Agarose Sols and Gels Revisited

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Summary: Agarose *sols* have been seen for long as solutions of flexible chains that, on cooling, produce thermoreversible gels through double-helix formation. Investigations of the chain conformation in the *sol state* by small-angle neutron scattering reveals instead a rigid chain with a very large persistence length ($l_p > 9$ nm). The chain cross-section radius and mass per unit length correspond to characteristics of helices as those described by Foord and Atkins. These results lead one to a reappraisal of the occurrence of double helices in the gelation process, as they rather suggest a transition of the type *loose-single helix⇒tight single helix*. Studies of gels from *agarose/water/cosolvent* where the cosolvent is Dimethyl Sulfoxide (DMSO), Dimethyl Formamide (DMF), and Methyl Formamide (MF) have led one to conclude on the formation of a*garose/water/ cosolvent* ternary complexes. The contrast variation method by neutron scattering gives further support to this assumption. Finally, determination of the gel nanostructure allows one to account for the two regimes observed for the variation of the elastic modulus vs concentration.

Keywords: agarose; gels; helices; rheological behaviour; sols; structure

Introduction

Agarose is a polysaccharide which is extracted from seaweeds. Although the thermoreversible gelation of agarose might be considered as an old problem, there are still some issues that are not settled. An important issue concerns the helical structure involved in the *GEL* state. Originally, the occurrence of double helices was suggested on the basis of diffraction patterns by poorly-ordered agarose gels samples[1]. The gelation mechanism was therefore seen as an intertwining of flexible chains. The paucity of the diffraction pattern led Foord and Atkins to question the very existence of double helices in these gels. On the basis of highly improved diffraction patterns[2], they insisted that single helices should rather be considered. As will be detailed below, we have obtained circumstantial evidence on the possible existence of single helices[3,4]. Another issue deals with the thermodynamic of the gelation process, and more particularly when cosolvent are dealt with[5]. Here we shall discuss these points together with the rheological properties of the gels, particularly the relation elastic modulus vs agarose concentration with respect to the gel structure.

Chain Conformation in the *SOL* State

Studies of the chain conformation in the *SOL* state in different solvents (water, water/DMSO, DMSO, and DMF) have revealed a worm-like chain behaviour[3,4,6] (Figure 1). This means that the chain is globally brownian, but locally rod-like. The persistence length l_p, namely the length over which the chain is rod-like, is larger than 9 nm.

This clearly means that agarose chains are intrinsically rigid, and not at all flexible as was first believed on the basis of polarimetry experiments (a flexible chain would have a persistence length of about

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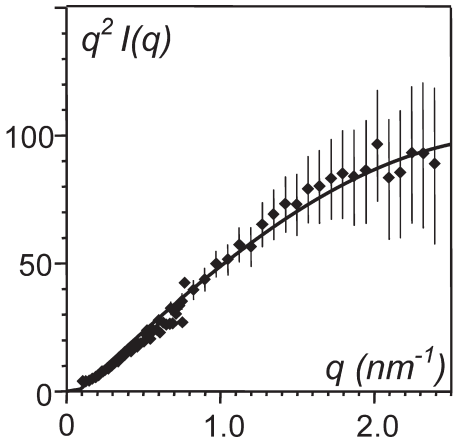

Figure 1.
Krakty-plot ($q^2I(q)$ vs q where $q = (4\pi/\lambda)\sin(\theta/2)$, λ = neutron wavelength, θ = scattering angle) for small-angle neutron scattering for agarose/$DMSO_D$ solutions. The solid line is calculated by considering a solid cylinder of cross-section $r_H = 0.45 \pm 0.15$ nm, and a mass per unit length in water $\mu_L = 360$ g/nmxmol. The same results are found in water at 90 °C or water/NaSCN 1 M medium at 20 °C. Results are from reference 4.

1 nm). Also, the results in water show that the chain-cross-section and the mass per unit length are close to those values obtained from some of the single helices put forward by Foord and Atkins. Note that the high rigidity of agarose chain was also suggested by ab-initio calculation[7].

