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Application of Gel Chromatography to Small Molecules. II

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Summary

This presentation is an extension of earlier work (*1*) in which liquid chromatographic separations of hydrocarbons, alcohols, triglycerides, and surfactants with molecular weights ranging from 100—1000 were made, using columns packed with porous polymeric beads. The fractionation capability of the porous gels was extended to smaller molecules through the development of small porosity gels. The gels separate according to molecular size and are therefore useful for both separation and identification purposes. Since porous gels require no stationary liquid phase, long column life is achieved without a saturated carrier.

In this work a high-resolution gel column system yielding greater than 180,000 theoretical plates was investigated, and the high-resolution capability of small-molecule gel permeation chromatography (GPC) was explored.

INTRODUCTION

Gel permeation chromatography (2) is an accepted technique of the polymer chemist for determining the molecular weight distribution of macromolecules. The technique, however, has found only limited use with small molecules and then largely as an extension of conventional gel permeation chromatography (3–7) work with oligomers of synthetic high polymers or the separation and determination of impurities in high polymers. A work of primary importance was reported by Hendrickson and Moore (5), who determined the size of a large number of small molecules in various solvents. Spell used the technique both to fractionate single species and oligomers for subsequent infrared analysis (7). Recent work in this

laboratory on the development of improved small porosity gels permitted the GPC technique to be used with small molecules for the analysis of single species. It is the purpose of this work to apply small porosity gel permeation chromatography at high resolution to single species in the low and intermediate molecular weight range as an analytical technique. The need for such work is evident. Gas chromatography is now useful for the analysis of materials up to molecular weights of perhaps 200–400. Conventional GPC is generally used in the range of 2000 to 2,000,000. In the intermediate molecular weight range, a multitude of materials are found which because of their volatility and stability cannot be fractionated by gas chromatography but can be separated by molecular size using gel permeation chromatography.

EXPERIMENTAL

Apparatus

All separations were made using a Waters Associates' Model 200 gel permeation chromatograph with a differential refractometer detector providing readout on a strip chart recorder.

Solvent

Tetrahydrofurane (THF) was used as the carrier solvent. Solvent flow rate as provided by the system's metering pump was set at a nominal flow rate of 1 ml/min, with up to 10 columns in series. With 40 columns in series, a flow rate of 0.4 ml/min was used. These flow rates provided linear velocities of 5 and 2 cm/min, respectively.

Columns

Standard GPC columns each $\frac{3}{8}$-in. diameter (0.775-cm i.d.) by 4 ft (121.9 cm) in length were packed with Styragel, a cross-linked porous polystyrene bead with a nominal 35-μ particle diameter. Pore size of the particle was controlled by the nature and amount of diluent used during polymerization. Pore size or porosity is specified as the end-to-end chain length in Angstroms of the extended polystyrene molecules excluded from the gel pore. Normal hydrocarbons and polystyrene standards were used to determine

the respective porosities. Porosities used are reported on the respective chromatograms.

Samples

Samples of 4 mg were introduced into the columns as 0.25–0.5% solutions by means of a loop injection valve. Materials used were

1. Synthetic mixtures of hydrocarbons, alcohols, triglycerides
2. Surfactants:
   a. Fatty acid esters of hexatol anhydrides
   b. Ethylene oxide adducts of nonylphenol

RESULTS AND DISCUSSION

Theory

The base-line separation of the hydrocarbons shown in Fig. 1 demonstrates the capability of the rigid cross-linked gels with small porosity to separate single species into discrete peaks, commonly expected by the practitioner of gas chromatography. While the separation could be done easily and in less time by gas chromatography, this work demonstrates that small molecules with molecular weights ranging from 80–226 can be separated as discrete bands according to molecular size by gel permeation chromatography.

The size separations by GPC may be described by

$$V_e = V_0 + KV_i \tag{1}$$

where V_e = elution volume of the solute
V_0 = interstitial volume (dead volume) of the column
V_i = internal solvent volume
K = the distribution coefficient, based on the relative concentrations between phases, or

$$K = \frac{\text{average concentration in the stationary solvent}}{\text{average concentration in the moving solvent}} \tag{2}$$

K may also be considered as the relative volume of internal solvent available to a molecule of a given size. It must be recognized that K is always between zero and 1 in gel permeation chromatography. When K is greater than 1, separation is not by molecular

