Development of Core-Shell Ion-Exchange Resin by Changing the Core-Shell Ratio and Its Elution Behavior with Carbohydrates

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Abstract
A novel core-shell ion-exchange resin composed of an ion-exchanging porous shell layer formed on a hard polymer core was prepared for application to HPLC. The effect of various core-shell ratios on the retention time and theoretical plate number \( (N) \) in the separation of carbohydrates was examined. A mixed aqueous sample of inositol, glucose, fructose, and sucrose was reasonably separated under alkaline conditions (100 and 150 mmol/L NaOH) at flow rates of 0.4-1.0 mL/min. The retention time was linearly related to the thickness of the porous layer. The values of theoretical plate number \( (N) \) of glucose, fructose, and sucrose depend on the shell thickness at a flow rate of 0.5 mL/min when using the 100 and 150 mmol/L NaOH eluent.

Keywords: HPLC; Core-shell ion-exchange resin; Carbohydrates; Retention time; Theoretical plate number

1. Introduction
The most commonly used resins in liquid chromatography are inorganic materials such as silica gel, alumina and apatite. Various core-shell silica resins, including those chemically modified on the surface with octadecylsilyl (ODS) groups, have also been developed [1-5]. However, silica-based resins are not compatible with high pH conditions as their adsorption ability decreases and the resin can become soluble in alkaline solutions, limiting the pH range of the mobile phase.

Styrene-divinylbenzene- and acrylamide-type polymers are frequently used as base materials for organic resins [6-8]. The physical properties of these polymer resins can be controlled by changing the monomer structure, crosslinking reagent, pore-controlling reagent and polymerization conditions, allowing the resin to be optimized for various analytes. Furthermore, control of the particle size and/or surface properties allows one to modify the resins for high speed and high-resolution miniaturization. However, most polymer resins developed to date are of the fully porous type, which has certain limitations for high-speed operation. The correspondence to improvement in the speed of analysis time is an essential viewpoint.

In order to solve these problems, the core-shell ion-exchange resin which is also made by precipitation polymerization around the core portion [9,10] and the latex type resin which is made with styrene as the base [6,11-13] are already put on market.

In food chemistry and biological processes, since the carbohydrates are one of major species, the development of new core-shell ion-exchange resins for high speed operation and high resolution are required. Furthermore, these resins that can be used under alkaline conditions will be useful because they are applicable to sensitive and selective analysis of carbohydrates by the electrochemical detection (ECD). As carbohydrates are electrochemically responsive but do not show UV adsorption, the ECD method has the advantage in selective detection of carbohydrates.

Therefore, we have prepared the core-shell ion-exchange resin (St-80) by controlling the weight ratio of the monomer for the core and the shell (20:80) and subsequent suspension polymerization as the other method [14-17]. The fully porous resin was also prepared by same method (monomer ratio = 100) for comparison with St-80.

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Received: 16 May 2021
Accepted: 11 September 2021
J-STAGE Advance Published: 7 October 2021
DOI: 10.15583/jpchrom.2021.013
The HPLC analysis of carbohydrates for St-80 provided shorter retention time with similarly good resolution compared to the fully porous resin [17]. The porous shell layer and fully porous resin have the same degree of cross-linking (55%). Since the retention time of carbohydrates for the core-shell ion-exchange resins is short, these resins are valid tool for analyzing carbohydrates contained in sweet potatoes, fruits and many foods. There are two factors to be considered in the development of such resins, namely the thickness and the degree of cross-linking in the porous layer.

In this study, we synthesized core-shell ion-exchange resins with various core-shell ratio, and investigated their separation characteristics. Moreover, these resins are also evaluated from the theoretical plate number (N) of glucose, fructose, and sucrose. The thickness of the shell was adjusted by controlling the weight ratio of the starting monomer for the core and shell (50:50 (St-50), 40:60 (St-60), 30:70 (St-70)). These core-shell ion-exchange resins have the same degree of cross-linking (55%) in the porous layer.

It is expected that the resins having thinner porous shell layer would show the shorter retention time of carbohydrates compared with St-80 while maintaining high resolution. In addition, these resins having polymer-based hard core and shell portion exhibit high alkali durability and mechanical strength.

