



Dedicated to Professor Bogdan C. Simionescu
on the occasion of his 65th anniversary

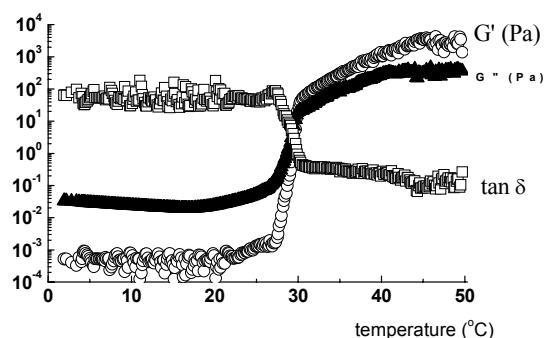
RHEOLOGICAL INVESTIGATION OF XANTHAN/PLURONIC F127 HYDROGELS

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The paper investigates different formulations based on xanthan and Pluronic F127 in water in order to design elastic hydrogels with good stability at 37 °C. Sol-gel transition was investigated through the viscoelastic parameters as a function of temperature. By adding small amounts of xanthan into a Pluronic F127 solution, the transition point is shifted to higher temperatures and the elasticity is improved. Pluronic F127 aqueous systems are fully thermoreversible and the answer to thermal stimulus is very fast. In the presence of long xanthan chains, the gel state is reached in a longer period of time and the system is no longer thermoreversible, showing a high yield stress value.



INTRODUCTION

Xanthan gum (X) is an anionic heteropolysaccharide produced by the *Xanthomonas campestris* microorganism, the main chains consisting in (1→4) β -D-glucopyranosil units substituted at C-3 with a charged trisaccharide chain formed by a D-glucuronic acid unit between two D-mannose units.¹ In aqueous solutions, the xanthan macromolecules undergo a thermally induced transition from an ordered (helical conformation) to a disordered conformation. At room temperature, due to the electrostatic repulsions of the charged groups, the xanthan chains are highly extended and they can form random helix structures by hydrogen bonding. As the temperature increases, the conformation changes to a coil-like one and, as a consequence, the

rheological properties change.² Xanthan gum forms highly viscous solutions even at low concentrations, being widely used as an effective stabilizer or a suitable thickener for various kinds of water-based systems in various applications including food, pharmaceutical, cosmetic, agricultural, textile, ceramic, and petroleum industries.³ Xanthan gum can play a successful role in matrix formulations for oral controlled-release drug delivery, it can form stable gel in conjunction with suitable biocompatible polymer systems.⁴⁻⁶ In hydrophilic matrix formulations, xanthan gum has demonstrated economic and pharmaceutical advantages as compared with other water-soluble biopolymers.⁷

Pluronics are nonionic surfactants with excellent wetting, antifoaming properties, non biodegradability and weak mechanical strength. To avoid some inconveniences, Pluronics can be used in

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physical mixtures with other polymers. Pluronic F127 (PI) is a triblock copolymer, poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (PEO-*b*-PPO-*b*-PEO), consisting of PEO units (70%) and PPO units (30%), which is transformed from a low-viscosity aqueous solution to a physical network upon heating to body temperature at concentrations higher than 12%.⁸⁻¹¹ The sol-gel temperature is around 20 °C – 25 °C (as a function of copolymer concentration^{10,11}) and this is an inconvenience during the summer or in the tropical environments. With increasing the concentration and/or the temperature, the Pluronic micelles come in contact with one another causing the entanglements among the hydrophilic corona of PEO chains and thus the gel structure is formed.¹² On the other hand, the micelles which form the network can be transformed reversibly in sol state by changing some physical environments, in particular the temperature.

It has been estimated that nearly 50% of new potential drugs have low solubility in water, which leads to poor bioavailability and makes the control of their release the main task. In order to overcome these difficulties, different formulations are tested. Very recently it was investigated how the polymeric non-ionic surfactants change the enzymatic degradation.¹³ Under intravenous conditions, the lipolysis rate can be altered by changing the mixture composition of different surfactants.

