6) core-shell ion-exchange resins, which had a core-shell monomer weight ratio of 50:50 and two, four, and six methylene groups, respectively (Table 3). Good chromatograms were obtained using St-50(Me:4) and St-50(Me:6), but not with St-50(Me:2). Unlike the case for St-70, the number of methylene groups in St-50 had no apparent effect on the carbohydrate retention times.

Table 3: Retention times (min) of glucose, fructose, and sucrose using St-50(Me:2), St-50(Me:4), and St-50(Me:6) resins with 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min.

Flow rate (mL/min)	Ion-exchange resin	Glu	Fru	Suc
0.5	St-50(Me:2)*	—	—	—
	St-50(Me:4)	9.8	11.3	13.0
	St-50(Me:6)	8.8	10.1	11.5

*No good chromatogram was obtained for St-50(Me:2).

Next, we compared the performance with that of a fully porous resin (i.e., with no dense core) with six methylene groups (Me:6). The cross-linking degree (55%) was the same as that for the porous shells of the St-70(Me:2), St-70(Me:4), and St-70(Me:6) core-shell resins. Notably, the core-shell resins all exhibited shorter retention times for glucose, fructose, and sucrose than the fully porous resin (Me:6) at all flow rates and NaOH eluent concentrations (Tables 1 and 2). Because the core-shell resins only contain a thin shell of porous polymer, the interaction time between the carbohydrates and porous region is lower than that for the fully porous resin (Me:6), resulting in significantly shorter retention times [32].

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 4), which were adjacent in the chromatograms. When using the 0.10 mol/L NaOH eluent, resolutions of ≥ 1.5 were achieved for the St-70(Me:4) and St-70(Me:6) core-shell resins at flow rates of 0.3 and 0.5 mL/min, indicating that they had good separation performance [36]. By contrast, the resolutions of St-70(Me:2) at flow rates of 0.3 and 0.5 mL/min were 1.4 and 1.3, respectively. Furthermore, at a flow rate of 0.7 mL/min, only St-70(Me:6) exhibited a resolution of ≥ 1.5 , whereas those of St-70(Me:2) and St-70(Me:4) were 1.2 and 1.4, respectively [36]. Therefore, St-70(Me:6) exhibited good separation performance with the 0.10 mol/L NaOH eluent at all flow rates. When the eluent concentration was increased to 0.15 mol/L NaOH, resolutions of ≥ 1.4 were achieved for St-70(Me:4) at flow rates of 0.3 and 0.5 mL/min and for St-70(Me:6) at all flow rates, demonstrating that they had good separation performance. By comparison, 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 4: Resolution between glucose and fructose using St-70(Me:2), St-70(Me:4), St-70(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 (mL/min)	Ion-exchange resin	0.10 mol/L NaOH	0.15 mol/L NaOH
0.3	St-70(Me:2)	1.4	1.3
	St-70(Me:4)	1.6	1.4
	St-70(Me:6)	2.2	1.9
	Fully porous (Me:6)	3.0	2.3
0.5	St-70(Me:2)	1.3	1.1
	St-70(Me:4)	1.5	1.4
	St-70(Me:6)	1.9	1.7
	Fully porous (Me:6)	2.6	1.9
0.7	St-70(Me:2)	1.2	1.0
	St-70(Me:4)	1.4	1.3
	St-70(Me:6)	1.6	1.5
	Fully porous (Me:6)	2.3	2.0

Electrostatic Charge and Ion-Exchange Capacity

The ion-exchange capacities of St-70(Me:2), St-70(Me:4), and St-70(Me:6) are shown in Table 5, 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. St-70(Me:2), which had the fewest methylene groups, had the highest positive charge on the nitrogen ion, the lowest ion-exchange capacity, and the shortest retention times of 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 and then decreased. Thus, it is difficult to explain the constant increase in retention time based on the trends in the electrostatic charge and ion-exchange capacity.

Table 5: Glucose retention times, theoretical plate numbers, electrostatic charges on N^+ atoms, and ion-exchange capacity of St-70(Me:2), St-70(Me:4), and St-70(Me:6). (Eluent: 0.10 mol/L NaOH; flow rate: 0.5 mL/min.)

Ion-exchange resin	Glu retention time (min)	Theoretical plate number	Electrostatic charge on N^+	Ion-exchange capacity (meq/mL)
St-70(Me:2)	8.6	3050	+0.720	0.224
St-70(Me:4)	10.7	2300	+0.637	0.308
St-70(Me:6)	12.3	4440	+0.668	0.232

Theoretical plate numbers (N) using St-70(Me:2), St-70(Me:4), and St-70(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). The N values were highest for St-70(Me:6) at all flow rates and lowest for St-70(Me:4). Thus, as the number of methylene groups in the porous shell increased from two to six, the N values 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). Similarly, to the results using the 0.10 mol/L NaOH eluent, the N values of all carbohydrates were lowest for St-70(Me:4) at each flow rate.

