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Smart Reactivity in Gelatine/PNIPAM Mixtures: Control of the Cross-Linking and Microheterogeneities

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Summary: Chemical cross-linking of gelatine was achieved by using a thermo-sensitive reactive poly(N-isopropylacrylamide) as a cross-linker. The reaction occurs only below the LCST. Controlled micro-heterogeneities were created inside the gel without macroscopic volume transition. Acceleration of the drying process indicates faster water diffusion in the micro-heterogeneous gel.

Keywords: gelatine; gels; heterogeneities; poly(N-isopropylacrylamide); stimuli-sensitive polymers

Introduction

Gelatine is a denatured, biodegradable protein that can be used for the preparation of hydrogel matrices in drug delivery applications.[1] The drug release depends, among other factors, on the cross-linking density and homogeneity of the hydrogel. Further control of the matrix properties can be achieved by combining gelatine with a thermo-sensitive polymer like, for instance, poly(N-isopropylacrylamide), PNIPAM.[2,3] PNIPAM exhibits inverse solubility behaviour (LCST, Lower Critical Solution Temperature) in aqueous solution. At the LCST ($\sim$32 °C) the polymer chain undergoes a coil-to-globule transition and this thermo-sensitive behaviour is of interest for the development of stimuli responsive aqueous formulations, surfaces and gels.[4–8] The properties of gelatine and PNIPAM have been combined in graft copolymers[9,10] and interpenetrating networks.[2,3]

In this work we explore the possibility to control the chemical cross-linking and properties (local heterogeneity) of gelatine gels by using a NIPAM-based copolymer as a thermo-sensitive cross-linker. The copolymer bears 5 mol% acrylic acid (AA) units. The cross-linking occurs by amide bond formation between the AA groups of the NIPAM-based copolymer and the amino groups of gelatine in the presence of a water soluble carbodiimide (EDC).[11] For comparison purposes we also used a non-thermo-sensitive copolymer (LCST > 100 °C) based on dimethylacrylamide (DMAM) and containing the same fraction of AA units (5 mol%).

Experimental Methods

Gelatine (bovine, $M_w \approx 180000$ g/mol) was provided by Kodak Industrie (France). The copolymers ($M_w \approx 30000$ g/mol) were synthesized by radical copolymerization in aqueous solvent.[12] The fraction of AA units, 5 mol%, corresponds to the monomer feed composition and it was confirmed by elemental analysis and potentiometric titration of the resulting copolymers. The NIPAM and DMAM-based copolymers will be referred to as PN-5 and PD-5 respectively (Figure 1). As water-soluble carbodiimide we used 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC) from Aldrich.

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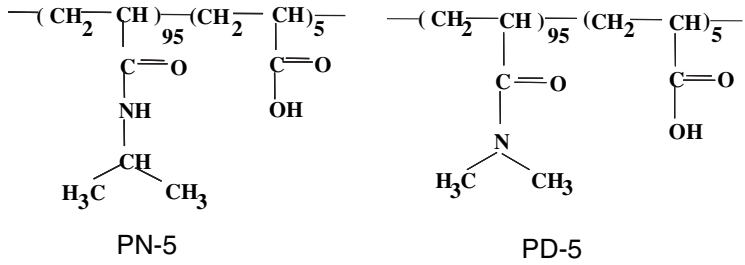

Figure 1.
Formula of the random copolymers used in this study. For convenience the AA units are shown in their acid form although most of them (≈70–75%) are in the neutralized sodium salt form.

Stock solutions of gelatine (15 wt%), synthetic copolymer (3 wt%) and EDC (30 mM) were prepared and equilibrated at 30 °C. The final solutions were prepared by mixing equal volumes of the stock solutions under vigorous stirring for a very short period of time (∼15s).

Rheological measurements were performed on an ARES controlled strain rheometer equipped with a 100FRT transducer and Couette geometry. The liquid mixture was transferred in the Couette cell, set at the desired temperature, immediately after mixing. The elastic and viscous modulus, G′ and G″, were measured as a function of time at constant temperature (frequency = 7 rad/s, strain = 1.5%).

Thin films were prepared by spreading the liquid mixture (thickness ∼0.25 or ∼0.10 mm) on polyester or glass slides placed on a temperature controlled stage (20 °C). The sample was kept at 20 °C during 10 min to achieve the cross-linking reaction. The films were dried at 60 °C in a humidity analyzer (Sartorius MA100) and the loss of water was recorded as a function of time.

