

Study of the mechanical properties of agarose gel chemically modified

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Abstract

In this paper, we perform a mechanical study of pure and chemically modified agarose to better understand the intermolecular interactions and the choice of solvent underlying. This study is to calculate the elastic modulus of three agarose samples chemically modified M_1 , M_2 , M_3 . The goal of this work is to present a theoretical study based on the theory of "De Gennes" [7] to calculate the elastic modulus of agarose gel as a function of polymer concentration. These theoretical results will then be confronted with the experimental results performed on the agarose gel.

1. Introduction

Agarose is a natural polymer extracted from the red marine alga, found in California and eastern Asia. It is one of the two major components of the polygalactoside agar, together with agaropectine. Agarose is frequently used in molecular biology for the separation of large molecules, especially DNA, by electrophoresis [1,3]. The agarose is a linear polysaccharide composed of repeating basic unit: the agarobiose.

The basic disaccharide repeating units of agarose consists of (1,3) linked β -d-galactose (G) and (1,4) linked α -1-3,6-anhydrogalactose (A) (Fig. 1) (Araki, 1966) [2].

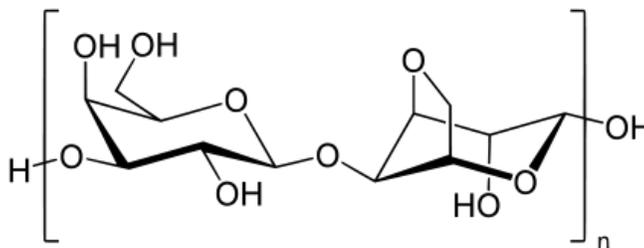


Figure 1: The structure of an agarose polymer [1].

The structure of the agarose is maintained by hydrogen bonds and may be disrupted by heating to a liquid state. The melting temperature is different from the temperature of gelation, in effect the gelation temperature of the agarose in the order of 40 ° C and its melting temperature is about 90 ° C [3,4]. The gelation of the biopolymer, that has been studied, among others, via calorimetry and a statistical study corresponds to the transformation of the macromolecular chains in coil form (diluted) in a helical conformation (gel) [1,5].

Agarose has been chemically modified by randomly replacing hydrogen of the hydroxyl groups OH by OCH₃ groups on the sites listed below (fig.2) [1,5,6].

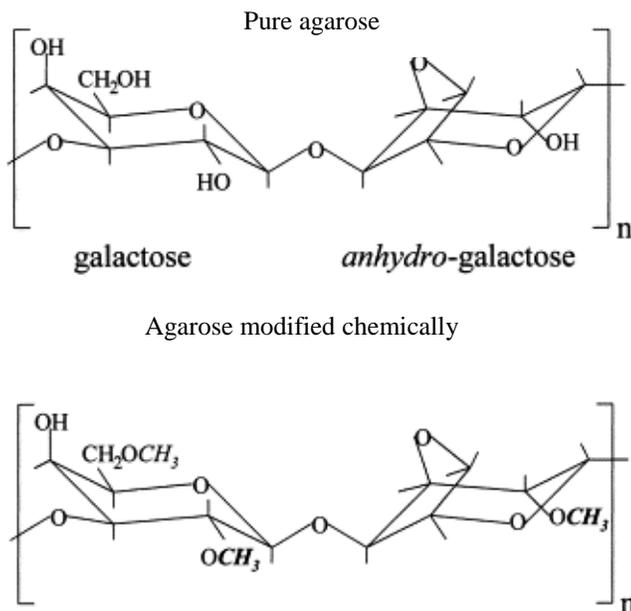


Figure 2: Chemical structure of agarose (top) and chemically modified agarose (bottom). Bold-face-type for the CH₃ group is used for indicating the sites that are most modified.

