ELSEVIER

Journal of Photochemistry and Photobiology
A: Chemistry 85 (1995) 285–289

Temperature dependence of the photobleaching process of fluorescein in poly(vinyl alcohol)

D. Dibbern-Brunelli, M.G. de Oliveira, T.D.Z. Atvars*

Departamento de Físico-Química, Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, CEP 13083-970, Campinas, SP, Brazil

Received 15 December 1993; accepted 28 June 1994

Abstract

The fluorescence spectrum of fluorescein dissolved in poly(vinyl alcohol) (PVA) was recorded at various temperatures. These fluorescence spectra indicate that there is an enhancement of the photobleaching process of the dye at $T > 350$ K. This temperature corresponds to the glassy transition of PVA which involves rupture of hydrogen bonds. The rupture of these bonds has also been followed by near-IR spectroscopy. The coincidence between these temperatures suggests that the glass transition and the enhancement of the photobleaching process are correlated, and that the former is strongly dependent on the mobility of the polymer chains. The determination of this limit temperature is important for technological applications of the PVA–fluorescein system.

Keywords: Photobleaching; Fluorescein; Poly(vinyl alcohol)

1. Introduction

Fluorescein belongs to the family of xanthene dyes whose applications are dependent on the nature of the medium owing to complex dissociation and ionization equilibria [1–5]. All of these applications are based on the photophysical properties of fluorescein and its derivatives, which are dependent on the physicochemical parameters of the solvent [6–10] and, in the case of solid polymers, on the optical properties and polymer–dye interactions [9,11–14].

The photophysical deactivation processes of fluorescein [9,15] and other molecular probes have been studied recently in order to determine polymer relaxation temperatures [16,17]. The potential of fluorescein and other xanthene dyes as probes for following polymer relaxation processes and studying non-linear optical properties in devices is based on the property of these molecules to exhibit a photobleaching reaction in many solvents and polymers [7,12–14,18–20]. Leclere et al. [20] reported that xanthene-dye-doped poly(vinyl alcohol) without an electron donor did not present photobleaching on the time scale of their investigation with the irradiation intensity (Ar$^+$ laser) used to obtain the bleaching profiles. They also indicated that the presence of halogen atoms in the fluorescein derivatives enhanced the photobleaching quantum yields.

In earlier work, we have observed efficient energy transfer processes from fluorescein and its halogenated derivatives to some polymers (poly(vinyl acetate), poly(ethylene glycol) 600 and poly(vinyl alcohol)). The energy transfer processes were dependent on the internal heavy atom effect and on the hydrogen-bonding interaction in the polymer–dye systems [11].

In this work, we present the results of the dependence of the photobleaching process of fluorescein dissolved in poly(vinyl alcohol) (PVA) on temperature, using fluorescence and near-IR spectroscopy. Fluorescein dissolved in PVA was chosen as the molecular probe since it is the most stable xanthene dye in the polymer medium, the relaxation process of polymers can be followed by the strong fluorescence emission of this dye, and this dye dissolved in PVA forms a very attractive photosensitive material for non-linear applications such as holographic recording.

2. Experimental details

Fluorescein (FL) (Merck) was purified by a process known as lactonization [5].

---

*Corresponding author.

SSDI 1010-6030(94)03915-H

Poly(vinyl alcohol) (PVA) (Aldrich Co.; average molecular weight $M_w = 124\,000–186\,000$, 87%–89% hydrolysed) was used as received. FL was incorporated in the PVA film by casting an aqueous solution containing both the polymer and the dye on a glass plate. The final concentration of the dye dissolved in PVA was estimated to be 10^{-5} M since the fluorescence spectrum, centred at 520 nm, corresponds to the monomeric form of the dye.

Electronic absorption spectra were recorded at room temperature in an Intralab DMS-100 spectrophotometer using a home-built support for the polymer films (thickness, 100 μm).

Fluorescence spectra were recorded as described earlier [9]. The photomultiplier signal was collected by a lock-in amplifier (Stanford Research Systems, model SR-530) and the data were stored in a PC-type computer.

