Characterization of the Swelling of a Size-Exclusion Gel

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The swelling of a dextran gel, Sephadex G-75, was observed in an aqueous environment at room temperature by a noninvasive technique that uses light microscopy coupled to an image analysis system via a video camera. The rate of swelling was found to follow the Tanaka and Fillmore theory, from which the overall gel diffusion coefficient was estimated as 8.5 × 10^{-9} cm^{2}/s. In addition to giving a quantitative measure of gel swelling that could be useful in the mechanical design of liquid chromatography columns, this approach provides data on wet particle size and particle size range, which is needed for the modeling of diffusion and mass transfer effects in size-exclusion chromatography. In this context, key observations are that the gel particles are nearly spherical with an elliptical shape factor of 0.98 (perfect sphere = 1) and that there is little difference between sizes of particles obtained in water, 50 mM Tris-glycine buffer (pH 10.2), and buffer containing 1 mg/mL protein. The diameter of the dry material ranged from 20 to 100 μm, while the hydrated particles had diameters of 40–350 μm. The rate of swelling is rapid, with 50% swelling occurring in about 10 s and swelling to 98% of the final wet particle size being obtained in less than 90 s.

Introduction

An important component in the design of a size-exclusion chromatography (SEC) column is the packing material (Yarmush et al., 1985), of which particle size is a key factor since it has a major effect on column size, throughput, and separation (Rudge and Ladisch, 1986; Purath, 1968). Moreover, SEC design equations should reflect the wet particle size of the packing material (Ladisch and Wankat, 1988), because an increase in the particle size increases the plate count and therefore column length for a given separation (Rudge and Ladisch, 1986). The swelling of a cross-linked dextran gel in water was chosen for this study because of the common use of aqueous gel filtration in purification of proteins (Dolan-Heitlinger, 1981; Wetzel, 1986; MacMillan et al., 1987).

The measurement of wet particle size by light microscopy is not new. Particle size before and after swelling of styrene/divinylbenzene in toluene has been determined by using an optical microscope equipped with a micrometer (Pickup et al., 1980). Dimensional changes and solvent front penetration have also been recorded at 5-min intervals for poly(2-hydroxyethyl methacrylate) hydrogel by taking photographs via an optical microscope (Lee, 1983). Other experimental measurements of gel swelling as a function of time include those of Patel et al. (1989), Skjak-Braek et al. (1989), and Komori et al. (1988). Tanaka and Fillmore (1979) reported the radius of a 5% polycrylamide gel swelling in water by visual observation of the spheres as a function of time under a microscope with a calibrated scale. Their results indicated that a sphere having a 510-μm radius achieves 50% swelling in about 100 min, with swelling to 99% of the final wet particle size requiring more than 10 h. We report a different approach in which a light microscope coupled to an image analysis system and VCR via a video camera allows the continuous and noninvasive monitoring of individual gel particles as a function of time so that the swelling process for rapidly hydrating gels can be followed on a second-to-second basis. The image analysis system also enabled facile analysis of samples containing up to 150 particles per slide to obtain the average particle size, particle size distribution, and shape. These parameters are necessary to develop mechanistic models for diffusion and dispersive processes, which cause band broadening in SEC, and have a significant impact on scale-up (Ladisch and Wankat, 1988; Rasmussen and Neretnieks, 1980).

Materials and Methods

Materials. Sephadex G-75, a dextran gel cross-linked with epichlorohydrin, was studied. Sephadex was obtained from Pharmacia (lot no. KP86158) and had a nominal particle diameter range of 40–120 μm. Swelling experiments were carried out with Sephadex in deionized water, in a Tris-glycine buffer, and in Tris-glycine buffer plus bovine serum albumin (BSA) at a concentration of 1 mg/mL. The Tris-glycine buffer, made of 3.9 mM Tris and 47 mM glycine, had a pH of 10.2. BSA (catalog no. A0281 from Sigma) was essentially fatty acid free and was stored in a freezer below 0 °C. Slides were prepared by using silicone vacuum grease to form a seal between the slide and the coverslip. The samples were viewed with a 2.5X lens and 100-W halogen illuminator under bright field conditions.

