Thermal Transitions of Poly(vinyl alcohol) Hydrogel Sensed by a Fluorescent Probe

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ABSTRACT: Fluorescence from the fluorescein molecule has been used to study of phase transition processes in five poly(vinyl alcohol) (PVA) hydrogels in the temperature range 75–350 K. These hydrogels were prepared with PVA with molecular weight = 124,000–186,000, 99+% hydrolyzed, by repeated freezing–thawing cycles in five compositions: 9.9, 11.1, 13.3, 16.5, and 20.9 PVA w/w %. Plots of total fluorescence intensity versus temperature, normalized with the fluorescence signal at 75 K, and of the emission wavelength maximum versus temperature indicated that the dynamic of the guest deactivation was dependent on the hydrogel thermal transitions. These thermal transition processes were compared to three model systems: uranine (fluorescein di-sodium salt) in water, fluorescein in water/ethanol (85%/15% v/v), and fluorescein/PVA homopolymer. Small spectral shifts were obtained for these model systems over the entire temperature range attributed to the hot band effect. Nevertheless, larger spectral shifts were obtained for hydrogels, indicating that shifts of the chemical dissociation equilibrium was occurring in this last case. Slope changes of both intensity and wavelength maximum plots versus temperature occurred at the same temperature values, and we attributed the onset of the hydrogel thermal transition as the sol–gel transition occurring at $T \cong 370$ K; at 320 K we observed the glass transition temperature of PVA plasticized by water molecules; at $T \cong 240 \pm 5$ K we attributed the melting point of water molecules bonded to the PVA chains (freezable bond molecules); at $T \cong 270 \pm 5$ K we observed the melting point of free water molecules (bulk water); and, at 135–145 K the water glass transition. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 75: 815–824, 2000

Key words: poly(vinyl alcohol) hydrogels; states of water; phase transitions; fluorescence spectroscopy

1. INTRODUCTION

A polymer gel is a multicomponent system composed by a continuous network of chains crosslinked by chemical or physical junctions. In particular, the linkage points in poly(vinyl alcohol) (PVA) hydrogels are small crystals of either the semi-crystalline polymer or water molecules and hydrogen-bonded water/macromolecular segments. They are classified as a physical gel exhibiting a thermo-reversible phase transition processes.[1,2]

PVA hydrogels are important in the biomedical field because of their compatibility with living tissues at high water content.[3–5] Their biocompat-

ibility has been associated with their interfacial properties, which are related to the content and state of water in hydrogels.[6]

Both the thermal and the mechanical properties of hydrogels depend on several factors: molecular weight, concentration of aqueous solution, temperature and time of freezing steps, number of freezing–thawing cycles during the preparation steps, and how the water molecules are organized inside the material.[3–7] PVA hydrogels prepared using aqueous solutions subjected to repeated freezing–thawing cycles present the following characteristics: stability at room temperature, highly rubber elasticity, retention of its original shape, and high mechanical strength.

Several experimental methods have been used to study how the water molecules are organized in synthetic hydrogels, including ultrasonic measurements,[7] differential scanning calorimetry (DSC),[4,5,8–14] nuclear magnetic resonance,[4,11–15] infrared and Raman spectroscopy,[15] and differential thermal analysis.[16] Physical states or different states of water have been used to nominate the different types of ordered water molecules. Nevertheless, the description of the states of the water remains controversial. While some authors classified them in three states: free water, nonfreezable bound water and freezable bound water[6,9–11,14,15]; some others considered only two states: freezable and nonfreezable water[4,7,8,12–14]; and others considered at the same results can be explained without postulating these different states.[17]

Luminescence methods are now being considered a very useful tool in studying several polymer properties because of their sensitivity and selectivity ability, providing a nondestructive analytical method. Several types of polymer properties have been studied including polymer relaxation and secondary transitions,[18–23] miscibility and phase separation,[18,24,25] dynamics,[22] polymerization reactions,[26] cure reaction of polymeric resins,[27] microstructure of polyacrylamide gels,[28] transition involving helix–coil structures,[29] and others. Considering that several studies of polymer relaxation processes using fluorescent and phosphorescent molecules have been reported,[18–23] it seems reasonable to apply the same technique to study relaxation or phase transition of hydrogels. Furthermore, luminescence (fluorescence or phosphorescence) intensity is supposed to be strongly dependent on the temperature and on the microenvironment where the guest is located.