In order to obtain the fluorescence spectra at various temperatures, the FL–PVA sample was placed in an FMX-1E optical vacuum shroud assembly of a Displex model DE-204S cryo-system, pumped by a closed-cycle low-pressure helium gas compressor module (APD Cryogenics, model HC-4 MK1). The temperature of the samples was changed from 10 to 400 K by a digital temperature controller (Scientific Instruments, model 9650). The optical sample holder in the cryogenic refrigerator was positioned in order to obtain a 90° angle between the incident light and the emission beams which were focused by a cylindrical lens on the entrance slit of the spectrometer.

Near-IR spectra at various temperatures (300–470 K) were recorded using a Nicolet spectrophotometer, model FT-IR 60SX-B, and the polymer films were placed in a standard heated cell (Perkin–Elmer) with a temperature controller.

X-Ray diffraction scatterings were recorded as described earlier [16].

3. Results and discussion

As reported earlier [11], the electronic spectra of FL in PVA may be correlated with those obtained for an aqueous solution (pH 6.4) for the absorption process and for an aqueous solution (pH 8.2) for the fluorescence process. We observed an ionization equilibrium of FL dissolved in PVA involving the quinonoid and monoanionic forms in the electronic ground state and the dianionic form in the electronically excited state. The fluorescence emission is centred at 520 nm.

PVA is a polar polymer with the general formula $[\text{-CH}_2\text{CH(OH)-}]_n$ if the sample is 100% hydrolysed. This polymer is, in general, prepared by a hydrolysis reaction from poly(vinyl acetate), which can be controlled in order to obtain a well-defined percentage of hydrolysis. The general formula is $[\text{-CH}_2\text{-CH(OH)-}]_n\text{-}[\text{-CH}_2\text{-C(COOCH}_3\text{)H-}]_m$, for a partially hydrolysed polymer. Therefore PVA is a hydroxylated polymer and the macromolecules show different types of intermolecular and intramolecular interactions through hydrogen bonds, which cause the polymer chain to have a somewhat regular crystalline morphology [21,22]. It forms a semicrystalline solid, and films of this polymer exhibit a very complex morphology with supermolecular structures, including domains with very different characteristics: a crystalline phase that can be described by many methods, including X-ray diffraction patterns [22] and IR spectroscopy [23], an amorphous phase with a size distribution of free volumes, and an amorphous–crystalline interphase.

The X-ray scattering pattern of the sample is shown in Fig. 1. This pattern is typical of a semicrystalline sample, with a profile similar to that reported by many workers, and indicating that our sample contains the same unit cell as in the crystalline structure, i.e. two chain segments in the unit cell held together by hydrogen bonds [22].

The glass transition temperature of this polymer is dependent on the molecular weight and for the sample used in this work it was determined by differential scanning calorimetry (DSC) as $T_g = 360$ K.

Near-IR spectroscopy is a very convenient technique for studying hydrogen-bonding interactions in polymers produced by water molecules or even by intermolecular and intramolecular hydroxyl groups [24,25]. In general, these spectroscopic studies were recorded over a near-IR range from 4500 to 8000 cm^{-1} where two water-sensitive bands at around 5100 cm^{-1} (a combination band of $\nu\text{OH} + \nu_{\text{asOH}}$) and 6900 cm^{-1} (a combination band of $\nu_{\text{asOH}} + \nu_{\text{sOH}}$) were observed. This latter band was formed by three components assigned to three different kinds of water species, involving different degrees of hydrogen bonding from singly to doubly hydrogen-bonded water. Nevertheless, if the water molecules are in the bulk form, the band is shifted to the red region of the spectrum. The near-IR spectrum of PVA (Fig. 2) exhibits some characteristic bands centred at 4800 cm^{-1} (assigned to the stretching CO), 5200

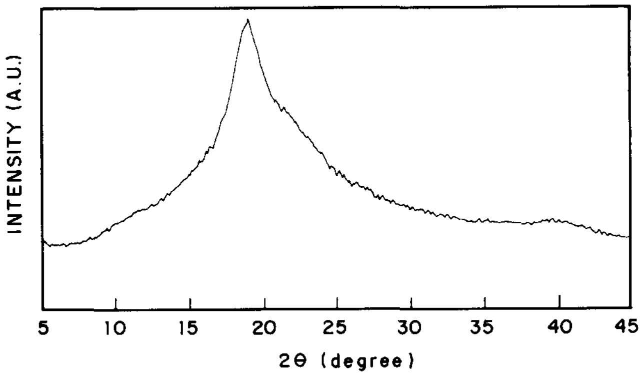

Fig. 1. X-Ray diffraction pattern for PVA.

