Thermoreversible Gelation of Agarose in Water/Dimethyl Sulfoxide Mixtures†

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ABSTRACT: The thermoreversible gelation of agarose in different water/dimethyl sulfoxide (DMSO) mixtures has been studied by polarimetry and small-angle neutron scattering, and the modulus–concentration relations have been determined. In the *sol state* it has been found that the chain is fairly rigid, independent of the solvent composition, with a persistence length exceeding 9 nm. Theoretical fits of the scattering patterns are consistent with conformations close to *single helices*. The mass per unit length, which decreases with increasing DMSO content, is accounted for by considering the formation of an agarose/DMSO complex. The mechanical properties of the *gel state* depend little upon the solvent composition. The notion of double helices in the gel state is discussed in the light of these results.

Introduction

Investigations into the conformation of polysaccharides have led Rees and co-workers to come up with the notion of the double helix.[1-3] The existence of a double-helical structure is the keystone to most of the current explanations of the formation of thermoreversible gels from these biopolymers. The notion of the double helix was first derived from X-ray studies on ι-carrageenan gels.[2] In this special case, the X-ray diffraction pattern is unambiguous as the number of observable reflections narrows down the possible helical structures. This combined with energy calculations is why the double helix has gone unchallenged for the past 20 years.

Yet, the case of agarose is subject to discussion as the number of reflections is much lower than with carrageenan gels. Foord and Atkins invented a series of single-stranded helices that can equally well account for the experimental data.[4] Other studies have also called into question the very existence of double helices,[5,6] and, correspondingly, the mechanism put forward by Arnott et al.[3] is no longer universally accepted as a correct depiction of the steps involved during the gelation process. For instance, it has been shown by small-angle neutron scattering that the chain in the sol state is far more rigid than expected,[5] which is at variance with previous models that consider a *flexible coil–helix* transition. Also, conformational analysis led Jimenez-Barbero et al. to conclude that the agarose chain is fairly rigid.[6] Clearly, additional experiments are needed to throw some light on the gelation mechanism as well as on the molecular structures involved. As with other systems an approach which considers the solvent as a parameter[7] seems now timely.

The purpose of this paper is to report on an investigation carried out in water/DMSO mixtures by several techniques. The gelation behavior (gel-setting temperature), the molecular structures in the sol state, and the gel modulus have been determined in the different mixtures.

Experimental Section

(1) Materials. The agarose sample was kindly supplied by Sobigel (Hendaye, France). The molecular weight determined by viscometric measurements is $M = 1.23 \times 10^5$.[8] The sulfate content provided by the manufacturer is 0.27%. The methyl content was found to be 0.7% by ^{1}H NMR. By means of ^{1}H and ^{13}C NMR, the presence of L-galactose 6-sulfate was not detected whereas the content of 3,6-anhydro-L-galactose was found to equal the galactose content.

DMSO (dimethyl sulfoxide) and NaSCN were purchased from Fluka, and freshly distilled water was used. Deuterated DMSO and heavy water, both with a deuteration content of 99.8%, were purchased from EURISO-TOP (Saclay, France).

(2) Polarimetry. Optical rotation measurements were carried out at 365 nm with a Perkin-Elmer 241 polarimeter using 1-cm^3 thermostated quartz cells. The gel-setting temperature was determined at 40 and 5 °C/h.

(3) Mechanical Testing. Modulus measurements were performed on a 4301 Instron device by recording the stress–strain curve on compression. The gel modulus is then obtained from

$$\sigma = E\epsilon \qquad (1)$$

in which σ is the stress, ϵ is the strain ($l - l_0/l_0$, where l_0 is the initial height), and E is the Young's modulus. For every agarose concentration and water/DMSO mixture, ten samples were tested to evaluate the experimental scatter.

Gels were obtained by pouring hot, homogeneous solutions into a cylindrical glass tube of 17-mm diameter. After the gels were cooled to room temperature, cylindrical pieces of gel of 17-mm diameter and 17-mm height were guillotined out of the original gel with a razor blade. A special setup was used for obtaining cylinders with highly parallel faces.

