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Dedicated to Professor Valer Farcasan
on the occasion of his 95th anniversary

IN-SITU GELLING SYSTEM BASED ON PLURONIC F127 AND POLY(VINYL ALCOHOL) FOR SMART BIOMATERIALS

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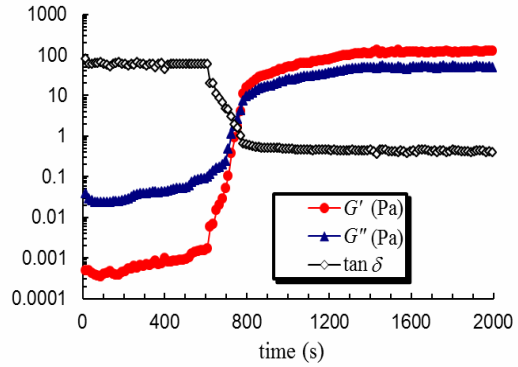
The paper is focused on the investigation of thermal induced gelation of Pluronic F127 and poly(vinyl alcohol) mixtures in aqueous solutions. *In-situ* thermal induced gelation process was investigated through rheological measurements, evidencing the optimum conditions for which the sol-gel transition occurs near the physiological temperature. The gelation in the presence of a globular protein (bovine serum albumin) was followed. The particle size, zeta potential and conductivity at different temperatures were also investigated and discussed. It was observed that the addition of bovine serum albumin determines a delay of gelation, an increase of the conductivity and absolute value of zeta potential.



INTRODUCTION

Stimuli responsive polymers which are able to form reversible networks by physical interactions present a high scientific interest because of their potential uses in biomedical and pharmaceutical applications, especially for controlled drug-delivery systems, cell encapsulation, tissue repair, bioseparation, etc. The drugs are easily *in vitro* mixed with the aqueous polymer solutions and, after *in vivo* administration, the solutions are *in-situ* transformed into hydrogels. Thus, these materials present the advantage of simple drug formulation and administration procedures, no organic solvent or other toxic components are involved, a high site-specificity, a sustained drug release behaviour, and ability to deliver both hydrophilic and hydrophobic drugs, etc.[1] Different strategies were developed by using the concept of physical or chemical interactions in both natural and synthetic polymer systems in order to design targeted biomedical formulations.[2-6]

One of the most popular polymer used for biomaterials is poly(vinyl alcohol) (PVA). The hydrophilic character controlled by the degree of hydrolysis recommends PVA as a thickening and wetting agent. The chemical gels were used as prostheses for a variety of plastic surgery (including breast augmentation), diaphragm and bone replacement. The main disadvantage observed after long term use was the calcification of the PVA chains.[7] Some efforts were devoted to

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prepare physical PVA hydrogels by repeated freezing/thawing cycles,[8] when the formation of a semicrystalline dense structure ensures the stability of the network structure. The lack of biodegradability for long PVA chains determines the researchers to find out other solutions to use this polymer in preparing different biomaterials.

Another polymer extensive investigated during the last decade was Pluronic F127 (PL), a linear triblock copolymer consisting of polyoxyethylene units (PEO) and polyoxypropylene blocks (PPO) (PEO_{99}–*b*–PPO_{69}–*b*–PEO_{99}). This polymer was used, for example, for preparing gels in protein delivery and skin burn treatments. Inside the body this gel slowly dissolves and the polymer can be removed and its use for some biomedical applications was considered improper.[7] Different methods were proposed to overcome this inconvenient;[9] some of them involve chemical reactions, but in many cases physical gelation is preferred.[3-8] In some recent papers,[10-12] gels with improved mechanical properties and good stability were obtained from mixtures of Pluronic F127 with natural and synthetic polymers. These polymer mixtures in aqueous solution prepared at low temperatures (5 °C – 10 °C) have the ability to undergo a sol-gel transition when a thermal stimulus is applied. The formulations with low enough viscosity in sol state become interesting for injectable biomaterials because they can be easily introduced into the human body and under physiological conditions the gel formation occurs.

In the present paper, aqueous mixtures of Pluronic F127 and PVA (15% polymer concentration in the system) are investigated in order to obtain temperature responsive hydrogels with the gelation point near the body temperature. Bovine serum albumin was used as a model protein and its influence on the gelation was investigated.

