Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Factors Affecting the Gel-Permeation Chromatographic Fractionation of Low-Molecular-Weight Compounds

Jack Cazes[a]; David R. Gaskill[a]
[a] Central Research Laboratory Mobil Chemical Company, Metuchen, New Jersey

To cite this Article Cazes, Jack and Gaskill, David R.(1967) 'Factors Affecting the Gel-Permeation Chromatographic Fractionation of Low-Molecular-Weight Compounds', Separation Science and Technology, 2: 4, 421 — 430
To link to this Article: DOI: 10.1080/01496396708049713
URL: http://dx.doi.org/10.1080/01496396708049713

SEPARATION SCIENCE, 2(4), 421–430 (1967)

Factors Affecting the Gel-Permeation Chromatographic Fractionation of Low-Molecular-Weight Compounds

JACK CAZES and DAVID R. GASKILL
CENTRAL RESEARCH LABORATORY
MOBIL CHEMICAL COMPANY
METUCHEN, NEW JERSEY

Summary

The effects of polarity and stereochemistry of oxygen-containing, low-molecular-weight substances upon gel-permeation chromatographic separations are examined. The basis for these separations appears to be differences in molar volume, together with some small adsorptive effects. Recommendations are made for the application of this size-separation technique to the identification of commercially important glycols and to the assignment of stereochemical configurations to low-molecular-weight compounds. It is also suggested that GPC might be useful for the preparative resolution of enantiomer mixtures by first converting them to diastereomers and then making use of the fact that diastereomeric substances can have different molar volumes.

Gel-permeation chromatography (GPC) has been used, increasingly, during the past few years, to solve a variety of polymer problems relating to molecular size (or weight) and/or molecular size (or weight) distribution. These have included studies of polymer synthesis and degradation and blending operations. Many of these could not be attacked by other means with any degree of facility or, even in some cases, at all. It is surprising that only a relatively small amount of work has appeared in the GPC literature involving low-molecular-weight, nonpolymeric substances, because it is in the region of small molecular sizes that the greatest resolution can be achieved with the GPC technique. This may be due, in part, to the observation that adsorptive forces can

play a major part in determining the elution volume of some polar, low-molecular-weight substances in a GPC column; these are forces that one tries to minimize, when working with polymers, by careful choice of solvent.

Studies aimed at the elucidation of the basis for GPC separations have involved examination of polymeric materials having different sizes and shapes (*1*) and attempts to correlate their GPC behavior with some parameter involving molecular size. These parameters include hydrodynamic volume [this hydrodynamic volume might be the same as that which is responsible for behavior of molecules flowing through a capillary viscometer (*2*)] or radius of gyration (*3*), or an extended chain length determined either by measurement of molecular models or from a table of experimentally determined effective contributions to chain length by various atomic groupings (*4*). It should be realized that any attempt at correlation of GPC data with the dimensions of a polymer, linear or volumetric, must be statistical in nature. This is due both to the fact that most polymers contain molecules covering a range of molecular sizes and to the fact that polymer molecules in solution are in constant motion and can assume a variety of conformations having a broad range of molecular dimensions.

Small, nonpolymeric molecules, on the other hand, offer an almost ideal situation for the study of various factors influencing the behavior of molecules in the GPC column. Smith and Kollmansberger (*5*) have shown that GPC elution volumes of some hydrocarbons and halogenated aromatics can be related to molar volumes. They did not examine very polar oxygen-containing molecules, however, and therefore did not observe any gross adsorptive effects.

Hendrickson and Moore (*4*) reported GPC results for a large number of low-molecular-weight compounds, both polar and nonpolar, and related their GPC elution volumes to their effective carbon numbers, i.e., the number of carbon atoms contained in the normal hydrocarbon having a GPC elution volume the same as that of the substance in question. The contributions of various atomic groupings to the effective carbon number were determined by examining several compounds containing a particular atomic grouping.

