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Size-exclusion chromatography dimension for rod-like macromolecules

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ABSTRACT

A phenomenological approach has been taken to determine the macromolecular dimension that controls peak migration in size-exclusion chromatography. For macromolecules representative of the class of rigid rods, flexible rods, random coils, and compat ellipsoids, the dependence of the chromatographic partition coefficient, K_{SEC}, on the viscosity radius, the radius of gyration, and the contour length, respectively, was determined. Since none of these dimensions appears to control retention uniquely, a phenomenological definition of R_{SEC} was provided. This parameter progressively deviates from the hydrodynamic radius with increasing macromolecular asymmetry.

INTRODUCTION

Theoretical and experimental studies have led to a number of suggestions concerning the macromolecular dimensional parameter that controls size-exclusion chromatography (SEC). Theoretical treatments[1,2] have indicated that peak migration in SEC should be governed by the mean projection length, $\bar{X}$. However, the prediction that $\bar{X}$ controls retention has not been borne out by experiment. Thus, for example, random coil polymers appear to co-elute with globular proteins of identical $[\eta]M$, where $[\eta]$ is the intrinsic viscosity, and M the molecular weight[3,4]; and since $[\eta]M$ has different dependences on $\bar{X}$ for macromoleculaes of different shapes[5], the observation

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of such "universal calibration"[6] for chain polymers and globular macromolecules is not in accord with a fundamental role for $\bar{X}$. In our previous studies[7], rod-like polymers were found to be eluted earlier than random coils of identical $[\eta]M$; however, no better congruence was achieved with plots of elution volume *vs.* $\bar{X}$.

The identification of a single dimensional parameter that controls SEC, if this "R_{SEC}" indeed exists, demands comparison of data for widely varying macromolecular shapes. If studies are confined to a single structural type, virtually any dimensional parameter will prove successful. Put differently, the dependence of the chromatographic partition coefficient, K_{SEC}, on log "size" will be uniform within a particular class of conformations (*e.g.* flexible chain macromolecules) regardless of the size parameter chosen. Thus, as pointed out by Potschka[8], the preference among biochemists for the Stokes radius, R_S[9], in contrast to the viscosity radius $R_\eta \approx ([\eta]M)^{1/3}$, employed by polymer chemists[10], may be more traditional than fundamental.

In addition to $\bar{X}$, R_S and R_η, the radius of gyration R_G has also been proposed as a fundamental SEC parameter[10]. With regard to the last three quantities, it should be noted that there is no compelling reason to assume that R_{SEC} must correspond to any dimension measured in dilute solution. The earlier notion that SEC is controlled by translational diffusion appears unlikely[10] so that the choice of a diffusion-related dimension is solely a matter of convenience. It is generaly accepted that partitioning between mobile phase and pore is an equilibrium process, but this observation does not lead to the identification of R_{SEC}.

In this work, pullulan, globular proteins, DNA, and schizophyllan are chosen as representative of (non-ionic) random coil, compact ellipsoid, wormlike chain, and (non-ionic) rigid rod, respectively. The selection of the column packing (Superose) and mobile phase (pH 5.5, 0.38 *M* $NaCl$–NaH_2PO_4, 9:1) is dictated by the need to avoid electrostatic or hydrophobic solute–packing interactions, *i.e.* to ensure "ideal" SEC[11]. Comparisions of the behavior of macromolecules with different structures under such conditions allow us to examine in more detail the nature of R_{SEC}

EXPERIMENTAL

Chromatography was carried out on a Superose 6 column (Pharmacia, Uppsala, Sweden). The samples employed were globular proteins, namely thyroglobulin, apoferritin, catalase, bovine serum albumin (BSA), ovalbumin, myoglobin, and cytochrome *c*; commercial pullulan molecular weight standards; and fractions of DNA and of schizophyllan.

The chromatographic partition coefficient, K_{SEC}, was obtained as

$$K_{SEC} = (V_e - V_0)/(V_t - V_0) \tag{1}$$

where V_e is the peak elution volume, V_0 the interstitial volume, determined by the elution of Blue Dextran, and V_t the total column volume, determined from the retention of ^2H_2O.

