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Study of dichlophenac polymeric derivative with 12-DOXYL spin probe

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Abstract

Continuous wave (CW) electron paramagnetic resonance (EPR) studies of the 12-doxylmethyl stearic acid methyl ester (12-DOXYL) spin probe were made in the solutions of a triblock copolymer of acrylamide, vinylchloroethyl ether and an ester of the vinylchloroethyl ether with 2-[(2,6-dichlorophenyl)amino] benzene acetic acid, "dichlophenac" (DPH). Light scattering experiments show that the hydrophobic DPH group influences the clustering of the polymer in solution. The EPR evidence suggests that when the polymer is dissolved in buffer solutions of 12-DOXYL, the spin probe undergoes a partitioning between the solution, the polymer clusters and a minor third environment. The rate of the 12-DOXYL partitioning from the solution into the polymer cluster is fast compared with the time scale of the changes observed in light scattering and exhibits a dependence on the concentration of the polymer in solution and the pH. The 12-DOXYL environment in the polymer cluster is characterized as restricting 12-DOXYL to slow molecular motion with rotational correlation time $\tau = 1.3 \times 10^{-8}$ s/rad and permitting relatively infrequent collisions between the 12-DOXYL molecules. The solution spectrum of the 12-DOXYL is dominated by the spin exchange narrowing expected for concentrated assemblies of the 12-DOXYL molecules, such as a micelle or bilayer. Partitioning of a hydrophobic spin probe in an aqueous solution is shown to be a sensitive measure of the presence of stabilizing hydrophobic regions in a polymer aggregate.

Keywords: 12-DOXYL; Electron paramagnetic resonance; Hydrophobic spin probe

1. Introduction

The last few decades have witnessed concerted efforts to enhance the effectiveness of drugs used in therapeutic, diagnostic and preventive medicine. Conventional drugs are characterized by inaccessibility of targets, premature loss through excretion, allergic reactions, etc. Many of the problems associated with conventional drug therapy may be circumvented by the use of delivery systems, which in a variety of ways will optimize drug action. The concept of targeted drug delivery was first aired early this century and entails the use of carrier systems to deliver drugs to where they are needed or facilitate their release there. Among the systems being investigated, formulations with the polymeric drug carrier covalently bonded to the drug hold considerable promise [1–9]. The latter release the active ingredient during hydrolysis. For the anti-inflammatory drug polymeric derivatives in which a biological substance is chemically bound to a carrier, two directions of synthesis can be distinguished. Firstly, one may obtain polymeric salts; secondly one may synthesize polymeric derivatives with an adibiotic, covalently linked with a polymeric chain. Such systems with controlled release of the active ingredient due to hydrolysis of the active bond between the carrier and the drug are of great interest. The main advantages are considered to be the possibilities of controlling the water solubility and of using them in the form of water solutions.

The anti-inflammatory drug polymeric formulations of the present study are polymeric systems in which the bioactive compound unit is attached to the polymeric chain by a hydrolyzable chemical bond. Previously we have performed static and dynamic light scattering measurements in a series of water soluble polymeric derivatives containing the same polymeric backbone as in the present electron paramagnetic resonance (EPR) study but with different side groups of varying hydrophobic character [1,3]. The light scattering data displayed changes and trends in the dynamics and scattering intensities that were discussed in terms of the concentration of the side groups present in the parent polymer and also as a function of time. It was proven that these systems undergo association in aqueous solution. The solution properties and the ultimate performance of these polymeric

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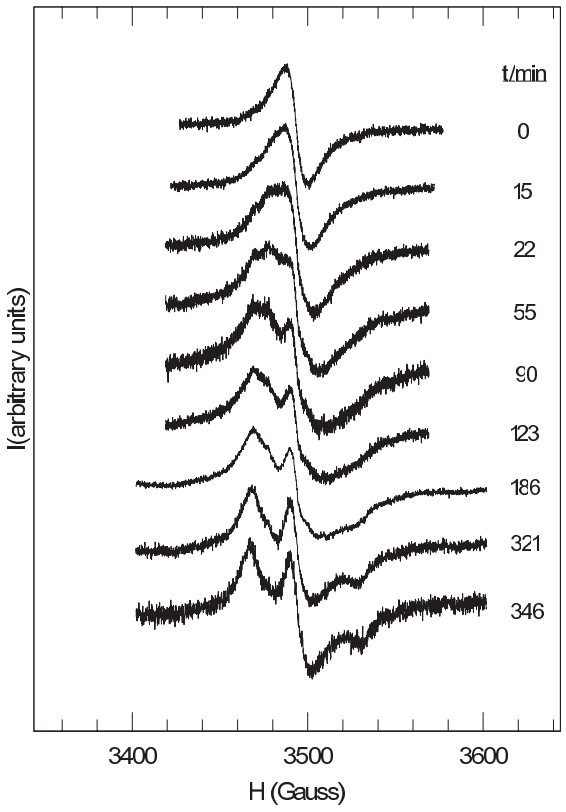


Fig. 1. Room temperature EPR spectra of 0.6 mM 12-DOXYL in an aqueous pH 9 buffered solution, as a function of time after bringing the solution to 2.7% by weight in polymer. The spectra were recorded at 100 mW of power at which no appreciable saturation was shown to occur.

