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Klaus H. Altgelt[a]
[a] CHEVRON RESEARCH COMPANY RICHMOND, CALIFORNIA

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Gel Permeation Chromatography with High Loads*

KLAUS H. ALTGELT
CHEVRON RESEARCH COMPANY
RICHMOND, CALIFORNIA 94802

Summary

High loads on GPC columns usually lead to poor efficiency because of steep viscosity gradients. A great difference in density between solution and solvent can also cause excessive band broadening. However, under certain conditions good separations are achieved with loads of 150 mg/100 cc column volume and higher. Two mechanisms are proposed to explain this phenomenon. Secondary exclusion is caused by obstruction of pores to larger molecules by the more rapidly diffusing small molecules. It takes place predominantly with molecules of less than 2000 molecular weight in small pore gels. Incompatibility is caused by repulsive interaction between solute molecules and the polystyrene gel. It is observed with solutes which are chemically quite different from polystyrene, e.g., with polyvinyl acetate, and in a low to intermediate molecular weight range.

THE EFFECT OF LOAD ON COLUMN EFFICIENCY

Generally speaking, GPC columns should not be overloaded. High sample viscosity and ensuing pressure gradients lead to drastic drops of column efficiency. This was first pointed out by Flodin in 1961 (*1*) and was confirmed by many others. The sample load is restricted by two limitations: (a) the sample viscosity should not be different from the solvent viscosity by a factor greater than 2 (*1–3*); (b) the sample volume must be small since it increases linearly the width of a zone, i.e., the peak width (*4–5*). Taking these two effects into account, most researchers limit their sample size to about 15 mg/100 cc column volume.

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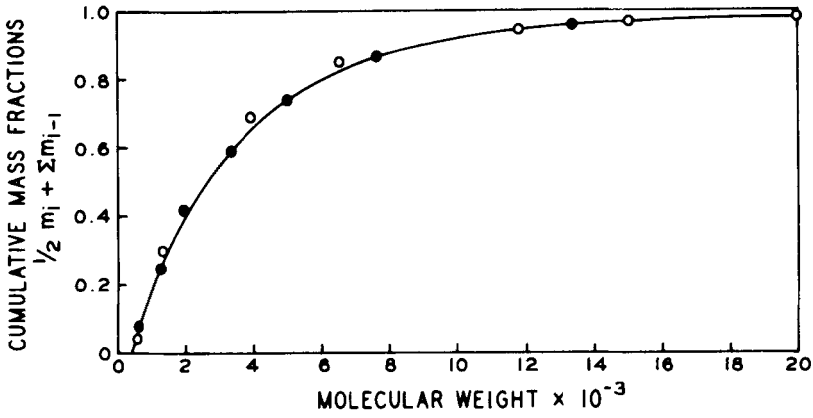

FIG. 1. Molecular weight distribution of a polybutene determined by GPC (○) and gradient elution chromatography (●).

On the other hand, we obtained surprisingly good separations with loads 10 times as great (*6*). Figure 1 compares the molecular weight distribution of a polybutene sample obtained by GPC with that obtained by gradient elution chromatography. The agreement is very good. Column load in the GPC separation was 1 g polybutene in 10 cc benzene + 10% methanol on a column with 600 cc total volume. Such good separation with high load is not a contradiction to previous findings but primarily a consequence of the low molecular weight of our samples (1200) as opposed to those of Flodin's (*1*) and others (100,000–2,000,000). Because of the relatively low intrinsic viscosities of our samples, we could use 10% solutions, in some cases even 20% solutions (*7*).

SECONDARY EXCLUSION

Some of our separations with highly loaded columns were so good that we searched for an explanation beyond that of undistorted flow due to low sample viscosity. Thus we came upon a separation mechanism in GPC which we named "secondary exclusion" (*5*, *8*).

