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Fluorescence polarization and rheological studies of the poly(*N*-vinyl-2-pyrrolidone) hydrogels produced by UV radiation

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Abstract

Poly(*N*-vinyl-2-pyrrolidone) hydrogels produced by direct ultraviolet irradiation of PVP aqueous solution leads to crosslinking through pyrrolidinone moiety photolysis. Generally, hydrogel physical properties, like crosslinking density, pore size, swelling capacity, storage and loss moduli are obtained by swelling and rheological tests. However, relations between anisotropy obtained by fluorescence polarization studies and these properties have not been addressed for hydrogel systems. In this work we show that there is a correlation between the data obtained from anisotropy and rheological experiments, since both of them are related with crosslinking density of the hydrogels. These results reveal that fluorescence polarization spectroscopy is a promising tool for understanding the structure of hydrogels.

Keywords: Hydrogel; Rheological behavior; Fluorescence polarization

1. Introduction

Since, the pioneering work of Wichterle and Lim in 1960 on crosslinked poly(HEMA) [1], hydrogels have been of great interest to biomaterial scientists [1–12], mainly due to their hydrophilic character and potential biological compatibility. Hydrogels are polymeric networks, which absorb and retain large amounts of water. Hydrophilic groups or domains are present in the polymeric network, which are hydrated in an aqueous environment thereby creating the hydrogel structure. Aqueous solution of hydrophilic polymers at low or moderate concentrations shows a Newtonian behavior when no substantial entanglement of chains is present. This rheological behavior changes when crosslinks between the polymer chains are introduced. Hydrogels morphological and rheological properties such as crosslinking density, pore size, swelling capacity, storage and loss moduli are needed for understanding the performance of these materials [12–14], and can be evidenced by a proper rheological analysis.

Fluorescence polarization spectroscopy is a tool that can determine microviscosity of different systems through steady state or time resolved anisotropy measurements [15–20]. The polarization or anisotropy measurements reveal the average angular displacement of a probing fluorophore, which occurs between absorption and subsequent emission of a photon. This angular displacement is dependent upon the viscosity of the environment. Polymers labeled with fluorescent moieties have been used to probe system anisotropy [21]. The combination of fluorescence anisotropy and rheology techniques have been recently addressed [22,23].

In the present work, the objective is to compare data from the rheological behavior of PVP hydrogels produced by UV radiation with the results obtained by fluorescence polarization in order to investigate macromolecular structure of these hydrogels.

2. Experimental

2.1. Materials

The PVP used in this study, known as Luviskol® K-90 ($M_w = 1.2 \times 10^6$) was supplied by BASF. The poly(*N*-vinyl-2-pyrrolidone-*co*-vinyl-anthracene) copolymer VP–VA was used as fluorescent probe. Its M_w was determined as 1.4×10^5 by SEC analysis, against PEO standards. The emission and excitation spectrum of the VP–VA is shown in Fig. 1. The polymerization reaction was run in ethanol as solvent, using

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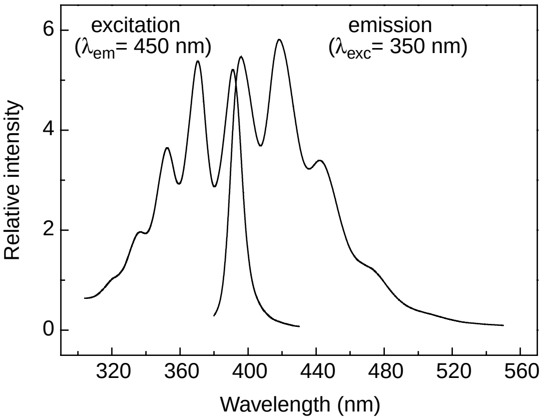


Fig. 1. Fluorescence spectra of the VP–VA copolymer.

N-vinyl-2-pyrrolidone and 9-vinyl-anthracene as monomers (VP/VA = 10^3 w/w), and 2,2′-azobis(isobutyronitrile) (AIBN) as initiator, at 60 °C for 48 h. The initiator concentration used was 0.1% (w/w).

2.2. Irradiation

Irradiation was carried out using a PCQ-X1 reactor from ultra violet products (Cambridge, UK) with three low-pressure doughnut-type Hg lamps with maximum emission λ_{max} = 254 nm (17.2 W/lamp). The solution was placed in quartz tube of 1 cm diameter positioned in the center of the lamp set. This setting produces 5.85 mW/cm^2 maximum emission.