There are several luminescent probes very sensitive to micro-environmental properties such as viscosity (molecular rotors[20,22]), polarity (spectral shifts or Ham effect),[30] or mobility (inhomogeneous spectral broadening).[31] Thus, the adequate choice of a luminescent probe for phase transitions or relaxation studies should involve some criteria: (1) sensitivity to temperature changes, (2) high emission intensity in the entire temperature range, (3) high solubility in both water and in the isolated polymer, (4) luminescence spectrum dependent on the micro-environmental properties, and (5) photochemical stability. Fluorescein is a xanthene dye that satisfies all of these conditions, including a reasonable photochemical stability in the present experimental condition.[32]

The aim of this work is the study of thermal phase transitions of some PVA [molecular weight (M_w) = 124,000–186,000, 99+% hydrolyzed] hydrogels with several compositions. Since this polymer is not fluorescent, we added fluorescein as a fluorescent probe. Fluorescein is a high-fluorescent dye molecule that exhibits six possible dissociation forms, whose stability is strongly dependent on the dye–solvent (or polymer) interactions (Scheme 1).[33] Therefore, this molecule should be an interesting probe to describe molecular interactions between PVA chains and water molecules. As a comparison, we also studied the thermal phase transitions of pure water and water/ethanol (85%/15% w/w) mixture using the photophysical properties of uranine (fluorescein di-sodium salt) and fluorescein, respectively, at the same temperature range.

2. EXPERIMENTAL

Fluorescein (Merck) was purified by lactonization process.[18,20,24] Uranine (Carlo Erba) was purified by recrystallization from an ethanolic solution. PVA [Aldrich Chemical Co., average M_w = 124,000–186,000, 99+% hydrolyzed] was used as received.

These solutions were heated at 110–120°C in sealed glass tubes for a period of 3 h, and then they were slowly cooled to room temperature. Immediately after this cooling step, a bi-phase hydrogel was formed in samples **1** and **3.** Although each sample was submitted to the same thermal cycle, neither phase separation nor gel formation was detected for sample **2.** After this thermal cycle, every sample was submitted to a quenching process from room temperature to 1°C and then they were kept at 1°C for 15 h. Finally, they were
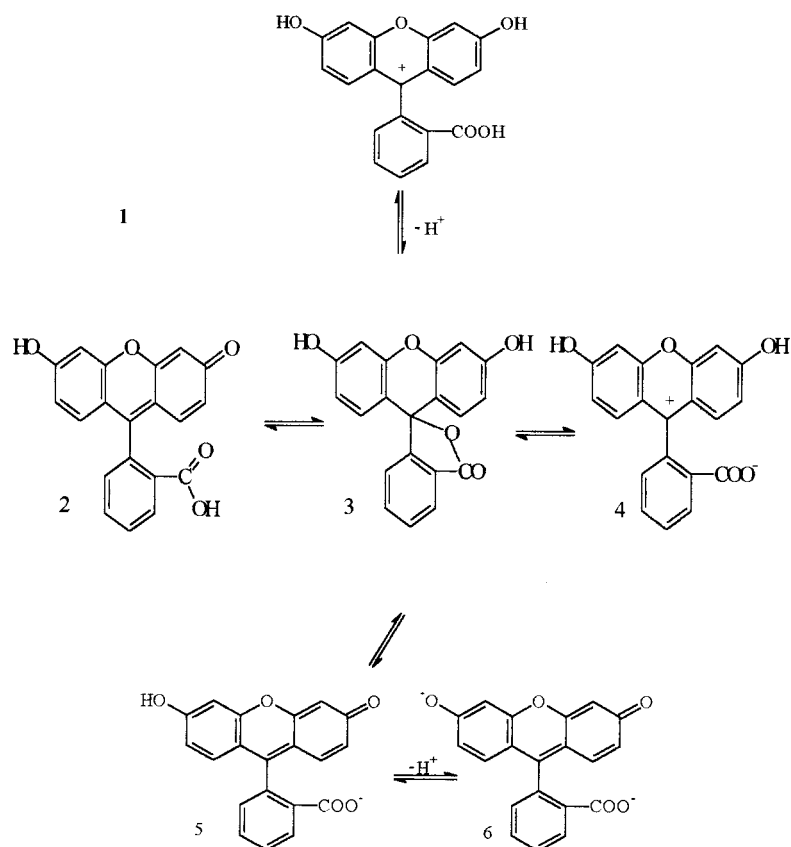
Scheme 1 Dissociation and ionic forms of fluorescein: (**1**) cation; (**2**) quinonoid; (**3**) lactone; (**4**) zwitterion, (**5**) monoanion, and (**6**) dianion.