The interest of the present study was to obtain low viscosity formulations at ambient temperature that undergo a transition to gel under physiological conditions. In-situ gelation was investigated through rheological measurements. The results suggest that microstructure and elastic properties of polymer mixtures in water as well as the hydrogel stability can be tuned by varying the system composition and by changing temperature.

RESULTS AND DISCUSSION

Fig. 1 illustrates the sol-gel transition of an aqueous solution containing 0.1% xanthan (X) and 12% Pluronic F127 (PI). At low temperatures, the system presents a liquid-like behaviour: loss modulus (G'') is much higher than storage modulus (G') and the loss tangent ($\tan \delta$) is high. G' , G'' and $\tan \delta$ change abruptly around the transition point. The sharp discontinuity is associated with a very fast response of the macromolecular chains at the thermal stimulus. In a narrow range of temperature, G' increases faster than G'' , its value increases with more than 5 order of magnitude. For G'' the increase is smaller, only 3 order of magnitude in the same temperature interval, when the loss tangent shows a minimum value. A first crossover point, for which $G' = G''$ and $\tan \delta = 1$, is observed at 30 °C and the network formation evolves with increasing the temperature, the minimum G''/G' ratio being around 0.1.

The sol-transition process is influenced by the system composition, as it is shown in Fig. 2. Dilute aqueous solutions of xanthan show continuous decrease of the complex viscosity (η^*) as a function of temperature, whereas concentrated Pluronic F127 solutions undergo a sol-gel transition above 20°C. In the presence of small amount of xanthan, the gelation point is shifted to higher temperatures and the elasticity is improved. Thus, the transition point is located near the body temperature and G' increases with one order of magnitude by adding only 0.2% xanthan into 13% Pluronic F127 in water.

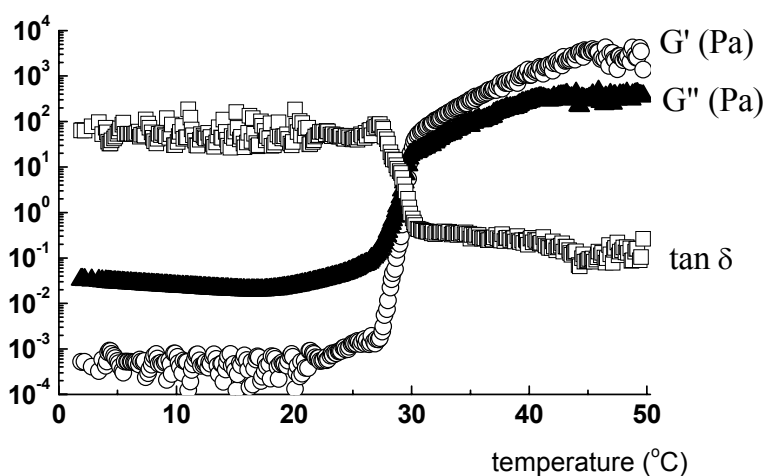


Fig. 1 – Evolution of the viscoelastic parameters during the temperature induced gelation for a system containing 0.1% X/12% PI (heating rate of 0.5 °C/min).

