Hydraulic Permeability of Agarose Gels

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A new technique was developed to measure the hydraulic permeability of reinforced gel membranes and allows calculation of the Darcy permeability (κ) of the gel. It was applied to agarose with concentrations ranging from 2.0 to 7.3%. To create membranes that would be thin enough to yield easily measured filtration rates at modest applied pressures and yet withstand handling, gels were cast on woven polyester meshes. The resulting membranes had thicknesses of 70–100 μm and a fractional open area of 0.32. To correct for the presence of the mesh, finite-element solutions were obtained for the pressure field in the 3-D region occupied by the gel. For the meshes used here, the hydraulic permeability of the reinforced membrane was calculated to be 0.47–0.55 times that for a layer of pure gel, the exact value depending on the thickness of the composite membrane. The principal determinant of κ was the agarose concentration, but there was a secondary effect of applied pressure. The Darcy permeability extrapolated to zero applied pressure (κ_0) varied from 616 nm^2 for 2.0% agarose to 22 nm^2 for 7.3% agarose. At a given gel concentration, the value for κ_0 was as much as twice the value for κ measured at the maximum pressure difference of 20 kPa. This method can be adapted to a variety of other gel materials.

Introduction

Hydrogels have numerous applications in biomedical engineering and biotechnology, and are important also as components of body tissues. Therapeutic devices such as contact lenses, breast implants, and drug-delivery capsules are composed of gels (Peppas, 1987). One strategy for developing implants for organ replacement is to encapsulate cells in gels that do not significantly interfere with the transport of nutrients or desired products (e.g., insulin from pancreatic islets) (Lacy et al., 1991). Because of their high water content, hydrogels provide an ideal matrix for cell growth and are commonly used to propagate bacteria and mammalian cells. Gels are also used to separate macromolecules in size-exclusion and ion-exchange chromatography and in electrophoresis. Connective tissue, vascular and epithelial basement membranes, and vitreous humor are examples of normal body tissues that have gel-like characteristics.

Fluid flow through gels or other porous media is usually modeled using Darcy's law,

$$v = -\frac{\kappa}{\mu}\nabla P, \tag{1}$$

where v is the fluid velocity, μ is the viscosity, P is the pressure, and κ is the Darcy permeability. For a pressure drop ΔP imposed across a slab of thickness, L, the superficial velocity obtained by integrating Eq. 1 is

$$v = \frac{\kappa \Delta P}{\mu L}. \tag{2}$$

In the membrane literature "hydraulic permeability" usually refers to the proportionality constant relating v to ΔP, namely $\kappa/(\mu L)$, and that is how that phrase is used here.

In an application such as protein separation by electrophoresis, where the main purpose of the gel is to suppress flow, the magnitude of κ is unimportant as long as it is sufficiently small. In other situations, however, the amount of water flow induced by a given applied pressure is of special interest. For example, the rates of fluid flow through renal capillary basement membrane and the extracellular matrix of tumors are critical for maintenance of normal kidney function (Drummond and Deen, 1994) and the delivery of anticancer drugs (Jain, 1987), respectively. Even when diffusion is the only mechanism for solute transport, κ is useful for predicting the hydrodynamic effects of a gel on the mobility of a

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macromolecular solute (Kosar and Phillips, 1995; Johnson et al., 1995). The value of κ has also been used to infer information on the microstructural characteristics of gels (Tokita and Tanaka, 1991).

Although there are a variety of reasons for measuring the hydraulic permeability (or κ) of hydrogels, limited experimental information is available. The hydraulic permeabilities of agar and gelatin have been determined by measuring water flow through a gel column (Pallman and Devel, 1945; Signer and Egli, 1950). Various approaches have been used also to measure κ for cross-linked polyacrylamide (Tokita and Tanaka, 1991; White, 1960; Weiss and Silberberg, 1976). In general, researchers have employed relatively thick samples (L ranging from ~ 1 mm to several cm), requiring the use of fairly large applied pressures and/or the measurement of very small water flow rates. The experimental difficulties encountered are not trivial, as evidenced by values of κ for a given concentration of polyacrylamide that span two orders of magnitude (Tokita and Tanaka, 1991; White, 1960; Weiss and Silberberg, 1976).

