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A Synthesis of Novel Ion-Exchange Resin, St-80 and its Evaluation by the Cross-Linking (40%) and Number of Methylene Group in the Porous Shell

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ABSTRACT

The development of novel core–shell resins with high sensitivity, resolution, and tunable separation performance is increasingly important for advanced chromatographic applications. A particular challenge is the complex relationship between ion-exchange resin structure and carbohydrate elution, which complicates polymer design for high-performance liquid chromatography.

To investigate the influence of cross-linking and methylene group number in the functional chain of the shell, core–shell ion-exchange resins with a monomer weight ratio of 20:80 (St-80) and a fixed cross-linking degree of 40% were synthesized. The length of the functional chain in the porous polymer shell was varied from two to six methylene groups St-80(40% Me: 2, 4, and 6) and evaluated for carbohydrate separation under strongly alkaline conditions. A mixture of inositol, glucose, fructose, and sucrose was separated using 0.10 or 0.15 mol/L NaOH eluents at flow rates of 0.3–0.7 mL/min. Resins with four methylene groups St-80(40% Me:4) exhibited the highest retention times and theoretical plate numbers, particularly at 0.7 mL/min with 0.10 mol/L NaOH, and consistently outperformed resins with two or six methylene groups under 0.15 mol/L NaOH. These findings demonstrate that St-80(40% Me:2, 4, and 6) resins provide efficient carbohydrate separations and are well suited for use under strongly alkaline conditions with electrochemical detection.

Keywords

High-performance liquid chromatography, Core–shell ion-exchange resin, Carbohydrates, Retention time, Theoretical plate number, Density functional theory, Hartree–Fock.

Abbreviations

St-80(40% Me:2, Me:4, and Me:6): constant core–shell monomer weight ratio of 20:80 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.

Introduction

The selection of an appropriate ion-exchange resin plays a key role in the efficiency and resolution of high-performance liquid chromatography (HPLC). Many different types of core–shell resins have been developed for this purpose [1,2]. Examples of core–shell ion-exchange resins commonly used in analytical applications includesilica-based resins, such as octadecyl-functionalized silica resins [3-8]. However, their limited stability under strongly alkaline conditions remains a significant challenge. Styrene–divinylbenzene- and acrylamide-type polymers, which are frequently used as base materials for organic resins [9-13], offer an alternative with improved chemical resistance.

To address these limitations associated with fully porous structures, core–shell ion-exchange resins composed of a dense core surrounded by a porous polymer shell have been synthesized. These resins offer enhanced chemical stability and durability under high pH conditions. Among commercially available options, two types of core–shell ion-exchange resins have been reported: those

prepared via precipitation polymerization around the core [14,15] and latex-type resins based on a styrene matrix [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 analysis [19]. As the retention time increases with the thickness of the porous shell, minimizing the shell thickness contributes to reducing the retention time. Furthermore, it is necessary to take into account the effect of the degree of cross-linking as an additional factor 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. These resins consist 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] 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 with a constant cross-linking degree of 55% [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]. When using St-60, St-70, and St-80 resins with two, four, and six methylene groups in the functional chain 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) and 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 St-60(40% Me:2, 4, and 6) and St-70(40% Me:2, 4, and 6) [38,39].

In this study, we evaluated the carbohydrate elution behavior by using new core–shell resins, St-80 (monomer weight ratio: 20:80) with a cross-linking degree of 40% in the porous shell and two, four, or six methylene groups St-80(40% Me:2), St-80(40% Me:4), and St-80(40% Me:6), respectively. This study investigates the factors influencing carbohydrate elution behavior in HPLC, complemented by analyses using computational chemistry methods. It provides a comprehensive assessment of carbohydrate elution, integrating experimental results with computational insights.

Materials and Methods

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.

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].

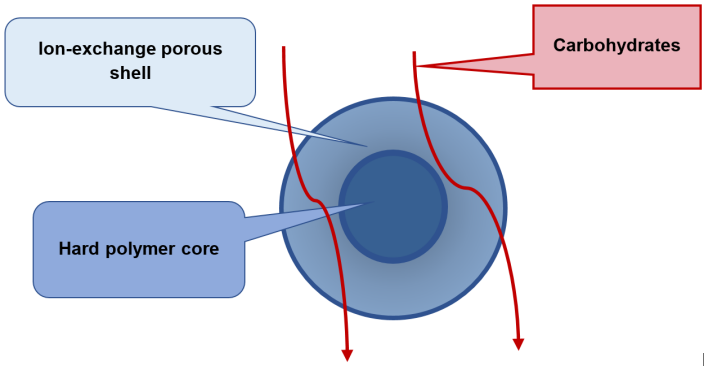

Figure 1: Schematic structure of the core–shell ion-exchange resin consisting of a dense polymer core and an ion-exchange porous polymer shell.

