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Chromatographic Separation of Maltopentaose from Maltooligosaccharides

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Abstract An experimental study on the chromatographic separation of maltopentaose from a mixture, including glucose, maltose, maltotriose, and maltopentaose, was carried out in a nonionic polymeric sorbent column while varying the operating conditions, such as the solution pH, buffer contents, and isopropyl alcohol (IPA) concentration. Unlike the pH and buffer contents, the IPA concentration had a significant impact on the single component chromatograms for maltopentaose. The retention times of the maltooligosaccharides with the nonionic polymeric sorbent SP207 were in the following order: glucose < maltose < maltotriose < maltopentaose. From the experimental binary, ternary, and quaternary chromatograms, gradient chromatographic separation with a changing IPA concentration as a function of time was required to obtain high-purity maltopentaose and reduce the elution time.

Keywords: chromatographic separation, gradient elution, maltooligosaccharides, maltopentaose, polymeric sorbent

INTRODUCTION

Recently, many biologically active oligosaccharides have been developed from raw materials, such as starch, sucrose, lactose, xylan, agar, chitin, and chitosan [1-3]. Among them, maltooligosaccharides, which have an α -1,4 glucosidic linkage with 2 to 10 glucopyranosyl units, have been used in a number of food industries as a low sweetener, anti-hygroscopic agent, truncating agent, or humectant. In particular, maltopentaose, which has five glucopyranoses, is in high demand as a high-value-added material in the medical field, as pure maltopentaose can be used as a diagnostic reagent to examine the activity of α -amylase in serum [4].

As such, the development of a separation and purification process for obtaining pure maltopentaose from a reaction mixture, including raw materials in the enzymatic hydrolysis of starch, is crucial for practical application. Although various separation techniques, such as gel filtration, adsorption by activated carbon, ion-exchange, and solvent precipitation have been applied, the chromatographic separation of maltooligosaccharides using a fine particle packing and ion exchange resin is most commonly used. However, since separation techniques using packing materials have a high operating cost and low separation efficiency [5], a more effective and convenient process is needed for obtaining a pure component from a mixture.

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Various reports have already been published on the separation of monosaccharides and disaccharides, yet only a few studies have focused on the separation of maltooligosaccharides, including maltotriose, maltotetraose, and maltopentaose [6-9]. Abe *et al.* presented an adsorption isotherm for several saccharides and polyhydric alcohols from an aqueous solution onto activated carbon in terms of the physical properties of the adsorbates, such as the molecular refraction or parachor. They also discussed the adsorption mechanism of activated carbon from an aqueous solution [6,10]. Moon and Cho reported that activated carbon could be employed as an alternative adsorbent to enhance the selectivity of maltopentaose from an aqueous reaction mixture coexisting with other maltooligosaccharides, such as glucose, maltose, maltotriose, and maltotetraose [11]. They also investigated the enzymatic hydrolysis conditions of starch by commercial α -amylase for the selective production of maltopentaose over other oligosaccharides. Three different commercial activated carbons were utilized to enhance the purity of maltopentaose after the enzymatic hydrolysis. When increasing the adsorption cycle with the selected activated carbon, the selectivity of maltopentaose was enriched in the mixture solution because the adsorption capacities of glucose and maltose by the activated carbon were so high compared with maltotriose, maltotetraose, and maltopentaose. Therefore, an effective separation process to obtain high-purity maltopentaose directly from a reaction mixture could be developed by combining the two processes of adsorption and chromatography, whereby the glucose and maltose are initially removed from a

mixture by activated carbon adsorption, then the remaining maltooligosaccharides, including maltotriose, maltotetraose and maltopentaose, are effectively separated by chromatography.

Several investigators have already studied the use of nonionic polymer sorbents to purify process streams and recover valuable compounds from aqueous solutions [12-16]. The key advantages to using polymeric sorbents for such applications are the ease with which they can be regenerated and their selectivity. Unlike activated carbon, nonionic polymeric sorbents typically have a uniform surface chemistry and can be synthesized with a controlled pore structure. Such uniformity reduces the heterogeneity of adsorption and often facilitates regeneration under milder conditions.

