

Multiple Peak Resolution in Gel Permeation Chromatography

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Synopsis

A new, graphic method of the resolution of a chromatogram into its component peaks is presented. From a mathematical description of the chromatogram of a monodispersed sample, a practical method of peak resolution of a multicomponent sample is derived. From this, the constituents may be characterized as to size and their weight fractions determined. As an example the final product in the synthesis of N,N-diglycidyl tribromoaniline is analyzed graphically and compared with results obtained using the du Pont 310 Curve Resolver. The agreement appears to be quite satisfactory.

INTRODUCTION

Since its introduction a few years ago, gel permeation chromatography (GPC) has been used intensively to characterize organic materials ranging in size from low molecular weight compounds to high polymers. Separation is effected based on the molecular "size" of the molecule. Each molecular species that is eluting is detected on the chromatogram as a bell-shaped curve which can be adequately represented as a Gaussian function.¹

The purpose of this paper is to present a graphic method of resolving these Gaussian functions for the case where a number of compounds are present. Tung^{2,3} has resolved this problem for the case where a continuously distributed polymer is eluting. Smith⁴ has modified Tung's method to include a discontinuous distribution. Smith's method is suitable for a sample containing molecular species which are all homologues of the same monomer. However, for the case of a sample with varying molecular and chemical constituents one would obtain poor agreement.

EXPERIMENTAL

The Waters Associates' gel permeation chromatograph, used for this analysis, is adequately described in the literature.⁵ The instrument was equipped with a series of four columns having nominal upper separating limits of 1000, 1000, 100, and 60 ångströms. Freshly distilled tetrahydrofuran solvent was used at a flow rate of 1 ml/min and ambient temperature. N,N-Diglycidyl tribromoaniline, $1/12$ ml of a 10 rot-% solution in tetra-

hydrofuran, was injected onto the columns. The resulting chromatogram was then resolved, using an analog computer, the du Pont 310 Curve Resolver. Subsequently, the chromatogram was subjected to the graphic solution presented in this paper.

THEORETICAL

It is well known that the weight concentration of a solute in a dilute solution is in many cases found to be⁶

$$C_w = \frac{n_s - n_1}{n - n_1} \quad (1)$$

where C_w is the weight concentration in g/cm³ of solution, and n_s , n_1 , and n are the refractive indices of the solution, the solvent, and the solute, respectively.

Inherent in eq. (1) is the assumption that the densities of the solution, solute, and solvent are similar and that the Gladstone-Dale equation holds for the system.⁶

The difference between the index of refraction of the solution and the solvent, $(n_s - n_1)$, is proportional to the height of the chromatogram, $R(V)$. This assumes a linear relationship between the input signal and the output of the recorder.

From this, the area under a chromatogram peak may be expressed as

$$A = \int_0^{\infty} R(V)dV = kw_i(n - n_1) \quad (2)$$

In general, the output signal of a chromatograph, $R(V)$, due to a mono-dispersed solute appears as a Gaussian peak, which can be expressed as

$$R(V) = k' \frac{A}{\sigma} \left(\frac{1}{2\pi} \right)^{1/2} \exp [-(\bar{V} - V)^2/2\sigma^2] \quad (3)$$

where the constant A has been evaluated in eq. (2); σ^2 is the variance of the peak and varies with the elution volume⁷; \bar{V} is that elution volume where the peak occurs.

