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ABSTRACT

It is important to clarify the relationship between the molecular structure of functional groups and the elution of carbohydrates in high-performance liquid chromatography. Herein, ion-exchange resins were synthesized with different numbers of methylene groups (two, four, or six) in the functional unit. The core-shell monomer weight ratio was 40:60 (St-60) and the degree of cross-linking was constant at 55%. The effect of the number of methylene groups on the separation of carbohydrates was examined 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. The retention times for St-60 variants with different numbers of methylene groups (two, four, or six) in the porous layer were found to be nearly the same. The theoretical plate number for glucose and fructose tended to show larger values at flow rates of 0.3 and 0.5 mL/min as the number of methylene groups increased.

Key words: Carbohydrates, core-shell ion-exchange resin, high-performance liquid chromatography, retention time, theoretical plate number

INTRODUCTION

Choosing an appropriate structural resin is essential for high-performance liquid chromatography (HPLC), a critical analytical tool. Among core-shell resins,[1,2] various silica-based resins have been developed with surface chemical modifications of the octadecyl group.[3-8] However, silica-based resins are not suitable for use under strongly alkaline conditions because their adsorption ability decreases and the resins become soluble. Styrene-divinylbenzene- and acrylamide-type polymers are frequently used as base materials for organic resins.[9-13] Regardless, the use of most polymer resins is limited in high-speed HPLC operations owing to their fully porous structure. To overcome the aforementioned problems, researchers have synthesized core–shell ion-exchange resins composed of a polymer shell and a core. These resins can provide superior durability under strongly alkaline conditions. Two commercially available examples of core-shell ion-exchange resins are those fabricated through 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 in the shell. Because a thicker porous shell tends to prolong the retention time, 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. Therefore, it is important to identify an optimal combination of shell thickness and degree of cross-linking in the porous layer for HPLC analysis.[19]

In our previous series of studies, we prepared a core-shell ion-exchange resin (St-80) by controlling the core-shell monomer ratio at 20:80 w/w before suspension polymerization.[19-27] The degree of cross-linking in the shell of St-80 was 55%. In addition, a fully porous resin with a cross-linking degree of 55% was prepared using the same method (monomer ratio = 0:100 w/w) for comparison. In the HPLC analysis of carbohydrates, St-80 (55%) demonstrated a shorter retention time compared with the fully porous resin.[28-30] We also examined the effects of the shell cross-linking degree and core-shell monomer ratio. First, resins were prepared at a fixed core-shell monomer ratio of 20:80 w/w and various shell cross-linking degrees (10%, 40%, and 55%). Both the

separation performance of the carbohydrates through HPLC and the theoretical plate number (*N*) were examined.[31] Second, we prepared three resins with the same shell cross-linking degree of 55% and various core-shell monomer weight ratios (50:50, 40:60, and 30:70 w/w, denoted as St-50, St-60, and St-70, respectively), and compared the retention time and *N*-value for separating carbohydrates through HPLC.[32] Third, the elution behavior of carbohydrates using St-50 with different shell cross-linking degrees (10%, 40%, and 55%) was compared.[33] Finally, to clarify the effects of different factors (shell thickness, shell cross-linking degree, and concentration of NaOH eluent) on the resin performance, we studied the elution behavior of carbohydrates using St-70 with different shell cross-linking degrees (10%, 40%, and 55%).[34]

In this study, we focused on the effect of the number of methylene groups in the functional unit on the elution behavior of carbohydrates. Novel core-shell ion-exchange resins based on St-60 (core-shell monomer ratio: 40:60 w/w) were prepared with two, four, or six methylene groups (denoted as St-60 [Me:2], St-60 [Me:4], and St-60 [Me:6], respectively). The degree of cross-linking in the shell was constant at 55%.

MATERIALS AND METHODS

Materials

Myo-inositol, sucrose, and NaOH were obtained from Fujifilm Wako Chemicals Co.; and 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.

