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Analysis of fibrous network fluid permeation data using the theory of ultracentrifugation: application to fibrin gels

F. Henry M. Nestler*

331 East 14th Street, New York, NY 10003, USA

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Abstract

A new and alternative method for calculating the strand diameter of fibrous gel networks from fluid permeation data is developed and used to analyze and compare previous Darcy constant measurements of fibrin gels. The calculated diameters from the various sets of experimental data using this method gives for a coarse fibrin clot a strand diameter of approximately 1000 Å and for a fine fibrin clot a strand diameter of 170 Å.

Keywords: Fibrin gels; Darcy constant; Model; Strand diameter

1. Introduction

Over the past years a number of papers containing experimental data obtained by various methods have been applied to the determination of the general structural properties, principally strand diameter, of fibrin gel networks. For example, Roberts et al. [1], Carr and Hermans [2], Blombäck et al. [3] and recently Thurston and Henderson [4] have made measurements of the strand diameter or pore size either by gel permeation through the use of the Darcy constant or by light scattering. A comparison of the two methods in Blombäck's [3] extensive and interesting paper leaves something to be desired with respect to the diameters of the network strands of fibrin gels formed under various conditions that provide a broad range of strand sizes.

In light of the fact that the calculation of strand diameters from permeation data, with the

* Corresponding author.
E-mail address: fhm.nestler@erols.com (F.H.M. Nestler)

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methods of Kuhn [5] and Emersleben [6] in Signer [7], is based on empirical macroscopic models, one wonders if a microscopic model and less empirical theory might not produce a more realistic method for the determination of network parameters.

Within this context the following model and theory, which is based on a microscopic collection of fibers and an essentially non-empirical theory, is developed as a method for calculating the diameters of strands from network permeation data.

2. Model and theory

The model used in the following calculation is an abstraction of a fibrous network where all junctions have been removed to leave a uniformly random angular distribution of cylindrical fibers with assumed length much greater than their diameter which approximates the network strands and represents an average of all the strands. Removing the junctions should not change the friction coefficient. A group of fibers under a force will undergo sedimentation such as in an ultracentrifuge and have a sedimentation coefficient s and an infinite dilution coefficient s_o if concentration dependence is considered. With the observation that fibers moving through a fluid (sedimentation) is equivalent to a fluid flowing through a group of fibers (permeation) one can apply the theory and practice of sedimentation in the ultracentrifuge to the analysis of permeation data.

From Tanford [8], the velocity U of the fluid flow under pressure P through a group of fibers of area A and thickness h is

$$U = \frac{AP}{f_n}, \tag{1}$$

where f_n is the friction coefficient of the fibers. One can define the number of fibers n in volume V, each of which has a material (protein) density ρ_s, by equating the grams of material of concentration c in the total volume to the grams of material in the fibers.

$$n = \frac{Ach}{2\pi ab^2\rho_s} \tag{2}$$

Approximating the cylindrical fibers with length $2a$ and diameter $2b$ as ellipsoids of revolution and discarding the square root terms ($\simeq 1$), the relative friction coefficient for an ellipsoid of axial ratio $\varrho = a/b$, (see Perrin [9] as quoted by Tanford [8]), is given in Eq. (3). f_i is the friction coefficient of the ith fiber and f_o is the friction coefficient for the equivalent Stokes sphere of radius r_o in a fluid of viscosity η_s.

$$\frac{f_i}{f_o} = \frac{\varrho^{2/3}}{\log_e(2\varrho)} \tag{3}$$

$$f_o = 6\pi\eta_s r_o \tag{4}$$

$$r_o = \sqrt[3]{ab^2} \tag{5}$$

and

$$f_n = nf_i \tag{6}$$

Combining the above equations gives

$$U = \frac{b^2 P\rho_s \log_e(2\varrho)}{3ch\eta_s}, \tag{7}$$

which can be solved for b, the radius of an average fiber. The log term will be later approximated; see below.

