In Situ Chemical Analysis of Domains in Polymer Blends by Optical Fluorescence Microscopy

D. DIBBERN-BRUNELLI and T. D. Z. ATVARS*

Departamento de Físico-Química, Instituto de Química, Universidade Estadual de Campinas, Caixa Postal 6154, CEP 13084-100, Campinas, SP, Brazil

SYNOPSIS

Poly(vinyl alcohol) (PVA)/poly(vinyl acetate) (PVAc) blends in different proportions show phase separation in the solid state. The dimensions, shapes, and distributions of the domains produced by the phase separation process are dependent on the composition and can be observed by conventional optical microscopy. However, using fluorescence optical microscopy of polymer blends in different compositions containing anthracene and/or fluorescein molecules dissolved in the matrices we were able to do a chemical discrimination of each domain. We also observed that anthracene is mainly localized in PVAc domains and fluorescein in PVA domains, and that the diffusion process of these fluorophers within the matrices is controlled by specific polymer–dye interactions. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Studies of polymer miscibility are relevant from both fundamental and technological points of view, since the final properties of the materials are dependent on the phase separation processes. There are some criteria defining miscibility in solid polymer matrices. For example, amorphous polymers are usually regarded to be miscible when they maintain their transparency after blending. This can be satisfied when the size of the dispersed phase is smaller than 0.5 μm, or when its refractive index is similar to that of the matrix.[1]

In general, the dispersion of phases is dependent on a large number of different parameters, including relative viscosity influenced by shear rate, temperature, and other extrinsic experimental parameters, and by surface tension (dependent on molecular interactions). Moreover, as pointed out by Utracki,[2] when analyzing the composition as a function of the linear dimension l in immiscible blends one finds that between a domain of polymer A and that of polymer B there is an interfacial layer of a thickness Δl, described as an "interphase," with its own characteristic properties. Although molecular interactions and the dimensions of the interphase region are difficult to evaluate, they produce important effects on the reproducibility of performance, processability, and recyclability of the material.[2]

Fluorescence optical microscopy (FOM) is a technique that combines the fluorescent properties of some molecules with optical microscopy. Recent applications of this technique in polymer science include (1) determination of additive distribution in semicrystalline polymers, (2) studies of morphology, distribution of oxidative degradation regions, and macromolecular interpenetration processes,[3–5] and (3) miscibility in polymer blends.[6–8] FOM is also a convenient technique to observe distribution of molecular probes in polymer blends with phase separation as an additional information to support studies of secondary relaxation processes by the photoluminescence technique.[8–10] This technique allows the observation of domains with sizes larger than 0.1 μm and, if combined with other information such as specific intermolecular interactions or the presence of crystalline domains, allows the determination some morphological characteristics of the material.

Intermolecular interactions in polymeric systems can be studied by different methods, including NMR, infrared spectroscopy,[2] and scanning transmission

* To whom correspondence should be addressed.

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electron microscopy.[1] Infrared spectroscopy can be also used to study phase separation processes with a spatial resolution of approximately 50 μm using the spectrophotometer coupled with a microscope (M-FTIR).[11]

In a recent work we studied relaxation processes of poly(vinyl alcohol)/poly(vinyl acetate) blends using the photoluminescence technique.[8] As these polymers were not luminescent, we dissolved fluorophers (anthracene and/or fluorescein) in these blends, to follow the dependence of the fluorescence intensity on the temperature of the system. In the present work we attempt to identity different domains produced by the phase separation process of these blends in different proportions after the blending process by casting. This identification has been performed using FOM in order to determine the distribution of fluorescent molecules in the material and consequently to perform an *in situ* chemical analysis of each domain. This approach is possible since we obtained a very strong blue emission of anthracene dissolved in PVAc and a very strong green emission of fluorescein dissolved in PVA.

EXPERIMENTAL

Samples of poly(vinyl alcohol) (PVA), poly(vinyl acetate) (PVAc), and their blends in different proportions (9/1, 1/1, and 1/9), containing fluorescein (FL), anthracene (AN), or both fluorophers, were prepared in the form of films, by casting appropriate solutions, using ethanol/acetone as solvents, containing both homopolymers and the probes over a polystytrene plate covered by a polyethylene film. All the films prepared in this work were dried at room temperature and normal pressure and maintained in a desiccator under vacuum until the measurements. These samples were also characterized as described previously.[8]

We have obtained fluorescence photomicrographs of different parts of these samples for a complete description of the material. These samples may be divided into two parts: one more regular, thinner (30–40 μm), and homogeneous and the other rougher and thicker ($\cong$ 100 μm).