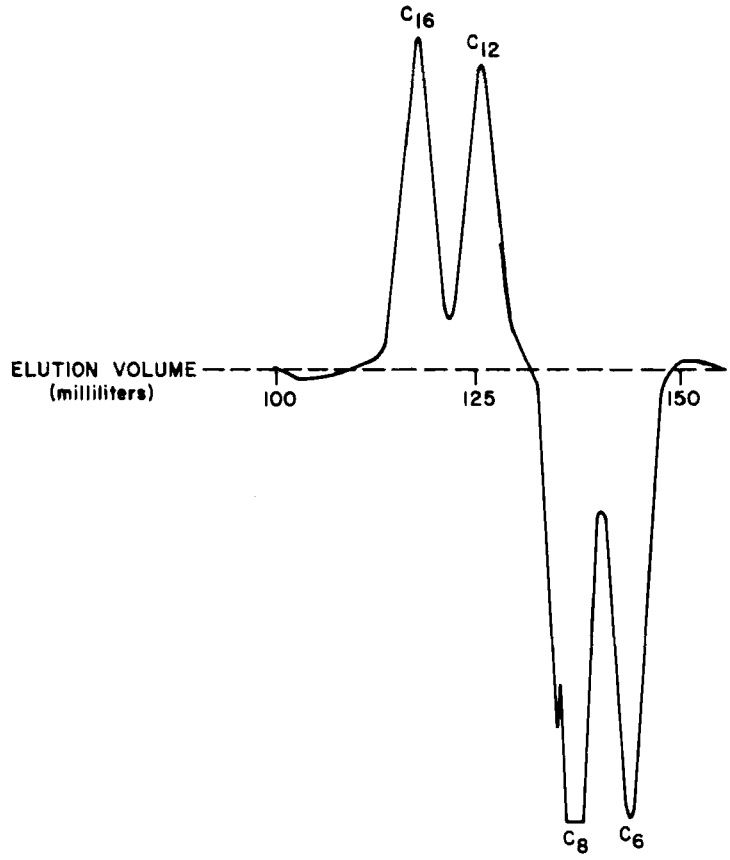

FIG. 1. Elution of *n*-hydrocarbons from Styragel. Analytical/operating conditions: sample, 2.5 mg/ml; injection time, 60 sec; columns, two 1.2-m columns of 60-Å Styragel; temperature, 29°C; solvent, THF; flow rate, 1.1 ml/min.

size but rather by a modified mechanism involving interaction with the gel matrix such as adsorption.

In Styragel columns, $V_i/V_0 \sim 1$. The capacity of the column for a given solute is expressed as K', where

$$K' = \frac{KV_i}{V_0} = \frac{V_e - V_0}{V_0} \tag{3}$$

Since K' is never greater than 1, relatively large column volumes

and high plate efficiencies are required to achieve high resolution. Fortunately, high-efficiency columns are readily attainable with the uniform spherical particles available in the cross-linked polystyrene gels.

Theoretical plate counts (N) of 5000 plates/m (HETP = 0.2 mm) are common in GPC and plate heights of 0.4 ml are minimal. With uniform columns, plate counts are directly additive, as shown by

$$N_{\text{total system}} = 0.97 \Sigma N_{\text{individual columns}} \quad (4)$$

Multiple-column systems may be used to obtain the resolution shown in this work.

Since V_i is not measured directly, it is expedient to convert Eq. (1) to use V_t:

$$V_t = V_0 + V_i \quad (5)$$

Equation (1) then becomes

$$V_e = V_0 + K(V_t - V_0) = V_0(1 - K) + KV_t \quad (6)$$

In this form, V_0 and V_t may be determined easily by an empirical calibration, as shown in Fig. 2, where V_0 is the exclusion volume and V_t is the elution volume of a material experiencing total penetration of the gel pore or retained by the total volume of internal solvent.

All GPC calibration curves contain the three elements shown in Fig. 2, that is, a vertical line of exclusion, a vertical line of total penetration, and a sloped region or partial permeation. The curve shown, however, is an ideal limit with sharp intersections of straight lines.

Since the gels possess a distribution of pore sizes and the molecules "flex" in solution, the calibration curve in practice is a continuous curve resembling the broken line in Fig. 2; the slopes are not linear and the intersections are not sharp. As indicated by Fig. 2, maximum resolution would be obtained when y/x is small.