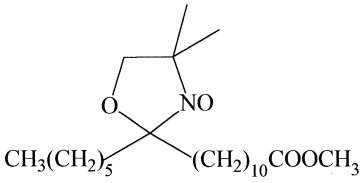
systems is determined by the specific structural characteristics of the solvated macromolecular backbone as well as on the presence of sufficient numbers of hydrophobic side groups. In order to develop an understanding of these structure–property relationships much more information at the molecular level is needed from probes of the local environment in aqueous media. Nitroxide free radicals have proven to be invaluable probes of chemical environment and molecular motion [10]. The literature on these applications is immense, so we give a few representative examples without any attempt to be comprehensive. These applications have given rise to the synthesis of many specialized nitroxide free radicals [11]. By means of nuclear coupling with the nitroxide electron spin, these probes have been employed to study enzyme site structure [12]. Organized structures such as the lipid bilayers of biological membranes can be probed with nitroxide spin probes [13], which may be targeted at particular depths in the bilayer [14]. The surfaces of catalytic materials have been probed with nitroxides [15,16]. Nitroxide free radicals have been applied previously as probes of polymer environments and motion [17–19].

In this study we examine the partitioning of the hydrophobic spin probe, 12-doxylmethyl stearic acid methyl ester (12-DOXYL) from an aqueous solution into aggregates of a polymer in aqueous solution which has a slowly hydrolyzing dichlophenac ester monomer group. The different superimposed forms present in the 12-DOXYL EPR spectrum corresponding to several 12-DOXYL environments in solution or polymer will be quantitatively decomposed. These decompositions will give a quantitative measure of the time course of 12-DOXYL partitioning into these environments.

2. Experimental section

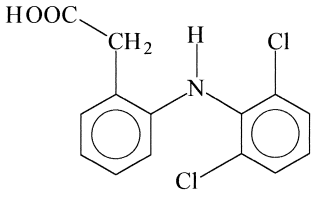
2.1. Materials

The spin probe 12-DOXYL was used as received from Sigma Chemical Co. All storage of 12-DOXYL and its solutions was done in the dark at −20°C. Preparation of the copolymer was



12-DOXYL

by the reaction of a 92 mol% acrylamide, 8 mol% vinylchloroethyl ether copolymer with the



Dichlophenac - DPH

dichlophenac (DPH) potassium salt in dimethylsulfoxide at 100°C for 8 h. The resulting mol% DPH monomer group is controlled with reagent proportions [20]. The mol% DPH in the polymer employed in the present study was 5.3% as determined spectrophotometrically. The average molecular weight was 9.5 kDa.

The EPR experiments were performed with buffered aqueous solutions of 0.6 mM in 12-DOXYL, which were brought to 2.7 and 4.9% by weight in the polymer. A control experiment in a 0.6 mM buffered 12-DOXYL solution without the polymer was also done. A concentration study was also performed by serial dilution of the polymer-free 0.6 mM 12-DOXYL solution down to 0.01 mM. Buffer solutions employed were pH 6 and pH 9 boric acid/potassium chloride/sodium hydroxide buffers. Limited studies were likewise made with 1,1′,4,4′-tetramethyl-piperidine-*N*-oxyl (TEMPO), used as received from Aldrich. Most of the EPR spectra were obtained at room temperature with a Brukker ELEXSYS X-band EPR spectrometer. The

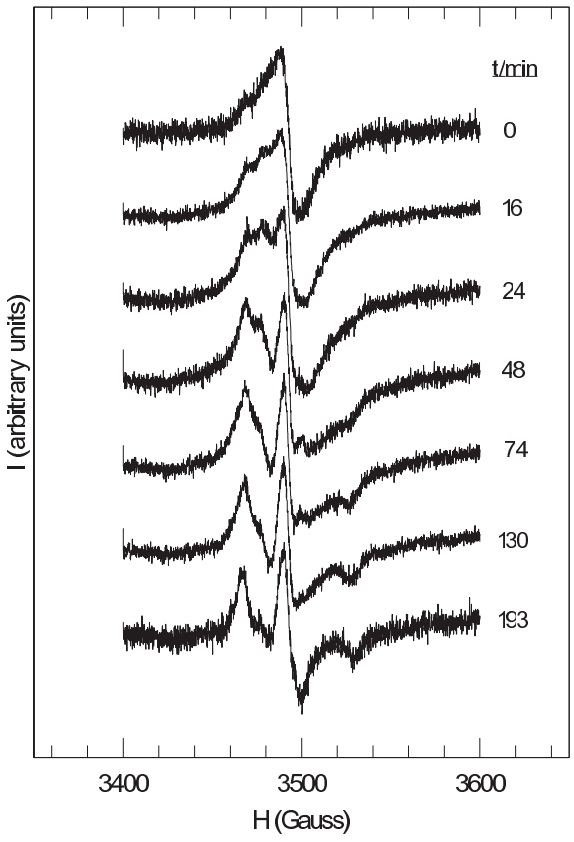


Fig. 2. Room temperature EPR spectra of 0.6 mM 12-DOXYL in an aqueous pH 9 buffered solution, as a function of time after bringing the solution to 4.9% by weight in polymer. The spectra were recorded at 100 mW of power at which no appreciable saturation was shown to occur.

spectrometer operates with a split-ring resonator at about 9.8 GHz with 100 kHz field modulation. The spectra were examined at a range of microwave power levels between 10 and 400 mW in order to establish where microwave power saturation of the spectra was negligible. High powers were employed to selectively saturate particular spectra effectively out of the spectrum. Samples were placed in 1.5 mm capillary tubes. A few experiments were performed below room temperature with an Oxford Instruments CF935 cryostat and ITC502 temperature controller.