Accordingly, the main purpose of the current study was to develop an economic separation and purification process for obtaining high-purity maltopentaose from a reaction mixture containing many maltooligosaccharides. In particular, the current study focused on how to separate and purify maltopentaose effectively from a mixture when using the nonionic polymeric sorbent SP207 as an alternative packing material for the ion-exchanger. As such, the influence of the solution pH, buffer contents, and IPA concentration on single component chromatograms of maltooligosaccharides was examined. Binary, ternary, and quaternary chromatograms were also obtained under the optimal mobile phase determined from the single component chromatography. Plus, isocratic and gradient elution modes were both applied for the binary elution of maltotriose and maltopentaose.

MATERIALS AND METHODS

The adsorbent used in the current study was the nonfunctional macroreticular polymeric sorbent, SP207, manufactured by Mitsubishi Chemical Ind. Co, Japan. The surface area and pore size distribution of the resin were measured using an N_2 adsorption apparatus (Micrometotocs, model ASAP-2400, Australia). The measured properties of the sorbent and those obtained from the manufacturer's specifications are listed in Table 1 and shown in Fig. 1. The arithmetic average particle diameter was 412 μm, which was determined by sorting wet resin particles with the aid of an optical microscope. Prior to the experiments, the sorbent was leached with ethanol for 24 h to wet the internal pores. Thereafter, the sorbent particles were loaded in a 0.016 m ID glass column, then a ten-bed volume of NaOH (0.1 N) and HCl (0.1 N) was passed through the column at a flow rate of 1.0 mL/min to rinse out any impurities, followed by a twenty-bed volume of distilled and deionized water at the same flow rate to remove the HCl.

The glucose and maltose were supplied by Junsei Chemical Co. (Japan) and the other maltooligosaccharides (maltotriose, maltotetraose, and maltopentaose) by Sigma Chemical Co. (USA). The chemical properties of the adsorbates used in the current study are listed in Table 2. The adsorbates were all used without further

Table 1. Physical properties of nonionic polymeric sorbent, SP207

Properties	Value	Unit
Particle size	412	μm
Particle density	611	m^3/kg
Particle porosity	0.48	-
Moisture content	50	%
Surface area	672(627[a])	m^2/g
Average pore diameter	60.2(105.2[a])	Å

[a] From manufacturer's report

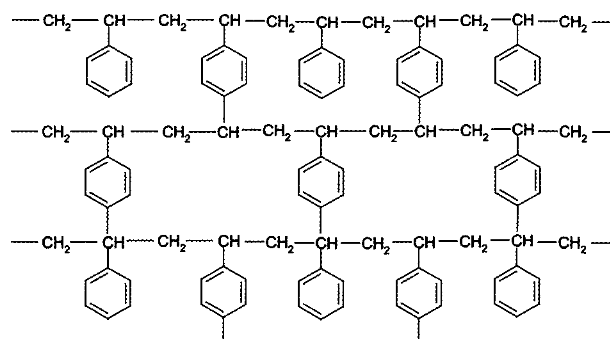

Fig. 1. Chemical structure of nonionic polymeric sorbent, SP207.

Table 2. Chemical properties of adsorbates

Adsorbate	Chemical formula	MW
D-(+)-Glucose (G1)	$C_6H_{12}O_6$	180.16
D-(+)-Maltose (G2)	$C_{12}H_{22}O_{11}$	342.31
Maltotriose (G3)	$C_{18}H_{32}O_{16}$	504.40
Maltopentaose (G4)	$C_{30}H_{52}O_{26}$	828.70

purification. Stock solutions of each species were made by dissolving the reagent-grade adsorbates in distilled and deionized water. The solution pH was adjusted using HCl and NaOH. Binary, ternary, and quaternary solutions were prepared by adding the stock solutions. The buffer was 2 M-Na_2HPO_4/0.2 M-NaH_2PO_4, pH 8.0. All other chemicals used in the experiments were of analytical reagent grade.

The single and multicomponent chromatograms were measured in a glass column, 0.016 m in diameter and 0.3 m in length. The column was lined with a water jacket to maintain a uniform column temperature of 20°C. A precision FMI pump (Rhocke, USA) was used to regulate the flow. The solutions were introduced to the column in a downward direction. To prevent channeling and enhance the distribution of the solution throughout the column, small glass beads were packed in the top and bottom regions of the column. The experimental conditions are all listed in Table 3.