The optimum curve would be produced with a gel in which all pores are of equal size at the best pore size for separating the solutes. It is evident that such a curve would be usable over only a very narrow molecular weight range. A common practice in gel permeation chromatography is to use gels with a pore-size range that extends the linear portion of the curve over as wide a range as is expedient to fractionate highly dispersed systems in a single

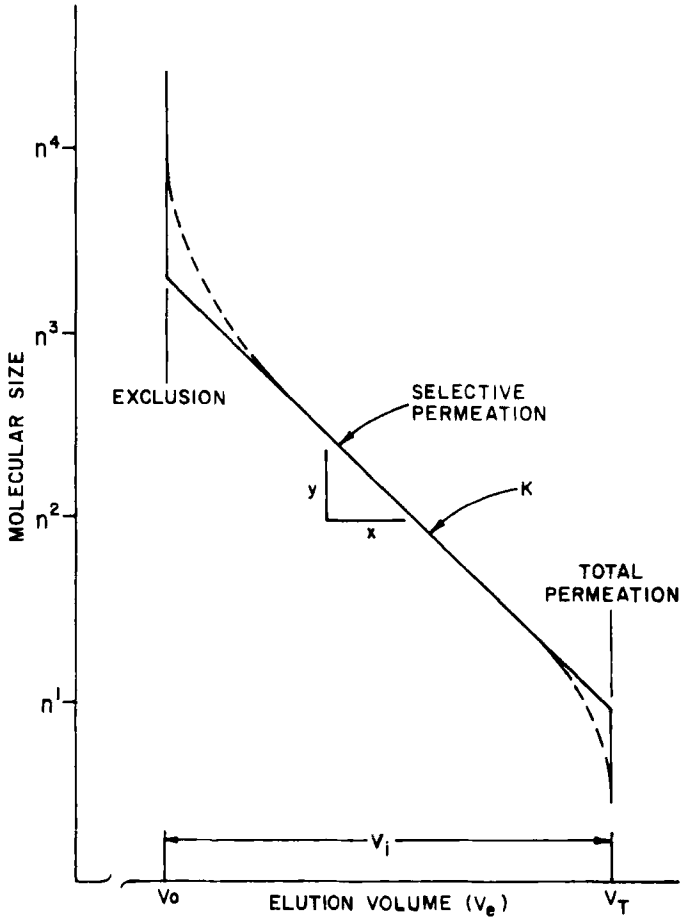

FIG. 2. Illustrative GPC calibration curve.

pass. In this work, both approaches were used. Column systems of 40-, 60-, 100-, and 500-Å gels were used for a range of molecular sizes, while 10 columns and 40 columns, all 500 Å, were used to give higher resolution of the triglycerides.

The separation of methanol from ethanol (MW 32 from 56), shown in the superpositioned chromatogram in Fig. 3, illustrates the separation capability of GPC by molecular size without evidence of adsorption or partition. The mixture of compounds shown in Fig. 4 covers a molecular weight range from 72 to 897. A plot of V_e versus

log molecular weight yields a straight line. Trilaurin is separated virtually to base line from tristearin by a six-column system. While 4.1 hr is required to cover the total molecular weight range in the mixture, the triglyceride separation could be made in less than 3 hr. By using only 500-Å porosity gel, greater resolution of the triglycerides could be obtained and the separation could be made in less time. This principle is illustrated in Fig. 5, where a 10-column series (12.2 m) is used to separate triglycerides. This system provided a base-line separation of tricaprin from trimyristin (ΔGMW = 168) in 5 hr.

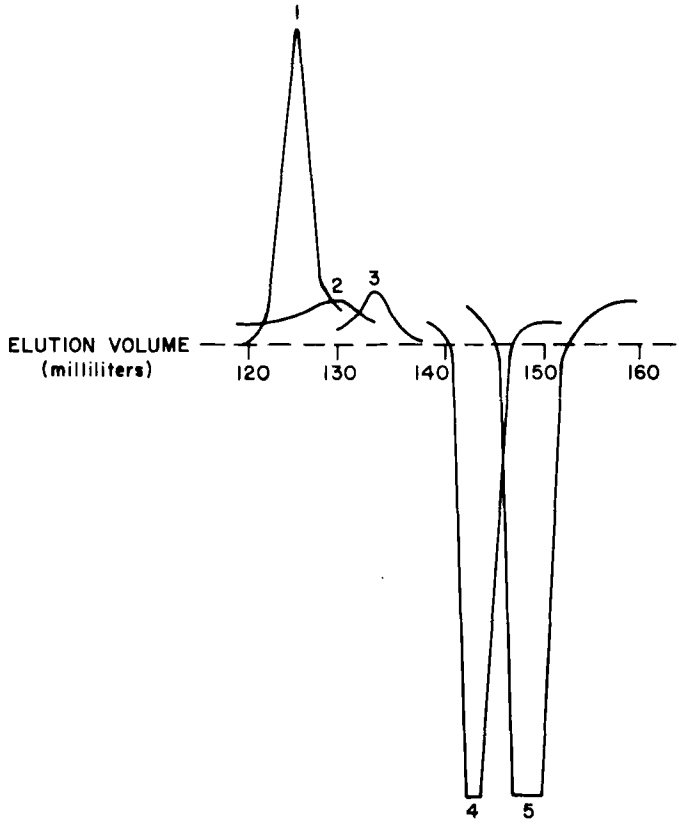

FIG. 3. Elution of alcohols from Styragel. Analytical/operating conditions: See Fig. 1. Peak identification: 1, octanol; 2, 4-methyl-2-pentanol; 3, pentanol; 4, ethanol; 5, methanol.