heated to 20°C and kept at this temperature for 1 h. After this freezing–thawing cycle, two hydrogel phases were formed in every sample. Each phase was removed from the original glass tube and cut in slices, and one part of them was inserted and sealed in a glass tube for fluorescence measurements. The composition was determined by gravimetric method and the PVA weight proportions were 9.9%, 11.1%, 13.3%, 16.5%, and 20.9% w/w.
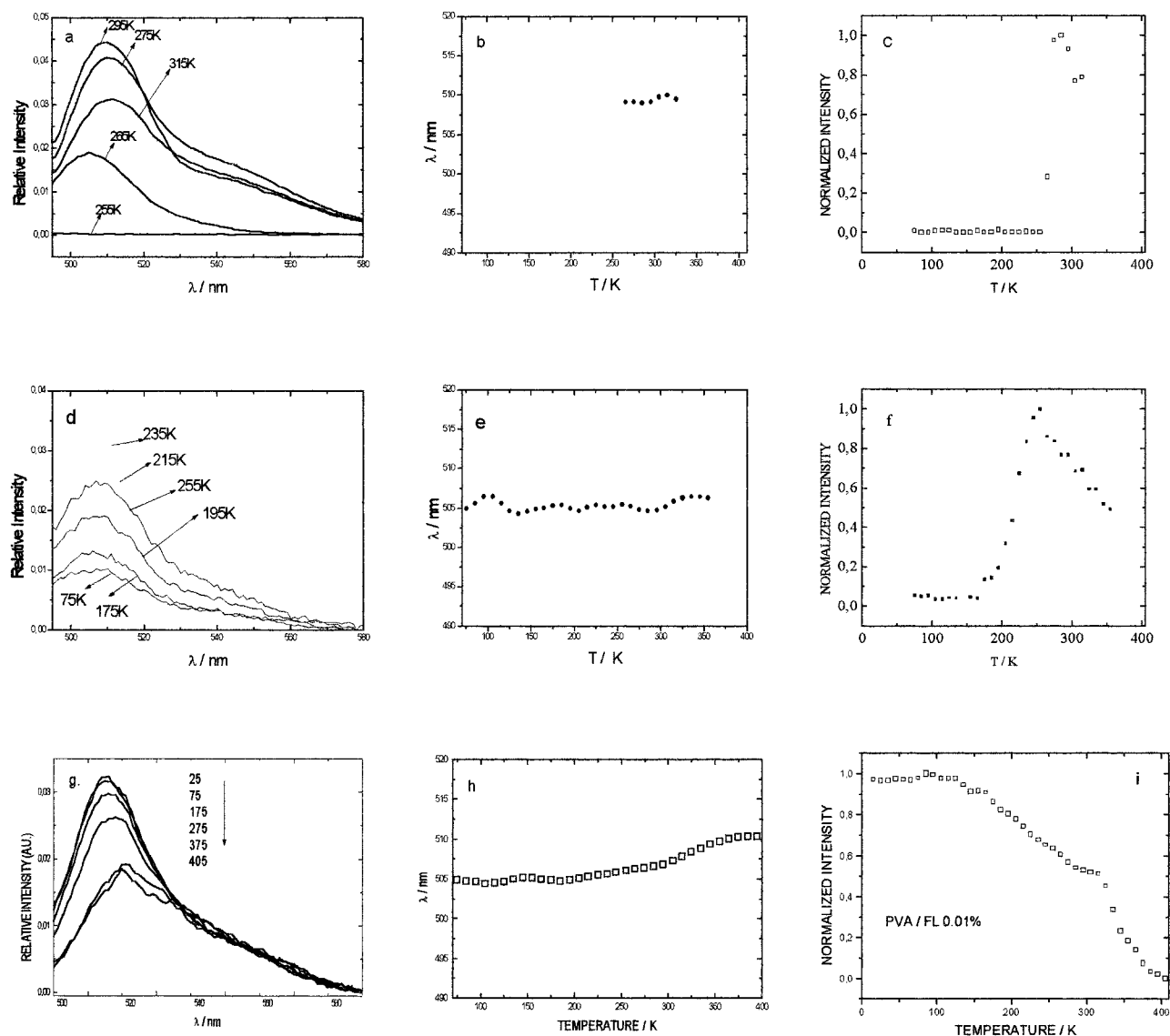
Fluorescence spectra at several temperatures were performed in a system described elsewhere.[19] The temperature was changed from 75 to 400 K by a digital temperature controller (Scientific Instruments, Model 9650), using previously established temperatures program and was kept constant during the spectrum recording. The samples were supported in the cryo-system by an optical holder positioned with the incident light and the emission beams at 90° angle. The fluorescence emission was collected in front-face geometry. A cylindrical lens on the entrance slit of the spectrometer focused the emission signal. Fluorescein was excited with a xe-

non lamp selected at 470 ± 10 nm by a monochromator, and the fluorescence spectrum was scanned from 480 to 580 nm.