Conditions for HPLC Analysis

HPLC was performed using a DKK-TOA SU-300 instrument equipped with an electrochemical detector and a gold electrode. The core-shell ion-exchange resin consisted of a hard polymer core and a porous shell containing functional groups, as shown in Figures 1 and 2.[31] The porous shell was synthesized by the reaction of a chloromethylstyrene-divinylbenzene copolymer carrier with a tertiary amine, as described previously.[19]

The thickness of the shell was kept constant by maintaining a constant initial core-shell monomer ratio of 40:60 w/w and a constant total mass of the monomers. The degree of cross-linking in the porous layer also kept constant at 55% by employing a styrene/divinylbenzene weight ratio of 45:55.[32] The number of methylene groups in the functional unit of the porous layer was adjusted using *N*,*N*,*N*′,*N*′-tetramethyl-1,6-hexa metylenediamine, *N*,*N*,*N*′,*N*′-tetramethyl-1.4-butanediamine, and *N*,*N*,*N*′,*N*′-tetramethyl ethylenediamine as the tertiary amines to produce core–shell ion-exchange resins with two, four, and six methylene groups (denoted as St-60 [Me:6], St-60 [Me:4], and St-60 [Me:2], respectively). For comparison, fully porous resins (i.e., with no core) were prepared through the reaction of the chloromethylstyrene-divinylbenzene copolymer (divinylbenzene weight ratio: 55%) carrier with a tertiary amine. The degree of cross-linking was 55%, and the number of methylene groups was two, four, or six. The prepared resins had an average diameter of 5 μm. We prepared 3 g of each core-shell and fully porous resin.

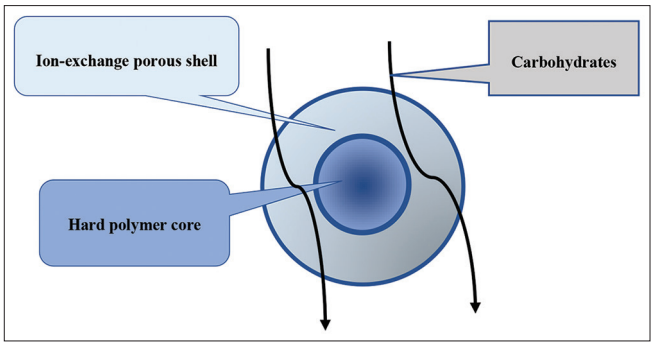

Figure 1: Structure of core-shell ion-exchange resin consisting of a hard polymer core and ion-exchange porous shell

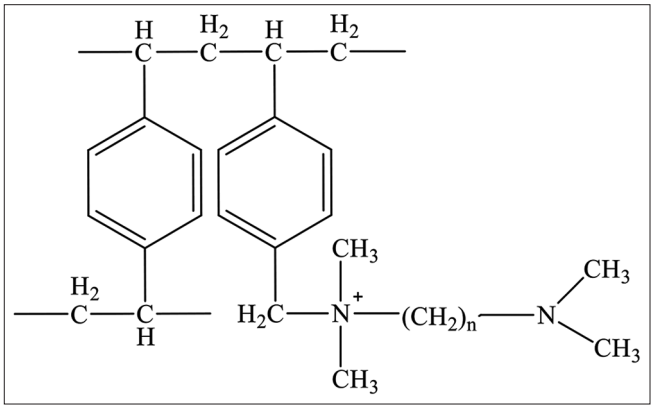

Figure 2: Chemical structure of the porous polymer shell in the ion-exchange resin (n = 2, 4, and 6)

For HPLC, 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 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 nitrogen atom in the functional group by density functional theory using the ωB97X-D density functional and 6.31G* basis set in Spartan'20 [Figure 3].

RESULTS AND DISCUSSION

The quantification of carbohydrate components can provide detailed information on food analytes. Because our core-shell ion-exchange resins St-60 (Me:2, Me:4, and Me:6) at a fixed core-shell monomer weight ratio of 40:60 and 55% cross-linking degree mainly target carbohydrate analytes, we used a standard solution containing inositol, glucose, fructose, and sucrose to evaluate their performance in HPLC. Notably, when an electrochemical detector was used, the solution did not require pretreatment and only the carbohydrates were analyzed.