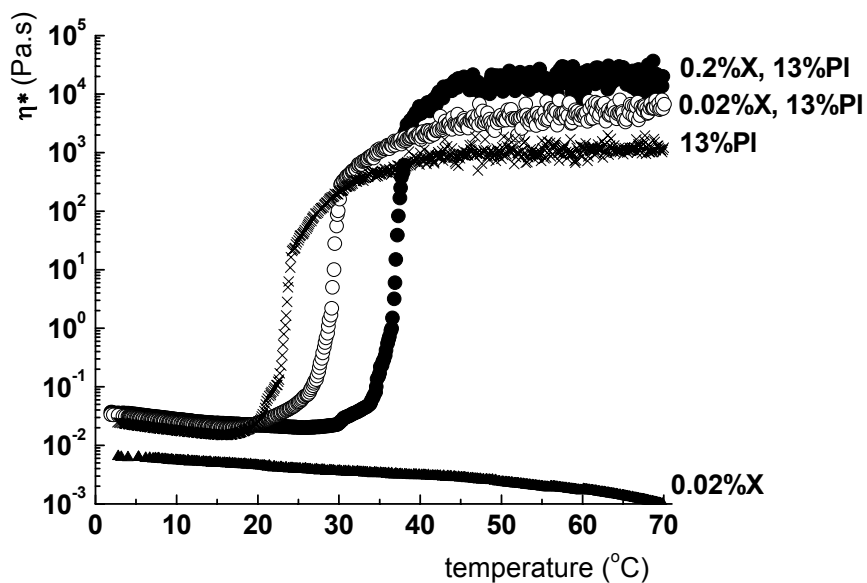


Fig. 2 – The dependence of the complex viscosity on temperature for 0.02% X in comparison with xanthan/Pluronic aqueous systems at indicated compositions (heating rate of 0.5°C/min, 1 rad/s, 1 Pa).

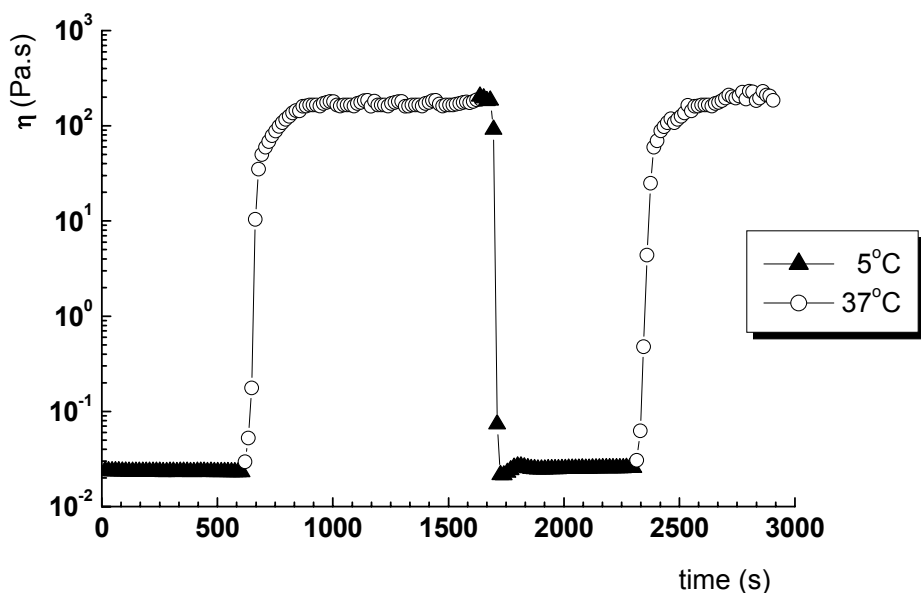


Fig. 3 – Viscosity of 13% PI at successive temperature changes (5 °C – 37 °C – 5 °C – 37 °C).

The high solubilizing capacity and non-toxic properties of Pluronic F127 make it suitable for biomedical applications. PI is more soluble in cold water than hot water. The solubility at low temperatures was attributed to excessive hydrogen bonding between water molecule and etheral oxygen of the polymer. The concentrated solutions are transformed from low viscosity solutions at 5 °C to solid gels by heating to body temperatures. The temperature-dependent gelling process is micellar in nature, being constructed from cubic orientation of micellar subunits. The micellar mode of association in a thermoreversible process makes

it useful as drug delivery systems for ophthalmic, parenteral, rectal and percutaneous use.^{8,9} An illustration of the thermoreversible behaviour of Pluronic aqueous systems is given in Fig. 3. One can see that the answer of the copolymer to thermal stimulus is very fast, the gel-sol and sol-gel transitions occur rapidly. In acidic medium, xanthan chains are always in double helix conformation, whereas in alkaline medium, xanthan chains exhibit partially conformation transition to coils which is favorable at temperatures superior to room temperature.¹⁴