The present work was undertaken to examine the effects of polarity and stereochemistry upon the GPC fractionation of low-molecular-weight substances and to develop a technique for the fractiona-

tion of commercially important glycols which would be expected to be present in polyester coating hydrolyzates. It would be expected that GPC should offer a valuable tool for the identification of these materials, especially since it would be nondestructive and therefore permit isolation and identification by other physical and chemical means. Peak elution volumes show that resolution is at least adequate for this purpose (see Fig. 1). Marked differences in elution volume result from small changes in size and shape.

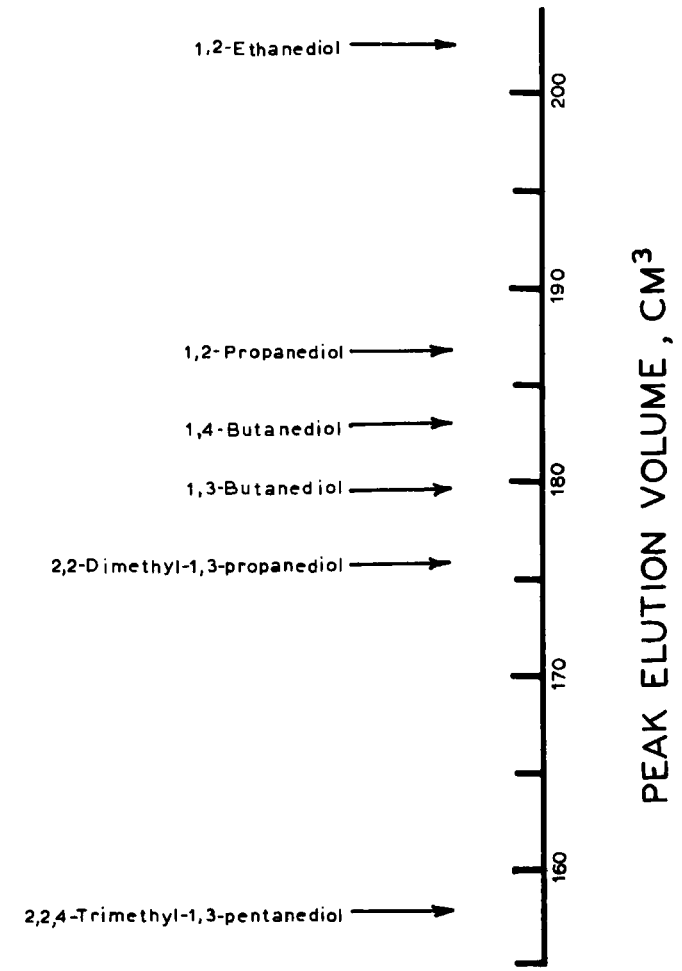

FIG. 1. Glycols.

EXPERIMENTAL

Gel-permeation chromatographic analyses were performed with a Waters Model 100 gel-permeation chromatograph equipped with an automatic sample injection system (manufactured by Waters Associates, Inc., Framingham, Mass.). Two milliliters of a 0.5 to 1.0% solution of the sample in *o*-dichlorobenzene was passed through four consecutive columns having exclusion limits of 3×10^3, 45, 45, and 45 Å, respectively. The columns were operated at 130°C and a flow rate of 1 ml/min. The eluting solvent was *o*-dichlorobenzene. The column substrate was a rigid, cross-linked polystyrene gel which was prepared and characterized by Waters Associates, Inc.

RESULTS AND DISCUSSION

An interesting observation is the emergence of 1,3-butanediol from the column earlier than 1,4-butanediol. This suggests that 1,3-butanediol is larger than its 1,4 isomer. Consideration of extended chain lengths would lead one to the à priori conclusion that the 1,4 isomer is the larger of the two and would emerge from the column before the 1,3 isomer. If one assumes that the concentration at which the GPC work was carried out (0.5% w/v) is low enough to make *intra*molecular hydrogen bonding more important than *inter*molecular hydrogen bonding, then the two glycols ought to assume the conformations shown in Fig. 2. It can be shown, with scale models, that the 1,3 isomer probably has the larger volume, owing to the bulky protruding methyl group. This is probably true in spite of the larger size of the "pseudo ring" in the *intra*molecularly hydrogen-bonded conformation for 1,4 isomer.