3. RESULTS AND DISCUSSION

3.1. Thermal Transitions in Water and PVA Homopolymer

Thermal transitions in water and PVA were initially studied to provide fundamental understanding for the PVA hydrogel behavior. Uranine (fluorescein di-sodium) was used for studies of water and water/ethanol (85%/15% w/w) solution due to restrictions of solubility, whereas neutral fluorescein was used for PVA and hydrogels studies. Neutral fluorescein is a xanthene dye with a complex ionization and dissociation equilibrium strongly controlled by the nature of the medium (Scheme 1).[33,34] As a consequence, this molecule, in one of its several forms, has been considered a

Figure 1 Fluorescence spectra, emission maximum, and normalized intensity versus temperatures: (a–c) uranine in water; (d–f) fluorescein in water/ethanol (85%/15%) solutions; and (g–i) fluorescein/PVA homopolymer.

suitable for micro-environmental studies and for hydrogel phase transitions.

In the first part of this work we studied the temperature dependence of the fluorescence spectrum of uranine (fluorescein di-sodium salt) dissolved in water. In this condition, uranine in water was always ionized in a dianion form (**6**), either in the electronic ground state or in the electronic singlet excited state. Thus, changes of the fluorescence emission (spectral intensity, band shape, and wavelength position) induced by the temperature must be attribute to other than the equilibrium changes. Fluorescence spectra for

some temperatures were shown in Figure 1(a). There was a lack of fluorescence emission at temperatures $T < 270 \pm 5$ K, and above this the fluorescence band was centered at $\lambda_{\mathrm{Em}} \cong 512$ nm, independently of the temperature [Fig. 1(b)] (Table I). This band was assigned to the emission of the dianion species (**6**).[33]

The plot of normalized fluorescence intensities (I_T/I_0) versus temperature (T) showed [Fig. 1(c)] an abrupt increase at the water melting temperature transition followed by a subsequent intensity decrease. The absence of the fluorescence emission at $T \leq 270 \pm 5$ K was explained by the

Table I   Absorption and Fluorescence Peaks for Fluorescein in Dilute Aqueous Solutions

Fluorescein[a]	λ_{Abs} (nm)	λ_{Em} (nm)
Cation (**1**)	436	480
Zwitterion (**4**)	439	508
Lactone (**3**)	275	
Quinonoid (**2**)	476	515
Monoanion (**5**)	476	515
Dianion (**6**)	489	520
Fluorescein in water/ethanol (**4**)		507
Uranine in water (**6**)		512

From refs. 29 and 30.
[a] See Scheme 1 for numeration.

strong light scattering produced by the microcrystalline solid-state phase, which did not allow the penetration of the excitation light beam into the sample. Water crystallization also produced nonfluorescent uranine crystals. Above the water melting temperature a nonviscous solution was formed, and the fluorescence intensity was controlled by a convolution of several deactivation pathways, including among others the internal conversion and vibrational relaxation processes. All of them led to a decrease of the fluorescence emission.[30]

Therefore, the melting temperature of the solid pure water was perfectly determined by the temperature dependence of the fluorescence emission. This also illustrated that the normalized fluorescence intensity represented a convolution among several effects, including the light-scattering ability of microscopic self-organized molecular assemblies, and the radiative and nonradiative processes ratio was related to the fluorescent probe in the electronic excited singlet sate.

The light scattering interfered with the fluorescence emission by two cooperative processes: (1) the depth of the light beam penetration in high scattering medium was shorter and few molecules located on the surface were excited; (2) the fluorescence emission suffered multiple scattering inside the micro-crystalline material, and the detectable signal was drastically reduced. The apparent increase of the fluorescence intensity after the phase transition produced an isotropic solution minimizing the light scattering and then eliminated the light-scattering effects.

The second model system used was neutral fluorescein dissolved in water/ethanol (85%/15% w/w). Some of its fluorescence spectra recorded

from 75 to 370 K is shown in Figure 1(d). The fluorescence band was centered at $\lambda_{Em} \cong 507$ nm, independently of the temperature [Fig. 1(e)], similar to the fluorescence spectra of fluorescein dissolved in pH 4.4 aqueous solutions. While uranine fluorescence centered at 512 nm was attributed to the dianion form, the band centered at 507 nm resulted from the absorption of the zwitterionic form (**4**) with subsequent emission by the neutral form (**2**) (Scheme 1) (Table I).[33,34] Similar to the uranine in water, the absence of spectral shifts over the entire temperature range [Fig. 1(e)] demonstrated that the temperature effect on the chemical equilibrium did not lead to the fluorescein ionization, which produced a new ionic form.