The presence of small amount of xanthan changes the viscosity profile at successive changes of the temperature (Fig. 4). By increasing the temperature to 37 °C one observe the fast response of PI during the first seconds. The network formation evolves the next 1000 s, the delayed answer being attributed to long macromolecules of xanthan. A further decrease of the temperature to 5 °C is not accompanied by gel-sol transition, the network structure is maintained by the xanthan chains which keep together the Pluronic micelles. Further increases of the temperature strengthen the physical network

The gel kept at 37 °C for 5 hours shows constant viscoelastic parameters in frequency sweep experiments (Fig. 5). The storage modulus exceeds the loss modulus and the loss tangent is lower than unity.

At 37 °C, Pluronic/xanthan hydrogels present high yield stress (Fig. 6). The yield stress (σ_0) is defined as the stress above which the material flows as a viscous fluid. It can be determined by following the shear viscosity as a function of shear stress (σ).

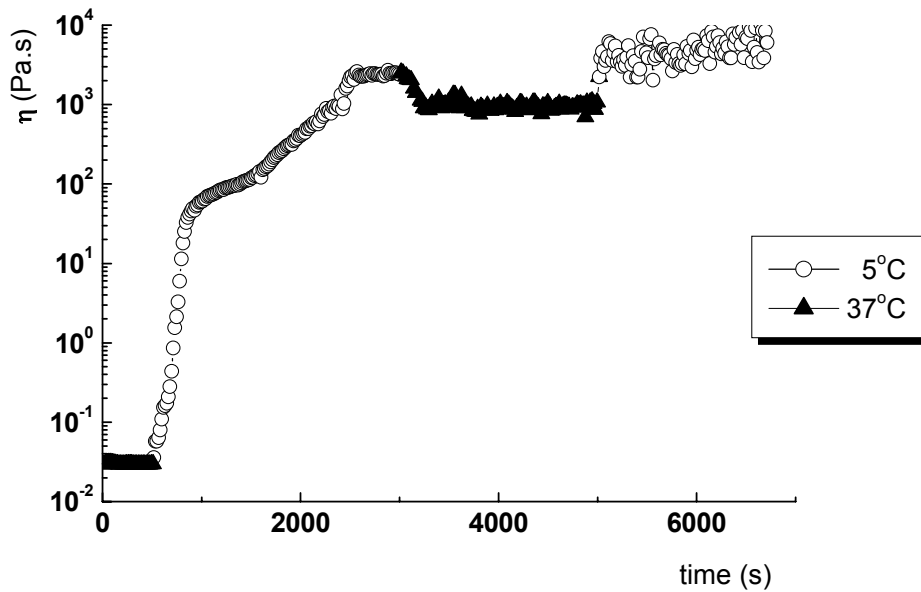


Fig. 4 – Viscosity of 13% PI in presence of 0.05% X at successive temperature changes (5 °C – 37 °C – 5 °C – 37 °C).