2.2. EPR measurements

The EPR spectra of pH 9 buffered solutions of 0.6 mM 12-DOXYL brought to 2.7 and 4.9% by weight in polymer were examined at a series of times from the time of polymer addition to over 300 min from addition. Selected spectra from these series at 100 mW of microwave power for the 2.7 and 4.9% samples are shown in Figs. 1 and 2, respectively. The spectra were shown not to be appreciably microwave power saturated in a saturation study. Results of this study are presented in Fig. 3, in which the peak-to-trough height of the central feature of the spectra measured 10 min after polymer addition in the 2.7 and 4.9% samples are plotted versus microwave power. The spectrum at $t = 193$ min from the 4.9% series in Fig. 2 is presented in Fig. 4 (a) for further consideration in Section 3.1. The spectrum observed at $t = 10$ min in both the 2.7 and 4.9% series is presented in Fig. 4 (b) for the former case for further consideration in Section 3.1.

The spectrum measured at 400 mW power both in the 2.7 and 4.9% solutions, 10 min after polymer addition is shown in Fig. 4 (c) for the latter case. In the simulation of the 4.9% solution spectrum in the next section, these unsaturable spectra are shown not to contribute significantly to the spectra observed at 100 mW. These measurements form the basis for the estimation of the relative amounts of the (two) spectra at 100 mW and the minor, non-saturated spectrum appearing at a high microwave power.

A series of experiments was performed in order to learn whether the time scale of the changes observed in Figs. 1 and 2 are associated with the movement of 12-DOXYL from the solution to the polymer or with hydrolysis of the polymer. In Fig. 5 a series of 12-DOXYL EPR measurements is presented on a polymer sample hydrolyzed for 15 days. The spin probe and the fully hydrolyzed polymer were mixed to 2.7% by weight polymer and 0.6 mM 12-DOXYL and spectra were recorded at time intervals from the time of mixing to 25 h. For comparison with this series and with the series of spectra in Figs. 1 and 2, the EPR spectrum of 0.6 mM 12-DOXYL in the polymer-free pH 9 buffer solution was measured for quantitative comparison with the first spectrum in Fig. 2 and with the essentially unchanging spectrum of Fig. 5. The spectra and their simulations to be discussed in Section 3.1 are presented in Fig. 4(d) and (e). We also verified that the 12-DOXYL spectrum in polymer-free pH 9 buffer does not change with the 12-DOXYL concentration. A change might be expected when there is a transition in the 12-DOXYL organization, such as at a critical micelle concentration. Measurements were made from 0.6 mM in the pH 9 buffer down to 0.01 mM, the lowest concentration that we could detect in the capillary sample tubes.

Another series of experiments was performed in order to address whether the spectral changes after the 12-DOXYL-polymer mixing is associated with the 12-DOXYL partitioning or with the hydrolysis. In this series the pH is adjusted to a value at which hydrolysis is inhibited. Polymer was added to a pH 7 buffer solution of 0.2 mM 12-DOXYL solution up to a level of 2.7% polymer by weight and the EPR spectra were recorded as a function of time. Selected spectra taken at 22 mW of microwave power from this series between the time of mixing and 321 min are shown in Fig. 6. A series of similar experiments during the early hydrolysis stages at pH 9 of the polymer with 3-indolbutyric acid ester monomer groups [3] showed only an unchanging solution spectrum of 12-DOXYL. The spectrum of 0.32 mM 12-DOXYL in a pH 9 buffer solution of 15 mM DPH in the absence of the polymer displayed a form essentially identical to the solution in the absence of DPH and the polymer.