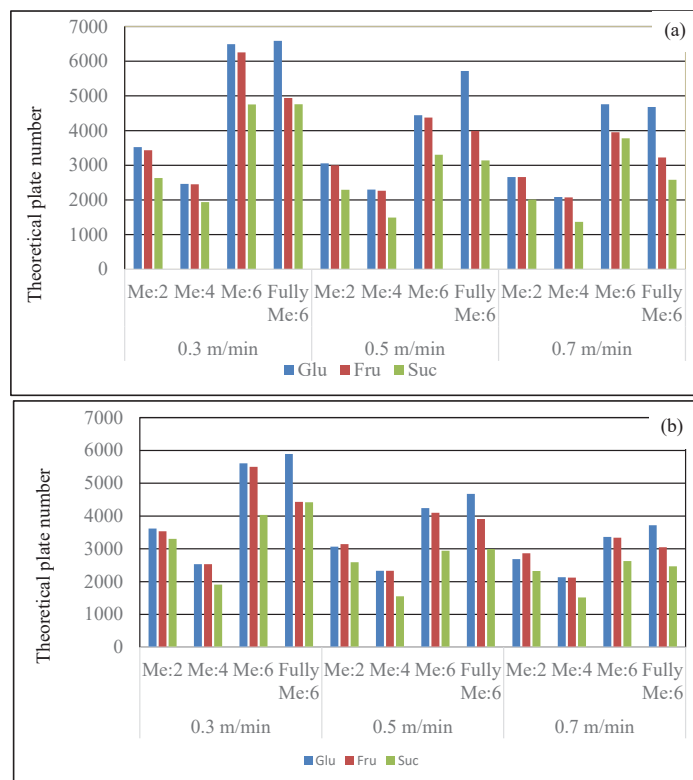


Figure 6: Theoretical plate numbers of glucose, fructose, and sucrose using St-70(Me:2), St-70(Me:4), St-70(Me:6), and the fully porous (Me:6) resins with (a) 0.10 and (b) 0.15 mol/L NaOH eluents at flow rates of 0.3, 0.5, and 0.7 mL/min.

Subsequently, we compared the N values with those for the fully porous resin (Me:6). With the 0.10 mol/L NaOH eluent, the N values for St-70(Me:6) were similar to those for the fully porous resin at flow rates of 0.3 and 0.5 mL/min (Figure 6a). Similarly, with the 0.15 mol/L NaOH eluent, the N values for St-70(Me:6) were similar to those for the fully porous resin at flow rates of 0.3 and 0.5 mL/min (Figure 6b).

Mechanism of Retention Time Variation

Table 5 summarizes the glucose retention times and N values for St-70(Me:2), St-70(Me:4), and St-70(Me:6) with the 0.10 mol/L NaOH eluent at a flow rate of 0.5 mL/min. It also shows the electrostatic charge on the N^+ atom and the ion-exchange capacity of the core-shell resins. As the number of methylene groups increased from two to six, the N values and electrostatic charge first decreased and then increased, whereas the ion-exchange capacity showed the opposite trend. Specifically, St-70(Me:4) had the smallest N value and electrostatic charge but the highest ion-exchange capacity. Consequently, there was no clear relationship between the number of methylene groups and these parameters. By contrast, the retention times increased steadily as the number

of methylene groups increased.

In our previous study on St-60 with different numbers of methylene groups, the ion-exchange capacities 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 had hypothesized that the 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. However, this hypothesis does not agree with the data for St-70. Consequently, other factors need to be considered in addition to the factors mentioned previously.

Discussion

In this study, we evaluated the performance of a core-shell ion-exchange resin, St-70, 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 carbohydrate analysis. Good chromatograms were achieved for glucose, fructose, and sucrose, regardless of the number of methylene groups. St-70(Me:4) and St-70(Me:6) displayed high resolutions (≥ 1.4) at flow rates of 0.3–0.7 mL/min and an eluent concentration of 0.10 mol/L NaOH, demonstrating that they had good carbohydrate separation performance. Increasing the number of methylene groups in functional chain increased the carbohydrate retention times, with St-70(Me:6) having the longest retention times of the core-shell ion-exchange resins at both 0.10 and 0.15 mol/L NaOH. However, all the core-shell ion-exchange resins demonstrated shorter carbohydrate retention times than a fully porous resin (Me:6) with no dense core.

