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A GEL CHROMATOGRAPHIC STUDY OF TRIMETHYLSILYL DERIVATIVES OF SEPHADEX WITH NON-POLAR SOLVENTS

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SUMMARY

Sephadex G gels were trimethylsilylated with trimethylchlorosilane–pyridine to extend their range of application, especially with regard to the polarity of the developing solvent.

Trimethylsilylated Sephadex G gels (TMS-Sephadex G) could be swelled sufficiently in non-polar solvents such as benzene and *n*-hexane, and their permeability limits on gel chromatography were increased on decreasing the degree of cross-linkage of the original Sephadex G gel. These gels were hydrolytically stable under the usual gel chromatographic conditions. It was found that TMS-Sephadex gels could be employed to separate solute mixtures either in straight-phase or in reversed-phase chromatography depending on the polarity of the developing solvent.

INTRODUCTION

Recently gel chromatography with organic solvents has often been employed to study the separation of oligomers and small molecules. At present Sephadex LH-20 (ref. 1), which is produced by the hydroxypropylation of Sephadex G-25 (ref. 2), is commercially available as one of the gels for use with organic solvents. However, Sephadex LH-20 gel still has a good many hydroxyl groups so that it will swell in polar solvents rather than in non-polar ones. Accordingly, gel chromatography on Sephadex LH-20 with non-polar solvents such as benzene and *n*-hexane is difficult. In addition, its upper permeability is limited, because it is derived from Sephadex G-25. Thus, the synthesis and gel chromatographic application of a lipophilic Sephadex derived from Sephadex G has been studied from the following two viewpoints: the first is to obtain a more lipophilic gel than Sephadex LH-20 and the second is to extend the upper permeability of the gel by comparison with Sephadex LH-20.

The substitution of the hydroxyl moiety in the polysaccharides by hydrophobic groups appears to be a suitable method of obtaining a lipophilic Sephadex. Based on such considerations, SJÖVALL *et al.* have already reported methylated[3-5] and hydroxyalkoxypropylated[6-8] Sephadex G, and their chromatographic properties. On the other hand, trimethylsilylation of polysaccharides with trimethylchlorosilane

has often been employed for obtaining hydrophobicity because of its good reactivity and the considerable stability of the trimethylsilylated products. Starch, amylose, cellulose, amylopectin and dextran have so far been trimethylsilylated by using trimethylchlorosilane–pyridine[9–11].

Trimethylsilylation was thus applied to Sephadex G as an easy means of obtaining a lipophilic gel which can be used in non-polar solvents. At the same time, Sephadex G gels with different degrees of cross-linkage were trimethylsilylated in the hope of obtaining gels having different upper permeabilities.

The present paper describes the fundamental and chromatographic properties of lipophilic Sephadex G gels which were obtained by reacting Sephadex G gels with trimethylchlorosilane.

EXPERIMENTAL

Materials and apparatus

Sephadex G-25, G-50, G-75, G-100, G-150 and G-200 were purchased from Pharmacia Fine Chemicals. It was confirmed by gas chromatography that trimethylchlorosilane (Katayama Chemicals) did not contain dimethyldichlorosilane[11]. Pyridine was distilled from potassium hydroxide before use. Toluene, the reaction solvent, was also distilled with sodium. Each of the methyl esters of normal fatty acids, normal fatty alcohols, and normal fatty amines used was a single component by gas chromatography and other solutes were of reagent grade. The developing solvents and the solvents used for the determination of solvent regain values were also of reagent grade.

A differential refractometer (Waters Associates, Model R-4) was used as a detector and the recorder used was an Electronic Polyrecorder EPR-2T (Toa Electronics). Infrared spectra were obtained with a Shimadzu Infrared Spectrophotometer IR-27G.

Preparation of trimethylsilylated Sephadex G-25 gel

Sephadex G-25 was washed thoroughly with methanol and dried overnight at 50° *in vacuo*. Forty grammes of dried Sephadex G-25 were placed into a three-necked 1-l round-bottomed flask containing 300 ml of dried pyridine and 300 ml of dried toluene. Then 125 g, trimethylchlorosilane were added dropwise under mild stirring. The temperature of the mixture was slowly raised to 80° with continuous stirring. After 2 h, the reaction mixture was cooled to room temperature and 100 ml of methanol were added to the reaction flask. The reaction product was filtered off on a funnel with a fritted glass filter and thoroughly washed with 200 ml of 2 % sodium acetate–methanol and then 300 ml of methanol. The gel was dried overnight *in vacuo*. The yield was 78 g and the content of silicon was 17.8 % (TMS-Sephadex G-25-17.8).

