Light scattering characterization of fibrin gels

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Abstract: The structure and growth kinetics of fibrin gels were studied by means of elastic light scattering. The gel mesh size and the fibers diameter were determined, and their dependence on fibrinogen concentration was investigated.

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1. Introduction

Fibrin gels are biological networks of fundamental importance in blood coagulation processes and in many other emorheological situations. They are grown from polymerization of fibrinogen, a high molecular weight macromolecule (MW = 340,000), rod-like in shape, ~50 nm long, ~5 nm thick. Basically, the molecule consists of a central globular domain which contains two pairs of the bonding sites A and B, and of two outer domains where the complementary sites $a$ and $b$ are located. When fibrinogen interacts with the enzyme thrombin, the sites A and B become active and the molecule starts to polymerize. The $aA$ interactions are responsible for linear aggregation, while the $bB$ enhance the lateral growth of the fibrin fiber. The activation mechanism of thrombin is, however, rather peculiar, unmasking first the sites A's, and only successively the B's. Thus, at the beginning, the monomers grow linearly, forming two-stranded, half-staggered chains known as protofibrils. Only successively, when the sites B start to be activated, the protofibrils aggregate to each other, and, depending on the physical-chemical conditions of the solution, they form fibers bundles, which eventually branch and give rise to a three-dimensional gel. A review on the fibrinogen structure and its polymerization processes can be found in Ref. [1] and references therein.

The aim of this paper is to present a light scattering study of fibrin gels grown under quasi-physiological conditions (0.1 M NaCl, 0.05 M Tris-HCl, 1 mM EDTA-Na$_2$, pH 7.4, $T = 25 \pm 0.1$ °C, molar ratio thrombin/fibrinogen = 0.01), but at different fibrinogen concentrations $c_F$. By combining Low-Angle Elastic Light Scattering (LAELS) and Classical Light Scattering (CLS), an overall wave-vector range of about three decades was spanned, from $q = 3.0 \times 10^{-2}$ to $q = 3.3 \times 10^5$ cm$^{-1}$. The scattered intensity distributions of all the gels were measured in absolute units and fitted to a single function, which was able to reproduce accurately the data over the entire wave-vector range. From the fitting it was possible to estimate the average diameter $d$ of the fibrin fibers, the average mesh size $\ell$, of the gel, and establish the fractal nature of the gel structure, with a measure of its fractal dimension $D_m$. The measure of $R(q)$ in absolute units also allowed to estimate the density of the fibrin fibers and provided an independent measure of their size. By varying $c_F$ between 0.03 - 0.81 mg/ml, gels with rather similar structures and with $\ell \sim 10 - 100 \mu$m, $d \sim 100 - 200$ nm and $D_m \sim 1.2 - 1.4$ were obtained. The kinetic of formation of these gels was also remarkably similar and was described in terms of a simple growth model.

2. Experimental results

Figure 1 shows the scattered intensity distribution $R(q)$ of five aged gels prepared at different concentrations as a function of the wavevector $q$. All the gels appear to be characterized by the same shape of $R(q)$, in which three different regimes delimited by the two wavevectors $q_1$ and $q_2$ are easily identified. $R(q)$ exhibits a peak for $q - q_1$, it decays as $\sim q^{-4.4}$ typical of mass fractals for $q_1 < q < q_2$, and crosses over to a faster decay as $\sim q^{-0.4}$ for $q > q_2$ where a surface-fractal behaviors is observed. The peak indicates the presence of a long-range order in the structure of the gel, which can be characterized in terms of a characteristic length scale or mesh-size $\xi$. This is related to $q_1$ by $\xi \sim 4.4/q_1$, the numerical coefficient 4.4 deriving from the model used for describing the data (see below). The crossover wavevector $q_2$ allows the estimate of the weight average diameter of the gel fibers, e.g. $d \sim 2.1 / q_2$. The
The growth kinetics of all these gels was also very similar and an example of its behavior is reported in Fig. 2 for a gel at c_F = 0.81 mg/ml. The curves refer to R(q) distributions taken with the LAELS instrument at different times after addition of thrombin. At the beginning, the scattered intensity is very low and no signal is observed until a time that we labeled as the "networking time", t_n, when R(q) starts to increase very fast and, contemporarily, a peak appears. Later on, the amplitude of R(q) keeps increasing, the peak moves toward smaller values of q's, and the power-law decay exponent grows up to a value of α_m ~ 1.2. This is the time period during which the gel structure ripens until R(q) attains its final steady-state shape at a time of ~ 200 s that we labeled as "ripening time", t_r. Then, for t ≥ t_r, only an increase of the amplitude of R(q) is observed, with no change in its shape. This can be explained by supposing that, during this phase, the gel growth consists only in a thickening of its fibers, with no change in their relative positions. The time required for forming the final aged gel is t_r ~ 30-50 t_n, and the overall increase of R(q) with respect to its value at t_n is ~ 100.