Apparatus. The swelling of Sephadex G-75 was studied by using a Kontron IM inverted microscope and a Zeiss IBAS 2000 image analysis system. The microscope image was transferred to the image analysis system through a Dage-MTI Series 68 video camera. The digital image was enhanced by using software provided with the IBAS system to distinguish the particles from the background. The Sephadex particles were then identified and measured. Particle size was determined by measuring the diameter.
Figure 1. Comparison of wet particle diameter over long time periods of swelling of Sephadex G-75 in water, Tris-glycine buffer (pH 10.2), and buffer containing protein at 1 mg/mL. Measurements reported are based on average particle size obtained from 100-120 particles.

\[ d_p = 2(A/\pi)^{1/2} \]

where \( A \) denotes area. Particle shape was determined from the elliptical shape factor, \( E \):

\[ E = b/a \]

where

\[ a = (4I_x/A)^{1/2} \]

and

\[ b = (4I_y/A)^{1/2} \]

Parameters \( I_x \) and \( I_y \) in eqs 3 and 4 represent the moments (IBAS 2000 Reference Manual, 1985):

\[ I_x = \frac{I_x + I_y}{2} + \left[ \left( \frac{I_x + I_y}{2} \right)^2 - (I_x - I_y)^2 \right]^{1/2} \]

\[ I_y = \frac{I_x + I_y}{2} - \left[ \left( \frac{I_x + I_y}{2} \right)^2 - (I_x - I_y)^2 \right]^{1/2} \]

\[ I_x = \int x^2 \, dA \quad I_y = \int y^2 \, dA \quad I_{xy} = \int xy \, dA \]

All distances are measured with respect to the centroid.

Methods. Initially, particle measurements were taken over a long time period at long time intervals. After the initial uptake of water, there appeared to be very little change in the particle size when periodically observed over a period of several days. Therefore, further experiments were performed at shorter time intervals over smaller time periods so that the initial swelling which occurs when water comes into contact with the Sephadex particles could be observed and recorded.

The swelling of Sephadex was first observed with particle measurements obtained 4-8 times during a 24-h time period with the same microscope slide. Slides were prepared of Sephadex in water, in buffer, and in buffer plus BSA at concentrations of 1 mg/mL. The sizes of 100-150 particles were measured per slide so that the average particle size could be monitored as a function of time.

In the second set of experiments, particle size was measured twice a day for 2.5 days. Measurements started approximately 3-5 min after the water or buffer solution was added. The Sephadex diameter was measured in water and in buffer at concentrations of 50 mg/mL. New slides were prepared for each measurement, and the sizes of approximately 200 particles were measured.

Figure 2. Swelling of two representative individual particles of Sephadex G-75 in water, buffer, and protein (conditions as in Figure 1). Panel a shows relatively small particles, while panel b shows larger particles.

Figure 3. Diameter of a typical Sephadex G-75 particle as a function of time during swelling in water. Diameter of dry particle at time \( t = 0 \) is 98.1 \( \mu \)m. The solid line is the Tanaka-Pillmore prediction.

Table I. Average Diameter of Sephadex G-75 in Water and Buffer

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Diameter, ( \mu )m</th>
<th>Std Deviation, ( \mu )m</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.6</td>
<td>186</td>
<td>69</td>
</tr>
<tr>
<td>24.6</td>
<td>191</td>
<td>65</td>
</tr>
<tr>
<td>33.6</td>
<td>166</td>
<td>64</td>
</tr>
<tr>
<td>49.5</td>
<td>171</td>
<td>57</td>
</tr>
<tr>
<td>buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>172</td>
<td>68</td>
</tr>
<tr>
<td>24.0</td>
<td>179</td>
<td>67</td>
</tr>
<tr>
<td>32.0</td>
<td>167</td>
<td>50</td>
</tr>
<tr>
<td>49.0</td>
<td>178</td>
<td>52</td>
</tr>
</tbody>
</table>

* Based on 200-230 particles.

