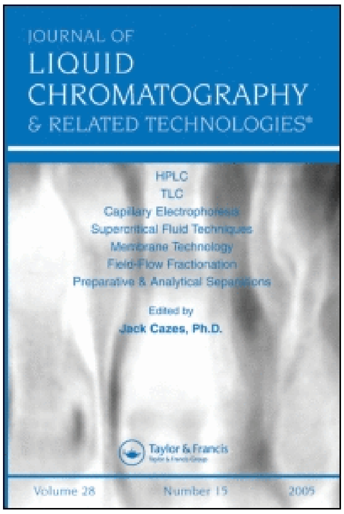

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Robert B. Walter (1)[a]; Julian F. Johnson[a]
[a] Institute of Materials Science and Department of Chemistry University of Connecticut Storrs, Connecticut

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DIMENSIONAL SEPARATION OF LOW MOLECULAR WEIGHT COMPOUNDS

Robert B. Walter (1) and Julian F. Johnson
Institute of Materials Science and
Department of Chemistry
University of Connecticut
Storrs, Connecticut 06268

ABSTRACT

The current status of separation of low molecular weight compounds by size exclusion chromatography and the hybrid modes size partition and size adsorption chromatography is reviewed.

INTRODUCTION

Size exclusion chromatography fills a void created by other separation methods which require, for resolution, sufficient differences in volatility, solubility, polarity, adsorptive behavior, or ionic character between the components, of interest, in a mixture. Selective retention is instead accomplished when differences between the components' solvated volume exist, as this defines the fraction of inert porous column packing available to each species. All components therefore elute within a characteristic volume range (the column's "peak capacity"), which is bounded by a total permeation volume and a total exclusion volume.

The method was initially applied by Porath and Flodin to the fractionation of water-soluble polymers on cross-linked dextran gels (2). Vaughan (3) extended the concept to non-aqueous systems by swelling cross-linked polystyrene beads with benzene which allowed the resolution of polystyrene fractions. The technique's application to non-polymeric, low-molecular weight compounds was pioneered by Cortis-Jones (4). The table below, lists the observed behavior with benzene as the solvent and cross-linked polystyrene gels as the stationary phase.

The guidelines within which exclusion chromatography could be used to characterize non-oligomeric systems were recognized early as summarized in the author's following comments. "The results suggest that gel filtration, as described by Porath and Flodin, is part of the mechanism involved in fractionation. However, the nature of each substance appears to be at least as important as its molecular weight (4).

The purpose of this review is to pragmatically assess the method's current status as an analytical tool for characterizing non-polymer, low molecular weight compounds.

HISTORICAL

Hendrickson (5,6) using tetrahydrofuran (and later benzene) as the mobile phase and polystyrene-divinyl benzene gel as the substrate, compiled extensive amounts of data for the purpose of

TABLE 1

Effluent Volumes At Peak Concentrations (4)

Substance	Molecular Weight	Volume (ml)
Acetaldehyde	44	165
Ethylamine	45	160
Ethanol	46	150
n-Pentanol	88	153
Furfural	96	167
Resorcinol	110	200
1-Naphthol	144	172
Alizarin	240	165
Quinalizarin	272	152
Cholesterol	387	126
Thymolphthalein	430	132

developing the method as a quantitative "liquid phase spectrometer". The elution behavior of several compound classes was related to that of n-alkanes by calculating an effective chain length of carbon atoms. For a number of compounds, it was possible to resolve the contribution of the different structural features of a molecule to its retention volume. While the effective volume was the determining parameter, the relative measurement of effective chain length allowed a more quantitative evaluation of structural contributions. The linear correlations obtained, however, were limited to specific solvent/substrate systems.

Cazes et al (7,8) studied the effect of molecular structure in a different manner. They chose to relate a component's elution behavior with its molar volume, calculated by the method of Lydersen (9). Using o-dichlorobenzene at 130°C as the mobile phase, the effects of solute-solvent and solute-substrate interaction, on a component's elution behavior were resolved. Solute-solvent association caused earlier departure from the column than an n-alkane of like molecular weight and conversely, solute-substrate interaction retarded its elution. Thus by judicious choice of solvent and a knowledge of the solute's functional group character, one could either amplify or degenerate a column's inherent resolving power by taking advantage of non-exclusion effects.

Dubin (10) illustrated how selectivity could be altered with the same solvent, DMF, by changing column packings (Table 2).

Using dimethylforamide again as the mobile phase and styragel as the exclusion medium, Feurer and Gourdenne (11) studied the solute-solvent interactions for a recurrent series of nitrogeneous aromatic species. As expected, it was found that solvation, hence a molecule's elution volume, increased as the unpaired electrons became less delocalized.