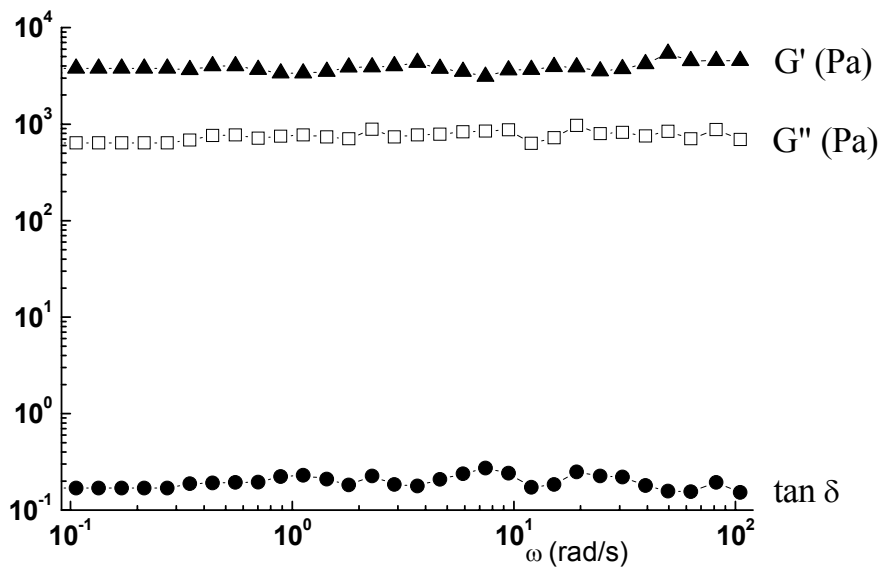


Fig. 5 – The evolution of the viscoelastic parameters in frequency sweep test carried out at 37 °C for a system containing 0.1% X and 12% PI.

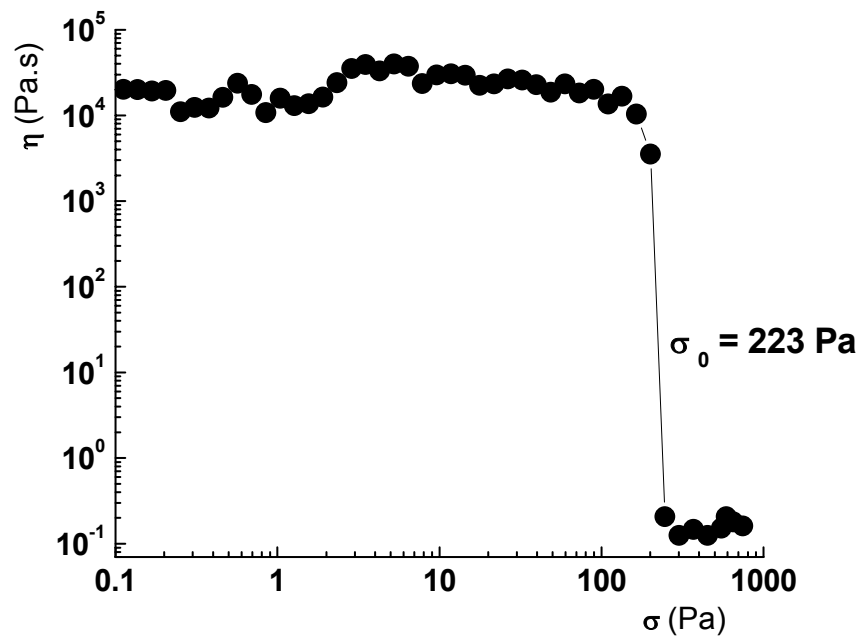


Fig. 6 – The yield stress for a system containing 0.3% X and 15% Pl (37 °C).