To understand the secondary exclusion mechanism, we must examine how the sample molecules diffuse into and out of the gel pores. The diffusion rate of a molecule in a gel depends not only on its size as it does in bulk liquid, but furthermore, on the ratio of the molecular size to the pore size. This is illustrated in Fig. 2. If we put a concentrated solution of small and large molecules onto a gel, then the small ones

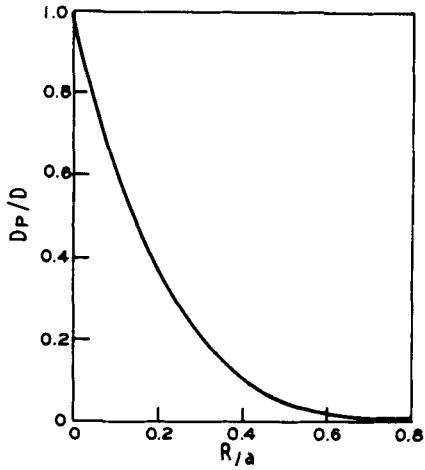

FIG. 2. Dependence of diffusion coefficient, D_p, of a spherical molecule in a pore on the ratio of particle radius, R, to pore radius, a. D is the diffusion coefficient in free solution. Reproduced from Ackers (*9*).

will rapidly diffuse into the available pores. The large ones, since they diffuse more slowly, will find more pores occupied. The probability of their diffusing into an occupied pore will be reduced depending on the reduction of available pore size and on the motion of the smaller molecule in the pore. If the small molecule is moving away from the oncoming larger molecule, it will have no effect. If it is moving toward it or lateral to it, it will obstruct the other molecule. In case of obstruction, the larger molecule will move on until it finds an unobstructed pore. Thus, it is effectively excluded from a pore which otherwise would have been accessible. This is secondary exclusion.

We tested our hypothesis with mixtures of a straight chain hydrocarbon, octacosane (C_{28}), and a narrow polystyrene standard of 900 molecular weight in chloroform on a polystyrene gel with an exclusion limit of about 3000 molecular weight. The materials were chosen such that (a) both molecular weights were low enough to permit high loads at moderate viscosities, (b) the low molecular weight sample consisted of molecules distinctly larger than the solvent molecules, and (c) the large molecules were small enough to fit into about 30% of the total gel pore volume. Figure 3 shows the elution pattern at low loading conditions. The limiting peaks, i.e., the first and the last ones, indicate the void volume, $V_0 = 44$ ml, and the total available column volume, $V_0 + V_i = 100$ ml, respectively. At this concentration, 15 mg/column

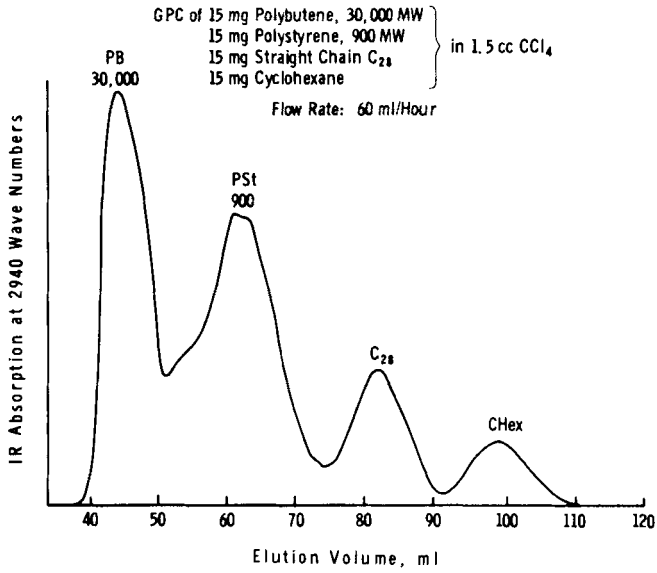

FIG. 3. Elution pattern of Polystyrene 900 and of octacosein under low load conditions.

volume (100 ml), the elution volume of the octacosane was 82 ml and that of the PSt 900 was 62 ml. Figure 4 demonstrates what happened at high loads, viz., 300 mg C_{28} and 50 mg PSt 900. The elution volume of PSt 900 shifted from 62 to 47 ml, i.e., almost all the way to V_0. Most of the C_{28} eluted with a peak at 65 ml leaving only a shoulder at its previous elution volume of 82 ml. At this high load not only the polystyrene had experienced secondary exclusion, but also part of the octacosane. An interesting detail is the fact that in this case the polystyrene had been injected not together with, but after, the octacosane. Thus it had literally to overtake the C_{28} molecules.

