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ANALYSIS OF SUCROSE ESTERS OF LONG-CHAIN FATTY ACIDS ON SEPHADEX LH-20

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SUMMARY

The sucrose esters of long-chain fatty acids were separated into their components by gel chromatography on Sephadex LH-20. The conditions for gel chromatography were investigated and adjusted as follows: column, 2.0 × 230 cm; column temperature, 50°; developing solvent, DMF; flow rate, 60 ml/h; sample size, 50 mg. Mono-, di-, tri- and higher sucrose esters, sucrose and methyl esters of fatty acids were separated. Reproducibility was satisfactory. The time required for a single determination was about 12 h.

INTRODUCTION

Sucrose esters, nonionic surfactants, formed by reaction of sucrose with methyl esters of long-chain fatty acids are usually mixtures of sucrose, methyl esters of fatty acids, mono-, di-, tri- and higher sucrose esters. Many analytical methods[1–8] have been studied to examine the conditions necessary for production and quality control. However, most of these methods were thin-layer (TLC) and paper chromatography (PC), and they were, if anything, insufficient for accurate quantitative determination and for collection of the components, pure samples of which are needed for further studies of physico-chemical properties of the nonionic surfactant.

Gel chromatography in which separation is based on molecular size has been widely applied in biochemistry and in synthetic polymer chemistry. In recent publications[9,10], gel chromatography has frequently been used in studying oligomers and small molecules and is useful for analysis of non-volatile substances that cannot be determined by gas chromatography. Thus this technique appears to be promising for separation of sucrose, mono-, di-, tri- and higher sucrose esters from sucrose esters of long-chain fatty acids.

The present paper describes the separation and determination of sucrose esters of long-chain fatty acids by gel chromatography on Sephadex LH-20.

EXPERIMENTAL

Apparatus

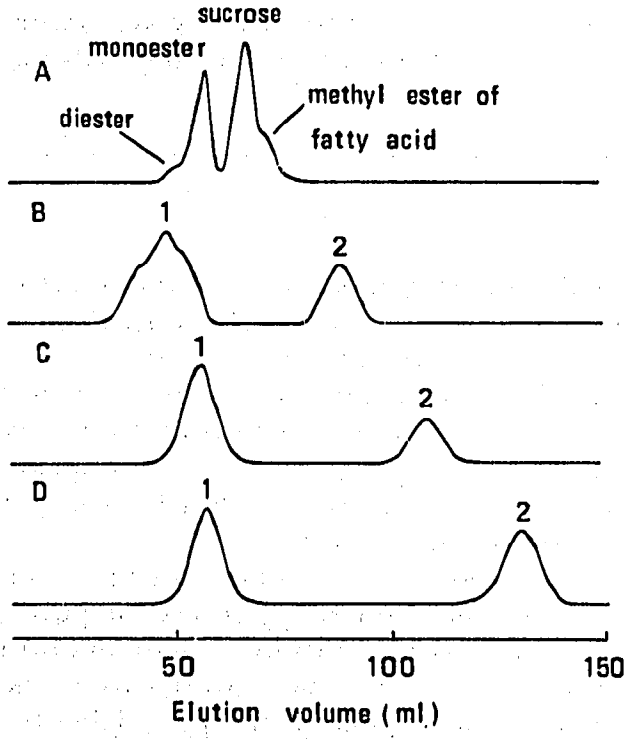
A glass column (2.0 × 230 cm) equipped with a jacket to control the column temperature was used. A differential refractometer (Waters Associates, Model R-4) was employed as a detector and the elution chromatogram was recorded automatically by a recorder. The flow rate was controlled with a pump (Japan Electron Optics Laboratory Co., Ltd., Model JLC-P2). A polyethylene tube (1.8 mm O.D.) was used as a connection between each apparatus.

Reagent and samples

For selecting a developing solvent, analytical grade dimethylformamide (DMF) and distilled water were used. Sephadex LH-20 gel (Pharmacia Fine Chemicals) was used as a column substrate. Commercially available sucrose esters of long-chain fatty acids were used as samples.