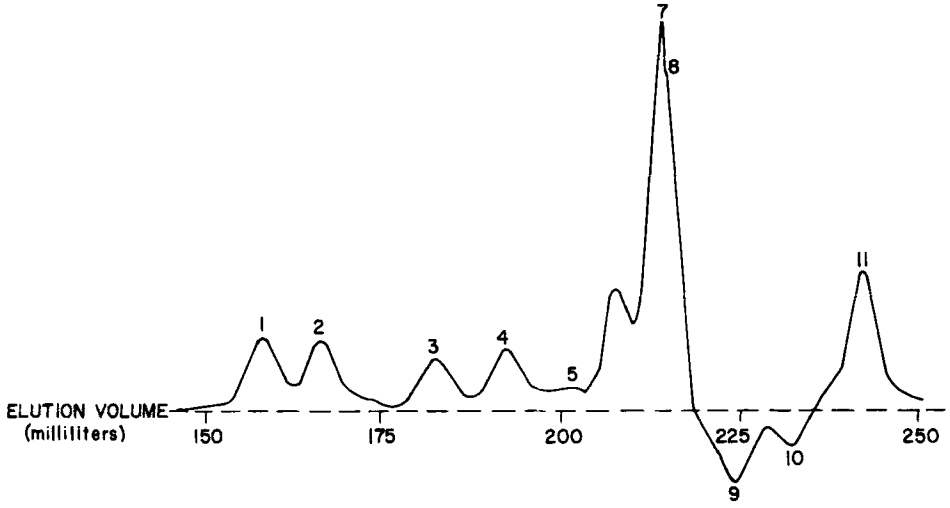

FIG. 4. Elution of complex organic mixture from Styragel. Analytical/operating conditions: columns, six 1.2-m columns (in series) of Styragel (1, 60 Å; 2, 60 Å; 3, 100 Å; 4, 250 Å; 5, 500 Å; 6, 1000 Å). Other conditions: See Fig. 1. Peak identification: 1, tristearin; 2, trilaurin; 3, tricaproin; 4, actyl ether; 5, dodicane; 6, catechol; 7, 2,4-xylene; 8, phenol; 9, hexane; 10, butraldehyde; 11, benzene.

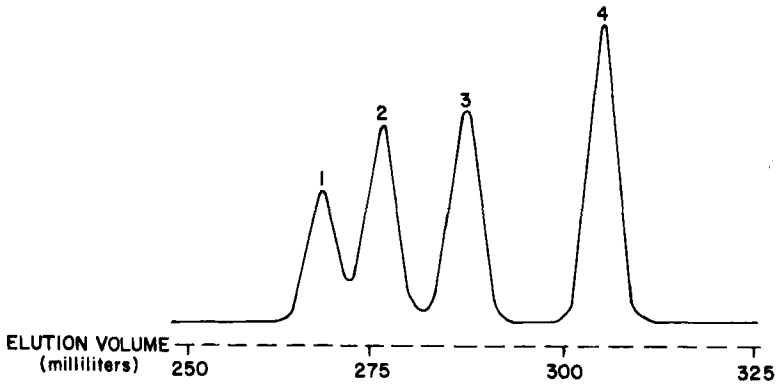

FIG. 5. Elution of triglycerides. Analytical/operating conditions: columns, ten 1.2-m columns of 500-Å gel in series; temperature, 36°C. Other conditions: See Fig. 1. Peak identification: 1, tristearin; 2, trimyristin; 3, tricaprin; 4, tricaproin.

High-Resolution GPC

The high-resolution capability of GPC is demonstrated by the C_{33}, C_{39}, C_{45}, C_{51}, C_{57}, and C_{63} triglyceride separation shown in Fig. 6. The triglycerides were separated on a 160-ft × $\frac{3}{8}$-in. o.d. column system which yielded 180,840 theoretical plates. A summary of the data obtained from the chromatogram is shown in Tables 1 and 2.