While I believe I have demonstrated the existence of the effect of secondary exclusion, I must point out that it seems to be restricted to a relatively narrow range of experimental conditions. We were not able to see it in gels of greater pore size. This restriction to small pores may perhaps be explained by the structure and "roughness" of the pore walls. Close examination of a polystyrene gel, Fig. 5, reveals that its walls consist of dense clusters which resemble grapes. The large pores are much less confined in that they have more connections with neighboring pores than do the smaller ones. In the extreme, the smallest

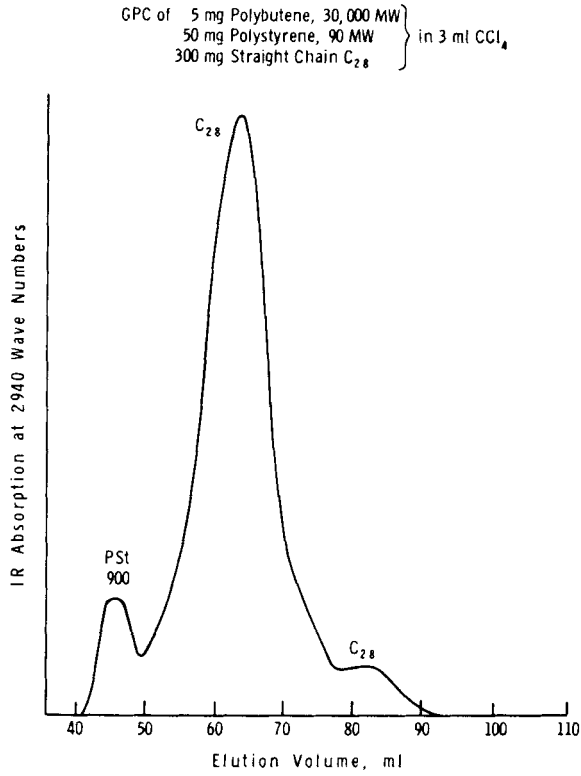

FIG. 4. Elution pattern of Polystyrene 900 and of octacosein under high load conditions.

pores are just crevices or holes in a wall and have just one opening. In pores with many openings a molecule has that many more ways of leaving the pore and thus not being in the way of an oncoming molecule.

In another paper given at this Symposium (*10*), Albaugh et al. also describe peak shifts toward V_0 in cases of high loads with no appreciable loss in separation power.

INCOMPATIBILITY

Another effect that may be encountered under conditions of high load is additional exclusion due to incompatibility between sample and gel. Incompatibility of two types of polymers has been described by various authors (*11–15*). It is caused by the low entropy of mixing

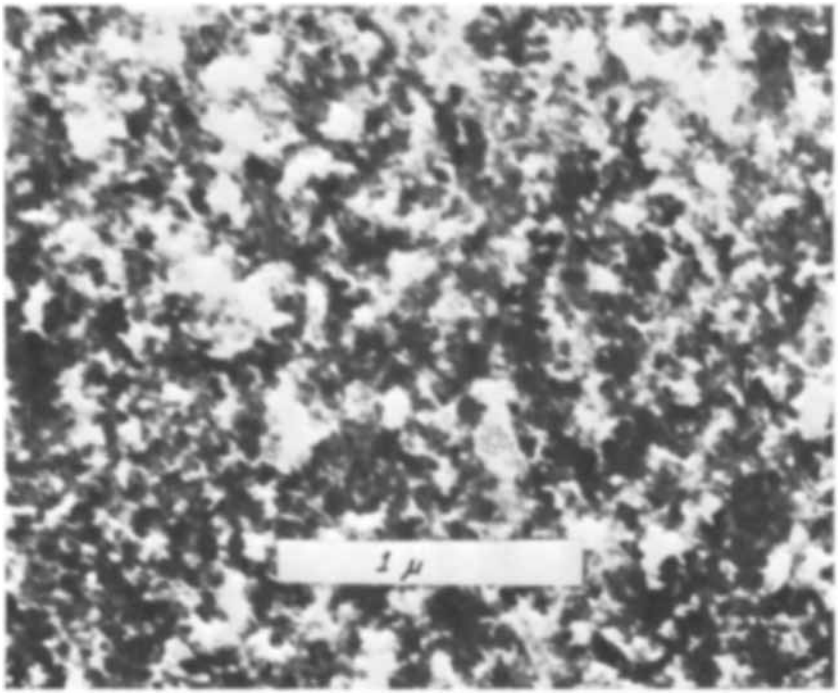

FIG. 5. Electron micrograph of large pore polystyrene gel.

between different macromolecules which cannot overcome even relatively weak positive heats of mixing and which thus leads to positive free energies of mixing. As a consequence, mixing does not take place, i.e., phase separation occurs at sufficiently high polymer concentration. The occurrence of phase separation is only dependent on the structure, size, and concentration of the two polymers; it is not affected by the solvent. Phase separation due to incompatibility takes place in good as well as in poor solvents.