Agarose is a polysaccharide derived from red seaweed (*Rhodophyceae*), and is used extensively in chromatography and electrophoresis. Agarose gels are formed by a reversible, physical association of the polysaccharide chains. At high temperatures (generally $> 80°C$, depending on the agarose type), the agarose chains are soluble in water. As the temperature is lowered, the agarose chains join to form double-helical fibrils (Arnott et al., 1974), which aggregate further to form larger fibers. Gelatin usually occurs by the time the solution reaches 40°C. Because the gels are formed by physical linkages among multiple chains, there is a distribution of fiber radii. Electron microscopy (Amsterdam et al., 1975; Spencer, 1982; Waki et al., 1982; Whytock and Finch, 1991) has demonstrated a range of fiber radii of 1–20 nm, without quantifying the distribution of fiber sizes. Light-scattering results (Obrink, 1968) suggest an average fiber radius of 1.5–2.0 nm. Data from small-angle X-ray scattering (SAXS) (Djabourov et al., 1989) indicate a bimodal distribution, with a radius of 1.5 nm for 87% of the fibers and a radius of 4.5 nm for 13%, yielding a number-average radius of 1.9 nm. Despite its widespread use, there appear to be no previous measurements of κ for agarose.

We report here a simple method for measuring hydraulic permeabilities of gels at low pressures, which we used to obtain values of κ for agarose with concentrations ranging from 2 to 7%. Membranes that were thin, but that had sufficient mechanical strength, were prepared by casting gels on a woven polyester mesh. Gel thicknesses as small as 70 μm were obtained in this manner.

Methods

Preparation of gels

Gels were made by first adding 10 mL of buffer to a measured amount of agarose powder (Type VI, high gelling; Sigma, St. Louis, MO) in a 20-mL glass vial, and placing the resulting slurry in a 90°C oven for 2–5 h. The buffer consisted of 0.01-M sodium phosphate and 0.1-M potassium chloride at pH 7. The vial, which was sealed to prevent evaporation, was rotated by hand periodically to ensure adequate mixing. Two glass plates were also heated. To cast the membranes, a 2.5-cm-diameter piece of woven polyester mesh (Spectrum Medical, Houston, TX) was placed on one of the glass plates. The mesh formed a square pattern with a fiber radius of 20 μm, a center-to-center fiber spacing of 93 μm, and a thickness of 70 μm. The hot agarose was quickly poured onto the mesh and the second plate used to form a sandwich. Care was taken to ensure that air bubbles were not trapped in the gel. The glass plates were then clamped together and allowed to cool to room temperature. Finally, the gel was immersed in phosphate/KCl buffer and stored (usually overnight) at 4°C.

Hydraulic permeability measurements

The gel membrane was mounted on a porous frit inside a 3-mL ultrafiltration cell (Model 3, Amicon, Beverly, MA), which was then filled with the phosphate/KCl buffer. The solution was forced through the membrane at a constant pressure using compressed nitrogen. The transmembrane pressure drop was monitored using a pressure transducer (Model DP15, Validyne Engineering, Northridge, CA), while the flow rate was determined by collecting and weighing the filtrate. The height of the water in the ultrafiltration cell was measured during the experiment and a correction was made for the hydrostatic pressure. To minimize transient effects, filtrate collections were begun only after at least 100 μL had passed through the system. The time for this depended on the agarose concentration and the applied pressure drop. For a 2% agarose gel at 20 kPa this equilibration period was about 20 s, whereas for a 7.3% agarose gel it was at least 15 min. After the equilibration period, two consecutive filtrate collections were made, each about 500 μL. The variation in permeability values between these two measurements was always less than 2%, suggesting that gel relaxation or other transient effects were negligible. After the flow measurements were completed, the membrane thickness was determined by placing the sample between two microslides and using a micrometer to determine the thickness both with and without the membrane present. The uncertainty in this measurement was estimated as ± 3 μm.

Hydraulic permeability measurements were made for agarose concentrations of 2.0, 3.9, 5.6, and 7.3% (w/v). The volume fraction of fibers was calculated by dividing the weight fraction by 1.025 (Johnson et al., 1995). To investigate the effects of compression of the membrane, results were obtained at five different pressures: 3, 7, 10, 13, and 20 kPa. The effects of heating and cooling time in gel preparation were also examined.

