# Mesoscopic Gel at Low Agarose Concentration in Water: A Dynamic Light Scattering Study

Donatella Bulone and Pier L. San Biagio
Institute for Interdisciplinary Applications of Physics, National Research Council, and Department of Physics and GNSM and INFM, University of Palermo, Palermo, Italy

ABSTRACT Previous work in our laboratory has shown that at very low agarose concentration in water gelation still occurs within mutually disconnected, high concentration regions generated by spinodal demixing. The freely diffusing particles obtained in these conditions are studied in the present work by depolarized dynamic light scattering and probe diffusion experiments. These particles are found to behave as large (in fact, mesoscopic) polymer fibers entangled in a continuously rearranged mesh with scaling parameters typical of partially flexible, neutral chains. The present results allow specifying the notion of mesoscopic gelation. They also reveal that the same symmetry-breaking mechanism that allows macroscopic gelation at polymer concentrations well below the threshold for random cross-link percolation generates additional and unexpected phenomena.

## INTRODUCTION

Previous studies at our laboratory have addressed the problem of self-assembly of agarose gels from aqueous sols at low to moderate concentration, well below the estimated threshold for random (continuum) cross-link percolation. Solute-solute correlations, allowing channeling, that is, nonrandom cross-linking and correlated percolation, are necessary in these cases (San Biagio et al., 1986; San Biagio and Palma, 1992; Sciortino et al., 1993; Bulone et al., 1993). Our previous work has indeed shown that in such cases percolation is preceded and promoted by a preliminary break of symmetry that is the occurrence of a spinodal demixing of the sol as such. This thermodynamic transition precedes and allows the topological phase transition of gelation (Bulone and San Biagio, 1991; Emanuele et al., 1991; Emanuele and Palma-Vittorelli, 1992; Leone et al., 1987; San Biagio et al, 1986, 1989, 1990, 1994). Both the sol instability and gelation lines in the T,c plane for agarose sols have been determined, and gelation at different concentrations has been related to such complete phase diagrams (Bulone and San Biagio, 1991; Emanuele et al., 1991; Emanuele and Palma-Vittorelli, 1992; Leone et al., 1987; San Biagio et al., 1986, 1989, 1990, 1994), although it must be taken with a note of warning as elsewhere discussed (San Biagio et al., 1994). Quenching of the agarose sol in its instability region causes demixing in lower and higher than average concentration regions. In the latter, the threshold value for gelation can be reached even when the average agarose concentration is much lower.

At agarose concentrations lower than 1 g/L, high concentration regions generated by spinodal demixing remain mutually disconnected. Inside these regions (rich in agarose monomers and where random percolation can occur) elongated agarose particles are formed and observed to diffuse freely in the specimen that, as a whole, maintains its liquid (free-running) character (Bulone and San Biagio, 1991). A closer microscopic view of the system in these conditions is provided in the present work by depolarized dynamic light scattering (DDLS) and dynamic light scattering (DLS) measurements. Our experiments give two distinct types of information: the size of elongated agarose particles as a function of agarose concentration and the structural features of the liquid solution of Agarose particles, along with its scaling properties.

## MATERIALS AND METHODS

Agarose is an essentially uncharged biostructural polysaccharide obtained from marine red seaweeds (*Rhodophyceae*). It has a molecular mass of ~100,000 Da. Its structure consists of an alternating copolymer of 3-linked-*D*-galactopyranose and 4-linked 3,5-anhydro-$\alpha$-*L*-galactopyranose residues (Arnott et al., 1974). In the present work, agarose was Ultrapure Seakem HGT(P) from FMC BioProducts, Rockland, ME, lot 22537, with a nominal sulfate content of <0.15%. Water was Millipore Super Q filtered with 0.22-$\mu$m filters. An agarose water solution in the concentration range 0.05 g/L to 0.5 g/L was put into a sealed tube and kept in boiling water for 20 min with occasional gentle mixing (autoclaving at higher temperature was avoided to prevent breaking of agarose chains, as previously reported (San Biagio et al., 1986)). A cylindrical (1 inch) cuvette was then washed several times with the filtered solution at 80°C before the final filling. Samples were then quenched to 20°C and kept at this temperature to allow gelation. In the concentration range chosen for the present experiments (specified above), gelation occurred within the initially disconnected mesoscopic regions where polymers had clustered as a result of spinodal demixing (Bulone and San Biagio, 1991). The end of the sequence of spinodal demixing and gelation processes was observed by DLS measurements monitoring the existence of freely drifting mesoscopic gel particles (Bulone and San Biagio, 1991).