RESULTS AND DISCUSSION

Sol/gel transition through rheological measurements

Fig. 1 shows the evolution of the shear viscosity, η, as a function of the temperature for different heating rates for a sample of PL/PVA mixture with the PVA weight fraction in the polymer mixture (w_2) of 0.75, submitted to a constant shear rate of 0.3 s^{-1}. In the present paper, when we refer to PL/PVA binary mixture, the index 1 corresponds to PL, whereas the index 2 corresponds to PVA. At low temperatures (below 19 °C) the viscosity is very small (between 0.15 Pa·s and 0.3 Pa·s) and the system presents a liquid-like behaviour, $G' < G''$ (as shown in Fig. 2). The heating rate influences the evolution of the rheological parameters, thus above 19 °C a sharp increase is observed for very low change in temperature. At higher temperatures, G' becomes higher than G'' and the shear viscosity and dynamic moduli tend to constant values. The temperature increase determines a sol-gel transition and the transition point is influenced by the heating rate. As for example, for 0.3 °C/min the sol-gel transition occurs around the physiological temperature. The gelation takes place in a larger temperature range as the heating rate is increased. For a given composition of the polymer mixture, the elastic modulus reaches a constant plateau at high temperatures, showing that the network structure is independent on the thermal history.[12] In continuous shear conditions, in the investigated temperature range, the viscosity tends to reach a constant value, but the heating rate influences the viscosity value above 19 °C (Fig. 1), i.e., lower heating rates give higher viscosities.

Due to the formation of a network structure at 37 °C, the Cox-Merz rule is not obeyed (Fig. 3). In continuous shear, the value of Newtonian viscosity was determined as η_o = 970 Pa·s. For the pseudoplastic region, the following dependences were observed: $\eta \sim \dot{\gamma}^{-0.81}$ and $\eta^* \sim \omega^{-0.82}$.

The sol-gel transition of PL/PVA mixtures was investigated when the sample is heated at the body temperature (37 °C). Firstly, the samples were introduced into the geometry of the rheometer at 4 °C, heated at 37 °C and the evolution of the viscoelastic parameters was then followed at constant oscillation frequency (1 rad/s) and shear stress (1 Pa). As for example, Fig. 4 presents the variation of G', G'' and $\tan\delta$ for the sample with w_2 = 0.75 during gelation at 37 °C. It can be noticed that the gelation occurs very fast ($G' = G''$ after approx. 80 s, as can be better seen in the inset of Fig. 4) and the stationary state is reached after about 1,200 s.

In the presence of BSA, the stationary state is also observed after 1,200 s, but the gelation is delayed, the sol-gel transition occurs in approx. 800 s (Fig. 5). This behaviour could be due to the hydrophobic interactions between PPO and BSA which weaken the PPO-PPO interactions and prevent the block copolymer micelle formation.

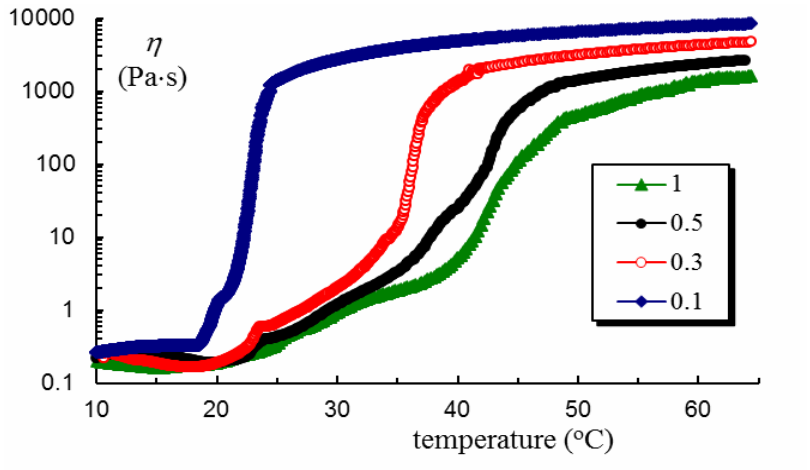

Fig. 1 – Shear viscosity as a function of temperature at different heating rates (°C/min) for PL/PVA mixture with w_2 = 0.75.

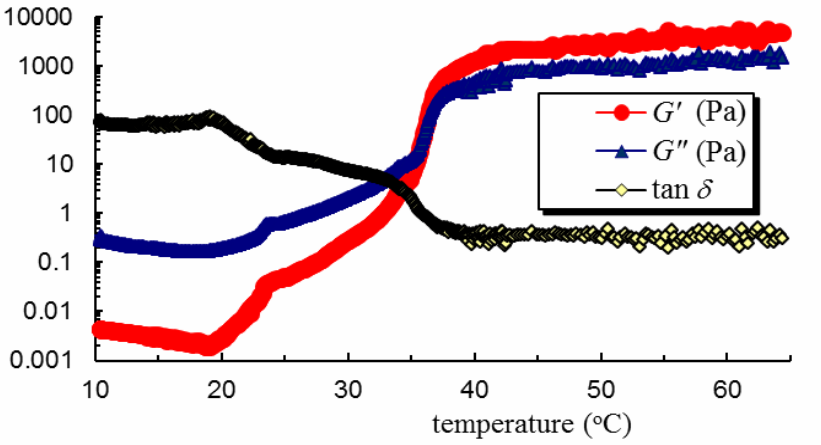

Fig. 2 – Evolution of the viscoelastic parameters during a temperature sweep test with a heating rate of 0.5 °C/min at 1 rad/s and 1Pa for PL/PVA mixture with w_2 = 0.75.