These results, in view of Foord and Atkins findings, that clearly discard the existence of the double helix, lead also one to question the existence of double helices in the *GEL* state. As a matter of fact, how could parts of two rigid chains intertwine with one another to form double helices? It seems offhand that, on cooling, they can simply align with respect to one another and is so doing give birth to the gel fibrils.

Ternary Agarose/Water/Cosolvent Complexes in the Gel State

Watase and Nishinari[5] have published a temperature-composition phase diagram of water/DMSO of the type drawn in Figure 2a, which shows that both the gel

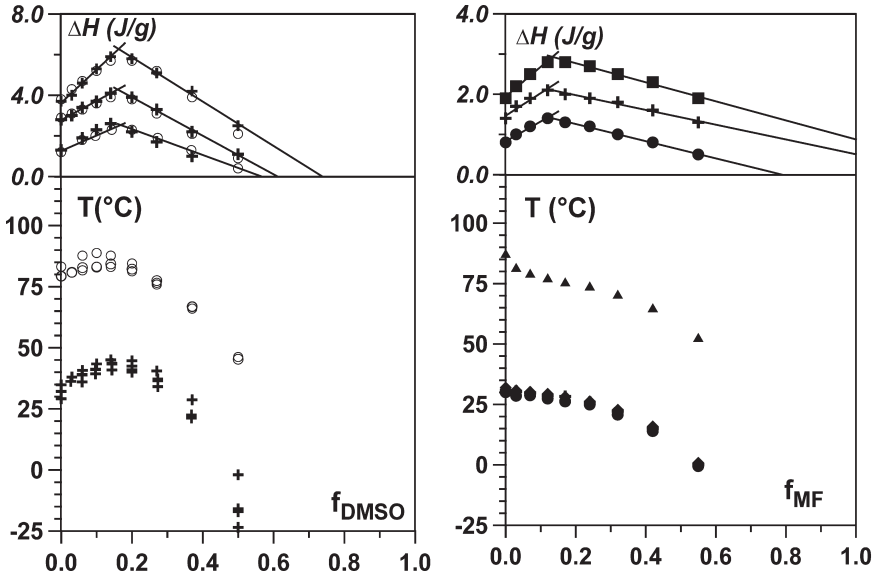

Figure 2.
Temperature-composition phase diagrams and Tamman's diagrams for *left*) water/DMSO systems. (○): gel melting (+) = gel formation; concentrations are 5%, 10%, 15%, molecular weight of agarose sample is $M_w = 3.5 \times 10^4$ for all and also $M_w = 1.12 \times 10^4$ for 5%. *right*) for agarose/water/N-methyl formamide systems for $C_{agarose} = 3\%$ (●), 5% (+) and 7% (■). In the T-C diagram, triangles stand for the melting temperature (independent of agarose concentration), the other symbols for the formation temperature; molecular weight of agarose sample is $M_w = 1.12 \times 10^4$. Results taken from reference 8.

melting temperature, T_m, the gelation temperature, T_{gel}, and the gel melting enthalpies display a maximum for a DMSO fraction of about $f_{DMSO} = 0.17$ (w/w).

These authors have interpreted the maxima as resulting from a change of solvent quality (increase of T_m), together with an increase of the gelling material (more agarose chains would participate in the gel structure by increasing the fraction of DMSO). The latter point is not borne out by rheological experiments that show that the gel modulus remains virtually unchanged when increasing DMSO content (see below in *Rheological behaviour vs gel structure*). More gelling material should entail a subsequent increase of this parameter. More recently, Ramzi et al.[8] have studied the gelation in Dimethyl formamide (DMF) and Methyl formamide (MF). The results are basically the same with DMF as with DMSO, while in DMF a maximum is still seen for the gel melting enthalpy but no longer for the meting temperature (see Figure 2b). Ramzi et al. have suggested to consider the occurrence of a binary complex *agarose/water* and a ternary complex *agarose/water/cosolvent* for explaining these results. Indeed, while the gel melting enthalpy can increase because the complex possess a melting enthalpy higher than binary complex *agarose/water*, the melting point does not necessarily follows the same trend (see for instance ref. 9).

