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PHYSICAL NETWORKS BASED ON GELATIN AND AZO-POLYSILOXANES

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Abstract

Novel systems based on modified azo-polysiloxanes and gelatin with potential biological applications were prepared and characterized. Simultaneous rheological and UV irradiation studies allowed evidencing structural modification inside the gelatin matrix. For all analyzed samples a shift in the temperature corresponding to the physical network destructuration, from 30˚C (corresponding to gelatin) to 40˚C (for the composite) was noticed. The experimental results proved the existence of interactions between gelatin and polysiloxanes intensified after UV irradiation. The increase in the values of G' and G" is a consequence of system restructuration leading to more arranged architectures able to release the included active principle. As a function of the azo-polysiloxane structure, the destructuration temperature of the composite can be tuned in the domain 30- 40°C.

Key words: biopolymers, dynamic moduli, gelatin, nucleobases, rheology

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1. Introduction

Drug delivery systems have been intensively studied in the last period, representing a rapidly growing area of interest for researchers all over the world. The possibility of controlling the time dependent release of the active principle and to target it to the right place represents a challenge accepted by scientists for many years and different classes of materials have been produced, analyzed and tested to be used as drugs carriers (Gupta et al., 2012; Kim et al., 2009; Kost and Langer, 2012; Machín et al., 2012; Malafaya et al., 2007; Oh et al., 2009; Qiu and Park, 2012; Vashist et al., 2012; Wang and Grayson, 2012; Zhang et al., 2013). Considering their low toxicity, biodegradability and chemical stability in the physiological environment, polymers have found multiple applications in this field. A lot of synthetic polymers have been used as drug carriers and among the most frequently studied we can mention polylactide/polyglycolide (Panyam and Labhasetwar, 2003), poly(ε-caprolactone) (Potineni et al., 2003), poly(ester-anhydride) (Pfeifer et al., 2005), polyaspartamides (Caldwell et al., 1997; Machado et al., 1992), poly (amidoamines) (Ferruti et al., 2002), poly(hydroxypropyl-methacrylate) (Hovorka et al, 2006) etc. In addition to synthetic polymers, naturally derived components such as chitosan (Janes et al., 2001), collagen (Lee et al., 2001) and gelatin (Young et al., 2005) are very attractive materials used in drug delivery systems. Due to its biocompatibility, biodegradability without toxic degradation products and high physiological tolerance, gelatin presents multiple advantages as drug carrier. Gelatin is derived

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from collagen contained in animal tissues by hot water extraction following an acid or alkaline pre-treatment and has distinctive sol-gel behavior forming 100% thermo-reversible gels. From more than 25 collagen types, only three can be used to prepare gelatin (collagen type I, II and III) (Schrieber and Gareis, 2007). Function of the preparation method, gelatin is characterized by different physical properties and isoelectric points. The ability of tuning the electric properties of gelatin has contributed to the understanding of the concept of sustained release of proteins from polymer matrices (Young et al., 2005). Because the physical network of gelatin hydrogel is destroyed around 30°C, crosslinking agents are necessary for 3D-system stabilization. There are three important methods to prepare gelatin carriers: gelatin microspheres, gelatin block hydrogels and porous gelatin block hydrogels (Hosaka et al., 2004; Kang et al., 1999; Yamamoto et al., 2000). Two types of crosslinking agents can be used: non-zero length and zero-length (Kuijpers et al., 2000). A major problem arising when chemical crosslinking agents are used is the total elimination of the unreacted products.

Here in we propose a new system based on gelatin and different polysiloxanes containing azo-benzene groups in the side-chains, with potential applications as drug release systems. Due to the presence of azo-polysiloxanes sequences the modification of the diffusion rate inside the gelatin matrix under UV light irradiation is expected. The azo-benzene groups undergo a specific behavior as a consequence of the azo-benzene groups' trans-cis isomerization capacity under UV/VIS irradiation (Moleavin et al., 2011; Rau, 2003). The geometrical modifications of the azo-groups are accomplished by strong alteration of the dipole-moment (in the case of unsubstituted azobenzene from 0.1 D to 3.5 D) that will also influence the diffusion process inside the material.

