interaction with the polyion does take place in the Debye–Hückel atmosphere, the theory overemphasizes the electrostatic effect of the coion charge.

From the theory of Manning, counterions condense onto the polyion if $\xi > \xi_c$ and the uncondensed counterions interact with the polyion. The equation for monovalent counterions is

$$D_1/D_1^0 = [(\xi^{-1}X + 1)][X + 1]^{-1}[1 - \tfrac{1}{3}A(1;\xi^{-1}X)] \quad (3)$$

where the subscript 1 refers to the counterions. From Figure 7 it can be seen that the solid line predicted from Manning's theory is in good accord with the experimental results below $X = 1$, and for the two highest concentrations of simple salt, 0.005 00 N and 0.010 0 N, over the whole range of X. Since limiting laws are derived from the theory, it would be expected that at the lowest polyelectrolyte and simple salt concentrations, i.e., at low X values, the best correlation of the experimental findings with the theory would result. While such is generally manifest in Figure 7, the experimental results show negative deviations from the theory for the lowest NaCl concentration by as much as 20%.

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Critical Permeation Size of Dextran Molecules

W. Haller[1]

Institute for Applied Physical Chemistry, University of Heidelberg, Germany.
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ABSTRACT: The chromatographic behavior of dextran fractions on glass of narrow pore size distribution has been studied. Since the pore size of such glasses can be measured very accurately by mercury intrusion, the permeation behavior of macromolecules into the pores of such glass allows their characterization by a new size parameter, "the critical permeation size". The "critical permeation size" is defined as the pore size of the controlled pore glass at which the elution coefficient becomes zero. Two plotting procedures are shown which effectively linearize the molecular weight vs. elution coefficient and the pore diameter vs. elution coefficient relations. These plotting procedures can be used for extrapolations when the calculation of the critical permeation size from a limited number of chromatographic determinations is desired.

Gel permeation chromatography[2,3] has, over the past 15 years, become a well established procedure for the fractionation and characterization of high polymers of synthetic and natural origin. Characterization consists usually in comparing the elution behavior of the unknown sample with the behavior of substances with known molecular weight. Within several years, controlled pore glass[4] has joined the rank of substances for permeation chromatography. Rigidity and chemical inertness make it particularly useful for applications where high speed,[5] sterilizability,[6,7,8] long life,[9] resistance against chemically reactive[10–13] or hot eluants,[14] and the ability of covalent surface derivatization[15–17] are of importance. As a result of its rigidity and the fact that its pore size is not affected by the presence of gases, liquids, or vacuum, the pore size of controlled pore glass (CPG) can be measured by electron microscopy and mercury intrusion analysis.[4] This has been found very valuable in the reproducible preparation of CPG, in comparing experimental results, and, to a lesser extent, in the investigation of the mechanism underlying permeation chromatography itself.[3,18–22]

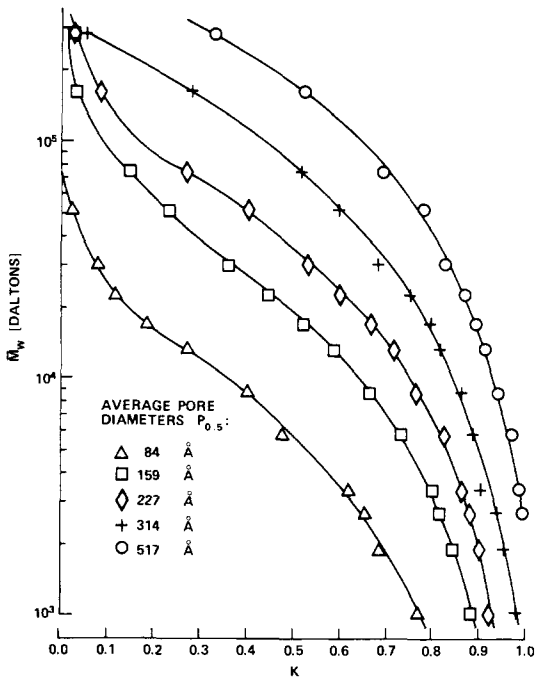

Figure 1. Normalized elution volume K vs. log of molecular weight ($\overline{M}_w$) of narrow molecular weight distribution dextrans.