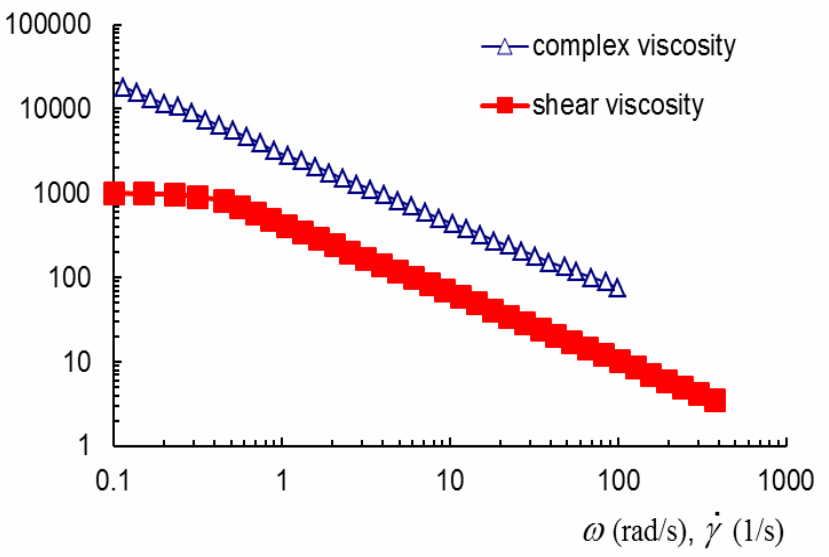

Fig. 3 – Flow curves obtained in continuous and oscillatory shear experiments near the sol-gel transition point at 37 °C (w_2 = 0.75, 1Pa).

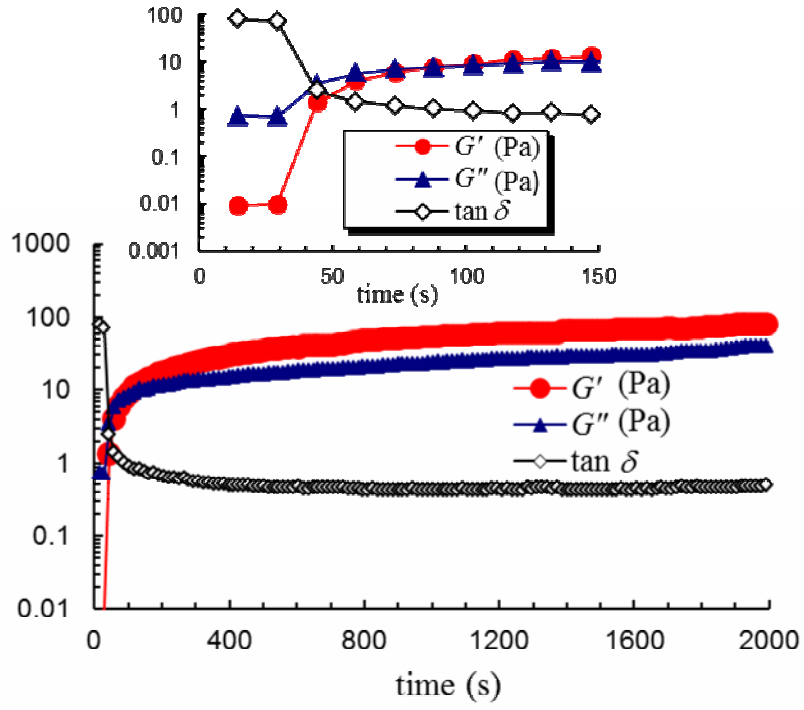

Fig. 4 – Gelation at 37 °C for PL/PVA mixture (w_2 = 0.75, 1 rad/s, 1 Pa).
The inset shows the variation of the viscoelastic parameters during the first 150 s.