Solvent regain value

Solvent regain values were determined[12,13] by swelling the gel in a glass tube with a sintered glass followed by excluding the external solvent from the swollen gel by means of centrifugation (1000 r.p.m. for 10 min).

Column preparation and gel chromatography

A glass column, 100 × 1.4 cm I.D., was used in this experiment. The elution chromatogram was automatically recorded by a recorder. A polyethylene tube (1 mm I.D.) was used as a connection between each apparatus. TMS-Sephadex gel was equilibrated with developing solvent for several hours and carefully poured into the column. When chloroform was used as developing solvent, a round filter paper was put on the top of the swollen gel bed so as to prevent the gel from floating. Furthermore the swollen gel bed was allowed to settle overnight by passing developing solvent through the column. By maintaining the reservoir, which contained the developing solvent, at a set height, the flow rate was kept constant throughout the experiment.

5–30 mg or 5–30 μl of the sample were chromatographed at room temperature as follows: The solute was dissolved in 0.3 ml of the developing solvent and carefully applied on the top of the column. Eluate was collected in a measuring cylinder and the elution volume of each solute was directly determined from the chromatogram. The elution data were expressed in terms of the ratio of the elution volume to the total column volume: the relative elution volume (REV).

RESULTS

Trimethylsilylation

Trimethylsilylation of Sephadex G was rapidly completed and the main factors influencing the degree of the substitution were found to be the reaction temperature and the amount of the reagent, trimethylchlorosilane. The effect of the reaction temperature on the weight increase of the reaction product, TMS-Sephadex G-25, was investigated (Fig. 1). From Fig. 1, it appears that constant substitution could be obtained above 60°. The influence of the amount of reagent on the degree of substitution was also studied, as shown in Fig. 2. In this case toluene was added as a reaction solvent and the reaction temperature was set at 80°. By employing toluene, the degree of substitution was markedly increased and good reproducibility could be obtained. When the weight ratio of the reagent to Sephadex G-25 was 2 or above, constant substitution was obtained (Fig. 2).

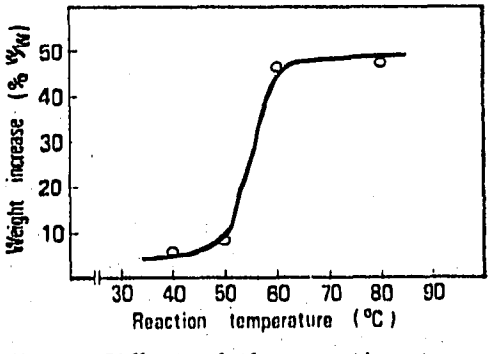

Fig. 1. Effect of the reaction temperature on the degree of substitution. Sephadex G-25, 3 g; pyridine, 30 ml; trimethylchlorosilane, 8 ml.

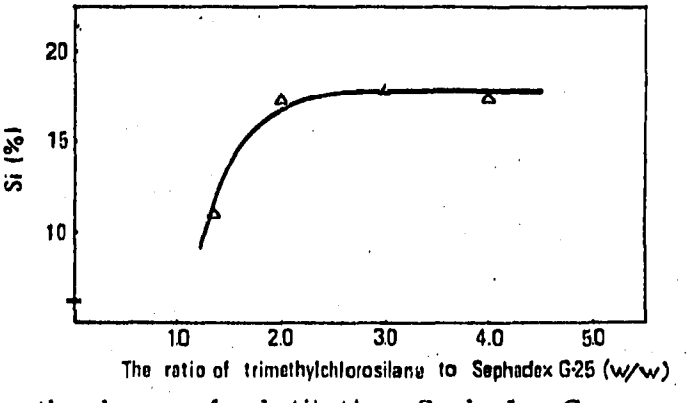

Fig. 2. Effect of the amount of trimethylchlorosilane on the degree of substitution of Sephadex G-25. Sephadex G-25, 3 g; pyridine, 30 ml; toluene, 100 ml. Reaction temperature: 80°. Development time: 2 h.