Fluorescence optical microscopy (FOM) was performed using a standard Zeiss Jena Universal Microscope. The excitation wavelength of the UV source was selected using an appropriate dichroic mirror ($\lambda_{exc} < 410$ nm), and magnification was chosen as 100 times for all of the micrographs. Samples for FOM were the same as those reported earlier.[8]

We have performed FOM of PVA/PVAc blends using two types of microscope configurations for the light beams. In the first case we have used the reflection fluorescence technique where the UV excitation beam is mounted above the sample plane. In this case any light that is not absorbed passes harmlessly through the sample and does not enter the imaging system. The specimen fluorescence is captured and imaged by the objective, which is used as a condenser. Only the area of sample being viewed is excited, giving improved sensitivity at higher magnifications. In this case, illumination and imaging take place on the same side of the specimen and the maximum fluorescence is produced in the layer being observed.[3–5,11] Therefore, in this microscope configuration we can obtain good information about fluorescent sites of PVA/PVAc samples covering all of the deep profiles of light penetration. We demote this configuration as reflection optical fluorescence microscopy (R-FOM).

In the second case we have combined the relection FOM technique (using a UV light beam) with conventional transmission optical microscopy (TOM) using a white light beam. Consequently, we obtain information related to both the bulk and the surface of the samples. In this configuration the emission intensity in the fluorescent region is lower than the white transmitted beam and consequently only those very strong fluorescent regions appear colored.

RESULTS AND DISCUSSION

Characterization of Samples

As shown earlier[7,8] PVA/PVAc blends exhibit a solid phase separation producing, on the optical microscopic scale, two types of domains whose dimensions and distributions are dependent on the composition. The dispersion of one polymer in the other has been observed for blends containing fluorophore by fluorescence spectroscopy. We show in Figure 1 fluorescence spectra of AN dissolved in PVAc, FL dissolved in PVA, and both fluorophore dissolved in different parts of samples of the 1/1 PVA/PVAc blend, at a temperature of 45K. Similar spectra were also obtained for blends in other proportions. The most important differences were the relative intensities of the fluorescence bands assigned to AN (380–480 nm) and FL (500–550 nm) in each part of the samples. The fluorescence spectrum of AN dissolved in both PVAc homopolymer and PVA/PVAc blends is composed of a vibronic progression and may be

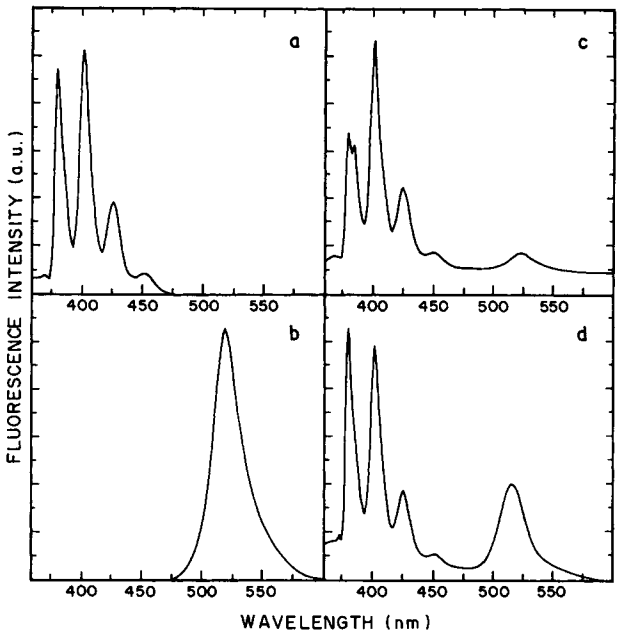

Figure 1 Fluorescence spectra at 45K of (a) AN dissolved in PVAc, (b) FL dissolved in PVA, (c) FL and AN dissolved in different parts of a 1/1 PVA/PVAc blend.

assigned to the isolated molecule, as indicated by the relative intensities of the bands centered at $\lambda \cong 380$ nm and $\lambda \cong 402$ nm.[12] The fluorescence spectrum of FL dissolved in PVA homopolymer and PVA/PVAc blends is composed of a strong band centered at $\lambda \cong 520$ nm and may be assigned to the fluorescence emission of its dianionic form in the electronically excited singlet state.[13] The intensities of these fluorescence bands are dependent on the temperature and can be used to determine relaxation processes of the homopolymers themselves or of their blends.[8]