At high pH, carbohydrates become more ionized, and their interaction with the porous layer increases. Thus, the elution sequence of the carbohydrates (glucose followed by fructose) was the same as their pK_a sequence [23]. The following aspects 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 increases from two to six, the carbohydrate retention times also increase. The trend in retention times, which increased constantly as the number of methylene groups increased, could not be explained solely by the electrostatic charge on the N^+ atom in the functional chain, which decreased and then increased as the number of methylene groups increased, or the ion-exchange capacity, which increased and then

decreased. 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-70(Me:6) resin. In addition, as the number of methylene groups increased, the N values first decreased and then increased. The N values of St-70(Me:6) were similar to those of the fully porous resin. Out of the three types of St-70 reported here, St-70(Me:6) was the most suitable packing material for separating carbohydrates, based on the retention time, resolution, and theoretical plate number results. In the present study, the characteristics of the resin were clarified based on three factors (NaOH concentration, the electrostatic charge on the N^+ atom, and ion-exchange capacity). However, since the factors alone could not explain the results comprehensively, further investigations are required.

Conclusion

The results suggest that the St-70 core-shell ion-exchange resins are highly efficient for carbohydrate analysis. 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.

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References

1. Kirkland JJ. Superficially porous silica microspheres for the fast high-performance liquid chromatography of macromolecules. *Anal Chem.* 1992; 64: 1239-1245.
2. Kirkland JJ, DeStefano JJ, Langlois TJ. Fused core particles for HPLC columns. *American Laboratory.* 2007; 39: 18-20.
3. Nagae N, Enami T, Doshi S. The retention behavior of reversed-phase HPLC columns with 100% aqueous mobile phase. *LCGC North America.* 2002; 20: 964-972.
4. Ruta J, Zurlino D, Grivel C, et al. Evaluation of columns packed with shell particles with compounds of pharmaceutical interest. *J Chromatogr A.* 2012; 1228: 221-231.
5. Ahmed A, Abdelmagid W, Ritchie H, et al. Investigation on synthesis of spheres-on-sphere silica particles and their assessment for high performance liquid chromatography applications. *J Chromatogr A.* 2012; 1270: 194-203.
6. Nagae N, Tsukamoto T, Gaitonde VD. Evaluation of six core shell columns based on separation behavior and physical properties. *Chromatogr Today.* 2015; 8: 18-21.
7. Aljehni R, Andre C, Lethier L, et al. An HPLC chromatographic framework to analyze the β -cyclodextrin/solute complexation mechanism using a carbon nanotube stationary phase. *Talanta.* 2015; 144: 226-232.
8. Zhao X, Zhang H, Zhou X, et al. Preparation of core-shell silica-carbon composite microspheres stationary phase and application in saccharide separation. *Chin J Chromatogr.* 2020; 38: 1357-1362.

