Scattering Properties of Agarose Gels

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Abstract

Static light scattering and small angle neutron scattering measurements are reported for agarose hydrogels prepared under various conditions of concentration and temperature. For the wide range of transfer wave vector explored, these measurements show that the gels do not display fractal behaviour. Their structure is better described by a stretched exponential form, in which the value of the exponent is n = 0.2.

As found by other authors, a maximum in the scattering intensity is observed in the light scattering spectra. The position of the maximum, q_{max}, depends on the concentration and on the thermal history of the sample. The inverse length $1/q_{max}$ is in good agreement with published measurements of the pore size D in this system.

Preliminary measurements by small angle scattering indicate that the sol-gel transition is not of spinodal type.

Introduction

Agarose gels are widely used commercially by the food industry as well as by the pharmaceutical industry as a growth medium for bacterial cultures. Over the last few years their importance in gel chromatography has motivated numerous investigations into their structure and properties,[1-7] but their behaviour is still not fully understood. In particular, the distribution of pore sizes, and the nature of the sol-gel transition, which has been attributed to spinodal decomposition, remain unresolved.

In this paper we describe observations on a series of agarose gels using small angle neutron scattering (SANS) and static light scattering (SLS). These techniques, which detect an average of the sample structure in reciprocal space, in principle allow models of the gel to be constructed in real space.

Experimental

SANS measurements were made both at the D11 instrument of the Institut Laue Langevin, Grenoble, and at the PACE instrument in the Laboratoire Léon Brillouin, Saclay.

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Both these instruments were operating with an incident wavelength of 6 Å. Standard procedures were used for radial averaging of the data and corrections for the empty cell and background subtraction. Conversion of the results to an absolute scale was performed using light water as a standard together with the measurements of Ragnetti et al.[8)]

The light scattering measurements were made with a SP 162 argon ion laser working at 488 nm and a Malvern goniometer, with a specially adapted motor mounting to rotate the sample for spatial averaging. Toluene was used as the standard to convert to absolute units.

The agarose was graciously provided by R. Armisen (Hispanagar, Spain). Its molecular weight, determined by viscometry [9)], is $M_W = 1.2 \times 10^5$. The sulphate content specified by the manufacturer is 0.1%. The methyl content was found by ^1H NMR to be 0.6%.

The samples were prepared by mixing the appropriate weight of agarose in de-ionized water, and heating the mixture to 100°C. The resulting solutions were stirred until complete dissolution, and then transferred to cylindrical glass tubes of 10 mm outer diameter and sealed. The samples were melted again at 100° C and two cooling protocols were used: 1) the hot samples were allowed to cool freely in air to room temperature; 2) the hot samples were quenched in a water-ice mixture at 0°C. All measurements were made in a temperature controlled bath at 25°C.

Results and Discussion

Figure 1a shows the reduced intensity I(q)/c, measured by light and small angle neutron scattering, for two sets of agarose gels at different concentration c, prepared under similar conditions. These measurements are expressed as a function of the transfer wave vector $q = \frac{4\pi n_0}{\lambda} \sin\frac{\theta}{2}$, where n_0 is the refractive index, λ the wavelength of the incident radiation and θ the scattering angle. In this figure the units are those of neutron scattering; the light scattering data have therefore been multiplied by the ratio of contrast factors, $(\rho_p-\rho_s)^2/KN_Ad^2$. Here $K = 4.42°10^{-7}$ cm^2 mole g^{-2} is the light scattering contrast factor for the agarose-water system at λ = 488 nm, N_A is Avogadro's number, d=1.7 g cm^{-3} is the density of agarose and $(\rho_p-\rho_s) = 2.85°10^{10}$ cm^{-2} is the difference in neutron scattering length densities of agarose and heavy water respectively.[10)] As all the data are in absolute units, no adjustment has been applied to match the different parts of the curve.

In the lower wave vector range of Figure 1a, a maximum in the intensity can be distinguished for the 3% sample. The corresponding maximum for the 1% sample is shifted to lower q, but is unresolved in this measurement. Similar observations have been reported previously. [1-3)] The existence of such a maximum at $q=q_{max}$ indicates a deficit in the density correlation function for distances greater than $1/q_{max}$; $1/q_{max}$ can thus be regarded as the largest mesh size in the system.