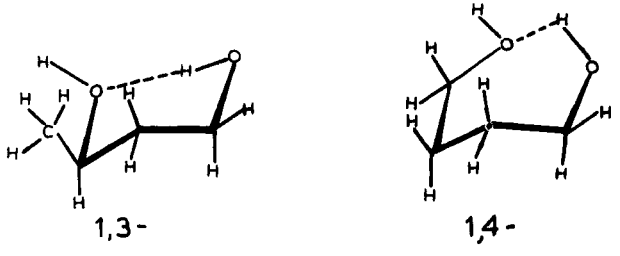

FIG. 2. Intramolecular hydrogen bonding in butanediols.

It seems useful, at this point, to compare the molar volumes of these compounds with their GPC elution volumes. Whereas molar volumes of most compounds at 130°C (the operating temperature of the gel-permeation chromatograph) are not normally available, they can be readily estimated by techniques discribed in the literature. The method of Lydersen (6) was chosen since it is relatively simple and reliable. Briefly, one first estimates the critical temperature, pressure, and volume for a compound using empirically determined group contributions for the various atomic groupings in the molecule, and then substitutes the resultant values in the appropriate equations for T_c, P_c, and V_c. The critical compressibility, Z_c, is then calculated:

$$Z_c = \frac{P_c V_c}{R T_c}$$

Referring to a table of reduced densities for substances having a given critical compressibility, one extracts a reduced density ρ_r at the temperature of interest (130°C). Molar volume is then calculated:

$$V_{130} = \frac{V_c}{\rho_r}$$

Whereas an estimate of molar volume based on such an involved series of steps might be expected to provide poor correlations, the values obtained by Lydersen for various types of compounds, both polar and nonpolar, agree quite well with experimentally determined values. Even better agreement should be obtained when experimentally determined critical constants are available for direct substitution into the critical compressibility equation above.

The glycols included in this work along with their GPC elution volumes and estimated molar volumes at 130°C are listed in Table 1. A series of straight-chain hydrocarbons is included to represent nonpolar molecules which should not be involved in hydrogen bonding and/or adsorption onto the column substrate. A series of straight-chain aliphatic acids was also examined to serve as representative of polar molecules which would be expected to show adsorptive properties involving the column substrate.

The computed molar volume of 1,3-butanediol given in Table 1 is larger than that of 1,4-butanediol. This is in agreement with the observed order of elution from the GPC column. A plot of elution volume versus the logarithm of the molar volume for the glycols

TABLE 1

Gel-Permeation Chromatographic Elution Volumes and Molar Volumes for Various Compounds

Compound	Elution vol.,[a] cm^3	Molar vol.,[b] cm^3/g mole
Glycols		
1,2-Ethanediol	202.5	65
1,2-Propanediol	186.7	88
1,4-Butanediol	182.9	101
1,3-Butanediol	179.7	108
2,2-Dimethyl-1,3-propanediol	175.8	125
2,2,4-Trimethyl-1,3-pentanediol	157.8	180
Hydrocarbons		
Styrene	171.2	106
Nonane	152.5	190
Eisosane	127.5	389
Hexatriacontane	113.8	624
Acids		
Acetic	194.4	62
Propionic	184.6	80
n-Butyric	178.4	98
n-Valeric	174.7	116
n-Hexanoic	168.9	133
n-Heptanoic	165.3	151
n-Octanoic	160.2	169
n-Nonanoic	157.1	185
n-Decanoic	154.0	204
n-Undecanoic	150.8	221
n-Dodecanoic	148.0	238
Myristic	143.1	271
Palmitic	139.6	305
Stearic	132.8	340

[a] All work was carried out with four columns in series having exclusion limits of 3×10^3, 45, 45, and 45 A, respectively (assigned by Waters Associates, Inc.) with *o*-dichlorobenzene at 130°C as the eluting solvent and a flow rate of 1 ml/min.