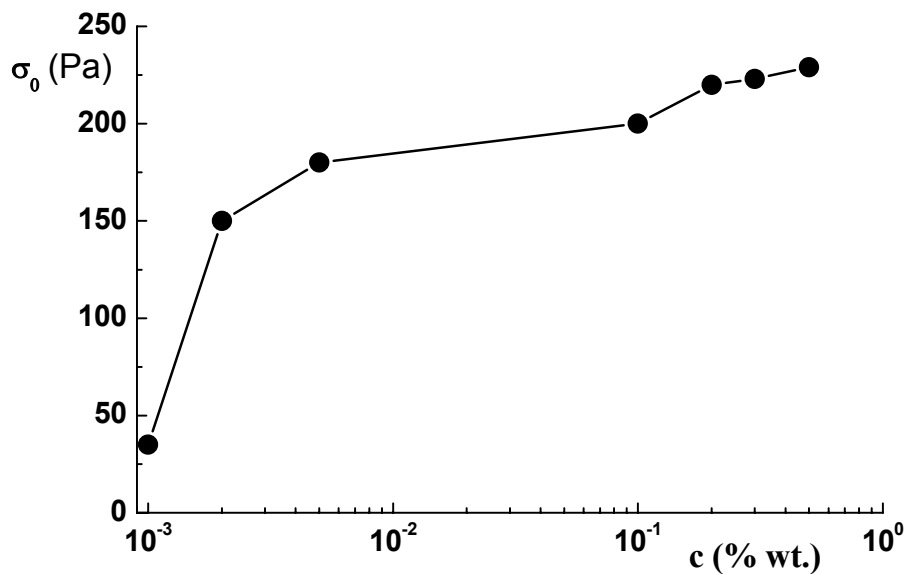


Fig. 7 – The yield stress as a function of xanthan concentration (c) in X/Pl/water mixtures (37 °C).

Pluronic gels present a yield stress of maximum 12.57 Pa (for 20 % copolymer concentration¹⁰). By adding xanthan, the yield stress increases with increasing the xanthan content (Fig. 7).

Based on the present investigations, Fig. 8 gives a suggestive picture of Pluronic F127 gels, dilute solutions of xanthan and xanthan/Pluronic hydrogels at 37°C. Pluronic gel is composed by micelle subunits, where the core is formed by PPO

hydrophobic blocks and the shell is formed by hydrophilic PEO chains. At this temperature, long xanthan macromolecules adopt a coil conformation. In xanthan/Pluronic F127 hydrogel, the long chains of xanthan penetrate between different copolymer micelles and due to the interactions with PEO corona assure a higher elasticity to the resulting gel which is stable at further temperature changes.