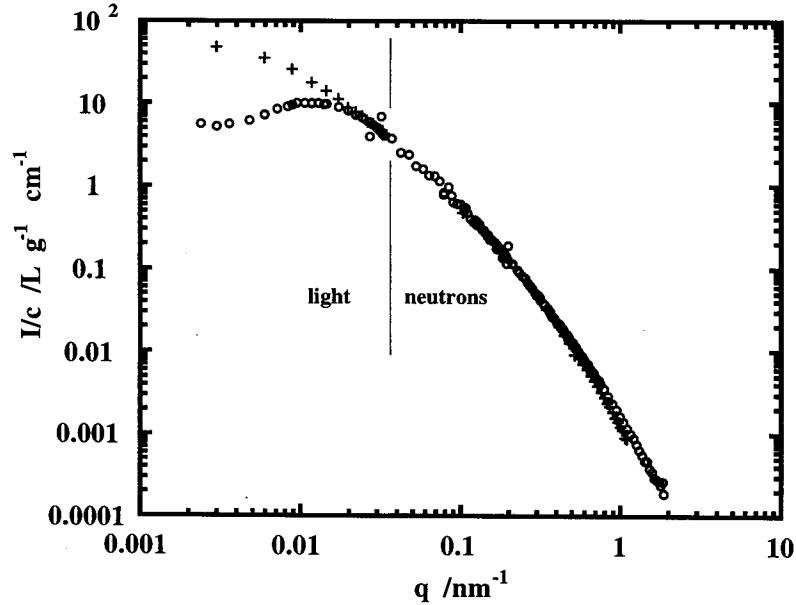

Figure 1a Reduced absolute intensity I(q)/c from SLS and SANS for agarose-water gels at c=10 g/L (+) and c=30 g/L (o). The light scattering data are multiplied by $(\rho_P-\rho_S)^2/KN_Ad^2$ = $1.06°10^3$ to convert to neutron scattering units (see text).

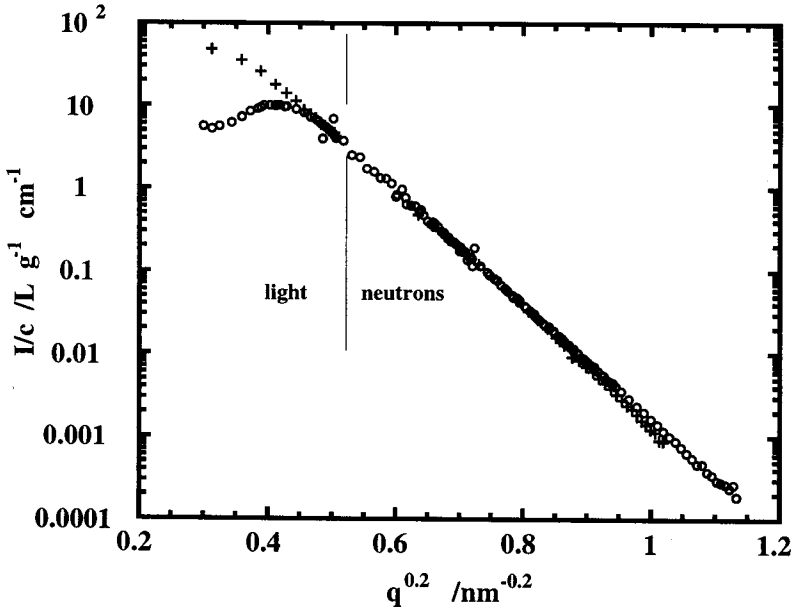

Figure 1b Same data as Figure 1a in a stretched exponential representation with exponent n=0.2.

For values of q greater than q_{max}, the data for the two concentrations in Figure 1a coincide in a continuous curve. The absence of straight line region in this double logarithmic plot demonstrates that the structure of these gels is not fractal. When the same data are plotted in the representation of a stretched exponential, however,

$$I(q) = I(0) \exp\{-(qR)^n\} \qquad (1)$$

with n = 0.2, straight line behaviour appears over an extended range of q (Figure 1b). It may be mentioned that stretched exponential distributions are not confined to agarose gels: analogous behaviour is also observed in fumed silica aerogels,[11] albeit with a different exponent n. The fitting function (1) is, however, empirical. In particular, it cannot continue indefinitely, since surface scattering must become significant at large q, giving rise to a power law[12] of the form q^{-4}. As for the characteristic length R, the value calculated from Figure 1b is approximately 1 mm. It is possible that such large distances correspond to the granularity that can be observed by the naked eye to develop in the structure when the solutions are cooled very slowly through the gel transition temperature.

