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Programmed Exclusion Chromatography: A Method for the Continuous Control of Retention

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COMMUNICATION

Programmed Exclusion Chromatography: A Method for the Continuous Control of Retention

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Summary

Means are proposed for controlling retention and thus for instituting retention programming in exclusion chromatography. Control is to be gained by the addition of a high molecular weight polymer to the incoming solvent. Preliminary data confirm that such a polymer strongly affects retention.

INTRODUCTION

One of the chief shortcomings of exclusion (gel filtration and permeation) chromatography is that retention is limited to the range between the interstitial or mobile phase volume, V_m, and the total solvent volume, $V_m + V_s$, which is roughly $2.5V_m$ (*1*, *2*). By contrast most GC and LC systems have an unlimited maximum retention time because the stationary phase shows a variable affinity, not just an exclusion effect, toward solute. Fewer solute peaks can be fit into the limited retention range of exclusion chromatography; with a column of similar dimensions and theoretical plates the peak capacity is reduced about four times (*1*). In order to separate the same number of peaks the column length would need to be increased an impractical $4^2 = 16$-fold beyond that used for GC or LC.

Not only is the retention range limited in exclusion chromatography, but particular retention times within that range are difficult to influence. A retention shift ordinarily occurs only with changes in the dimensions of the solute. These dimensions, if they change at all with

solvent and temperature, may change discontinuously. Thus it is difficult to use solvent composition and temperature as parameters for the control retention (*3*).

PROPOSED METHOD

Proposed here is a method for the control of retention and for extension of the retention range. This control is to be gained by adding to the incoming solvent a certain percentage level of a high molecular weight polymer whose molecules are too large to penetrate the pores of the gel. In this way the thermodynamic properties of the mobile phase would be selectively controlled by variations in polymer percentage and composition. The stationary phase, within the pores, would presumably remain free from the dissolved polymer and retain its original solution properties. (The conditions needed to achieve this are somewhat less than obvious, and require further investigation.)

This proposed method is related to the observations of Edmond et al. (*4*) (extended in the Experimental Section) that a background polymer will alter retention values. However, these authors attributed the change to the osmotic shrinking of the gel which would have an effect opposite to the mechanism proposed here.

If the added polymer species and the solvent interact equally with the solute species, the alteration of the equilibrium constant will be determined by exclusion effects in the mobile phase (originating with the reduced entropy of molecules restrained by the presence of neighboring molecules) (*5*, *6*). This effect depends solely upon molecular dimensions, so that the method, like gel filtration chromatography itself, will achieve size-dependent separations. Such could be obtained, in fact, without the gel, as in combination with adsorption chromatography, partition chromatography, or column fractional precipitation.

One can also imagine a polymer with groups designed to provide a certain selective chemical interaction. Similarly polyelectrolytes might be useful in providing a "salting out" effect. This would also decrease the effective volume of the mobile phase relative to that of the stationary phase, an increase which theory shows to increase both peak capacity and resolution (*3*).

A programmed variation in the polymer percentage would, of course, yield a programmed retention, and would therefore show all the advantages of programmed chromatography in general. One would ordinarily start with a polymer percentage high enough to cause the strong retention of most species. Upon the reduction of this percentage

the various species would acquire, one at a time, a significant migration rate.

Because a high molecular weight polymer solution behaves toward a solute species much like a cross-linked gel (*5*), this system provides an effective gel–gel partitioning, with the "pore size" of the mobile phase "gel" continuously variable and programmable. However, as will be noted in the Experimental Section, ordinary gels shrink with the addition of polymer because of osmotic effects (*4*). Therefore an addition of polymer intended to decrease the effective pore size in the mobile phase will also somewhat decrease pore size in the stationary phase. Since the stationary phase differs from the mobile phase by the restraint of cross-linking, its pores will ordinarily remain smaller than those in the stationary phase. With this system it would be difficult to make the distribution coefficient, K_D, greater than unity, as desired. However, if the stationary phase porous network were rigid, as with porous glass, no shrinkage would occur, and providing the added polymer truly remained outside the pores, K_D could exceed unity. Present evidence suggests that added polymer does remain excluded (*4*), but more investigation is needed to determine the limits of validity of this assumption with regard to polymer size and type.

Also worth mentioning is the fact that programming might be more successful with a rigid network since the particles and thus the gel bed would not shrink or swell during the run due to the osmotic effects.

A basic advantage of this method is that retention can be continuously controlled using only steric (or exclusion) forces. These forces are especially appropriate for macromolecules because they are much more gentle than the "chemical forces" used in most chromatographic systems. The fine-tuning of retention for macromolecules is exceedingly difficult when "chemical forces" are used (*7*). Yet all present programming methods entail systems based on "chemical forces." The use of "steric programming," as proposed here, should be correspondingly useful for systems of macromolecules.

EXPERIMENTAL METHOD AND RESULTS

Sephadex G-100, Lot No. 226, from Pharmacia Fine Chemicals, Inc., was used in these experiments. The gel was swollen overnight in a 0.01 *M* Tris buffer with pH adjusted to 8.0 with HCl and made 0.2 *M* with respect to NaCl. It was then degassed and packed in a column of 0.88 cm diameter. Samples were layered on top of the column and eluted by force of gravity.