In the following, it is attempted to utilize the well-defined, easily measurable and sharply distributed pore size of CPG as a means for determining the size of macromolecules. Since a well-accepted and quantitatively all-satisfying theory relating elution position, molecular size, and pore size of substrate has not yet been established, the following proposed methods rely only upon the easily understood and agreed fact that a molecule or particle appears in the exclusion volume if it is too large to enter the pores of the substrate. Conversely, for a given molecule a critical pore size exists at which the molecule is just unable to penetrate. This critical permeation pore size is believed to be close to the largest diameter of the molecule and, while certainly subject to charge effects[23] and other restrictive influences, represents a new experimental size parameter for high-polymer molecules, viruses, and small particles which relate their size to mercury intrusion pressure measurements of a rigid pore structure.

In the present work, the sketched method has been applied to dextrans of different molecular weights. The particular choice of this biopolymer was made because of its near-ideal theta behavior in aqueous solutions around room temperature, the absence of strongly charged groups in its carbohydrate structure, and, most important, because of the fact that extremely well characterized and monodisperse dextran fractions have become available.[24]

Experimental Section

Materials. Commercial dextran was fractionated by multiple permeation chromatography. The molecular weight distribution of the individual fractions was measured chromatographically on controlled pore glass columns and the fractions were further characterized by ultracentrifuge sedimentation equilibrium measurements ($\overline{M}_w$), endgroup analysis ($\overline{M}_n$), and viscosity measurements (η). A detailed account of these procedures as well as a listing of the used dextran fractions and their properties can be found in the publication by Basedow.[24] Fraction number 4 of the cited publication was not used in the present work because of its being very close in molecular weight to fraction number 5. The molecular weight average $\overline{M}_w$ is believed to be accurate within ±1.5%. The dextran fractions had been processed for storage by alcohol precipitation and vacuum drying. The precipitation apparently caused some further fractionation and disproportionation into grains of slightly different molecular weight averages. These became apparent through small systematic plotting scatter in this work when single grains of dried dextran were dissolved and run repeatedly on columns of different pore size.

The controlled pore glass was prepared and characterized as described elsewhere.[4] Five different types were used. They had average pore diameters ($P_{0.5}$) of 84, 159, 227, 314, and 517 Å. The average pore diameter $P_{0.5}$ is the pore diameter read from the integral pore distribution curves at one-half of the total pore volume.[4] All CPG had 75 to 125 mm particle size (120 to 200 mesh ASTM).

Chromatography. The narrow molecular weight dextran fractions were chromatographed on controlled pore glass columns of 8 × 750 or 11 × 1000 mm size. Columns were of a commercial cartridge type design where the CPG is permanently sealed between stainless steel porous disks and the column exits are closed by elastomer septums. Columns, once packed by strong vibration in an orbital column vibrator, maintained their parameters indefinitely. Eluant was a 0.1 M glyzine buffer containing 0.076 M NaCl and 50 ppm sodium azide, adjusted with NaOH to a pH of 8.2. The eluant pump was of the peristaltic type. Flow rates were 15 cm³/h for the 8 × 750 mm columns and 30 cm³/h for the 11 × 1000 mm columns. For column flushing, equilibration with buffer, etc., a flow rate of 315 cm³/h (maximum speed of the pump) was used. Monitor was a differential refractometer. Dextran samples of 1 mg/cm³ concentration were injected through the septum at the column head with a syringe. Sample volume was 0.5 or 1.0 cm³ respectively for the two different column sizes. All columns were calibrated for exclusion volume (V_0) with tobacco mosaic virus. The total volume of liquid in the columns (V_t) was calculated from the volume of the column and the weight of CPG used in packing of the column. More experimental details, considerations of flow-rate effects, correction of exclusion volume for sample volume and monitor delay, etc., are to be found elsewhere.[25]

Results and Discussion

All elution volumes (V_e) after correction for sample volume and monitor delay (volume in system between the end of CPG packing and center of the monitor cell) were normalized[26] with respect to V_0 and V_t by the equation $K = (V_e - V_0)/(V_t - V_0)$. The values of the normalized elution volumes K (elution coefficient) are plotted in the customary way against the log of the molecular weight $\overline{M}_w$ in Figure 1.