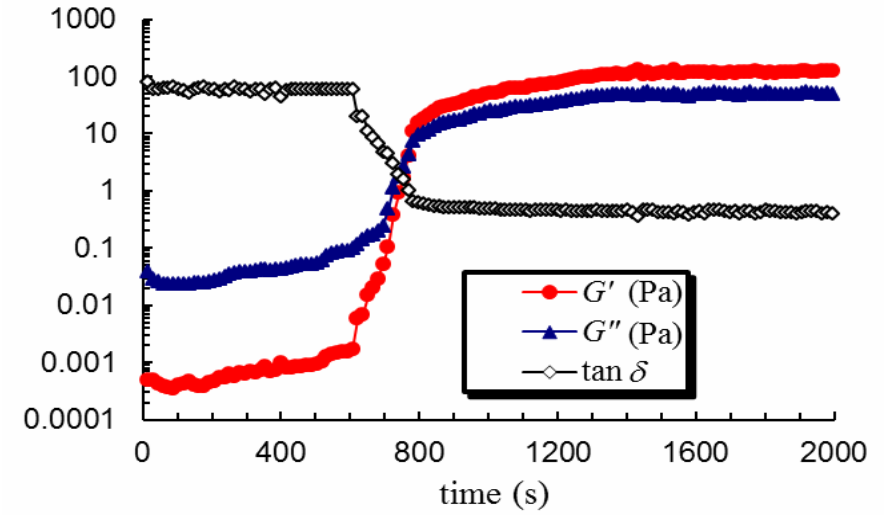

Fig. 5 – Gelation of PL/PVA mixture (w_2 = 0.75) in the presence of BSA (the weight ratio BSA/polymer = 1/1), at 37 °C.

Above a certain concentration, aqueous solutions of Pluronic F127 undergo a fully reversible sol-gel transition as a function of temperature. Guo *et al.*[13] investigated the micellization of PEO-PPO-PEO block copolymers with different PPO content in the presence of BSA by fluorescence spectroscopy and they observed that the addition of BSA into Pluronics/water systems shifts the critical micellization temperature toward to higher temperature and alters the self-assembly in aqueous medium. The increase of the PPO content into Pluronics or BSA concentration will impede the micelle formation.[13]

According to Winter and Mours,[14] at the gelation point $G'(\omega) \sim G''(\omega) \sim \omega^n$ or $G''(\omega)/G'(\omega) = \tan\delta = \tan(n\pi/2)$. The values of the relaxation exponent n are from 0 and 1 and at the transition point the shear viscosity diverges. The case of n = 0 corresponds to the limiting behaviour of a Hookean solid when the relaxation modulus is constant.

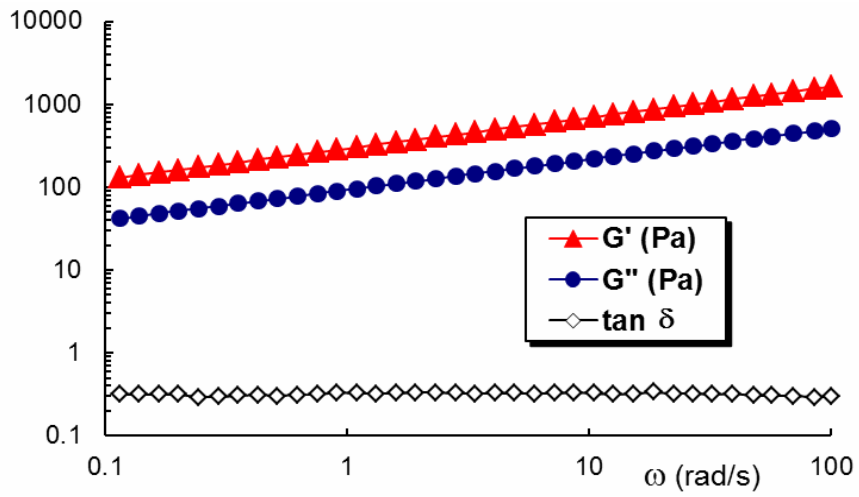

Fig. 6 – The viscoelastic parameters in frequency sweep experiments near the gelation point at 37 °C after the addition of BSA to PL/PVA mixture with w_2 = 0.75. The experimental data give the following dependences: $G' \sim \omega^{0.372}$ and $G'' \sim \omega^{0.368}$.

In order to evaluate the gelation time, the scaling exponent n was experimentally determined for PL/PVA mixtures. Thus, the dependence of the viscoelastic parameters in frequency sweep tests was followed at constant temperature and different aging times. Fig. 6 shows the dependence of G', G'' and $\tan\delta$ around the transition point at 37 °C for PL/PL mixture in the presence of PVA, from which a value of 0.37 was determined for n. The network structure at the gel point can be represented by the fractal dimension (FD), which is defined by $R^{FD} \sim M_c$, where R is the radii of gyration and M_c is the molecular weight of a cluster. FD can be calculated as $(10n - 15)/(2n - 6)$ and, according to Fig. 6, FD = 2.15. The values of parameters FD and n are in agreement with other values reported for physical gels.[15-17] The relaxation exponent, n, near the gelation point is relatively low as compared with chemical crosslinked systems,[18] indicating that the system is highly entangled. The value of fractal dimension suggests that the physical network in this system is very dense although the gelation occurred without crosslinkers.