Samples used in DLS experiments contained monodisperse polystyrene latex spheres (PLS) of 60 nm diameter from Polyscience, Inc., Warrington, PA. PLS were added at 80°C, before temperature quenching. Under these conditions, the light scattered by PLS was at least 20 times larger than that from agarose aggregates. Sticking of agarose molecules to PLS can be ruled out as shown by previous work at even higher agarose concentration (San Biagio et al., 1986).

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Address reprint requests to Dr. Donatella Bulone, CNR-IAIF, Via Archirafi 36, I-90123 Palermo, Italy. Tel.: 011-39-91-6233-221; Fax: 011-39-91-6233-224; E-mail: bulone@iaif.pa.cnr.it.



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## DLS and DDLS experiments

### Theoretical background

Let us consider a beam of vertically polarized laser light scattered by a solution of noninteracting uniform spherical particles. When observing the scattered light on the same polarized plane, the normalized field autocorrelation function can be written as

$$g^{(1)}_{VV}(K, t) = A \exp(-\Gamma t), \quad (1)$$

where $\Gamma = D_T K^2$. Here, $D_T$ is the translational diffusion coefficient of the spheres and $K$ is the propagation vector, given by

$$K = |K| = |k_s - k_i| = \left(\frac{4\pi n}{\lambda}\right)\sin\left(\frac{\theta}{2}\right) \quad (2)$$

where $k_s$ and $k_i$ are the wave vectors of the scattered and incident light, respectively, $n$ is the refraction index of the solution, $\lambda$ is the wavelength of the laser light, and $\theta$ is the scattering angle.

For noninteracting optically asymmetric particles, the scattered light includes also a horizontally polarized component. When observing this horizontally scattered light, the normalized field autocorrelation function can be written as (Berne and Pecora, 1976; Schmitz, 1990)

$$g^{(1)}_{VH}(K, t) = A \exp(-\Gamma_{VH} t) \quad (3)$$

where $\Gamma_{VH} = 6D_R + D_T K^2$, so that information on both the translational ($D_T$) and rotational ($D_R$) diffusion coefficients of the scatterers can be obtained, respectively, from the slope and intercept (at $K = 0$) of linear plots of $g_{VH}^{(1)}$ versus $K$.

### Probe diffusion experiments

In this type of experiment, DLS is used for studying PLS diffusion in solution. The field autocorrelation function obtained in this case can yield information on the trapping of scatterers within a flexible boundary (Madonia et al., 1983). If the time scale of wall motion is much longer than that of particle diffusion, the following relationship exists between the normalized amplitude of the intensity correlation function and the fraction ($f_m$) of particles for which motion occurs on the time window selected on the correlator (Madonia et al., 1983; Newman et al., 1991):

$$f_m = 1 - \left\{1 - \frac{[G^{(2)}(0) - G^{(2)}(\infty)]}{[G^{(2)}(\infty) A]}\right\}^{1/2} = 1 - \left\{1 - \frac{A'}{A}\right\}^{1/2} \quad (4)$$

Here, $A$ is the amplitude of the normalized intensity autocorrelation function that would be observed if all probes were freely diffusing, and $A'$ is the experimentally observed amplitude. Qualitatively, the observed decrease of $f_m$ versus $(1/K)$ indicates the transition region of the scatterers from mobility to immobility and can be used to estimate a relevant scale length that characterizes the structure of the solution in which the PLS are diffusing.

### Experimental setup

The cuvette containing the sample prepared as described above and kept for up to 4 days at 20°C was put into the thermostatted cell compartment of a Brookhaven Instruments 200-SM goniometer system. The goniometer was mounted on a Newport Research vibration isolation table and the autocorrelation analysis of polarized and depolarized light was performed with a 128-channel Brookhaven Instruments 2030-AT correlator. Usually, a DLS measurement took 5 min; in DDLS experiments, the signal was accumulated for at least 2 h, the intensity being ~50 times lower. The cell was temperature controlled within 0.1°C by a circulating-fluid thermostat. All experiments were performed at 20°C over angles from 15° to 130°. An argon ion laser (Spectra Physics, Mountain View, CA) was used as a light source. The laser beam (514.5 nm) was vertically polarized. The beam intensity could be varied from 50 to 200 mW, depending upon the observation angle and sample concentration.