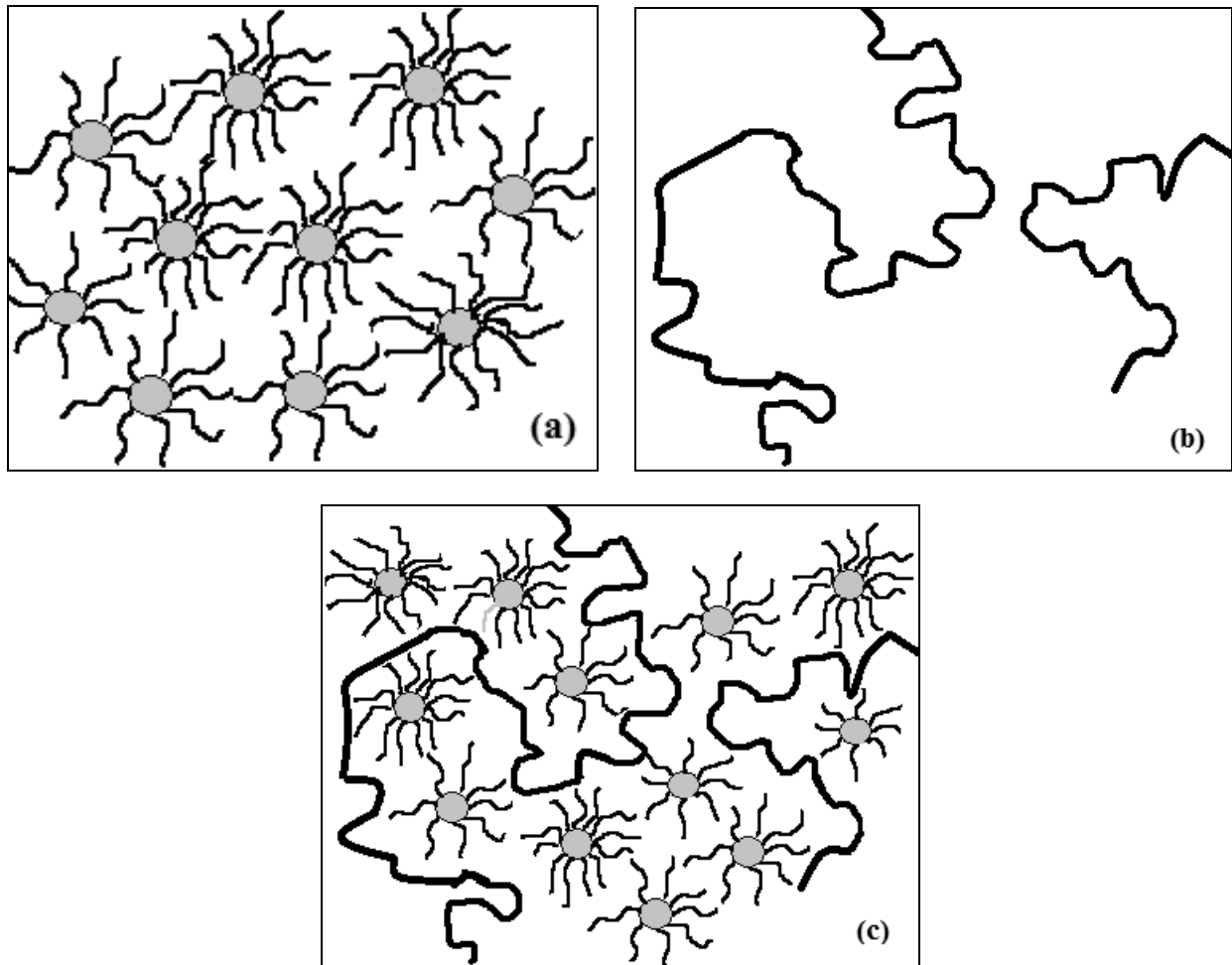


Fig. 8 – Schematic presentation of (a) Pluronic F127 hydrogel, (b) xanthan dilute aqueous solution and (c) xanthan/Pluronic F127 hydrogel, at 37 °C.

EXPERIMENTAL

Materials

Commercial polymer samples of Pluronic F127 (PI) and xanthan (X) were used as received. Pluronic F127 ($M_w = 1.26 \times 10^4$ g/mol) was purchased from BASF. Xanthan sample ($M_w = 2 \times 10^6$ g/mol) was purchased from Fluka. As solvent it was used deionized water treated with Millipore-Q water purification system. Pluronic F127 was dissolved in cold Millipore water under gentle stirring at 4 °C in order to obtain a clear solution. After dissolution, each Pluronic containing solution was equilibrated overnight at 4 °C. The solution of xanthan was prepared one day before the experimental measurements by gentle stirring at room temperature. Each polymer mixture system of a given composition was prepared before testing by mixing 60 minutes at 200 rpm.

Methods

Dynamic oscillatory investigations were carried out with a shear stress controlled Bohlin CVO rheometer equipped with a Peltier device for temperature control. The measurements were performed by using parallel plate geometry, the upper plate having a radius of 30 mm (gap of 500 μ m). The behaviour of the samples was followed in temperature sweep

tests at temperatures between 0 °C and 80 °C and the characteristics of the gels were determined at 37 °C. In order to prevent the water evaporation, an anti-evaporation device was used.

In the oscillatory tests, a sinusoidal stress (or strain) was applied to the upper plate of the rheometer and along with the resulting strain (or stress) lead to the components of the complex modulus, G^* , which are in phase with the input (elastic response) and $\pi/2$ out of phase with the input (viscous response):

$$G^* = G' + iG'' \quad (1)$$

where G' (storage modulus) and G'' (loss modulus) give information on energy storage and energy dissipation during the flow, respectively. For a perfectly elastic solid, $G'' = 0$ and $G^* = G'$. For a Newtonian fluid, $G' = 0$ and $\eta^* = G''/\omega$, where η^* is the complex viscosity and ω represents the angular frequency of oscillation.

The loss tangent, $\tan \delta$, is a measure of the ratio of lost to stored energy in a cyclic deformation:

$$\tan \delta = \frac{G''}{G'} \quad (2)$$

A low value in $\tan \delta$ indicates a high degree of elasticity (more solid-like). For dilute solutions $\tan \delta$ is very high

because both solvent and polymer contribute to G'' but only the polymer contributes to the G' value.