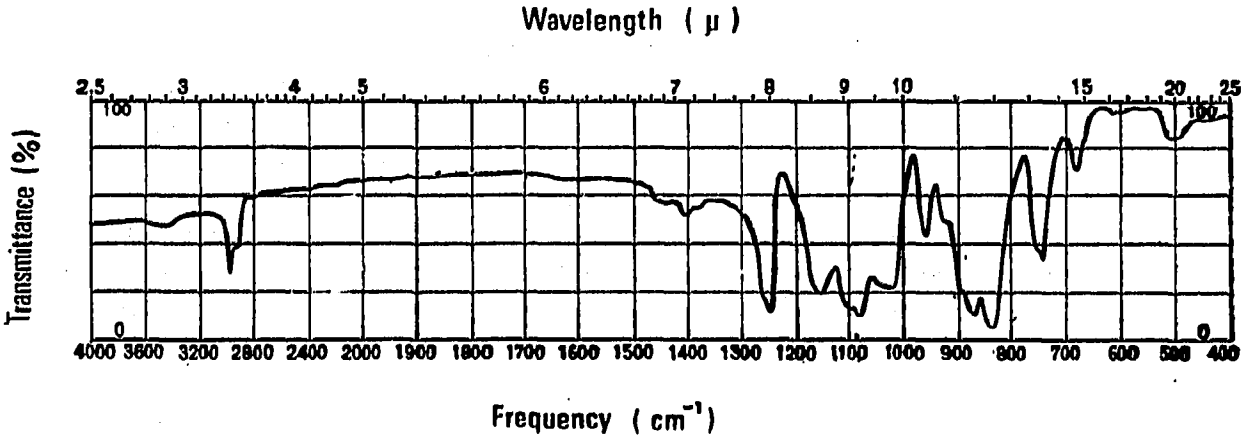

Fig. 3. Infrared spectrum of TMS-Sephadex G-100–19.0 in KBr.

The infrared spectrum of TMS-Sephadex G-100 gel shows characteristic[9,11] peaks of compounds with silicon-methyl groups (Fig. 3).

Hydrolytic stability

The hydrolytic stability of TMS-Sephadex G gel was studied in 50% aqueous methanol solution at room temperature (Fig. 4). Even after 72 h, this gel appeared to be quite stable. However, in acidic solution, cleavage of the silyl ether bond was easily achieved.

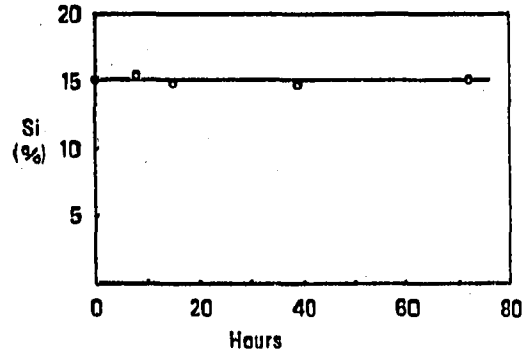

Fig. 4. Hydrolysis of TMS-Sephadex G-25–15.1 in 50% aqueous methanol at room temperature.

Solvent regain

The solvent regain (*Sr*) of TMS-Sephadex G-25 gels with various degrees of substitution and some TMS-Sephadex G gels with different degrees of cross-linkage were measured, respectively, and the results are shown in Tables I and II. As can be seen from Table I, the *Sr* values of TMS-Sephadex gel with the non-polar solvent became greater as the degree of substitution was increased. With regard to the difference of the degree of cross-linkage of the Sephadex gel, the higher the cross-linkage, the smaller the *Sr* with the studied solvents. It was found that TMS-Sephadex G gel has the best affinity to chloroform in the experimental range.

Gel chromatography

Polypropylene glycols and polyethylene glycol were eluted on TMS-Sephadex G-25–16.4, G-50–19.3 and G-100–17.2 using chloroform or toluene as developing

TABLE I

SOLVENT REGAIN OF TMS-SEPHADEX G-25 GELS WITH DIFFERENT DEGREES OF SUBSTITUTION

Solvent regain = ml solvent/g dry gel.

Solvent	*Silicon content (%)*			
	8.3	*11.0*	*16.4*	*18.0*
Dimethylformamide	2.38	1.94	0.45	0.38
Methanol	1.23	1.45	0.35	0.33
Ethanol	0.91	1.45	0.55	0.54
Acetone	0.58	1.09	0.62	0.65
Ethyl acetate	0.55	1.15	0.94	1.08
Chloroform	0.65	1.28	1.36	1.48
Toluene	0.24	0.66	1.16	1.33
n-Hexane	0.11	0.31	0.98	1.16

TABLE II

SOLVENT REGAIN OF TMS-SEPHADEX G GELS WITH DIFFERENT DEGREES OF CROSS-LINKAGE

Solvent regain = ml solvent/g dry gel.