PROCEDURE

Sephadex LH-20 (175 g) was allowed to swell for 24 h in contact with DMF and



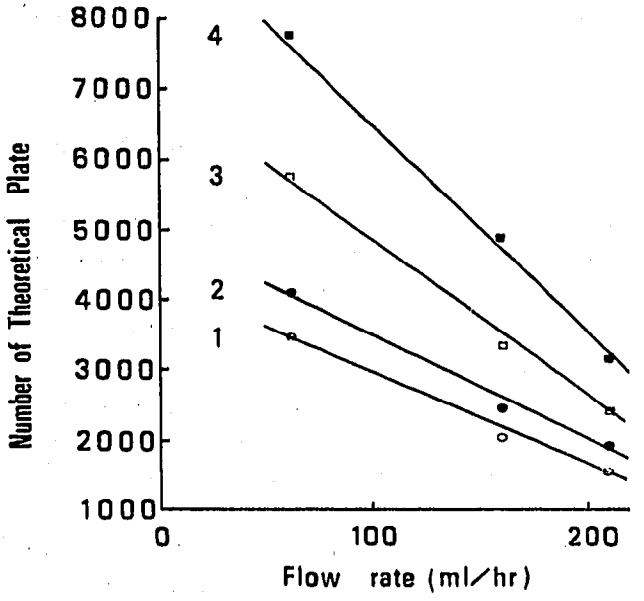


Fig. 1. Gel chromatograms obtained during preliminary tests for developing solvents. (A) Sephadex LH-20–DMF; (B) Sephadex LH-20–water; (C) Sephadex G-25–water; (D) Sephadex G-50–water. Chromatographic conditions: sample size, 50 mg; column size, 1.5 × 100 cm; column temperature, *ca.* 25°. 1, ester part; 2, sucrose.

Fig. 2. The relationship between flow rate and number of theoretical plates. (1) sucrose diester; (2) sucrose monoester; (3) sucrose; (4) methyl ester of fatty acid. Chromatographic conditions: sample size, 50 mg; column size, 2.0 × 230 cm; solvent, DMF; column temperature, 43°.

then was carefully poured into the glass column. Thereafter, the gel bed was allowed to settle for 24 h while DMF flowed through the column. The column temperature was maintained at 50° and the flow rate was controlled at 60 ml/h by a pump. 50 mg of a sample were diluted to 1 ml with DMF and carefully applied on top of the gel bed. The chromatogram was recorded automatically with a differential refractometer and a recorder. Each component fractionated was identified by its IR spectrum and the molecular weight was determined using a vapour-pressure osmometer. Peak resolution, R, was calculated as in a previous paper[10].

RESULTS AND DISCUSSION

The chromatographic conditions such as developing solvents, samples size, column temperature and flow rate were investigated. The R was calculated by using the monoester, diester and sucrose peaks.

Developing solvent

The developing solvents, about twelve, generally used in gel chromatography on Sephadex LH-20 cannot all be employed in analyses of sucrose esters of long-chain fatty acids because part of the sucrose esters is insoluble in methanol, ethanol, isopropanol, *n*-butanol, dioxane, acetone, ethyl acetate, tetrahydrofuran, chloroform and toluene. Dimethylformamide (DMF) and distilled water are good solvents for sucrose esters and can also be employed in gel chromatography on Sephadex LH-20 as developing solvents. The results are shown in Fig. 1. In this case, in order to shorten the length of the experiment, a glass column (1.5 × 100 cm) was used because sufficiently good results were obtained to choose the developing solvent.