It is apparent from Figure 1 that in this type of plot the results produce curves which are concave toward low M except for values close to $K = 0$. For low K, the data show an "upturn" in the curve. In other investigations[10,25] it was observed that this "upturn" relates in magnitude to the broadness of the pore-size distribution and in particular to a small "foot" in the integral pore size distribution curve. In gels, this upturn is much more pronounced and the K vs. log M curves are generally remembered as being s shaped with a "straight" portion in the middle. This "straight" portion, in fact not called for by any existing theory, is taken as a consequence of this plotting technique. It can easily be shown that artificial broadening of the pore-size distribution by mixing several pore sizes of glass produces resolution curves with more "upturn". This extends the working range of such a column, but at a cost of differential resolution.

It shall be pointed out that the column to column reproducibility of the K values depends much upon the precise determination of V_0 and V_t and also upon the shape of the peaks. For highly monodisperse substances, as have been used in this study, the peaks are narrow and symmetric. Under these conditions, the V_e of the peak is unique. This does not necessarily hold for broad molecular weight distribution samples.

Plotting Procedures for Homologous Series (Procedure I). Rather than using the K vs. log M plot, it is proposed to display elution data in a log M vs. $-\log(1-K)$ plot. This is a similarly empirical plot but gives extended straight lines as shown in Figure 2. The figure gives all the experimental data, except for small K in the region of the "upturn" and for $\log(1-K)$ larger than 0.85, where this function magnifies scatter due to mathematical distortion of the experimental values. The straight lines have been produced by least mean square linear regression of the sets of data shown for the in-

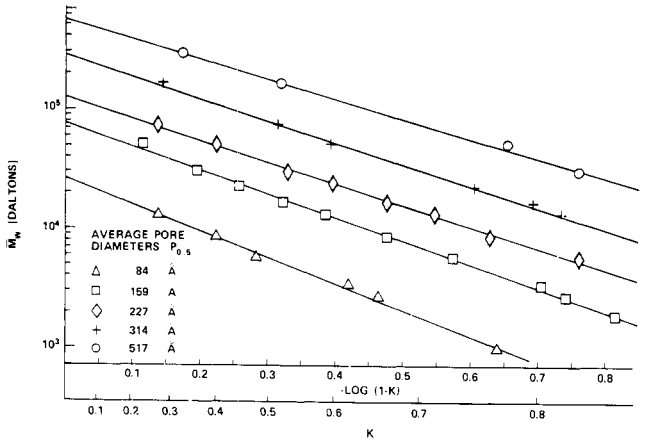

Figure 2. Extrapolation procedure I.

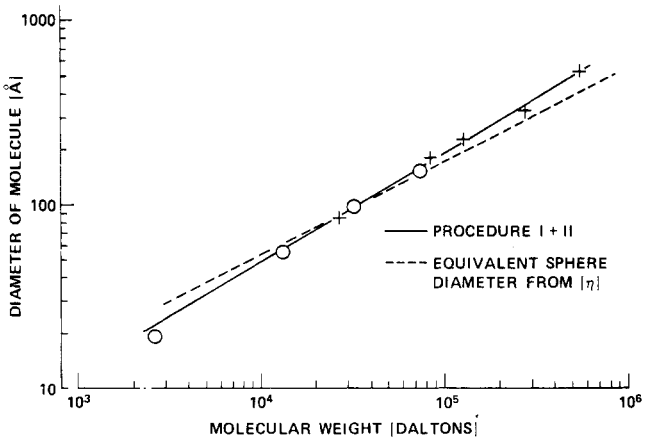

Figure 3. Diameter of dextran molecules vs. molecular weight; (+) procedure I; (○) procedure II.