2. Experimental

2.1. Reagents

Inositol, sucrose (FUJIFILM Wako Chem. Co. Tokyo, Japan), fructose, glucose (Kanto Chem. Co. Tokyo, Japan) and sodium hydroxide (FUJIFILM Wako Chem. Co.) were obtained from commercial sources. Ultrapure water (ELGA LabWater, High Wycombe, UK) was used for the preparation of the eluent and sample solutions. The sample solutions were prepared by sequentially mixing and diluting stock solutions (500 or 1000 mg/L). The concentration of each carbohydrate in the sample solution ranged from 12.5 to 50 mg/L.

2.2. Conditions for HPLC analysis

HPLC analysis was performed using a TOA-DKK SU300 instrument (DKK-TOA Co. Tokyo, Japan) equipped with a pulsed amperometry detector (with a gold electrode). The resin used in this study consisted of a hard polymer core and a shell with functional groups in its porous layer. The porous layer shell was synthesized via the reaction of a chloromethylstyrene-divinylbenzene copolymer carrier with a tertiary amine [14,18-20]. The thickness of the shell was adjusted by varying the weight ratio of the starting monomer for the core and shell (50:50, 40:60 and 30:70), while keeping the total mass of the monomers constant. After the reaction, 3 g of particles (about 5 μm in size) were mixed with 10 mL of eluent (100 mmol/L NaOH). The obtained homogeneous slurry was poured into a packer that connected to a 4.6 × 150 mm stainless steel column, which was filled with 100 mmol/L NaOH eluent under 120 kg/cm².

The sample solution (20 μL) was injected using an AS8020 autosampler (Tosoh Co. Tokyo, Japan) and eluted with either 100 mmol/L (pH 13.0) or 150 mmol/L NaOH (pH 13.2) eluent at room temperature. The flow rate ranged from 0.5 to 1.0 mL/min. The theoretical plate numbers (N) of each carbohydrate in the sample were determined using the built-in data processing program.

3. Results and discussion

3.1. Evaluation of separation properties of St-50, St-60 and St-70 ion-exchange resins

The sugar content of fruits is usually evaluated by their total carbohydrate content measured with a saccharimeter. Moreover, this is necessary to quantify each component of carbohydrates for the detailed analysis and understanding in food chemistry. Because carbohydrates are the possible main analytes of the present core-shell ion-exchange resins, a solution containing the major carbohydrates in fruits and foods, namely, inositol, glucose, fructose and sucrose, was used as a standard solution to evaluate the HPLC performance of the prepared resins. Under all elution conditions, the retention times of these carbohydrates followed the order of inositol, glucose, fructose and sucrose.

Chromatograms of these carbohydrates for St-50 at flow rates of 0.5 and 0.7 mL/min eluted with the 100 mmol/L NaOH eluent are shown in the Figs. 1a-b, respectively. The retention time of sucrose, which was longest among those of the four carbohydrates, was approximately 10 min at a flow rate of 0.5 mL/min (Fig. 1a) and approximately 7 min at 0.7 mL/min (Fig. 1b). The chromatograms of these carbohydrates for St-70 at flow rates of 0.5 and 0.7 mL/min showed retention times of 15 and 12 min, respectively, for sucrose (Figs. 2a-b). At 0.5 mL/min, the retention time of sucrose using St-50 was approximately 5 min shorter than that of sucrose using St-70. This result was likely caused by the smaller shell thickness of St-50, which allows the eluent to pass through the shell more quickly.

The core-shell ratio and associated retention times of sucrose are plotted in Figs. 3a-b. When using the 100 mmol/L NaOH eluent, the retention time of sucrose for St-50 was 12-16 min shorter than that of the fully porous resin (Fig. 3a), while the carbohydrates remained well separated (Fig. 1a). At a higher eluent concentration (150 mmol/L NaOH), the retention times of sucrose for core-shell ion-exchange resins were similarly shorter than those of the fully porous resin (Fig. 3b).

The following aspects could be reasons for understanding the remarkable separation properties of the core-shell ion-exchange resin. First, the core suppresses solute diffusion, mainly in the direction column axis. Second, the moving
Fig. 1. Chromatogram obtained for the separation of inositol, glucose, fructose and sucrose using the St-50 resin with 100 mmol/L NaOH eluent at 0.5 mL/min (a) and 0.7 mL/min (b).