Next, particle sizes were measured 16-24 times during a 24-h time period with a single screen on the image analysis system, so that swelling of individual particles...
could be monitored. Measurements started with time intervals of 2 min. The time intervals were then increased as the rate of swelling decreased. A run was started by adding Sephadex particles to obtain a 5 mg/mL (dry basis) concentration in water, buffer, and buffer plus BSA.

Finally, a fourth set of experiments were carried out in which the initial swelling of the Sephadex particles was captured on videotape by using a Sony SLO 350 video recorder (Beta format) and viewed on an Ikegami FM20A 20-in. black and white monitor. Dry Sephadex was placed between two circular coverslips in a Dvorak-Stottler culture chamber. The swelling of the Sephadex particles was then captured on videotape as water was injected between the coverslips. Later, the selected microparticle images, as recorded on videotape, were converted to digital form and analyzed at 3-s intervals to determine particle sizes by using the IBAS image analysis system.

**Results**

Measurements of particle size for Sephadex in water, Tris-glycine buffer (pH 10.2), and Tris-glycine buffer containing 1 mg/mL BSA showed that the diameter changes only slightly over a long period of time (see Figure 1). The distribution of particle diameters for all three cases (indicated by the bars) spanned a range of 90–250 μm. The particle size for each of the three slides was monitored for a period of up to 1600 min. The apparent difference in particle size between water (150 μm), buffer (176 μm), and buffer with BSA (164 μm) is probably due to differences in sampling for the three respective slides, each of which was based on 100–150 particles. More representative samples were prepared of Sephadex particles in water and buffer in which 200–250 particles were measured at 8, 24, 32, and 48 h by using multiple slides. Little difference was noted between the water and buffer samples (Table I). Hence, from Table I and Figure 1 we conclude that the differences between water, buffer, and buffer containing a low protein concentration are at most small, with fine differences being masked by the broad particle size distribution. From these measurements of Sephadex, it was found that the particles were approximately spherical, with an elliptical shape factor of 0.98 (1.0 being perfectly spherical), and the wet particle diameters ranged from 40 to 350 μm, while the initial dry material had a diameter range of 20–100 μm. On the basis of these results, further development of the measurement techniques was carried out and resulted in procedures for tracking individual particles (i.e., experiments 3 and 4). Measurements were taken of individual particles ranging from 68 to 354 μm in diameter for 24–24 h starting at 3 or 4 min. The results of Sephadex in water (shown in expanded scale in Figure 3) indicate that particle size changes are essentially complete after 3 or 4 min. Therefore, it was necessary to record the swelling on videotape, followed by digitizing of the image and subsequent image analysis. Results from a typical run show a rapid initial swelling followed by a slower approach to the final size (Figure 3). Swelling data for three representative particles are shown in Table II.

**Discussion**

We tested the kinetic theory of Tanaka and Fillmore (1979) for the swelling of gels against these experimental data. Here the swelling process is attributed to the net effect of two phenomena: the expansion of the polymer network, modeled as a diffusive process, and the frictional resistance of the liquid found within the gel's pores against the swelling. It will be shown later that thermal effects and motion of the solvent molecules may be neglected.

Tanaka and Fillmore described the expansion of the gel in terms of the displacement vector $\mathbf{u}(t, \mathbf{x})$, defined so that $\mathbf{q} = 0$ as $t \to -\infty$. The governing equation for $\mathbf{u}$ has been shown (Tanaka and Fillmore, 1979; Tanaka et al., 1973) to be

$$\frac{\partial \mathbf{u}}{\partial t} = K \nabla^2 \mathbf{u} + \frac{1}{\eta} \Delta \mathbf{u} \quad (8)$$

where $K$ is the osmotic bulk modulus and $\eta$ the shear modulus of the gel, $f$ is the friction coefficient of the liquid within the pores of the gel, and $\Delta$ denotes the Laplacian.

