# ESR Spectroscopy as a Probe of the Morphology of Hydrogels and Polymer–Polymer Blends[1]

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ABSTRACT: The use of spin-labeled solutes to determine the morphology of polymer–water blends (hydrogels) and polymer–polymer blends was evaluated. The ESR spectra of the nitroxides 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO were measured in poly(2-hydroxyethyl methylacrylate) (PHEMA), poly(vinyl alcohol) (PVA), and polyacrylamide (PAA) at pH 4.45 and 10.5. The spectra in PHEMA were independent of pH and could be simulated as single species using the theory of Freed et al. developed for slow-tumbling species. Similar results were obtained with the nitroxides in poly(vinyl alcohol) and polyacrylamide. There was no evidence of either bulk or ordered water existing as phase-separated domains within the hydrogels. The ability to detect aqueous domains was verified by measurement of the spectra of the nitroxides in a porous form of PHEMA; in this case, the spectra were pH dependent and could only be simulated as the sum of the spectra in water and nonporous PHEMA. The ability of ESR spectroscopy to detect phase separation in polymer–polymer blends was tested using blends of PVA with poly(glycolic acid-*co*-lactic acid (PGLA) and poly(ϵ-caprolactone) (PCL). Only single nitroxide species were observed in a miscible 20:80 blend of PGLA and PVA, whereas composite spectra with pH-dependent line shapes and rotational correlation times were observed for the phase-separated 80:20 blend. The method showed that both 80:20 and 20:80 blends of PVA and PCL were miscible. Three block copolymers of poly(ethylene glycol) (PEG) and poly(lactic acid) were similarly evaluated, and in one case partial phase separation was observed. The advantages and limitations to this method of characterizing blend miscibility are discussed.

## Introduction

The compatibility of polymer blends is commonly assessed by dynamic mechanical analysis, differential scanning calorimetry, and microscopic observation of phase separation. Spectroscopic methods have been used to examine the compatibility of polymer blends at the molecular level.[2] None of these methods is unambiguous. Some of the difficulties of interpretation are illustrated by the literature on hydrogels, which can be considered as blends of polymer and water. Early studies of hydrated poly(2-hydroxyethyl methacrylate) (PHEMA) and structurally related hydrogels using DSC and NMR spectroscopy led to the proposal that at least three types of water may coexist within a hydrogel: water bound to the polymer skeleton by hydrogen bonding, an interfacial or partially ordered phase, and bulk water.[3] This model of hydrogel morphology, which corresponds to phase separation of a polymer–water blend, in turn led to the concept that hydrophilic solutes are primarily associated with the bulk water phase, while hydrophobic solutes partition to a greater extent into the polymer phase. As an extension of this model, it was proposed that diffusion of hydrophilic solutes in hydrogels occurs primarily via aqueous pores, while lipophilic solutes diffuse within the polymer phase.[4]

Recently, the existence of different water phases in hydrogels has been challenged and much of the original experimental data has been reinterpreted.[5–7] For example, adiabatic and isoperibolic calorimetry measurements of the melting of frozen water in PHEMA and other hydrogels failed to detect the endotherm that had previously been attributed to an ordered water phase. Notwithstanding this report, it can be argued that calorimetry is not an appropriate probe of hydrogel morphology; freezing constitutes a nonequilibrium perturbation of the system which may induce phase separation not present in the unfrozen state. NMR spectroscopy, conducted at ambient temperatures, is not subjected to this criticism but recent relaxation studies with oxygen-17 labeled water also conflicted with earlier $^1$H-NMR measurements by failing to detect more than a single water phase. However, rapid exchange of water between different phases may not be detectable on an NMR time scale, and the oxygen-17 studies, while questioning the correctness of earlier data,[8] did not disprove the existence of multiple water types.

We have used a different experimental method to probe the equilibrium morphology of hydrogels and polymer–polymer blends. The method is based on the sensitivity of the ESR spectrum of nitroxides to their environment. The rotational correlation time ($\tau_c$) of nitroxides, determined from the line shape of their ESR spectra, has frequently been employed to assess molecular motion in the solid and liquid crystalline state, e.g., biological membranes, liposomes, latexes, and macroporous polymeric resins.[9] This analytical method should also be relevant to blend morphology for, clearly, the presence of a nitroxide in more than a single phase will be evident by an increased complexity in the ESR spectrum. The reliability of this method has been improved by using the amino-substituted nitroxides 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO. The lipophilicity of these reporter molecules is changed significantly by protonation of the amino group. If more than a single phase exists within a hydrated hydrogel, a pH change will result in redistribution of the spin probe between phases (Figure 1), manifested by a change in line shape of the ESR spectrum.