Relative permeation values (α) were calculated as follows:

$$\alpha = \frac{V_2 - V_0}{V_1 - V_0}$$

Effective plates (n_{req}) (8,9) and theoretical plates (N_{req}) required for separation were calculated using Eqs. (7), (8), and (9):

$$n_{req} = 36\left(\frac{\alpha}{\alpha - 1}\right)^2 \tag{7}$$

When relative retention (α') is used, Eq. (7) becomes

$$N_{req} = 36\left(\frac{\alpha'}{\alpha' - 1}\right)^2 = 36\left(\frac{\alpha}{\alpha - 1}\right)^2\left(1 + \frac{1}{K_2'}\right)^2 \tag{8}$$

where

$$\alpha' = \frac{V_2}{V_1}$$

$$K' = \frac{KV_i}{V_0} = \frac{V_e - V_0}{V_0} \tag{9}$$

The theoretical plate requirement (N_{req}) for separation compared with the actual calculated plate number (N) for the respective peaks is shown in Table 1. Also shown is the resolution (R) in sigmas, as calculated using Eq. (10) and assuming that $W = 4\sigma$:

$$R_\sigma = \frac{8(V_2 - V_1)}{(W_2 + W_1)} \tag{10}$$

As is apparent from the chromatogram of Fig. 6, the C_{63}-C_{57} and C_{57}-C_{51} triglycerides are nearly, but not completely, resolved. The plate count shown in Table 1 indicates the system is adequate to resolve the C_{45}-C_{39} and C_{39}-C_{33} pairs. The system is just under the 6σ resolution required to separate the C_{51}-C_{45} pair and about 50% under that for the C_{63}-C_{57} pair. It is evident from Fig. 6, however,

TABLE 1
GPC Separation Data for Triglycerides

Component	Carbon number (C_{no})	Molecular weight	Elution volume (V_e), ml	Peak width (W), ml	Theor. plates (N)	Relative retent. (α')	Theor. plates (N_{req})	Resolution (R), σ
Polystyrene		867,000	873	14.50				
Triarachidin	63	976	1132	14.30	100,240	1.0134	205,900	4.2
Tristearin	57	892	1147	14.60	98,750	1.0141	186,220	4.6
Tripalmitin	51	807	1163	13.35	121,460	1.0165	136,630	5.6
Trimyristin	45	723	1183	14.30	109,380	1.0178	117,700	6.1
Trilaurin	39	639	1203	13.10	135,010	1.0202	91,830	7.3
Tricaprin	33	555	1228	13.55	131,350			
O-Dichlorobenzene		147	1579	14.85	180,840			

TABLE 2
Effective Plate Calculations for Triglycerides

	Elution volume		Peak width (W), ml	Effect. plates (n_{eff})	Theor. plates (N)	K'	Relative permeat. (α)	Effect. plates (n_{req})	Theor. plates (N_{req})	Resolution (R), σ
	V_e, ml	$V_e - V_0$, ml								
Polystyrene	873.2		14.50							
Triarachidin	1131.8	258.6	14.30	5,234	100,240	0.296	1.0586	11,630	206,760	4.2
Tristearin	1147.0	273.8	14.60	5,627	98,750	0.313	1.0589	11,630	187,320	4.6
Tripalmitin	1163.2	290.0	13.4	7,548	121,460	0.332	1.0662	9,340	136,340	5.6
Trimyristin	1182.3	309.1	14.3	7,478	109,380	0.354	1.0679	9,130	120,521	6.1
Trilaurin	1203.4	330.2	13.1	10,162	135,010	0.378	1.0738	7,580	91,000	7.3
Tricaprin	1227.7	354.5	13.6	19,952	131,350	0.405				
O-Dichlorobenzene	1578.08	705.6	14.8	36,118	180,840					

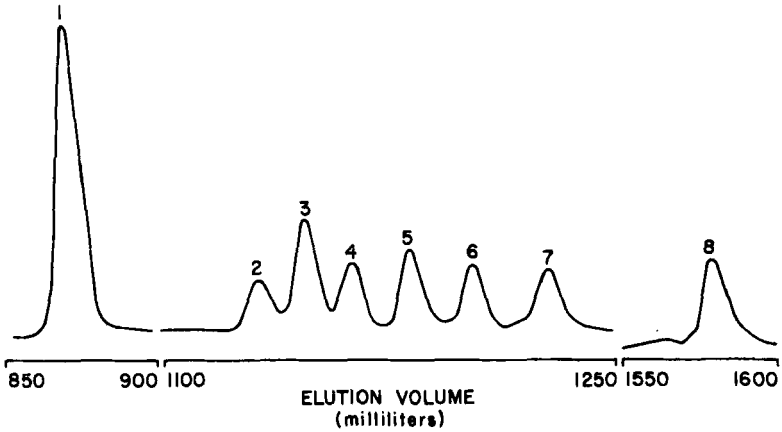

FIG. 6. High resolution GPC separation of triglycerides. Analytical/operating conditions: columns, 160-ft × ⅜-in. o.d. column of 500-Å gel; flow rate, 0.4 ml/min. Peak identification: 1, polystyrene; 2, triarachidin; 3, tristearin; 4, tripalmitin; 5, trimyristin; 6, trilaurin; 7, tricaprin; 8, O-dichlorobenzene.

that the 4σ resolution provided is more than adequate for any type of analytical separation. These data indicate the "high price" of base-line separation (i.e., 6σ) over 4σ resolution. Since N is proportional to $1/\sigma^2$, increasing the resolution from 4.2 to 6σ requires a system that provides twice the number of theoretical plates.