The oscillatory shear measurements were carried out within the linear viscoelastic regime for each sample, under shear stress for which the storage and loss moduli as well as the loss tangent are independent of the shear stress. According to Fig. 9, which shows for example an amplitude sweep test for the sample having 0.1% X and 12% Pl, the linear viscoelasticity is ensured above 0.2 Pa, in a large domain of shear stress, such a behaviour being characteristic to structured samples.¹⁵⁻¹⁷ For the present study, a shear stress of 1 Pa was

selected. The temperature sweep tests at different heating rates and constant angular frequency ($\omega = 1$ rad/s) in the linear viscoelastic regime (1 Pa) were used to determine the evolution of the viscoelastic characteristic functions during the gelation process. The viscoelastic parameters in conditions close to physiological ones were determined in time sweep tests at 37 °C and at constant angular frequency (1 rad/s) and shear stress (1 Pa). In addition, the thermoreversible behaviour was investigated by successive cycles of temperature (5 °C – 37 °C – 5 °C – 37 °C).

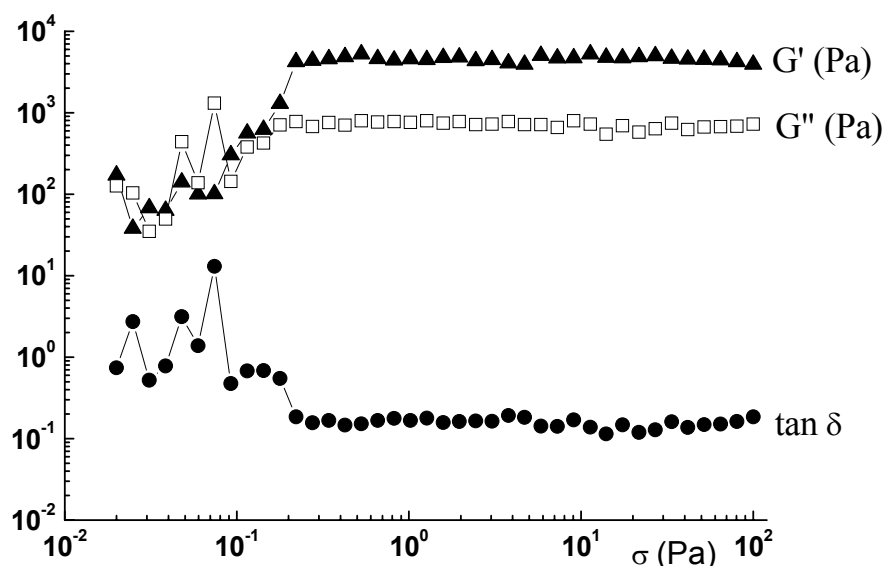


Fig. 9 – The evolution of the viscoelastic parameters in amplitude sweep test carried out at 37 °C for an aqueous system containing 0.1% X and 12% Pl.

CONCLUSIONS

Polymer matrices able to change inside the body into supramolecular gels represent an attractive approach, especially when there is a minimally invasive and patient friendly administration. Different formulations based on Pluronic F127 in combination with xanthan were modulated in order to have the gelation temperature around 37 °C to ensure the gelation at physiological temperature after the administration. The observations concerning the gel formation resulted from the evolution of the viscoelastic parameters as a function of temperature and shear conditions. In the presence of long xanthan chains, the Pluronic F127 gels lose their thermoreversibility, the elastic properties are improved and the yield stress increases considerably.

The synergistic interaction between xanthan and Pluronic F127 favours the formation of non-thermoreversible gels due to the intermolecular binding between xanthan and copolymers which are not sensitive to temperature changes. Thus, the natural and synthetic polymers can be used in

mixture in order to obtain biomaterials which combine advantageously chemical, physical and biological properties of the individual components.

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