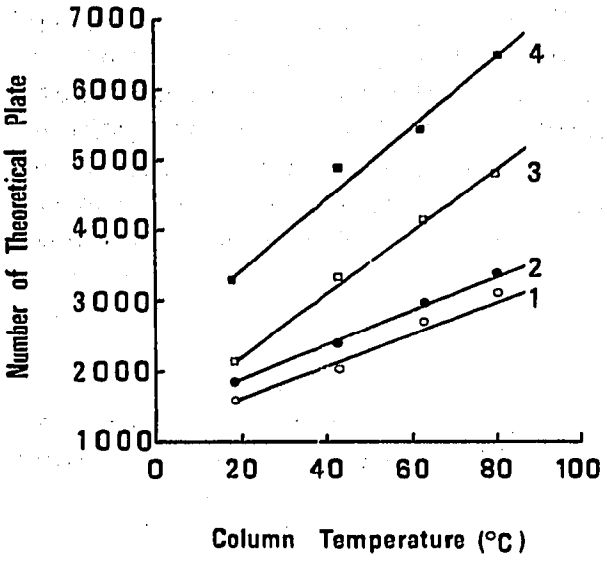
When DMF was employed, the separation of each component of sucrose esters was satisfactory, while the resolution of sucrose esters was inadequate using distilled water as developing solvent. However, using distilled water, the separation between sucrose esters and sucrose was very good, especially on Sephadex G-25 or G-50 instead of Sephadex LH-20. Therefore, in order to remove sucrose from sucrose esters, distilled water is suitable for a developing solvent. DMF, in which the sucrose esters readily dissolved and with which the peak resolution R was best, was selected as the developing solvent.

Flow rate

The effect of flow rate, from 40 to 210 ml/h, on the separation was investigated using DMF and a sample, and the number of theoretical plates was plotted as presented in Fig. 2. The lower was the flow rate, the better was the separation. From these results, the flow rate was fixed at 60 ml/h.

Column temperature

The effect of column temperature on the separation was investigated from 20 to 80° (Fig. 3). As shown, the higher was the temperature, the better was the separation within the experimental range. It is considered that diffusion of solute into the gel increases with rising temperature and consequently that the equilibrium rate of partition is improved at each theoretical plate. Thus the column temperature was fixed at 50°.



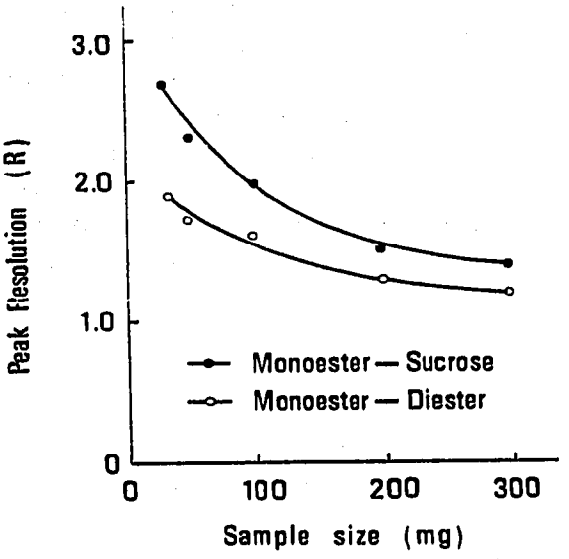


Fig. 3. The relationship between column temperature and number of theoretical plates. (1) sucrose diester; (2) sucrose monoester; (3) sucrose; (4) methyl ester of fatty acid. Chromatographic conditions: sample size, 50 mg; column size, 2.0 × 230 cm; solvent, DMF; flow rate, 156–174 ml/h.

Fig. 4. The relationship between sample size and peak resolution. Chromatographic conditions: column size, 2.0 × 230 cm; solvent, DMF; flow rate, 60 ml/h; column temperature, 50°.

Sample size

The relationship between peak resolution and sample size, from 30 to 300 mg/ml of DMF, was studied (Fig. 4). 30–100 mg of sample gave the best separation; however, because of the requirements of the recorder, a sample size of 50 mg was selected. For quantitative analysis, a 300-mg sample was selected for convenience.

TABLE I

ANALYTICAL RESULTS AND REPRODUCIBILITY

Chromatographic conditions: sample size, 50 mg; solvent, DMF; column size, 2.0 × 230 cm; flow rate, 60 ml/h; column temperature, 50°.