The normalized fluorescence intensity (I_T/I_0) versus temperature (T) curve for fluorescein in water/ethanol (85%/15% w/w) solution [Fig. 1(f)] was more complex than that obtained in pure water [Fig. 1(c)]. A very well-defined onset temperature was obtained for pure water, coincident with the water melting temperature, while a lower onset temperature was noted for ethanol/water mixture. In this mixture two well-defined slope changes were observed (150 ± 5 and 260 ± 5 K), but no abrupt phase transition was detected between them, suggesting that in addition to the melting transition another thermal-induced structural process is present.

Thermal transitions of water were studied by several techniques, and these processes were assumed strongly dependent on the sample's thermal history. Angell et al.[35] and Sugisaki et al.[36] reported that the glass transition temperature of water and the ice cubic to hexagonal crystal transformation occurred at 135 and 160–210 K, respectively. On the other hand, Johari et al.[37] obtained an S-endotherm at the temperature range of 134–152 K, which was attributed to the water glass–liquid phase transition of hyperquenched water. In general, glassy transition is detected in hyper-quenched liquids producing a completely amorphous vitreous solid state. The samples, in the present experiments, were submitted to a cooling rate of ~ 5°/min, which does not characterize a hyper-quenching processes. Thus, we are assuming that the solid water/ethanol (85%/15% w/w) solvent contains both amorphous (vitreous) and crystalline phases and the onset of the glass transition based on the lower temperature slope change is 150 ± 5 K (Table II).

It was noteworthy that the increase of the normalized fluorescence intensity in the temperatures between $180 \pm 5 \leq T \leq 260 \pm 5$ K was

Table II   Glass Transition (T_g) and Melting (T_m) Temperatures for Water and Water/Ethanol (85%/15%) and PVA/Homopolymer from Slope Changes in Figure 1

Molecule	T (K)	Assignment	
Water/fluorescein	150 ± 5	T_g	
	180 ± 5	Water solid transition	
	260 ± 5	T_m	
Water/ethanol/uranine	270 ± 5	T_m	
PVA/fluorescein	This work	Ref. 35	
	125	130–150	$T\gamma$
	250–270	250–270	$T\beta$
	340	330–350	T_g

followed by a monotonic decrease of the fluorescence intensity for temperatures > 260 ± 5 K. This later process refers to the melting temperature of the solvent mixture, producing an enhancement of the radiationless process efficiencies of the dye in the electronic excited state in an isotropic nonviscous liquid medium.[32,34]

In an attempt to explain the apparent increase of the fluorescence intensities in this temperature interval, we may address to the decrease of the light-scattering signal, as noted by the tail of the base line at $\lambda \cong 480$ nm, which becomes lower with the temperature increase. Thus, a decrease of the light-scattering effect produced by the crystalline phase of the semi-crystalline medium precedes the melting transition (260 ± 5 K). Furthermore, since there is a change of the light-scattering signal, it must involve the crystalline phase of the system (Table II).

The third system studied was the thermal transitions of PVA homopolymer in form of films containing fluorescein. The fluorescence spectra exhibited almost the same behavior as the water/ethanol (85%/15%) solution, with the spectral maximum centered at 505 nm [Fig. 1(g,h)]. In addition they were almost independent of the temperature, as were the other systems. In earlier works we showed that fluorescein in PVA exhibits an electronic absorption band and fluorescence emission comparable to pH 6.4 and pH 8.2 aqueous solutions, respectively.[17,34] We attributed this spectral behavior to the existence of a dissociation equilibrium involving the quinonoid (**2**) and monoanionic (**5**) forms in the electronic ground state, which were ionized to the dianion form (**6**) in the electronic excited singlet state (Scheme 1).[33,34]

The normalized fluorescence spectra versus temperature show two slope changes. The onset of the first change was ca. 130 ± 5 K and can be attributed to the small segment movements of the polymer chain located at the amorphous phase and crystalline–amorphous interface.[35] The onset of the second change was at 340 ± 5 K assigned to the PVA glass transition process.[17]

Considering the results obtained for these three different systems, we assume that the spectral shifts exhibited by fluorescein resulted in the ionic or dissociation forms present in every medium. Nevertheless, the temperature did not shift the dissociation equilibrium. There are at least two effects leading to small spectral red-shift of the fluorescence spectrum at higher temperatures: one of them is the relaxation processes of the solvent cage surrounding the excited-state fluorescent molecules. If this relaxation time is faster than the fluorescence emission, the fluorophore decay originates from the relaxed medium, which is common in nonrigid solutions. The second is produced by the Franck–Condon coupling, involving excited rotational and vibrational states that can be populated by thermal activation, and consequently, the emission energy is lower at higher rather than at lower temperatures. This last process is called the hot-band effect. Finally, the apparent increase of the fluorescence intensity, in a certain temperature range, was attributed to the light-scattering effects.