The potential of this method for determining the morphology of polymer–polymer blends has been explored using blends of poly(vinyl alcohol) (PVA) with poly(glycolic acid-*co*-lactic acid) (PGLA) and poly(ϵ-caprolactone) and block copolymers of poly(ethylene glycol) and poly(DL-lactic acid).

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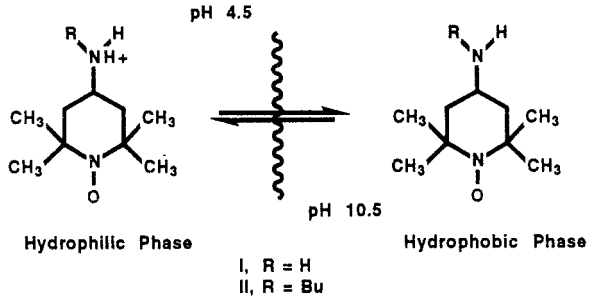


**Figure 1.** Distribution of unprotonated and protonated 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO between polymer (hydrophobic) and water (hydrophilic) phases as a function of pH.

## Experimental Procedures

Nonporous PHEMA was prepared by azobis(isobutyronitrile)-initiated polymerization of 2-hydroxyethyl methacrylate at 60 °C between high-density polyethylene plates separated by 0.8-mm spacers; after 24 h unchanged monomer was removed by soaking the resulting membrane in water for several days. Porous PHEMA was prepared by the method of Ronel et al.[10] using a mixture of 24.88% HEMA, 0.12% ethylene glycol dimethacrylate, and 75% water. Polymerization was initiated by addition of 0.25% $(NH_4)_2SO_4$ and 0.25% $Na_2S_2O_5$, and the mixture was maintained at 5 °C for 24 h and then room temperature for 24 h. Poly(vinyl alcohol), 99.7% hydrolyzed, molecular weight 78 000, was obtained from Polysciences Inc. Aminoethylated polyacrylamide was purchased from Bio-rad (Bio-Gel P-100) and was prepared according to the method of Inman.[11] Poly(glycolic acid-*co*-lactic acid), a 1:1 molar copolymer, $M_w$ = 18 000, $M_w/M_n$ = 2.4, was prepared by stannous octoate catalyzed ring-opening polymerization of diglycolide and DL-dilactide (Boehringer Ingelheim). Blends of PVA and PGLA were prepared by dissolving different proportions of the two polymers in hexafluoro-2-propanol (bp 59 °C, Aldrich). Films, approximate thickness 0.3 mm, were cast on Teflon (low PVA content) or glass (high PVA content) plates; residual solvent was removed in vacuo at 60 °C for 3 h and then at room temperature for 12 h. Poly(ethylene glycol-*b*-DL-lactic acid) was prepared by PEG-initiated ring-opening polymerization of dilactide in the presence of stannous octoate.[12,13]

**Synthesis of 4-(*N*-Butylamino)-TEMPO.** Butylamine (6.5 g, 0.090 mol) in methanol (40 mL) was adjusted to pH 7 with hydrogen chloride gas before addition of 4-oxo-TEMPO (2.2 g, 0.013 mol). After 30 min, sodium cyanoborohydride (0.6 g, 0.01 mol) and methanol (80 mL) were added. The pH was maintained at pH 7 with HCl in methanol. After 48 h, methanol was removed in vacuo, and the pH of the residual oil was adjusted to pH 5 with 1 N HCl. The aqueous phase was extracted with ether (3 × 15 mL), adjusted to pH 10, and again extracted with ether (3 × 15 mL). The latter extracts were dried, concentrated, and eluted from neutral alumina with ethanol in toluene, to give 2.3 g (79%) of the product. The hydrochloride salt was prepared by adjustment of a methanol solution to pH 5 with HCl and crystallization of the concentrate from an ethanol/ethyl acetate/cyclohexane mixture; mp 201–203 °C. Anal. Calcd for $C_{13}H_{28}ClN_2O$: C, 59.18; H, 10.70; N, 10.62. Found: C, 59.19; H, 10.81; N, 10.55.