HIGH EFFICIENCY COLUMNS

With the advent of small diameter (microparticulate) high-performance packings, much of the previous work involving non-

TABLE 2

Elution Volumes Low Molecular Weight Solutes[a] (10)

	toluene	dioxane	N-methyl pyrrolidone	pyridine	phenol	aniline
untreated glass[b]	60.5	60.3	62.0	60.8	60.5	59.8
silanized glass[b]	61.8	61.5	61.5	61.8	60.6	60.8
Styragel[c]	69.5	63.5	--	--	58.4	56.2
Styragel (THF)[c]	58.4	60.0	60.0	60.0	52.8	53.7

[a] All column sets comprised of four 1/4 in. OD x 40 in. columns.

[b] 700Å + 350Å + 170Å + 75Å CPG-10

[c] 10^5Å + 10^3Å + 10^2Å + 60Å

exclusion effects was neglected in favor of optimizing the practical aspects of the technique, those of speed and simplicity. Majors (12) has done an excellent job of categorizing the various columns available on the market. These columns are usually obtained prepacked from the vendor because packing them for maximum efficiency is an art.

Krishen (13,14) has reviewed the application of the high-efficiency, semi-rigid, U-Styragel columns to the characterization of small molecules. These supports are very efficient micro particulate packings. However, their fragile nature usually limits the choice of solvents to the one in which they are shipped. As a consequence, the user is limited, in most cases. to tetrahydrofuran as the mobile phase. While not as critical for molecular weight characterization of homologous polymers, this limitation is an important consideration when dealing with mixtures containing non-oligomeric compounds of varying character. To compensate for this, an empirical "size factor", F, was introduced to correct for non-exclusion effects (13). However, upon close examination, it was observed that F varies considerably, even between members of the same functional group. In addition, the peak capacity of microparticulate columns is ten times less than conventional exclusion columns, 5mL vs 50 mL. As a result, attempts to correlate elution volume with molecular weight for other than simple mixtures probably should be avoided.

Rigid silica-based supports permit the variation of mobile phase as well as temperature to maintain exclusion behavior. Originally applications were limited to the controlled pore size glasses. However, the development of the more efficient microparticulate packings for adsorption and partition chromatography has been extended into the size exclusion mode with the advent of porous microspheres, PSM (15,16) and the application of silica glasses (17,18,19). Of these two, the PSM substrates are the most interesting because they are available with pore sizes under 100Å. These microspheres are produced by the aggregation of uniform-size colloidal silica particles to form interconnecting three-dimensional lattices. Their versatility is offset somewhat by possible solute adsorption on the surface silanol groups. In addition this packing exhibits sample overloading behavior in that retention volumes increase with loading (18).

FUNDAMENTAL STUDIES

In reviewing the literature it became apparent that separation of non-oligomeric mixtures via size exclusion chromatography alone was the exception rather than the norm. Non-exclusion behavior was reported on organic gels (19-24) as well as on inorganic media (10,18,26).

The elution volume, V_R, of a solute molecule in exclusion chromatography is characterized by $V_R = V_o + K_{SEC} V_P$, where V_o, K_{SEC} and V_P are the interstitial volume, the partition coefficient, and the pore volume, respectively.

To account for other possible retention mechanisms, the partition coefficient needs to be expanded so that $K_{SEC} = K_{\Delta S}K_{\Delta H}$, where $K_{\Delta S}$ is the entropic and $K_{\Delta H}$ is the enthalpic contribution (24). When equilibrium between the mobile and stationary phases exist; $\Delta G = -kT \ln K_{SEC} = \Delta H° - T\Delta S°$, so that $K_{\Delta S} = e^{\Delta S°/K}$ and $K_{\Delta H} = e^{-\Delta H°/kT}$. Pure size exclusion behavior ($K_{\Delta H} = 1$) is thus observed only in the absence of solute interactions with the stationary phase. Hence, the nature of the stationary phase determines the secondary mechanism which will supplement size exclusion.

Non-exclusion behavior on organic gels has been explained in terms of partition chromatography (21,25) and on inorganic gels by adsorption chromatography (18,26). Both partition and adsorption have been observed on inorganic gels using mixed solvents (26). One investigator was even able to relate Snyder's (27) equation for adsorption chromatography to his observations of partitioning between benzene and styrene/divinyl benzene gel (19).

Freeman (25) has dubbed non-exclusion behavior on organic supports as "gel partition chromatography". He evaluated the behavior observed with n-alkanes partitoned between copoly(isodecylmethacrylate/DVB) gel and various solvents, with respect to the following equation:

$$\ln K = -AL + \frac{\bar{V}_i}{RT}[(\delta_i-\delta_m)^2-(\delta_i-\delta_g)^2] + \ln(\bar{V}_g V_p/\bar{V}_m V_S).$$

A is the specific pore area, L is the mean external length of the solute, $\bar{V}$ is the molar volume, $V_S = V_o + V_P$, and δ is the Hildebrand solubility parameter, where i, m and g refer to the solute, mobile phase and gel respectively. Figure 1 exhibits the excellent correlation obtained between experimental and calculated values when solvents of various polarities were employed.