3.2. Thermal Transitions of PVA Hydrogels

The fluorescence spectra and the respective temperature dependence of fluorescein dissolved in five PVA hydrogels (9.9%, 11.1%, 13.3%, 16.5%, and 20.9% PVA/water w/w) are showed in Figure 2. Although the PVA hydrogel samples were prepared with low fluorescein concentrations, the fluorescence spectra exhibit two overlapping fluorescence bands: one centered at 515–520 nm, assigned to the totally isolated dye molecule, and other centered at 540 nm, probably due to dimers and higher molecular aggregates.[32,33] This concentration effect suggests that there is a population of water molecules that is not available for the dye hydration, producing an apparent concentration increase.

It is also noteworthy that the fluorescence maximum is almost constant for fluorescein in all PVA hydrogel at low temperatures, with a significant shift for lower energy at $T > 250$ K. The temperature effect on the fluorescence band

Table III   Assignments for the PVA Hydrogel Thermal Phase Transitions from Figure 2

Temperature (K)	Thermal Phase Transitions
135–145	Water glass transition
245	Melting transition of freezable water bond to PVA
275	Melting transition of freezable bulk water
320	Glass transition of plasticized PVA
375	PVA sol–gel transition

shape is postulated to be dependent on the balance of several contributions. Among them the hot-band effect is expected with a small spectral shift toward lower energy, and changes of the medium polarity alter the fluorescein dissociation equilibrium among its different forms. Unlike fluorescein/water, uranine/water/ethanol, and fluorescein/PVA, which exhibited slight fluorescence spectral shifts attributed to the hot-band effects, fluorescein/hydrogels exhibited larger spectral shifts that must be explained by the dissociation equilibrium shifts.

The curves of normalized fluorescence intensity versus temperature (I_T/I_0 versus T) [Fig. 2(k–o)] and λ_{max} versus T [Fig. 2(f–j)] exhibit slope changes at (a) 135–150 K, (b) 250–275 K, (c) 320 ± 5 K, and (d) 370 ± 5 K (Table III). The magnitude and the temperatures where the slope changes were observed depend on the hydrogel composition and will be analyzed, taking into account the slope changes for the model systems: water, water/ethanol, and PVA homopolymer.

The lower temperature slope change was observed at 135–150 K, and it is more evident for hydrogel with higher water content (9.9%, 11.1%, and 13.5%) (Fig. 2). Regardless, a similar lower temperature transition (135–150 K) detected for water/ethanol solution [Fig. 1(f)].

Hofer et al.[39] reported that a reversible glass–liquid transition should be resolved in two endotherm steps at $\approx$ 132 K and $\approx$ 162 K for hydrogels of poly(2-hydroxyethyl methacrylate). They attributed these endotherm peaks, supported by dielectric and conductivity studies, to transitions involving nonfreezing and freezable water fractions, respectively. They also suggested that the glass transition temperature (T_g) at $\approx$ 132 K was due to configurational degrees of freedom of water molecules in local regions of strong hydrophobic interactions and that at $\approx$ 162 K due to local

motions where hydrophilic interactions among water molecules bonded to the polymer chains.[38] Considering that we do not expect hydrophobic interactions between water molecules and PVA macromolecules, we attributed this low temperature transition (135–145 K) to the glass transition freezable molecules (Table III).

Very complex changes of the fluorescence intensity were obtained for fluorescein/hydrogel systems at the temperature range ca. 245–270 K. An initial decrease of the fluorescence intensity was obtained at 250 ± 10 K, followed by a subsequent fluorescence increase (Fig. 2). Slope changes of the plots in Figure 2 occurring at 250–275 K are very close to that obtained for water transitions in both pure water (270 ± 5 K) and water/ethanol mixture (260 ± 5 K) (Table II). It seems also remarkable that the light scattering is reduced much more in PVA hydrogels than in pure water and water/ethanol mixture, suggesting that the water molecules crystallized into different forms and/or in smaller sized crystals. For the uranine in PVA, a sharp transition was detected at 270 ± 5 K, attributed to the water melting temperature, which decreased to 260 ± 5 K for water/ethanol solution containing fluorescein.