$$b = \sqrt{\frac{3ch\eta_s U}{\rho_s P\log_e(2\varrho)}} \tag{8}$$

Next, the quantity U/P which represents the velocity of fluid flow per unit pressure is related to the Darcy constant $\mathscr{D}_o$ (Signer and Egli [7]) defined in Eq. (9), where, in the original variables

Table 1
See Table I of Blombäck [3]; Coarse gels at various concentrations (pH 7.4, μ 0.16)

C (g/l)	D ($cm^2 \times 10^9$)	d(Å) (Blombäck [3])	d(Å) (This paper)
4.0	2.48	2230	1250
3.5	3.59	2480	1360
3.0	3.19	2090	1140
2.5	8.83	3090	1650
2.0	8.70	2630	1390
1.5	12.39	2570	1360
0.5	106.83	3560	1990

Q is the volume of fluid flowing through a volume of height L and cross-sectional area F under pressure P in time t.

$$\mathscr{D}_o = \frac{L\eta_s Q}{FPt} \tag{9}$$

A change of variables for convenience gives

$$\mathscr{D}_o = \frac{h\eta_s U}{P}, \tag{10}$$

which is solved for U/P and substituted into the previous equation for b [Eq. (8)] to give the diameter $d(=2b)$ of a fiber.

$$d = 2\sqrt{\frac{3c\mathscr{D}_o}{\rho_s \log_e(2\varrho)}} \tag{11}$$

The concentration dependence can now be introduced. From the practical application of sedimentation velocity experiments where a relation between s and an infinite dilution sedimentation coefficient s_o is defined one can as well define a similar relation between $\mathscr{D}$ and $\mathscr{D}_o$ in an analogous manner to account for the second order concentration dependence of the fibers.

$$\mathscr{D}_o = \mathscr{D}(1 + ck_s) \tag{12}$$

To approximate the log term and account for the change in the axial ratio of the strands in the network with volume fraction or concentration, the above equation for the number of fibers n [Eq. (2)] is combined with the fact that the network can be modeled as a cubic lattice with three fibers per cube and a volume of $n/3$ times the volume of each cube ($8a^3$).

$$\varrho \simeq 1/2\sqrt{\frac{3\pi\rho_s}{c}} \tag{13}$$

Incorporating the concentration dependence of the Darcy constant and the axial ratio dependence on the volume fraction of the network gives the final working equation for the diameter of an average fiber.

Table 2
See Table III of Blombäck [3]; ionic strength (μ) dependence (pH 7.4)

C (g/l)	μ	D ($cm^2 \times 10^9$)	d(Å) (Blombäck [3])	d(Å) (This paper)
	0.15	10.92	1970	1070
	0.19	6.48	1540	820
1.15	0.24	3.04	1060	565
	0.28	0.93	590	310
	0.33	0.31	330	180
	0.38	0.26	280	170

Table 3
Data from Roberts [1]; gels formed under various thrombin concentrations and degree of fibrin ligation (pH 7.5, μ 0.15). Column 4 is a recalculation of his data using $\rho_s = 0.4$ g/ml

C (g/l)	D ($cm^2 \times 10^9$)	d(Å) (Roberts [1])	d(Å) (Roberts [1]) $\rho_s = 0.4$	d(Å) (This paper)
9.2	2.5	400	740	810
9.4	7.1	670	1260	1390
9.4	1.8	340	640	700
9.4	3.3	470	860	950
9.4	1.3	290	540	590

$$d = 2\sqrt{\frac{3c\mathscr{D}(1+ck_s)}{\rho_s \log_e\left(\sqrt{3\pi\rho_s/c}\right)}} \tag{14}$$

This equation can be rewritten in terms of the volume fraction $\varphi_2 = c\rho_s$ leaving a single empirical parameter, the second order network volume fraction dependence $\rho_s k_s$, and should be used for volume fractions of less than a few percent.