The internal structure of the films was observed by Laser Scanning Confocal Microscopy, LSCM (Zeiss LSM 510) operating in the fluorescence mode (λ_{ex} = 488 nm). To enhance the contrast, rhodamine-labeled copolymers were used. These copolymers where obtained by grafting a very low faction of rhodamine (less than one rhodamine reside per chain) on the AA groups of PN-5 and PD-5.[13] Such a low degree of labeling suffices for the purpose of the present study. The surfaces heterogeneities of the dry films were visualized by AFM (Park Scientific Instrument operating in the tapping mode).

Results and Discussion

Influence of the Temperature on the cross-Linking Reaction

The formation of the mixed chemical gel was followed by rheology. Figure 2 shows the variation of elastic modulus (G′) as a function of time for mixtures of gelatine with PN-5 (or PD-5) in the presence of EDC, at 30 and 45 °C. At these temperatures gelatine is in the coil conformation and the increase in G′ is attributed exclusively to chemical cross-linking. At 30 °C, i.e. below the LCST (≈34 °C) of PN-5, both systems exhibit a fast and pronounced increase in G′. On the other hand, at 45 °C the mixture containing PD-5 is by far more efficient than the PN-5 system (Figure 2). At this temperature the PN-5 chains have a compact globule conformation and their carboxylic groups can not contribute to the cross-linking reaction. As a consequence, the reactivity of the system is substantially reduced. The observed smooth increase in G′ is due to the self-cross-liking of gelatine in the presence of EDC. This point is discussed in detail elsewhere.[14] A schematic representation of the gelatine/PN-5 system, below and above the LCST, is shown in Figure 3.

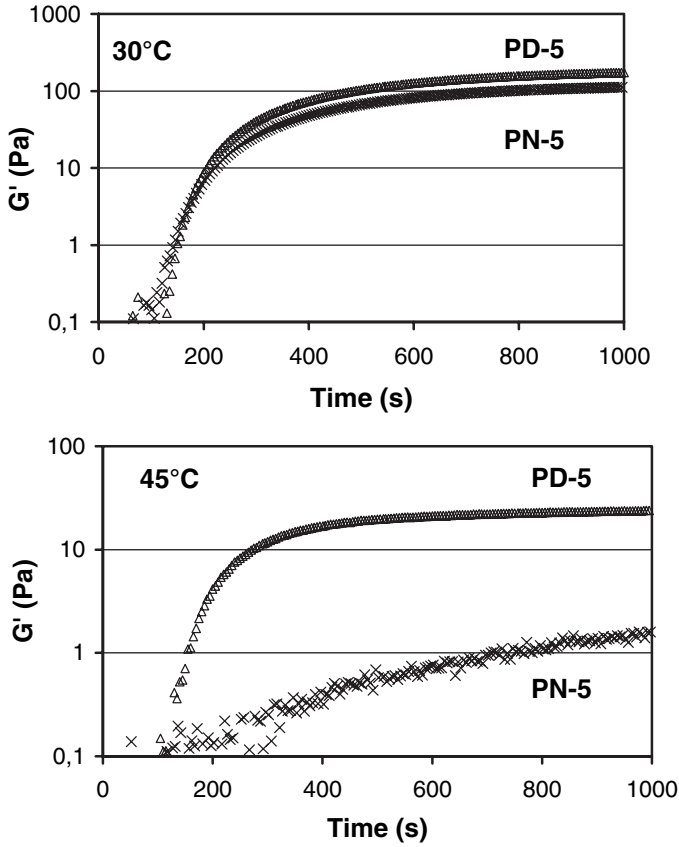

Figure 2.
Evolution of G′ as a function of time at 30 °C and 45 °C for mixtures containing 5% gelatine, 1% copolymer and 10 mM EDC.