Correction factor for the effect of the fiber mesh

Because the open area was reduced considerably by the fiber mesh support, it was necessary to use a correction factor in computing κ. This factor (β) was defined by rewriting Eq. 2 as

$$v = \beta \frac{\kappa \Delta P}{\mu L}. \tag{3}$$

Thus, in general $0 \leq \beta \leq 1$, with $\beta = 1$ corresponding to the hypothetical case of no mesh. As shown in Figure 1, we mod-

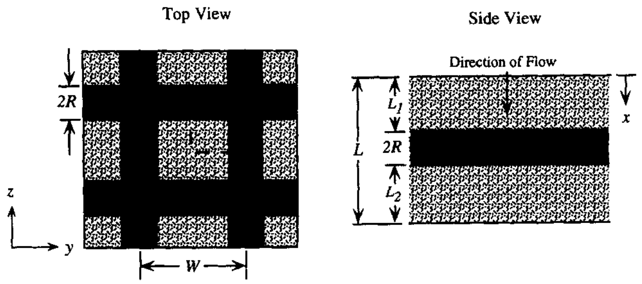

Figure 1. Model geometry used for calculating the pressure field and correction factor for hydraulic permeability for mesh-supported gels.

The woven mesh is represented as a coplanar network of intersecting cylinders of radius R, with a center-to-center distance W. The side view shows the total thickness of the membrane, L, and the thickness of gel layers upstream and downstream from the fibers, L_1 and L_2, respectively. Because there are two planes of symmetry, the finite-element calculations used only 1/4 of a unit square, as indicated by the dashed box in the top view.

eled the mesh structure as a square array of intersecting cylinders. Four independent dimensions are the mesh fiber radius (R), the center-to-center spacing of the fibers (W), the thickness of the gel on the upstream side of the fibers (L_1), and the total thickness of the membrane (L). With four dimensional lengths, the correction factor β must be a function of three dimensionless groups. These were chosen as

$$\alpha \equiv \left(1-\frac{2R}{W}\right)^2 \tag{4}$$

$$\lambda \equiv \frac{L}{R} \tag{5}$$

$$\gamma \equiv \frac{L_1}{L_1+L_2} = \frac{L_1}{L-2R}. \tag{6}$$

Thus, $\beta = \beta(\alpha, \lambda, \gamma)$, where α is the ratio of open area to total area, λ is the ratio of membrane thickness to fiber radius, and γ is the fraction of the "excess" gel that is upstream of the fibers.

The governing equation for the three-dimensional pressure field, $P(x, y, z)$, was obtained by combining the continuity equation with Darcy's law to give

$$\nabla^2 P = 0. \tag{7}$$

Taking x as the direction normal to the membrane surfaces, the square mesh provides symmetry in both y and z. Accordingly, the domain chosen corresponded to only one-quarter of a unit cell, as shown by the dashed square in the top view of Figure 1. The boundary conditions used were

$$P = \Delta P \quad \text{at } x = 0 \tag{8a}$$

$$P = 0 \quad \text{at } x = L \tag{8b}$$

$$\boldsymbol{n}\cdot\nabla P = 0 \quad \text{at fiber surfaces, symmetry planes,} \tag{8c}$$

where $\boldsymbol{n}$ is a unit normal vector. The pressure field and β were computed using a commercial finite-element package (FIDAP, Fluid Dynamics International, Evanston, IL) on a Silicon Graphics Indigo workstation. The calculations were based on the Galerkin method with quadratic basis functions. As the number of nodes was increased, the average pressure gradients at the surface converged quadratically, as expected. The factor β was calculated as the pressure gradient averaged over the downstream surface, $\langle -(\partial P/\partial x)|_{x=L}\rangle$, divided by the macroscopic pressure gradient, $\Delta P/L$. Using 5,769 nodes in these three-dimensional simulations yielded errors of $<0.1\%$ in β.

Results and Discussion

Correction factor

In calculating the correction factor β needed to compute κ from data obtained using mesh-reinforced gels, a simplifying assumption was that the mesh fibers were intersecting cylinders. The actual mesh was woven, so that the fibers were not uniformly coplanar. To estimate the error introduced by this assumption, we varied the parameter γ, which corresponds to the fraction of the excess gel that is on the upstream side of the fibers. For $0.1 \le \gamma \le 0.9$ there was less than a 3% change in β. This indicates that the exact location of the cylindrical fibers in the x-direction is relatively unimportant. Although it was not practical to calculate β using an exact representation of the woven mesh, the intersecting cylinder model appears to be a reasonable approximation. Having found that $\beta \cong \beta(\alpha, \lambda)$ only, the dependence of β on the remaining two parameters was determined using simulations where $0 \le \alpha \le 1$ and $\lambda = 2.50$, 3.75, and 7.50 (with $\gamma = 0.5$). Figure 2 shows the results, together with a power-law curve fit for each value of λ. For the actual membranes used $R = 20$ μm, $W = 93$ μm, and $70 \le L \le 100$ μm. Thus, $\alpha = 0.32$ and $3.5 \le \lambda \le 5.0$. For those particular conditions it was found that β was linear in L, varying from 0.466 at $L = 70$ μm to 0.548 at $L = 100$ μm.