Molecular weights were determined by measuring the intrinsic viscosity at a temperature of 35 ° C. dosing methyl is obtained by hydrolysis of agarose. the samples have the following characteristics:

M ₀	M _w =1,12×10 ⁵	C ₁₂ H ₁₈ O ₉
M ₁	M _w =9,87×10 ⁴	C ₁₂ H _{17,46} (CH ₃) _{0,54} O ₉
M ₂	M _w =1,03×10 ⁵	C ₁₂ H _{17,32} (CH ₃) _{0,68} O ₉
M ₃	M _w =1,02×10 ⁵	C ₁₂ H _{17,07} (CH ₃) _{0,93} O ₉

This modification definitively removes the possibility of a hydrogen bond, while promoting the polarization of covalent bonds also leads to the appearance of fractional electric charges on different atoms. The groups OCH₃ may be crosslinked eventually leading to a network structure. The formation of a gel of agarose modified results from the combination of physical and chemical gelation [1,6].

The study of mechanical properties of the gels is the study of flow and deformation of these materials under the constraint action by calculating the elastic modulus of these gels. In the case of agarose gels, this parameter depends on the polymer concentration according to a power law $E \approx KC^n$.

Where E is the elastic modulus, C is the polymer concentration, n is an exponent and k is a constant.

Finally, we did this study mechanical of three agarose samples chemically modified M₁, M₂, M₃ to better understand intermolecular interactions.

2. Theoretical Formulation

2.1. Introduction

The theoretical model we have adopted to calculate the elastic modulus E, is based on the description of polymer solutions for predicting the distribution of macromolecules [7,8]. The physical system that we consider in this work is a solution of macromolecule interpenetrated polymerization degree N in a good solvent.

It is supposed that the gel is obtained from its macromolecules that reticle by physical processes. It is also assumed that the gel structure is close to that of the solution. The gel can be considered as a stack of ζ size; blobs are introduced by "De Gennes" (Fig. 3).

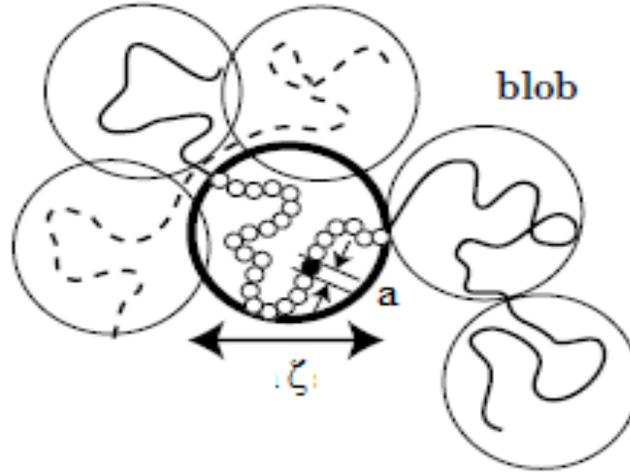


Figure 3: Representation of a gel as a domain ζ stack size.

The concentration of gel C is proportional to the concentration of recovery C^* :

$$C = K(z)C^* \quad (1)$$

$K(z)$ is a constant which depends on the number of crosslinking and z represents the functionality of the crosslinking agents.

The concentration of recovery C^* varies with the degree of polymerization N , according to the law [9,10]:

$$C^* = V^{-\frac{3}{5}} a^{-\frac{6}{5}} N^{1-3\nu} \quad (2)$$

Where N , ν and a are respectively the number of monomers between two points of crosslinking, the volume parameter excluded (coefficient of swelling of the chain) and the size of the monomer.

The screen length or size of blob decreases with the concentration according to the law [7,9]:

$$\zeta \propto C^{\frac{\nu}{1-3\nu}} \quad (3)$$

$\nu = \frac{3}{2+d}$ is the critical exponent of swelling.

3.2 The elastic modulus of the gel

If we denote by $C(r)$ the local concentration at r , the pair correlation function is given by [7,10]:

$$g(r) = \frac{1}{C} \left[\langle C(r)C(0) \rangle - C^2 \right] \quad (4)$$

Where $\langle C(r)C(0) \rangle$ is the average value of the concentration.