Generally, the gelation occurs as a result of three-dimensional network formed between the components. In case of PL/PVA system, the physical bonds are generated between the existing functional groups of the two polymers. The BSA presence, with more functional groups, increases the possibility of establishing different types of interactions. The formation of BSA/PVA complex under physiological conditions was evidenced by Wang *et al.*[19] by using different methods, such as: fluorescence spectrophotometry, differential scanning calorimetry or Fourier transform infrared spectrophotometry. The intermolecular interactions between PVA and BSA determine a reduction of intramolecular interactions of BSA or PVA. It was also shown that the enthalpy and entropy of micellization for Pluronics in water decrease by adding BSA due to the hydrophobic interactions, leading to a delay of the micellization process in aqueous solutions.[13] Therefore, there is a balance between different types of interactions (influenced as well by the environment conditions) which determine the formation of the most stable physical bonds for the network with a direct consequence on the gelation time.

Particle size, zeta potential and conductivity

Laser diffraction and scattering techniques are non-invasive methods with high reproducibility and flexibility for measuring the particles size, typically in the submicron region.[20-22] In the present study, the dynamic light scattering measurements were carried out in order to evidence the gel formation in correlation with rheological measurements.

Fig. 7 illustrates the results obtained for PL/PVA mixture with w_2 = 0.75 in the presence or absence of BSA as compared with PL and PVA solutions at 25 °C and 37 °C. In the case of polymer mixture solution, the unique feature of the system is that it displays a large dimensional change within the supramolecular structure appearance and the competition between hydrophobic-hydrophilic interactions and the thermal motion influences its behaviour. The differences between the molecular weights of PL and PVA explain also the difference in size for w_2 = 0 and w_2 = 1.

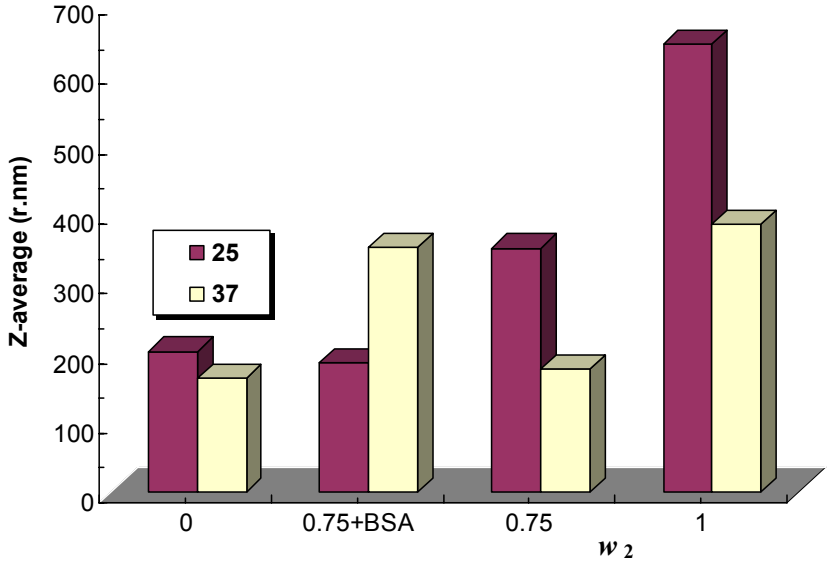

Fig. 7 – The particles size registered at 25 °C and 37 °C for systems with different PVA weight fractions in PL/PVA mixtures.

The presence of BSA determines an increase in particle size as temperature is raised from 25 °C to 37 °C. With increasing the temperature, due to the thermal motion and the conformational transitions, the intermolecular interactions between BSA and the two types of polymer chains can occur and the gelation process starts. This phenomenon explains as well the increase of size of the polymers/BSA mixtures at 37 °C.

Temperature influences significantly the BSA conformation and, as a consequence, its feature and characteristics, as it was recently shown in the literature.[23] In neutral aqueous solutions, below 20 °C, BSA only exists in a single conformation state A. As the temperature increases above 20 °C, two different conformation states of BSA coexist: A and B, and around 50 °C the state B accounts approx. 25% of all BSA conformations. The conformation change from A state (more extended) to B one (more compact) is endothermic, nonspontaneous and mainly entropy-driven.[23] In addition, it was observed that the adsorption of BSA to hydrophobic stationary phases could lead to its conformational change.[24]

Zeta potential value is slightly influenced by the polymer composition and temperature (Fig. 8) and a minimum was registered for w_2 = 0.75. An interesting feature is observed for w_2 = 0.50 when zeta potential value is closed to zero. This behaviour suggests the formation of a supramolecular structure with the functional group distributed inside of the formed structure.

For PVA solution, the conductivity is nearly constant in the temperature range 3 °C to 40 °C (Fig. 9). By increasing the PL content into the mixture, the conductivity increases and the temperature influence becomes important. The BSA addition determines an increase of the absolute value of ζ (Fig. 8) and of conductivity (Fig. 9) due to the system enrichment in the functional groups.