(4) Neutron Scattering. Neutron scattering experiments were carried out at the Laboratoire Léon Brillouin (Saclay, France) on three different cameras, namely,

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PAXY, PAXE, and PACE. PAXY and PAXE are equipped with two-dimensional-sensitive counters, while PACE consists of a series of concentric rings that are regularly spaced (further details are available on request). In all cases a wavelength of $\lambda_m = 0.6$ nm was used with a wavelength distribution characterized by a width at half-height $\Delta\lambda/\lambda_m \approx 10\%$.

Counter calibration was achieved by using the incoherent spectrum scattered by *cis*-decalin. As usual, spectra were corrected for thickness and transmission. As agarose is a hydrogenated material, the extra incoherent scattering must be removed from the total scattering. This is achieved by using a blank sample which contains the same number of protons per unit volume as the agarose sample. These additional protons are introduced under the form of light water. The normalized intensity, $I_N(q)$, scattered by the agarose material is given by

$$I_N(q) = \frac{\dfrac{I_s(q)}{T_s\delta_s} - \dfrac{I_b}{T_b\delta_b}}{\dfrac{I_d}{T_d\delta_d} - \dfrac{I_e}{T_e\delta_e}} \tag{2}$$

in which T and δ are the transmission and the thickness and the subscripts s, b, d, and e stand for the sample, the background, decalin, and empty cell, respectively.

It is worth noting that the intensity scattered by the blank sample contains an additional coherent term, which does not exist in the agarose sample, due to the difference in scattering amplitude between the solvent and light water. This term is very small in the case of a solvent which contains heavy water, as there is proton–deuteron exchange. As a result, this coherent term is between HOD and D_2O. Theoretical estimates of this additional term have shown that it represents less than 10% of the total blank signal. While this can be ignored in the low-angle region, these few percent have a definite effect in the wide-angle domain. Consequently, this has been taken into account in the calculation of the error bars.

The absolute intensity, $I_A(q)$, is then obtained from

$$I_A(q) = I_N(q)/K = C_pS_p(q) \tag{3}$$

in which $S_p(q)$ is the agarose coherent intensity and C_p is the agarose concentration. K is a calibration constant given by

$$K = \frac{[a_p - (v_p/v_D)a_D]^2(4\pi)\delta_d N_A T_d}{g(\lambda_m)(1 - T_d)m_p^2} \tag{4}$$

in which a_p and a_D are the scattering amplitudes of agarose and the solvent, respectively; v_p and v_D are the molar volumes of agarose and the solvent, respectively; N_A is Avogadro's number; and m_p is the molecular weight of the agarose repeat unit. $g(\lambda_m)$ is a correction factor which depends upon the neutron wavelength, λ_m, and the camera. In the present case, this factor was determined by means of Cotton's method.[9]

For calculating the scattering amplitude of agarose, the following parameters were used: (1) in mixtures containing heavy water, the exchange of 4 agarose protons was taken into account, which gives for the chemical formula $C_{12}H_{14}D_4O_9$; (2) the molar volume of agarose was taken to be $v_p = 180 \pm 5$ cm^3/mol (taking $d = 1.7 \pm 0.05$ g/cm^3).

Results and Discussion

(1) Polarimetry. The gel-setting temperatures determined on cooling agarose solutions ($C_p = 0.97 \times 10^{-3}$ g/cm^3) prepared in different water/DMSO mixtures (X_{DMSO} = DMSO volume fraction) are given in Figure 1. As can be seen, the gel-setting temperatures go through a maximum for a DMSO content of about 40% (v/v) while gelation capability vanishes beyond a DMSO content of 70% (v/v). Interestingly, the gel-setting temperature is some 14 °C higher in the 50% DMSO/water mixture than in pure water.

