Notes to the Editor

Spin-labelling of poly(acrylic acid)

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INTRODUCTION

The molecular motion of macromolecules in both the liquid[1] and solid state[2] has been studied by attaching nitroxide containing spin-labels to the macromolecules and monitoring the resulting e.p.r. spectrum. From these spectra it has been possible to study their behaviour adsorbed at interfaces[3] as well as their interaction with small molecules such as surfactants[4]. Spin labels have been attached to both natural (proteins and enzymes) and synthetic macromolecules. Of the latter, it has been mainly non-ionic, organic soluble molecules that have been labelled. Apart from sodium carboxymethyl cellulose[5], synthetic polyelectrolytes have not been spin-labelled although their adsorption and solution properties are of considerable scientific and industrial importance. In this note, a method is outlined for attaching nitroxide labels to polyacids such as poly(acrylic acid) in aqueous systems, using Woodward's reagent K (WRK, *N*-ethyl-5-phenylisoxazolium-3′ sulphonate). Alternative methods for labelling polyacids (e.g. using dicyclohexyl carbodiimide) have been reviewed[6], though the present one is probably more simple and convenient.

EXPERIMENTAL

The poly(acrylic acid) (PAA) samples were prepared by polymerizing acrylic acid in water using potassium persulphate as initiator and *i*-propanol as a chain-transfer agent. The polymers were dialysed for 20 days and freeze dried. The polymers had molecular weights of 51 000 and 75 000 (determined by viscosity) and were spin-labelled by a method described by Patel *et al.*[7] as follows. PAA (1 g) was dissolved in H_2O (40 ml) and NaOH (1.1 mol/dm^3) added to give a pH of 9.7. The solution was cooled to 273K and WRK (0.29 g) (Merck) added. The pH dropped to 7.4, but after 5 minutes, drifted back to 7.7. 4-amino 2,2-6,6 piperidinooxy (*I*) (0.172 g Aldrich Chemical Co.) were added and the mixture allowed to stand at 277K overnight. The mixture was dialysed to remove unreacted label (*I*) and WRK. This dialysis step is normally complete after a week (with non-ionic polymers) though in the case of PAA, it took much longer at pH 7–8. The reason for this is discussed below, but dialysis under acid (pH 3–4) conditions and in the presence of strong neutral electrolyte improves the efficiency of the dialysis. After removal of the label, the polymer was dialysed at pH 7–8 and freeze dried. The spectrum from the labelled polymer is shown in *Figure 1*. From the intensity of the spectrum, the density of labelling was calculated