Two different azo-polymers structures mixed with gelatin were investigated: azo-polysiloxane modified with adenine or thymine and azo-polysiloxanes grafted with poly(dimethyl acrylamide) and poly(butyl acrylate) segments. A rheological method was used to evaluate the composites behavior under UV light irradiation. Following the UV irradiation a shift in the temperature corresponding to the gel network destructuration, from 30°C (corresponding to gelatin) to 40 °C (for gelatin-polymer composite) was evidenced.

2. Experimental

2.1. Materials

Tetrahydrofurane, ethyl acetate, dichloromethane, nucleobases (adenine and thymine), dimethyl sulfoxide, methanol, chloroform, acryloyl chloride, dicyclohexylcarbodiimide, 4-N,N dimethylaminopyrydine and 4-phenylazophenol were purchased from Aldrich, Steinheim, Germany and used without supplementary purification.

The ((chloromethyl) phenylethyl) methyldichlorosilane was purchased from ABCR GmbH & Co. KG, Karlsruhe, Germany and also used without supplementary purification. The azo-methacrylate monomer was obtained through Sterlich esterification of acryloyl chloride with 4-phenylazophenol in the presence of 4-N,N-dimethylaminopyrydine and dicyclohexylcarbodiimide (Neises and Sterlich, 1978). The compound was purified by thin layer chromatography technique on silica gel using a mixture of 20/1 dichloromethane and ethyl acetate as eluent. Type A gelatin (G2500) was acquired from Aldrich, Steinheim, Germany and used as received.

The linear polysiloxane was prepared in agreement with ref. (Kazmierski et al., 2004). A two-step reaction was employed for the modification of azo-polysiloxanes with nucleobases, starting from a polysiloxane containing chlorobenzyl groups in the side-chain (Fig. 1). In the first reaction step, the polysiloxane was modified with sodium salt of 4-hydroxyazobenzene (50–60% substitution degree) and, in the second step, the unreacted chlorobenzyl groups were substituted with adenine (Sample 1) or thymine (Sample 2). The polymers were characterized by ^{1}H-NMR, details concerning polymer synthesis and characterization being previously reported (Hurduc et al., 2007). The ^{1}H-NMR spectra were recorded on a Bruker 400 MHz apparatus. SET-LRP (single electron transfer - living radical polymerization) technique (Fig. 1) was used to obtain the graft azo-polysiloxanes (Rusu, 2012a; 2012b; 2013). In a typical reaction 0.09 g bipyridyl were added to 0.1 g cyclic polysiloxane macroinitiator previously dissolved in 1.5 mL DMSO. The reaction mixture was introduced in a 25 mL flask where monomers (0.1 g of azo-methacrylate (AZOMA), 0.5 g of N,N-dimethyl acrylamide (DAM), 0.5 g of butyl acrylate (BA)), and Cu wire (0.25 g) were added. The system was degassed by five freeze–pump–thaw cycles. The flask was heated at the reaction temperature (80 °C) and the synthesis was performed under nitrogen atmosphere and stirring, for 8 h. At the end of the reaction, the mixture was diluted with $CHCl_3$ and the catalyst was removed by passing the solution through a basic alumina column. The purified solution was then precipitated in diethyl ether, washed three times with the same solvent, and dried under vacuum (Sample 3).

2.2. Methods

The polymer composites were prepared by mixing a 1% azo-polysiloxane solution in THF with a 5 % gelatin solution in water.

The gelatin solution in water was prepared at 90°C in agreement with the literature data (Benton et al., 2009). Following the complete dissolution of the gelatin the solution was cooled at 60 °C and the azo-polysiloxane solution was added drop by drop under intense agitation. After the complete addition of the azo-polysiloxane, the mixture was heated to 66 °C for another 30 minutes to eliminate the THF.