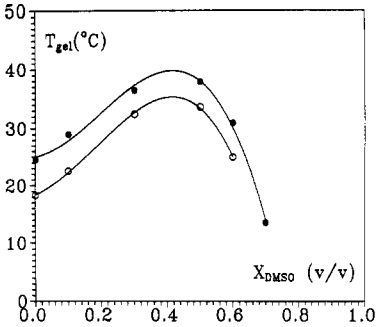

Figure 1. Gelation temperatures as obtained from polarimetry experiments on agarose solutions of different water/DMSO compositions: (○) cooling rate = 40 °C/h; (●) cooling rate = 5 °C/h. $C_{agarose} = 0.97 \times 10^{-3}$ g/cm^3.

These results are in contradiction with previous hypotheses that considered gelation mechanisms involving either liquid–liquid phase separation or, at least, poor solvent effects, which, in both cases, were supposed to promote chain aggregation. Yet, DMSO is known to behave as a good solvent toward agarose as gelation does not take place in this solvent. Allegedly, adding DMSO to water should improve solvent quality, and, accordingly, decrease the gel-setting temperature.

In fact, as has been observed for other systems, poor solvent quality is far from being a prerequisite for gelation to take place. In many systems gelation also occurs in so-called good solvents[7] (e.g., PMMA in bromobenzene and syndiotactic polystyrene in benzene).

Manifestly, when DMSO is added to water, something happens that enhances the gelation propensity of agarose. Similar effects have been reported by Watase and Nishinari[10] from DSC experiments carried out on the same system: with increasing content of DMSO, the gel melting point first increases and then decreases. The maximum occurs for $X_{DMSO} \approx 0.3$. However, these authors assumed these effects to arise only from the peculiarity of DMSO/water mixtures. As a matter of fact, water forms several complexes with DMSO in the solid state, of which one is a congruently-melting compound possessing a water/DMSO composition (v/v) of 0.44/0.56.[11,12] Watase and Nishinari[10] consider, on the basis of Cowie and Toporowski's findings,[12] that there exists a memory effect of this complexed form in the liquid state which causes a reduction in the available amount of water for agarose. As a result, they claim that agarose chains behave as if they were in a more concentrated state; hence the maximum of the gel melting point. This approach overlooks another possibility: if DMSO can form a complex with water, its propensity to form a complex with agarose should be also considered.

In what follows, we shall give a series of arguments derived from small-angle neutron scattering performed on agarose sols that suggest the existence of a DMSO/agarose complex possessing properties that differ from those of pure agarose.

(2) Neutron Scattering. The sol state was obtained by heating 3% solutions at 130 °C and then investigating them at 70 °C. Only one diluent, i.e., pure DMSO, in which gelation is absent, was studied at 20 °C.

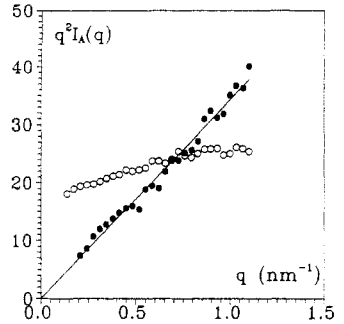

Figure 2. Scattered intensity in a Kratky representation ($q^2I(q)$ vs q) for a 3% agarose solution in heavy water at 70 °C: (○) solution heated at 100 °C and then quenched to 70 °C; (●) solution heated at 130 °C and then quenched at 70 °C.

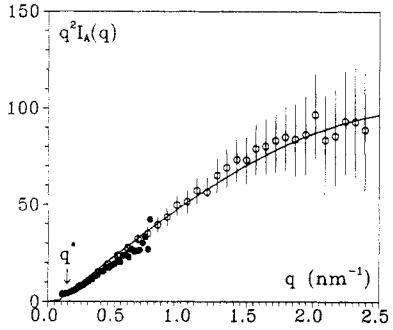

Figure 3. Scattered intensity in a Kratky representation for agarose chains in perdeuterated DMSO. Open and filled circles stand for two different sample–detector distances. The solid line is calculated from relation 13 with r_H = 0.45 nm.