ESR measurements were made on a Varian E-109B spectrometer operating at 9.5 GHz. Samples were introduced into the cavity in a quartz aqueous flat cell or in a cylindrical glass tube, diameter 2 mm. Spectra were recorded using the following instrumental parameters: scan range, 100 G; time constant, 0.25 s; scan time, 4 min; modulation amplitude, 0.33 G; microwave power, 10 mW; modulation frequency, 100 Hz. Samples were prepared by immersing the polymer in a $10^{-4}$ M solution of the nitroxide in 50 mM phosphate solution at the specified pH. Samples were maintained at room temperature for 4–24 h before washing extensively to remove any surface-bound spin probe. The rotational correlation times ($\tau_c$) of 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO were calculated from their ESR spectra using equations derived from the theory of Kivelson[14] for weakly immobilized molecules undergoing isotropic motion, or by simulation of the line shape using the method of Freed et al.[15] The following **g** and **A** tensors were used in the computer simulation of spectra by the method of Freed et al.[15]: $\mathbf{g}_{xx}$ = 2.0093, $\mathbf{g}_{yy}$ = 2.0062, $\mathbf{g}_{zz}$ = 2.0062; $A_{xx}$ = 5.8, $A_{yy}$ = 7.0, $A_{zz}$ = 35.5 G. Other parameters were as described by Schneider and Freed.[15] A linear correlation (eq 1) existed between the values of $\tau_c$ calculated

$$\tau_c(\text{Kivelson}) = 0.20\tau_c(\text{Freed}) - 0.0009 \ (r = 0.997) \quad (1a)$$

$$\tau_c(\text{Kivelson}) = 0.22\tau_c(\text{Freed}) - 0.002 \ (r = 0.998) \quad (1b)$$

from Kivelson theory and from the computer simulations of Freed et al. over the range of interest from $\tau_c$(Freed) = 0.025 to 3.3 ns. That is, the value of $\tau_c$(Kivelson) was 5 times smaller than the number derived from the computer simulation but the same overall trend was observed. The computer simulation of the experimental line shape was used to determine the values of $\tau_c$.

The ESR spectra of protonated and unprotonated 4-(*N*-butylamino)-TEMPO were measured in an ethylene glycol:water (85:15) mixture at a series of temperatures from −20 to −54 °C to compare the spectra of the species in a homogeneous medium; no difference in the spectra of the protonated and unprotonated probe over the range $\tau_c$ = 1 − 100 ns was observed. This result demonstrated that a change in the line shape of the spectrum (and $\tau_c$) was not a reflection of a change in the degree of protonation, but a change in the microenvironment of the probe.

The distribution of 4-amino- and 4-(*N*-butylamino)-TEMPO between 50 mM phosphate solution and PHEMA or octanol at different pHs was determined by UV spectroscopy (246 nm) using the standard shake method. Measurements were in triplicate. The partition coefficients of the base ($K_B$) and the conjugate acid ($K_{BH}$) were calculated from the observed distribution coefficients ($D$) at pH 10.5, 7.0, and 4.45 using the relationship (eq 2) between the p$K_a$, $D$, and pH

$$D(1 + F) = K_{BH} + K_B F \quad (2)$$

where $F$ is antilog(pH − p$K_a$).[16,17] Scanning electron microscopy (SEM) was concluded with a JEOL model JSM-T220 instrument (20 kV, magnification 5000; samples were sputter-coated with Au–Pd).

## Results

**Poly(2-hydroxyethyl methacrylate).** PHEMA was insoluble in organic solvents and, in agreement with literature data, contained 40% water after hydration at room temperature. The surface and a cross-section of the dry polymer were featureless and devoid of pores when examined by SEM at a resolution of 0.1 μm. The ESR spectra of the nitroxides 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO in PHEMA were measured after equilibrating the swollen hydrogel with $10^{-4}$ M solutions of each amine in 50 mM phosphate at pH 4.45 for 24 h. There were no changes in the characteristic nitroxide triplet (Figure 2A) between 4 and 24 h. The spectra of both nitroxides were broadened relative to the spectra in water (Figure 2B) but each could be simulated as single species using a theoretical model and computer program developed by Freed et al.[15] for slow-tumbling nitroxides in isotropic liquids. The values of $\tau_c$(Freed) of 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO in PHEMA were 2 and 3 ns, respectively, consistent with rotation in a viscous isotropic fluid. There is no evidence of a second component to the spectrum. No differences in the spectra or $\tau_c$ were observed when the pH of the buffer was changed from 4.45 to 10.5.