Similar experiments were attempted with TEMPO in the

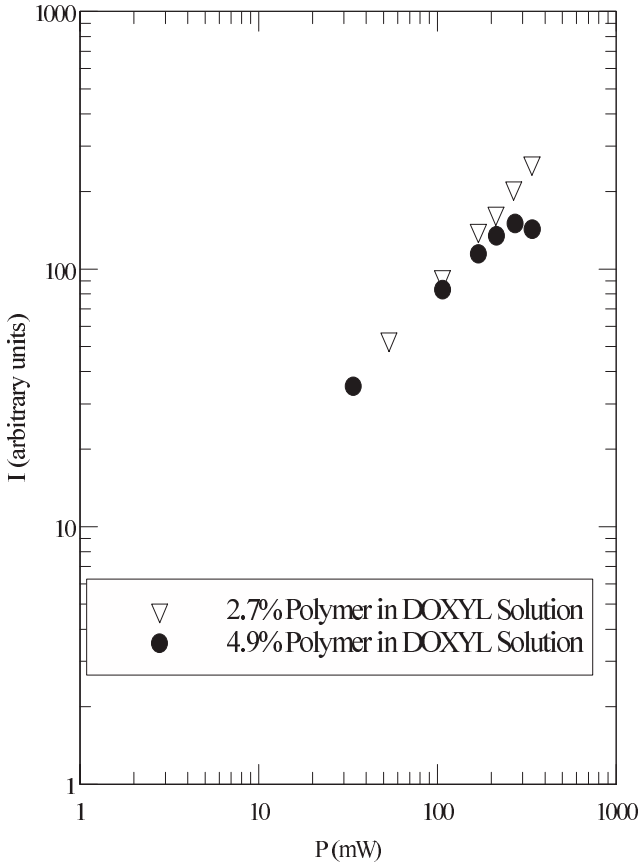


Fig. 3. Microwave power saturation study. Peak-to-trough height of the central feature of the EPR spectra of 0.6 mM 12-DOXYL in an aqueous pH 9 buffered solutions 10 min after bringing the solutions to 2.7 and 4.9% polymer by weight. Data is presented as a function of microwave power.

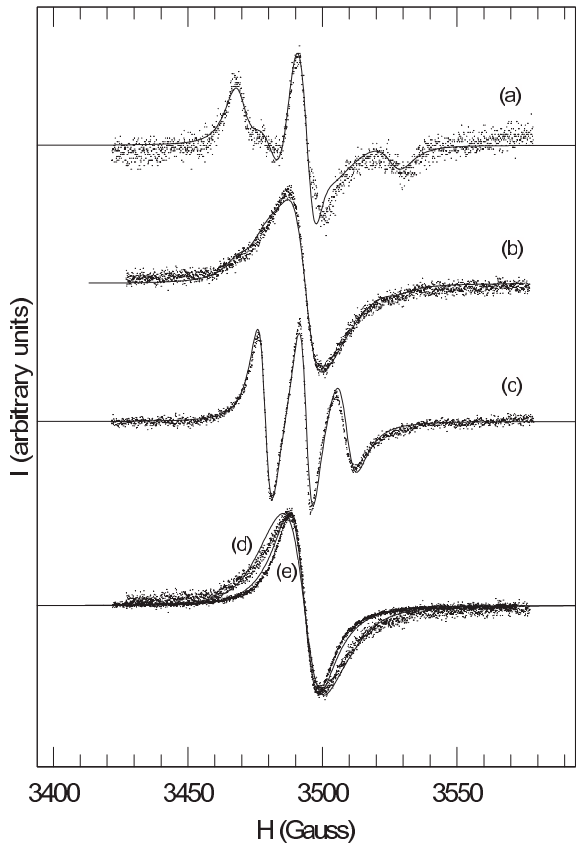


Fig. 4. Simulations and comparisons of selected 12-DOXYL spectra: (a) microwave power unsaturated spectrum 193 min after mixing the pH 9 buffered solution to 4.9% by weight polymer; (b) microwave power unsaturated spectrum 10 min after mixing the pH 9 buffer solution to 2.7% by weight polymer; (c) microwave power saturated spectrum 10 min after mixing the pH 9 buffer solution to 4.9% by weight polymer; (d) microwave power unsaturated spectrum in fully the hydrolyzed polymer at 2.7% by weight in the pH 9 buffer; (e) microwave unsaturated spectrum in the polymer-free pH buffer solution.

presence of the polymer but no changes from the solution spectrum were observed at room temperature under any circumstances. Cooling the solutions did force a change in the spectrum toward a form exhibiting slower molecular motion and/or increased Heisenberg exchange. These changes were not observed in polymer-free preparations. The solution spectrum of TEMPO exhibited a nearly equal intensity ^{14}N hyperfine triplet with little broadening or hyperfine line coalescence.

3. Results and discussion

3.1. Analysis of data

Representative 12-DOXYL EPR spectra from the 2.7 and 4.9% polymer series shown in Figs. 1 and 2 were decomposed into linear combinations of two spectra corresponding to two distinct environments of the 12-DOXYL molecule. The two spectra were: (a) the $t = 193$ min spectrum from the 4.9% series and (b) the $t = 10$ min spectrum in the particular series being decomposed. See Fig. 4(a) and (b). The spectrum of (b) is slightly dependent on the polymer concentration for reasons to be considered in Section 3.2. The spectrum of (a) displays no significant dependence on the polymer concentration. Each of these spectra was simulated with a program written by Freed and co-workers [21,22]. The simulation program is designed to account for the molecular tumbling of the 12-DOXYL in its environment and the extent of Heisenberg spin exchange resulting from collisions between the 12-DOXYL radicals or from close proximity of the 12-DOXYL radicals. The simulated spectra are shown in Fig. 4 and the simulation parameters are given in Table 1. Linear combinations of spectra (a) and (b) were then determined which best fit the spectra from Figs. 1 and 2. Representative examples of these decompositions are presented in Fig. 7. The decrease in the fraction of spectrum (b) with time is plotted in Fig. 8 for the 2.7 and 4.9% samples.