Before detailing and discussing the results further, it is necessary to assess the effect on the agarose conformation of the temperature at which the gel samples have been turned into solutions prior to being investigated at 70 °C. Heating at 100 or 130 °C does not yield the same results as reported by Dormoy and Candau,[13] who noticed the presence of aggregates in gels melted at 100 °C. Here, the existence of aggregates after heating at 100 °C is revealed through the scattered intensities on comparison with a gel melted at 130 °C (Figure 2). Whereas a rodlike behavior is seen for the 130 °C molten sample, this behavior is absent for the 100 °C molten sample. The intensity scattered by the 100 °C molten sample is consistent with the presence of aggregates. As these aggregates may be of various forms, of which we have no clear picture, no attempt to fit this curve will be made here.

Once the gels are properly molten at 130 °C so as to erase all possible traces of aggregates, one of the most salient outcomes from the results obtained by neutron scattering is that the chain conformation in the sol state does not depend markedly upon solvent composition. A typical scattering curve is given in Figure 3. As can be seen, a q^{-1} behavior, typical of rodlike structures, is observed in the small-q region. Departure from this behavior is seen at larger q. As a rule, the chain is wormlike and characterized by high rigidity.

For wormlike chains the crossover from the q^{-2} asymptote to the q^{-1} behavior occurs for a value q^* in a Kratky representation ($q^2I(q)$) that can be calculated from the analytical expressions obtained by Des Cloizeaux:[14]

first asymptote

$$q^2I_A(q) = \frac{6\mu_L}{l_p} \tag{5}$$

second asymptote

$$q^2I_A(q) = \mu_L\left[\pi q + \frac{2}{3l_p}\right] \tag{6}$$

q^* then reads

$$q^* = \frac{16}{3\pi l_p} = 1.7l_p^{-1} \tag{7}$$

in which l_p is the chain persistence length and μ_L is the mass per unit length. If one considers that the plateau observed in the very small q range corresponds to the asymptote described by relation 5, then from these experiments a minimum persistence length of about 9–12 nm can be derived by considering 0.15 nm^{-1} ≤ q^* ≤ 0.2 nm^{-1}.

Alternatively, the solutions studied here are rather concentrated ((3–8) × 10^{-2} g/cm^3) so that one may wonder whether the chains are not in a semidilute state, which entails screening effects of the type described by Edwards.[15] In this situation the q^{-2} plateau arises from Lorentzian broadening due to a screening length ξ:

$$I(q) \sim [q^2 + \xi^{-2}]^{-1} \quad \text{for } q < q^* \tag{8}$$

$$I(q) \sim q^{-1}(1 + 2q^{-1}\xi^{-1}) \quad \text{for } q > q^* \tag{9}$$

Such a crossover would be observed for $l_p > \xi$. If $\xi > l_p$, then the analysis with a wormlike chain holds. However, for such concentrations it is likely that $l_p > \xi$, so the values of l_p derived above would be very conservative.

A third case may also be contemplated: formation of a nematic-like arrangement. Under these conditions the q^{-2} behavior has another origin, such as heterogeneities in the medium. Again, this would mean that the above values would be higher in reality.

To summarize, the values of l_p are most probably lower limits. Only experiments carried out at lower q values and different concentrations might eventually give a more accurate value.

The overall results indicate unambiguously that agarose is an intrinsically rigid chain independent of the solvent. This is again confirmed by experiments on sols at 70 °C in water containing the hydrogen-bond breaker NaSCN (Figure 4). In this medium, gelation is strongly impeded[16] and, as a result, does not occur at room temperature. Despite the high content of NaSCN, the chain conformation does not differ significantly from that in pure water or in pure DMSO. Possibly, the chains are more flexible in this medium as the persistence length calculated from eq 7 yields $l_p \approx 6.8$ nm.