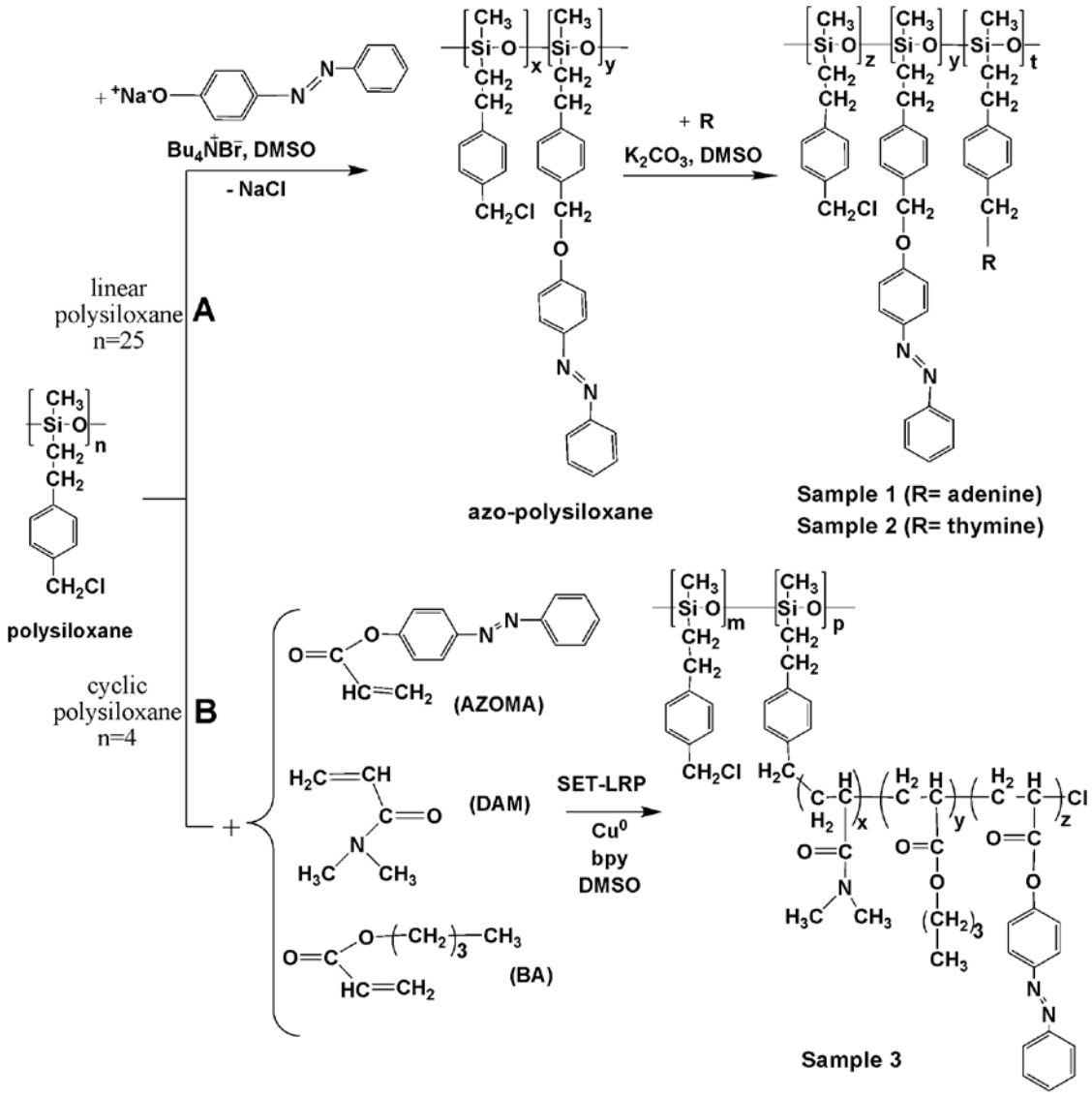

Fig. 1. Synthesis of azo-polysiloxanes modified with nucleobases (A) and of graft polysiloxanes (B)

Afterwards, the mixture was cooled at 35 °C and introduced between the plates of the rheometer measuring system. The rheological properties were investigated using an Anton Paar Physica MCR 501 rheometer equipped with a Peltier system for the temperature control. The temperature can be adjusted between -40 and 200 °C. Measurements were carried out using parallel-plate geometry (Danu et al., 2012). Firstly, a strain sweep was performed to establish the limits of the linear viscoelastic range (LVE) and then temperature tests were carried out at constant oscillation amplitude (γ = 0.5% situated in linear viscoelastic range) and frequency (f = 1Hz). The temperature had been varied between 10 and 100 °C with a heating rate of 0.5 °C /min. Simultaneous UV irradiation and rheological studies could be performed using an advanced UV system, under a Peltier controlled P-PTD-UV chamber on Physica MCR 501 rheometer. As measuring system a 43 mm parallel glass plate fixture was used. The UV source was the Omnicure Series 1000 manufactured by EXFO (Vanier, QC, Canada), using a 100 W lamp able to deliver UV light of 365 nm using a Mercury bulb.