Evaluation of Separation Properties for St-60 (Me:2, Me:4, and Me:6) Ion-Exchange Resins

The chromatograms illustrating the separation of saccharides in columns packed with the St-60 (Me:2, Me:4, and Me:6) resins eluted at a flow rate of 0.5 mL/min with a 0.10 moL/L NaOH eluent are shown in Figure 4a-c. Table 1 presents the retention times of glucose, fructose, and sucrose for these resin eluents at flow rates of 0.3–0.7 mL/min with 0.10 moL/L NaOH. Interestingly, the retention times for each carbohydrate were almost the same for all three ion-exchange resins at all flow rates when using the 0.10 moL/L NaOH eluent.

The eluent concentration was then increased to 0.15 moL/L NaOH. The chromatograms are illustrated in Figure 5a-c, and the retention times are listed in Table 2. As for the 0.15 moL/L NaOH eluent, the retention times for each carbohydrate were almost the same for all three ion-exchange resins at all flow rates.

Thus, the number of methylene groups did not significantly affect the retention times of these carbohydrates during elution using 0.10–0.15 moL/L NaOH eluent at flow rates of 0.3–0.7 mL/min. Each carbohydrate demonstrated good peak shapes in the chromatograms, regardless of the number of methylene groups.

For comparison, we examined the retention times of carbohydrates using St-50 (Me:2, Me:4, and Me:6) (core-shell monomer weight ratio: 50:50) with a 0.10 moL/L NaOH eluent, again focusing on differences due to the number of methylene groups in the functional unit of the porous shell. St-50 (Me:4 and Me:6) produced good chromatograms, whereas St-50 (Me:2) did not [Table 3]. Nevertheless, there was no apparent effect of the number of methylene groups on the retention times of each carbohydrate. This was ascribed to the thinness of the shell layer.

We also compared the performance to that of a fully porous resin (i.e., with no core) with six methylene groups (Me:6). The cross-linking degree (55%) was the same as that for the porous shell layer of the core-shell ion-exchange resins St-60 (Me:2, Me:4, and Me:6). The core-shell ion-exchange resins St-60 (Me:2, Me:4, and Me:6) 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 ion-exchange resins only contained a thin layer of porous resin, the interaction time between the carbohydrates and porous layer was lower than that for the fully porous resin (Me:6), resulting in significantly shorter retention times.[32]

To investigate the separation properties of these resins, we focused on the resolution between glucose and fructose in the chromatograms, as these analytes demonstrated adjacent peaks [Table 4]. When using the 0.10 moL/L NaOH eluent, a resolution of over 1.5 was achieved for all core-shell resins (St-60 [Me:2, Me:4, and Me:6]) at all flow rates.[35] Therefore, they exhibited good separation performance for glucose and fructose with 0.10 moL/L NaOH at flow rates of 0.3–0.7 mL/min. When the NaOH eluent concentration was increased to 0.15 moL/L, the resolutions for St-60 (Me:2, Me:4, and Me:6) were above 1.3 at flow rates of 0.3 and 0.5 mL/min. By comparison, when eluting the fully porous resin (Me:6) with the 0.10 moL/L NaOH eluent, the resolutions between

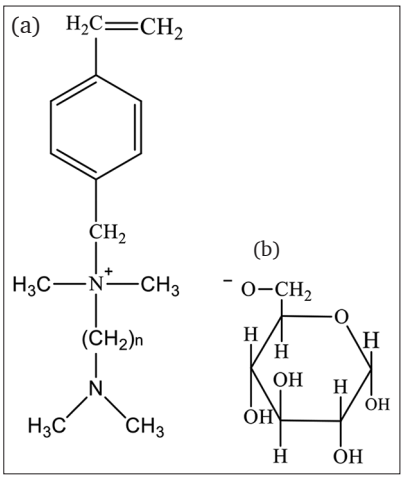

Figure 3: Structures used for optimization of the electrostatic charge on N^+ in Spartan'20: (a) functional unit of the ion-exchange resin and (b) representative carbohydrate molecule