The lipophilicities of 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO and their phosphate salts were determined by measurement of their distributions in octanol–water and PHEMA–water phases at pH 4.45, 7.0, and 10.5. Based on the p$K_a$'s of 4-amino-TEMPO and 4-(*N*-butylamino)-TEMPO of 8.89 and 9.29, respectively, the amino groups are >99% protonated at pH 4.45 and

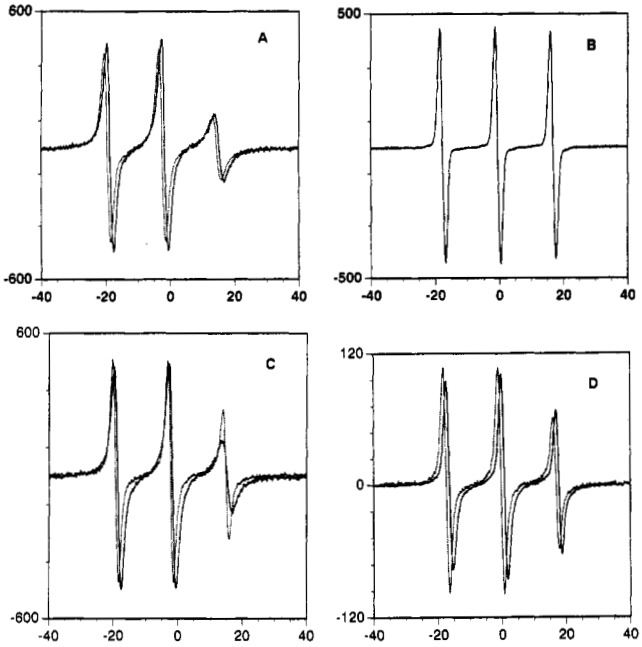


**Figure 2.** (A) ESR spectra of 4-(*N*-butylamino)-TEMPO in PHEMA after equilibration with solute in pH 4.45 and pH 10.5 buffers; spectra are offset to allow comparison. (B) ESR spectrum of 4-(*N*-butylamino)-TEMPO in buffer (identical at pH 4.45 and 10.5). (C) Comparison of ESR spectra of 4-(*N*-butylamino)-TEMPO in PHEMA (broader spectrum) and macroporous PHEMA (narrower spectrum), external buffer pH 4.45. (D) ESR spectrum of 4-(*N*-butylamino)-TEMPO in macroporous PHEMA versus sum of spectra of 4-(*N*-butylamino)-TEMPO in water and nonporous PHEMA, pH 10.5.

**Table I**
**Octanol–Water and PHEMA–Water Partition Coefficients of Spin Probes**

| | octanol–water | | PHEMA–water | |
|---|---|---|---|---|
| | protonated | free base | protonated | free base |
| 4-amino-TEMPO | 0 | 6.6 | 0.4 | 4.7 |
| 4-(*N*-butylamino)-TEMPO | 0.2 | 347 | 2.3 | 19.4 |

>90% unprotonated at pH 10.5. The partition coefficients (Table I) reflect this change from a hydrophilic ionic species at the low pH to a relatively hydrophobic species at the high pH. Based on the measured partition coefficients, if PHEMA is at least partially phase separated into polymer and water domains, the change in the pH from 4.45 to 10.5 will result in at least a ten fold change in the relative concentrations of the spin probe in the aqueous and polymer phases. The failure to detect any pH-dependent change in the ESR spectra, coupled with the ability to simulate the spectra as single species, is strong evidence of only a single phase in PHEMA.

Ronel et al.[10] have reported that a macroporous form of PHEMA may be prepared by radical-catalyzed polymerization of HEMA in dilute aqueous solution. This afforded an opportunity to test the ability of the ESR method to detect the presence of phase-separated water domains. The water content of the swollen porous gel prepared by the method of Ronel et al.[10] was 74 wt %. Based on the water content of nonporous PHEMA, this corresponds to a pore content of 34%. The pore size was determined by SEM to be in the range of 1–5 $\mu$m. The spectra of both nitroxides in porous PHEMA at pH 10.5 consisted of a broadened triplet, the line shape of which was sensitive to the external pH (Figure 2C). The spectra could not be simulated as a single species by the theoretical treatment of Freed et al.[15] but could be satisfactorily reproduced as the sum of the spectra of the nitroxides in water and nonporous PHEMA in the ratio of 1:4 at either pH (e.g., Figure 2D). The signals were narrower at pH 4.45, consistent with the formation of a greater proportion of the more hydrophilic protonated amine at the acidic pH and its partitioning into the more mobile aqueous phase. It was possible to calculate the distribution of the nitroxides in the polymer and water phases of porous PHEMA using the experimentally determined PHEMA:water partition coefficients of the nitroxides at each pH (Table I) and the pore content of porous PHEMA. Good agreement was observed when these calculated distributions were compared with the values found experimentally to simulate the spectra in porous PHEMA (Table II).