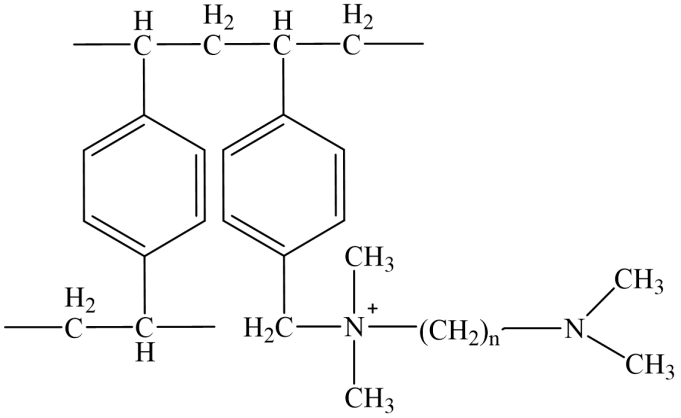

Figure 2: Chemical structure of the porous polymer in the ion-exchange resin shell (n = 2, 4, and 6).

The shell thickness was maintained consistently by ensuring a constant core–shell monomer weight ratio of 20:80, 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 St-80(40% Me:2), St-80(40%

Me:4), and St-80(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 (Fully(40% Me:6)). The average diameter of the prepared resins particles was 5 µm. We prepared 3 g of each core–shell and fully porous resin.

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 (The pH is approximately 13) 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. The electrostatic charge on the N^+ atom in the functional chain and O^- atom in the carbohydrate model compounds, as well as the stable configurations of their complexes, were calculated using density functional theory (DFT) with the ωB97X-D functional and 6.31G* basis set in Spartan'20 (Figure 3). pH of about 13.

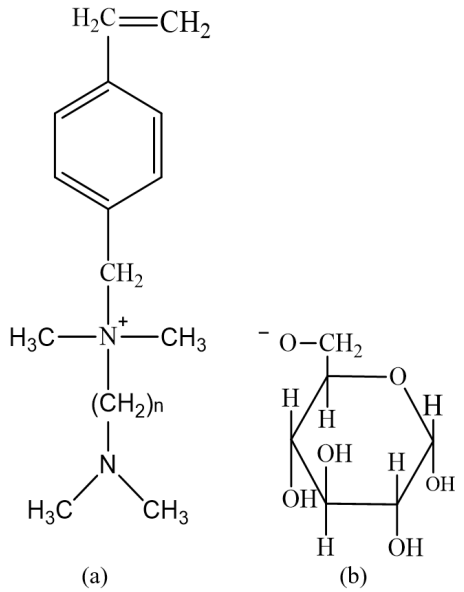

Figure 3: Structures used for optimization of the electrostatic charge on the N^+ atom in the functional chain and O^- atom in carbohydrate using Spartan'20: (a) functional chain of the ion-exchange resin and (b) representative carbohydrate molecule.

Results

Carbohydrate separation performance of St-80(40% Me:2), St-80(40% Me:4), and St-80(40% Me:6) ion-exchange resins

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 with St-80(40% Me:2), St-80(40% Me:4), and St-80(40% Me:6) core–shell ion-exchange resins, which had a 20:80 core–shell monomer weight ratio and contained two, four, and six methylene groups, respectively. We evaluated the carbohydrate separation performance of columns packed with St-80(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). Chromatograms of St-80(40% Me:4, and 6) at these flow rates are shown in Figure 4a–f. The separation performance of columns packed with St-80(55% Me:6) resins under the same conditions is also summarized in Table 1. Retention times of glucose, fructose, and sucrose at the three flow rates with the 0.10 mol/L NaOH eluent are presented in Figure 5a–c.