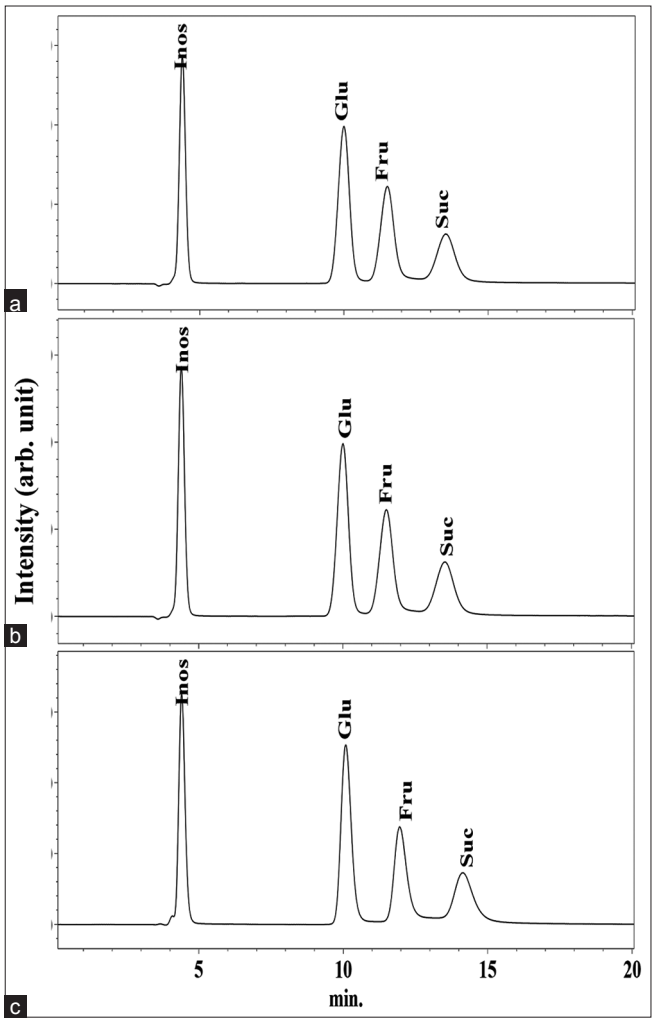

Figure 4: Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-60 (Me:2), (b) St-60 (Me:4), and (c) St-60 (Me:6) with 0.10 moL/L NaOH eluent at a flow rate of 0.5 mL/min