It is worth examining these results in the light of equations developed for scattering by helices. Pringle and Schmidt[17] have considered helix conformation possessing a cross-section as drawn in Figure 5. This cross-section represents a double helix if ϕ differs from 0, while it can be used for a single-stranded helix if $\phi = 0$. Pringle and Schmidt end up with the following relation:

$$I(q) \sim (\pi\mu_L/q)\sum_{n=0}^{\infty} \epsilon_n \cos^2(n\phi/2)\frac{\sin^2(n\omega/2)}{(n\omega/2)^2} g_n^2(qr_H,\gamma) \tag{10}$$

in which ω and ϕ are related to the helix cross-section, μ_L is the mass per unit length, $\epsilon_0 = 1$ and $\epsilon_n = 2$ for $n \geq 1$,

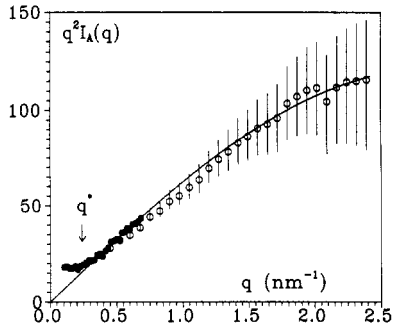

Figure 4. Scattered intensity in a Kratky representation for agarose chains in solution in heavy water containing 1 M NaSCN. Open and black circles stand for two different sample–detector distances. The solid line is calculated from relation 13 with r_H = 0.45 nm.

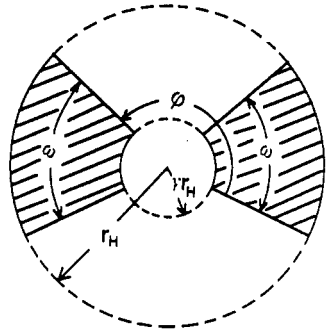

Figure 5. Helix cross-section considered by Pringle and Schmidt.[17]

and $g_n(qr_H,\gamma)$ is

$$g_n(qr_H,\gamma) = \frac{2}{(1-\gamma^2)r_H^2}\int_{\gamma r_H}^{r_H} rJ_n[qr(1-a_n^2)^{1/2}]\,dr \quad (11)$$

in which J_n is the Bessel function of the first kind and order n and with the following conditions on the a_n terms dependent on the pitch P of the helix:

$$a_n = 2\pi n/qP \quad \text{for } q > 2\pi n/P$$

$$a_n = 1 \quad \text{for } q \le 2\pi n/P$$

Pitches usually considered in such structures are not larger than 3.0 nm.[4] Taking this as an upper value and complying with the above conditions, up to q = 2.1 nm^{-1} only the term for which $n = 0$ contributes since $a_0 = 0$ and $a_n = 1$. For q = 2.1 nm^{-1} to q = 2.5 nm^{-1}, one should consider $a_1 \neq 1$, which would introduce an additional $g_1(qr_H,\gamma)$ term. It can be shown, however, that this term is only a small correction in this q range, so it can be ignored, particularly if one keeps in mind that the accuracy in this range is rather poor (cf. Figures 3 and 4). Equation 10 reduces therefore to

$$I(q) \sim (\pi\mu_L/q)\left[\frac{2}{(1-\gamma^2)qr_H}[J_1(qr_H) - \gamma J_1(q\gamma r_H)]\right]^2 \quad (12)$$

In turns out that in this q range, eq 12 is simply the same as that calculated by Mittelbach and Porod[18] for hollow cylinders of outer radius r_H. Similarly, if $\gamma = 0$, then relation 10 reduces to the well-known form for solid cylinders of outer radius r_H:

$$I(q) \sim (\pi\mu_L/q)\frac{4J_1(qr_H)}{q^2r_H^2} \quad (13)$$

In the case of an assembly of such cylinders, the intensity, plotted in a Kratky representation, reads

$$q^2I_A(q) = C_p\left[\pi q\mu_L\frac{4J_1^2(qr_H)}{q^2r_H^2} + \text{const}\right] \quad (14)$$

The constant term is related to the number of rod–rod contacts, sharp or soft kinks, and rod ends per unit length. In semidilute solution this term is usually dominated by rod–rod contacts. Here, we note that for $q > q^*$ the constant terms can be neglected.