The correlation between reactivity and chain conformation is shown in Figure 4 where the elastic modulus G′ (measured at 1000s after the mixing) is plotted as a function of temperature. For the system based on PN-5 a pronounced decrease in G′ is observed just above the LCST (indicated by an arrow on the figure). Concomitantly

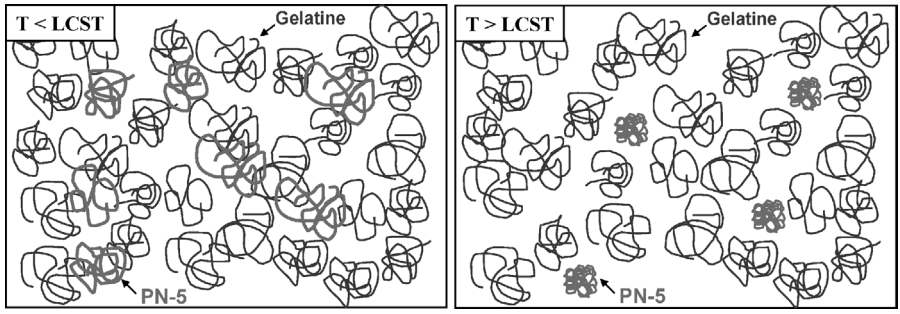

Figure 3.
Schematic representation of the gelatine/PN-5 mixtures below and above the LCST. Cross-linking occurs only at T < LCST when the PN-5chains are in the coil conformation.

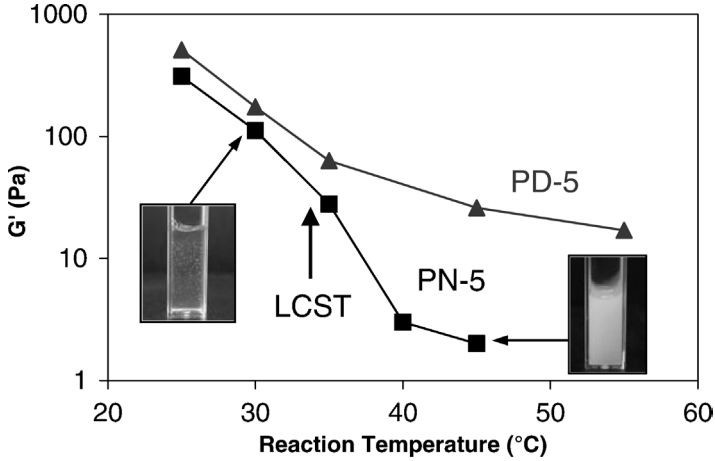

Figure 4.
Evolution of G′, measured at t = 1000s, as a function of temperature for mixtures of 5% gelatine, 1% copolymer and 10 mM EDC. Photographs show the PN-5 based systems prepared at 30 and 45 °C.

the mixtures prepared at T > LCST exhibit turbidity which is a further confirmation of the globular conformation of PN-5 (for observation, see photographs on Figure 4).[15]

An interesting consequence of this thermo-sensitive behavior is that the reactive mixture can be prepared and handled in the liquid state, i.e. at T > LCST, and then chemically cross-linked by decreasing temperature below the LCST.[14] Such systems can be used for in-situ chemical cross-linking after application or injection of the liquid formulation. Another important property of these systems relays on the possibility to adjust the LCST at the desired value by copolymerizing NIPAM with more hydrophilic or hydrophobic monomers.[16] This flexibility opens numerous routes for the preparation of smart reactive systems.

Influence of the Heterogeneities on the Properties of Films

The data presented in the previous section show that it is possible to prepare gelatine gels, cross-linked with a thermo-sensitive polymer, provided that the reaction is performed at temperatures below the LCST. Such mixed gels exhibit thermo-sensitive character due to the presence of PNIPAM strands in their structure. The ability of the PNIPAM strands to undergo the coil-to-globule transition was confirmed by DSC and ^{1}H NMR experiments.[13]

Local micro-heterogeneities are formed when the gelatine/PN-5 gel (prepared at 20 °C) is heated at 60 °C. These heterogeneities are evidenced by LSCM using rhodamine-labeled PN-5 (data not shown). Note that these mixed gels, in which the thermo-sensitive polymer is the minor component (17% of the total polymer concentration), do not exhibit macroscopic volume transition at the LCST.

Heterogeneities persist in the dry material (film) obtained after evaporation of water. The surface and the bulk heterogeneities are visualized by AFM and LSCM respectively (Figure 5B and D). Under the same conditions, the films containing the non-thermo-sensitive PD-5 look homogeneous (Figure 5A and C).