For a multicomponent solute of n components, the chromatogram can be described by the equation

$$R(V) = \frac{k'}{(2\pi)^{1/2}} \sum \frac{A_i}{\sigma_i} \exp [-(\bar{V}_i - V)^2/2\sigma_i^2]. \quad (4)$$

The method of resolution used here is a modification of a method developed by Bhattacharya.⁸ Assuming that the component peaks are sufficiently separated so that there is a finite region, for each component, where the effect of all the other components is comparatively negligible, then the ordinate in this region may be expressed as

$$R(V) \cong \frac{k'}{(2\pi)^{1/2}} \frac{A_r}{\sigma_r} \exp [-(\bar{V}_r - V)^2/2\sigma_r^2] \quad (5)$$

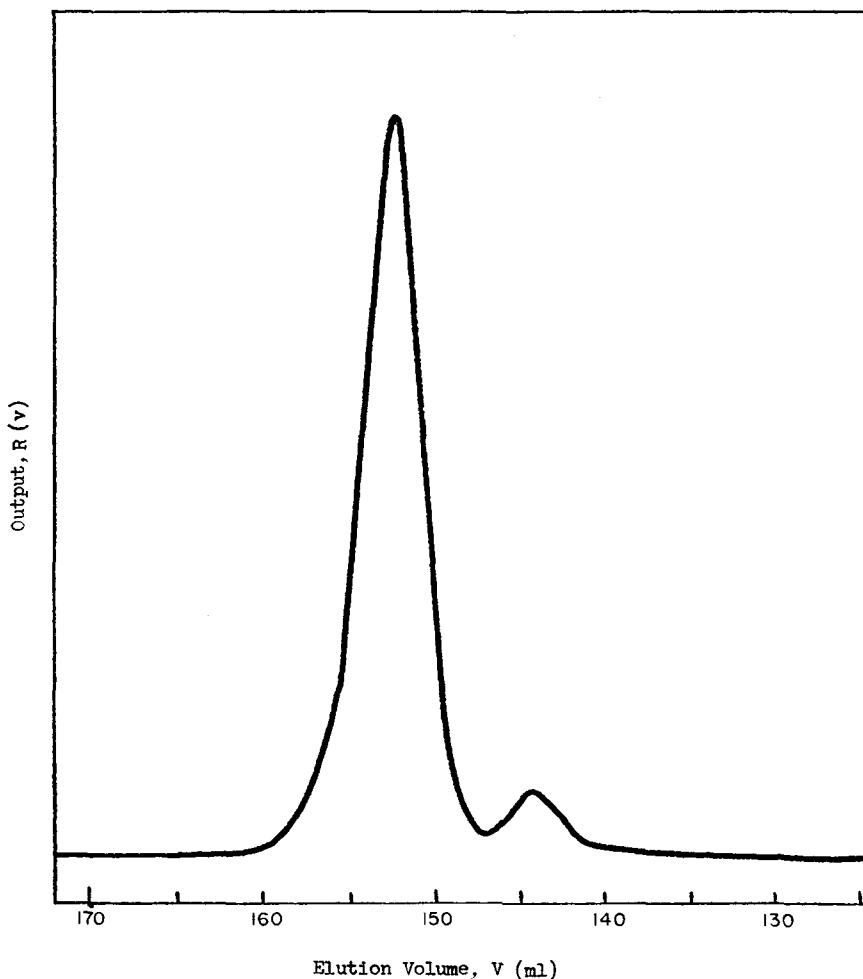


Fig. 1. GPC curve of N,N-diglycidyltribromoaniline.

In eq. (5), only the r -th component contributes to the ordinate value. This assumption, although appearing drastic, is actually generally valid for a small number of components (i.e., five or so). Taking the logarithm of the above yields

$$\ln R(V) = \ln \left[\frac{k' A_r}{(2\pi)^{1/2} \sigma_r} \right] - \frac{(\bar{V}_r - V)^2}{2\sigma_r^2}. \quad (6)$$

Differentiating eq. (6) results in

$$\frac{1}{R(V)} \frac{dR(V)}{dV} = \frac{1}{\sigma_r^2} (\bar{V}_r - V). \quad (7)$$

The quantity $[1/R(V)] [dR(V)/dV]$ can be measured directly from the chromatogram by measuring the slope, $[dR(V)]/dV$, and height, $R(V)$, at a given elution volume.