Table 2 shows the effective plate requirement (n_{req}) for 6σ resolution (R_σ), the effective plate numbers (n_{eff}) of the respective peaks, and also the resolution (R) provided by the system. A comparison of n_{req} with the average of n_{eff} shows complete agreement with the resolution values obtained and with the proportionality of the theoretical plate values (N and N_{req}) reported in Table 1. It is evident that either system is usable and provides meaningful results. The large difference between n_{eff} and the theoretical values (N and N_{req}) may be shocking until it is realized that at $K = 1$,

$$K' = \frac{KV_i}{V_0} = \frac{V_i}{V_0} = 0.8$$

for this system.

From the agreement between the calculated plate requirement and the actual plate count, it is evident that column requirements for difficult separations by GPC can be calculated from α values

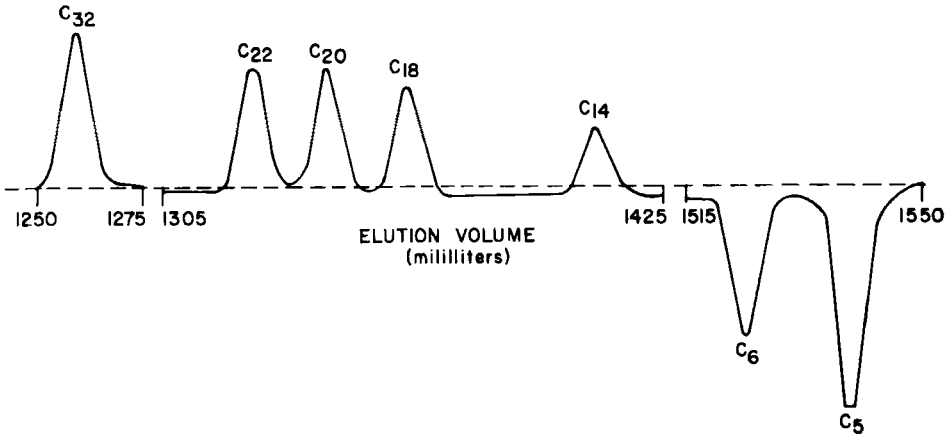

FIG. 7. High-resolution GPC separation of hydrocarbons. Analytical/operating conditions: See Fig. 6.

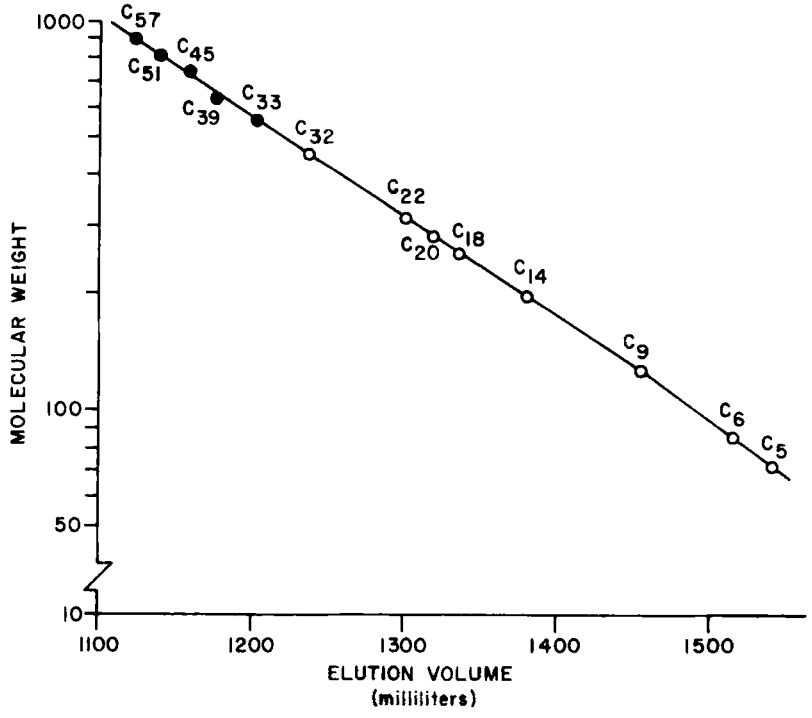

FIG. 8. High-resolution GPC system: calibration curve ($V_0 = 883.0$ ml). Standards: *n*-hydrocarbon, ○, tristearins, ●.

using Eq. (9) and V_e values determined separately on short columns of proper porosity and of similar K' value.