Other spectra observed in this study were also simulated with the Freed program [21,22]. The spectrum, observed at long times in the sequence, that was isolated by microwave power saturating out components (a) and (b), and its

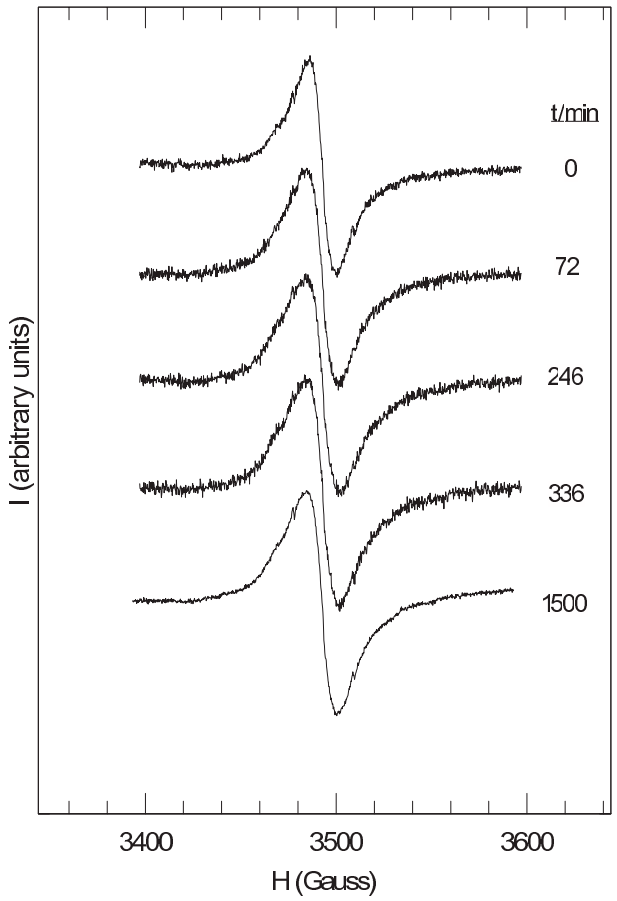


Fig. 5. Room temperature EPR spectra of 0.6 mM 12-DOXYL in an aqueous pH 9 buffered solution, as a function of time after bringing the solution to 2.7% by weight in polymer that was previously hydrolyzed for 15 days. Spectra were recorded at 100 mW of power at which no appreciable saturation was shown to occur.

simulation are shown in Fig. 4(c). A comparison of the simulation of 0.6 mM 12-DOXYL in polymer-free, pH 9 buffer solution with the simulation in the presence of 2.7% polymer is presented in the curves of Fig. 4(d) and (e) The parameters of the simulations of the spectra shown in Fig. 4 are tabulated in Table 1.

It was also demonstrated that at microwave powers above 100 mW, the longest time spectrum in the 4.9% series could be decomposed approximately into linear combinations of spectra (a) and (c). The quality of these decompositions (not shown) showed some imperfections in the wings. This was possibly due to our imperfect ability to reject dispersion components from the spectra at these high microwave powers.

The simulations of partially microwave saturated spectra yield an approximately 0.83 fraction of the unsaturable spectrum (Fig. 4(c)) at 400 mW of microwave power, 0.41 (c) at 290 mW and 0.17 (c) at 180 mW, the lowest power at which we were able to simulate the spectrum. Thus at 100 mW, where measurements are effectively free of saturation, Fig. 4(c) is around five times weaker than spectrum (a). These spectra are taken at the longest times recorded in the series shown in Figs. 1, 2 and 8. One can estimate from the data in Fig. 8 that at these longest times the spectrum comprises around 0.5 (a), 0.4 (b) and 0.1 (c).

3.2. Discussion

The observed, systematic time dependence of the 12-DOXYL EPR spectra, shown in Figs. 1, 2 and 6, appears to be primarily from the partitioning of the 12-DOXYL molecule between its initial environment in solution and an environment associated with the DPH moiety in the polymer aggregates. The time scale of several hundred minutes over which these changes take place is at least an order of magnitude shorter than the time scale of the changes in polymer aggregation observed in light scattering experiments [1–3]. Thus the changes observed here over a few hundred minutes by EPR are by-and-large not associated with changes in polymer aggregation. The similarity of the short-term changes in pH 9 (Figs. 1, 2 and 8) and in pH 7 (Fig. 6) buffer solutions is further evidence that these changes are independent of whether hydrolysis can take place or not. However, when 12-DOXYL and the polymer fully hydrolyzed for 15 days are mixed, the unchanging 12-DOXYL spectrum remains essentially identical to the initial 12-DOXYL solution spectrum (see Fig. 5). It is known that the DPH copolymer employed in this study undergoes relatively minor changes in polymer aggregation in the course of hydrolysis [3]. Thus we conclude that when the hydrophobic DPH monomer groups are present in the polymer aggregate, the 12-DOXYL spin probes are stabilized in the aggregate. When the polymer is fully hydrolyzed and no longer contains DPH groups, 12-DOXYL does not enter the polymer aggregate to an appreciable extent, as evidenced in Fig. 5. Thus it is the hydrophobic nature of the DPH group rather than the degree of polymer aggregation that governs 12-DOXYL partitioning into the aggregate. The present study illustrates how the partitioning of a hydrophobic spin probe in an aqueous solution can be used as a sensitive measure of the presence of stabilizing hydrophobic regions in a polymer aggregate.