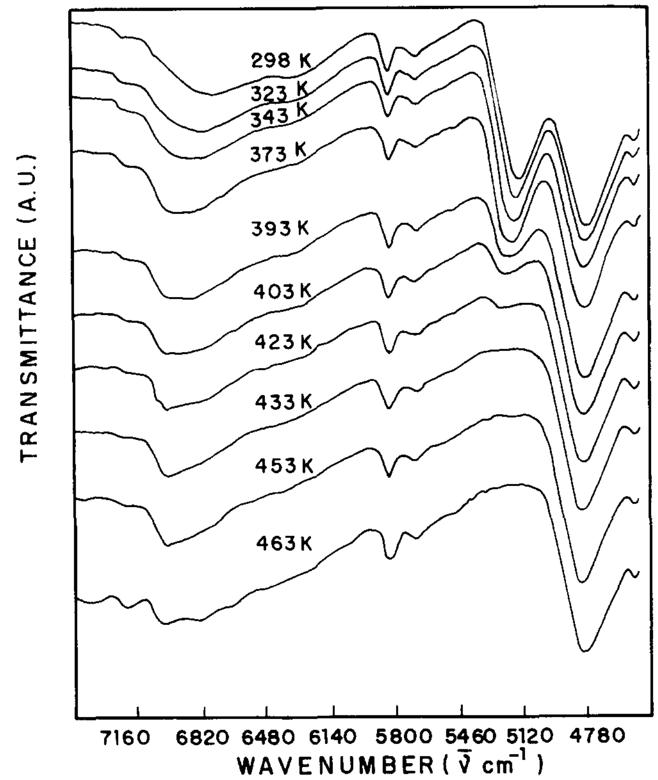

Fig. 2. Fourier transform near-IR spectra of PVA film at different temperatures.

cm^{-1} (a combination band of νOH + ν_{OH}), a doublet at 5800 cm^{-1} (a combination band of symmetric and antisymmetric CH stretching) and a very broad and complex band from 6700 to 7200 cm^{-1}, attributed to a combination of $2\nu_{OH}$ + νOH and an overtone of $2\nu_{OH}$ bands. These absorption bands were resolved into three lines, which are assigned to the hydroxyl groups associated with intramolecular, intermolecular and no hydrogen bonding, in order of increasing wavenumber.

The relative intensities of these bands are dependent on the temperature, as shown in Fig. 2. For example, if we consider the first overtone of the CH stretching modes (the doublet at 5800 cm^{-1}) as the internal standard, we observe that the relative intensities of the other bands are altered: the intensity of the combination band centred at 5200 cm^{-1} decreases, but its wavenumber remains constant, while the intensity of the band centred at 6810 cm^{-1} decreases and is shifted to a higher wavenumber with a simultaneous increase in the intensity of the band at 7010 cm^{-1}.

A plot of the intensity of the band at 5200 cm^{-1} vs. temperature is shown in Fig. 3. Significant changes in intensity are observed at T = 350 K, which is coincident with the PVA glass transition temperature, a process that occurs in the amorphous domains of the polymers: (1) a decrease in the intensity of the band centred at 5200 cm^{-1}, which is assigned to the hydroxyl groups interacting by hydrogen bonds; (2) a decrease in the intensity of the band at 6800 cm^{-1}; (3) an increase in the intensity of the band at 7010 cm^{-1}; this suggests that the former (2) may be assigned to the hydroxyl groups bonded by hydrogen bonds and the latter (3) may be assigned to free hydroxyl groups. This blue shift of the wavenumber of the bands attributed to free hydroxyl groups was also obtained for water in the vapour phase, compared with the bulk [25].