As has been shown previously, our experiments were carried out on particles that were spherical. For a spherical particle, eq 8 becomes a scalar relationship:

$$\frac{\partial u}{\partial t} = D \frac{1}{r_0} \left[ \frac{1}{r_0^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial u}{\partial r} \right) \right] \quad (9)$$

where $D$, first called the diffusion coefficient of the gel by Tanaka et al. (1973), is given by

$$D = (K + \frac{f}{2} \eta) \eta / E \quad (10)$$

where $E$ is the longitudinal elastic modulus of the gel (Geisser and Hecht, 1980).

If $a$ is the final radius of the gel particle in its fully swollen (equilibrium) state and $\Delta a$ is the total increase in the radius of the particle over the swelling process, the initial condition for the governing equation (9) can be written as

$$u(r_0, t) = \Delta a / a \quad (11)$$

One boundary condition derives from the fact that the center of the sphere suffers no displacement throughout the swelling process, so that

$$u(0, t) = 0 \quad (12)$$

The other boundary condition expresses the absence of radial stress at the surface of the gel at all times. This would give rise to a moving boundary value problem; since, as will be seen presently, we are primarily interested in the latter stages of the swelling process (i.e., at long $t$), we may approximate this by the fixed boundary condition
\[ (K + \frac{1}{2} \mu) \frac{\partial u(t)}{\partial t} + (K - \frac{1}{2} \mu) \frac{u(t)}{p} = 0 \]  

The general solution of eqs 9-13 has been derived (Peters and Candau, 1986, 1988). Tanaka and Fillmore (1979) obtained the solution for the special case where the shear modulus, \( \mu \), is negligible relative to the elastic bulk modulus, \( K \), and a review of the available solutions was offered by Peters et al. (1989). It will be shown later that the restricted solution of Tanaka and Fillmore is adequate for our data. This solution can be expressed as

\[ u(r,t) = -6\Delta a_0 \sum_{n\pi/r} \left( \frac{1}{n\pi/r} \frac{\cos \left( \frac{n\pi}{r} \right) - \sin \left( \frac{n\pi}{r} \right) n\pi/r}{k_n^2 - \left( k_n^2 - \frac{n^2}{r^2} \right)^2} \right) e^{-\alpha_n \sqrt{r}} \]  

where

\[ k_n = n\pi/a \]  

(14)

Equation 14 represents the solution to a vector Laplace equation in spherical coordinates and thus differs from analogous solutions of the diffusion equation in spherical coordinates for scalars such as given by Crank (1975). Equation 14 can be rewritten as

\[ u(r,t) = -6\Delta a_0 \sum_{n\pi/r} \left( \frac{1}{n\pi/r} \frac{\cos \left( \frac{n\pi}{r} \right) - \sin \left( \frac{n\pi}{r} \right) n\pi/r}{X_n - X_n^2} \right) e^{-a_n \sqrt{r}} \]  

where

\[ X_n = n\pi/r \]  

and

\[ \alpha_n = \sigma n^2 \]  

(17)

where \( r \) can be regarded as the (longest) relaxation time. For \( r/t < 0.25 \), eq 14 is dominated by its first term, so that for long times (defined by the above condition)

\[ u(r,t) \approx -\frac{6}{r} \Delta a_0 \left[ \frac{\cos \left( \frac{n\pi}{r} \right) - \sin \left( \frac{n\pi}{r} \right) n\pi/r}{X_n - X_n^2} \right] e^{-a_n \sqrt{r}} \]  

(18)

Finally, the gel surface as a function of time is given by

\[ a - r(t) = u(t) = \frac{6}{r^2} \Delta a_0 e^{-a_n \sqrt{r}} \]  

(19)

where \( r(t) \) is the radius of the gel at any time \( t \). Similarly

\[ \Delta a_0 = a - r_0 \]  

(20)

where \( r_0 \) is the initial radius (at \( t = 0 \)). Then, for long times

\[ a - r(t) = \frac{6}{r^2} \Delta a_0 e^{-a_n \sqrt{r}} \]  