We distinguish two regimes:

- The short regime: $r < \zeta$

In this case, the correlation function is dominated by interactions within the same chain, and follows the law of Edward [1]:

$$g(r) \propto a^{-3} \left(\frac{a}{r} \right)^{4/3} \quad (5)$$

- The long regime: $r > \zeta$

In this case, the fluctuation of the density is limited by the macroscopic rigidity of the gel and the behavior of $g(r)$ is provided by the Ornstein-Zernike theory [7]:

$$g(r) \propto C^*(\zeta / r) \exp(-r / \zeta) \quad (6)$$

The free energy that accounts for interactions within the system is given by:

$$F = \frac{1}{2} E \left(\frac{\delta C_q}{C} \right)^2 + LV \left(\frac{\delta C_q}{C} \right)^2 \quad (7)$$

Where δC_q is a local variation of the concentration, E is the elastic modulus of the system, L represents a higher order correlation. Since the only available length is ζ , we must have [7]:

$$L \approx E\zeta^2 \quad (8)$$

In reciprocal Fourier space, the equation of energy F (eq.7) becomes:

$$F = \sum_q \left(\frac{\delta C_q}{C} \right)^2 (E + Lq^2) \quad (9)$$

Where q is the wave vector. By applying the equipartition theorem for each mode q, there will be:

$$\frac{1}{C^2} \langle (\delta C_q)^2 \rangle = \frac{T}{E + Lq^2} \quad (10)$$

Note that this equation is only applicable for $q\zeta < 1$. In real space R, the pair correlation function (eq.6) becomes:

$$g(r) = \frac{CT}{4\pi L r} \exp \left[-r \left(\frac{E}{L} \right)^{1/2} \right] \quad (11)$$

$$g(r) \approx \frac{N}{\zeta^2} \frac{1}{r} \exp(-A \frac{r}{\zeta})$$

By identifying both sides of this equation:

$$E \cong \frac{L}{\zeta^2} \cong \frac{C^*}{N} T \cong \frac{C}{N} T \quad (12)$$

Knowing that:

$$C^* = V^{-3/5} a^{-6/5} N^{1-3\nu}$$

The elastic modulus then follows the scaling law:

$$E \cong TV^{\frac{3}{5(3\nu-1)}} a^{\frac{6}{5(3\nu-1)}} C^{\frac{3\nu}{3\nu-1}} \quad (13)$$

Here, C represents the concentration of monomer; the concentration is related to the mass concentration C_m of the polymer through the relationship:

$$C = \frac{C_m}{m}$$

Where m is the mass of a monomer agarose ($m=5,1 \cdot 10^{-26}$ Kg).

Therefore, the elastic modulus varies with the mass concentration of the polymer according to the equation:

$$E \propto C_m^{\frac{3\nu}{3\nu-1}} \quad (14)$$

3. Results and discussion

The mechanical study consist to calculate the elastic modulus E of compression. Ramzi et al [1] were established elastic modulus-concentration relationships for different binary solvent for a wide concentration range between 3 and 50 g / l. they found that the mechanical properties do not depend on the choice of binary solvent and its composition. The variation of the elastic modulus as a function of the pure water in the polymer concentration for three agarose chemically modified samples M_1 , M_2 , M_3 and M_0 pure agarose. These results are represented in figure4. This figure shows that we are in the presence of two concentrations regimes:

- A low concentration regime ($3 < C < C^*(M(i))$ where $i=0, 1, 2,3$. Where the scale law is of type:

$$E \propto C^{2.2 \pm 0.1}, \text{ similar to determine by Rochas et al [5].}$$

Where: $C^*(M_0)=20$ g/l ; $C^*(M_1)=24$ g/l ; $C^*(M_2)=29$ g/l; $C^*(M_3)=37$ g/l.

- A high concentration regime ($C > C^*(M(i))$) or the law of scale is of type: $E \propto C^{1.5 \pm 0.1}$.