dividual pore sizes. The lines have the equation

$$\log \overline{M}_w = \log M_0 + S \log (1 - K) \quad (1)$$

It suggests immediately that the molecular weight M_0 at the intercept of the straight lines at $K = 0$, $-\log (1 - K) = 0$, represents the critical molecular weight of dextran molecules having the same dimensions as the pore size of the particular column.

Thus, in Figure 3, the experimentally found critical molecular weights (M_0) and their pore sizes are shown in the usual log molecular weight (M) vs. log molecular diameter (D) plot. A straight line through the data has the equation

$$D = 0.215M^{0.587} \quad (2)$$

where D is in Å and M in dalton units. Figure 3 shows also the curve found for plotting the $\overline{M}_w$ of the dextran fractions vs. the equivalent sphere diameter $D_{[\eta]}$ of the fractions, where the $D_{[\eta]}$ of the fractions were calculated[27] from the Einstein equation

$$D_{[\eta]} = 1.08\sqrt[3]{M_w[\eta]} \quad (3)$$

where $[\eta]$ is the experimentally determined intrinsic viscosity (from Table 2 of ref 24).

Again, constructing the best straight line through the latter data gives

$$D_{[\eta]} = 0.53\overline{M}_w^{0.5} \quad (4)$$

The power of 0.50 in eq 4 indicates ideal solution behavior of a random coil-type high polymer (random flight model), while the power of 0.587 in eq 2 indicates coil extension.

Plotting Procedure for Single Substances (Procedure II). The systematic variation of the slopes as a function of P (Figure 2) could be expressed by the empirical relation

$$S = 4.53P_{0.5}^{-0.164} \quad (5)$$

The above relation suggests another extrapolation procedure, usable for single substances, not necessarily belonging to a homologous series which consists of measuring the K of a substance with columns of different pore size P and plotting these values (for K larger than 0.25 and smaller than 0.85) in the form $-\log (1 - K)\, P^{-0.164}$ against the log of the used pore size $P_{0.5}$. This plot is shown for several pore sizes and dextran fractions in Figure 4. The lines again represent the best fit for the experimental data.

The intercepts P_0 (for $K = 0$) represent the critical exclusion pore sizes of the particular substances. These were plotted into Figure 3. Figure 3 shows the curve for the equivalent sphere diameter and the values for critical permeation parameters of the dextran according to the two methods postulated in this paper.

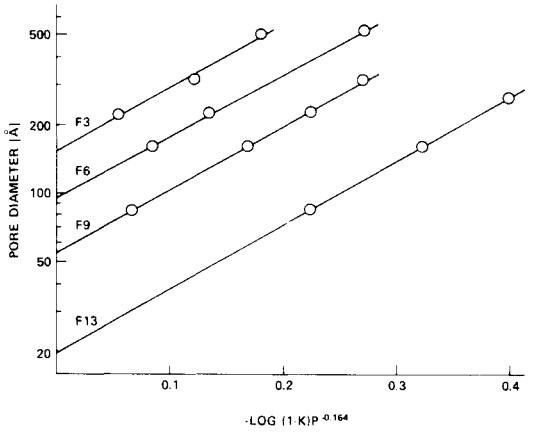

Figure 4. Extrapolation procedure II. Values for dextran fraction number 3, 6, 9, and 13.

Summary

Based upon experimental work with narrow molecular weight distribution dextrans on controlled pore glass, a plotting procedure (I) is proposed whereby the function $-\log (1 - K)$ is plotted against the log of the molecular weight. This produces linear curves extending from $K \cong 0.25$ to 0.85. When the curve for a CPG column of a given pore size is extrapolated to $K = 0$, the resulting intercept M_0 is defined as the critical molecular weight of the homologous polymer series for which the critical permeation pore size is the pore size of the particular column.