The peak width (W) shown in Tables 1 and 2 contributes valuable information on peak spreading. It is important to note that the excluded polystyrene peak at V_0, the ODCB peak at V_t, and all solute peaks across KV_i are of equal width, indicating that peak spreading is occurring in the transport phase and not inside the gel pore. The significance is enhanced by the realization that the excluded polystyrene was polydispersed, whereas all other materials were single species.

The high-resolution hydrocarbon separation shown by the chromatogram of Fig. 7 indicates base-line resolution between C_{14}, C_{16}, C_{18}, C_{20}, and C_{22} hydrocarbons. Greater than 8σ resolution was obtained between pentane and hexane. A plot of log molecular weight versus V_e for the hydrocarbons and the triglycerides is shown in Fig. 8. It is interesting to note that all points fall on a straight line. It is reasonable to assume from these results that the system required to effect a needed separation can be calculated directly from the calibration curve using Eq. (9) and appropriate plate number.

APPLICATIONS

Surfactants

A five-column system containing 500-Å gel was used to examine a number of industrial surfactants. The results are shown in Fig. 9. The elution diagram of triglycerides for the same column system is included in Fig. 9 to show comparative molecular size. From the chromatograms the presence of mixed esters is evident. Also, the presence of the monoester in the tri and the tri in the monoester is evident, as would be expected in a competitive rate reaction.

Ethylene Oxide Adducts of Nonylphenol

A high-resolution system was used to separate these components. The chromatogram of Fig. 10 shows the distribution of components in Triton X100 and Triton X45. These materials are separated by molecular size, with the successive peaks representing a material with one less ethylene oxide unit.

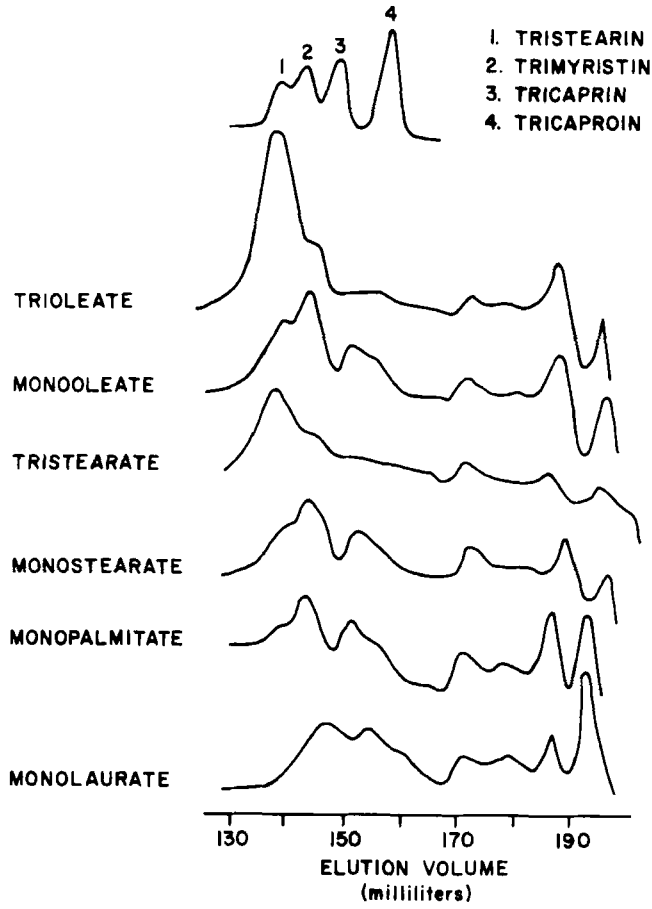

FIG. 9. Fatty acid esters of hexatol anhydride eluted from Styragel. Analytical/operating conditions: columns, five 1.2-m columns of 500-Å Styragel in series; solvent, THF; temperature, 43°C; flow rate, 1.1 ml/min.

Crude Oil

The GPC chromatogram of Fig. 11 shows the size distribution of Kuwait crude oil before and after distillation. The separation was made on a five-column system using 500-Å gel. The sharp peak at 110 ml indicates the exclusion of a portion of the crude oil compo-

nents by the small porosity gel. Curve B clearly shows loss of the low molecular weight material as a result of removing the gasoline oil by distillation.