This two-regimes behavior was interpreted taking into account the existence of a free chain low polymer concentration ($C < C^*(M_i)$ such as $C^*(M_0)$ for pure agarose, $C^*(M_1)$ for sample M_1 , $C^*(M_2)$ for sample M_2 , $C^*(M_3)$ for sample M_3), the proportion decreases by increasing the agarose concentration (chain not gelled). Taking into account the decrease of the hydrogen bonds in the modified agarose, it is necessary to increase the polymer concentration for gel. These conclusions have received additional support from neutron scattering experiments [6]. Figure 4 indicates that gels the M_3 sample always contain free channels even at high concentration. This is consistent with presence of an ungelled material for the range of studied concentrations.

From the figure 4, we observed a decrease in the elastic modulus M_0 to M_3 , and we observe that the concentration with the increases of the modification degree. This is again compatible with the increase in the fraction of ungelled chain when increasing the degree of modification. It is quite possible that these free channels may possibly freeze when increasing the concentration of polymer simply because they become less and less soluble (solubility in the liquid-solid transition sense).

According to the figure 4, looks at the critical concentration varies from sample to another such as $C_0(M_0) < C_1(M_1) < C_2(M_2) < C_3(M_3)$. This variation depends on changes made to the pure agarose and also returns to the chemical bond.

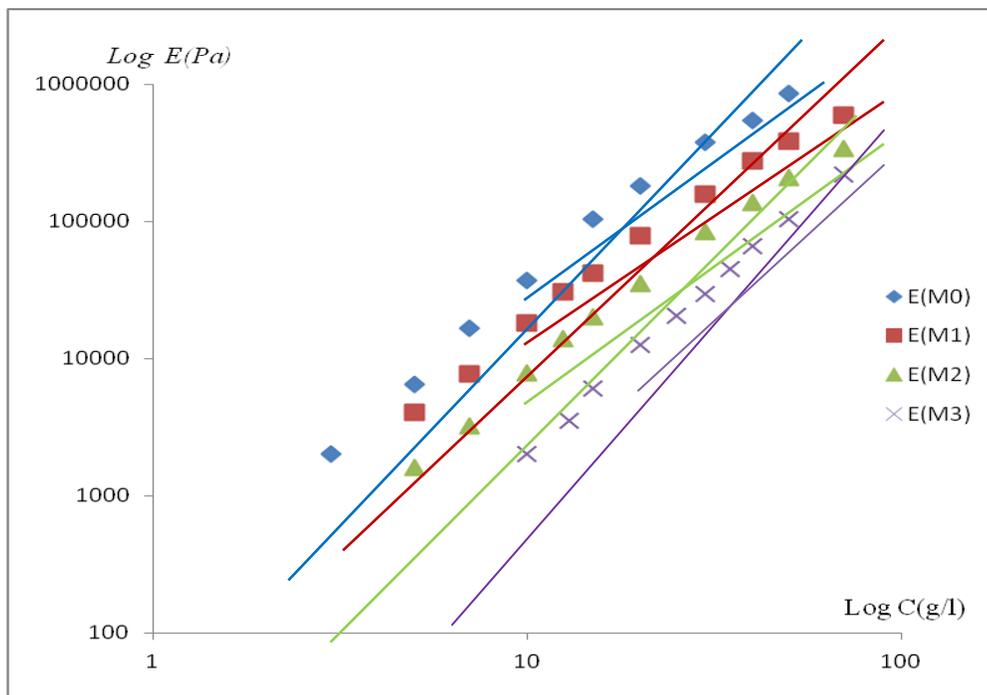


Figure 4 : Variation of elastic modulus of the gel as a function of concentration for the various samples chemically modified agarose M_1 , M_2 , M_3 and a sample agarose M_0 .

They concluded from the scaling laws that the value 1.5 is a real exponent which characterizes the elasticity of the network while 2.2 is an exponent apparent due to the presence of free chain. Such exponent 1.5 corresponds objects fractal dimension equal to 1 (the chains are sticks), but in the context of an entropy elasticity. As objects, the fibers of the gel, can only be governed, we concluded that the junctions are flexible and it is not crystalline. The fibers thus entangled together by crossing each other, the place where they cross is the junction that is amorphous. For our part, we have established the modulus-concentration relationship for a binary solvent (DMSO) and different compositions (see Figure 5).