Figure 1 suggests that q_{max} shifts to lower q as the agarose concentration decreases. This conclusion is confirmed in Figure 2, in which the light scattering functions of agarose gels are shown for five different concentrations. These samples were obtained by free cooling of the agarose solutions to room temperature. It can be seen that the position of the maximum is inversely related to the concentration. Equivalent measurements made on agarose gels in the same concentration range, but quenched to 0°C, are shown in Figure 3. Inspection of these two figures shows clearly that the position of q_{max} is temperature sensitive: quenching to a lower temperature causes q_{max} to increase.

The above results are summarized in Figure 4, where the characteristic distance $1/q_{max}$ is displayed as a function of concentration c. Over the limited concentration range explored, for each quench temperature T, the values of $1/q_{max}$ lie to a good approximation on a straight line in the double logarithmic representation. This yields

$$1/q_{max} = A(T)\, c^{-1.2} \qquad (2)$$

where the pre-factor A(T) is an increasing function of the quench temperature. This property of A(T) is typical of an arrested ripening mechanism, in which larger structures develop if the rate of cooling is slowed.

In Figure 4 are also shown the results of permeability measurements of agarose gels by Righetti.[13] In reference 13 no details are given about the temperature treatment to which the samples were subjected. There is, however, remarkable agreement between the maximum mesh size $1/q_{max}$ measured by light scattering and the pore size D estimated by electrophoresis. Given the sensitivity of the system to the rate of cooling at gelation, it seems likely that the differences in these two measurements are entirely attributable to differences in sample preparation and/or in the fine chemical structure of the samples used. We therefore conclude that the position of the intensity maximum $1/q_{max}$ can be identified with the pore size of the gel relevant to electrophoresis.

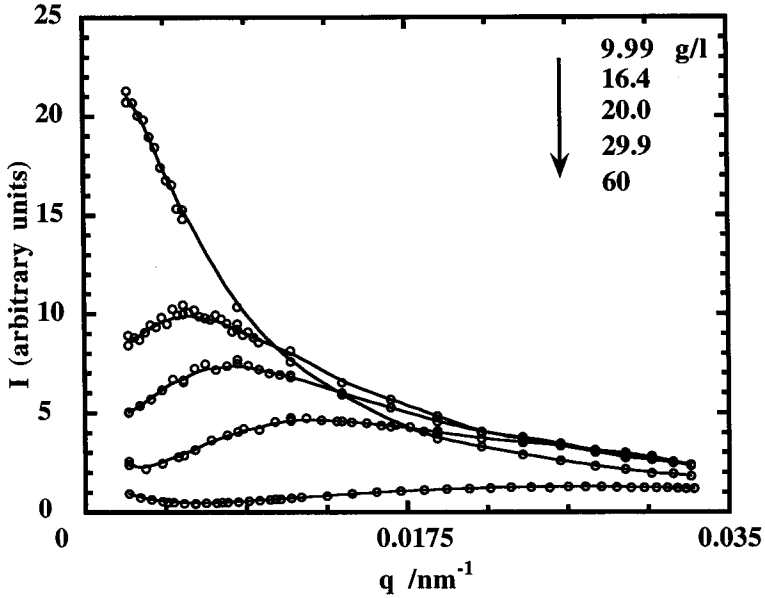

Figure 2 Light scattering spectra of agarose gels freely cooled to room temperature. The concentrations are indicated in the figure.