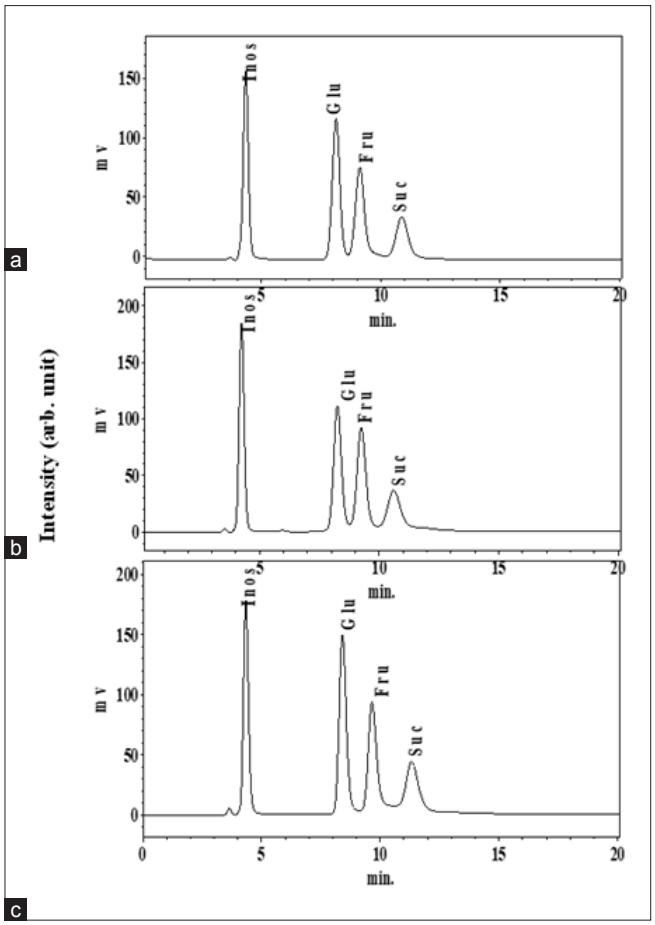

Figure 5: Chromatographs obtained for the separation of inositol, glucose, fructose, and sucrose using (a) St-60 (Me:2), (b) St-60 (Me:4), and (c) St-60 (Me:6) with 0.15 moL/L NaOH eluent at a flow rate of 0.5 mL/min

the glucose and fructose peaks at flow rates of 0.3, 0.5, and 0.7 mL/min were 3.0, 2.6, and 2.3, respectively, indicative of good separation performance.

At high pH levels, carbohydrates become more ionized. Therefore, their interaction with the porous layer should increase. The elution sequence (glucose followed by fructose) was the same as their pK_a sequence.[23] The following aspects are considered important for understanding the separation properties. (1) The core suppresses solute diffusion along the column axis. Because the porous layer is thin, the solute moves a shorter distance within the shell to reach the core. (2) The concentration of the NaOH eluent plays a critical role in the separation of these carbohydrates. (3) The retention times of these carbohydrates were almost the same regardless of the number of methylene groups in the functional unit of the shell layer.

To explain why the retention time was the same when the number of methylene groups changed, two factors need to be considered. (a) In general, the retention time for ion-exchange resins is large because the ion-exchange capacity is large.[31] The ion-exchange capacities of St-60 (Me:2, Me:4, and Me:6) are shown in Table 5. Although St-60 (Me:6) had the largest ion exchange capacity, it did not have the highest retention time. Therefore, the retention time cannot be explained by the ion exchange capacity alone. (b) The positive electrostatic charge of the nitrogen atoms in the functional groups of the resin can also affect the retention time owing to the increased interaction between the positively charged nitrogen atoms and anionic carbohydrate molecules, with higher electrostatic charges corresponding to longer retention times. We therefore calculated the positive electrostatic charge of the nitrogen atoms for these resins using Spartan'20; the results are shown in Table 5. St-60 (Me:6) exhibited the lowest positive charge on the nitrogen atom in the functional group; therefore, if all other factors were equal, it should exhibit the lowest retention time. However, it did not have the lowest retention time and, in fact, the retention times for all three resins were almost the same. It is suggested that these opposing factors resulted

Table 1: Retention times (min) of glucose, fructose, and sucrose using St-60 (Me: 2), St-60 (Me: 4), St-60 (Me: 6), and fully porous resin (Me: 6) with 0.10 moL/L NaOH eluent at flow rates of 0.3–0.7 mL/min.

Flow rate (mL/min)	No. of me groups	Glu	Fru	Suc
0.3	2	16.5	19.0	22.3
	4	17.2	19.6	23.1
	6	16.9	20.2	23.8
	Fully porous, 6	26.9	32.5	44.2
0.5	2	10.0	11.5	13.5
	4	10.4	11.8	13.8
	6	10.1	11.9	14.1
	Fully porous, 6	16.4	19.6	27.2
0.7	2	7.2	8.3	9.8
	4	7.5	8.5	10.2
	6	7.0	8.2	9.6
	Fully porous, 6	16.4	19.6	27.2

Table 2: Retention times (min) of glucose, fructose, and sucrose using St-60 (Me: 2), St-60 (Me: 4), St-60 (Me: 6), and fully porous resin (Me: 6) with 0.15 moL/L NaOH eluent at flow rates of 0.3–0.7 mL/min.