EXPERIMENTAL

Sample preparation

Among the Pluronic series, Pluronic F127 (PL) has the lowest gelation concentration and the simplest phase behaviour. A sample with M_w = 12.6 kg/mol was purchased from BASF and used without any further purification.

The poly(vinyl alcohol) (PVA) sample 98-99% hydrolyzed was purchased from LOBA Feinchemie AG (Austria Chemical Companies) and used as received. The molecular weight of 51 kg/mol was determined by viscometry in water at 30 °C with the intrinsic viscosity – molecular weight relationship previously established.[25]

The de-ionized water used was treated with Milipore-Q water purification system. Concentration of polymer is expressed in weight percentage (% w/w).

Bovine serum albumin (BSA) was purchased from Sigma-Aldrich and is composed of 583 amino acid residues; it is a single, nonglycosylated protein and its molecular mass is about 6.65×10^4 g/mol.[26]

Homogeneous solution of 15% PVA was obtained by dissolving the polymer in Milipore water at 200 rpm and 90 °C for 1 h. In the same time, Pluronic F127 was dissolved in cold Milipore water under gentle magnetic stirring at 4 °C in order to obtain a clear solution of 15%. After dissolution, both solutions were equilibrated overnight at 4 °C.

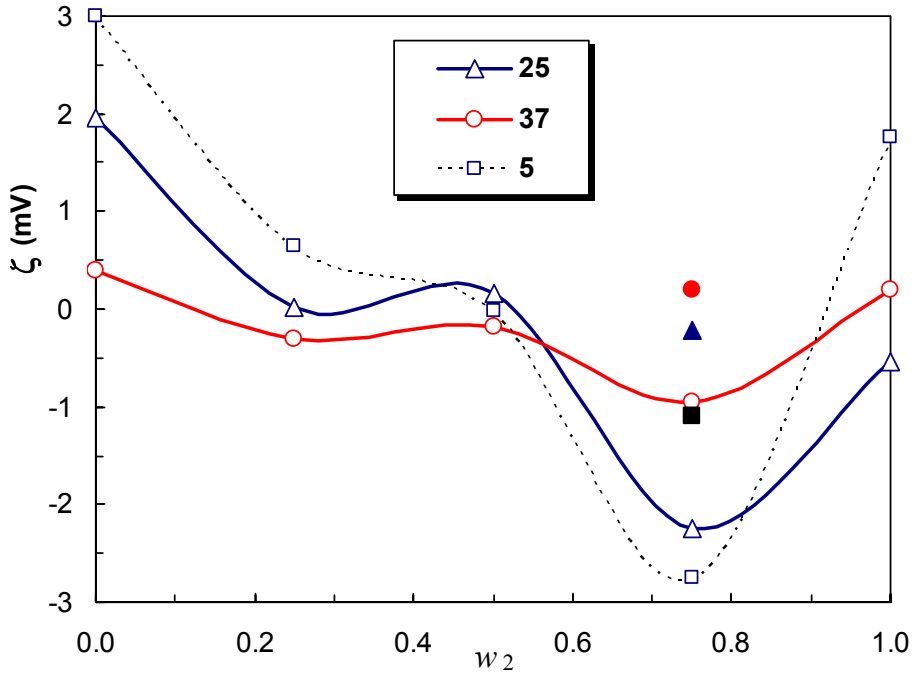

Fig. 8 – Zeta potential as a function of PL/PVA mixture composition, registered at 5 °C, 25 °C and 37 °C. Full symbols represent the values obtained in the presence of BSA.

