reported shear-dependent diffusion coefficients from falling film data wherein a power-law analysis was used, and our calculations can demonstrate that perhaps 30% of the shear dependence in $\mathcal{D}$ is removed when the more realistic Ellis model is employed.

The perturbation method proposed here is not limited to any particular rheological model or to the film problem. It should be useful in more complex flows and in those in which penetration theory is not appropriate. In general it would be expected that the number of dimensionless parameters appearing in the convective diffusion equation would be one less than the number of parameters in the model.

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Kinetics of Swelling of Dextran Gels in Aqueous Protein Solutions

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Sephadex, a cross-linked, spherical dextran gel that has been widely used in liquid chromatography, has been demonstrated to be effective in concentrating aqueous solutions of high molecular weight (MW) solutes. Upon contact with aqueous solutions, dry Sephadex imbibes relatively large quantities of solvent. Solutes which are too large to enter the gel are left behind in the external solution and are thereby concentrated. Low MW solutes, which can freely enter the gel, show essentially unchanged or only slightly higher external concentrations.

Some of the properties of Sephadex are given in Table 1. While both types listed exhibit relative rigidity in the swollen state, the G-25 gel is more extensively cross-linked (with epichlorohydrin) than the G-50 gel. The exclusion property of the Sephadex gels gives them many biological and biomedical applications.

Solutions containing macromolecules (for example, many biological fluids) can be quickly and efficiently concentrated two- or three-fold in a single step without changing *p*H or ionic strength (repeated separation of the supernatant and treatment with more dry gel can give greater and greater concentrating effects). The ability of dextran gels to concentrate sensitive biological fluids makes the gel hydration process an attractive alternative to methods such as evaporation, freeze drying, and ultrafiltration. No applied pressure is needed since the high internal osmotic pressure of the beads provides a strong solvent-uptake driving force, and extreme temperatures are unnecessary. Sephadex is convenient for fast concentration of moderately-sized dilute samples to quite small volumes for further use and/or analysis, for example, samples of biological fluids can be easily concentrated for subsequent separation in chromatographic columns. Gel hydration is especially useful when viscous solutions need to be concentrated, since the large specific surface area of the beads (for example, 10 cc of dry 200 micron beads has a total surface area of about 2000 cm^2) gives reasonable solvent removal rates even when solvent diffusivities are rather low.

Another application relates to treating the localized swelling (edema) of body tissues which occurs in various pathological conditions. Draining the excess fluid from the body alleviates the edema but causes a significant loss of protein. Sephadex could be used to recover these proteins via the concentration process, after which they could be reinfused.

Since Sephadex is sold commercially in the dry form and must be hydrated prior to use in aqueous systems, it is important to know the characteristics of the gel expansion. For example, packing a chromatographic column with Sephadex before complete swelling equilibrium has been achieved can result in either severe bed compaction or column rupture. It is clear that knowledge of the kinetics of gel hydration is crucial to all of the applications cited.

PREVIOUS WORK

Flodin et al. (1960) were the first to use the gel hydration method. Working with the G-25 type gel, it was found that 10 minutes were needed to obtain complete swelling of the gel in contact with solution, after which the material was centrifuged to effect separation. Recoveries of 90% at a 10- 20-fold concentration were common. The Sephadex was able to be effectively regenerated by washing with water, suspension in ethanol, and subsequent drying.

Vavruch (1965) later reported results of a detailed study of the swelling kinetics of Sephadex G-25, G-50, and G-75 in water. The volume change of the gel as a function of time was measured by an involved and delicate system of mercury displacement. Unfortunately, in Vavruch's apparatus uniform instantaneous wetting of the bed of dextran could not be achieved and the experimental results were found to be dependent on the size of the sample and the dimensions of the apparatus itself.

This dependence is clearly an artifact.