Darcy permeabilities

Duplicate measurements of hydraulic permeability with a given membrane were usually within 1% of one another, and

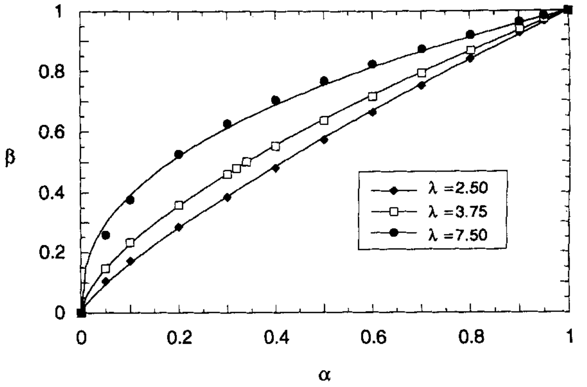

Figure 2. Effects of α, fractional open area, and λ, ratio of membrane thickness to fiber radius, on correction factor, β.

The symbols show the finite-element results, and the curves are power-law fits of the form $\beta = \alpha^m$. For $\mu = 2.50$, 3.75, and 7.50 the exponents are $m = 0.787$, 0.642, and 0.409, respectively. All results are for $\gamma = 0.5$.

always within 2%. Membrane-to-membrane variations in κ tended to be much larger, so that statistics were calculated on the basis of the number of membranes examined. There was no significant effect of varying the heating time in gel preparation from 2 to 5 h, or of varying the refrigeration time before study from 1 to 24 h. We found, however, that if the solution was not carefully mixed during the several hours of heating, inhomogeneities in the gel could cause the permeability to vary by a factor of 2 or 3, especially at the higher gel concentrations. Even with careful attention to mixing, the variation in κ between nominally identical membranes was as much as 27%.

The hydraulic permeabilities were very sensitive to the gel concentration. As shown in Figure 3, the Darcy permeability at $\Delta P = 20$ kPa decreased by more than 20-fold as the volume fraction of agarose (ϕ) was increased from 0.019 to 0.072. As indicated by the straight line, there was approximately a power-law relationship between κ and ϕ, with an exponent (slope) of -2.4. This contrasts with the slope of -1.5 that is predicted from scaling theory for semidilute polymer solutions, and that has been observed experimentally for cross-linked polyacrylamide (Tokita and Tanaka, 1991). The difference in these slopes is not surprising, given the very different cross-linking mechanisms (physical association of agarose fibrils vs. chemical cross-linking of polyacrylamide).

There was an effect also of the applied pressure, κ decreasing for any given membrane as ΔP was increased. Figure 4 shows κ as a function of ΔP, as determined using one membrane at each agarose concentration. The permeabilities decreased by 14–31% when the pressure drop was increased from 3 to 20 kPa, the relationship between κ and ΔP being approximately linear. Darcy permeabilities extrapolated to zero pressure drop (κ_0) were computed by applying the slopes from the data in Figure 4 to the mean values of κ measured for all membranes at $\Delta P = 20$ kPa (Figure 3). The values of κ and κ_0 for the various gel concentrations are shown in Table 1. Also shown in Table 1 are values of the correlation length, calculated as $\xi = \kappa_0^{1/2}$, which is a measure of interfiber spacing in the gel. Assuming that the average fiber radius is ~ 2 nm, at the lowest gel concentration the interfiber spacing greatly exceeded the fiber radius, whereas at the highest gel concentration the fibers were only a few radii apart.