A graph can now be constructed of $[1/R(V)] [dR(V)/dV]$ versus V . Typically this is evaluated for increments of 1 ml; regions should appear where the curve through the data approaches a straight line with a negative slope. The slope of the line is $-1/\sigma_r^2$. Thus, by this graphical procedure the components of a multicomponent solute can be obtained.

RESULT AND DISCUSSION

As an example, a chromatogram of the final product in the synthesis of N,N-diglycidyl tribromoaniline is shown in Figure 1. Figure 2 represents the results obtained from the analysis of Figure 1 using the du Pont 310 Curve Resolver (E.I. du Pont de Nemours, Wilmington, Delaware).

The results of the numerical analysis, as described above, are presented numerically in Table I and graphically in Figure 3. A straight line has been drawn through those regions where the points seem to form a straight line with a negative slope. The data seem to indicate that there are four components present. From Figure 3, the peak elution volume, \bar{V}_r , is found to

TABLE I
Data Calculated From Figure 1

V , ml	$R(V)$, divisions	$dR(V)/dV$, divisions/ml	$1/R(V) \cdot dR(V)/dV$, ml^{-1}
135	0		
137	1.0	-0.462	-0.462
138	1.3	-0.462	-0.355
139	1.6	-0.288	-0.18
140	1.9	-0.300	-0.158
141	2.3	-1.23	-0.535
142	4.0	-4.0	-1.0
143	7.0	-3.76	-0.537
144	9.0	-1.85	-0.206
145	8.6	+3.7	+0.43
146	6.0	+3.7	+0.617
147	3.8	+1.82	+0.48
148	4.8	-3.31	-0.69
149	10.5	-24.3	-2.31
150	22.0	-63.7	-2.9
151	48.0	-92	-1.92
152	82.0	-112	-1.37
153	101	0	0
154	75.5	+75	+0.995
155	52.0	+110	+2.12
156	18.1	+28	+1.55
157	14.7	+16.9	+1.15
158	5.3	+5.93	+1.10
159	2.2	+4.31	+1.95

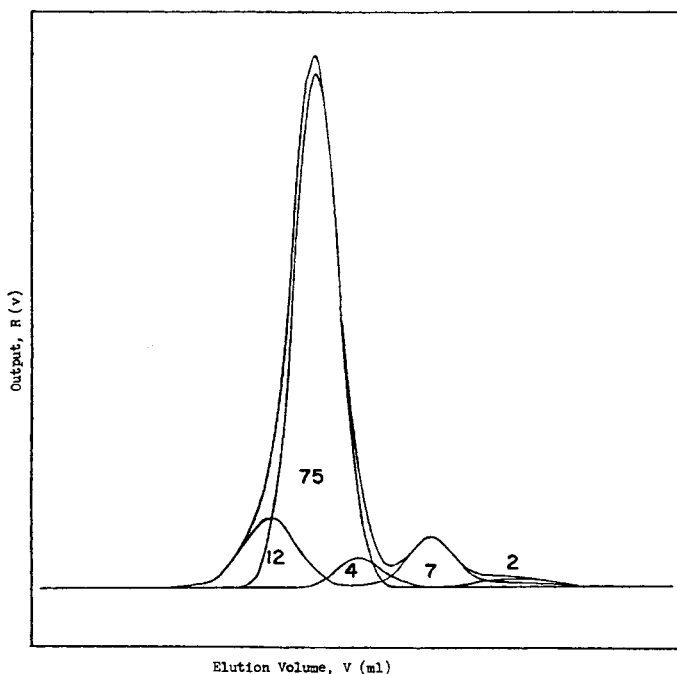


Fig. 2. Resolution of Figure 1 into its components by use of Curve Resolver.

be the value of V_r at the point where the straight line crosses the abscissa. The variance, \bar{V}_r^2 , is equal to the negative of the inverse of the slope of the straight line.