Vavruch's data indicate that total uptake versus time follows S-shaped curves on linear plots. In his explanation of such curves, Vavruch introduced the concept of an induction period, during which the solvent fills the void space, wets the surface of the particles, and some sort of complex solvation process begins to effect the gel expansion itself. This induction period was found to last somewhat less than 30 sec. for G-25 and about 2 min. for G-50. Vavruch tried to fit his data to a model based on a first-order rate expression:

$$\phi = \frac{v - v_0}{v_f - v_0} = 1 - e^{-kt} \qquad (1)$$

This formula was found to agree with the middle part of his G-50 expansion curves, but not with any part of his G-25 curves. Equation (1) is derived by assuming that the rate of swelling at any instant is proportional to the difference between the prevailing solvent content of the gel and the solvent content which exists at swelling equilibrium, that is, $dv/dt = k\ (v_f - v)$.

APPARATUS AND PROCEDURE

Our system consisted of a Unitron compound microscope with four objective lenses and an internal light source. Placed directly over the eyepiece of the microscope was a Bolex H-8 reflex movie camera mounted on a tripod. Specially constructed glass microscope slides were used to hold the Sephadex beads.

Runs involving pure distilled water and albumin solutions of various concentrations were carried out. Albumin, although fairly insoluble in water, is easily dissolved in salt solutions. Water brought to 0.15 N with sodium chloride was therefore used in preparing the protein solutions. This electrolyte content approximates that of body fluids (plasma, lymph, intracellular fluids).

To obtain the best viewing results, the following combination of lenses was employed: a 5.5mm focal length camera lens, a ten-power objective microscope lens, and an internal light source fitted with a polarized filter. To begin each run, several beads of Sephadex were placed upon the glass slide. By viewing through the camera-microscope, the beads were then inspected for sphericity. Once a suitable single bead was found, it was isolated by clearing the others from the glass slide.

A few drops of protein solution or water (a large excess relative to the bead, however) were then placed on the slide and, at the instant the solution contacted the bead, a timer was started and one frame of film was exposed. Thereafter, one frame was exposed every ten sec., while the microscope was constantly refocused on the ever enlarging bead. The experimental runs lasted a minimum of 10 min. each.

The analysis of the developed film (Kodachrome 8 mm film) was performed on a Vangard Motion Analyzer, which is equipped to measure the size of the screen image by means of a pair of hairlines. Each bead image diameter was measured horizontally and vertically and the results averaged to obtain the mean diameter of the bead. This value was then used to compute the bead volume, which was then made dimensionless according to the formula $\phi = (v - v_0)/(v_f - v_0)$. The value of v_f was an asymptotic one chosen by inspection of the data. Graphs of dimensionless volume versus time were prepared on both linear and semilogarithmic paper.

The Sephadex G-25 beads which we used swelled between 4.2 and 5.6 times their initial volume, with a mean value of 4.7 times their initial (dry) volume. The Sephadex G-50 beads, on the other hand, swelled between 7.5 and 9.8 times their initial volume, with a mean value of 8.7 times the dry bead volume.

The temperature was that of the room and was 25 ± 2°C in all runs. No precise control was attempted since auxiliary experiments indicated that small temperature variations do not significantly affect the swelling kinetics.

The thesis of Pratt (1972) gives a detailed discussion of all of the experiments.

RESULTS AND DISCUSSION

Upon examination of the data, it was decided to attempt to fit the data to Equation (1) (Vavruch's model). For each experimental run, the k-value which gave the best overall fit to the data was estimated by fitting the data to straight lines on semilogarithmic paper. In most cases, Equation (1) was able to very closely describe the swelling of the dextran gel beads although significant mismatching between theory and data occasionally occurred for large values of time (those corresponding to the final 20% of the expansion). Figure 1 gives data for some of our better runs (that is, those in which good focus was always maintained and in which the initial contacting of bead and solution went smoothly), along with a plot of Equation (1) for the k value which best characterizes those runs.