From the fluorescence spectra obtained for both homopolymers and different parts of the 1/1 PVA/PVAc blend we obtained a significant change of the relative intensities of FL and AN spectra. In other words, we observed that there were parts of the samples more concentrated in AN (high blue fluorescence emission) and others more concentrated in FL (high green fluorescence emission). FL interacts more strongly with PVA domains while AN will interact more strongly with PVAc domains of the materials, then we expect a diffusion of FL toward PVA rich domains and of AN toward the PVAc rich domains. These diffusion processes of the fluorophore dissolved in different homopolymers during blending are induced by specific dye–polymer interaction forces, such as hydrogen bonding between FL and PVA and dispersive forces between AN and PVAc. Therefore, differences in the relative intensities of AN and FL fluorescence spectra in different parts of PVA/PVAc blends indicate that these parts present different compositions of both homopolymers, produced by a phase separation process.

As reported earlier[8] we could consider that each polymer blend studied in this work is composed of at least four complex phases: the crystalline phase has been assumed to be constituted purely of crystallizable PVA macromolecules without any fluorophore; there is an interphase between PVA and PVAc homopolymers, whose composition is not easily determined, but probably presents a gradient of concentration between the two amorphous domains and probably will also exhibit a gradient of fluorophore concentration; and there are two amorphous phases that should be formed by both pure noncrystallizable PVA and PVAc homopolymers localized in the interfibrillar space. These amorphous regions should also contain the fluorophores.

If different fluorophores occupy different amorphous or interphase regions, depending on the specific dye–polymer interactions, all of the relaxation processes of polymers and polymer blends measured using fluorescence spectroscopy are occurring at specific locations in the matrices.

Fluorescence Optical Microscopy (FOM)

We have performed R-FOM using FL, AN, and both fluorophores dissolved in the polymer blends. Photomicrographs were obtained using both microscopy configurations: reflection fluorescence optical microscopy (R-FOM) and reflection combined with conventional transmission optical microscopy (TOM). Photomicrographs using both techniques are shown in Figures 2–4 for different parts of PVA/PVAc blends containing fluorophores.

R-FOM of PVA/PVAc blends containing one of those fluorophores may be represented by photomicrographs with colored regions (where fluorophores are located) and dark regions (where they are absent). Different colors might be observed depending on the fluorescent molecules if UV light is able to excite the molecules to their electronic excited states. In our case we observe green regions containing FL and blue regions containing AN (Figs. 2–4 top). The presence of regions with these different colors may be explained by the different solubilities of AN and FL in PVAc and PVA richer domains, respectively. These figures are organized in sequence of higher PVAc composition (from a to c); 1 represents the thinner parts of the samples while