Chromatographic conditions and procedures, and the preparation and characterization of the DNA and schizophyllan fractions are described elsewhere[7].

RESULTS AND DISCUSSION

As previously noted, "universal calibration" plots for the polymers of this study systematically diverge with increasing asymmetry, so that R_η is not the governing parameter for separation. Similarly, macromolecules with different shapes but identical $\bar{X}$ fail to co-elute[7]. Fig. 1 illustrates that the radius of gyration R_G also is not a unifying dimension. Lastly, one might speculate that rapid tumbling of rod-like solutes, such as the schizophyllan fractions, could lead them to partition as if they were spheres with effective radii $L/2$ where L is the contour length; but Fig. 2 shows this to be erroneous.

The foregoing remarks show that no previously suggested dimension uniformly governs K_{SEC} for macromolecules of widely differing shape. We now consider whether a phenomenological approach could help to identify a universal dimension, if such a concept is indeed appropriate. Geometric considerations[2,12] suggest that, for spherical macromolecules in pores of well-defined shape

$$K_{SEC} = (1 - R/r_p)^\lambda \tag{2}$$

where R and r_p are dimensions of solute and pore respectively, and λ is a constant dependent on pore geometry, namely $\lambda = 1$ for slabs, 2 for cylinders, and 3 for spherical pores. While hypothetical pore dimensions may thus be envisioned, it should be pointed out that microscopy and other techniques reveal little resemblance between the structure of real gels and such idealized models[13]. For spherical solutes, the quanity R is unambiguous; for asymmetric solutes we identify it with R_{SEC}, which is not *a priori* defined. However, as noted above, we believe that R_{SEC} does not, in general, correspond to R_η, R_G, or $\bar{X}$. The relationship between R_{SEC} and some familiar dimension, say R_η, can be defined in a very general way as

$$R_{SEC} = \alpha R_\eta^\beta \tag{3}$$

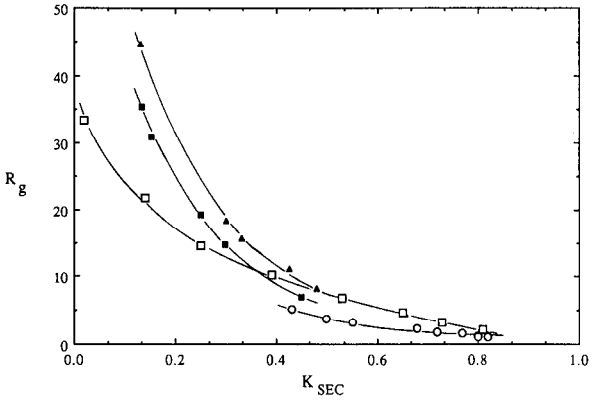

Fig. 1. Dependence of K_{SEC} on radius of gyration for pullulan (□), DNA (▲), schizophyllan (■) and proteins (○).

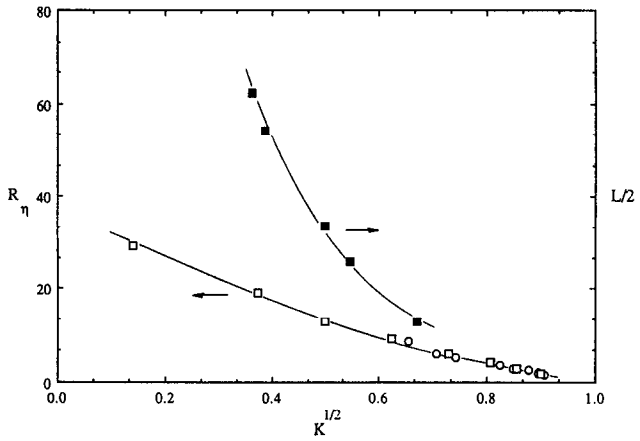

Fig. 2. Dependence of K_{SEC} of schizophyllan on $L/2$ (■) (right axis), compared to K_{SEC} *vs.* R_η for pullulan (□) and proteins (○) (left axis).

where α and β will depend on the shape of the macromolecule. Combining eqns. 2 and 3

$$K_{SEC} = [1 - (\alpha/r_p)R_\eta^\beta]^\lambda \tag{4}$$

The prediction of K_{SEC} thus depends on several unknown parameters. Identification of these by experiments requires the elimination of at least one unknown first. Thus, for example, if λ were known, a plot of $\ln(1 - K_{SEC}^{1/\lambda})$ *vs.* $\ln R_\eta$ yields β as the