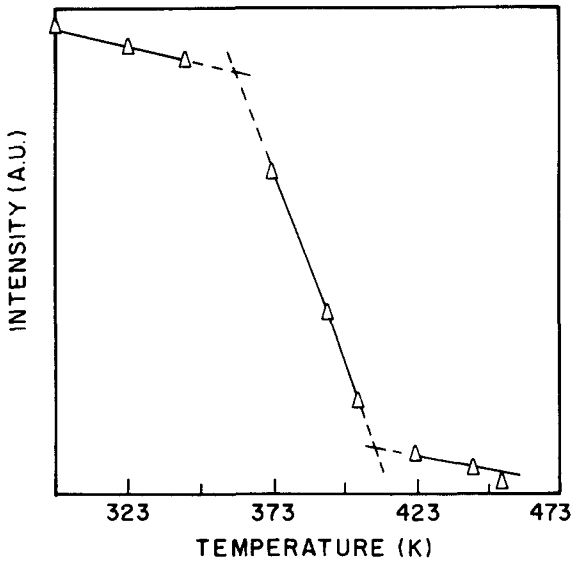

Fig. 3. Intensity of the 5200 cm^{-1} band of PVA at different temperatures.

From these results, we can conclude that thermal motions of the polymer chains at $T > T_g$ produce a significant decrease in the amount of associated hydroxyl groups which become free to move by the glass relaxation process. Furthermore, a certain amount of the crystalline phase remains in the solid phase and could explain the significant intensity of the band at 5200 cm^{-1}, present in this temperature range. It is expected that groups interacting by hydrogen bonds in the crystalline phase will only behave as free groups at temperatures higher than the melting temperature.

From the data of Fig. 3, we observe that the band at 5200 cm^{-1} disappears at temperatures around 430–450 K, suggesting that hydrogen bonds between the hydroxyl groups have been destroyed. This temperature range could be assigned to a PVA relaxation process associated with the crystalline domains, as determined by the mechanical dynamic relaxation method [26]. It has been reported that the melting point of semicrystalline PVA is observed in the range 475–513 K and that it is dependent on the synthetic method, degree of hydrolysis and molecular weight [23]. However, it is very difficult to determine this temperature since the polymer suffers thermal degradation processes occurring in two steps: (1) there is water loss in the temperature range 440–520 K, producing a polymer chain with a polyenic structure; and (2) there is an elimination reaction of aromatic hydrocarbon molecules at $T=600$ K. We do not observe thermal degradation, since our study was limited to a temperature of 460 K. Nevertheless, from our data we estimate that the α relaxation process temperature is around 430 K, from the decrease in intensity of the band at 5200 cm^{-1} prior to the PVA melting point.

Fluorescence spectra of FL dissolved in PVA at various temperatures were obtained. These spectra may be assigned to the monomeric form of the dye in its dianionic form and exhibit a red shift compared with the emission in water. There are many results indicating that the incorporation of a dye in a semicrystalline polymer occurs mainly in the amorphous and interphase domains [15–17,27,28]. Consequently, all the photophysical and photochemical processes produced by luminescent molecules dissolved in polymers should be restricted to those regions of the microheterogeneous solid polymers.

The integrated intensity of FL–PVA fluorescence bands decreases as the temperature increases (Fig. 4). Moreover, the decay profile is not monotonic, but a pronounced inflexion of the intensity is observed at temperature $T=350$ K, which is also coincident with the PVA glass transition temperature. It is noteworthy that the fluorescence intensity is drastically reduced after the polymer glass transition, resulting in an irreversible dye photobleaching quantum process on the film surface at the excitation focal point. Therefore, we can associate the increase in the mobility of the polymer chains with an increase in the dye photobleaching yield. Consequently, if the hydrogen bonds are destroyed, as observed by near-IR spectroscopy, this process changes both the photophysical and photochemical processes of the molecules in the electronically excited state. Considering that FL molecules are localized either in the amorphous or crystalline–amorphous interphase regions, but never inside the PVA unit crystal cell, the photobleaching phenomenon must also occur mainly in these regions.