2.3. Gel properties

The gel content, g(%) and crosslinking density, ρ_x, were calculated as described elsewhere [10].

2.4. Fluorescence polarization

When a fluorescent sample is illuminated by plane polarized light under steady-state conditions the anisotropy, r, is defined as [24]:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where I_{VV} corresponds to vertically polarized excitation and vertically polarized emission, I_{VH} corresponds to vertically polarized excitation and horizontally polarized emission, and G is a factor obtained from I_{HV}/I_{HH} with I_{HV} being the horizontally polarized excitation and vertically polarized emission and I_{HH} corresponding to horizontally polarized excitation and horizontally polarized emission. The higher value of r relates to a more restricted motion of the fluorophore during its fluorescence lifetime.

Fluorescence intensity values were determined by fluorescence polarization using a Spex-Fluorolog-2 model FL-111 fluorometer equipped with an L-format polarization assembly using Glan–Thompson polarizers, model 1935B. Excitation was performed at 360 nm and the emission was detected at 418 nm.

2.5. Rheological behavior

Rheological measurements were performed on a cone-plate Rheometer—Physica MCR 300—Paar Physica. All rheological measurements were carried out at 25 °C. The samples used on these experiments are disc shaped PVP hydrogel samples with diameter 50 mm and thickness ~1 mm.

The storage (G') and loss (G'') moduli were measured in the linear viscoelastic regime, through frequency sweep experiments, with frequencies ranging from 0.1 to 100 s^{-1}, at maximum strain, γ, of 1%. This γ value was determined by preliminary strain sweep experiments, in which the complex modulus was measured as a function of strain at a fixed frequency value of 10 Hz to check if the deformation imposed on the hydrogel structure by the rheological experiment was entirely reversible. A strain value, γ, of 1% was found to yield reversible deformation for all samples investigated.

3. Results and discussion

The feasibility of producing PVP hydrogels has been previously demonstrated by direct UV irradiation of the polymer aqueous solution using either deep-UV radiation (λ_{exc} < 260 nm) [10] or photo-Fenton conditions ($\lambda \approx 360$ nm in the presence of H_2O_2) [12]. The special achievement then presented was the ability to produce PVP hydrogels in the total absence of vinylic monomers by using an inexpensive radiation source (as opposed to high-energy radiation sources that are typically used).

Table 1 shows the effect of radiation dose on gel content obtained for the PVP concentrations used here. It can be observed that the gel content increases to values higher than 90% at doses above 2 kJ, but shows very little dependence with PVP concentration. As it has been established previously [10], this is true for concentrations above 8%, with a sharp drop of gel content at concentrations below 6%.

Table 2 shows the results of radiation doses, crosslinking density ρ_x and the anisotropy r-values obtained for three different PVP concentrations. An increase of the crosslinking density with radiation dose can be observed, but in a way much less pronounced than that of gel content (Table 1).

These results can be explained from the standpoint of Peppas–Merrill model for hydrogels (a modified Flory–Rehner model) where ρ_x is related to the reciprocal of $\bar{M}_C$,

Table 1
Effect of radiation dose and PVP concentration on the gel content

[PVP] (g/L)	Gel content (%)		
	80	90	100
Dose (kJ)			
0.61	13 ± 1.7	19 ± 1.5	22 ± 0.8
1.21	63 ± 2.8	66 ± 5.1	64 ± 4.8
1.81	82 ± 2.0	78 ± 1.6	80 ± 2.8
2.42	93 ± 1.4	95 ± 0.6	96 ± 1.6

Table 2
Effect of radiation dose and PVP concentration on crosslinking density and polarization *r*-values