Comparison among St-80(40% Me:2, 4, and 6), St-80(55% Me:2, 4, and 6), and Fully(40% and 55% Me:6)

Each carbohydrate for St-80(40% Me:4 and 6) showed longer retention times than those of St-80(55% Me:4 and 6) at flow rates of 0.3, 0.5, and 0.7 mL/min with 0.10 mol/L NaOH eluent. Each carbohydrate for St-80(40% Me:2) showed shorter retention times than those of St-80(55% Me:2) at all flow rates with 0.10 mol/L NaOH eluent. (Table 1) Each carbohydrate for Fully(40% Me:6) showed the larger retention time than those for St-80(40% Me:2, 4, and 6) at all flow rates. The chromatograms of St-80(40% Me:2, 4, and 6) showed clean peaks. Next, the eluent concentration was increased to 0.15 mol/L NaOH. The retention times of glucose, fructose, and sucrose for St-80(40% Me: 2, 4, and 6) and St-80(55% Me:2, 4, and 6) at flow rates of 0.3, 0.5, and 0.7 mL/min are summarized in Table 2. For St-80(40% Me:4) retention times were consistently longer than those of St-80(55% Me:4) at all flow rates, whereas St-80(40% Me:6) and St-80(55% Me:6) exhibited nearly identical retention times under the same conditions. 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 and 0.15 mol/L NaOH eluent (Table 1 and 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-80(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-80(40% Me:2, 4, and 6) were 1.9, 2.6, and 2.1 at flow rate of 0.5 mL/min, respectively [40]. When using the 0.15 mol/L NaOH eluent, St-80(40% Me:4 and 6) core–shell resins showed a higher resolution of ≥1.5 at all flow rates. When using Fully(40% Me:6) with 0.10 mol/L NaOH eluent, the resolutions between the glucose and fructose peaks were 3.4, 3.2, and 3.0, respectively, at flow rates