The UV/Vis kinetic studies in THF solution were carried out using a Shimadzu spectrophotometer (Shimadzu UV 1700 Pharma Spy, Kyoto, Japan). The azo-polymer solutions were irradiated using a UV lamp (50 W) equipped with 365 nm filter. The isomerization kinetics of azobenzene can be investigated due to the strong absorption peaking at 350 nm, attributed to the π-π* transition of the azobenzene chromophore. During UV irradiation at 365 nm the absorbance corresponding to the π-π* transition strongly decreases, permitting to calculate the *trans* (respectively *cis*) content of the azo-groups.

3. Results and discussion

The main characteristics of the synthesized modified azo-polysiloxanes are presented in Table 1. The polymers' molecular weights (M_n) were calculated using ^{1}H-NMR spectra.

The chemical structure of the obtained polymers was confirmed by NMR spectroscopy using $CDCl_3$ as a solvent, at room temperature. If we refer to Sample 3 in a typical ^{1}H-NMR spectrum we can identify: the signal corresponding to the methyl groups (CH_3-Si-) from polysiloxane (reference signal for polysiloxane structural unit) at 0.1 ppm, the characteristic signal for the methyl groups (-CH_3) corresponding to the poly (butyl acrylate, BA) segment at 0.9 ppm, the signal for (-CH_2-CH_2-) groups at 1.5 ppm and (-CH_2-O-CO-) signal present at 4 ppm . The signal corresponding to the methyl groups of dimethyl acrylamide (DAM) is present at 2.9 ppm. The signals corresponding to the azo-groups are found in the domain 7.5 - 8.0 ppm.

Firstly, the photochromic behavior of the azo-polymers under UV irradiation was investigated. Fig. 2 presents the kinetic curves of the irradiation process. Evidently, the polymers response in solution is very fast, less than 2 minutes of UV irradiation being necessary to obtain the maximum value of *cis*-group. In these circumstances 5 minutes of UV exposure were selected for the rheological experiments.

It was also noticed that the *trans-cis* conversion rate of the azo-groups is influenced by the substituted molecule. Likely due to the smaller size of thymine comparing with the adenine, the structures of Sample 2 are possibly more compact than Sample 1. The 60% conversion rate of sample containing thymine may be due to the steric hindrance caused by the network density (Resmerita et al., 2010). The evaluation of the rheological properties as a function of temperature evidenced the existence of interactions between gelatin and azo-polysiloxanes, the addition of modified polymers having significant influence on the network formation and stability. A 5% gelatin solution in water was used as reference for the temperature sweep tests. From Fig. 3a the destructuration process of the physical network corresponding to gelatin (5% in water) can be evidenced at 30°C, in agreement with literature data (Benton et al., 2009). For temperatures under 30°C, a stable gel structure is developed with the storage modulus *G'* higher than the loss modulus, *G"*. Increasing temperature, the physical bonds between chains are broken and the system turns liquid with *G'<G"*.