**Table II**
**Calculated and Observed Distributions[a] of 4-Amino-TEMPO and 4-(*N*-Butylamino)-TEMPO in the PHEMA and Water Phases of Porous PHEMA Necessary To Simulate the ESR Spectra**

| | pH 4.45 | | pH 10.5 | |
|---|---|---|---|---|
| | calcd | obsd | calcd | obsd |
| 4-amino-TEMPO | 0.4 | 0.3 | 3.4 | 3.4 |
| 4-(*N*-butylamino)-TEMPO | 2.1 | 1.6 | 11.2 | 13.9 |

[a] Nonporous PHEMA/water distribution ratio.

**Poly(vinyl alcohol) and Polyacrylamide Hydrogels.** A cross-linked polyacrylamide gel (95 wt % water content) and 99.7% hydrolyzed PVA (90 wt % water content) were examined as examples of hydrogels with greater degrees of hydration relative to PHEMA. The spectroscopic behavior of 4-amino- and 4-(*N*-butylamino)-TEMPO in these hydrogels was essentially identical to that in nonporous PHEMA. That is, the ESR spectra were independent of pH (4.45 and 10.5) and could be simulated as single species using the computer simulation method of Freed et al.[15] The $\tau_c$ values of 4-amino- and 4-(*N*-butylamino)-TEMPO in PVA derived from the computer simulation were 0.3 and 1 ns, respectively, reflecting the higher water content of PVA relative to PHEMA and the correspondingly greater free volume. The $\tau_c$ values of 4-amino- and 4-(*N*-butylamino)-TEMPO in polyacrylamide were 0.1 and 0.2 ns, respectively, and were almost indistinguishable from the spectra in water. There was no evidence of separate polymer and water phases for either hydrogel.

**Blends of PVA with PGLA.** Based on DSC and electron microscopy measurements, it had previously been proposed that blends of PVA and PGLA are only miscible when the PVA content is greater than 70 wt %.[18] To test this conclusion using the ESR method, the spectrum of 4-(*N*-butylamino)-TEMPO was measured in two PGLA:PVA blends (80:20 and 20:80) and compared with the spectra in the unblended polymers. The ESR spectrum of 4-(*N*-butylamino)-TEMPO in unblended PGLA was a broadened triplet (Figure 3A), characteristic of a highly immobilized spin probe in a glassy polymer. The spectrum could be computer simulated as a single species with a $\tau_c$ value of 50 ns (Figure 3B); no pH dependence was observed. Consistent with this observation, recent studies[19] have shown that PGLA absorbs about 3 wt % of water in a 24-h period and, after this degree of hydration, the $T_g$ decreases from 45 °C to about 24 °C; that is, the polymer is in the glassy state at room temperature. The spectrum in PVA, described above, was a sharp triplet with a $\tau_C$ value of 1.0 ns.

The ESR spectrum of 4-(*N*-butylamino)-TEMPO in the 20:80 blend of PGLA and PVA (Figure 3C) closely resembled the spectrum in PVA (Figure 3D). The spectrum was independent of pH (4.5 vs 9) and could be