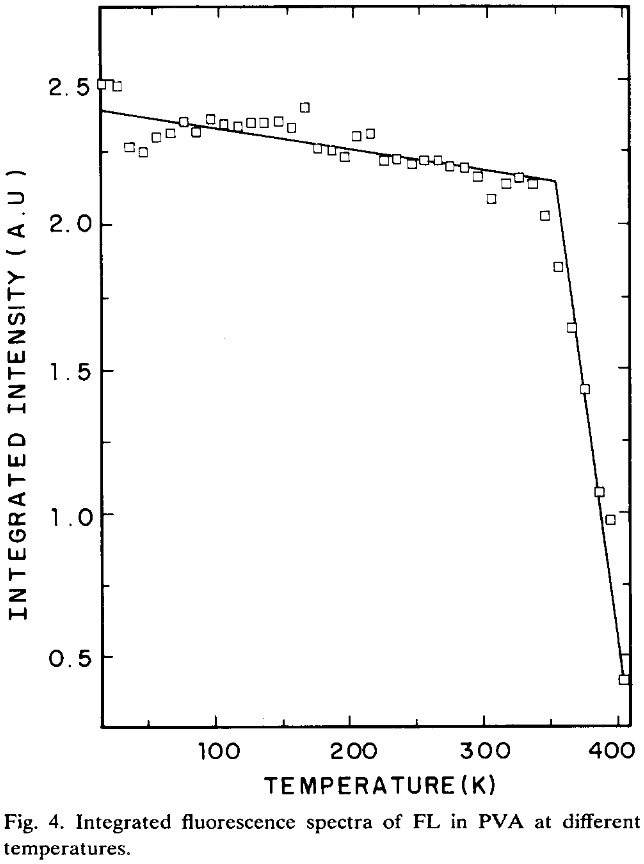

Fig. 4. Integrated fluorescence spectra of FL in PVA at different temperatures.

Leclere et al. [20] reported that the photobleaching of this kind of dye, under visible excitation at room temperature, involves a PVA matrix that behaves as an electron donor and the dyes change into the leuco form. They suggested that the mechanism occurs in a similar manner as that obtained if an electron donor molecule (such as an amine) is added to the matrix. In this case, the photochemical reduction of the xanthene dyes in the presence of an amine is a triplet process and the dye reduction by oxygen or by the partner amine competes with energy transfer to acceptor oxygen. Other reports also indicate that the photobleaching reactions of FL and other xanthene dyes involve electron transfer to give a semireduced dye radical which suffers a disproportionation to a leuco dye [29].

As indicated earlier, we observed a remarkable increase in the photobleaching yield of FL in PVA at temperatures higher than T_g ($T>350$ K), the temperature which produces an increase in the mobility of the polymer chains induced by the breaking of intermacromolecular and intramacromolecular hydrogen bonds. Although we do not know, at this moment, the details of the photobleaching mechanism of FL in PVA, we have also observed, for some vinyl polymers, such as PVA and poly(vinyl acetate), an enhancement of the polymer fluorescence emission centred at 430–450 nm in the presence of xanthene dyes, mainly if the xanthene dye contains halogen atoms (e.g. erythrosin and rose bengal) and if there are strong polymer–dye interactions, e.g. hydrogen bonds [11]. This emission in the UV region of the spectrum has been assigned to chromophores present in these polymers, such as $-(CH=CH)_n-CO$ for $1<n<3$ [30]. The presence of these electron donor groups may be responsible for the FL photobleaching. Considering that the amount of these groups also increases with the thermal decomposition of PVA, we must expect an enhancement of the photobleaching process at higher temperatures.

4. Conclusions

PVA is a very interesting polymer for many technological applications since it forms a transparent film, is commercially available in different degrees of hydrolysis and different molecular weights, is soluble in water, as are many interesting dyes, and is very attractive as a host for non-linear optical material. If the guest molecule is FL, as in the present work, the PVA–FL system is stable over a large temperature range. In this work, we have shown that the PVA–FL system is photochemically stable only for temperatures lower than 350 K.

Nevertheless, FL dissolved in PVA is a very convenient probe to study the relaxation processes of the polymer matrix, as indicated in other work [15]. Specifically, in this work, we demonstrated that the intensity of the fluorescence of this dye may be utilized to determine the PVA glass transition temperature, also obtained by DSC measurements. We have also demonstrated by near-IR spectroscopy that the glass transition involves mobility of the polymer chain produced by the breakage of hydrogen bonds at T_g, and that this process induces changes in the photophysical and photochemical processes of the dye, which is decomposed to the leuco form.

Acknowledgements

D.D.B. acknowledges the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for a fellowship. Financial support from CNPq/PADCT, Financiadora de Estudos e Projetos (FINEP) and Fundação de Amparo a Pesquisas do Estado de São Paulo (FAPESP) is acknowledged.