Table 1. Characteristics of the modified azo-polysiloxanes

Spl. no.	***Azo groups content (%)***	***Nucleobases or other monomers Content (%)***	***Mn***	***Azo-units groups/ polymeric chain***	***Nucleobase or monomers-units /polymeric chain[a]***
1	50	15	7,650	12[a]	4
2	50	11	7,530	12[a]	3
3	12	22(DAM+BA)	4,200	3[b]	12DAM + 10BA

[a] Average polymerization degree of the polysiloxanic chains = 25; [b] Average polymerization degree of the polysiloxanic chains (cyclic) = 4; DAM = dimethyl acrylamide; BA = butyl acrylate

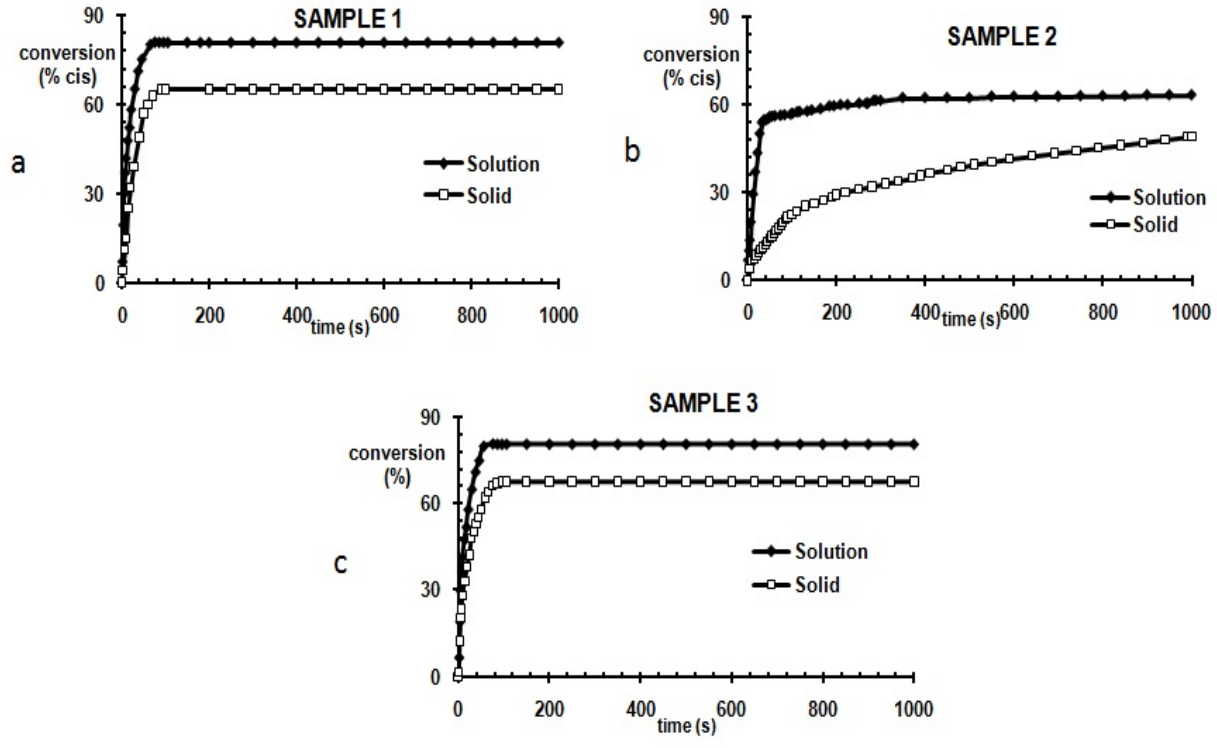

Fig. 2. Polymer response after UV irradiation: (a) Sample 1; (b) Sample 2; (c) Sample 3