3. Modeling, data analysis and discussion
The data presented in Fig. 1 suggest that we can describe the structure of an aged gel in terms of a simple model in which the gel is imagined as a dense collection of spatially correlated fractal blobs of average size ξ and mass fractal dimension D_m. Suppose that each blob is made of an assembly of n segments or "building blocks" which can be sketched as cylindrical objects with an average diameter d and an average length ℓ. Thus, n ~ (ξ/ℓ)^D_m, and the blob molecular weight M is given by

\[ M = N_A \frac{4}{\pi} \alpha \xi^{D_m} d^2 \]

where \( \rho \) is the segment density and \( N_A \) is the Avogadro number. The intensity distribution R(q) scattered by such a system can be written [2] as the product of a structure factor S(q) which describes the spatial correlation between each blob's center of mass and a form factor P(q) which describes the internal structure of each blob

\[ R(q) = K c_F M \left[ 1 - \beta e^{-(\xi q)^2} \right] \frac{1}{1 + (q \xi / \pi)^2} \frac{D_m}{\xi^{D_m}} \left( 1 + q^2 \right)^{-\alpha_m/2} \]

where the product A(q) B(q) plays the role of the "particle" form factor P(q), c_F is the fibrinogen concentration, and K is the usual optical constant [2]. Equation (2) was first proposed in Ref. [3], in which a detailed description of the meaning of the many parameters \( \rho, \beta, \gamma, \xi, D_m, d, \) and \( \alpha_m \) is reported. Of these, only \( \rho, \xi, D_m, \) and \( d \) were actually left to be floating in the fitting, the remaining ones being fixed to values \( \beta = 1, \gamma = 0.28, \alpha_m = 4, \) and \( \xi = 0.4 \mu m \)
determined according to physical considerations based on the model [3]. In particular, the value assigned to $\gamma$ derives from having supposed that the average distance $\xi_0$ between blobs is comparable to their size $\xi$, i.e. $\xi_0 \approx \xi$. This is equivalent to say that $\xi_0$ is approximately equal to the “overlap concentration” $M/\xi_0^2$ and implies that there is a scaling between $\xi_0$ and $\xi$, with denser gels giving rise to smaller blobs. This is shown in Fig. 3a, in which $\xi \sim \xi_0^{\beta}$ with $\beta = 0.6$. Since for all the gels $D_m = 1.3$, the value of $\beta$ appears to be consistent with $\beta = (3-D_m)^2$, a relation found to hold also for colloidal gels [4]. As to the other parameters, one may notice that the recovery of $\rho$ is a direct consequence of measuring $R(q)$ in absolute units, because $\rho$ appears only in the amplitude prefactor of (1). By analyzing the behavior of $R(q)$ around the crossover region at $q \sim q_2$ it is possible to show [3] that $d$ is actually a weight average root mean square diameter, e.g. $d \sim \sqrt{<d_2^2>_w}$, and that is related to $q_2$ by $d \sim 2d_2 / q_2$. Moreover, since $R(q)$ is measured in absolute units, an independent estimate of $d$ is possible. Indeed, in the fractal region ($\xi_2 < q < \ell^{-1}, d^2$), Eq. (1) can be approximated to [3]

$$R(q) = \frac{\pi^{1-D_m}}{4} K c N_A \rho \sqrt{<d_2^2>_w} q^{-D_m} \tag{3}$$

Thus, provided that the gel parameters $\rho$ and $\ell$ are known, the fiber diameter $d$ can be recovered even if only LAELS data are available. The dependence of $d$ on $c_F$ obtained by fitting LAELS data to Eq. (3) is reported in Figs. 3b, in which it is shown that the fiber diameter scales as $d \sim c_F^\delta$ with $\delta = 0.16$. We have no interpretation for this behavior.

In conclusion, the light scattering data presented in this work have shown that fibrin gels at different fibrinogen concentrations $c_F$, prepared under the same chemical-physical conditions, exhibit quite similar structures, characterized by the same mass and surface fractal dimensions, but different mesh sizes and fibers diameters. The growth kinetics of these gels are fairly similar as well, and can be described in terms of a very simple growth mechanism: the gel scaffold is formed very soon in the course of the gelation process, at a time that is much smaller than the time required to form the aged gel; its successive growth consists basically only in a ripening of this structure and in a thickening of its fibers. Moreover, since the concentration utilized for building the initial scaffold is only a small fraction (~ 10-20%) of the total fibrinogen mass available in solution (see Ref. [3]), we expect that the concentration would play a minor role in determining the structural properties of these gels. Thus, gels prepared under the same physical-chemical conditions, but at different fibrinogen concentrations, would exhibit similar structures and kinetics. This is consistent with what we have observed and reported in this work.

4. References