Solvent	*TMS-Sephadex G*				
	G-50–18.2	*G-75–17.0*	*G-100–19.0*	*G-150–19.6*	*G-200–19.3*
Dimethylformamide	0.62	1.62	0.78	0.86	1.13
Methanol	0.50	1.07	0.75	0.92	1.05
Ethanol	0.84	1.33	1.35	1.41	1.98
Acetone	0.81	1.68	1.31	1.18	1.53
Ethyl acetate	1.48	2.51	1.85	2.54	2.17
Chloroform	3.56	5.24	3.99	9.61	7.78
Toluene	2.95	4.03	2.84	7.86	3.52
n-Hexane	1.58	1.98	2.19	2.65	2.11

solvent (Figs. 5 and 6). From Figs. 5 and 6, it was found that the upper permeability of trimethylsilylated gels increased as the G number of Sephadex increased. Approximate upper permeation limits of TMS-Sephadex G-25–16.4, G-50–19.3 and G-100–17.2 were, respectively, 2000, 4000 and 7000 in both solvents.

Hydrocarbons and other solutes which have approximately the same molecular weight but with different functional groups were chromatographed on TMS-Sephadex G-25–16.4 gel in chloroform or toluene. The results are shown in Table III.

Both aliphatic compounds and mono-substituted benzenes were eluted with larger elution volumes than other solutes when the functional group was carboxyl, hydroxyl or amino and the elution volumes of these compounds were different with different functional groups. On the other hand, ethers, aldehydes, ketones and esters had almost the same elution volumes. In the case of some hydrocarbons, it could be observed that analogous solutes were eluted in order of their molecular weights. However, alicyclic compounds were eluted more slowly than the corresponding aliphatic and aromatic ones. All the solutes mentioned in Table III were eluted more rapidly with chloroform than when toluene was used as developing solvent.

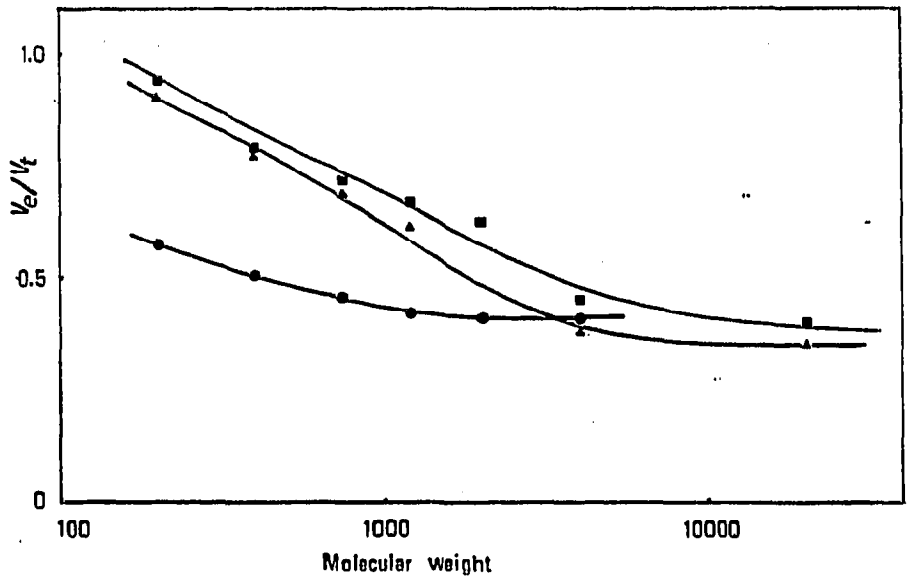

Fig. 5. Correlation between relative elution volume and molecular weight for polypropylene glycols and polyethylene glycol (mol. wt. 20000) in chloroform on TMS-Sephadex G gels with different degrees of cross-linkage. ●, TMS-Sephadex G-25–16.4; ▲, TMS-Sephadex G-50–19.3; ■, TMS-Sephadex G-100–17.2.