Unlike the model systems, including fluorescein/ethanol/water, fluorescein/hydrogels exhibited the phase transition at 250 ± 10 K, suggesting the existence of a population of water molecules that are organized and structured different from the usual freezable molecules, with a phase transition at 270 K. However, based on molecular dynamic simulations, the existence of freezable bound, freezable free, and nonfreezable water was seriously questioned.[17] This questioning arises because PVA is always a hydrophilic medium, and there is no sense in the assignments of distribution in hydrophobic and hydrophilic microenvironments. It has been estimated that, on average, each PVA hydrogen is bound to 2.5 water in its solvation shell.[40] The average time correlation for hydrogen bonds between PVA and water ($\approx$ 1.8 ps) is similar to the pure water ($\approx$ 1.1 ps) at 300 K, and both of them become longer at lower temperatures. The result showed that the mobility of water molecules bound to the polymer chain was slower than that in the bulk. In addition, the orientational correlation time distribution is bimodal only below the freezing point of bulk water, demonstrating the evidence of two states of water (bulk and bond). No evidence for the third state, nonfreezing, was obtained. Taking into account these data, we attribute the transition at 245 ± 5 K to the melting transition of freezable water
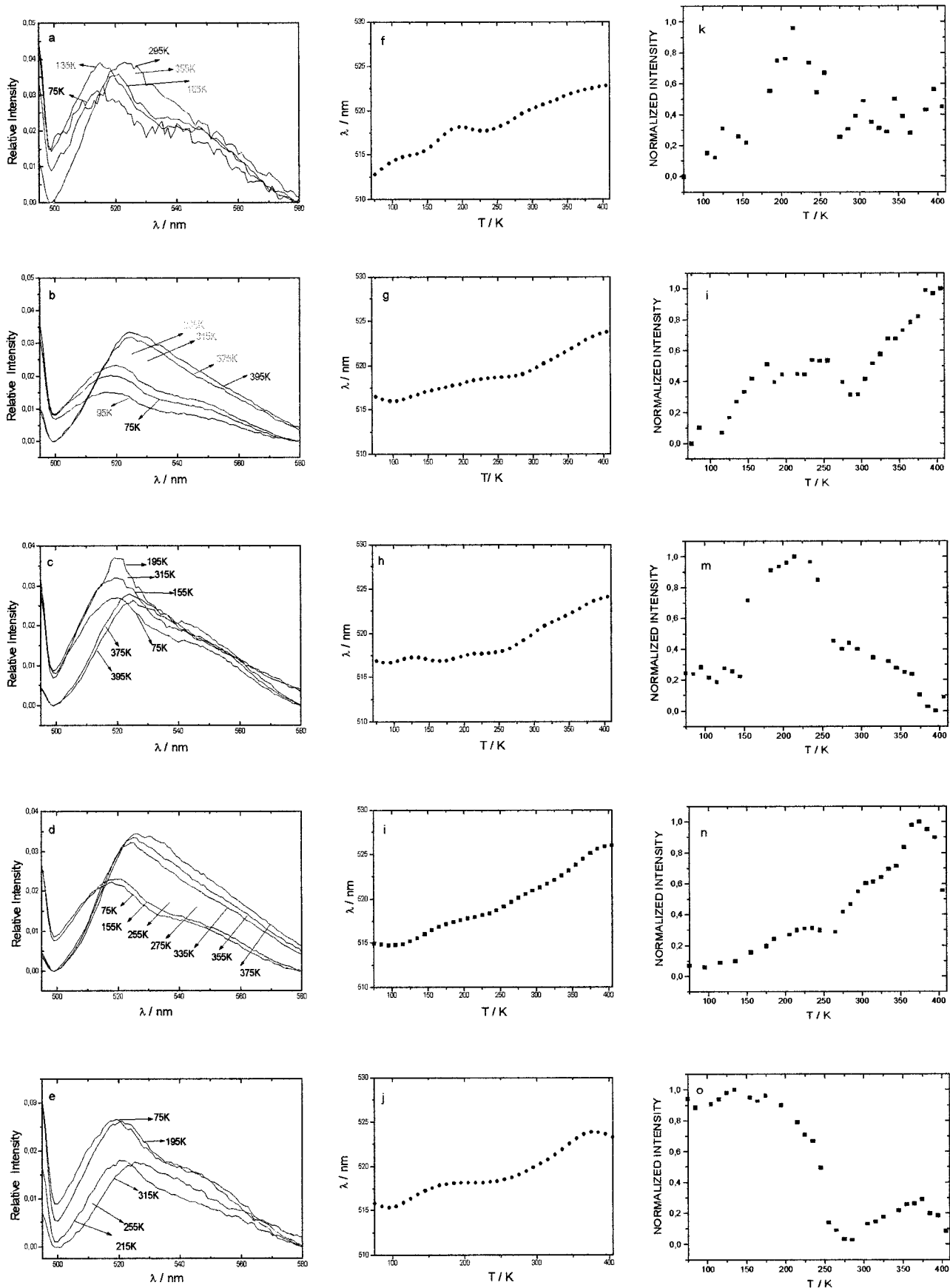
bonded to PVA chains and that at 270 ± 5 K to the melting transition of bulk water (Table III).