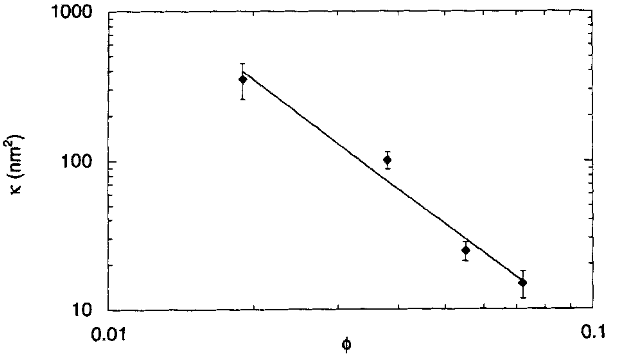

Figure 3. Darcy permeability for agarose gels, κ, as a function of the volume fraction of fibers, ϕ.

The symbols show the mean ± SD for $n = 5$ membranes at each agarose concentration, with $\Delta P = 20$ kPa. The best-fit line is given by $\kappa = 0.0244\ \phi^{-2.45}$.

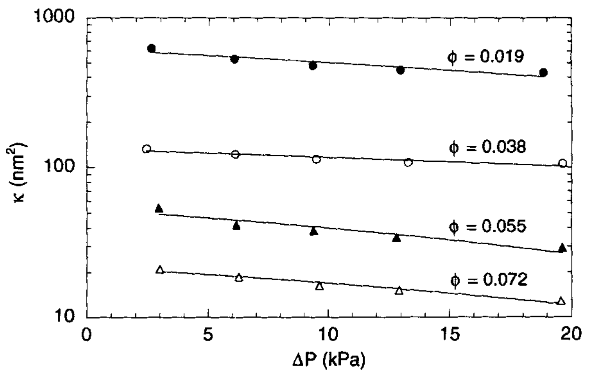

Figure 4. Effect of applied pressure, ΔP, on Darcy permeability, κ, for single membranes at each of four agarose concentrations.

The fitted curves are of the form, $\kappa = \kappa_0 - c\Delta P$. The intercepts ($\kappa_0$) are given in Table 1, and the slopes (in nm²/kPa) are $c = 11.5$, 1.59, 1.35, and 0.488 for $\phi = 0.019$, 0.038, 0.055, and 0.072, respectively.

Jackson and James (1986) reviewed theoretical predictions for κ based on hydrodynamic calculations for various arrangements of cylindrical rods, and examined Darcy permeability data obtained from various types of fibrous media. They concluded that κ for random, three-dimensional arrays of fibers could be predicted reasonably well by

$$\frac{\kappa}{a^2} = -\frac{3}{20\phi}(\ln \phi + 0.931), \tag{9}$$

where a is the fiber radius and ϕ is the volume fraction of fibers. Using Eq. 9 with $a = 1.9$ nm, as suggested by the SAXS data cited earlier, we obtain the predicted values of Darcy permeability shown in Table 1. The values from Eq. 9 were smaller than either of the experimental measures of Darcy permeability (κ or κ_0), the discrepancies being greater at the lower gel concentrations. At the three highest gel concentrations, at least, the extent of agreement between Eq. 9 and the values of either κ or κ_0 (up to fourfold differences) is no worse than what one might expect, given the variability in the experimental results reviewed by Jackson and James. Nonetheless, the increasing discrepancies at lower gel concentrations might be due to changes in the agarose microstructure. For example, the trend could be explained by a change in average fiber radius with gel concentration, a increasing as ϕ decreases, although we are not aware of any structural data that suggest such variations in fiber radius. Another possibility is that the interfiber spacings became more heterogeneous at the lower gel concentrations. The ef-

Table 1. Darcy Permeability of Agarose Gels*

ϕ	κ nm²	κ_0 nm²	κ (Eq. 9) nm²	ξ nm
0.019	353±95	616	86	25
0.038	101±13	132	33	12
0.055	25±4	53	19	7
0.072	15±3	22	13	5

*Values of κ are mean ± SD for five membranes at $\Delta P = 20$ kPa.

fect of unequal fiber spacings would be to increase κ for given values of a and ϕ.

Conclusion

A novel technique was developed to measure the hydraulic permeability of fiber-reinforced gel membranes, allowing calculation of the Darcy permeability for the gel. The method was applied to agarose with concentrations ranging from 2.0 to 7.3%, providing what appear to be the first measurements of Darcy permeability for this material. A particular advantage in using this methodology is that, because the membranes are very thin (~ 100 μm), easily measured volumes of filtrate are obtained with modest applied pressures. This simple approach should be adaptable to a variety of other gel materials.

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