The existence of the agarose/water binary complex and the agarose/water/DMSO ternary complexes has received further support from neutron scattering experiments using the contrast variation method[10]. As a matter of fact, one can calculate the fraction of the different isotopic components in mixtures of solvent

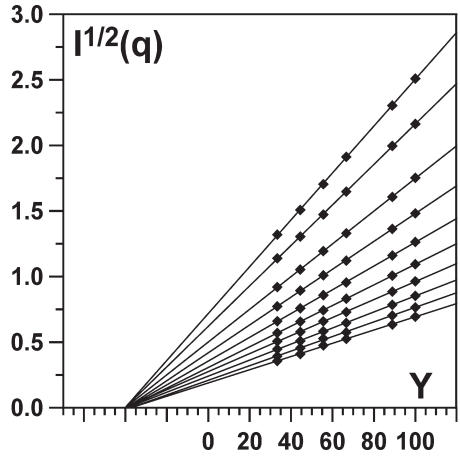

Figure 3.
Variation of the square root of the scattered intensity as a function of the fraction *Y* (%) of deuteriated DMSO ($DMSO_D$) in a 9/1 mixture *water/DMSO* (70/30 in w/w). The data points at constant *Y* stand for the different values of *q*. The value required for matching the coherent scattering of agarose in agarose gels would be $Y_o = -0.36 \pm 0.04$ while the calculated value is $Y_{theo} = 0$. From reference 10.

that is needed for matching the coherent scattering of agarose chains (for instance the fraction of deuteriated DMSO with respect to hydrogenous DMSO). If no complex occurs, the calculated value should be equal to the experimental one. Conversely, if complexes are formed, then the calculated value should differ significantly from the experimental one. Clearly, there are only two possibilities. In Figure 3 are shown the experimental curves obtained from the contrast variation method by either varying the content of $DMSO_D$ and $DMSO_H$ in the water/DMSO mixtures or the fraction of D_2O with respect to H_20. The results are gathered in Table 1. As can be seen the strong discrepancy between the calculated values and the experimental

Table 1.
Experimental values of the fraction of deuteriated material needed to match the coherent intensity of agarose chains, X_o (when the ratio H_2O/D_2O is varied), Y_o (when the ratio $DMSOD_6/DMSOH_6$ is varied) against the theoretical values calculated by ignoring complex formation. From reference 10.

water/DMSO fraction (mol/mol)	X_o	X_{theo}	Y_o	Y_{theo}
9/1 D_2O/DMSO ($DMSOD_6/DMSOH_6$)			-0.36 ± 0.04	0
9/1 H_2O (H_2O/D_2O)**/DMSOD_6**	0.16 ± 0.03	-1.00		