In GPC one polymer is the gel, the other the sample. Here phase separation means complete exclusion. Complete exclusion would be an extreme case and is unlikely to be encountered in practice. However, partial exclusion due to incompatibility (*3*, *6*) does take place and can be demonstrated. The calibration curve in Fig. 6 was established with polystyrene and with straight chain polymethylenes (C_6–C_{32}). The two sets of points representing high and low concentrations of polyvinyl acetate fractions are shifted toward smaller elution volumes.

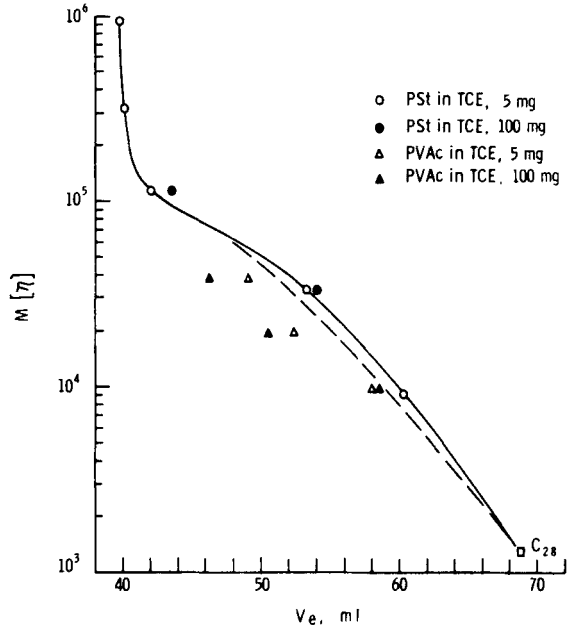

FIG. 6. Calibration curve of the polystyrene gel used for the present investigation. The shift of the PVAc points to the left is interpreted as incompatibility between the PVAc and the polystyrene gel.

This shift cannot be due to swelling or chain stiffness since the ordinate in our plot is not the molecular weight but the molecular size in terms of $M[\eta]$ (*5*, *16*). Also, it can not be secondary exclusion in this case as the concentration dependence of the polystyrene samples on this gel was normal and opposite to that of the polyvinyl acetate fractions, see Fig. 6. An effect arising from repulsive interaction between solute and gel, i.e., incompatibility, seems to be the only explanation under these conditions.

The effect of incompatibility too is restricted to a limited set of conditions. While the molecular weight of the sample must be low enough to permit high loads, it must, on the other hand, be high enough to lower the entropy of mixing sufficiently to overcome the enthalpy term in the equation

$$\Delta F = \Delta H - T\Delta S$$

According to Fig. 6 the effect is quite small in the case of the 500 mol wt polyvinyl acetate and can be expected to be negligible at lower molecular weights for this polymer–gel combination.

VISCOSITY AND DENSITY EFFECTS

The detrimental effect of viscous samples in columns was described in the early days of GPC by Flodin (*1*). Less well known is the tailing that is caused by high viscosity and originates in tubings of the kind used for connections and sample loops in GPC systems. This is the subject of other presentations in this Symposium (*17*, *18*) and need not be elaborated here.

Highly concentrated samples may be quite different in density from the solvent. Examples are hydrocarbons in chlorinated solvents. In such cases, broad peaks may result from two causes. First, fingering due to steep density gradients may occur in the column bed. We had occasion to observe this with colored samples such as azulene in carbon tetrachloride. Again, less well known is the second cause, viz., the layering which can take place in tubings. Initially, we used ⅛-in. tubing for our sample loops. With colored samples, e.g., asphalt solutions, we could see how chloroform or carbon tetrachloride did not displace the solution as a plug but instead took off layer after layer starting at the bottom and slowly working its way to the top of the horizontally positioned sample loop. This problem was easily solved by replacing the ⅛-in. by $\frac{1}{16}$-in. tubing.

CONCLUSIONS

Reasonably good separations can be achieved with over-loaded columns if care is taken to avoid relative viscosities above 2 and to minimize density effects. Secondary exclusion and incompatibility enhance separation but may lead to great errors if calibration curves are used indiscriminately. Secondary exclusion predominates in the low molecular weight range, incompatibility at higher molecular weights. It should be possible to exploit both effects for improving separations on a preparative scale.

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