The first gel studied was Sephadex G-50, swelling in distilled water. Experimental runs were performed using a significant range of dry bead sizes. Figure 2 shows the values of the rate constants determined in this manner (the bead radius given here is a relative measurement of a photographic image and has no specific units). It was evident from these runs (and was also true in all of our other runs) that the initial dry bead size is inversely related in some form to the speed of swelling. Sephadex G-25 was then investigated in the same manner as the G-50 gel. Distilled water was again the solvent, and a characteristic range of bead sizes was examined. The much faster swelling of G-25 made accurate measurements somewhat more difficult to obtain, however. Figure 2 includes the k values determined in these runs.

The relationship between bead size and value of the rate constant noted before also showed up in the G-25 runs. The data further demonstrated another important property of Sephadex: the swelling rate of G-25 in water is much faster than that of G-50—roughly twice as fast for beads of the same size. An explanation as to why the more highly cross-linked G-25 gel has the greater rate of swelling is that, even though the porosity of the G-25 gel is lower than the G-50 gel and this lessens the diffusion rate of solvent, a much smaller amount of solvent is needed for G-25 to reach the same degree of swelling as the G-50 gel. For example, Table 1 shows that the equilibrium water regain of G-25 is only one-half that of G-50.

The next phase of the project involved the study of the swelling of Sephadex beads in protein solutions. These studies related to the practical applications of Sephadex

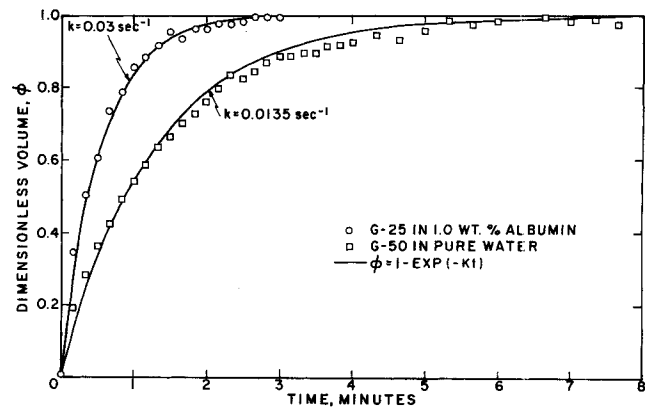

Fig. 1. Typical swelling curves for sephadex.

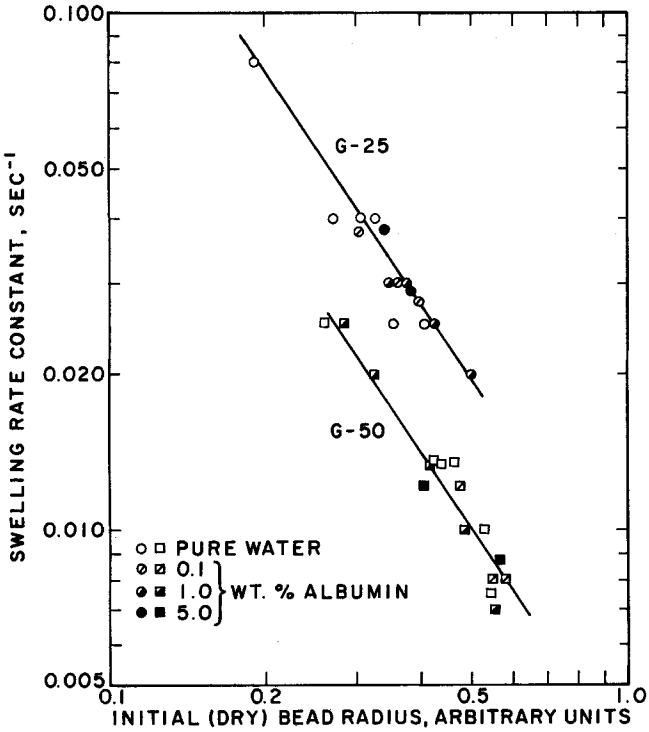

Fig. 2. Dependence of swelling rate on bead size.