Sucrose ester	*Monoester*	*Diester*	*Triester*	*Tetra-ester*	*Penta-ester*	*Sucrose*	*Others*
1	70.7	18.6	2.4			8.3	
	68.4	19.2	2.7			9.6	
	67.7	18.9	2.8			10.5	
	69.4	18.3	3.0			9.3	
	69.0	17.9	2.2			10.8	
	68.1	19.0	2.6			10.3	
	70.0	17.5	1.9			10.6	
Average	69.0	18.5	2.5			9.9	
$\sqrt{V}$	1.1	0.62	0.38			0.89	
2	28.3	5.3	0.1			57.4	8.9
3	50.5	21.5	3.7			20.6	3.7
4	69.6	27.6	2.7				
5	13.6	14.2	6.6	2.4	1.6	61.7	

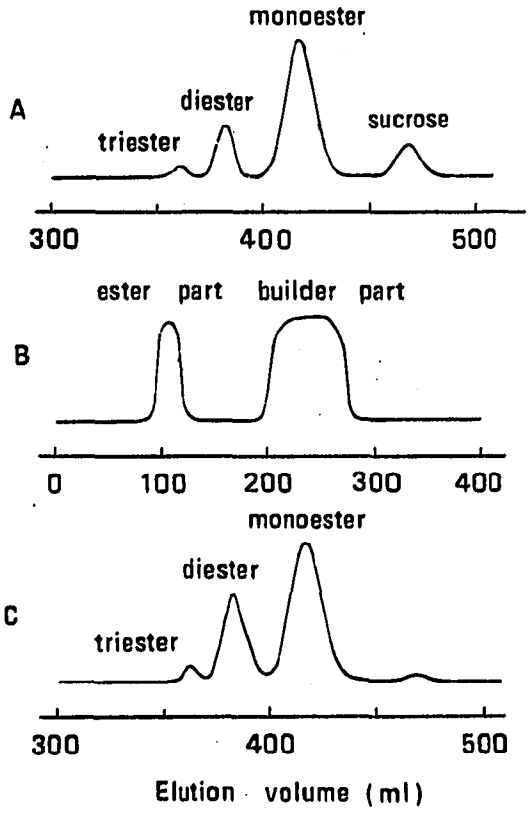


Fig. 5. Typical gel chromatogram. (A) sucrose esters; (B) a washing-up preparation; (C) sucrose ester part of a washing-up preparation. Chromatographic conditions: column size, 2.0 × 230 cm (A and C), 2.5 × 42 cm (B); solvent, DMF (A and C), water (B); Sephadex, LH-20 (A and C), G-25 (B); sample size, 50 mg (A, B, and C); flow rate, 60 ml/h (A and C), 235 ml/h (B); column temperature, 50° (A and C), 24° (B).

Gel chromatogram

A typical gel chromatogram obtained under the above-mentioned conditions is shown in Fig. 5A. It appears that the separation of mono-, di-, tri- and higher sucrose esters, sucrose, and methyl esters of fatty acids is satisfactory. Commercially available sucrose esters were analysed and the results are presented in Table I. The reproducibility which was satisfactory is also summarised in Table I.

Practical application of the method

The method described above was applied to the analysis of a washing-up preparation, in which sucrose esters of long-chain fatty acids are present as detergent. Because the washing-up preparation is a mixture of detergents and several kinds of builders, phosphate, phosphoric acid and low-molecular-weight organic sulphonates, etc., these materials must first be removed from the sucrose esters. Thus, gel chromatography using a Sephadex G-25–water system was carried out. The gel chromatogram obtained by this method is shown in Fig. 5B in which it appears that the separation between sucrose esters and the builders is satisfactory. However, in this case, sucrose in the sample was included in the builders. Part of the sucrose esters was collected quantitatively and the fraction was dried at 50°. Then sucrose esters thus obtained were chromatographed using Sephadex LH-20–DMF as described under EX-

PERIMENTAL. The gel chromatogram is shown in Fig. 5C which shows that each component of the sucrose esters is separated as well as when only sucrose esters are chromatographed, except that no sucrose peak appears in the chromatogram.

In analysing sucrose esters of long-chain fatty acids, in some respects, gel chromatography on Sephadex LH-20 is considerably more effective than TLC or PC. For example, in gel chromatography, conversion of sample to derivatives is not necessary, compounds are eluted more rapidly than by other chromatographic techniques, for a given flow rate, and the column has no tendency to prolong retention of samples and may be repeatedly used. Also, reproducibility is satisfactory when chromatographic conditions are constant, and components separate very conveniently. However, when the carbon number distribution of alkyl groups of a sample is large, this analytical method could not be applied to the sample because the resolution of each component of sucrose esters is unsatisfactory.

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