The rates at which 12-DOXYL partitions into the polymer aggregate shown in Fig. 8 are complicated functions of time, neither first nor second order in 12-DOXYL solution concentration nor limited by the number of polymer aggregate sites available. If the rate were proportional to the presence of DPH monomer groups, one might expect the initial rates to be proportional to polymer concentration, all other things being equal. The ratio of polymer concentrations for the data in Fig. 8 is 1.6 and the corresponding ratio of initial rates is 2.1. This does not appear to be in very good agreement. However, it may not be all that bad given the evidence that the degree of polymer aggregation depends on polymer concentration [3] and that the activity of the 12-DOXYL in solution may also depend on polymer concentration. Evidence suggesting that the 12-DOXYL activity could depend on polymer concentration will be

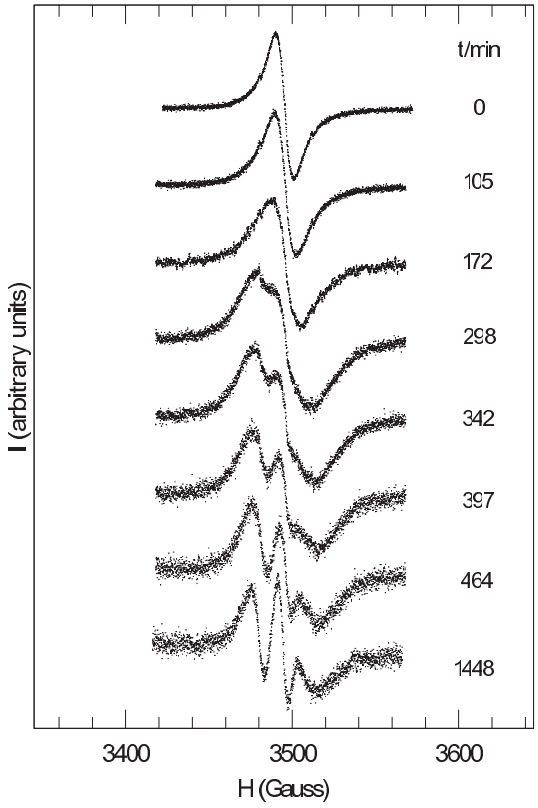


Fig. 6. Room temperature EPR spectra of 0.2 mM 12-DOXYL in an aqueous pH 7 buffer solution, as a function of time after bringing the solution to 2.7% by weight in polymer. Spectra were recorded at 22 mW of power at which no appreciable saturation was shown to occur.

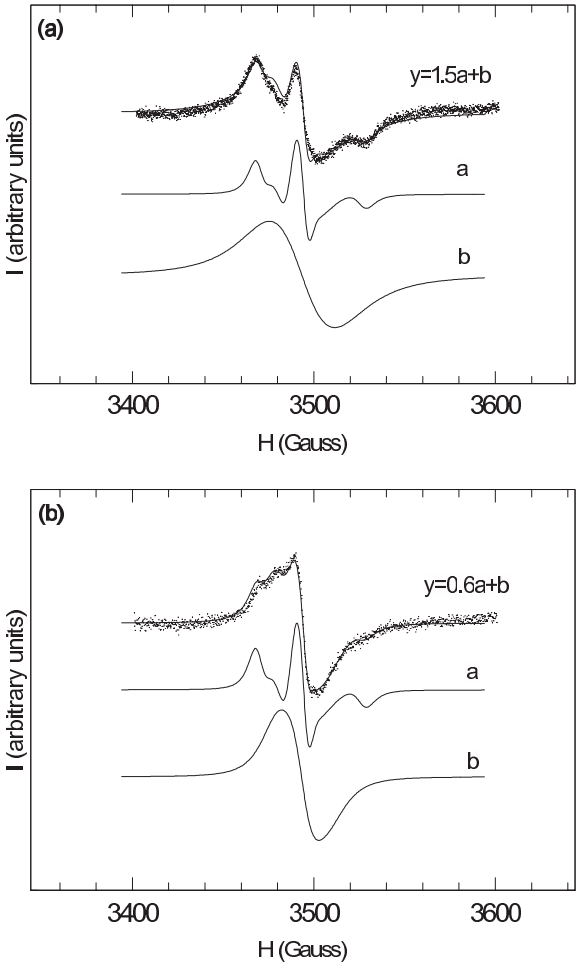


Fig. 7. Representative examples of the decomposition of the 12-DOXYL room temperature EPR spectra into immobile and mobile spectra (a) and (b) respectively in Fig. 4: (a) microwave power unsaturated spectrum 266 min after mixing the pH 9 buffered solution to 2.7% by weight polymer; (b) microwave power unsaturated spectrum 16 min after mixing the pH 9 buffered solution to 4.9% by weight polymer.

presented later in this section. The increase of the 3% data in Fig. 8 may be from the approach to equilibrium and possibly from a small loss in hydrophobic regions in the aggregates due to the loss of DPH from hydrolysis. This is probably still an overly simplistic view, however, in view of the fact that the rate of DPH penetration in the pH 7 system is about three times slower than at pH 9 in the 2.7% system (cf. Figs. 1 and 6).