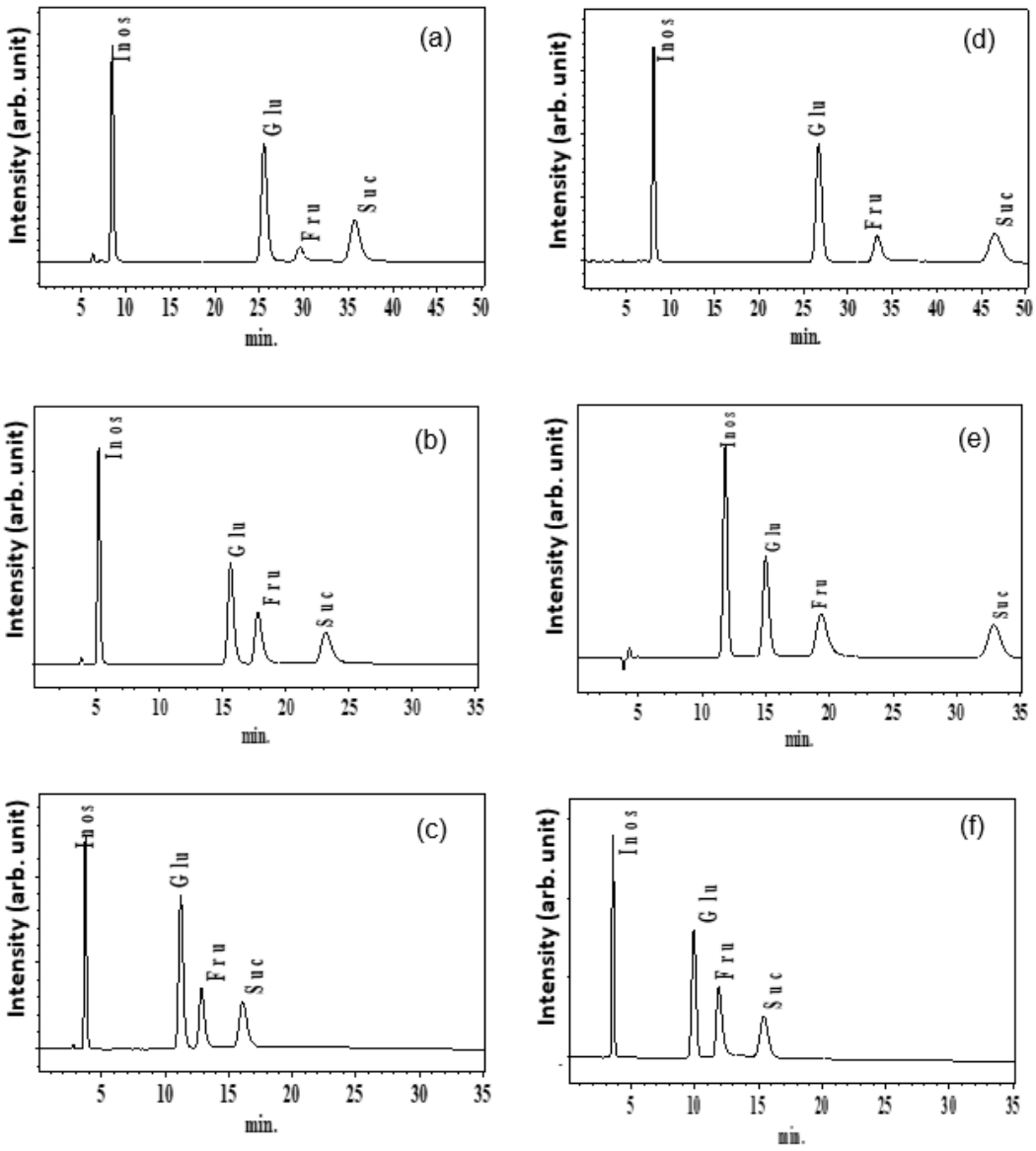

Figure 4a-f: Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using St-80(40% Me:4) with a 0.1 mol/L NaOH (a) 0.3 mL/min, (b) 0.5 mL/min, (c) 0.7 mL/min, and St-80(40% Me:6) (d) 0.3 mL/min, (e) 0.5 mL/min, (f) 0.7 mL/min.

Table 1: Retention times (min) of glucose, fructose, and sucrose using St-80(40% CH2-2, 4, and 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 CH2	Glu		Fru		Suc	
	Cross-linking	40%	55%	40%	55%	40%	55%
0.3 mL/min	2	18.6	20.5	21.0	24.6	24.5	27.3
	4	25.5	21.8	29.6	25.9	35.7	31.5
	6	26.7	19.3	33.3	22.1	46.5	26.7
	Fully porous 6	34.9	26.9	43.5	32.5	58.6	44.2
0.5 mL/min	2	11.0	12.4	12.4	14.8	14.3	16.9
	4	15.6	13.1	17.9	15.6	22.3	18.9
	6	15.0	12.9	19.3	15.0	32.9	18.6
	Fully porous 6	21.1	16.4	26.6	19.6	35.5	27.2
0.7 mL/min	2	7.9	8.9	8.8	10.6	10.2	12.2
	4	11.2	9.5	12.8	11.2	16.0	13.6
	6	9.9	9.2	11.9	10.7	15.2	13.2
	Fully porous 6	15.3	11.8	19.2	14.2	25.7	19.9

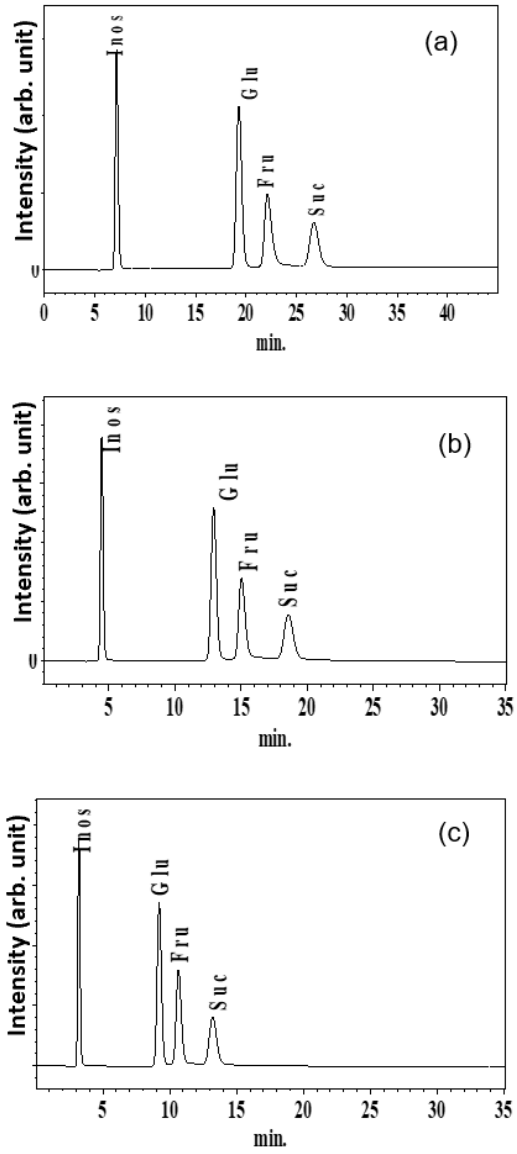

Figure 5a-c: Chromatograms obtained for the separation of inositol, glucose, fructose, and sucrose using St-80(55% Me:6) with a 0.1 mol/L NaOH (a) 0.3 mL/min, (b) 0.5 mL/min, (c) 0.7 mL/min.