The effluent samples were analyzed using a Shimadzu

Table 3. Experimental conditions for chromatography

Variables	Range	Unit
Column dimension	1.6x30	cm
Packing porosity	0.45	-
Packing density	360	kg/m^3
Flow rate	1.0~2.0	mL/min
Maltooligosaccharides conc.	0.5, 1.0	moL/L
Isopropyl alcohol (IPA) conc.	0.25, 0.5, 1.0, 2.0	voL %
pH	2, 7, 11	-
Buffer	10, 40	%

model LC-6A HPLC(Japan) system equipped with a refractive index detector, RID-10A(Shimadzu, Japan), CLC-NH_2 column (Shim-pak, Japan) and data acquisition device, NI-DAQ converter (National Ins., USA). A mixture of deionized water and analytical grade isopropyl alcohol (IPA) was used for the mobile phase.

RESULTS AND DISCUSSION

In a previous report, the enzymatic hydrolysis conditions for starch were investigated when using commercial α-amylase for the selective production of maltopentaose over other oligosaccharides [11]. The determined conditions were a 29.6 KNU (kilo Novo α-amylase unit) enzyme loading in 150 mL of a 0.3% starch solution at a pH level of 5 at 40°C for 30 min. A maltopentaose selectivity of about 40% was attained under these optimum conditions. To further enhance the maltopentaose selectivity, three different commercial activated carbons (Norit-Netherlands, Union-Korea, Shamchully-Korea) were utilized after the enzymatic hydrolysis. Among them, the activated carbon from Samchully Co. exhibited the best results. When increasing the adsorption cycle with the selected activated carbon, the maltopentaose selectivity was enriched up to 75%.

The ultimate goal of the current study was to develop an economical separation and purification process for obtaining high-purity maltopentaose from a reaction mixture containing many maltooligosaccharides. When glucose and maltose are effectively removed from a mixture by activated carbon adsorption, as reported in previous studies, the remaining maltooligosaccharides, including maltotriose, maltotetraose, and maltotriose, can be more efficiently separated using a chromatographic technique. Thus, as a systematic approach for chromatographic separation, the single and multicomponent elution characteristics of maltooligosaccharides when using the nonionic polymeric sorbent SP207 were investigated with different pHs, buffers, and IPA concentrations to determine the optimal mobile-phase conditions for the separation of maltopentaose. The reason for selecting a nonionic polymeric sorbent was based on the fact that the separation in an ion-exchange column generally entails difficulties related to the elution of the adsorbate and regeneration of the adsorbent.

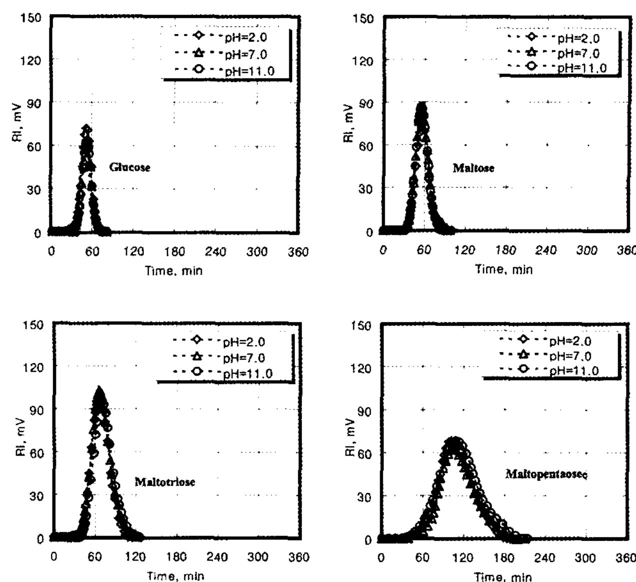

Fig. 2. Effect of pH on single component chromatogram.

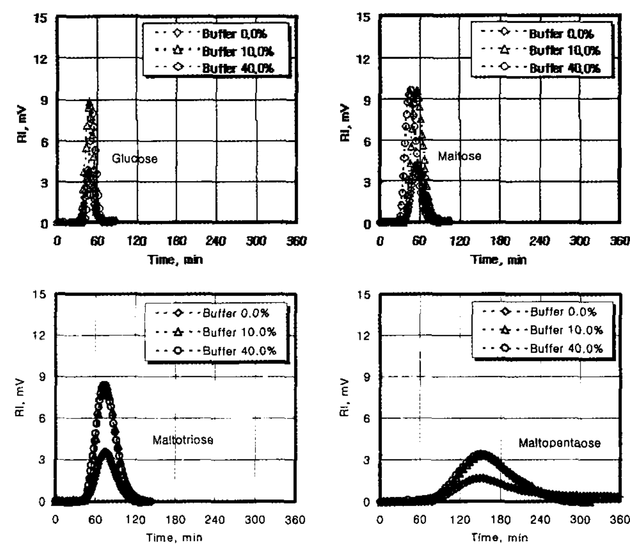

Fig. 3. Effect of buffer contents on single component chromatogram.