Table 1
Parameters employed to simulate the spectra of Fig. 4. The spin Hamiltonian parameters were held fixed throughout at the values given by Berliner [9], $g_{xx} = 2.0088$, $g_{yy} = 2.0061$, $g_{zz} = 2.0027$, $A_{xx}/h = 6.3$ MHz, $A_{yy}/h = 5.8$ MHz, $A_{zz}/h = 33.6$ MHz. The Heisenberg exchange parameter is abbreviated HE, $R_{\|}$ and $R_{\perp}$ are the molecular rotational rate parameters, parallel and perpendicular, respectively, to the long DOXYL axis. ΔH is the homogeneous EPR line width parameter

	HE (MHz)	$R_{\|}$ (10^6 rad s^{-1})	$R_{\perp}$ [a] (10^6 rad s^{-1})	ΔH [a] (Gauss)
Fig. 4(a)	0	13	2.6	3
Fig. 4(b)	300	13	2.6	3
Fig. 4(c)	10	130	2.6	3
Fig. 4(d)	300	13	2.6	3
Fig. 4(e)	400	13	2.6	3

[a] These values were optimized for the spectrum of Fig. 4(a) and kept fixed at these values in the other optimizations, which displayed little dependence on these parameters.

The environment of the 12-DOXYL in solution is one in which the radicals are in rapid Heisenberg spin exchange with one another, coalescing the ^{14}N triplet of hyperfine lines to a single line. This is observed either from the rapid diffusion of the paramagnetic species in low viscosity solvents, an unlikely explanation in the present case, or from the formation of very concentrated assemblies containing the radicals such as micelles or bilayers. Molecules containing long aliphatic chains with a hydrophilic functional group on them are well known to form such assemblies and the family of DOXYL spin probes is often used to probe such assemblies [4,13,14,17–19]. The Heisenberg exchange interaction parameters in Table 1 are very characteristic of the fast exchange limit. The spectra in Fig. 4(b), (d) and (e) and in Fig. 5 and their Heisenberg exchange parameters in Table 1 indicate some sensitivity of these assemblies of 12-DOXYL molecules in solution to the presence of the

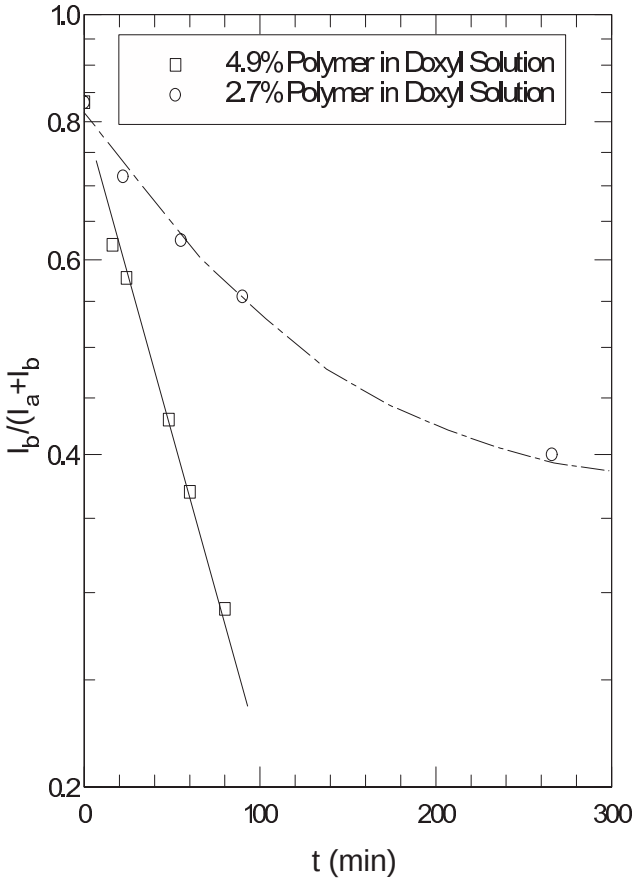


Fig. 8. Fraction of the mobile 12-DOXYL spectrum of Fig. 4 (b) as a function of time after mixing the polymer with the buffered 12-DOXYL solution to 2.7 and 4.9% polymer by weight.

polymer. In general the Heisenberg rate parameters are somewhat less (the lines are broader) in the presence of the polymer than for 12-DOXYL in the polymer-free solution. This would appear to indicate that some part of the polymer inserts into the micellar or bilayer assemblies of 12-DOXYL without changing their basic character but reducing the frequency of spin exchanging radical–radical encounters.