Table 2: Retention times (min) of glucose, fructose, and sucrose using St-80(40% CH2-2, 4, and 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 CH2	Glu		Fru		Suc	
	Cross-linking	40%	55%	40%	55%	40%	55%
0.3 mL/min	2	14.6	15.7	16.1	18.3	18.8	21.3
	4	19.0	17.1	21.3	19.8	27.2	24.9
	6	16.2	16.3	18.2	18.6	24.0	23.7
	Fully porous 6	26.7	20.0	33.2	23.3	46.1	33.6
0.5 mL/min	2	8.7	9.6	9.6	11.1	11.4	13.2
	4	11.5	9.4	12.9	10.8	16.5	13.9
	6	9.9	10.1	11.2	11.6	14.7	14.5
	Fully porous 6	16.3	11.9	20.1	13.8	28.3	19.2
0.7 mL/min	2	6.3	6.9	6.9	8.0	8.2	9.5
	4	8.3	7.4	9.3	8.6	11.9	11.0
	6	7.1	7.4	8.1	8.5	10.3	10.9
	Fully porous 6	11.8	8.9	14.5	10.5	21.1	14.6

of 0.3, 0.5, and 0.7 mL/min, respectively, indicative of good separation performance. When using Fully(40% Me:6) with a 0.15 mol/L NaOH eluent, the resolution between glucose and fructose was comparable to that obtained with Fully(40% Me:6) under 0.10 mol NaOH eluent.

Table 3: The resolution between glucose and fructose using St-80(40% Me:2, 4, and 6), St-80(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	CH2-2		CH2-4		CH2-6		Fully CH2-6	
(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	2.3

Table 4: The resolution between glucose and fructose using St-80(40% Me:2, 4, and 6), St-80(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	CH2-2		CH2-4		CH2-6		Fully CH2-6	
(mL/min)	40%	55%	40%	55%	40%	55%	40%	55%
0.3	1.4	2.6	2.0	2.3	1.8	2	3.1	2.3
0.5	1.3	2.2	1.8	2.1	1.5	1.9	2.8	1.9
0.7	1.1	1.9	1.6	2.0	1.5	1.7	2.6	2.0

Theoretical plate numbers (*N*) using St-80(40% Me:2), St-80(40% Me:4), and St-80(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) and (b)). With 0.10 mol/L NaOH eluent, St-80(40% Me:4) exhibited larger theoretical plate numbers for glucose, fructose, and sucrose than St-80(55% Me:4) across all flow rates, whereas St-80(40% Me:2) showed smaller plate numbers than its 55% counterpart under the same conditions. As the number of methylene groups in the porous shell increased from two to six, the *N* values of glucose for St-80(40% Me:2, 4, and 6) increased at flow rates of 0.3 and 0.5 mL/min with 0.10 mol/L NaOH eluent. In this time, the *N* values of fructose for St-80(40% Me:2, 4, and 6) increased and then decreased at flow rates of 0.3, 0.5, and 0.7 mL/min. As the number of methylene groups increased, the *N* values of glucose, fructose, and sucrose for St-80 (40% Me:2, 4, and 6) increased and then decreased at flow rates of 0.5 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.

Theoretical plate numbers of glucose, fructose, and sucrose for Fully(40% Me:6) and Fully(55% Me:6) with 0.10 mol/L NaOH