[b] Estimated by the method of Lydersen at 130°C [cf. (6)].

closely fits a straight line (see Fig. 3) which falls above a similar line obtained for the hydrocarbons. It may be that this noncoincidence of the two lines in the observed direction is due, at least in part, to adsorption of the glycols onto the column substrate and consequent increase in their elution volumes. Support is lent to

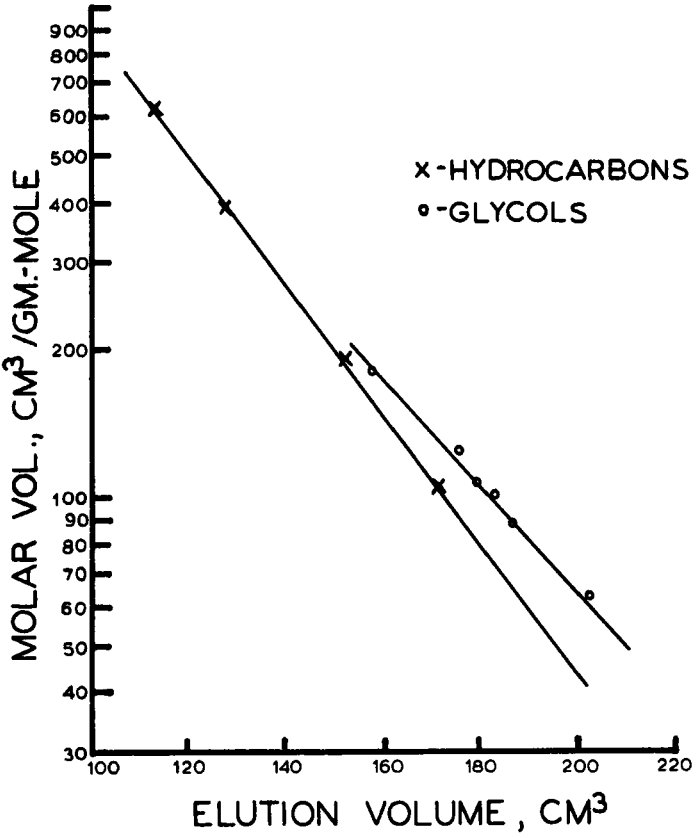

FIG. 3

this hypothesis by the fact that the correlation line for the glycols approaches the linear correlation line for the hydrocarbons at higher molecular weight; adsorption would be expected to be diminished with the increasing molecular weight of the glycols, since they become more hydrocarbon-like and less glycol-like as molecular weight increases.

A similar plot for the normal carboxylic acids is given in Fig. 4. These data also fall on a straight line *above* the hydrocarbons, and the acid line approaches the hydrocarbon correlation line at higher molecular weights in a manner similar to that observed for the glycols. In fact, the highest member of the series studied, stearic acid, falls directly on the hydrocarbon correlation line. Apparently,