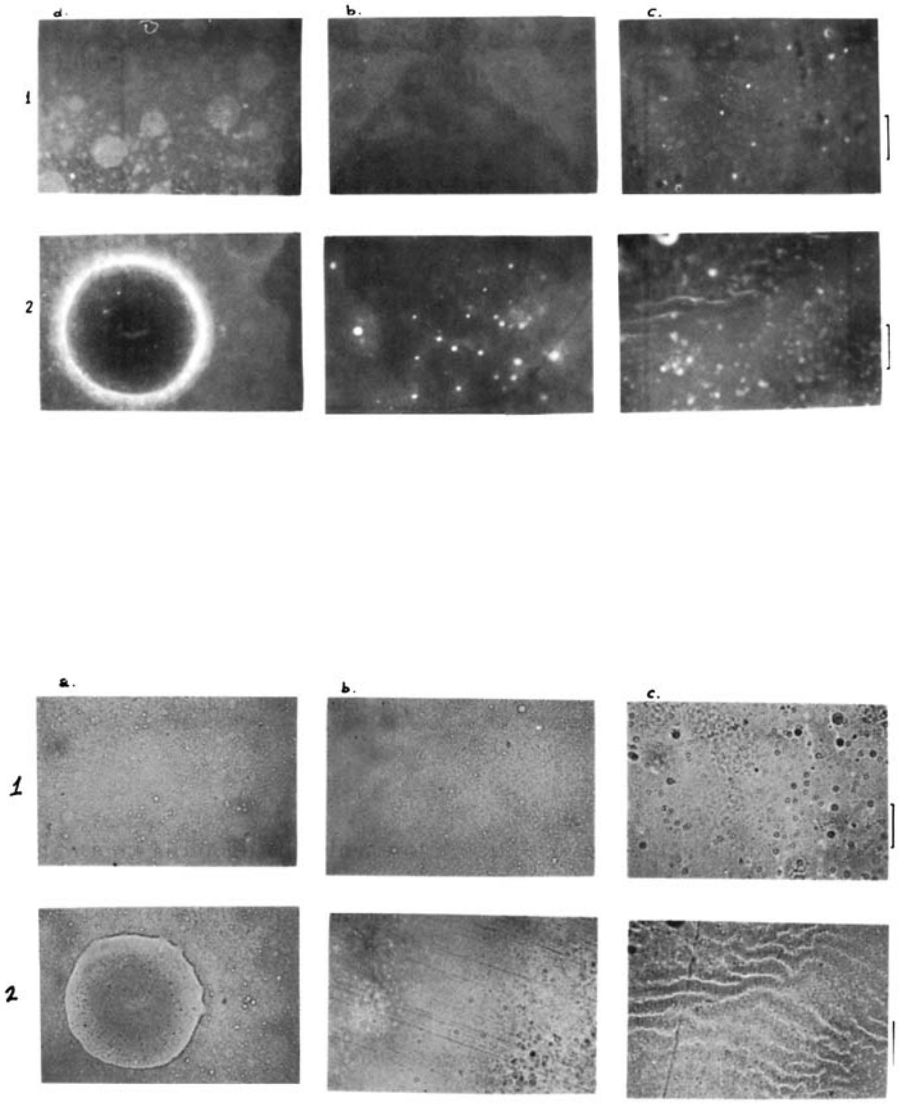

Figure 2 Photomicrographs of different parts of PVA/PVAc blends containing FL, in proportions (a) 9/1, (b) 1/1, (c) 1/9 (top, R-FOM; bottom, R-FOM/TOM). Sequence 1: thinner part of the samples; sequence 2: thicker parts of the samples (scale $\cong$ 200 μm).

2 represents the thicker parts of samples of each initial composition.

In the second case (Figs. 2–4 bottom) we have combined the R-FOM technique (using a UV beam) with TOM using a white light beam. Although in the case of FL dissolved in the samples (Fig. 2) we also observe green regions, with less contrast, we obtain better visual evidence of domains without fluorescent FL as almost red regions in the central part of the circular domains which are produced by FL crystallization due to its lower solubility in PVAc-rich domains. Moreover, the interphase regions are defined by a very strong green emission around each domain indicating a gradient of fluorophore concentration. Using these two combined techniques we can conclude that

1. there is a phase separation in these blends for all initial compositions, producing a heterogeneous material containing parts whose composition is different from the initial composition;
2. the phase separation process produces a biphasic system, composed of one richer in PVA (sequence 1) and one richer in PVAc (sequence 2);
3. photomicrographs of different parts of the blends prepared in 9/1, 1/1, and 1/9 PVA/