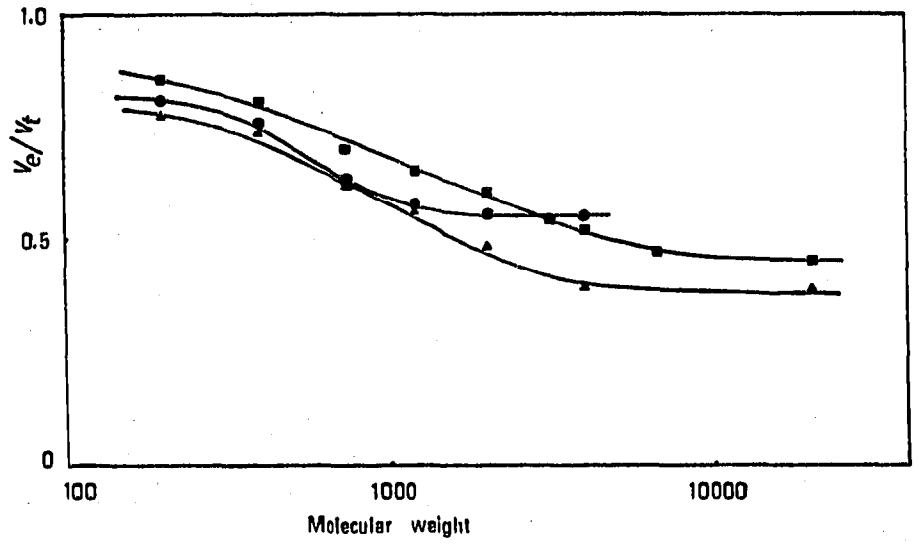

Fig. 6. Correlation between relative elution volume and molecular weight for polypropylene glycols and polyethylene glycol (mol. wt. 20000) in toluene on TMS-Sephadex G gels with different degrees of cross-linkage. ●, TMS-Sephadex G-25–16.4; ▲, TMS-Sephadex G-50–19.3; ■, TMS-Sephadex G-100–17.2.

The relative elution volume (V_e/V_t) of methyl esters of fatty acids, normal fatty alcohols and normal fatty amines were plotted against the logarithm of the molecular weight, as shown in Fig. 7, and a linear relationship was found. It can be seen that in chloroform the elution order was, in turn, esters, amines and alcohols, but in toluene the elution order between the amines and alcohols was reversed. As an example, a chromatogram of a series of normal fatty alcohols is shown in Fig. 8.

When alcohols or mixed solvent systems containing alcohol were used as developing solvents, it was clearly observed that the elution of the methyl esters of

TABLE III

RELATIVE ELUTION VOLUMES OF SOME CHARACTERISTIC COMPOUNDS ON TMS-SEPHADEX G-25–16.4 GEL

Column: 100 × 1.4 cm; flow rate, 30–38 ml/h.

Compound	*Solvent*		*Compound*	*Solvent*	
	Chloroform	*Toluene*		*Chloroform*	*Toluene*
n-Butyric acid	78.5	—	Nitrobenzene	61.8	68.5
n-Butyl alcohol	75.0	100.7	Phenyl acetate	60.9	67.6
n-Butylamine	71.1	107.9	Acetophenone	59.5	69.2
Diethyl ether	66.8	76.0	Benzene	67.8	—
n-Butyraldehyde	64.8	73.7	Cyclohexane	73.4	75.0
Methyl ethyl ketone	64.5	75.0	*n*-Hexane	71.7	74.3
Methyl acetate	63.2	73.7	*n*-Nonane	66.7	69.7
Phenol	125.7	184.9	Naphthalene	62.8	69.7
Benzoic acid	84.2	—	Decalin	68.4	70.4
Aniline	66.4	80.3	Phenanthrene	59.9	65.0
Benzaldehyde	62.2	69.9			