Flow rate (mL/min)	No. of me groups	Glu	Fru	Suc
0.3	2	13.3	15.0	17.7
	4	13.6	15.3	17.5
	6	13.8	15.9	18.6
	Fully porous, 6	26.9	32.5	44.2
0.5	2	8.1	9.1	10.9
	4	8.2	9.2	10.6
	6	8.4	9.7	11.3
	Fully porous, 6	16.4	19.6	27.2
0.7	2	5.9	6.6	7.9
	4	6.0	6.7	7.6
	6	6.1	7.0	8.2
	Fully porous, 6	16.4	19.6	27.2

in the retention times being almost the same. In addition, because of the thin shell, these resins all have relatively short retention times, which may have minimized any differences between them.

Effect of the Number of Methylene Groups on the Theoretical Plate Number *N* for St-60(Me:2, Me:4, and Me:6)

The characteristics of the resins were further compared in terms of their *N*-values. We evaluated the *N*-values of glucose, fructose, and sucrose with the 0.10 moL/L NaOH eluent. The *N*-values for St-60 (Me:6) were higher than those for St-60 (Me:2 and Me:4) at all flow rates [Figure 6a].

At a flow rate of 0.3 mL/min, increasing the number of methylene groups in the functional unit increased the *N*-values of glucose and fructose. At flow rates of 0.5 and 0.7 mL/min, St-60 (Me:4) demonstrated smaller *N*-values for all carbohydrates as compared to St-60 (Me:2 and Me:6). Table 5 summarizes the retention times and *N*-values of glucose for St-60 (Me:2, Me:4, and Me:6) at a flow rate of 0.5 mL/min with the 0.10 moL/L NaOH eluent. The *N*-values of glucose, fructose, and sucrose were also evaluated using the 0.15 moL/L NaOH eluent [Figure 6b]. At flow rates of 0.3 and 0.5 mL/min, increasing the number of methylene groups in the functional unit increased the *N*-values of glucose and fructose.

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

Flow rate (mL/min)	No. of me groups	Glu	Fru	Suc
0.5	2*	-	-	-
	4	9.8	11.3	13.0
	6	8.8	10.1	11.5

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

Table 4: Resolution between glucose and fructose using St-70 (Me: 2), St-70 (Me: 4), St-70 (Me: 6), and fully porous resin (Me: 6) with 0.10 and 0.15 moL/L NaOH eluents at flow rates of 0.3–0.7 mL/min.

Flow rate (mL/min)	No. of Me groups	0.10 moL/L NaOH	0.15 moL/L NaOH
0.3	2	1.4	1.3
	4	1.6	1.4
	6	2.2	1.9
	Fully porous, 6	3.0	2.3
0.5	2	1.3	1.1
	4	1.5	1.4
	6	1.9	1.7
	Fully porous, 6	2.6	1.9
0.7	2	1.2	1.0
	4	1.4	1.3
	6	1.6	1.5
	Fully porous, 6	2.3	2.0

At a flow rate of 0.7 mL/min, the *N*-values of all carbohydrates were lower for St-60 (Me:4) than St-60 (Me:2 and Me:6). Subsequently, we compared the *N*-values of glucose, fructose, and sucrose with 0.10 and 0.15 moL/L NaOH eluents to those for the fully porous resin (Me:6; 55% cross-linking degree). St-60 (Me:6) exhibited similar results to the fully porous resin (Me:6) for all carbohydrates using each flow rate and eluent, demonstrating its good performance.