Admittedly, one cannot claim that in the sol state, despite a very rigid conformation, the chain takes on a well-defined helical form. Yet, as relation 13 works for both a helix and a cylinder, the transverse radius of the chain can therefore be determined. In addition, relation 9 allows one to measure the mass per unit length, μ_L.

In all cases, a typical radius r_H = 0.45 ± 0.15 nm is derived from the scattering curves. This value differs quite markedly from that expected for the double-stranded helix (r_H = 1.35 nm).[3,4] While the value of r_H is independent of the solvent considered, within the experimental uncertainty, this is not so for the mass per unit length, μ_L. Results are listed in Table 1.

In *water*, $\mu_L \approx 360$ g/(mol nm), a value that is at variance with what one would expect either for a double-helical form ($\mu_L \approx 970$ g/(mol nm))[3] or even for a single strand of this double helix. Such a mass per unit length corresponds in fact quite closely to rather a stretched conformation such as those described by Foord and Atkins[4] ($\gamma = 0$ for these helices). For instance, different single-stranded 3_1-helical forms put forward by these authors are characterized by μ_L = 314–326 g/(mol nm) and $r_H \approx$ 0.45–0.54 nm. Similar conclusions as to the helical structure were postulated by Jimenez-Barbero et al. from conformational analysis in solution.[6] These authors suggest a threefold helical form with a pitch of 2.85 nm. Thus, not only does the experimental mass per unit length in water at 70 °C agree with single helices but so does the experimental radius.

To summarize, these results point toward an *intrinsically rigid chain* whose conformation closely resembles a near-extended chain. One question comes to mind as to the helical forms involved in the gel state: how can very rigid chains intertwine rather than simply align when undergoing gelation? Unless some appropriate mechanism is invented to solve this question, the helical conformations put forward by Foord and Atkins[4] may prove serious candidates to account for the chain in the gel state. As a result, this would lead one to dismiss the notion of double helices in agarose gels.

While the chain transverse radius remains virtually unaffected on modifying the composition of the DMSO/water mixture, the mass per unit length is seen to decrease on increasing the DMSO content. In pure DMSO, this parameter, which does not vary with temperature within the range 20–70 °C, is even *lower than what could be achieved by totally stretching the agarose chain.* Such a situation is often encountered when apparent values are involved and means that the calculated contrast factor is not appropriate. This in turn strongly suggests the presence of a DMSO/agarose complex as this appears to be the only way of altering the agarose contrast with respect to the surrounding solvent. Indeed, in the case of a complex, the intensity in the limit of small q is

Table 1. Mass per Unit Length of Agarose Chains as a Function of the Solvent Composition[a]

% DMSO/D_2O	100/0	30/70	50/50	30/70	0/100
μ_L (g/(nm mol))	230 ± 23	256 ± 20	293 ± 20	341 ± 20	360 ± 36

[a] Densities reported by Cowie and Toporowski[11] were used for calculating the scattering amplitude.

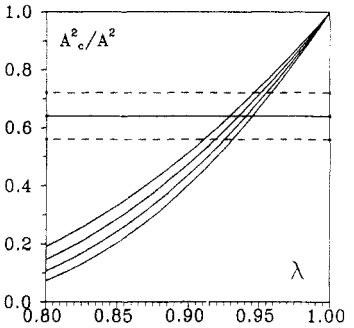

Figure 6. Squared ratio of the calculated scattering amplitude of an agarose/DMSO complexed form over the calculated scattering amplitude of the noncomplexed form as a function of λ (see text for further details). Curves are for different numbers of DMSO molecules per agarose residue: from top to bottom, n = 1, 2, 3, and 4. The horizontal line stands for the ratio μ_{LDMSO}/μ_{Lwater} (see text), and the two horizontal dashed lines represent the estimated experimental uncertainty on this ratio.

$$q^2I(q) = K_cC_c\pi\mu_{Lc}q \tag{15}$$

in which the subscript c relates to the parameters of the agarose/DMSO complex form.