(21)

The left-hand side can be replaced with the corresponding diameters (rather than radii) to get

\[ \frac{d_v}{d_p} = -\frac{d_v}{d_p} = \frac{6}{r^2} e^{-a_n \sqrt{r}} \]  

(22)

Equation 23 has the same form as the leading term in the solution to the analogous scalar diffusion equation (Crank, 1975) (we are indebted to a reviewer for pointing this out). This is because the temporal dependence is of the same form in both solutions, and while the radial dependence in the two solutions is different, they tend to the same limit at the particle boundary; consequently, the results for the particle diameter as a function of time become identical. This does not invalidate the distinction drawn earlier between the solutions of the scalar and vector diffusion equations, which is reflected in the differing radial dependencies.

When \( d_v^0 \) and \( d_p^0 \) are known, linear regression may be used with the logarithmic form of eq 23 to extract \( r \). However, in many experimental runs, there was a good deal of fluctuation in the measured value of \( d_v \) for very long times, so that \( d_v^* \) became difficult to estimate. If the mean value of the fluctuations were chosen, the left-hand side of eq 23 would become negative for some values of \( d_v^* \), making it impossible to use the logarithmic form in the linear regression. Consequently, \( d_v^* \) was regarded as an additional parameter, and a nonlinear regression was carried out on eq 23 by using the program MARLIN in the statistical package SAS to obtain values of \( d_v^*, r \), and \( C \). Here, the constant \( b_0 \) was replaced by \( b \) as a constant \( C \) in order to examine the applicability of the Tanaka-Fillmore model to our data. In all cases, only data for \( t \geq 12 \) were used. It will be shown that this meets the long-time condition.

The nonlinear regressions were based on the Gauss-Markovardt method (SAS User's Guide, 1985). Sample output from SAS for one representative gel particle is shown in Table III, and the fit of the regressed curve as obtained from eq 16 to the experimental points is depicted in Figure 3. It can be seen that a good fit is obtained. The F-value was 2.34; the probability of the null hypothesis that the data are Gaussian is 0.05. The results for particles ranging in initial diameter from 78 to 157 um are given in Table IV. For \( s = d_v^*/2 \), calculated values of \( \bar{c} \) can be plotted against those of \( r \) to give a straight line according to eq 18, the slope of which can be used to extract the diffusion coefficient of the gel, \( D \). This is shown in Figure 4, from which the value of \( D = 0.3 \times 10^{-9} \) cm^2/s is obtained. The regression value of \( C \) from the various experiments is shown in Figure 5, where the solid line represents the theoretically predicted result of \( 6\pi/7 \). As shown by Peters and Candau (1986, 1988), if the shear modulus were not negligible relative to the elastic bulk modulus, the value of the experimentally derived constant \( C \) would be significantly less than \( 6\pi/7 \), and their more involved calculations would have to be invoked. Figure 5 shows that \( C \) is remarkably close to \( 6\pi/7 \), which justifies our earlier neglect of the shear modulus.

The magnitude of the mass diffusivity \( (10^{-10} \text{cm}^2/\text{s}) \) is much smaller than that characteristic of thermal fluctuations; e.g., the thermal diffusivity for water is about \( 1.4 \times 10^{-9} \text{cm}^2/\text{s} \) (Tanaka et al., 1973). Thus, thermal fluctuations were neglected. A recent paper by Li and Tanaka (1989) also indicates that the temperature relaxation time is more than an order of magnitude smaller than the volume swelling) relaxation time. Another effect that could alter the temperature locally is the heat of mixing accompanying the swelling process. Preliminary experiments carried out in an insulated flask indicate that the temperature rise was 0.5-1°C for 10 g of Sephadex in 200 mL of water. Further, since the bulk of this temperature increase is probably associated with the rapid swelling of the gel in the first few seconds, the assumption of isothermal conditions for the long-time data used to calculate the gel diffusion coefficient seems reasonable.
Table IV. Regressed Values of $d_i^2$, C, and r for Nine Different Particles