CONCLUSION

In this study, we evaluated the performance of a core-shell ion-exchange resin, St-60, with different numbers of methylene groups (two, four, and six) in the functional units of the shell. Good chromatograms were achieved for glucose, fructose, and sucrose regardless of the number of methylene groups and NaOH eluent concentration. St-60 (Me:2, Me:4, and Me:6) displayed high resolutions above 1.5 and good separation performance at flow rates of 0.3–07 mL/min with eluent concentrations of 0.10 and 0.15 moL/L NaOH. St-60 (Me:2, Me:4, and Me:6) all demonstrated shorter retention times for these carbohydrates as compared to a fully porous resin (Me:6) with no core. However, increasing the number of methylene groups did not significantly affect the retention times. The stability in retention times was linked to two opposing factors: As the number of methylene groups increased from two to six, the positive charge on the nitrogen atoms in the functional unit decreased, whereas the ion-exchange capacity increased.

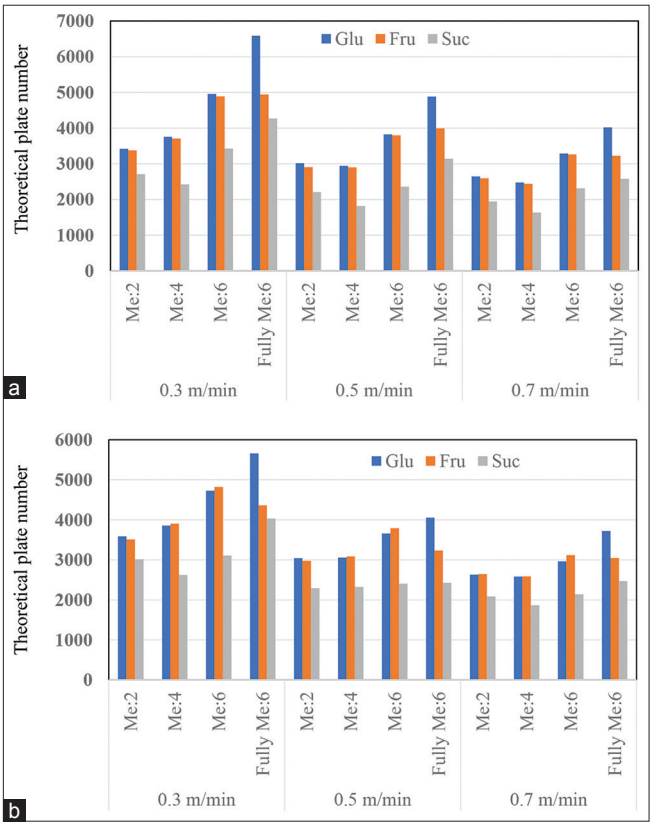

Figure 6: Theoretical plate numbers N of glucose, fructose, and sucrose using St-60 (Me:2), St-60 (Me:4), St-60 (Me:6), and fully porous resin (Me:6) with (a) 0.10 moL/L and (b) 0.15 moL/L NaOH eluents at flow rates of 0.3, 0.5, and 0.7 mL/min

Table 5: Retention time and theoretical plate number *N* of glucose, electrostatic charge on N^+, and ion-exchange capacity when St-60 (Me: 2), St-60 (Me: 4), and St-60 (Me: 6) were used with 0.10 moL/L NaOH as the eluent (flow rate, 0.5 mL/min).

No. of Me groups	Retention time (min)	Theoretical plate number	Electrostatic charge on N^+	Ion-exchange capacity (meq/mL)
2	10.0	3020	+0.720	0.294
4	10.4	2950	+0.637	0.353
6	10.1	3820	+0.668	0.360

Furthermore, the shell layer was thin. It may be that the combined effects of these opposing factors resulted in the retention times being similar. When the number of methylene groups increased, the *N*-values of glucose and fructose increased at a flow rate of 0.3 mL/min with the 0.10 moL/L NaOH eluent. Compared to sucrose, glucose demonstrated larger *N*-values at flow rates of 0.3–0.7 mL/min with the 0.10 and 0.15 moL/L NaOH eluents. At each flow rate, the *N*-values of glucose, fructose, and sucrose for St-60 (Me:6) with the 0.10 moL/L NaOH eluent demonstrated good results, similar to those of the fully porous resin (Me:6).

These results suggest that 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 due to their polymer-based core and shell.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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