The environment of the 12-DOXYL in the polymer aggregate is one that is dilute and one in which the radical undergoes relatively slow molecular tumbling or reorientational motion. The spectrum of the 12-DOXYL in this environment shown in Fig. 4(a) resembles the powder spectrum of a nitroxyl radical and was simulated with the parameters given in Table 1. The rotational correlation constant about the 12-DOXYL aliphatic chain, $R_{\|} = 13$ MHz, and the corresponding correlation constant, $R_{\perp} = 2.6$ MHz, for end-to-end tumbling indicates a relatively immobilized 12-DOXYL molecule in a very slowly tumbling polymer aggregate. The spectrum is adequately simulated with a zero Heisenberg exchange parameter (see Table 1). Given a 3 G line width, this probably puts an upper limit of about 10 MHz on the Heisenberg exchange rate. Thus the 12-DOXYL environment in the polymer is sufficiently more dilute than in solution so as to have one to two orders of magnitude less frequent spin-exchanging 12-DOXYL encounters.

The origin of the minor 12-DOXYL spectrum shown in Fig. 4(c), i.e. the spectra that can be isolated from the other spectra at high microwave powers, is not altogether clear. Its simulation parameters point to a molecule in fast rotation about its long axis and with a moderately large Heisenberg exchange rate. Thus this minor 12-DOXYL environment is one with a moderately high concentration and one in which a high frequency of 12-DOXYL motion is allowed. The small amplitude nature of this signal makes systematic studies difficult. We have ruled out two environments that might display these characteristics. One is the isolated 12-DOXYL molecules in aqueous solution below a critical concentration for agglomeration, such as the critical micelle concentration. We have examined the polymer-free buffer solutions of 12-DOXYL and do not see this spectrum appear down to concentrations at the limit of our ability to observe 12-DOXYL EPR signals. In polymer solutions, however, we have never observed this spectrum until several hundred minutes into the series depicted in Figs. 1 and 2, where appreciable hydrolysis may have begun. It is possible that the series depicted in Fig. 5 displays this signal from the beginning. Note that in Fig. 5 the small triplet of signals whose outside members are the most pronounced near the peak and trough of the main signal. In this series a fully hydrolyzed polymer solution is exposed to 12-DOXYL. These observations of the signals are both circumstances in which DPH may have begun to appear in solution, most likely in ionic form at pH 9. However leading this evidence may be, spectra of 12-DOXYL in polymer free pH 9 buffer solutions do not display this spectrum.

4. Conclusions

In this study we examined the partitioning of the hydrophobic spin probe, 12-DOXYL from an aqueous solution into aggregates of a copolymer of acrylamide and vinylchloroethyl ether which has a slowly hydrolyzing dichlophenac ester monomer group. The EPR spectra of the 12-DOXYL spin probe reflect different environments in a given sample with different motional characteristics. The different superimposed forms present in the 12-DOXYL EPR spectrum corresponding to several 12-DOXYL environments in solution or polymer have been quantitatively decomposed. These decompositions give a quantitative measure of the time course of 12-DOXYL partitioning into these environments.

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References

[1] Rizos AK, Tsatsakis AM, Shtilman MI, Brown W. J Non-Cryst Solids 1998;235–237:625.
[2] Rizos AK, Tsatsakis AM, Shtilman MI, Brown W. Polymer 1998;39:4729.
[3] Rizos AK, Tsatsakis AM, Shtilman MI, Brown W. Polymer 1998;39:1753.
[4] Allan GG, Chapra CS, Neogi AN, Wilkins RM. Nature 1971;237:349.
[5] Shtilman MI. Immobilization on polymers. Tokyo: VSP, 1993.
[6] Kydonieus AF. Control release technologies: methods, theory and applications. Boca Raton, FL: CRC Press, 1980.
[7] Tsatsakis AM, Shtilman MI. Plant Growth Regulation 1994;14:69.
[8] Wilkins RM. Chemical manipulation of crop growth and development. In: McLaren JS, editor. London: Butterworths, 1982. p. 116.
[9] Tsatsakis AM, Paritsis KN, Shtilman MI, Shashkova IB, Alegakis AK, Roubelakis-Angelakis KA. Plant Growth Regulation 1995;17:167.
[10] Berliner LJ, editor. Spin labeling: theory and applications. New York: Academic Press, 1976.
[11] Volodarsky L, Reznikov V, Ovcharenko V. Synthetic chemistry of stable nitroxide. Boca Raton, FL: CRC Press, 1994.
[12] Mustafi D, Makinen MWJ. Am Chem Soc 1995;117:6739.
[13] Marsh D, Watts A. In: Jost PC, Griffith OH, editors. Lipid–protein interactions. New York: Wiley Interscience, 1982. p. 253.
[14] Pinheiro TJT, Bratt PJ, Davis IH, Doetschman DC, Watts AJ. Chem Soc Perkin Trans 1993;:2113.
[15] Lunina EV. Applied Spectroscopy 1996;50:1413.
[16] Doetschman DC, Thomas G. Chemical Physics 1998;228:103.
[17] Pilar J, Sikora A, Labsky J, Schlick S. Macromolecules 1993;26:137.
[18] Szajdzinska-Pietek E, Schlick S. Langmuir 1994;10:2188.
[19] Szajdzinska-Pietek E, Pilar J, Schlick S. J Phys Chem 1995;99:313.
[20] Shtilman MI, Tsatsakis AM, Khachanyan AA. Polym Sci, Ser B. 1995;38:268.
[21] Schneider DJ, Freed JH. Adv Chem Phys 1989;73:387.
[22] Freed JH. Plant Growth Regulation 1995;17:53.