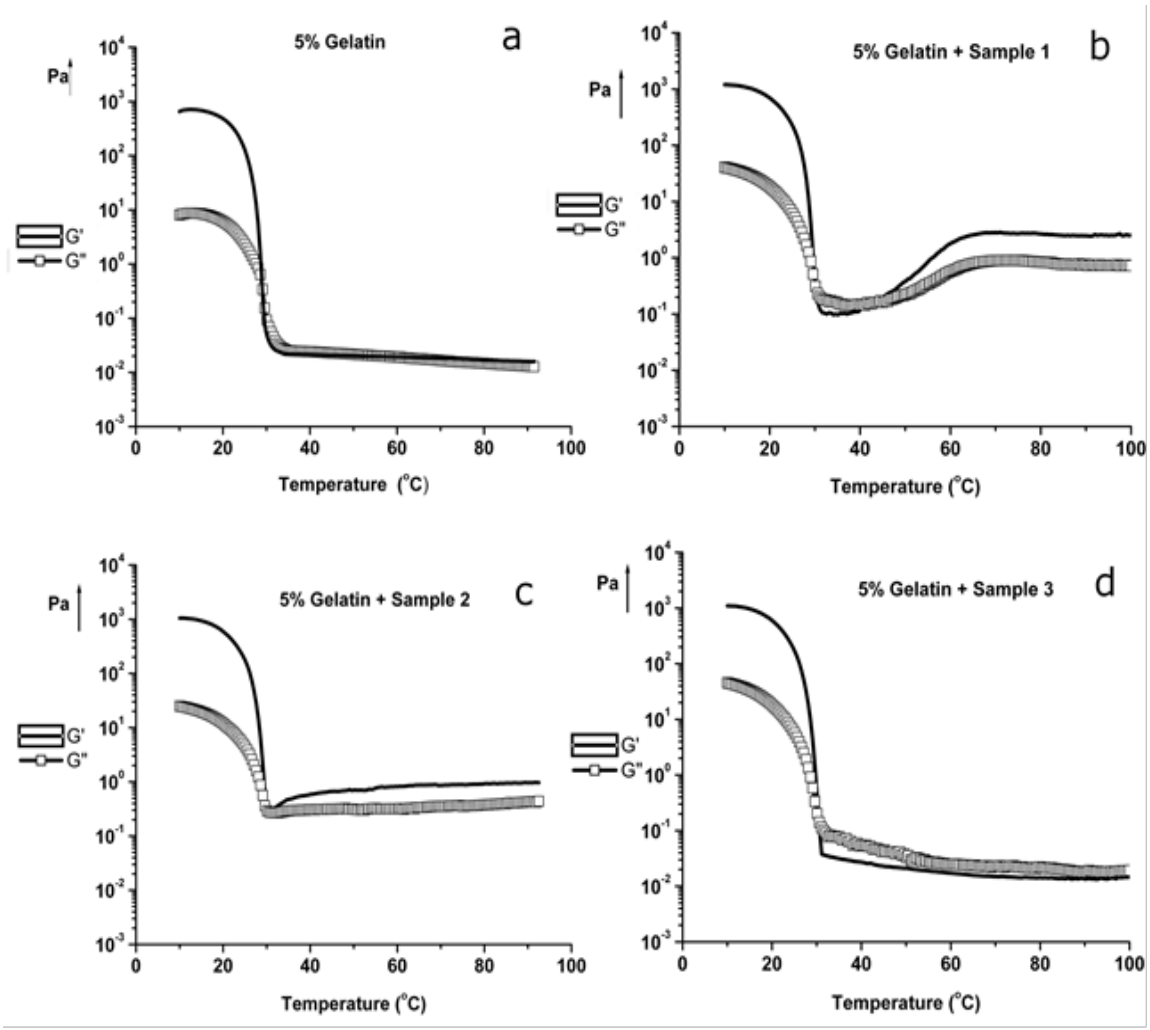

Fig. 3. Temperature sweep for a 5% gelatin solution in water:
(a) and mixtures of gelatin with Sample 1 (b), Sample 2 (c) and Sample 3 (d)

This particular behavior of gelatin networks turning from solid-like to liquid-like behavior by changing temperature can be exploited for the controlled release of active principles, but the characteristic temperature of phase transition is too low. The idea is to bring this temperature closer to the physiological temperature.

The polymer addition into gelatin network does not significantly modifies the destructuration temperature, but in the case of Sample 1 considerable differences in curves profile were evidenced (Fig. 3b): an increase in both dynamic moduli can be observed starting with 40°C, followed by a stabilization of the values, two orders of magnitude lower as compared with the initial one. Over 60°C some kind of stable week gel structure is formed. The purine structure of adenine may be responsible for the complex rheological behavior of Sample 1.

In the case of Sample 2 added into gelatin network (Fig. 3c) more stable and flexible structure at higher temperatures can be observed. The rheological behavior of the gelatin network incorporating a polysiloxane grafted with thermo-sensitive segments (Sample 3) is very similar to pure gelatin, but the flexibility and stability of the resulted system is increased (Fig. 3d).