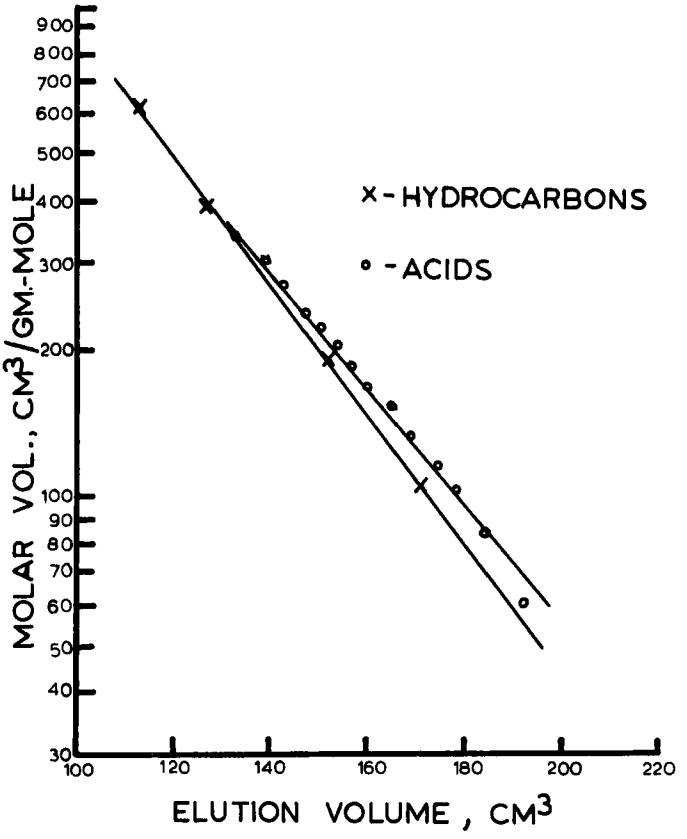

FIG. 4

the C_{17} alkyl chain of stearic acid makes it behave enough like a hydrocarbon to eliminate the absorptive property of the carboxyl group.

Several interesting and useful conclusions can be drawn here:

1. The basis for GPC separations is probably molar volume rather than some linear dimension of the molecule. One would instinctively expect this to be the case. It would be difficult to envision a situation where molecules would line themselves up in the proper orientation for entry into the gel pores. Therefore, consideration of chain length as the basis for GPC separations is probably not valid.

2. Adsorption onto the column substrate probably plays a significant part in determining the elution volume of a substance. This is evident from the observed locations of the linear correlation lines for polar molecules as compared to the line obtained for hydrocarbons.

3. This adsorptive phenomenon may be useful for studies of molecular structure. Estimation of molar volumes by GPC might be coupled with information regarding the presence or absence of certain functional groups and anticipated adsorptive properties to yield details on the structure of an unknown substance.

4. Stereochemical configurations of unknown substances might be determinable by GPC. For example, *cis* and *trans* configurations of cyclohexane derivatives might be assigned on the basis of GPC data. It has been observed in this laboratory that *trans*-1,2-dimethylcyclohexane emerges from the GPC column before the *cis* isomer, indicating the larger size of the *trans* compound. Molar volumes for these substances (determined by the method of Lydersen) are in agreement with the GPC results: *cis*-1,2,-dimethlycyclohexane, 150 cm^3/g mole, and *trans*-1,2,-dimethylcyclohexane, 171 cm^3/g mole.

5. Since diastereomeric substances have different molar volumes, GPC might be useful for the preparative resolution of racemic mixtures into their component enantiomers by conversion to diastereomers through appropriate chemical reactions, followed by GPC fractionation. A typical example might involve a racemic mixture of acidic enantiomers. A common method for the resolution of such a mixture is to treat the mixture with an optically active base, such as brucine, and then to separate the resultant diastereomeric mixture of salts by fractional crystallization. Another approach involves conversion of racemic mixtures of organic compounds to various molecular complexes, followed by, once again, physical fractionation. In all cases the object of the prior chemical treatment is to convert the mixture of racemates having identical molar volumes to a mixture of diastereomers having slightly different molar volumes. GPC offers a unique method for the fractionation of such mixtures, where sensitivity to minor differences in molecular architecture is required.

There is no doubt that gel-permeation chromatography will be used more and more in applications involving low-molecular-weight compounds, both organic and inorganic.

Acknowledgment

The authors wish to acknowledge the technical assistance of Mr. Thomas A. Kujawski in performing much of the experimental work for this study.

REFERENCES

1. H. Benoit et al., paper presented at the *3rd International GPC Seminar, Geneva, 1966.*
2. C. L. Rohn, *J. Polymer Sci.*, **A5**, 547 (1967).
3. S. Porath, *Pure & Applied Chem.*, **6**, 233 (1963).
4. J. G. Hendrickson and J. C. Moore, *J. Polymer Sci.*, **A4**, 167 (1966).
5. W. B. Smith and A. Kollmansberger, *J. Phys. Chem.*, **69**, 4157 (1965).
6. A. L. Lydersen, *Univ. Wisc., Eng. Expt'l. Sta. Rept. 3*, Madison, 1955. This method is described and critically discussed in R. C. Reid, and T. K. Sherwood, *The Properties of Liquids and Gases*, 2nd ed., McGraw-Hill, New York, 1966, Chap. 2.

Received by editor April 7, 1967
Submitted for publication May 8, 1967