Effect of Solution pH, Buffer, and IPA Concentration on Chromatogram

Adsorption chromatographic separation is defined as separation according to the difference in the adsorption affinity between the adsorbate and the adsorbent [17-19]. However, such separation techniques often encounter difficulties as adsorbates generally coexist as a mixture and also have similar chemical and physical properties. Yet, when the affinity differences between the adsorbates vary according to the solution pH, buffer, and IPA concentration, more effective separation can be achieved. Thus, examining the influence of the pH,

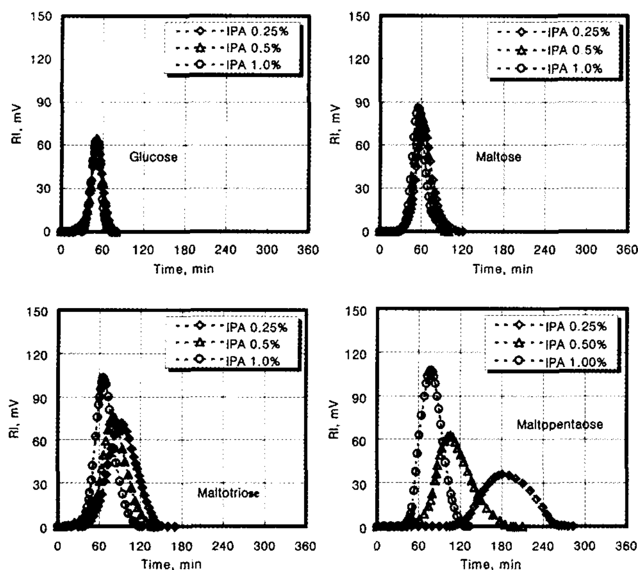

Fig. 4. Effect of isopropyl alcohol on single component chromatogram.

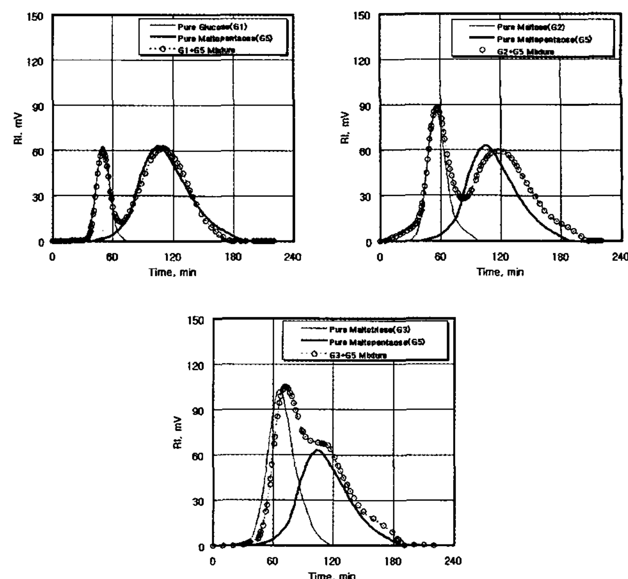

Fig. 5. Binary chromatogram of maltooligosaccharides with 1% isopropyl alcohol (glucose, maltose=0.1 mol/L, maltotriose=0.5 mol/L, maltopentaose=1.0 mol/L).

buffer, and IPA concentration is very important.