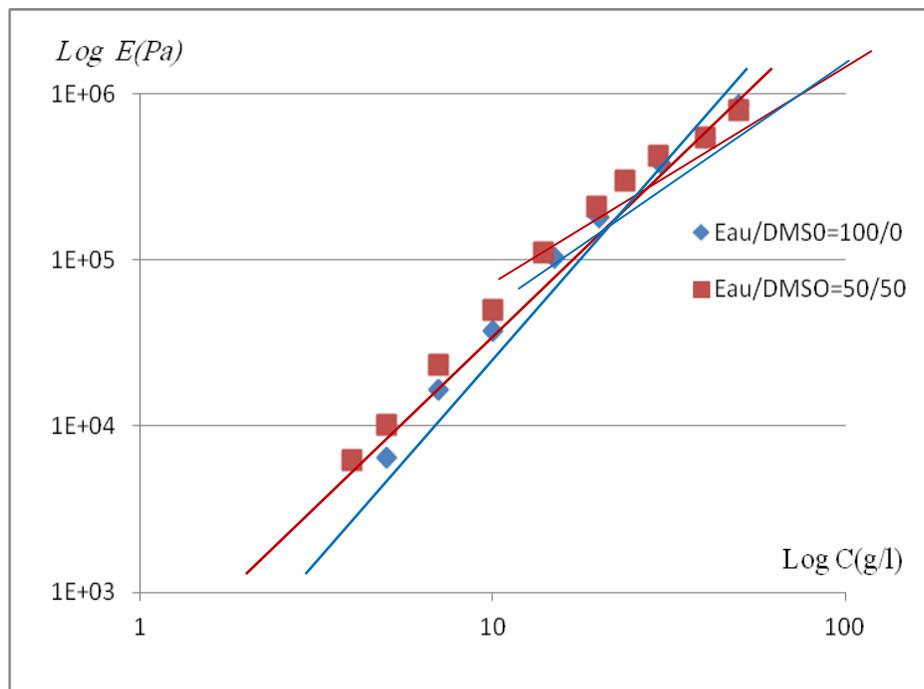


Figure 5: Example of the variation of the elastic modulus as a function of the concentration for the given binary solvent and different compositions.

We observed two regimes for the elastic modulus as a function of concentration: high concentration regime ($C > C_1^*$ such as $C_1^* = 20$ g/l for water / DMSO = 100/0 and $C_2^* = 32$ g/l for water / DMSO = 100/100) and low concentration regime ($C < C_1^*$) and we found the same laws of scales. These results presented in Figure 4 show that the elastic modulus varies linearly with the concentration to reach a critical concentration :

$C = 30$ g / l.

As concentration increases the elastic modulus also increases, so the macromolecules are highly interpenetrated and the forces emanating from the excluded volume become very important. Therefore, gelation becomes more efficient since the macromolecules are pulled and then takes a configuration "stick" means a swelling coefficient $\nu = 1$. And the low concentrations of agarose, there were free channels chains including their proportions are expected to degrade gradually, while increasing the concentration of polymer.

We found that the mechanical properties (elastic modulus) are very sensitive to binary solvent. So the behavior to both plans is independent of binary solvent and its composition.

The results for the study of the elastic modulus of agarose gels in the binary water / DMSO are already found by Rochas, Brulet and Guenet [5].

Conclusions

In this work, we proposed a model based on the description of the polymer solutions consider the agarose gels as homogeneous stack size blobs of ζ , this assumes that the gel structure is close to that of the solution.

Through this model, the elastic modulus follows a scaling law in two areas:

- A low concentration or the law of type: $E \propto C^{2,25}$
- A high concentration or the law of type: $E \propto C^{1,5}$

We concluded that the regime change in M_0, M_1, M_2, M_3 is achieved gradually as the concentration increases.

This model is then confronted with the experience, it turned out that it conforms to experimental reality. So the theoretical model is well suited to describe the mechanical properties of agarose [7].

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