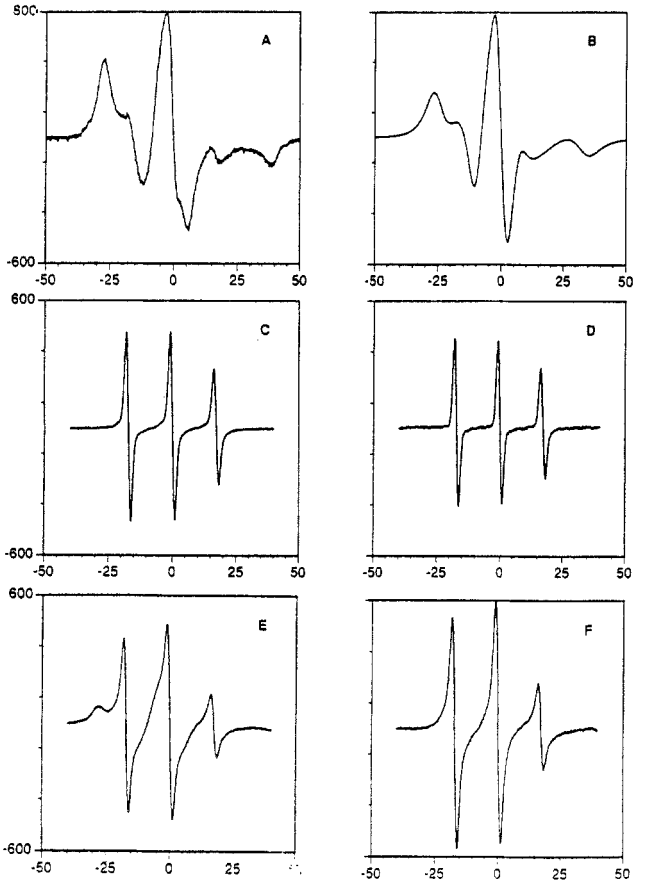


**Figure 3.** (A) ESR spectra of 4-(*N*-butylamino)-TEMPO in PGLA. (B) Computer-simulated ESR spectrum of 4-(*N*-butylamino)-TEMPO in PGLA. (C) ESR spectrum of 4-(*N*-butylamino)-TEMPO in a 20:80 blend of PGLA and PVA after equilibration with solute with pH 9. (D) ESR spectrum of 4-(*N*-butylamino)-TEMPO in PVA after equilibration with solute at pH 9. (E) ESR spectrum of 4-(*N*-butylamin)-TEMPO in an 80:20 blend of PGLA and PVA after equilibration with solute at pH 9. (F) Spectrum resulting from subtraction of the spectrum of 4-(*N*-butylamino)-TEMPO in PGLA from the spectrum of the same solute in the phase-separated blend of PGLA and PVA (80:20).

computer simulated as a single species with a $\tau_C$ value of 1.5 ns, slightly increased relative to the value of 1.0 ns in PVA alone. This small increase in the $\tau_C$ value is consistent with a small decrease in the free volume of the blend as a result of incorporation of the glassy PGLA into the PVA matrix. In contrast, the ESR spectrum of the nitroxide in the 80:20 blend of PGLA and PVA at pH 4.5 was clearly a composite of two spectra of the constituent polymers (Figure 3E). On changing the pH of the equilibrating buffer to 9 (the upper pH was limited to a value of 9 to minimize hydrolytic degradation of the polyester linkages that is known to occur at a higher pH), the weighting of the spectrum attributable to the nitroxide in the PGLA phase increased, reflecting the greater solubility of the free amine in the more lipophilic PGLA. These results confirmed the earlier conclusion that a decrease in the PVA content of PVA:PGLA blends results in phase separation.

Subtraction of the spectrum of 4-(*N*-butylamino)-TEMPO in PGLA from the spectrum of 4-(*N*-butylamino)-TEMPO in the phase-separated 80:20 PGLA:PVA blend afforded a triplet (Figure 3F) which corresponds to the PVA phase. However, the $\tau_c$ value of this triplet was 4.5 ns, which is greater than the value of 4-(*N*-butylamino)-TEMPO in unblended PVA. This discrepancy is explained by a partial mixing of the PVA phase with the PGLA phase. That is, the 80:20 PGLA:PVA blend consists of

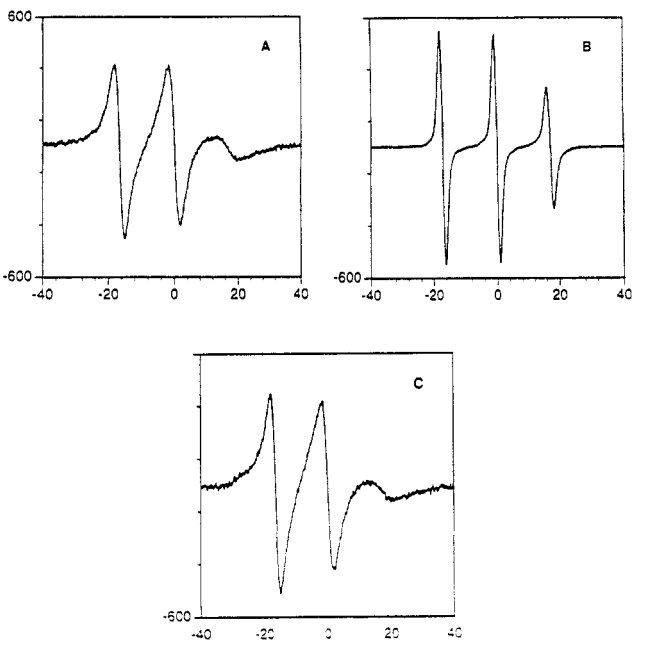


**Figure 4.** (A) ESR spectra of 4-(*N*-butylamino)-TEMPO in a 20:80 blend of PVA and PCL after equilibration with solute at pH 10.5. (B) ESR spectra of 4-(*N*-butylamino)-TEMPO in an 80:20 blend of PVA and PCL after equilibration with solute at pH 10.5. (C) ESR spectrum of 4-(*N*-butylamino)-TEMPO in PCL after equilibration with solute at pH 10.5.

two phases, a PGLA phase and a second phase which is PVA-rich but with a significant PGLA component. Such a blend composition was not evident from the earlier DSC and microscopy studies but is sensible if one recognizes that PGLA can mix with PVA up to 30 wt %.