Single component chromatograms of 4 maltooligosaccharides, including glucose, maltose, maltotriose, and maltopentaose, were measured with different solution pHs (2.0, 7.0, 11.0), buffer contents (0, 10, 40%), and IPA concentrations (0.25, 0.50, 1.0, 2.0%). All experiments were carried out under the conditions listed in Table 3. The reason for selecting glucose and maltose was to compare the adsorption affinity on SP207. Meanwhile, maltotriose and maltohexaose were not included in the current study due to their relatively low concentrations in the reaction mixture after adsorption treatment with activated carbon in a previous report [11]. The single component chromatograms of the 4 maltooligosaccharides relative to the IPA concentration, buffer contents, and solution pH, are illustrated in Figs. 2-4. Unlike the pH and buffer contents, the IPA concentration had a significant influence on the single component chromatograms of maltotriose and maltopentaose, as shown in Figs. 2,3, implying that the pH and buffer were ineffective for controlling the mobile phase as regards the separation of maltooligosaccharides in nonionic polymer resin columns. The retention time in a chromatography column means the adsorption affinity between the adsorbent and the adsorbate under a given mobile phase. The order of the retention times measured in the SP207 column at 1% IPA without any buffer content or pH adjustment was as follows: glucose < maltose < maltotriose < maltopentaose. Maltopentaose could be not easily eluted at 0.25% IPA due to the high affinity for SP207 and took about 300 min under the given experimental conditions listed in Table 3. The chromatograms for glucose and maltose relative to the IPA concentration were almost similar, whereas those for maltotriose and, especially, maltopentaose were quite different with IPA concentrations of 0.25, 0.5, and 1.0%. When increasing the number of glucopyranoses, the retention time generally increased. Since the influence of the IPA concentration on the retention times for maltotriose and maltopentaose was extremely sensitive, effective separation was feasible based on just a minor adjustment in the IPA concentration rather than changing the pH and buffer contents. From an engineering point of view, this has significant implications as regards reducing the cost of separation, thus a further examination was conducted on the influence of the IPA concentration on multicomponent chromatograms.

Multicomponent Chromatograms

From the experimental results of the single component system, the appropriate mobile-phase conditions for the chromatographic separation of maltopentaose were determined to be 1% IPA with no pH and buffer adjustment. Figs. 5-7 show the multicomponent experimental chromatograms of various binary, ternary, and quaternary systems under the determined mobile-phase conditions. One key concern in the current study was how to obtain high-purity maltopentaose, that is, a good resoluion of maltopentaose. Therefore, to investigate the influence of glucose, maltose, and maltotriose on the resolution of maltopentaose, 3 binary systems focusing on maltopentaose were considered as follows: glucose/maltopentaose, maltose/maltopentaose, and maltotriose/maltopentaose. As expected, the influence of maltotriose on the resolution of maltopentaose was greater than that of both glucose and maltose, as shown in Fig. 5.

Figs. 6,7 show the experimental chromatograms of 2 ternary systems of glucose/maltose/maltopentaose and

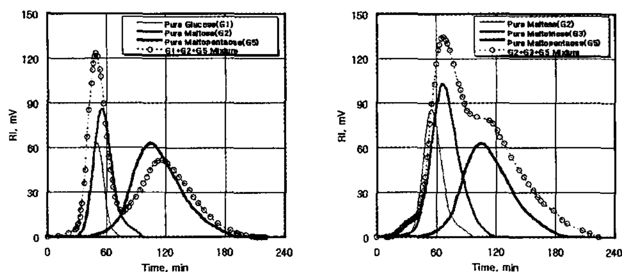

Fig. 6. Ternary chromatogram of maltooligosaccharides with 1% isopropyl alcohol (glucose, maltose=0.1 mol/L, maltotriose=0.5 mol/L, maltopentaose=1.0 mol/L).

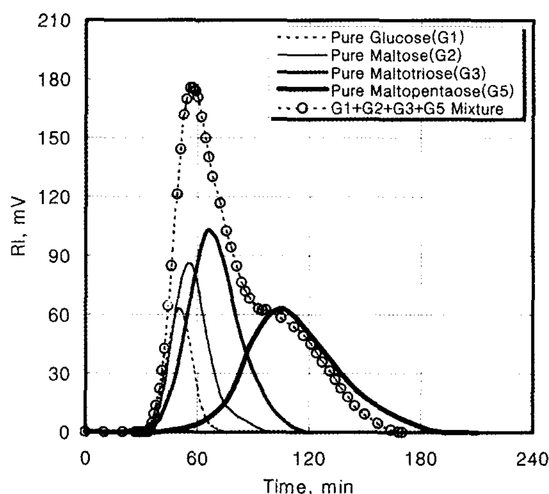

Fig. 7. Quaternary chromatogram of maltooligosaccharides with 1% isopropyl alcohol (glucose, maltose=0.1 mol/L, maltotriose=0.5 mol/L, maltopentaose=1.0 mol/L).

maltose/maltotriose/maltopentaose and a quaternary system of glucose/maltose/maltotriose/maltopentaose. The resolution of maltopentaose was not good in the isocratic-mode separation of a multicomponent system with maltotriose. In addition, the influence of the flow rate on the binary chromatogram of maltotriose and maltopentaose was also ineffective, as shown in Fig. 8. Since the composition of maltooligosaccharides in the enzymatic hydrolysis of starch can be adjusted according to the reaction conditions, the composition of maltotriose could possibly be reduced, even though the yield of maltopentaose is low.