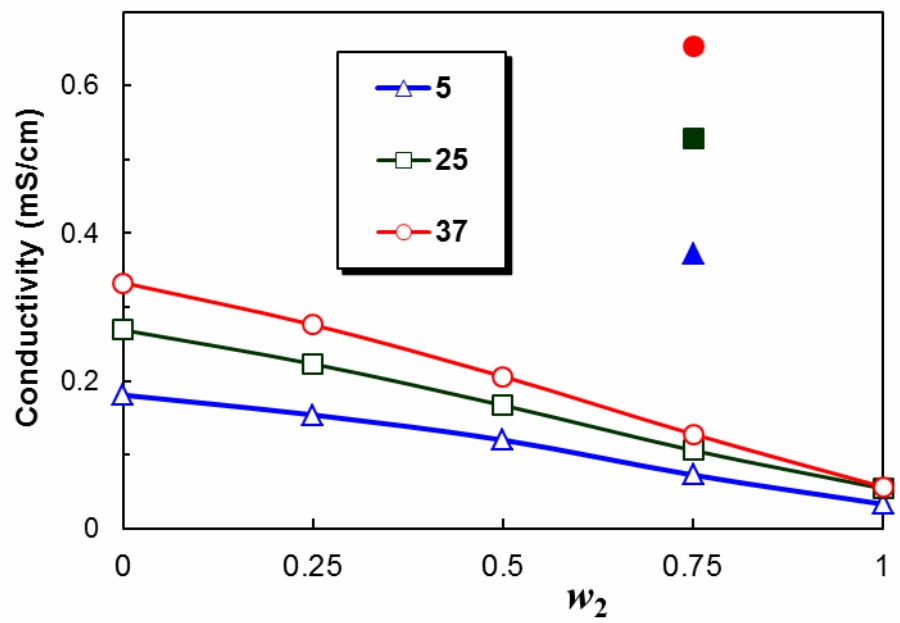

Fig. 9 – The conductivity as a function of composition of PL/PVA mixtures at three indicated temperatures (°C). Full symbols refer to the values of conductivity obtained in the presence of BSA.

Before each rheological test, the PL solution was gradually added to the PVA solution for obtaining a given weight fraction. In order to ensure that the polymers are well mixed and the system becomes homogeneous, the entire solution was mixed at 4 °C for 60 minutes at 200 rpm. Samples of PL/PVA mixtures with w_2 of 0.25, 0.5 and 0.75 were investigated after their preparation. In addition, samples of PVA and Pluronic F127 were stored and studied in the same experimental conditions. The effect of the addition of a globular protein was investigated for the sample having w_2 = 0.75 which was mixed with BSA, the BSA/polymer weight ratio being of 1/1.

Measurements

Solutions of PL, PVA and their mixtures in the above specified ratios were tested in continuous and oscillatory shear

regimes on a Bohlin CVO rheometer equipped with Peltier device for temperature control. The measurements were made by using a parallel-plane geometry with a gap of 500 μm, the upper plate having the radius of 30 mm. For each determination, approx. 2 mL of the homogeneous solution were poured on the lower plate of the rheometer thermostated at 4 °C. Before the oscillatory measurements, the amplitude sweep tests were made at a constant frequency (ω) of 1 rad/s at two temperatures, 10 °C and 37 °C, in order to establish the linear viscoelastic region for polymer solutions and gels. For polymer solutions, linear viscoelastic domain is reached for shear stresses between 0.1 Pa and 50 Pa and it is extended for gels from 0.04 Pa to 110 Pa. The large range of linear viscoelasticity is characteristic to structured samples.[27,28]

The gelation process was followed in temperature sweep experiments (shear stress of 1 Pa and oscillation frequency of 1 rad/s) at different heating rates from 0.1 °C/min to 1 °C/min, the starting temperature being 4 °C and the maximum reached temperature was 65 °C.

The gelation process was also investigated at the physiological temperature (37 °C) by using aqueous solutions prepared at 4 °C and kept at this temperature before the starting of the experimental tests. When the temperature of 37 °C was reached, the dynamic properties were followed as a function of time at a constant shear stress of 1 Pa and oscillation frequency of 1 rad/s.

In the oscillatory deformation tests, the following viscoelastic parameters were determined: the elastic (or storage) modulus, G', as a measure of the reversibly stored deformation energy; the viscous (or loss) modulus, G'', as a measure of the irreversibly dissipated energy during one cycle, and the loss factor, $\tan\delta$, as a measure of the ratio between the lost energy to the stored energy in a cyclic deformation.

The particle size was determined by using a dynamic light scattering technique (Zetasizer model Nano ZS, Malvern Instruments, UK) with red laser 633 nm (He/ Ne). The system uses non-invasive back scatter (NIBS) technology wherein the optics are not in contact with the sample, back scattered light being detected. The use of NIBS technology reduces multiple scattering effects and consequently size distributions in higher concentrations of sample can be measured.[20] The Mie method was applied over the whole measuring range (from 0.6 nm to 6 μm). The apparent hydrodynamic diameter (D_H), often expressed by symbol Z or z-average, was determined according to the following equation:

$$D_H = \frac{kT}{3\pi\eta D} \quad (1)$$

where k - the Boltzmann constant, T – the temperature, η – the viscosity and D – the diffusion coefficient.

Zeta potential, ζ, was calculated according to the Henry and Smoluchowski approximation:

$$\zeta = \frac{\eta\mu}{\varepsilon} \quad (2)$$

where μ represents the electrophoretic mobility determined with the Malvern Zetasizer ZS, and ε is the dielectric constant of the medium.

The electrical conductivity was determined in parallel with zeta potential measurements, on the same apparatus. Each measurement was performed 3 times and the average of the values was considered.