Similar spectroscopic results were obtained with 4-amino-TEMPO.

**Blends of PVA with Poly(ϵ-caprolactone).** The evaluation of the PCL:PVA blend provided an example where the ESR methodology was superior to microscopic and thermal methods. The crystallinity of PCL prevented optical and microscopic detection of phase separation. DSC evaluation was ambiguous. The $T_g$'s of blends of PCL and PVA in the ratio of 80:20 and 20:80 could not be detected by DSC. The $T_m$ and normalized heat of fusion ($\Delta H_f$) of PCL in the 80:20 blend were 65 °C and 68.0 J/g, respectively, compared with values of 61 °C and 60.8 J/g for unblended PCL. The 20:80 blend failed to exhibit a PCL melting endotherm. None of these data permitted firm conclusions about miscibility, although the suppression of the crystallinity of PCL in the 20:80 blend was suggestive of polymer–polymer interaction.

The ESR spectra of films of the 20:80 and 80:20 PVA:PCL blends were measured after equilibration with 4-(*N*-butylamino)-TEMPO in phosphate buffer at pH 4.45 and 10.5 (Figure 4A,B). The spectra were then compared with those obtained with unblended PCL and PVA. The spectrum in unblended PCL (Figure 4C) was characteristic of a partially immobilized spin probe in a low-$T_g$, rubbery polymer. The spectra in PCL ($\tau_c$ = 12.5 ns) were sufficiently different from the spectra in PVA ($\tau_c$ = 1 ns) that phase separation of PCL:PVA blends would have been easily detected. However, no signals attributable to a separate PCL or PVA phase were detected. Instead, broadened pH-independent spectra were observed that could be attributable to single miscible phases for both 80:20 and 20:80 compositions. This result demonstrated that PVA and PCL are completely miscible in both PVA-rich and PVA-poor blends, in contrast to the PVA–PGLA system.

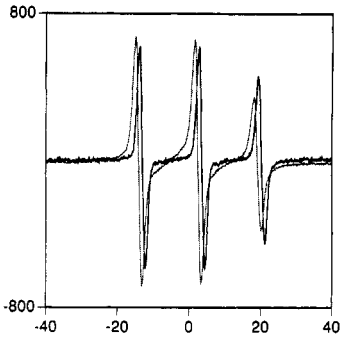


**Figure 5.** ESR spectra of 4-(*N*-butylamino)-TEMPO in the block copolymer PLA:PEG:PLA, $M_n$ ratio 1000:1000:1000, after equilibration with solute at pH 4.45 or pH 10.5. The spectra are offset to show the slight difference in line shapes, as evidence of some phase separation.

**Poly(DL-lactic acid-*b*-oxyethylene-*b*-DL-lactic acid).** Block copolymers of oxyethylene and lactic acid (both DL and L forms) have been studied by a number of laboratories as examples of biodegradable ABA systems derived from hard (A) and soft (B) polymer blocks.[13,20,21] It has been suggested that microphase separation of the blocks occurs, based on the observation of glass transitions assignable to the PEG blocks.[20] However, in the case of the poly(L-lactic acid) copolymer, depression of the $T_m$ of the PEG component was cited as evidence of partial miscibility.[21] In our hands, DSC of the unhydrated block copolymers of poly(oxyethylene-*b*-DL-lactic acid) revealed only a single $T_g$, which varied with the copolymer composition in accord with the Fox equation (eq 3). On swelling in water, films

$$(1/T_g)_{AB} = (W/T_g)_A + (W/T_g)_B \qquad (3)$$

of the copolymers showed a slight haziness, which was greater with higher PLA content. These observations suggested that the amorphous domains of the block copolymers were partly immiscible after hydration. To obtain further information on the morphology, the ESR spectra of 4-(*N*-butylamino)-TEMPO in a series of three block copolymers with the following $M_n$'s were measured: PLA:PEG:PLA = (a) 2000:1000:2000, (b) 1000:1000:1000, and (c) 2000:2000:2000. The mole ratio of PLA and PEG in this series was 4:1, 2:1, and 2:1, respectively; the latter two polymers differed in the block lengths but not the weight ratios of PLA and PEG. The water contents of the three polymers after 24-h immersion in water were 24, 36, and 66%. The lower water content (24%) of the 4:1 PLA:PEG copolymer reflected the greater proportion of the more hydrophobic PLA blocks. The water content of the 2:1 PLA:PEG copolymers depended on the PEG block length and increased from 36 to 66% when the PEG block length was increased from 1000 to 2000.