Equation (6) can now be solved by plotting $R(V)$ versus $(\bar{V}_r - V)^2$ on semilog paper. It is essential, however, to use only those points of the curve where the r -th component is the predominant one. Figure 4 is precisely this graph for the four component as indicated. Solving eq. (6) for the fractional area of the r -th component yields

$$A_r^f = \frac{y_r(0)\sigma_r}{\sum_i y_i(0)\sigma_r} \tag{8}$$

TABLE II
Resolution of the Chromatogram in Figure 1 Using the Curve Resolver, and the Graphical Technique

Component	σ^2	\bar{V}	\bar{V}	A_r^f	A_r^f
		from Fig. 4	from Curve Resolver	from Fig. 4	from Curve Resolver
1	1.21	156.5	156.6	0.12	0.12
2	0.85	153.0	153.0	0.73	0.75
3	2.27	144.4	144.3	0.11	0.07
4	6.87	140.0	138.5	0.04	0.02
			149.6		0.04

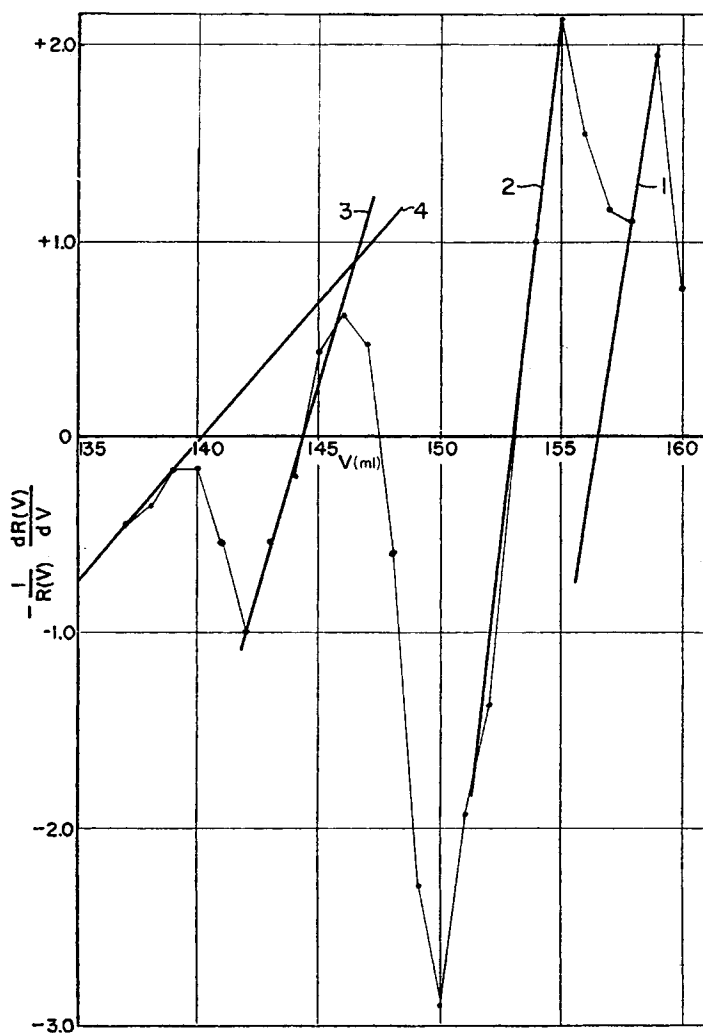


Fig. 3. Graphic presentation of data in Table I, to determine components of a chromatogram.

where $y(0)$ is that point where the line intercepts the y axis in Figure 4, and the summation sign is taken over the total number of components.

A comparison between the results found using the Curve Resolver and the graphic methods presented above is summarized in Table II.

Component 5 was detected on the Curve Resolver. Unfortunately, the graphic technique indicated above is not of sufficient sensitivity to adequately detect this peak. This is due primarily to the basic limitation of the procedure, i.e., the peaks must be "sufficiently" separated.

However, a comparison between the four remaining peaks shows that generally the agreement between the two methods is rather good.