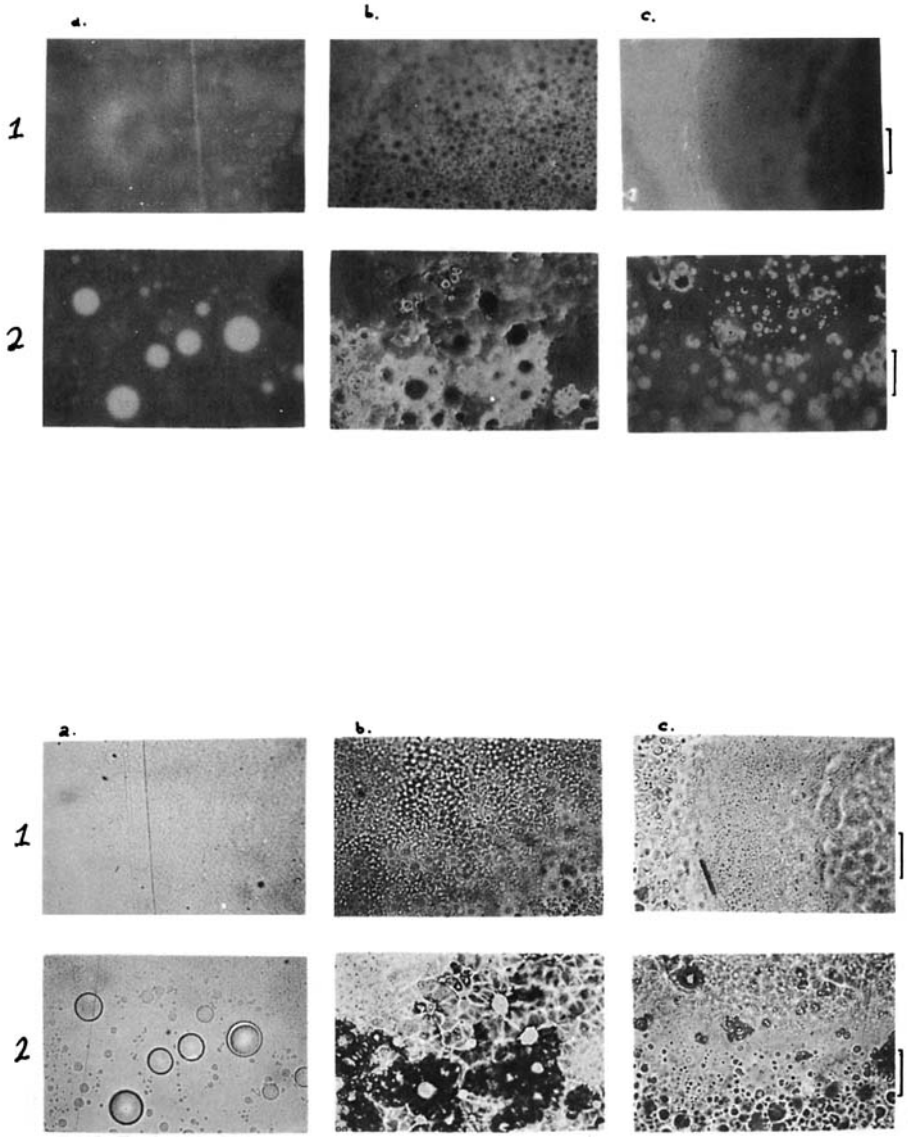

Figure 3 Photomicrographs of different parts of PVA/PVAc blends containing AN, in proportions (a) 9/1, (b) 1/1, (c) 1/9 (top, R-FOM; bottom, R-FOM/TOM). Sequence 1: thinner part of the samples; sequence 2: thicker parts of the samples (scale $\cong$ 200 μm).

PVAc initial concentrations containing FL or AN, present a set of fluorescent regions (blue or green depending on the fluorophore) and nonfluorescent regions, indicating the absence of the fluorophores (Figs. 2 and 3 top);

4. considering the specific interactions between the fluorophores and the homopolymers we can identify the green fluorescent domains as those richer in PVA and those with blue emission as those richer in PVAc.

To improve the contrast between the dark and fluorescent regions in those different blends we have prepared samples containing both fluorophores and using these samples we have performed both R-FOM (Fig. 4 top) and R-FOM/TOM (Fig. 4 bottom). Photomicrographs for these samples present regions with a blue emission (containing AN and consequently richer in PVAc) and regions with a green emission (containing FL and consequently richer in PVA). These photomicrographs follow the same sequence as those of Figures 2 and 3.

From the analysis of photomicrographs shown in Figures 2–4 we can observe that both dark (top) and red (bottom) domains of samples containing FL appear as blue domains in samples containing