The non-covalent interactions type π-π staking and hydrogen bonding (Mignon et al., 2005) are responsible for the different behaviors of the samples containing nuceobases moieties when geometrical conditions are accomplished. Different factors (nucleobases type, main chain conformation, network density) lead to the development of physical interactions makeing this mechanism more complicated than in case of solitary nucleobases. The possibility of thymine dimers formation (Li et al., 2008; Goodsell, 2001) explains the higher stability of Sample 2 as compared with Sample 1.

The next step was to investigate the influence of the *trans-cis* isomerisation of included modified azo-polysiloxanes on the rheological properties of the gelatin networks. The samples were irradiated for 5 minutes before running the temperature sweep tests, enough time for the complete isomerization of the azo-groups, according to the kinetic curves of the irradiation process. As expected a shift towards higher values in the specific phase transition temperatures was noticed for all samples (Fig. 4).

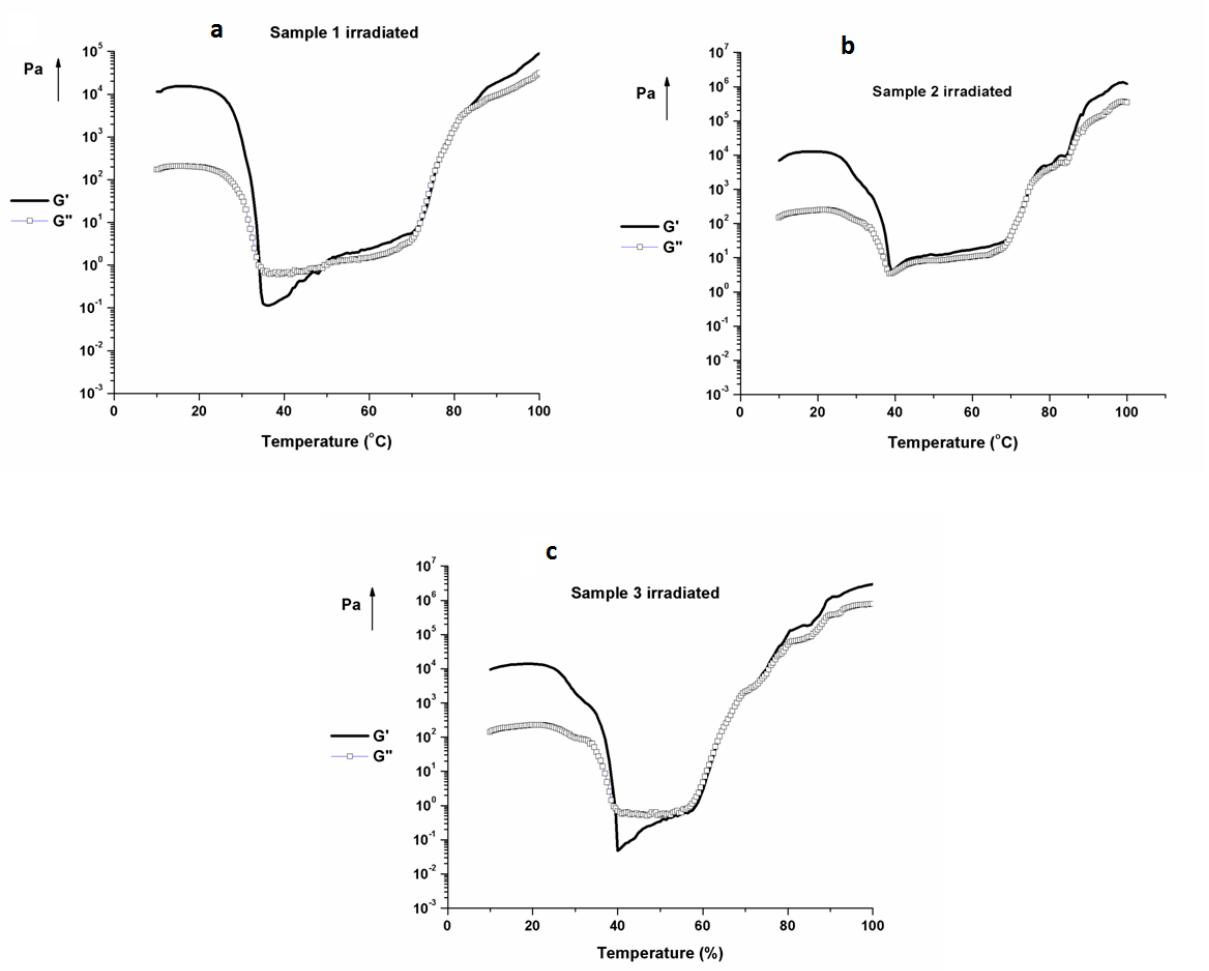

Fig. 4. The influence of UV irradiation on rheological properties of gelatin network incorporating modified azo-polysiloxanes; (a) 5% gelatin in water with Sample 1, (b) 5% gelatin in water with Sample 2, (c) 5% gelatin in water with Sample 3

The destructuration temperature increases at 34°C for gelatin with Sample 1, 39°C for gelatin with Sample 2 and 40°C when gelatin is mixed with Sample 3. If below the phase transition the rheological behavior is not modified by the incorporation of azo-polymers into gelatin network, things are changing after the irradiation process. The *trans-cis* isomerization is increasing the gel stability, G' being increased with one order of magnitude. When covalently bound to the polymer chains, the azo-molecule motion can have a strong impact on the conformation of the entire polymer chain and on its organization within a matrix. A remarkable example is given in the case of azo-polymer films subjected to a light interference pattern. In this case, the molecular isomerization process leads to large topographic modifications reproducing the intensity modulations (Rochon et al., 1995, Damian et al., 2014).

But more important modification of the rheological behavior takes place in the liquid state, even for the samples for which no polymer/polymer interactions were identified before the irradiation (Fig. 4a, b, c). Probably the higher mobility of both polymers (gelatin and azo-polysiloxane) in the liquid state and the strong modification of the dipole moment corresponding to the azo-group in the *cis* configuration (*cis* azophenol dipole-moment = 3.5D) are responsible for the intensification of the polymer/polymer interactions, leading to a system restructuration at high temperature.