Gradient Elution of Maltopentaose

Fig. 9 shows the influence of the IPA concentration on the binary chromatograms of maltotriose and maltopentaose under isocratic conditions (0, 1, 2% IPA). The binary chromatogram was overlapped at 1% IPA and highly overlapped at 2% IPA. As such, maltopentaose was not well separated in a binary system when using the isocratic-mode separation technique with IPA. In

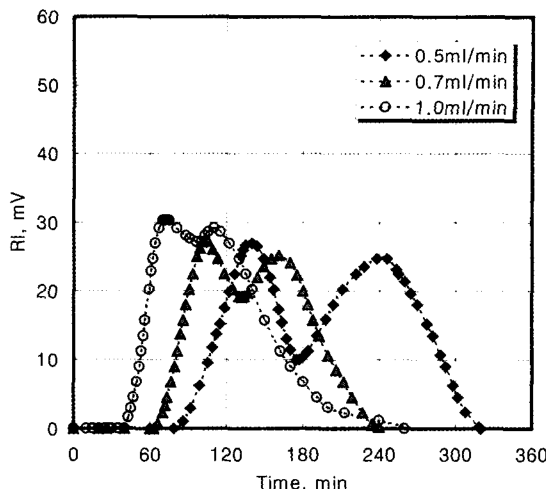

Fig. 8. Binary chromatogram of maltotriose and maltopentaose with 1% isopropyl alcohol relative to flow rate (maltotriose:maltopentaose=0.5:1.0 mol/L).

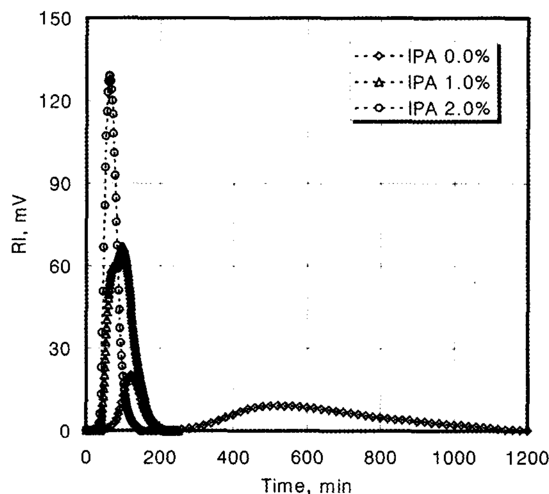

Fig. 9. Binary chromatogram of maltotriose and maltopentaose relative to isopropyl alcohol concentration (maltotriose:maltopentaose=0.5:1.0 mol/L).

contrast, a well-separated chromatogram was observed when pure water, i.e. 0% IPA, was used as the mobile phase, although a long separation time was required. Therefore, to reduce the separation time, maltotriose could be separated in a mobile phase of water (0% IPA), while maltopentaose separated in 2% IPA. Fig. 10 illustrates a comparison of the separation times for maltopentaose according to the use of IPA. The elution time was about 20 h when water was used as the mobile phase, whereas only 3 h was sufficient to elute maltopentaose when 2% IPA was used.

In a gradient elution, a modulator is often used in the mobile phase to adjust the eluent strength for better results in chromatographic separations. When compared with isocratic elution, the modulator concentration in

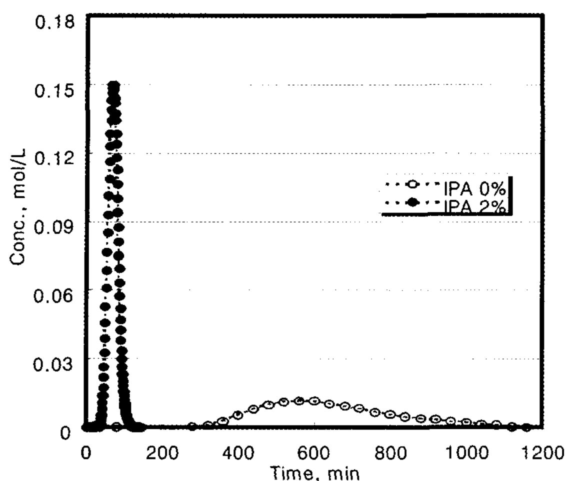

Fig. 10. Comparison of maltopentaose chromatograms with 0% and 2% isopropyl alcohol.