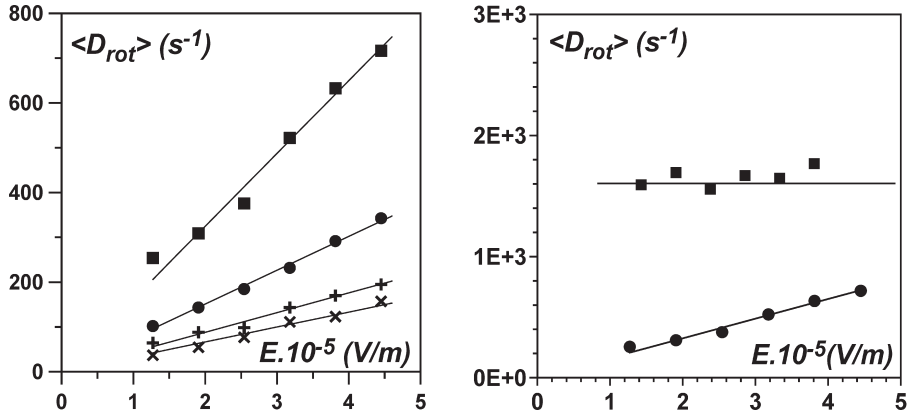

Figure 4.
left: variation of the rotational diffusion coefficient as a function of the applied electric field for agarose aggregates, $C_{agarose} = 0.03$ g/l (×); 0.05 g/l (+); 0.08 g/l (●); 0.1 g/l (■) ($f_{DMSO} = 0.2$); ***right***: comparison between poly[vinyl chloride] (PVC) aggregates, $C_{PVC} = 5$ g/l (■) and agarose aggregates $C_{agarose} = 0.1$ g/l and $f_{DMSO} = 0.2$ (●). Molecular weight of agarose $M_w = 1.23 \times 10^5$. From reference 11.

ones are clearly in favour of the occurrence of complexes. Negative values obviously mean that there is no way to match the coherent scattering of the complexes.

As has been discussed by Ramzi et al. the complex is thought to occur through electrostatic interactions between the negatively charged parts of the agarose residue and the positively charged parts of the DMSO, DMF or MF solvents[8].

Another piece of circumstantial evidence deals with the study by electric birefringence of aggregates, namely systems that are formed below the critical gelation concentration (see Figures 4). The rotational diffusion coefficient is seen to be dependent upon the applied electric field[11]. Comparison with poly [vinyl chloride] aggregates shows that this is germane to agarose aggregates. This implies that the size of the agarose aggregates decreases with increasing the magnitude of the electric field. It has been therefore suggested that the electric field perturbs the electrostatic arrangement of the ternary complex entailing its destruction, and correspondingly gradual disruption of the agarose aggregates.

Results obtained with modified agarose samples (OH groups are randomly replaced by OCH_3 groups have given further support to the existence of these complexes. It is even shown that DMSO molecules replace the hydrogen bonds that have disappeared due to the above modification[12].

Rheological Behaviour vs Gel Structure

Determination of the elastic modulus E as a function of agarose concentration[13] has shown the existence of two regimes (see Figure 5): below $C < 20$ g/l (region I) it was

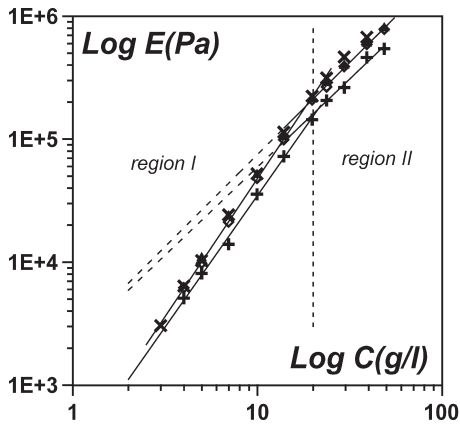

Figure 5.
Variation of the elastic modulus vs agarose concentration for three different water/DMSO compositions: (×) = 100%; (◆) = 70/20; (+) = 50/50. The vertical dashed line delimits either regime while the other dashed lines highlight the departure from the C^{2-3} behaviour. From reference 13.

observed that $E \sim C^{2.3}$ while for $C > 20$ g/l (region II) the modulus varies like $E \sim C^{1.5}$.

Such a behaviour was rather puzzling until investigations on the molecular structure of the gel gave some clue. As was shown by Sugiyama et al.[14] agarose gels consist of an array of fibrils with cross-section radii ranging from 2 to 20 nm. The terminal scattering behaviour for such systems should be of the Porod type[15], namely:

$$I(q) \propto q^{-4}$$

As can be seen in Figure 6[13], this behaviour is effectively observed for concentrations above 20 g/l while a strong upturn is seen for concentrations lower than 20 g/l. Ramzi et al. have interpreted the occurrence of this upturn as arising from the presence of free and/or dangling chains[13]. As a result, for C < 20 g/l the agarose concentration and the concentration of elastic materials differ. Conversely, for C > 20 g/l all the agarose chains are incorporated in the gel fibrils and these concentrations are nearly equal. This implies that the modulus-concentration relation describing the elasticity of the agarose network is that with the 1.5 exponent. The exponent 2.3 is only apparent due to the presence of the free and/or dangling chains.