TABLE 1. PHYSICAL PROPERTIES OF SEPHADEX

Type	Particle size, microns	Bed vol./g dry gel (ml)	Water regain, g/g	Wet density, g/ml	Exclusion limit
G-25	100-300	5	2.5 ± 0.2	1.13	MW > 5,000
G-50	100-300	10	5.0 ± 0.3	1.07	MW > 10,000

and constituted the main focus of the project. We investigated 0.1, 1.0, and 5.0 wt. % bovine albumin solutions (dissolved in a 0.15 N NaCl in water stock solution).

In Figure 2, we present all of the data for protein solutions along with the results of the previous pure water runs. It is obvious that the gel continues to follow an inverse size-rate relationship when swelling in the protein solutions.

Concentration polarization was expected to have some effect on the dynamics of dextran swelling. The data show, however, that this is not the case. When runs with pure water and albumin solutions are compared, no major differences are noted. This is brought out very clearly in Figure 2 where we have plotted k values versus initial dry bead radius. This figure exhibits a definite correlation between the swelling rate constants and initial bead radius which apparently does not depend on whether the solution involved is pure water or contains protein. The resistance to molecular diffusion offered by the gel itself must therefore be substantially greater than the resistance of any external protein-rich boundary layer. For, only if the gel's internal diffusion resistance is strongly rate controlling could concentration polarization have little or no effect on the kinetics of expansion. This is not really surprising since one would expect the diffusivity of water in the gel to be significantly less than the diffusivity of water in a protein solution, even a somewhat concentrated one. From the slopes of the lines drawn through the data, the rate constant is found to be roughly proportional to the initial bead radius to the minus three-halves power.

The fact that Sephadex G-25 swells faster than G-50 is also demonstrated in this figure. Since both lines have identical slopes of $-3/2$, the difference in the k-values between like-size G-25 and G-50 beads is a constant factor. The relation is $k_{G\text{-}25} \cong 1.9\ k_{G\text{-}50}$, where $k_{G\text{-}25}$ and $k_{G\text{-}50}$ denote the rate constants for Sephadex G-25 and G-50 beads (respectively) of equal size. Note that the rate constants for G-25 and G-50 are almost inversely proportional to the amounts of solvent that must be taken up to achieve equilibrium (see Table 1).

Vavruch obtained a rate constant for G-50 swelling in water of about 0.003 sec^{-1}, using 100-270 mesh beads (average diameter $\cong$ 100 microns). Our beads, taken from a 100 to 300-micron diameter lot, were undoubtedly larger in general and would thus be expected to swell somewhat more slowly than Vavruch's beads. Our G-50 rate constants are, however, 0.007 to 0.025 sec^{-1}—consistently much larger than Vavruch's. Problems encountered in efficiently and completely wetting the gel bed might explain the much slower swelling rate observed by Vavruch. With regard to G-25, no accurate comparison with Vavruch's data is possible since his k value (sec^{-1}) for G-25 drifted from about 0.050 at 30 sec. to 0.008 at 8 min. Our values (0.08 to 0.02) are again generally higher, however.

In this study no induction periods of the type found by Vavruch were noted. No bead studied showed any hesitation in actively swelling for even a few sec. (much less ½ to 2 min.). Even though some variations in initial swelling rates were found from run to run, no S-shaped behavior was ever detected. The nature of Vavruch's kinetic curves is probably caused by failure of his apparatus to quickly and completely contact the gel and solvent at time zero.

Our slowest swelling bead had a rate constant of 0.007 sec^{-1}. After 16 min., this particular bead would have accomplished all but one-thousandth of its volume change. For purposes of packing a laboratory column, this suggests that a swelling time on the order of 20 min. would be adequate to prepare the gel. The effective use of Sephadex to concentrate solutions does not, of course, require a close approach to equilibrium. Times of 10 min. are probably adequate for these applications.

NOTATION

k = swelling process first-order rate constant, s^{-1}
v = volume of bead at any time, cc
v_f = volume of bead at swelling equilibrium ($t \rightarrow \infty$), cc
v_0 = volume of bead at time zero, cc
t = time, s
T = temperature, °C
ϕ = dimensionless volume, equal to $(v - v_0)/(v_f - v_0)$

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