SUMMARY

The GPC separation is virtually unaffected by functional group activity or molecular composition. V_e in a given system is a function only of the size of the molecule in solution. Therefore, the tech-

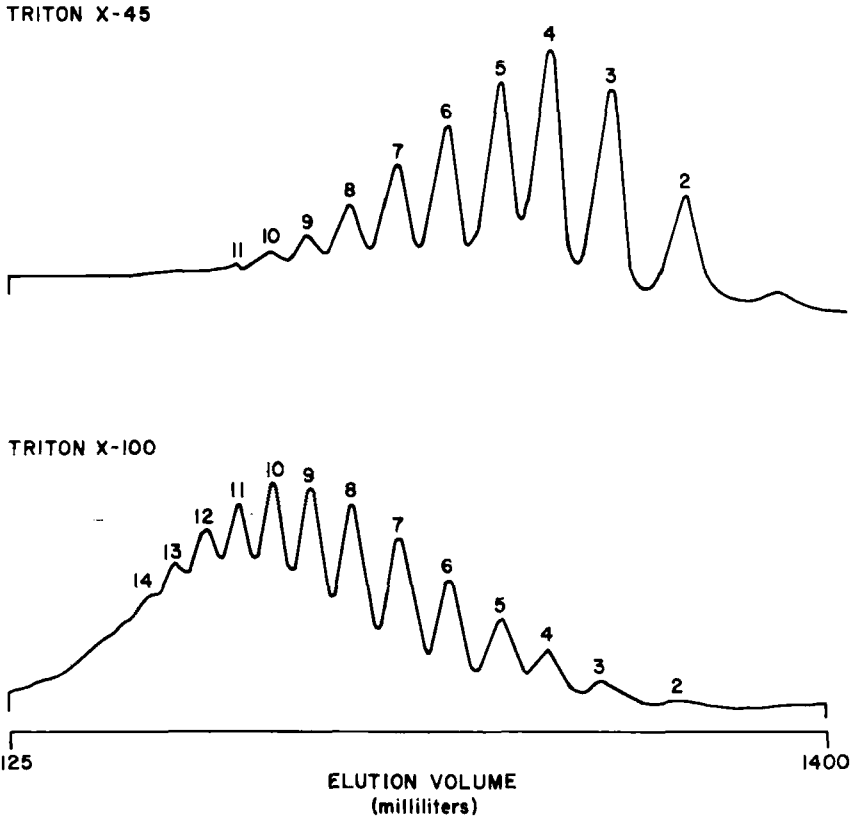

FIG. 10. High-resolution GPC separation of surfactants (nonylphenol–ethylene oxide adducts). Analytical/operating conditions: See Fig. 6. Note: Numbers 2 through 14 = moles of ethylene oxide per molecule of nonylphenol.

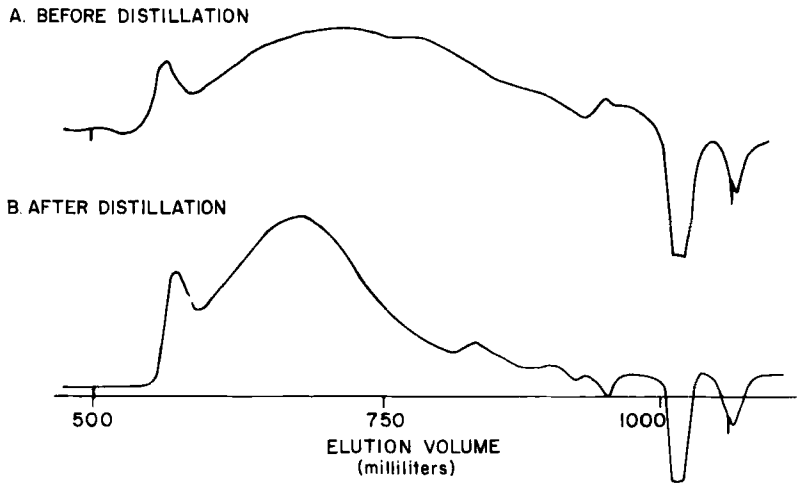

FIG. 11. GPC characterization of crude oil. Analytical/operating conditions: column, 20-ft × ⅜-in. o.d. column of 500-Å gel.

nique provides not only a method of fractionation but, in a system of uniform composition, also gives qualitative information on the molecule constituting the eluting band.

High resolution is not only possible with GPC techniques but is also practicable and may be necessary because of small α values (< 1.02). Another advantage of GPC is that system requirements for a given separation can be calculated from the calibration curve.

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