The enthalpic contribution to the separation can therefore be predicted by the solubility parameters of the participants. However, unlike pure liquid-liquid chromatography, partitioning diminishes with increasing molecular size because the pore structure of the gel limits the area available for solute-gel interaction. Hence a more accurate label for this approach would be "size-partition" chromatography of SPC.

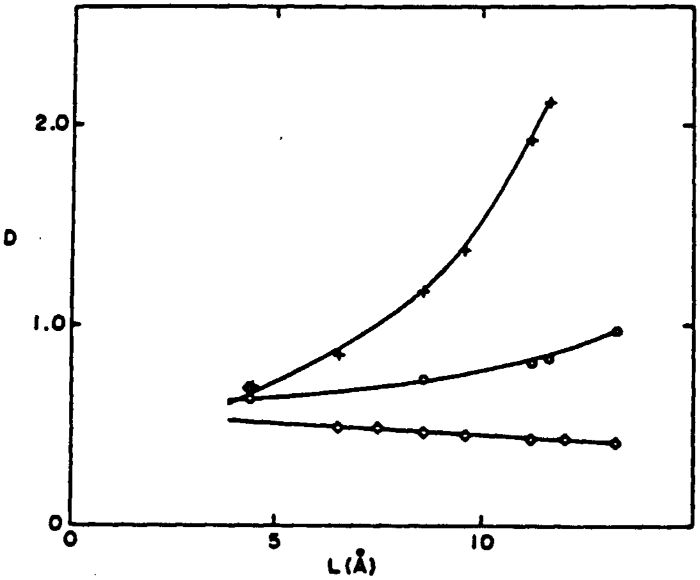

FIGURE 1 Chromatographic distribution coefficient measured for n-alkane solutes using different solvent systems: methanol (+), ethanol (O), and cyclohexane ( ) (25).

Klein and Treichel (18) developed an analogous equation for adsorption occuring during size exclusion on silica gel:

$$V_R = V_o + [(1 - \frac{a}{r})^2 + \frac{S_i'}{S}]V_P.$$

Exclusion behavior was described by $(1 - \frac{a}{r})^2$ which assumes spherical solutes of radius a, and cylindrical pores of radius, r. The relative amount of surface area available for adsorption is given by S_i'/Si. The net behavior resulting from the two mechanisms is shown in Fig. 2 for oligomers of polyethylene glycol.

As a result, a new chromatographic method (which we shall label S.A.C., "size-adsorption" chromatography) was proposed (18): "Given a proper choice of eluent and porous adsorbents with 'tailored' pore size distribution, the higher relative molecular mass, more strongly adsorbed molecules are excluded from the small pore with higher specific surface and are eluted earlier than from

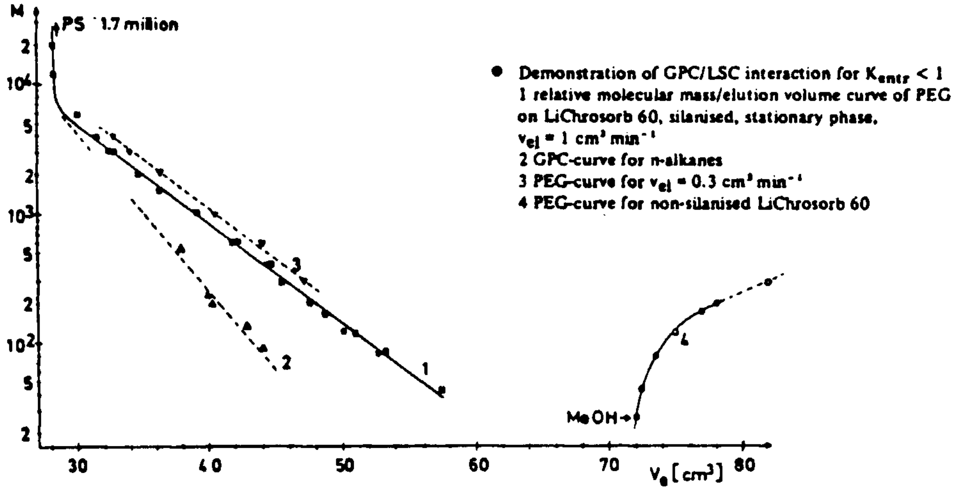

FIGURE 2 Demonstration of GPC/LSC interaction for K_{entr}<I.
I relative molecular mass/elution volume
curve of PEG on LiChrosorb 60, silanised, stationary
phase (18).
$v_{el} = 1\ cm^3\ min^{-1}$.
2GPC-curve for n-alkanes
3PEG-curve for $v_{el} = 0.3\ cm^3 min^{-1}$
4PEG-curve for non-silanized LiChrosorb 60

columns filled with adsorbents having the same surface but without steric exclusion."