As was discussed by Ramzi et al.[13] such an exponent implies the existence of *entropic elasticity*[16]. How can a network made up with rigid fibrils possess *entropic elasticity*? The only possibility is to envisage flexible junctions (see Figure 7).

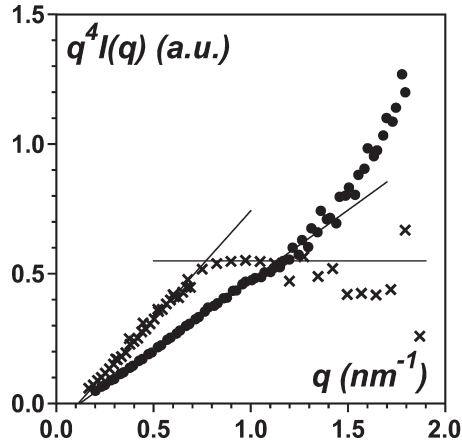

Figure 6.
Plot ($q^4I(q)$ vs q) in arbitrary units of the scattered intensity for agarose gels in water. For $q > 0.8$ nm^{-1} the Porod behaviour is seen for the gel of highest concentration ($C_{agarose} = 50$ g/l; ×), while an upturn shows up for the gel of lowest concentration ($C_{agarose} = 10$ g/l; ●). The straight solid lines drawn up to $q \approx 0.8$ nm^{-1} for (×) and up to $q \approx 0.8$ nm^{-1} for (●) stand for the *transitional behaviour* as calculated by Guenet for fibrillar systems with polydispersed cross-section radii[13,15]. From reference 13.

This entails that junctions between fibrils are rather disorganized as opposed to the crystalline nature commonly attributed to junctions in physical networks. It is worth emphasizing that agarose gels possess the unique behaviour among thermoreversible gels which is to display an increase of elastic

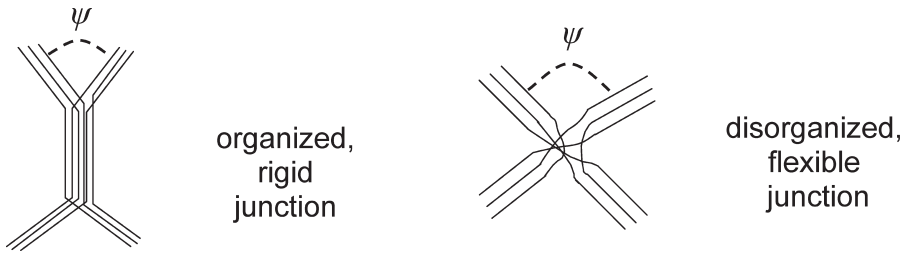

Figure 7.
Sketch of organized, rigid junctions (left), and disorganized, flexible junctions (right). If we consider the mean square fluctuation $\langle \delta\psi^2 \rangle$ of the angle ψ, then *entropic* elasticity occurs when $\langle \delta\psi^2 \rangle \neq 0$ and *enthalpic* elasticity for $\langle \delta\psi^2 \rangle = 0$.

modulus with temperature[17], as expected for entropic elasticity.

Concluding Remarks

Agarose chains in the *SOL* state are intrinsically rigid as the minimum persistence length can be estimated to be $lp > 9$ nm. The gelation is possibly due to a transition of the type *loose-helix* ⇒ *tight-helix*. One should therefore abandon the notion of double helix in view of the results by Atkins and Foord, and the high rigidity in the *SOL* state.

There exists ternary complexes agarose/water/cosolvent formed with solvents such as DMSO, DMF and MF due to electrostatic interactions rather than hydrogen bonds. Finally, DMSO or DMF replace H-bonds in agarose while repairing missing H-bonds in modified agarose[12].

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