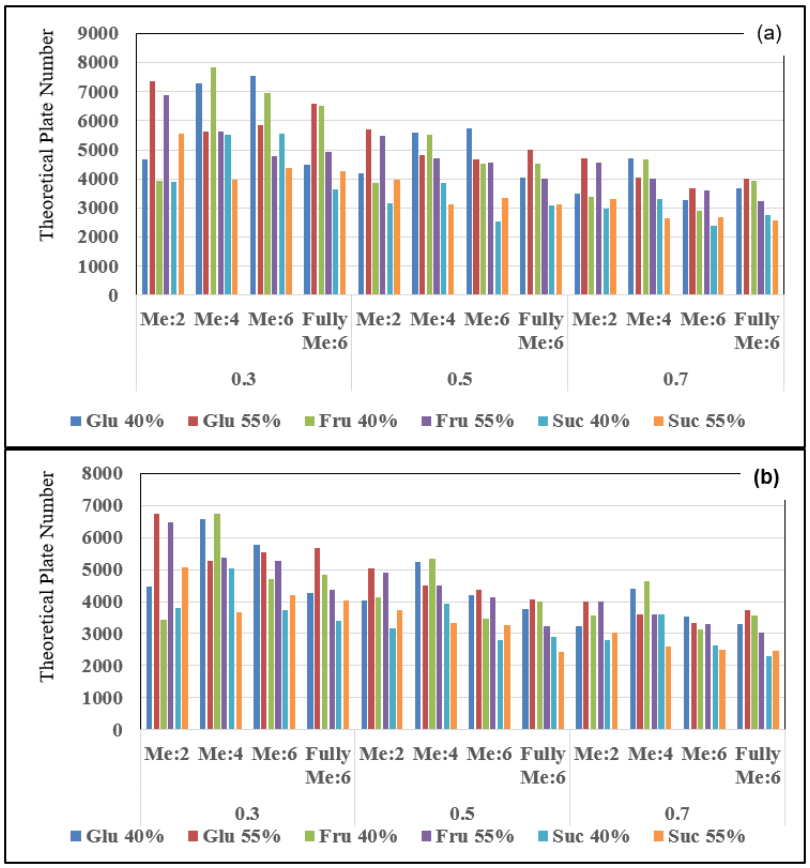

Figure 6a-b: Theoretical plate numbers N of glucose, fructose, and sucrose using St-80(40% Me:2), St-80(40% Me:4), St-80(40% Me:6), and Fully (40% Me:6) with (a) 0.10 mol/L and (b) 0.15 mol/L NaOH eluents at flow rates of 0.3, 0.5, and 0.7 mL/min.

eluent are shown in Figure 6a. At all flow rates, each carbohydrate for St-80(40% Me:2) exhibited smaller theoretical plate numbers than that of St-80(55% Me:2), whereas that for St-80(40% Me:4) showed the opposite trend. The corresponding data obtained with 0.15 mol/L NaOH are presented in Figure 6b.

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, the electrostatic charges on the N^+ atom and O^- atom, and the ion-exchange capacity of the core–shell resins. As the number of methylene groups increases from two to six, the electrostatic charges on the N^+ atom decreases, that on O^- atom initially decreases and then increases, and the ion-exchange capacity increases. Specifically, St-80(40% Me:2) had the largest electrostatic charge on the N^+ atom and St-80(40% Me:6) had the largest electrostatic charge on the O^- atom. When the number of methylene groups increased, the ion-exchange capacity increased. The retention times of glucose for St-80(40% Me:2, 4, and 6) are 11.0, 15.6, and 15.0 min, respectively as the number of methylene groups increased from 2 to 6.

In our previous study, the ion-exchange capacities of St-80(55% Me:2, 4, and 6) with 0.10 mol/L NaOH eluent also increased (Table 5). Meanwhile, the electrostatic charge on the N^+ atom decreased as the number of methylene groups increased from two to six. The retention time of glucose for St-80(55% Me:2, 4, and 6) exhibited a similar trend comparable to those observed with St-80(40% Me:2, 4, and 6) (Table 5). We hypothesized that the observed retention time results could be attributed to two opposing factors. For St-80(40% Me: 2, 4, and 6), the positive charge of the N^+ atom in the functional chain tended to decrease as the number of methylene groups increased, whereas the ion-exchange capacity increased with chain length. However, the ion-exchange capacity of St-80(40% Me: 2, 4, and 6) showed a trend similar to that of St-80(55% Me: 2, 4, and 6). Among these resins, St-80(40% Me:4) exhibited the longest glucose retention time.

The stable configuration of these molecules between the monovalent anion of a carbohydrate and the cation of an ion-exchange model compound was investigated using Spartan'20 (Figure 7a–c). The distances between N^+ and O^- in the model complexes were 3.938, 4.206, and 3.187 Å, respectively. These findings suggest that multiple factors must be considered to comprehensively explain the observed differences in carbohydrate retention times.