<table>
<thead>
<tr>
<th>particle no.</th>
<th>$d_i^2$ (μm)</th>
<th>$d_i^2$, cm</th>
<th>C</th>
<th>r, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78.1</td>
<td>182.9</td>
<td>0.35</td>
<td>15.6</td>
</tr>
<tr>
<td>2</td>
<td>85.6</td>
<td>181.7</td>
<td>0.42</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>95.5</td>
<td>145.9</td>
<td>0.58</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>93.0</td>
<td>148.2</td>
<td>0.92</td>
<td>9.3</td>
</tr>
<tr>
<td>5</td>
<td>95.1</td>
<td>165.0</td>
<td>0.49</td>
<td>11.1</td>
</tr>
<tr>
<td>6</td>
<td>105.4</td>
<td>181.1</td>
<td>0.58</td>
<td>13.9</td>
</tr>
<tr>
<td>7</td>
<td>107.3</td>
<td>235.6</td>
<td>0.38</td>
<td>21.0</td>
</tr>
<tr>
<td>8</td>
<td>127.6</td>
<td>216.9</td>
<td>0.67</td>
<td>17.2</td>
</tr>
<tr>
<td>9</td>
<td>127.0</td>
<td>248.4</td>
<td>0.69</td>
<td>22.8</td>
</tr>
</tbody>
</table>

The initial diameter, $d_i^2$, is also given. The particles are listed by increasing size.

Figure 4. Plot of regressed value of $(d_i^2)^2$ against relaxation time r. The slope of the resulting straight line is $\pi D/4$, where D is the diffusion coefficient of the gel.

Figure 5. Plot of the regressed constant C for the various experiments. The Tanaka-Fillmore value of this constant is 6/s, which is within 11% of the average experimental value. The particle numbers are as in Table IV.

The data in Table III show that the characteristic relaxation times r are of the order of 10-20 s, so that the long time assumption $t > 0.25r$ would require the neglect of experimental points obtained before 2-5 s. We have neglected all points before 12 s, so that the long time approximation may be safely assumed to hold.

Tanaka and Fillmore pointed out that their theory is strictly applicable only to "linear" systems, i.e., systems for which the Hookean elasticity relationship is valid and which result in a constant diffusivity. Their experiments were carried out with 5% polyacrylamide gels, and the relative degree of swelling was 10% - 14%, justifying the linear assumption (Tanaka and Fillmore, 1979). Our experiments on Sephadex particles show much greater swelling effects, the relative degree of swelling being 80% - 120% (cf. Table IV), and it may be argued that a linear model should no longer describe the data accurately.

Figure 6. Worst-case comparison of the Tanaka-Fillmore model with experimental data on the swelling of a Sephadex G-75 particle in water. Initial diameter of the particle was 78.1 μm.

However, the basic purpose of the Tanaka-Fillmore model was to describe the swelling process for long times, as previously discussed. The relative degree of swelling at long times in our experiments is of similar magnitude to that r. The correct initial Tanaka and Fillmore, consequently, given this approximation, the Tanaka-Fillmore model accurately described our data. In this context, it may be noted that the two smallest particles exhibit the greatest deviation from theory (Figure 5). This is to be expected, since the fractional increase in volume is largest for the smallest particles, making them the most likely to deviate from the assumption of constant diffusivity. If this had been a general feature of all the data, it would have been necessary to replace the present model by one in which the diffusivity becomes a function of concentration. However, since the majority of particles behave in accordance with the simple constant diffusivity theory, a more complex model was felt to be unnecessary.

An alternative formulation of the kinetics of gel swelling has been proposed by Komori and Sakamoto (1989). The process is described in terms of the interplay between the elastic and fluid draining of the gel and a swelling pressure due to fluid infiltrating the gel. While the resulting model differs from that of Tanaka and Fillmore, the modified equation expressing the swelling of a given particle at any time can be formally identical with eq 16, which is the expression of Tanaka and Fillmore. However, the application of the model of Komori and Sakamoto to experimental data permits the estimation of only the diffusivity of the fluid that penetrates the gel. Theoretically, with the caveat that considerably more complex theories are likely to be necessary in elucidating all of the relevant aspects of gel swelling, we retained the model of Tanaka and Fillmore as being suitable for the purpose of estimating characteristic swelling times in SFC.