In aqueous systems, Cooper and Matzinger (28) have demonstrated that the resolution of low molecular weight anionic solutes, on controlled pore glass (CPG), can be enhanced by ion exclusion as shown in Figure 3. Ion exclusion results from the electrostatic double layer lining the silica gel's surface. The thickness of the double layer, hence the accessibile pore volume, can be tailored for the desired molecular weight range by varying the ionic strength of the solvent. This approach differs from the secondary partition and adsorption mechanisms, previously described, in that manipulation is confined to within the column's inherent peak capacity, i.e. the total permeation volume (V_o+V_p) is not exceeded.

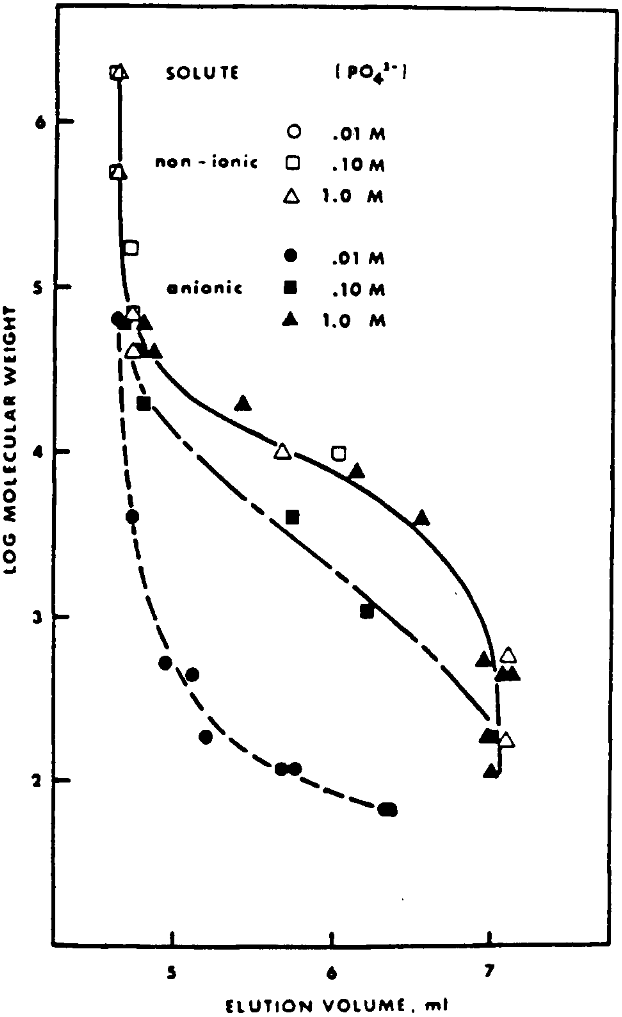

FIGURE 3 GPC calibration curves for the CPG 75-Å column (28).

APPLICATIONS

Table 3 presents applications of size exclusion chromatography to the characterization of non-polymeric low molecular weight compounds, reported in the literature.

TABLE 3

Applications of Exclusion Chromatography to Non-Oligomeric Compounds

Compounds	Reference
Aliphatic and aromatic alcohols	(5-7)
Antioxidants	(29-34)
Carbonyls	(5)
Diols	(8)
Organic acids	(5,6,8)
Organic halides	(5)
Organometallics	(35)
Pesticides	(36,37)
Plasticizers	(36,38,39)

CONCLUSION

The main objective of this review was not to duplicate previous literature compilations (14,40) but to critique the current status of the method. In this context, it is hoped that more questions were generated than answered.

The application of the hybrid modes, size-partition (SPC) and size-adsorption chromatography (SAC) to the analysis of complex mixtures should be of great interest. As chromatography is a serial method, late elution of unwanted compounds has always been a problem in the homogeneous enthalpic techniques. As a consequence, backflushing, column switching, or pre-injection clean-up procedures are employed to reduce analysis times. The fact that in SPC and SAC, the retentive surface is less for the larger components of a mixture should prove to be a strong advantage for the method's application.

In summary, while non-oligomeric size exclusion chromatography does not enjoy the inert, simple behavior found in homologous polymeric applications, the potential for further development of the technique seems to lie in its application to small molecules. Further work therefore should be directed towards relating the participating mechanisms to their base method. Thus, while SPC has been directly related to partition behavior through the solubility parameter (δ), SAC still needs as refined a treatment.

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