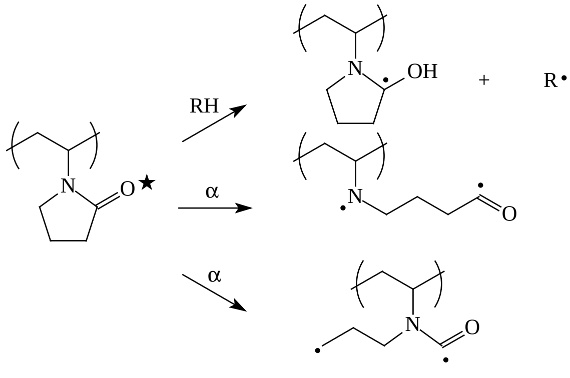
[PVP] (g/L)	ρ_x (10^{-6} mol/cm^3)			*r* value		
	80	90	100	80	90	100
Dose (kJ)						
0				0.064	0.080	0.086
0.61	–[a]	–[a]	–[a]	0.182	0.213	0.228
1.21	7.65 ± 0.06	7.79 ± 0.24	7.78 ± 0.19	0.204	0.228	0.231
1.81	8.30 ± 0.05	8.19 ± 0.13	8.48 ± 0.12	0.208	0.237	0.241
2.42	8.71 ± 0.14	8.91 ± 0.08	9.42 ± 0.19	0.230	0.241	0.242

[a] The hydrogel lacked sufficient structural stability.

the number-average molecular weight between crosslinks, and ξ, the mean pore size (in angstroms) [4]. These crosslinks are the product from recombination of macroradicals produced by UV direct photolysis of PVP (Scheme 1). While a single crosslinking is enough to hold a soluble macromolecule in the network, a much higher number is needed to change its overall density.

A combined analysis of the results from Table 2 leads to the conclusion that the crosslink density and anisotropy relate to polymer concentration and radiation dose in the same way. The correlation between *r*-values and crosslinking density shown in Fig. 2 evidences this supposition. The higher values of anisotropy are due to the restricted motion of the fluorophore during its fluorescent lifetime and, in this case, restricted motion is related to a smaller pore size.

Table 3 shows the dependence of storage G', loss G'' and complex G^* moduli with radiation dose and PVP concentration of the hydrogels in linear viscoelastic regime, when maintaining frequency at 10 Hz and at a strain, γ, of 1%. As an example, the frequency dependency of G' and G'' of the PVP hydrogel ([PVP] = 80 g/L), obtained at the two extremes of radiation doses are plotted in Fig. 3. In this case, it can be observed that the values of the G' and G'' are similar at 0.61 kJ of the radiation dose. However, the increasing of the G' values are bigger and farther of G'' when the radiation dose is increased to 2.42 kJ. The same behavior was observed for hydrogels produced from 90 and 100 g/L of PVP aqueous solution. Fig. 4 exemplifies the angular frequency dependence of G' for three different concentrations of PVP, with the highest radiation dose, 2.42 kJ.



Scheme 1.

These results can be analyzed from two aspects: elastic (storage modulus) and viscosity (loss modulus) contributions. At the same concentration G' and G'' moduli increase with the increase of the radiation dose. However, storage modulus, G', shows more rapid increase than viscous modulus G'', implying that the elastic contribution is more affected by the crosslink density increase. This behavior was obtained for all samples. These results were in agreement with those obtained by other gelation techniques and polymer systems which shows that an increase in the amount of crosslinker (typically, a bisvinyl monomer) leads to a linear increase of G'. Although, in general, hydrogels behave viscoelastically, their rheological properties are usually represented by its elastic modulus and described

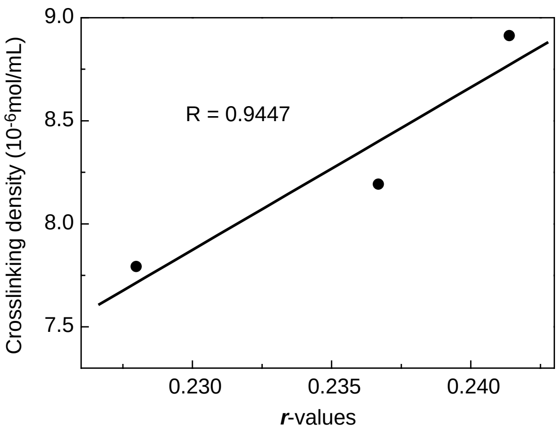


Fig. 2. Correlation between anisotropy and crosslinking density of the hydrogel obtained from PVP aqueous solution ([PVP] = 90 g/L).