References

[1] M.M. Martin and L. Lindqvist, *J. Lumin., 10* (1975) 381.
[2] H. Dihel and R. Markuszewiski, *Talanta, 32* (1985) 159.
[3] H. Dihel, *Talanta, 36* (1989) 413.
[4] P.R. Ojeda, I.A.K. Amashta, J.R. Ochoa and I.L. Arbeloa, *J. Chem. Soc., Faraday Trans. 2, 84* (1988) 1.
[5] D. Dibbern-Brunelli and T.D.Z. Atvars, *Spectrosc. Lett., 23* (1990) 627.
[6] G.R. Fleming, A.W.E. Knight, J.M. Morris, R.J.S. Morrinson and G.W. Robinson, *J. Am. Chem. Soc., 99* (1977) 4306.
[7] L.E. Cramer and K.G. Spears, *J. Am. Chem. Soc., 100* (1978) 221.
[8] O. Valdes-Aguilera and D.C. Neckers, *Acc. Chem. Res., 22* (1989) 171.
[9] C.A. Bortolato, T.D.Z. Atvars and D. Dibbern-Brunelli, *J. Photochem. Photobiol. A: Chem., 59* (1991) 123.
[10] K.K. Rohatgi and A.K. Mukhopadhyay, *J. Indian Chem., 49* (1972) 1311.
[11] T.D.Z. Atvars, C.A. Bortolato and D. Dibbern-Brunelli, *J. Photochem. Photobiol. A: Chem., 68* (1992) 41.
[12] K.P.B. Moosat, T.M.A. Rasheed, V.P.N. Nampoori and K. Sathianandan, *Appl. Opt., 29* (1990) 449.
[13] Y. Silberg and I. Bar-Joseph, *Opt. Commun., 39* (1981) 265.
[14] H. Fujiwara and K. Nakagawa, *Opt. Commun., 55* (1985) 386.
[15] T.D.Z. Atvars, C.A. Bortolato, D. Dibbern-Brunelli and E. Sabadini, *J. Appl. Polym. Sci., Appl. Polym. Symp., 49* (1991) 167.
[16] T.D.Z. Atvars, E. Sabadini and S.M. Martins-Franchetti, *Eur. Polym. J., 29* (1993) 1259.
[17] S.M. Martins-Franchetti and T.D.Z. Atvars, *J. Appl. Polym. Sci., 50* (1993) 1591.
[18] J.J.A. Couture, R.A. Lessard and R. Changkakoti, ICO 15, SPIE Vol. 1319, Optics in Complex Systems, 1990, 281.
[19] T. Todokorov, L. Nikolova, N. Tumova and V. Dragotinova, *IEEE J. Quantum Electron., 22* (1986) 1262.
[20] P. Leclere, G. Manivannan, S. Semal, R. Changkakoti, Y. Renotte, R.A. Lessard and Y. Lion, *3rd Int. Conf. on Polymer Photochemistry, 1993, Italy,* p. 80.
[21] A. Tager, *Physical Chemistry of Polymers,* MIR Publishers, Moscow, 1972.
[22] R.C.L. Mooney, *J. Am. Chem. Soc., 36* (1941) 2828.
[23] N.A. Peppas and P.J. Hansen, *J. Appl. Polym. Sci., 27* (1982) 4787.
[24] G. Herzberg, *Molecular Spectra and Molecular Structure,* Van Nostrand, New York, 2nd edn., 1950, p. 281.
[25] M. Fukuda, H. Kawai, N. Yagi, O. Kimura and T. Ohta, *Polymer, 31* (1990) 295.
[26] P.D. Garrette and D.T. Grubb, *J. Polym. Sci., Part B, Polym. Phys., 26* (1988) 2509.
[27] P.J. Phillips, *Chem. Rev., 90* (1990) 425.
[28] Z. He, G.S. Hammond and R.G. Weiss, *Macromolecules, 25* (1992) 1568.
[29] D.F. Eaton, in D.H. Volman, G.S. Hammond and K. Gollnick (eds.), *Advances in Photochemistry,* Vol. 13, Interscience Publication, Wiley, New York, 1986, p. 427.
[30] J. Mieloszyk, R. Drabent and Z. Siodmiak, *J. Appl. Polym. Sci., 34* (1987) 1577.