Fig. 2. Chromatogram obtained for the separation of inositol, glucose, fructose and sucrose using the St-70 resin and 100 mmol/L NaOH eluent at 0.5 mL/min (a) and 0.7 mL/min (b).

Fig. 3. Relationship between retention time of sucrose and shell layer thickness at 0.5 mL/min (open diamond) and 0.7 mL/min (filled squares). The eluents used were (a) 100 mmol/L and (b) 150 mmol/L NaOH.

Fig. 4. Chromatogram obtained for the separation of inositol, glucose, fructose and sucrose using the St-70 resin (used more than 1200 h) with 100 mmol/L NaOH eluent at 0.5 mL/min.
distance of the solute within the shell is shortened because the porous layer is thin. As a result, the core-shell ion-exchange resin exhibited shorter retention times as compared to the fully porous resin. In addition, the retention time was linearly related to the thickness of the porous layer. Furthermore, even after operating for more than 1200 h, St-70 provided the good chromatographic separation (Fig. 4).

3.2. Evaluation of theoretical plate number (N) of carbohydrates for St-50, St-60 and St-70 ion-exchange resins

When using the 100 and 150 mmol/L NaOH eluent, the effect of changing the thickness of porous shell layer was evaluated from the result of the values of N of glucose, fructose and sucrose at each flow rate.

Figures 5a-c show the values of N with the 100 mmol/L NaOH eluent. At a flow rate of 0.5 mL/min (Fig. 5a), glucose and fructose showed large values of N (3.6-4.4 × 10^3) for all resins. A larger value of N of each carbohydrate was observed as the shell thickness increased. No obvious relationship between the value of N and the shell thickness was observed at the flow rates of 0.7 and 1.0 mL/min as shown in Figs. 5b-c. On the other hand, the values of N of glucose and fructose decreased with increasing flow rate: 2.2-3.5 × 10^3 at 0.7 mL/min (Fig. 5b) and 1.8-2.5 × 10^3 at 1.0 mL/min (Fig. 5c). The values of N of glucose and fructose for each resin are larger than sucrose at all flow rates. It should be noted that St-60 exhibited a larger value of N of fructose compared to glucose and sucrose at flow rates of 0.7 and 1.0 mL/min.

With the 150 mmol/L NaOH eluent, the value of N of each carbohydrate increased with increasing shell layer thickness at a flow rate of 0.5 mL/min (Fig. 6a). The values of N of each carbohydrate showed the same tendency at flow rates of 0.7 and 1.0 mL/min (Figs. 6b-c). The values of N of glucose and fructose were larger than that of sucrose at all flow rates. The values of N of glucose and fructose in 100 mmol/L NaOH eluent was larger than those in 150 mmol/L NaOH eluent at flow rate of 0.5 mL/min.

The above results indicate that the values of N of these carbohydrates are strongly dependent on the shell thickness in 100 mmol/L and 150 mmol/L NaOH eluent at a flow rate of 0.5 mL/min. An obvious relationship between the values of N of glucose and fructose and the concentration of the NaOH eluent at a flow rate of 0.5 mL/min was observed when comparing with Fig. 5a and Fig. 6a.

4. Conclusion

Herein, we report that a core-shell ion-exchange resin exhibits a shorter retention time than that of a conventional fully porous resin. The novel core-shell ion-exchange resin composed of a hard polymer core and ion-exchanging porous shell developed herein efficiently separated a mixed aqueous solution of inositol, glucose, fructose and sucrose. Generally,
lower flow rates resulted in larger theoretical plate numbers. It should be noted that these resins shorten the retention time of carbohydrates compared with a fully porous ion-exchange resin; this could be explained by the structural effect of the core-shell ion-exchange resins, where the eluent passes only through the thin shell. The values of $N$ of glucose, fructose and sucrose for these resins are strongly dependent on the shell thickness in 100 mmol/L and 150 mmol/L NaOH eluent at a flow rate of 0.5 mL/min. It should be emphasized that novel core-shell ion-exchange resins exhibited excellent durability under highly alkaline conditions (pH >13.0) and was suitable for long-term operation. Further control of the thickness, functional group of the shell and degree of crosslinking may provide an excellent core-shell ion-exchange resin with a porous shell optimized for a variety of analytes.

Acknowledgement
The authors would like to thank Prof. Nobuharu Takai for valuable discussions.

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