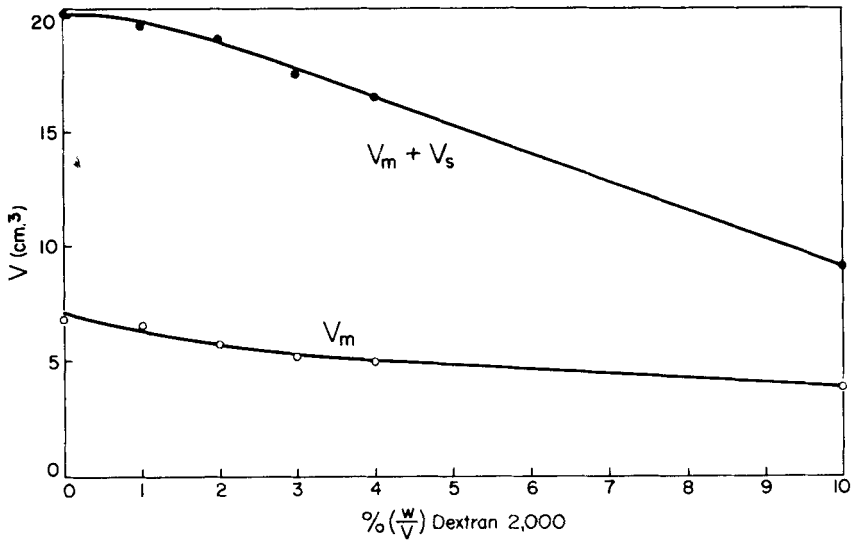

FIG. 1. Variation in the volume of mobile phase, V_m, and of total volume, $V_m + V_s$, with background concentration of dextran 2000. Values determined by elution of blue dextran and adenosine, respectively.

Markers for void volume, V_m, and total liquid volume, $V_m + V_s$, were blue dextran of molecular weight 2,000,000 and adenosine of molecular weight 267, respectively. The eluate was monitored by an UA-2 ISCO ultraviolet detector operating at 254 nm and was collected in a buret. Elution volume, V_e, was read from the buret as the recorder traced the peak maximum. The high molecular weight additive was dextran 2000, of molecular weight 2,000,000.

The test portions were bovine fibrinogen, fraction I; bovine γ-globulin, Cohn fraction II; bovine serum albumin, S 2281; and bovine hemoglobin, S 2607.

The concentration of dextran in the mobile phase is given as weight/volume percentage.

Since the gel bed was steadily decreasing in volume with increasing dextran concentration, V_m and $V_m + V_s$ were both determined for each particular dextran buffer. The column did not immediately obtain its equilibrium dimensions and consequently zones of blue dextran and adenosine were injected repeatedly until each showed identical successive readings for the elution volumes. The variations in V_m and $V_m + V_s$ with concentration of dextran 2000 are shown in Fig. 1.

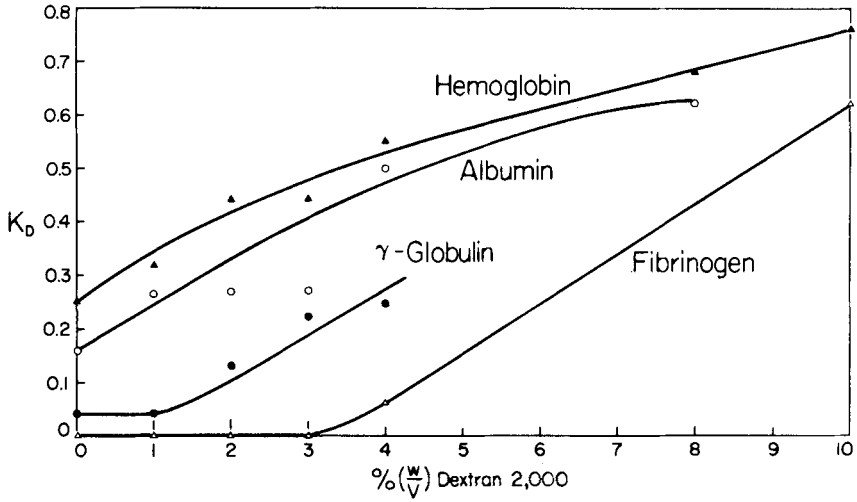

FIG. 2. Variation in K_D for different proteins with varying background concentration of dextran 2000.

The effect of dextran 2000 background concentration on the partition coefficient, $K_D = (V_e - V_m)/V_s$, of four different proteins is shown in Fig. 2. The values obtained for bovine serum albumin were somewhat uncertain due to aggregation; the maximum of the recorded peak was obtained by extrapolation of its rear profile.

Figure 2 shows that K_D values for all components do indeed increase significantly with dextran 2000 concentration. The increase is not uniform, thus giving changes in selectivity which may be useful. The increase occurs despite the osmotic shrinkage that would tend to decrease pore size and thus K_D. These preliminary experimental results confirm the basic phenomenon underlying the proposed method. More data will be presented subsequently.

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