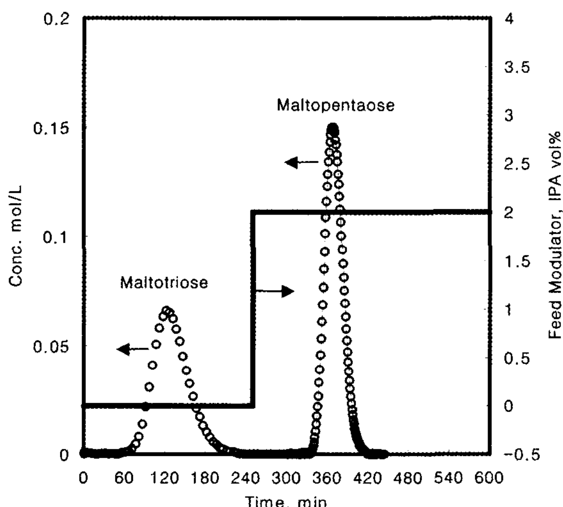

Fig. 11. Gradient elution of maltotriose and maltopentaose (maltotriose:maltopentaose=0.5:1.0 mol/L).

the mobile phase in a gradient elution increases or decreases continuously with time. Plus, a gradient elution is able to produce a high concentration in a shorter operation cycle compared with an isocratic elution. Fig. 11 shows the good resolution of maltotriose and maltopentaose with a stepwise gradient. The feed modulator in the mobile phase was only water for 250 min followed by 2% IPA. However, when the mobile phase was changed, the baseline became unstable. Therefore, 1min samples were taken periodically after 250 min and analyzed using a CLC-NH_2 column without data acquisition.

From the experimental results in the current study, the control of the mobile phase through the IPA concentration was found to be very important for the resolution of maltopentaose. Plus, the nonionic polymeric sorbent SP207 was found to be effective for the preparative separation of other oligosaccharides as an alternative adsorbent to ion-exchangers due to the fact that maltooligosaccharides could be easily eluted by either a low concentration of IPA or pure water. However, gradient-elution chromatographic separation was shown to be necessary to obtain high-purity maltopentaose from a mixture. Therefore, further experimental and theoretical studies on gradient elution according to various gradient modes, such as linear, stepwise linear, and nonlinear are required. In order to carry out a complete engineering study of gradient-elution chromatography, a mathematical model is needed that can realistically simulate the chromatographic process compared to conventionally employed models. Since a chromatogram is dependent on the adsorption equilibrium, mass transfer, and fluid dynamics, it is reasonable that these effects should all be considered together for a satisfactory simulation of a chromatogram. Based on previous studies [14,15,20,21], the modeling, simulation, and optimization of gradient chromatography processes are still continuing and will be reported on in the near future. Accordingly, based on the current results, the nonionic polymeric sorbent SP207 would appear to be effective for the separation of other oligosaccharides as an alternative adsorbent to ion-exchangers.

CONCLUSION

Single component chromatograms were measured under different solution pHs (2.0, 7.0, 11.0), buffer contents (0, 10, 40%), and IPA concentrations (0.25, 0.5, 1.0, 2.0%). Unlike the pH and buffer contents, the IPA concentration was found to have a significant effect on the single component chromatograms for maltotriose and maltopentaose. The order of the retention times in the SP207 column was as follows: glucose < maltose < malto-triose < maltopentaose. From the experimental results for binary, ternary, and quaternary chromatograms under the current experimental conditions, the resolution of maltopentaose was low in the presence of maltotriose when using the isocratic mode with IPA. However, a good resolution and short separation time were achieved when employing gradient elution-mode chromatography and high-purity maltopentaose was obtained from a pure binary mixture of maltotriose and maltopentaose. In order to simulate these experimental chromatograms, modeling and simulation results will be reported within the near future.

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