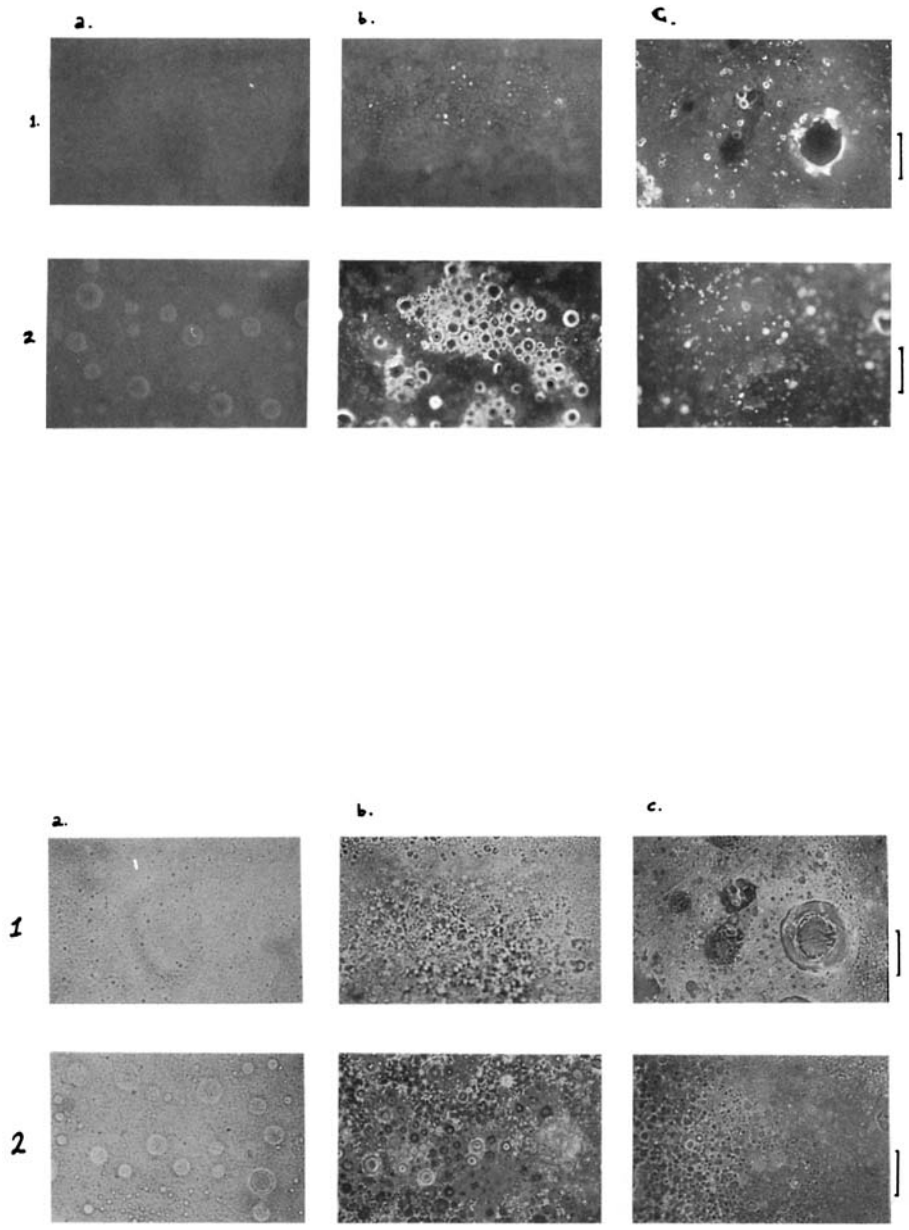

Figure 4 Photomicrographs of different parts of PVA/PVAc blends containing FL and AN, in proportions (a) 9/1, (b) 1/1, (c) 1/9 (top, R-FOM; bottom, R-FOM/TOM). Sequence 1: thinner part of the samples; sequence 2: thicker parts of the samples (scale $\cong$ 200 μm).

AN or both fluorophores, while green domains present in Figures 2 and 4 appear as dark domains in Figure 3.

Different parts of those blends also show interfaces with different morphological properties. In general, these interfaces are thicker and in some cases rougher than other parts of the samples. R-FOM/TOM photomicrographs of some parts of the FL containing samples exhibit a pronounced gradient of concentration of fluorophores across the interface. For example see Figure 2(a), bottom, where we can observe a decrease of the concentration outside of the domain, but the green emission is maintained, while inside the domain there is also a decrease of the green emission achieving a red color in the central part produced by the crystallized FL. Using information related to the solubility of FL in different media we can conclude that there is a continuous gradient of the composition of homopolymers across the interface. In other words, there is

some degree of miscibility of these homopolymers producing parts with different compositions from the initial proportions.

These gradients of concentration have also been observed in photomicrographs 2c, top and bottom, and in this case, are revealed by AN. There are parts of the material without AN (dark regions in the top and transparent regions in the bottom), very intense blue regions (richer in AN) which appear strongly colored even using the R-FOM/TOM technique, and moderately intense blue regions between those two. This interface may be represented by an "interphase" region between the two phases formed by the homopolymers. Figure 5 illustrates a sketch for those "interphase" regions in Figures 2(a), bottom and 3(c), bottom, showing the distribution of the fluorescent molecules across the interphase from a PVAc-rich phase to a PVA-rich phase.