### Data analysis

Autocorrelation functions (at least five for each angle) were analyzed by the standard cumulant method (Koppel, 1972). Both a second and a third order cumulant fit were performed. Very close values of amplitude of the correlation function and Γ-average (first cumulant) of the species in solution were obtained in the two cases, confirming the reliability of the data analysis procedure. When the specimen contained PLS, their diffusion coefficient at different agarose concentrations was calculated by the best linear fit of Γ versus the scattering vector magnitude squared. It has been observed that in well firm gels, partial heterodyning can be caused by trapped particles acting as local oscillators (Reina et al., 1990). In the extreme case of very strong gels, decay constants obtained under homodyne assumption can be even 40% smaller than the true value. The system here studied is very far from well firm gel conditions as it appears macroscopically liquid at all agarose concentrations studied. A weak heterodyne signal should affect PLS diffusion coefficients derived under homodyne assumption only at the higher agarose concentration and lower $K$ values studied. On the other hand, analysis of data collected from different regions of the same sample or different samples at the same agarose and PLS concentration gave consistently the same fractions of trapped PLS and the same diffusion coefficients for those PLS that remained freely diffusing. Probably as a result of mobility and flexibility of the microgel particles, it was not applicable to perform data analysis in terms of the Pusey-van Megen theory (Pusey and van Megen, 1989) for nonergodic media.

## RESULTS AND DISCUSSION

A first set of experiments concerned freely drifting agarose particles formed inside high concentration regions (generated by spinodal demixing) and obtained as specified in the previous section. DDLS measurements at 20°C were used to characterize their shape and concentration-dependent size (Bulone and San Biagio, 1991). Samples not containing PLS were prepared at agarose concentration from 0.1 to 0.5 g/L. The lower concentration limit was dictated by the need of dealing with a safely detectable signal. In Fig. 1 we report plots of the $\Gamma_{VH}$ (first cumulant) versus $K^2$ values for our lowest and highest concentrations used. Similar linear dependencies were observed at intermediate concentrations. The existence of $\Gamma_{VH}$ evidences the optical asymmetry of agarose particles detected by these experiments and allows the derivation of their rotational and diffusional coefficients

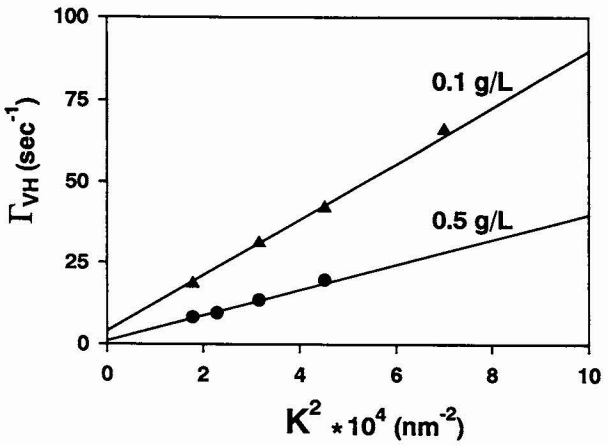


FIGURE 1 First cumulant of the autocorrelation function of depolarized light scattered by mesoscopic gel particles versus $K^2$ at two different agarose concentrations at 20°C. ▲, 0.1 g/L; ●, 0.5 g/L.

according to Eq. 3. A rod-like structure of these particles can be assumed as suggested by transient electric birefringence studies on the same system in similar conditions (Dormoy and Candau, 1991) and by the reported rigidity of the agarose chain (Rochas et al., 1994). Length ($L$) and diameter ($d$) can be calculated from the combined use of $D_T$ and $D_R$ values through the Broersma's relationships (Zero and Pecora, 1985). In our case the intercept (see Fig. 1) is too small to provide an accurate value for $D_R$, so we have assumed $d \approx 50$ Å, consistent with various experiments (Dormoy and Candau, 1991; Stellwagen and Stellwagen, 1990), to derive the $L$ value. From data in Fig. 1 we obtain $L = 3$ μm for concentration $C = 0.1$ g/L, and $L = 6$ μm for $C = 0.5$ g/L. Alternatively, if we consider the gel particles as partly flexible chains, we can use the Hearst relationship (Zero and Pecora, 1985) giving $D_T$ and $D_R$ in terms of monomer sizes and persistence length ($Q$), which can be thought of as the length of the rigid segments flexibly jointed into a chain of contour length $L$. Taking a length of 50 Å for the monomer, corresponding to the mean distance between kink sites (Di Stefano, 1991) and a diameter as above, we get $L = 3$ μm and $Q = 1.5$ μm for $C = 0.1$ g/L, $L = 7$ μm and $Q = 1.2$ μm for $C = 0.5$ g/L, according to sizes estimated in our previous work (Bulone and San Biagio, 1991).