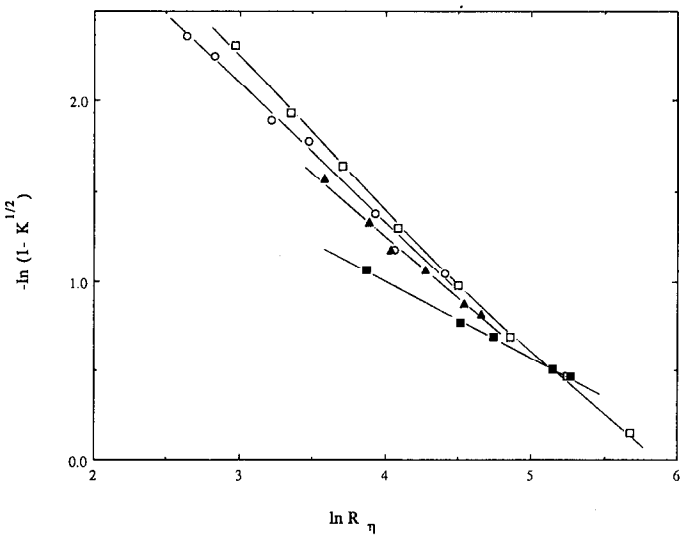

Fig. 3. Chromatographic behavior of (from right to left): pullulan (□), proteins (○), DNA (▲), and schizophyllan (■), plotted according to eqn. 4 with $\lambda = 2$.

TABLE I

FITS OF CHROMATOGRAPHIC DATA TO EQN. 4

	Pullulan	*Proteins*	*DNA*	*Schizophyllan*
β^a	0.85	0.75	0.69	0.43
r^b	0.998	1.00	0.996	1.00

[a] Slope of $\ln(1 - K^{1/2})$ *vs.* $\ln R_\eta$, yielding the exponent of $R_{SEC} = \alpha R_{SEC}^{\beta}$.
[b] Regression coefficient for eqn. 4.

slope. If the solute molecules were truly spherical, then $\alpha = \beta = 1$, and one may seek the value of λ which provides the best linear fit to $K^{1/\lambda}$ *vs.* R_η.

None of the solutes employed in this study approximate spheres, and the value of λ is difficult to define with the data in hand. We proceed with the assumption of $\lambda = 2$, for two reasons. First, we have found that the goodness of the fit of the data to the form $\ln(1 - K_{SEC}^{1/\lambda})$ *vs.* $\ln R_\eta$ is not very sensitive to the choice of λ. Second, our primary interest is to contrast the behavior of macromolecules with different degrees of asymmetry; since the data are all acquired on a single column, *i.e.* constant λ, useful comparisons may be made, even with residual uncertainty in λ.

Fig. 3 shows the chromatographic data plotted according to the logarithmic form of eqn. 4. Data points for $K_{SEC} < 0.1$, *i.e.* close to the exclusion limit, are omitted from this plot. The deviation of such data from the lines of Fig. 3 was attributed to the effect of pore-size distribution, *i.e.* that the mean pore diameter sampled by large solutes must be larger than the effective pore size in the middle of the calibraiton range. The linear correlation of the data is remarkably good. Values for β and the regression coefficients for the lines for pullulan, globular proteins, DNA, and schizophyllan are given in Table I. The values of β appear to deviate systematically from unity with increasing solute asymmetry, inasmuch as schizophyllan has the largest persistence length and pullulan the smallest. It is interesting to note that β for the globular proteins is intermediate between the values for pullulan and DNA. This effect may indicate that the overall structures of these proteins are better approximated by ellipsoids than spheres, but the possibility that weak non-ideal interactions with the packing distort the data cannot be ruled out.

The phenomenological treatment indicates two fruitful directions. First, the value of λ may be better defined when data are obtained with solutes of more nearly spherical structure. Second, direct insight into a separation model may be generated when comparisons are made with appropriate theoretical predictions. These issues are the subject of continued efforts.

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