Explanations for this complex behavior are very controversial. Hofer et al.[39] pointed out that the state of liquid water in the hydrogel is stable against crystallization at 230–240 K, and this temperature depends on the water content. Dielectric relaxation studies[36] also show that this crystallization process occurs in a wide temperature range and water molecules coexist with ice at temperatures of 217–267 K. Hatakeyama et al.[11] determined that the ice melting peaks are displaced to lower temperatures ($\cong 250$ K) when the degree of reticulation increases in several crosslinking densities of PVA hydrogels. In addition, Yoshida et al.[10] demonstrated that the melting temperature of ice in hydrogels is smaller than that of pure water. All of them postulated the existence of freezable free water and freezable bound water, with phase transitions at 273 K and 260–240 K, respectively.

The higher temperature slope change is obtained at 370 ± 5 K, which is more evident for higher PVA content hydrogels (16.5% and 20.9%) [Fig. 2(o,n)]. This process is followed by a significant decrease of the fluorescence intensity. No such high-temperature event has been observed for the model systems, including the fluorescein/PVA, whose temperature is higher than the PVA glass transition but lower than the PVA melting temperature (450 K). Actually, the effect of the water content on the PVA glass transition involves an opposite effect, since it should promote the PVA polymer plasticization by the increasing of the free volume cavity size and consequently with a decrease in T_g.[41,42] Thus, we can not assign this transition to any event occurring at the amorphous region of the PVA matrix.

On the other hand, PVA is a semi-crystalline homopolymer and in our case this PVA sample exhibits a melting temperature at 450 K. The presence of water molecules in the PVA hydrogels induces a reorganization of chains into crystallites due to the polymer plasticization by water molecules, which act as a crosslinking agent, keeping the polymer chains and the micro-crystals together by hydrogen bonds. Taking into account these data, we assume that the transition

at 345–375 K is due to the sol–gel transition of the PVA hydrogel, involving motions of the PVA chains, which interacts with water molecules by hydrogen bonds. Thus, we attributed this temperature transition to a sol–gel transition, supporting the evidence that the water molecules are simultaneously acting as a plasticizer (with a decrease of the PVA glass transition temperature) and a crosslinking agent (with a decrease of the PVA melting temperature).[41,42] A sharp endothermic peak has also been reported for PVA hydrogels at $T \cong 345$ K, related to the melting of crystalline regions during a sol–gel transition.[43,44]

4. CONCLUSIONS

PVA hydrogels containing fluorescein show fluorescence spectra dependent on both the PVA content and the temperature. The fluorescence peak of fluorescein dissolved in PVA hydrogels (9.9–20.9 w/w %) present a spectral shift to lower energy at higher temperatures. In spite of this spectral shift, none was observed for fluorescein dissolved in water/ethanol (85%/15%) and PVA homopolymer or for uranine (fluorescein di-sodium salt) dissolved in water at the same temperature range. Because of these results, we attributed this spectral shift to changes of the chemical dissociation equilibrium, instead of the presence of hot bands. This photophysical behavior suggests that the hydrogel thermal transitions simultaneously change the properties of the microenvironment where the fluorescein molecules are located, leading to a new dissociation form.

The formation of hydrogels involves structural organization of the water molecules, some of them organized near the polymer macromolecules and others, for example, in the water bulk. Thus, the fluorescein molecules are distributed over the entire sample but experiment at least two types of microenvironments: the hydrogel and the water bulk. The different types of dye–environment interactions stabilize different dissociation forms of fluorescein. The phase transitions change the medium organization and, consequently, the temperature dependence of both the spectral band and the fluorescence intensity reflects these changes.

Figure 2 Fluorescence spectra at several temperatures, emission maximum (λ_{max}), and normalized intensity (I_T/I_0) temperatures for PVA hydrogels: 9.9% (a, f, k), 11.1% (b, g, l), 13.3% (c, h, m), 16.5% (d, i, n), and 20.9% (e, j, o).

Although we did not succeeded in determining the PVA hydrogel thermal transitions by differential thermal analysis, we determined that both plots of normalized intensity and of fluorescence peaks versus temperature showed slope changes occurring at temperatures assigned to the PVA hydrogel thermal transitions. We considered the onset temperatures as the beginning of the hydrogel transitions. In an attempt to assign these transitions, we considered some model systems: uranine/water, fluorescein/water/ethanol, and fluorescein/PVA homopolymer. Taking into account the transitions for these model systems and the hydrogel transitions, we made the following assignments: the sol–gel transition occurs at $T \cong 370$ K; at 320 K we observed the glass transition temperature of PVA plasticized by water molecules; we attributed the transition at $T \cong 240 \pm 5$ K to the melting point of water molecules bonded to the PVA chains (freezable bond molecules); at $T \cong 270 \pm 5$ K, we observed the melting point of free water molecules (bulk water); and, at 135–145 K the water glass transition. Similar results have been reported for PVA hydrogels with other compositions.

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