Figure 6 shows the effect of the synthetic copolymer (PN-5 or PD-5) on the drying process. The loss of water is measured as a function of time for thin gels cross-linked at 20 °C and dried at 60 °C. Clearly the micro-heterogeneous gel, cross-linked with PN-5, exhibits faster drying presumably due to the faster water diffusion within the ''voids''

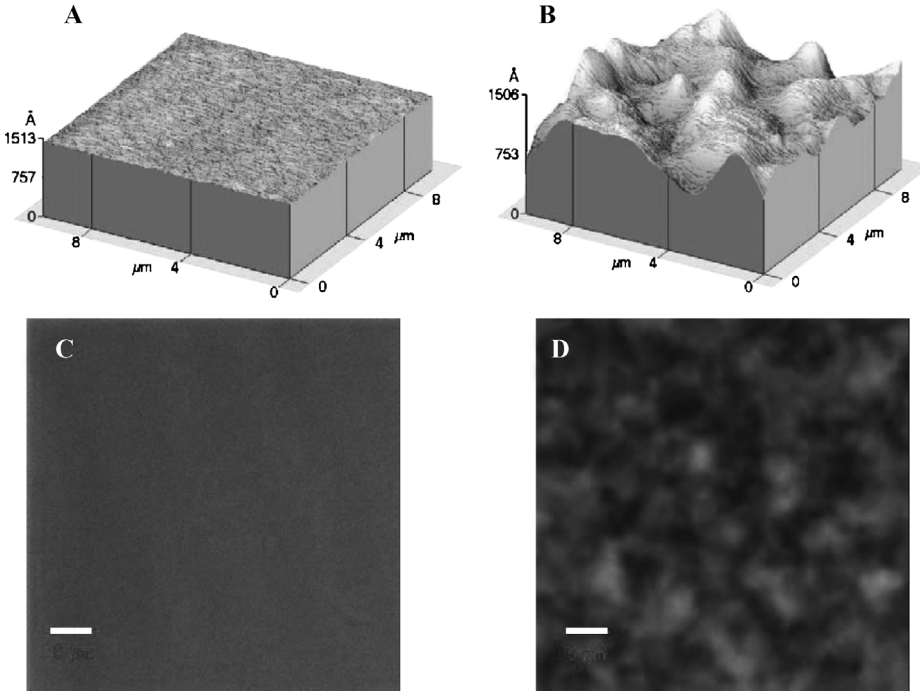

Figure 5.
Pictures of films dried at 60 °C. Surface topography observed by AFM (A, B) and internal structure visualized by Confocal Microscopy (C, D, Bars = 5 μm). A and C: films based on the non-thermo-sensitive PD-5 copolymer. B and D: films based on the thermo-sensitive PN-5 copolymer. Rhodamine-labeled copolymers are used for the Confocal Microscopy observations.

created around the collapsed PNIPAM strands. This suggests the possibility to control drug diffusion and release by adjusting the degree of heterogeneity of such mixed gels. Further studies are needed to understand the details of the drying mechanism and the role of the micro-heterogeneities.

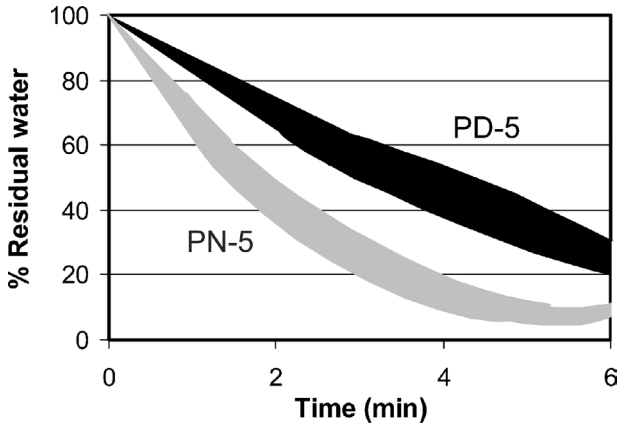

Figure 6.
Drying curves at 60 °C for thermo-sensitive (grey) and non-thermo-sensitive (black) systems. Experiments have been repeated 3 times. The thickness of the curves indicate the dispersion of the experimental data.

Conclusion

The chemical cross-linking of gelatine can be strongly temperature-dependant when using a thermo-sensitive polymer as cross-linker. Effective cross-linking occurs essentially below the LCST when the thermo-sensitive polymer is in the coil conformation. The resulting gelatine-rich gels exhibit thermo-sensitive behavior and micro-heterogeneities due to the presence of PNIPAM strands in their structure. The formation of heterogeneities accelerates the diffusion of water through the gel and is of potential use for controlled release of drugs.

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