The fractional areas found by any method in general are not necessarily proportional to either the weight or mole fraction present in the sample. This problem can be resolved by determining exactly what compound is eluting at a given \bar{V} . From this, n_r can be determined. Equation (2) can then be used to determine the number of grams of eluent.

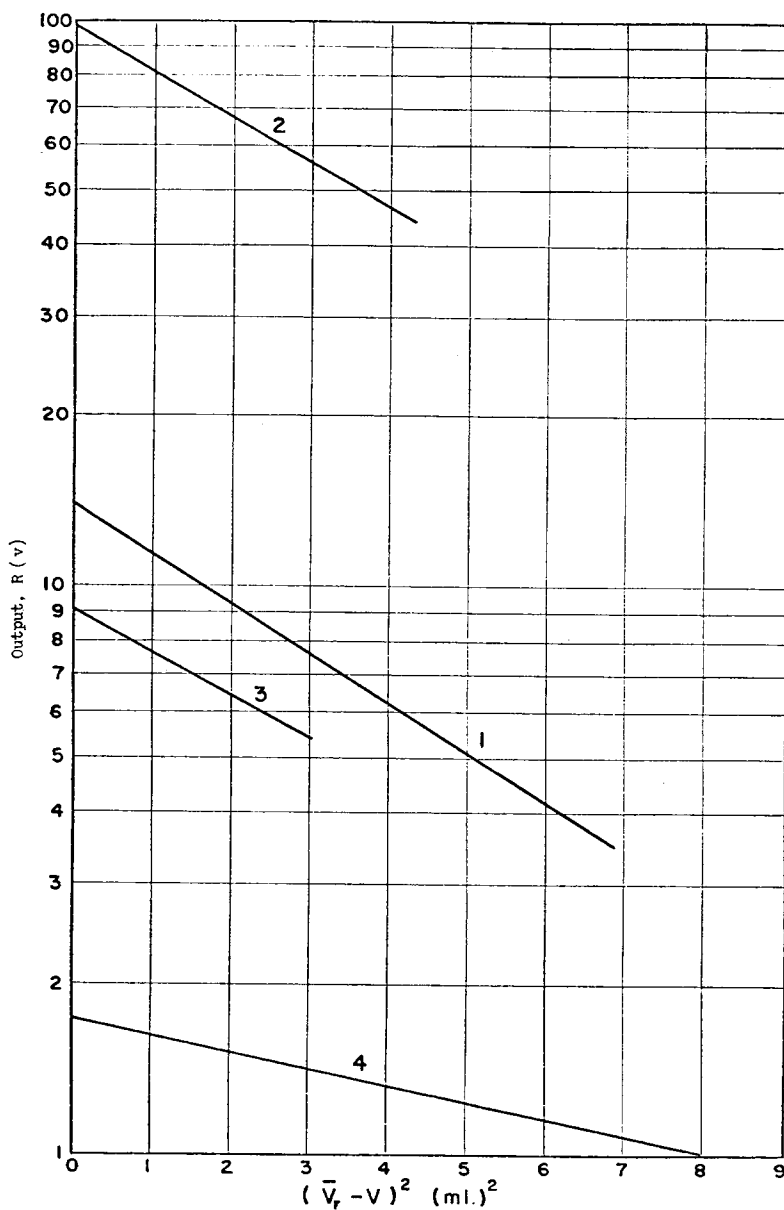


Fig. 4. Graphic method of determining fractional area of each component.

The weight fraction of component r , w_r^f can therefore be expressed as

$$w_r^f = \frac{A_r}{(n_r - n_1)} \bigg/ \sum_i \frac{A_i}{(n_i - n_1)} \quad (9)$$

where $A_i = y_i(0)\sigma_i$. For the case where all the components exhibit the same refractive index (as is approximately the case for polymer molecules that are homologues of the same monomer),

$$w_r^f = A_r \bigg/ \sum_i A_i.$$

SUMMARY

A graphic procedure has been outlined above, which is of general use in resolving chromatograms of multicomponent eluents.

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