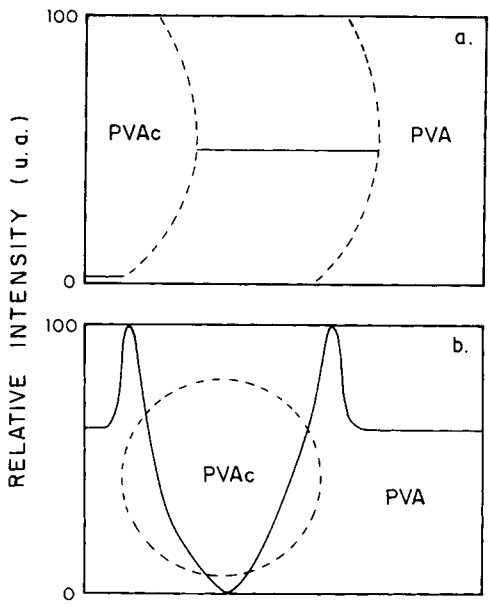

Figure 5 Sketch of the fluorescence intensity profile and R-FOM photomicrographs of an interphase of PVA/PVAc blend: (a) containing AN; (b) containing FL.

CONCLUSION

From the results reported in this work we conclude that there is a diffusion process of different fluorophores in polymer blends controlled by specific dye–polymer interactions. Using appropriate fluorophores we were able to define different chemical domains in a solid-state polymer blend. We assume that AN is preferentially located in PVAc-richer domains while FL is located in PVA-richer domains, as shown in the different photomicrographs.

Moreover, from the morphological results revealed by photomicrographs we can suggest a mechanism for the phase separation processes of these blends. The initial solution from which the films were cast contains four components: PVA (water soluble), PVAc (ethanol soluble), water, and ethanol. Phase diagrams for this system have not been determined. When enough ethanol is present a single phase exists. The ethanol vapor pressure is higher than that of water and its evaporation rate is also higher. As this solvent evaporates, the system passes into a two-phase region and begins to demix. Therefore, the system becomes formed by a PVA-richer solution and a PVAc-richer and swollen phase. The final morphology obtained in bulk will be determined by the rate of solvent evaporation compared to that of demixing,[14] which is dependent on the initial PVA/PVAc proportion.

The solution within the binodal composition produces nucleation and growth, which give rise to a dispersion of spheres of the minor component in the major component matrix. In the case of the PVA/PVAc system, PVAc is first demixed due to its lower solubility and the remaining solution is always richer in PVA. Therefore, the spherical shape of PVAc domains will be present in the PVA-richer substrate. The spherical shape of PVAc domains is a consequence of surface energy minimisation within each droplet, and reflects different "interphase" tensions.[14] Moreover, the most likely origin of the broad distribution of droplet sizes is phase separation by nucleation and growth, which are both dependent on the initial composition of PVA/PVAc solutions.

During film preparation, solvent evaporation from the surface is rapid. Then, phase separation begins at the surface after the surface composition passes into the spinodal region of the phase diagrams. The skin formed on the surface slows down the solvent rate evaporation from the bulk contributing to the crossover in phase separation. As PVAc is first demixed, this surface layer will probably be richer in PVAc and the droplets will grow in samples prepared with higher PVAc composition by coalescence of the droplets. Therefore, all PVAc-richer parts of the samples will be remarkably heterogeneous as a result of the higher solvent rate evaporation (nucleation of the droplets by demixing process) and PVAc droplets growth (by droplet coalescence). Moreover, for the higher initial composition of PVAc (Figs. 2–4, sequence 2) all of the material is more heterogeneous than for samples with lower PVAc composition (Figs. 2–4, sequence 1).

Solvent evaporation of the remaining PVA-rich solutions will be slower for many reasons: (1) there

is a skin formed by the PVAc-rich phase, (2) the remaining solvent is mainly composed of water, which has a lower vapor pressure than ethanol, and (3) this medium is continuously becoming viscous and then the diffusion rate of the PVAc droplets is lower. Therefore, parts of the sample richer in PVA are more homogeneous, exhibit a disperse phase containing spherical droplets of PVAc, and the size distribution of these droplets is also more homogeneous, suggesting that nucleation and growth of the droplets are not important processes.

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