Since in dilute solutions the ratio $C_c\mu_{Lc}/m_c^2 = C_p\mu_L/m_p^2$, whre μ_L is the partial mass per unit length of the polymer in the complex, the only relevant parameter remains the square of the difference of the scattering amplitudes, which, in the case of a complex, is

$$A_c^2 = \left[a_p + na_s - \frac{\lambda(v_p + nv_s)}{v_s}a_s\right]^2 \tag{16}$$

in which n is the number of solvent molecules complexing one agarose repeat unit and λ is an adjustable parameter that accounts for the variation of molar volume with complex formation. If λ = 1 this entails pure additivity of the molar volumes, which is not strictly compatible with complex formation. Besides, for λ = 1 the scattering amplitude would not be different from the pure polymer; hence the nonoccurrence of any apparent value.

In Figure 6 are plotted a series of curves representing the ratio A_c^2/A_p^2 as a function of λ with n as a parameter (A_p^2 is the contrast factor of agarose in the absence of complexation). As can be seen, if one considers that the true mass per unit length is that in pure water, then the correct ratio can be obtained. The experimental uncertainties allow us to bracket λ between 0.91 and 0.96 with n from 1 to 4. These seem reasonable values for molar volume reduction due to complexation.

Now, assuming that the same type of complex is formed independent of the DMSO/water composition, it is worth attempting to fit the variation of μ_L as a function of mixture composition. Obviously, the equation used for this fit does not hold for pure water nor for pure DMSO, in the latter case on account of the absence of isotopic exchange. Figure 7 shows that a good fit can be achieved by using λ = 0.975 and n = 2. These data are consistent with what has been derived above when experimental uncertainties are taken into account (for n = 2, 0.915 ≤ λ ≤ 0.95).

It is worth emphasizing that, if one is dealing with an apparent mass per unit length, the same should apply to the transverse radius. The fact that, experimentally, the radius is not sensitive to the DMSO/water composition does not constitute a counterproof. It is likely that the uncertainty on the value of the transverse radius, which is estimated to be 30%, precludes detection of apparent values. Strictly speaking, however, this has to be kept in mind.

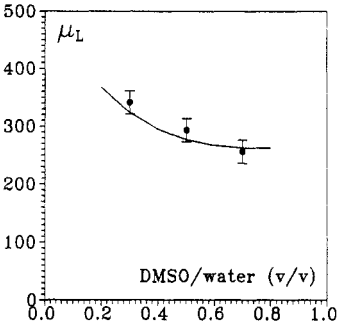

Figure 7. Experimental mass per unit length as a function of the water/DMSO mixture composition (v/v). The solid line stands for the variation calculated with λ = 0.975 and n = 2 by assuming that the stoichiometry of the agarose/DMSO complex is independent of solvent composition.

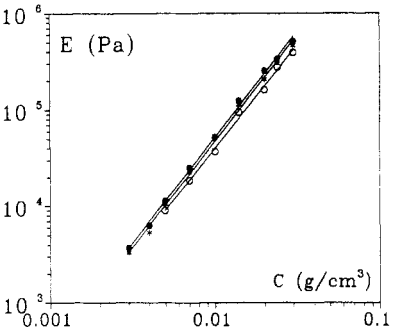

Figure 8. Young's modulus vs agarose concentration for different water/DMSO compositions: (○) 30/70; (★) 100/0; (●) 70/30.

(3) Mechanical Testing: Modulus vs Concentration. The relation between the gel modulus and the agarose concentration was determined in three water/DMSO mixtures at 20 °C for concentrations ranging from 3×10^{-3} to 3×10^{-2} g/cm^3.