CONCLUSIONS

The present study was carried out in order to design low viscosity formulations at low or ambient temperatures that undergo a transition to a gel state near physiological conditions. The investigated mixtures have shown thermal induced gelation and for specific conditions the transition can occur near the human body temperature. The thermal induced gelation of Pluronic F127, which occurs below 30 °C, can be shifted to higher temperatures by mixing its solution with an entangled PVA solution. The Pluronic micelles are hidden into the PVA entangled network and their packing process becomes slower. Thus, the system can be injected into human body in sol state and the gel formation ensures the release of the active principle from the formulation. By adding BSA, a model globular protein, we noticed that in situ gel-forming was delayed due to the competition between the hydrophobic interactions between PPO and BSA and gelation process. The BSA addition determines an increase of the absolute value of zeta potential and of conductivity due to the system enrichment in the functional groups.

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REFERENCES

1. C. He, S. W. Kim and D. S. Lee, *J. Control Release*, **2008**, *127*, 189-207.
2. I. Neamtu, A. P. Chiriac, L. E. Nita, M. Bercea and A. Stoleriu, *J. Optoelectron. Adv. Mat.,* **2007**, *9,* 981-984.
3. S. Morariu and M. Bercea, *J. Phys. Chem. B,* **2012**, *116*, 48-54.
4. L. M. Gradinaru, C. Constantin, S. Vlad, M. Bercea and M. Popa, *Ind. Eng. Chem. Res.*, **2012**, *51*, 12344-12354.
5. L. M. Gradinaru, C. Constantin, S. Vlad, M. Bercea and M. Popa, *Centr. Eur. J. Chem.*, **2012**, *10*, 1859-1866.
6. E. S. Dragan, *Chem. Eng. J.*, **2014**, *243*, 572-590.
7. A. Atala, D. J. Mooney, J. P. Vacati and R. Langer (Eds.), "Synthetic Biodegradable Polymer Scaffolds", Birkhäuser, Boston, USA, 1997.
8. M. Bercea, S. Morariu and D. Rusu, *Soft Matter*, **2013**, *9*, 1244-1253.
9. E. S. Dragan, *Pure Appl. Chem.*, **2014**, *11*, 1707-1721.
10. M. Bercea, R. N. Darie, L. E. Nita and S. Morariu, *Ind. Eng. Chem. Res.*, **2011**, *50*, 4199-4206.
11. M. Bercea, R. N. Darie and S. Morariu, *Rev. Roum. Chim.*, **2013**, *58*, 189-196.
12. M. Bercea, S. Morariu, L. E. Nita and R. N. Darie, *Polym. Plast. Technol.,* **2014**, *53*, 1354-1361.

13. C. Guo, J. Wang, X. Liang, L. Cheng and H. Liu, *Sci. China Ser. B: Chem.*, **2006**, *49*, 541-549.
14. H. H. Winter and M. Mours, *Adv. Polym. Sci.*, **1997**, *134*, 165-234.
15. J. H. Choi, S. W. Ko, B. K. Kim, J. Blackwell and W. S. Lyoo, *Macromolecules*, **2001**, *34*, 2964-2972.
16. M. Bercea, S. Morariu and C.-E. Brunchi, *Rev. Roum Chim.*, **2008**, *53*, 769-776.
17. M. Bercea, S. Morariu and C.-E. Brunchi, *Int. J. Thermophys*., **2009**, *30*, 1411-1422.
18. L. E. Nita, A. P. Chiriac, M. Bercea and I. Neamtu, *Rheol. Acta*, **2007**, *46*, 595-600.
19. G.-Z. Wang, J.-T. He, M.-Y. Feng and Q. Xia, *Chem. J. Chin. Univ.*, **2009**, *30*, 68-71.
20. A. P. Chiriac, L. E. Nita, I. Neamtu and M. Bercea, *Polymer Testing,* **2009**, *28*, 886-890.
21. L. E. Nita, A. P. Chiriac, M. Bercea and B. A. Wolf, *Coll. Surf. B*, **2013**, *103*, 544-549.
22. L. E. Nita, A. P. Chiriac and M. Bercea, *Coll. Surf. B*, **2014**, *119*, 47-54.
23. L. Bian, D. Wu and W. Hu, *Biomed. Chromatogr.,* **2014**, *28*, 295-301.
24. R. Ueberbacher, A. Rodler, R. Hahn and A. Jungbauer, *J. Chromatogr. A*, **2010**, *1217*, 184-190.
25. I. E. Lamatic, M. Bercea and S. Morariu, *Rev. Roum Chim*., **2009**, *54*, 981-986.
26. R. N. M. Weijers, *Clin. Chem.,* **1977**, *23*, 1361-1362.
27. S. Morariu and M. Bercea, *Rev. Roum Chim*., **2011**, *56*, 545-551.
28. V. Hurduc, M. Bercea, M. Lungu and I. Nor, *J. Macromol. Sci., Part B: Phys*., **2009**, *48*, 379-390.