Table 3
Effect of radiation dose and PVP concentration on values of G', G'' and G^* (Pa)

[PVP] (g/L)	G'			G''			G^*		
	80	90	100	80	90	100	80	90	100
Dose (kJ)									
0.61	1.6	–	–	3.6	–	–	3.9	–	–
1.21	8.7	16	15	7.3	16	15	11	23	21
1.81	67	65	65	18	27	39	70	73	92
2.42	105	95	134	41	41	49	113	103	143

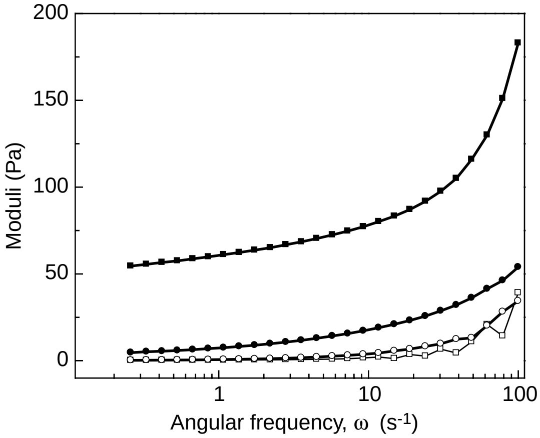


Fig. 3. Storage and loss moduli as function of angular frequency (ϖ) for hydrogels obtained to PVP aqueous solution at different radiation doses: G' at 0.61 kJ (−□−); G'' at 0.61 kJ (−○−); G' at 2.42 kJ (−■−); G'' at 2.42 kJ (−●−); [PVP] = 80 g/L.

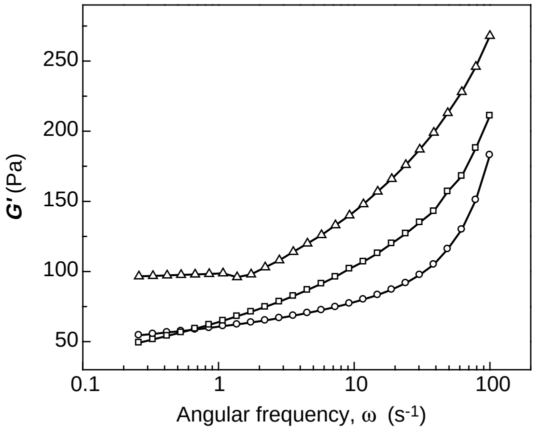


Fig. 4. G' as function of angular frequency (ϖ) for hydrogels obtained at different and PVP concentrations: 80 g/L (−○−); 90 g/L (−□−); 100 g/L (−△−); radiation dose = 2.42 kJ.

as $G' = n_e RT$, where n_e is the number of crosslinks in the network [25,26].

The comparison of G' and G'' values at different PVP concentrations, at the same radiation dose, showed a poor correlation as shown in Table 3. Again, this parallels the anisotropy behavior shown in Table 2 where the r-values have a better correlation with radiation dose than with the three PVP concentrations studied.

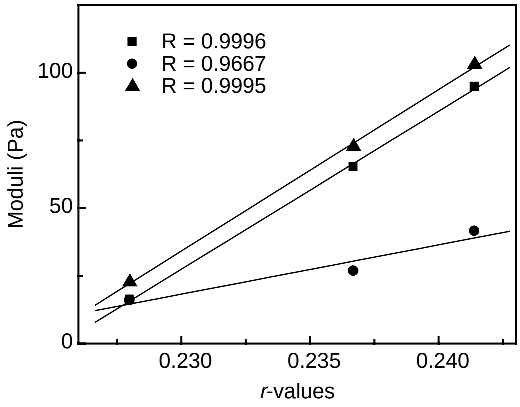


Fig. 5. Correlation between r-values and G' (−■−), G'' (−●−) and G^* (−▲−) of the hydrogels obtained from PVP aqueous solution ([PVP] = 90 g/L).

Finally, in order to check the relationship between macro-rheological properties and anisotropy, G', G'' and G^* were plotted against r-values, using hydrogels obtained from PVP aqueous solution at 90 g/L, as example (Fig. 5). The linear fit obtained for the three relations, all of them with high correlation coefficients, points to a true relation between anisotropy and rheological behavior of these hydrogels.

4. Conclusion

The results obtained in this work showed that the amount of crosslinking obtained during UV irradiation of PVP aqueous solutions has a strong influence over the rheological properties of the hydrogel formed. Storage modulus seems to be more sensitive parameter revealing the more pronounced elastic character of the hydrogel formed.

A fluorescence polarization study showed that, likewise, anisotropy values could also be correlated to the crosslinking density. A good correlation could be drawn between anisotropy and the shear moduli. Since anisotropy is a property related to microenvironment, this result is an evidence for a qualitative correspondence between microviscosity and structural characteristics of the hydrogel.

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