With the above sizes, the number of agarose fibers for unit volume, $C^A$, can be roughly evaluated by mass conservation law. For the extreme concentrations here used we obtain $C^A_{0.1\,g/L} = 3 \times 10^{11}$ and $C^A_{0.5\,g/L} = 6 \times 10^{11}$, respectively. Comparison with their overlap concentration ($C^*_{0.1\,g/L} = 7 \times 10^{10}$ and $C^*_{0.5\,g/L} = 6 \times 10^9$, respectively) shows that we are dealing with semidilute solutions. It should be noted that we have used equations valid for a dilute regime for a system that is, in fact, a semidilute regime. As a consequence, size values reported above are probably overestimated. Nevertheless, the overall interpretation of our results is not affected.

Properties and structure of semidilute solutions can be investigated by PLS probe diffusion experiments (Phillies, 1987; Ullmann et al., 1985). To this purpose, in a second set of experiments, DLS measurements were performed on samples containing PLS probes as described in materials and methods and subjected to the same thermal history as in the set of experiments just described. In this way we observed the diffusion of PLS in the macroscopically liquid solution of agarose fibers. In Fig. 2 the fraction of mobile PLS in solution ($f_m$), calculated from Eq. 4, is plotted versus the probing distance ($1/K$) at several agarose concentrations. A progressive decrease of $f_m$ is observed for increasing $1/K$ value and increasing concentration. Note that, even at the highest agarose concentration used, the inclusion of a measurable fraction of PLS probes within the mesoscopic aggregates can be ruled out by considering their respective dimension and the absence of sticking as previously reported (San Biagio et al., 1986). The observed reduced probe mobility evidences that, on the time scale of probe diffusion and in the concentration range studied here, the solution is seen by the probes as a mesh of agarose fibers. At a certain probing distance ($1/K$ value) it will be seen as mobile only that fraction of PLS diffusing through holes with size equal or larger than such probing distance plus PLS diameter. Increasing agarose concentration, hole size will be reduced and the same fraction of mobile PLS ($f_m$) will be observed at smaller $1/K$ value. So, we can choose a constant value for $f_m$ and look at the dependence upon agarose concentration of the hole size, $L_t$, defined as the corresponding $1/K$ value plus PLS diameter (60 nm in these experiments). In this way, the concentration dependence of mesh density is obtained. $L_t$ values, derived as just described, are plotted in Fig. 3 versus concentration for two different $f_m$ choices. The figure shows that the concentration dependence of $L_t$ in both cases is well represented by a linear log-log relationship, i.e., by a law:

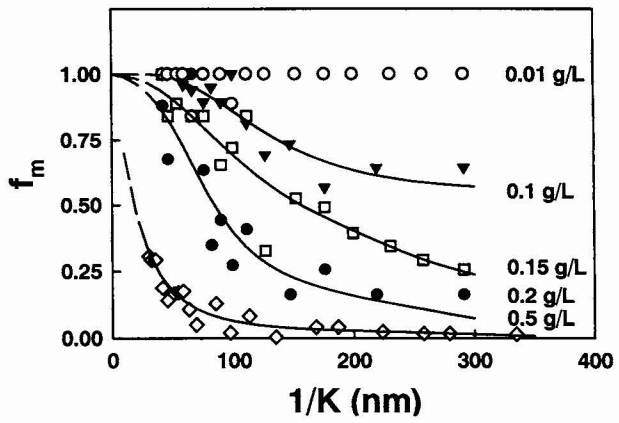


FIGURE 2 The mobile fraction ($f_m$) of 0.06-μm PLS probes diffusing in solution of mesoscopic gel particles, as calculated in the text, versus $1/K$ at different agarose concentrations at 20°C. ○, 0.01 g/L; ▼, 0.1 g/L; □, 0.15 g/L; ●, 0.2 g/L; ◇, 0.5 g/L.

$$L_t^{-1} = \text{Constant } C^y \qquad (5)$$

with the same $y$ value equal to 0.75, independently of $f_m$ choice.

Additional information is provided by agarose concentration dependence of the PLS mean diffusion coefficient, $D$.