A second plotting procedure (II) is proposed, which is applicable to a specific substance. The substance is chromatographed on several CPG columns of different pore size. The results are displayed by plotting the log of the CPG pore size against $-\log (1 - K)P^{-0.164}$. The resulting straight lines are extrapolated to $K = 0$ and the intercept P_0 is the critical permeation pore size of the investigated species.

In the case of dextran, the critical permeation size corresponds closely to the equivalent sphere diameter calculated from viscosity and ultracentrifuge measurements.

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Hydrodynamic Effect on the Coagulation of Porous Biopolymers

Peter G. Wolynes*[1a] and J. A. McCammon[1b]

Departments of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Harvard University, Cambridge, Massachusetts 02138.

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ABSTRACT: The rate of diffusional coagulation of globular macromolecules vanishes in hydrodynamic calculations which employ stick boundary conditions at the solvent–macromolecule surface. We show that finite coagulation rates are predicted by a theory which recognizes the porous or rough character of polymer surfaces. Approximate rates for the coagulation of neutral proteins are calculated. The theory is relevant to other macromolecular rate processes, such as large-scale internal motions in proteins.

Many biochemical processes involve the association of large subunits or the relative motion of two subunits in close proximity.[2a] The diffusion equations describing the kinetics of these processes contain diffusion constants which depend on the relative positions of the subunits.[2b] These diffusion constants are inversely related to the hydrodynamic drag coefficients for relative motion of the subunits.[3,4] It is well known that the ordinary hydrodynamics of impenetrable bodies with stick boundary conditions predict that the hydrodynamic drag coefficients diverge strongly as the bodies come into contact.[5] As noted by Honig, Roebersen, and Wiersema this leads to the unphysical result that the coagulation rate of colloids vanishes, if one does not assume infinitely strong attractive forces near contact.

Recently, it has been noted by several authors that the porous nature of biopolymers affects their hydrodynamic properties.[6] Porosity may arise from either the imperfect packing of subunits or the surface irregularities of otherwise close-packed structures, such as globular proteins. Here, we note that biopolymer porosity is of key importance in the hydrodynamics of relative motion of subunits. When protein porosity is taken into account the hydrodynamic drag coefficients are only weakly singular when the bodies are in contact and the coagulation rate, predicted from diffusion-controlled reaction rate theory, is nonvanishing.

Consider the coagulation of two equal-sized spherical particles with radius a and interaction potential $U(r)$. Diffusion-controlled reaction theory relates the rate constant k for coagulation and the position-dependent relative diffusion constant $D(r)$ where r is the distance between particle centers:[3]

$$k = 4\pi \left[\int_{2a}^{\infty} \frac{e^{U(r)/k_B T}}{r^2 D(r)} \, dr \right]^{-1} \tag{1}$$

If there were no hydrodynamic interactions between the particles $D(r)$ would be twice the free translational diffusion constant of the particles. Then the rate constant for coagulation of particles with no interaction potential is given by the Smoluchowski expression:

$$k_{SM} = 4\pi(2D)(2a) \tag{2}$$

The relative diffusion constant $D(r)$ is related by an Einstein relation to the drag coefficient for relative motion of the particles $f(r)$

$$D(r) = k_B T / f(r) \tag{3}$$

An exact series expansion of $f(r)$ has been obtained by Brenner for the case of relative motion of impenetrable spheres with stick boundary conditions. Honig, Roebersen, and Wiersema have shown that this exact result is very closely approximated by a rational function which fits the series expansion exactly at large and small separations. At large separations $f(r)$ can be obtained by the use of the Oseen tensor[3]

$$f(r) = \tfrac{1}{2} f_d \left(1 + \frac{3}{2u} + \ldots \right) \tag{4}$$

where $u = (r - 2a)/a$ and f_d is the translational friction constant of the particle. At small separations most of the drag is