9. Podzimek S. A review the application of HPLC and GCP to the analysis of synthetic resins. *Chromatographia*. 1992; 33: 377-384.
10. Lee YC. Carbohydrate analyses with high-performance anion-exchange chromatography. *J Chromatogr A*. 1996; 720: 137-149.
11. Cataldi TRI, Campa C, De Benedetto GE. Carbohydrate analysis by high-performance anion-exchange chromatography with pulsed amperometric detection: The potential is still growing. *Fresenius J Anal Chem*. 2000; 368: 739-758.
12. Inoue K, Yamazaki K, Kitahara K, et al. Synthesis of new di-cation type stationary phases for high performance anion-exchange chromatographic separation of carbohydrates. *Bunseki Kagaku*. 2011; 60: 959-964.
13. Hayes R, Ahmed A, Edge T, et al. Core-shell particles: Preparation, fundamentals and applications in high performance liquid chromatography. *J Chromatogr A*. 2014; 1357: 36-52.
14. Li W-H, Stöver HDH. Monodisperse cross-linked core-shell polymer microspheres by precipitation polymerization. *Macromolecules*. 2000; 33: 4354-4360.
15. Bai F, Yang X, Huang W. Synthesis of narrow or monodisperse poly(divinylbenzene) microspheres by distillation-precipitation polymerization. *Macromolecules*. 2004; 37: 9746-9752.
16. Showa Denko KK, Japanese patent. 4979059 (in Japanese).
17. Takashi N, Fukushi E, Onodera S, et al. Isolation and identification of novel tri- and tetra-saccharides synthesized by *Thermoanaerobacter brockii* kojibiose phosphorylase. *J Appl Glycosci*. 2007; 54: 195-200.
18. Pfeiffer P, Geyer H, Geyer R, et al. Separation of glycoprotein-N-glycans by high-pH anion-exchange chromatography. *Biomed Chromatogr*. 1990; 4: 193-199.
19. Miyashita A, Usui M, Takai N. Japanese patent 6218574 (in Japanese).
20. Masuda T, Nishimura Y, Tonegawa M, et al. High-performance liquid chromatographic separation of monosaccharides and disaccharides on stationary phases prepared from polystyrene-based resins and tertiary diamines. *Chem Lett*. 1997; 26: 1239-1940.
21. Masuda T, Nishimura Y, Tonegawa M, et al. High-performance liquid chromatographic separation of carbohydrates on stationary phases prepared from polystyrene-based resin and tertiary amines: Effect of chemical structure of anion-exchange sorbents. *J Chromatogr A*. 1999; 845: 401-408.
22. Masuda T, Kitahara K, Aikawa Y, et al. Determination of carbohydrates by HPLC-ECD with a novel stationary phase prepared from polystyrene-based resin and tertiary amines. *Anal Sci*. 2001; 17: i895- i898.
23. Masuda T, Kitahara K, Aikawa Y, et al. High-performance liquid chromatographic separation of carbohydrates on a stationary phase prepared from polystyrene-based resin and novel amines. *J Chromatogr A*. 2002; 961: 89-96.
24. Masuda T, Kawano A, Kitahara K, et al. Quantitative determination of sugars and myo-inositol in citrus fruits grown in Japan using high-performance anion-exchange chromatography. *J Nutr Sci Vitam*. 2003; 49: 64-68.
25. Hanada T. High-performance liquid chromatographic separation of carbohydrates on stationary phase. *J Human Environ Eng*. 2003; 5: 220-229.
26. Kitahara K, Okuya S, Yoshihama I, et al. Preparation of monodispersed vinylpyridine-divinylbenzene porous copolymer resins and their application to high-performance liquid chromatographic separation of aromatic amines. *J Chromatogr A*. 2009; 1216: 7409-7414.
27. Inoue K, Kitahara K, Aikawa Y, et al. HPLC separation of all aldopentoses and aldohexoses on an anion-exchange stationary phase prepared from polystyrene-based copolymer and diamine: The effect of NaOH eluent concentration. *Molecules*. 2011; 16: 5905-5915.
28. Mitomo S, Negishi Y, Mutai T, et al. Development of a novel surface porous polymer core-shell ion-exchange filler and its elution behavior with carbohydrates. *J Life Support Eng*. 2019; 31: 158-162.
29. Inoue Y, Komiya N, Murata I, et al. Analysis of patchouli alcohol by HPLC using core-shell column. *J Drug Res Dev*. 2017; 3: 1009-1135.
30. Inoue Y, Mitsumori A, Narumi S, et al. Quantitative analysis of α -glucosidase by ECD with a column of the ion exchange resin of core-shell type filler. *World J Pharm Sci*. 2018; 6: 47-54.
31. Mitomo S, Negishi Y, Mutai T, et al. Elution behavior of carbohydrates for core-shell ion-exchange resins with different degrees of cross-linking in porous shell layer. *J Ion Exchange*. 2021; 32: 40-45.
32. Mitomo S, Negishi Y, Mutai T, et al. Development of core-shell ion-exchange resin by changing the core-shell ratio and its elution behavior with carbohydrates. *Chromatography*. 2021; 42: 159-163.
33. Mitomo S, Negishi Y, Mutai T, et al. Elution behavior of carbohydrates for core-shell ion exchange resin St-50 with different degrees of cross-linking in the porous shell. *J Ion Exchange*. 2022; 33: 62-66.
34. Mitomo S, Inoue Y, Tanikawa T, et al. Elution behavior of carbohydrates using core-shell ion-exchange resin St-70 with different degrees of cross-linking in the porous shell. *Chromatography*. 2023; 44: 151-155.
35. Mitomo S, Kodama N, Inoue Y. Elution behavior of carbohydrates using core-shell ion-exchange resin St-60 with different number of methylene groups in the porous shell and a constant cross-linking of 55%. *Thai Journal of pharmaceutical sciences*. 2023; 47: e4.
36. JP XVII. The Japanese Pharmacopoeia Seventeenth Edition, General Tests, Processes And Apparatus. "2. Physical Methods Chromatography, 2.01 Liquid Chromatography, 8. Terminology, (iv) Complete separation of peak" (page43). <https://www.mhlw.go.jp/content/11120000/000945683.pdf>