3. Results

As an application of the theory, two different sources of permeation data for the fibrin gel will be used to calculate the average strand diameter in the network. The first example is an extensive set of experimental data where fluid under hydrostatic pressure was forced through a fibrin gel formed under varying fibrinogen, thrombin concentrations and ionic strength. Table 1 and Table 2 contain a partial reproduction of Blombäck's [3] Tables I and III where the diameter was denoted as Ø. The second source, Roberts [1], is the determination of the Darcy constant with the Birnboim transducer (Massa and Schrag [10]) where the dynamic mechanical properties of the fibrin gel were determined in a closed end geometry configuration which resulted in the forcing of fluid through the gel; Table 3.

The two parameters ρ_s and k_s used in the calculations are

$$\rho_s = 0.4\,\mathrm{g/ml} \text{ and } k_s = 200\,\mathrm{ml/g}, \tag{15}$$

where ρ_s has been determined from the weight fraction data ($\omega_2 \simeq 0.17$) along with the approximate volume fraction ($\varphi_2 \simeq 0.5$) of fibers in the fibrin films of Müller [11] (Fig. 7), and Roska [12], by writing out the ratio of the two quantities

$$\frac{\omega_2}{\varphi_2} = \frac{\rho_s}{\rho_f}. \tag{16}$$

ρ_f is just the density of the fibrin film, which is in the range $1.0 \le \rho_f \le 1.38$ (the dry weight density of fibrinogen). Choosing $\rho_f \simeq 1.1$ g/ml, then

$$\rho_s = \rho_f \frac{\omega_2}{\varphi_2} \simeq 1.1\frac{0.17}{0.5} \simeq 0.4\,\mathrm{g/ml}. \tag{17}$$

The parameter k_s is an average value of the sedimentation coefficient concentration dependence, 1–3 dl/g, for elongated molecules [13].

4. Discussion

In general, the diameters of the fibers maintain the same basic trends as originally determined using the method in Signer [7] and Roberts [1]. For example the strand diameters for coarse clots (Table 1) are still reasonably constant with concentration and the large value for the diameter at $c = 0.5$ g/l remains. However, the magnitude of the strand diameters is significantly different and in general agreement for coarse gels with the average value of 800–1800 Å determined by Weisel [14] and Baradet [15] from electron micrographs. Part of this difference is due to the dif-

ferent values for the strand protein density used in the two sets of data relative to the value used in the method of this paper. An example of this is given in Table 3 where the data of Roberts [1] is recalculated using his original application of the method of Signer and Egli [7] with $\rho_s = 0.4$ g/ml. The value for the strand protein density used in Blombäck's [3] calculations is less where as the value used in Roberts [1] is greater than the value of $\rho_s = 0.4$ g/ml used here.

One particularly interesting determination of the diameter is the value in Table 2 at $\mu = 0.38$ which should be close to the minimum possible diameter obtainable (170 Å). Using the electron density data of Rao [16] for crystals of proteolytically modified fibrinogen one can approximate these 170 Å diameter strands by constructing a 210×190 Å cell containing 16 (4×4) fibrinogen molecules. With a molecular weight of 330 000 this gives a value for the strand density of $\rho_s = 0.5$ g/ml, which should represent an upper bound of the density for strands in fibrinogen clots.

5. Nomenclature

U	=	Velocity of water/buffer through the network
A	=	Area of the network
P	=	Pressure
f_n	=	Friction coefficient of n fibers
f_i	=	Friction coefficient of the ith fiber
f_o	=	Friction coefficient of the equivalent Stokes sphere
h	=	Thickness of the network
n	=	Number of strands/fibers in the network
ρ_s	=	Density of protein in a strand/fiber
c	=	Concentration: g/l or mg/ml
a	=	Ellipsoid major axis
b	=	Ellipsoid minor axis
d	=	Average fiber diameter $(= 2b)$
ϱ	=	Ellipsoid axial ratio
r_o	=	Radius of equivalent Stokes sphere
$\mathscr{D}$	=	Darcy constant
η_s	=	Viscosity of water/buffer
μ	=	Ionic strength
k_s	=	Concentration dependence constant
φ_2	=	Volume fraction
ω_2	=	Weight fraction
ρ_f	=	Density of protein in a fibrin film

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