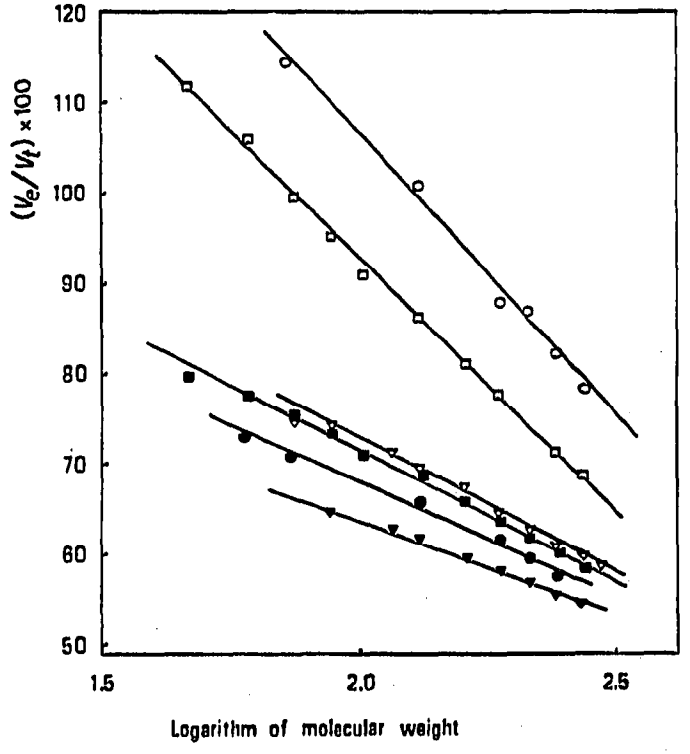

Fig. 7. Correlation between the relative elution volume and the logarithm of molecular weight on TMS-Sephadex G-25–16.4. Solutes: methyl esters of normal fatty acids (▽, ▼); normal fatty alcohols (□, ■); normal fatty amines (○, ●). Solvents: toluene (▽, □, ○); chloroform (▼, ■, ●). Column: 100 × 1.4 cm I.D. Flow rate: 30–38 ml/h.

normal fatty acids was in order of increasing molecular weight (Table IV). Furthermore, the alcohols were eluted more rapidly than esters which have the same carbon numbers. Table IV shows that the higher the polarity of the solvent, the larger the elution volume of the solute.

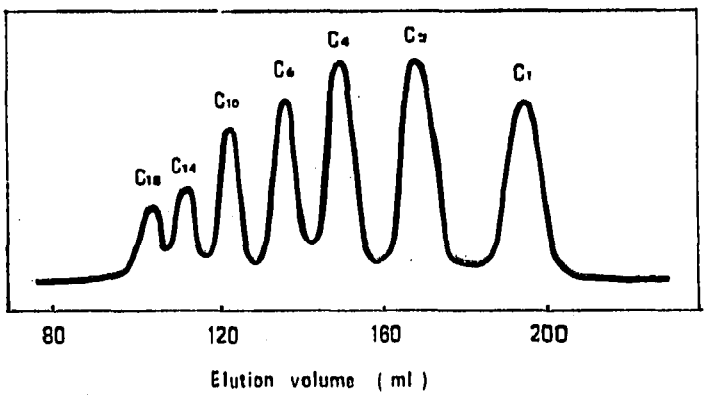

Fig. 8. Separation of normal fatty alcohols. Gel: TMS-Sephadex G-25–16.4. Solvent: toluene. Column: 93 × 1.4 cm. Flow rate: 38.8 ml/h. Room temperature.

TABLE IV

RELATIVE ELUTION VOLUMES OF METHYL ESTERS OF NORMAL FATTY ACIDS AND NORMAL FATTY ALCOHOLS ON TMS-SEPHADEX G-25–13.5 GEL IN POLAR SOLVENTS

Solvent	*Compound*	*REV*	*Column (cm)*	*Flow rate (ml/h)*
Methanol	Methyl caproate	100	39 × 1.2	38.7
	Methyl caprate	136		
	Methyl myristate	218		
Isopropanol	Methyl caproate	94	56 × 1.4	24.5
	Methyl caprate	106		
	Methyl myristate	153		
Methanol–acetone–*n*-heptane (4:2:1)	Methyl laurate	100	40 × 1.2	34.7
	Methyl stearate	131		
	Stearyl alcohol	86		
Isopropanol–*n*-heptane (2:1)	Methyl laurate	91	69 × 1.2	27.2
	Methyl stearate	93		
	Lauryl alcohol	72		
	Stearyl alcohol	72		

DISCUSSION

The trimethylsilylated gel was found to be sufficiently stable for use as a gel chromatographic packing with organic solvents. It can be presumed that this considerable stability to hydrolysis is due to the steric hindrance of three methyl groups[11].

It is considered that the characteristics of TMS-Sephadex gel permit its use in non-polar solvents which could not be used with Sephadex LH-20. Trimethylsilylation of Sephadex G-25, G-50, G-75, G-100, G-150 and G-200 also extends the upper permeability limit of Sephadex gel which can be used in organic solvents.

When polar solutes were chromatographed on TMS-Sephadex gel with non-polar solvents, the separation was thought to be by straight-phase chromatography. At the same time, the separation of hydrocarbons was presumedly due to only the molecular sieve effect. With these chromatographic conditions, it was considered

that either π-electron adsorption or other sorptions were not likely to be present[14]. On the other hand, when a polar solvent, such as alcohol, was employed as a developing solvent, solutes were thought to be eluted by reversed-phase chromatography.

Accordingly, from the discussion, it would appear that TMS-Sephadex gel can be arbitrarily employed in three separation mechanisms with the selection of any solvent or solvent system having different polarity.

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