The ESR spectra of 4-(*N*-butylamino)-TEMPO in the 1000:1000:1000 copolymer (36% hydration) at pH 4.45 and 10.5 were superimposable. The $\tau_c$ value was 1.25 ns, considerably smaller than the value in unblended PLA ($\tau_c$ = 50 ns), and the same as the $\tau_c$ value of the same solute in a 50% mixture of water and poly(ethylene glycol). These data are consistent with the hydrated copolymer existing as a single miscible phase. The ESR spectra of 4-(*N*-butylamino)-TEMPO in the 2000:2000:2000 block copolymer (66% hydration) were almost superimposable at the two pH's (Figure 5), but the spectra were different enough to suggest a small amount of phase separation and the presence of a PLA-rich phase. The spectra of the 4:1 PLA:PEG copolymer, 24% hydration, were more difficult to compare because of the low signal to noise ratio of the spectrum of 4-(*N*-butylamino)-TEMPO at pH 4.45. The reduced hydrophilicity and diffusivity of this copolymer resulting from the higher PLA content served to reduce the concentration of the protonated spin probe in the polymer. However, based on the ratio of the peak heights at pH 4.45 and 10, the spectra were identical. That is, there was no evidence of significant phase separation.

## Discussion

Spin labels and spin probes have previously been used to study the morphology of blends, copolymers, and IPNs.[22-26] Spin labeling, which requires covalent bonding to the paramagnetic species, is more laborious and may require chemical modification of one or both polymers. For example, virgin polyethylene has no reactive sites to which a label may be bound and can only be spin-labeled by introducing functional groups, e.g., 0.5–2.5% of carbonyl groups by copolymerization.[27] Spin labeling is not applicable to the study of the morphology of hydrogels, where the polymer phase but not the aqueous phase may be labeled.

The use of spin probes, involving physical incorporation or sorption of the paramagnetic species into the polymer is more straightforward than spin labeling. However, it has been noted that it is easy to confuse a composite spectrum with a motionally slowed spectrum.[28] Localization of the spin probe in defects or irregularities in the polymer matrix can also produce spurious results. The temperature dependence of the spectrum has proven to be a powerful means of verifying composite spectra arising from the contribution of more than one phase.[26,28] The present method, where the pH is used to change the lipophilicity of the spin probe and so cause its redistribution between two phases in a predictable manner, provides an alternative means of detecting phase-dependent spectra. The success of the method is contingent upon two conditions. First, the nitroxide reporter molecule must be present in each phase to a measurable degree. It is not necessary that the probe exist in both phases at the same time; a shift from one phase to a second on changing the pH is a sufficient condition. Second, the mobility of the nitroxide in each phase must be sufficiently different to result in measurably different line shapes for the solute in each phase (this condition applies to any spin label or probe method). The concentration of the nitroxide in a phase will be determined in part by its relative affinity for that phase. Provided the phases have different solubility parameters, the ability to change the nitroxide from a hydrophobic to a hydrophilic solute by changing the pH will generally ensure that the nitroxide changes its distribution between the two phases. Even if one phase is a minor component, the sensitivity of the method can be amplified by the use of nitroxides which have partition coefficients favoring the minor phase.

The ability to detect a change in the line shape of the nitroxide in different phases appears to be the limiting feature of the method. In the case of the PGLA:PVA blend, where PGLA is a relatively high $T_g$ polymer with a water content of only 10 wt % and PVA has a water content of >90 wt %, the line shapes of the nitroxide in each phase are clearly different. As a result, two distinct triplets in the phase-separated blends are very evident. It is similarly possible to make clear distinctions between the spectra of PVA:PCL blends and PLA–PEG block copolymers because of the difference in the line shapes of the probe in the parent polymers. On the other hand, the difference in the free volume of pore water and hydrated PHEMA in macroporous PHEMA is much less and two

distinct triplets associated with each phase are not discernible; here, only the difference in line shapes at the two pHs and the failure to computer simulate the spectrum as a single species reveal that the ESR spectrum is in fact a composite spectrum. For nonporous PHEMA prepared using standard polymerization conditions, the absence of any pH dependence of the spectrum and the ability to computer simulate the spectrum as a single species both indicate that the water and polymer exist as a single homogeneous phase.

It must be pointed out that the ESR results do not distinguish between different types of water, e.g., water that is hydrogen bonded versus non-hydrogen bonded to PHEMA chains; it is probably that such species must exist. Rather, the results show that there are not separate domains of water and polymer in which a hydrophobic or hydrophilic solute may exist or diffuse selectively.

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## References and Notes

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