The data are linear in a double-logarithmic scale (see Figure 8) and yield the following power relations (in these relations C is expressed in g/cm^3):

water/DMSO = 100/0 $\quad E = 1.16 \times 10^6 C^{2.2 \pm 0.03}$ kPa

water/DMSO = 70/30 $\quad E = 1.27 \times 10^6 C^{2.19 \pm 0.03}$ kPa

water/DMSO = 30/70 $\quad E = 0.74 \times 10^6 C^{2.13 \pm 0.03}$ kPa

The occurrence of exponents close to 2.2 does not mean that one is dealing with flexible gels as those prepared chemically, for which a value of $^9/_4$ (2.25) is predicted.[19] The fact that the chain is very rigid, which precludes the existence of flexibility even in the "amorphous" domains of the gel, together with electron microscopy pictures[20,21] that show an array of fibers indicates that theories specifically developed for rigid gels must be considered.

Recently, Jones and Marquès derived power laws for the modulus–concentration relation for rigid gels for which the junctions are connected by objects of longitudinal fractal dimension ν^{-1}.[22] These authors obtained the following relation:

$$E \sim \frac{er^4}{a^4}\left[\frac{\phi a^2}{nr^2}\right]^{(3\nu+1)/(3\nu-1)} \tag{17}$$

in which e is the intrinsic Young's modulus, a is the unit step so that N steps represent the longitudinal contour length L (Na = L), and r is the cross-section of the connecting object. n stands for the junction functionality (number of chains merging at the same junction).

Relation 17 can be used for the present data provided that the gel mesh size is larger than the object cross-section. Electron micrographs indicate that such is the case in the range of agarose concentrations investigated.[20,21]

If the fractal dimension of the fibers between two connections is $\nu^{-1} = 1$, then relation 17 reduces to

$$E \sim e\frac{\phi^2}{n} \tag{18}$$

As can be seen, the experimental exponents are slightly larger than 2, which suggests a fractal dimension slightly larger than 1 (ν^{-1} from 1.08 to 1.12). This may simply indicate that the fibers are not strictly straight, which is consistent with morphological observations.[20,21]

Interestingly, morphological studies on κ-carrageenan gels, which show an array of highly straight fibers,[21] suggest a fractal dimension of $\nu^{-1} = 1$, which is consistent with the exponent of 2.0 found for the modulus–concentration variation.[23] Jones and Marquès' analysis is therefore quite relevant for polysaccharide thermoreversible gels.

Further, while for $\nu^{-1} = 1$ the fiber section is theoretically not supposed to play any role, because these terms cancel, this is no longer true for $\nu^{-1} > 1$. For $\nu^{-1} = 1.12$ one obtains

$$E \sim \frac{e}{n^{1.2}}\frac{a^{0.4}}{r^{0.4}}\phi^{2.2} \tag{19}$$

If r differs from one mixture to another, then the front factor is also expected to vary. This may account for why there is a significant discrepancy observed for the different water/DMSO mixtures.

One may also attribute the variation of the front factor as arising from a change of the intrinsic Young's modulus, e, of the fibers. Since the agarose chain conformation is not significantly sensitive to mixture composition, we accordingly surmise e will not vary depending on whether one is dealing with the complexed or noncomplexed form.

Concluding Remarks

Results presented in this paper indicate clearly that agarose chains are fairly rigid, in contrast with previous hypotheses. It is worth emphasizing that SANS results are not at variance with polarimetry experiments from which the notion of the *flexible random coil–helix* has been deduced. As a matter of fact, polarimetry is sensitive to the glycosidic torsion angles so that a difference is also expected between the optical rotation of a *loose helix* and a *rigid helix*.

The fact that agarose chains are very rigid in the sol state inevitably raises the question about the helical conformation in the gel state. Can a very rigid chain possessing a conformation close to energetically viable single helices rewind with another chain instead of simply aligning? Single-helical models match our data, and unless some new information can be found to support double-helical structures for agarose, the latter should be dismissed.

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