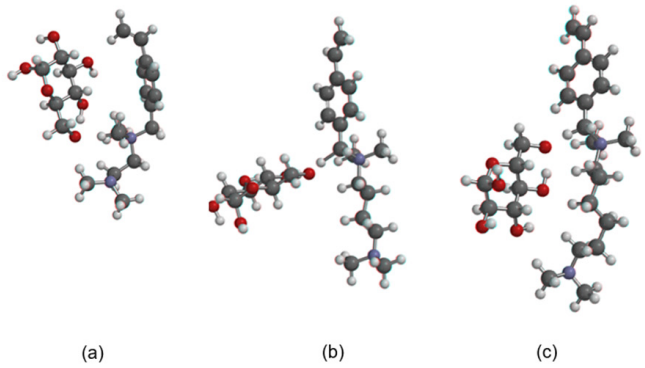

Figure 7a-c: The most stable configuration of model complexes between the monovalent negative carbohydrate ion and positive N^+ group in the porous shell portion for (a) Me:2, (b) Me:4, and (c) Me:6 (calculated using HF 3.21G Spartan' 20).

Discussion

The quantitative analysis of carbohydrates is critical for food chemistry and has broad applications across many scientific disciplines. In this study, we assessed the efficacy of a core–shell ion-exchange resin, St-80(40% Me:2, 4, and 6), which incorporates varying numbers of methylene groups (two, four, and six) in porous shell portion with constant cross-linking degree of 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, it is noted that a sample solution containing carbohydrates can be analyzed without any special pretreatment by using an electrochemical detector. St-80(40% Me:2, 4, and 6) displayed high resolution (≥1.5) at flow rates of 0.3–0.7 mL/min with 0.10 mol/L NaOH eluent, demonstrating good carbohydrate separation performance. While St-80(40% Me:4 and 6) also displayed high resolution (≥1.5) at flow rates of 0.3–0.7 mL/min with 0.15 mol/L NaOH eluent. Clear chromatograms were also obtained for glucose, fructose, and sucrose, regardless of the number of methylene groups, demonstrating effective carbohydrate separation performance. When increasing the number of methylene groups in the functional chain, the retention times of glucose, fructose, and sucrose for St-80 (40% Me:2, 4, and 6) with 0.10

Table 5: Retention time and theoretical plate number N of glucose, electrostatic charges on N^+ and O^-, ion-exchange capacity of St-80(Me:2), St-80(Me:4), and St-80(Me:6) (crosslinking 40%, 0.10 mol/L NaOH eluent at flow rate: 0.5 mL/min).

Ion exchange	Retention		Theoretical plate		Electrostatic	Electrostatic	Ion-exchange	
resins	time(min)		number		charge N^+	charge O^-	capacity(mEq/mL)	
Cross-linking	40%	55%	40%	55%	(a) in Fig 3	(b) in Fig 3	40%	55%
St-80(Me:2)	11.0	12.4	4180	5710	0.720	0.651	0.379	0.172
$\|ee'/d^2\|$					0.469			
St-80(Me:4)	15.6	13.1	5580	4820	0.637	0.648	0.458	0.346
$\|ee'/d^2\|$					0.413			
St-80(Me:6)	15.0	12.9	5730	4660	0.623	0.788	1.2	0.399
$\|ee'/d^2\|$					0.491			

*$|ee'/d^2|$ = electrostatic interaction magnitude between N^+ (resin) and O^- (carbohydrate); e, e′ = partial charges; d = distance (Å)

mol/L NaOH eluent at flow rate of 0.3 mL/min also increased. These carbohydrates for St-80 (40% Me:4) with 0.15 mol/L NaOH eluent at all flow rates showed the largest retention times.

St-80(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 increased. Thus, the elution sequence of carbohydrates (glucose followed by fructose) is consistent with the pK_a sequence [23]. The separation status is affected by the comprehensive influence of the following factors: First, in HPLC, the retention time is significantly influenced by solute diffusion along the column axis. When the porous layer is thin, the solute moves a shorter distance within the shell. As the resins evaluated in this study were 5 μm in size, effective separation is anticipated. Second, optimizing NaOH concentration plays a key role in the effective separation of carbohydrates. Third, St-80(40% Me:4 and 6) with 0.10 mol/L NaOH eluents at all flow rates showed longer retention times for carbohydrates than those of St-80(55% Me:4 and 6).

St-80(40% Me:4) with 0.15 mol/L NaOH eluents at all flow rates showed longer retention times for carbohydrates than those of St-80(55% Me:4). These findings clearly indicate that the degree of crosslinking has a substantial impact on retention time. As the number of methylene groups increased from two to six, the ion-exchange capacity increased. Glucose for St-80(40% Me:4 and 6) showed the longest retention times than that of St-80(40% Me:2). This suggests that ion exchange capacity is one of the factors affecting the retention time of glucose. Furthermore, other factors should be considered to explain the differences in carbohydrate retention times during the analysis of carbohydrates. From the discussion of factors influencing retention time, it can be concluded that optimal separation conditions are expected to involve ion-exchange resins that provide the shortest retention time for sucrose and high resolution.