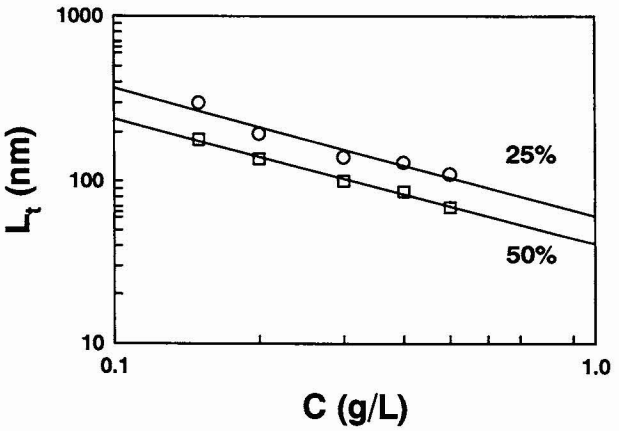


FIGURE 3 Hole size, $L_t$ (obtained as stated in the text), allowing diffusion of the same fraction of PLS as a function of agarose concentration, for two different choices of $f_m$. Continuous lines, fitting of the data by using Eq. 5 in the text with $y = 0.75$.

The latter is obtained, for each agarose concentration, from linear plots of the first cumulant ($\Gamma$) of the autocorrelation function of freely diffusing PLS versus $K^2$ (see Eq. 1). In Fig. 4 we plot $D/D_0$ versus agarose concentration, where $D_0$ is the diffusion coefficient of PLS in the pure solvent. Interestingly, this dependence can be described according to the following scaling law, similar to that used by Langevin and Rondelez (1978) in the case of probe diffusion in a polymer solution:

$$\frac{D}{D_0} = \exp(-\alpha C^{\nu}) \tag{6}$$

The best estimate for the $\nu$ parameter from the data above reported is 0.64, which is close to that obtained for $y$ (0.75) in Eq. 5 as predicted by the theory (de Gennes, 1979) for completely flexible polymeric chains. A closer agreement is found with the $\nu$ value (0.62) experimentally determined by Langevin and Rondelez (1978) in semidilute solutions of polyethylene oxide. As pointed out by the same authors, the difference from the theoretical $y$ value can be a result of incomplete flexibility of polymeric chains. Therefore, the system studied behaves on microscopic scale as a semidilute solution of partially flexible elongated structures. Similar behavior was recently found in a solution of elongated micelles obtained by increasing surfactant concentration and/or changing the solvent properties by adding salt or alcohol in solution (Appell and Porte, 1990).

## CONCLUSIONS

Previous studies at our laboratory (Bulone and San Biagio, 1991) had shown that, in very dilute aqueous agarose systems quenched in the instability region (as encompassed by the spinodal line), gelation occurs in the mutually disconnected high concentration regions generated by the spinodal demixing where random percolation can occur. In the present work we show that freely drifting agarose fibers are formed inside these high concentration regions. DDLS is used to estimate their concentration-dependent size. Probe diffusion results show that the solution of fiber aggregates behaves like a solution of entangled polymers arranged in a transient mesh. The mesh, of course, is not to be viewed as static but changing in time, with a time scale longer than diffusion of PLS spheres, allowing a continuous rearrangement of the sample that remains macroscopically liquid. Concentration dependence of mesh size and PLS diffusion coefficient are well described by scaling laws with an exponent typical for a network of partially flexible neutral polymers.

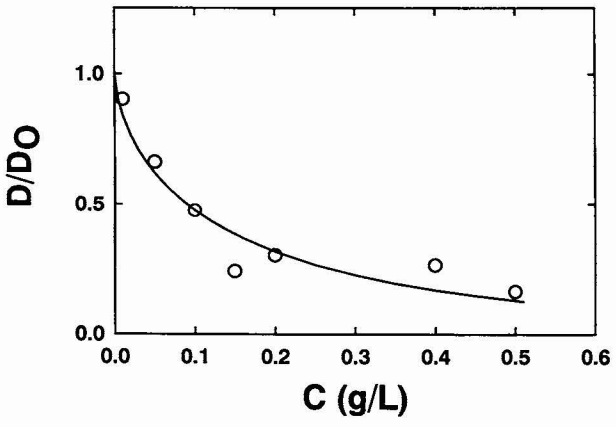


FIGURE 4 The normalized diffusion coefficient of PLS probes ($D/D_0$) as a function of agarose concentration. Continuous line, fitting of the data by using Eq. 6 in the text with $\nu = 0.64$.

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