Moreover, the temperature is inducing the azo-molecules relaxation (Garcia-Amorós et al., 2010), which combined with the dipole-dipole interactions and shear rate leads to an increase of viscoelastic moduli with six or seven orders of magnitude, becoming even higher than the moduli values at 15 °C. A pseudogel like system is formed. Comparing the three graphs (Fig.4) a delay between the destructuration temperature and the sharp increase of G' and G'' was noticed. The star shape polymer of Sample 3 (Fig. 4c) is letting the azo-groups more exposed and around 55°C both moduli are increasing.

In the case of linear azo-polysiloxanes substituted with nucleobases the sharp increase of the two moduli starts at around 65°C; because of the coil conformation, and due to the thermal relaxation of azo-molecules, some steric hindrance takes place**.**

All the results are an indication that the addition of the modified azo-polysiloxane in gelatin has noticeable influence on the overall rheological

temperature dependent behavior. Moreover, the UV irradiation of the modified systems allows the increase of the phase transition temperature bringing it closer to the physiological temperature. In this way the systems become suited for the use as drug release systems and the entities included in the azo-polymers can determine specific behaviors.

4. Conclusions

New systems based on gelatin and three different polysiloxanes containing azo-benzene groups in the side-chain with potential application as drug release systems were investigated by rheological methods combined with UV irradiation.

The experimental results proved the existence of interactions between gelatin and polysiloxanes intensified after UV irradiation. Due to its structure, gelatin is able to form multiple hydrogen bonds between the protein chains, thus generating a network which can be broken by chemical or physical stress and reformed after the stress is removed (Kozlov, 1983). By embedding azo-polysiloxanes substituted with nucleobases into this network new hydrogen bonds with significant effect onto cohesive forces between molecules are generated. Moreover, after the UV irradiation of the azo-molecules, dipol-dipol interactions are changing the physical proprieties of gelatin network. The increase in the values of *G'* and *G"* is a consequence of system restructuration leading to more organized architectures able to release the included active principle. As a function of the azo-polysiloxane structure, the destructuration temperature of the composite can be tuned in the domain 30-40 °C.

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