The resins with a short sucrose retention time of 20 min or less and resolution of ≥1.5 under 0.10 ml/L NaOH eluent were as follows: St-80(40% Me:4) at flow rate of 0.3 mL/min and St-80(40% Me:6) at flow rates of 0.3, 0.5, and 0.7 mL/min. The resins with a short sucrose retention time of 20 min or less and resolution of ≥1.5 under conditions of 0.15 mol/L were as follows: St-80(40% Me:4 and 6) at all flow rates. The performance of the resins could not be fully explained solely by the five factors evaluated in this study (concentration of NaOH eluent, thickness of the shell portion, cross-linking, electrostatic charge on the N^+ and O^- atom, and ion-exchange capacity).

One possible explanation is as follows: In HPLC, the retention time of a compound depends on the strength of its interaction with the ion-exchange resin and the carbohydrate. The stronger the electrostatic attraction between the positively charged sites (N^+) in the resin and the negatively charged sites (O^-) in the carbohydrate, the slower the elution of the carbohydrate. In other words, a larger value of $|ee'/d^2|$ is considered to correspond to a longer retention time. Furthermore, the stable three-dimensional structure of the complex formed between N^+ (Figure 3a) and O^- in the model compound (Figure 3b) was calculated using Spartan. As a result, the stereochemical configuration of the most stable complex was elucidated. These results may provide a new perspective for elucidating the relationship between different stereochemical configurations and retention time. From these results, this could provide a computational chemistry explanation for the differences in retention times observed experimentally.

Conclusions

Analyses of the retention time resolution and theoretical plate number under various numbers of methyl groups suggested that St-80(40% Me 2, 4, and 6) core–shell ion-exchange resins are highly efficient for carbohydrate analyses with respect to the retention time and resolution between glucose and fructose. In order to achieve optimal separation, we are capable of selecting the most appropriate packing material and establishing the best separation conditions. Specifically, St-80(25% Me:2, 4, and 6), the resins with a short sucrose retention time of 20 min or less and resolution of ≥1.5 under 0.10 ml/L NaOH eluent were as follows: St-80(40% Me:4) at flow rate of 0.3 mL/min and St-80(40% Me:6) at flow rates of 0.3, 0.5, and 0.7 mL/min. The resins with a short sucrose retention time of 20 min or less and resolution of ≥1.5 under conditions of 0.15 mol/L were as follows: St-80(40% Me:4 and 6) at all flow rates.

In order to account for the retention time, the interaction between N^+ in the resin and O^- in carbohydrate was considered as a novel explanatory variable. This could provide a computational chemistry explanation for the differences in retention times observed experimentally.

Their suitability under strongly alkaline condition allows their effective use in electrochemical detection without any special pretreatment. As a particularly noteworthy viewpoint, these resins also possess outstanding durability, owing to their polymeric cores and shells.

References

1. Kirkland JJ, Truszkowski FA, Dilks CH Jr, et al. Superficially porous silica microspheres for fast high-performance liquid chromatography of macromolecules. J Chromatogr A. 2000; 890: 3-13.
2. Kirkland JJ, DeStefano JJ, Langlois TJ. Fuse 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. Aljhni 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 WH, Stöver HDH. Monodisperse cross-linked core-shell polymer microspheres by precipitation polymerization. Macromolecules. 2000; 33: 354-4360.
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.
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.
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.
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, Inoue Y, 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 J Pharm Sci. 2023; 47: e4.
36. Mitomo S, Kodama N, Inoue Y. Elution behavior of carbohydrates using core-shell ion-exchange resin St-70 with different number of methylene groups in the porous shell and a constant cross-linking of 55%. Chem Pharm Res. 2025; 7: 1-7.
37. Mitomo S, Kodama N, Inoue Y. 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. Int J Pharm Chem. 2025; 11: 1-10.
38. 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 40%. Chem Pharm Res. 2025; 7: 1-7.
39. Mitomo S, Kodama N, Inoue Y. Elution behavior of carbohydrates using core-shell ion-exchange resin St-70 with different number of methylene groups in the porous shell and a constant cross-linking of 40%. Int J Pharm Chem. 2025; 11: 76-86.
40. https://www.pmda.go.jp/files/000217651.pdf