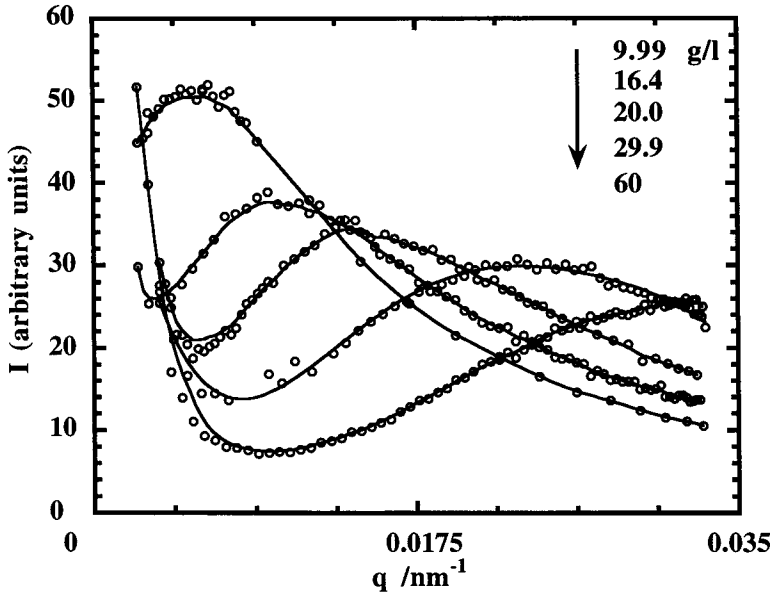

Figure 3 Light scattering spectra of agarose gels quenched to 0°C.

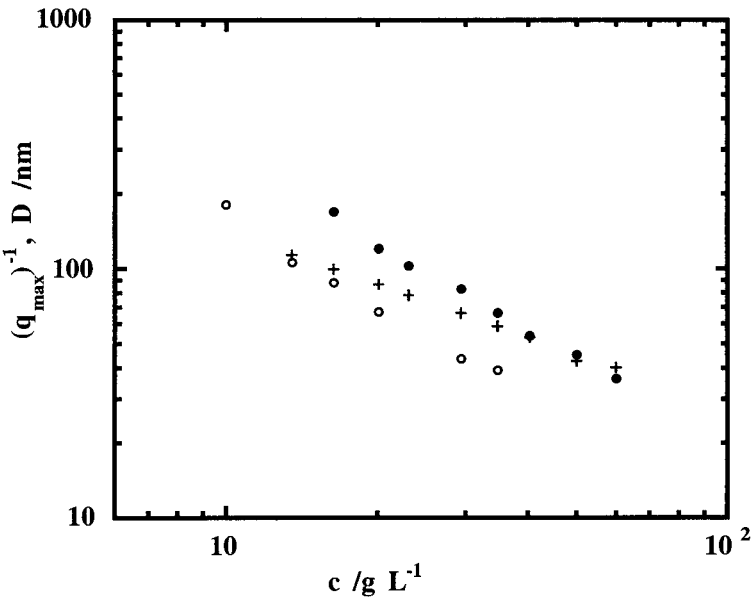

Figure 4 Concentration dependence of the characteristic length $1/q_{max}$; •: samples cooled to room temperature; o: samples quenched to 0°C; + : pore size D (ref. 13).

The above measurements yield little information about the nature of the sol-gel transition, which resembles a spinodal decomposition insofar as it possesses an intensity maximum. In a spinodal decomposition, since the second derivatives of the free energy are zero or negative, the surface tension and interfacial energy between the two separating phases vanishes, thus favouring bi-continuous structures that are similar to the rod-like network of agarose gels. If agarose gels are indeed formed through a spinodal mechanism, then it is reasonable to expect that any structural changes occurring in the gel will not be governed by surface tension effects, and in particular, thickening of the helix bundles should not be favoured. In this context, we report preliminary small angle light scattering measurements, performed as a function of time on an agarose gel maintained at a constant temperature just below the gel point. These measurements, not shown here, reveal that the intensity of the maximum increases strongly with time to a new equilibrium value, but that its position q_{max} is invariant. This result indicates that the pore size does not change, and that the intensity increase must therefore be related to a coarsening of the fibre bundles. Surface tension effects thus do play a strong role at this temperature. We conclude therefore that the gel transition in agarose is not of spinodal character, and that the superficial similarity is simply the result of the fact that surface tension effects are much weaker than the bending energy of the stiff rods that develop during the formation of the gel.[14)]

Conclusions

Light and small angle neutron scattering measurements from agarose water gels show that their static structure is not fractal, but instead is described by a stretched exponential. These systems display a maximum in their intensity, the position of which, $1/q_{max}$, is consistent with the pore size of the gel measured by electrophoresis.

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