Values of the gel diffusivity coefficient available in the literature are of the same order of magnitude as the present estimate. Tanaka and Fillmore (1979) calculated $D = 2.2 \times 10^{-7}$ cm²/s for a 5% polyacrylamide gel. Fiorestone and Sigel (1989), on the basis of the Tanaka-Fillmore theory, arrived at an order of magnitude estimate of $10^{-8}$ cm²/s for a slightly cross-linked, hydrophobic polyelectrolyte gel in water. Gehrze and Cussler (1988) used a different approach to estimate the gel diffusion coefficient of an acrylamide-based hydrogel at about $6 \times 10^{-7}$ cm²/s.

While the results in Figures 1 and 2 show that protein solutions do not significantly affect the rate of swelling, these results were for 1 mg/mL protein concentration. It
is possible that higher solute concentrations (particularly likely for smaller molecules, where solubility is less of a limitation) could result in concentration-dependent swelling.

With respect to how well the model predicts the experimental data at short times, it has already been shown in Figure 3 that it can be a good fit. This is fortunate, since eq 23 is only expected to hold for longer times. In general, the data may be expected not to agree with the theory for short times, and the case for which the fit at short time was the poorest is shown in Figure 6. The theory underpredicts the data, which is consonant with the notion that the diffusion coefficient is a function of position and time in this regime and is underpredicted by the "zeroth-order" constant diffusivity $D$ that is valid for long times.

In the context of design, the complex problem of attempting to describe the time and space dependence of the gel diffusion coefficient at short times is unlikely to arise. For systems such as those discussed by Tanaka and Fillmore, the total extent of swelling is not large enough to invalidate the linear model even in the short time regime, and the data over the entire range should conform to the Tanaka-Fillmore model. In these cases, the relaxation times are larger than or comparable to chromatographic separation times [the systems of Tanaka and Fillmore (1979) have relaxation times of several hours], and the swelling process could play a significant role in the separation.

For the Sephadex particles discussed here, the linear model is invalid for short times. However, the characteristic relaxation time is on the order of 15 s and is therefore very much smaller than characteristic separation times. The swelling process thus occurs so quickly relative to separation that it is unlikely to play a significant role in the overall process, thus obviating a detailed description of the diffusion coefficient in the short time region. These conclusions suggest that rapid packing of a stable column of Sephadex can be achieved by mixing the gel with water or the buffer, holding for about 2 min, and then pumping the slurry into the column.

The significance of the gel diffusion coefficient is that it can be used to estimate the effects of the swelling process and thus indicates when these effects could be significant in large-scale SEC columns. For instance, if experiments similar to those described above are carried out with small particles of a given SEC gel and the gel diffusion coefficient is estimated, the relaxation time for a large-scale column packed with particles of diameter $d_p$ (of the same gel material) can be estimated as

$$r^* = (d_p)^2/(4\pi D)$$  \hspace{1cm} (24)

Conclusions

Swelling of Sephadex G-75 occurs very rapidly and reaches a stable particle size within minutes. A novel combination of light-field microscopy and image analysis facilitates direct measurement of the swelling process and quantitation of swelling rates as well as final particle sizes. The Tanaka-Fillmore theory of gel swelling was found to be applicable to our data on Sephadex and was used to arrive at a diffusion coefficient of $6.3 \times 10^{-5}$ cm$^2$/s. This then allowed the calculation of the characteristic relaxation time for a given particle, which is on the order of 15 s. The results for this gel suggest that changes in particle size due to effective protein concentrations experienced inside a gel column will have a minimal effect on changing particle size when the samples are dilute. Changes in particle swelling, if they occur, are likely to happen quickly.

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