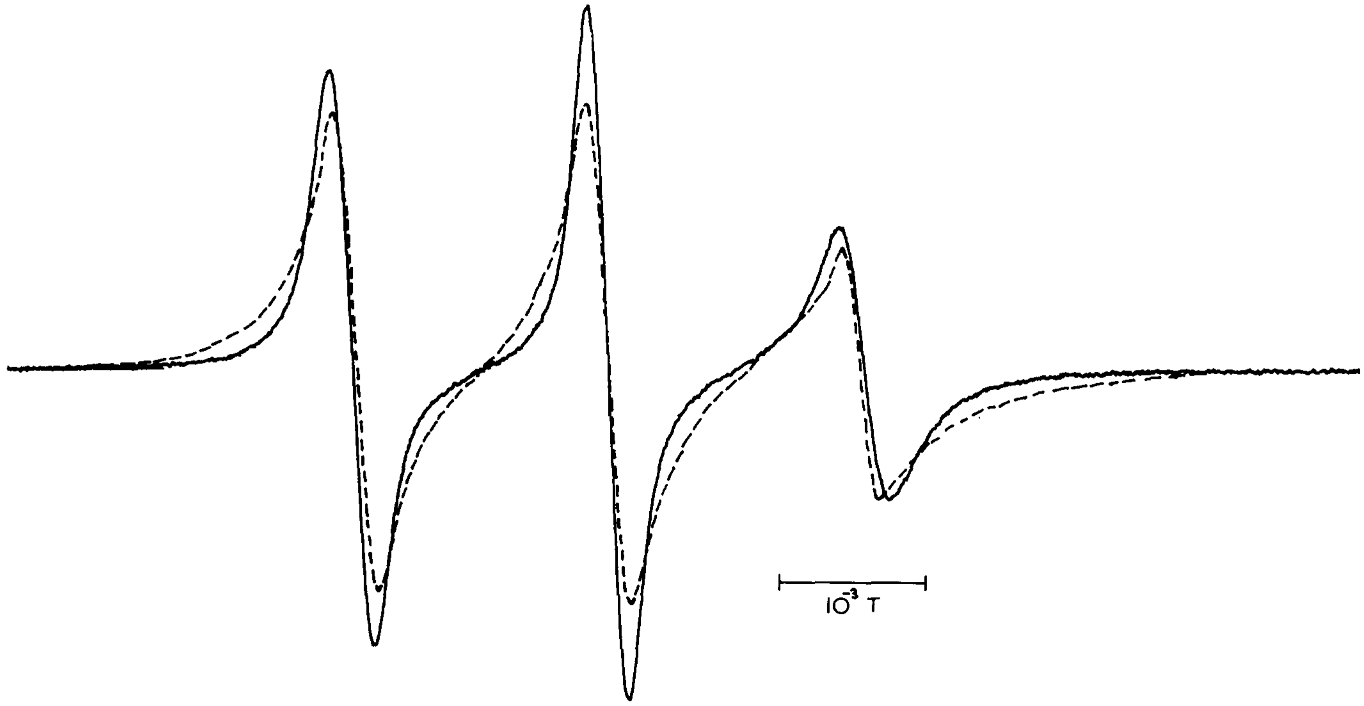

Figure 1 (i) Continuous curve; 10^{-2} mol/dm^3 labelled PAA, pH7. (ii) Dotted curve; 10^{-2} mol/dm^3 labelled PAA, pH2

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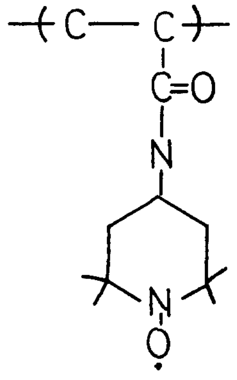

Figure 2 Structure of the spin-labelled PAA

to be 1 label per 18 monomer units. Spectra were recorded on a Varian E4 X-band spectrometer at 296 ± 2K. Viscosity measurements were made using the normal Ubbelohde dilution viscometer. The likely structure of the pendant group is given in *Figure 2*.

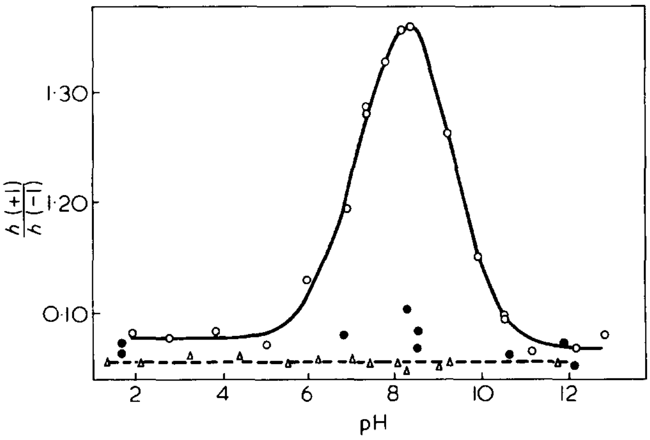

Figure 3 Ratio of the height of the low-field line [h(+1)] to that of the high field line [h(−1)], as a function of pH for: ○, unlabelled PAA + 4-amino 2,2-6,6-piperidinooxy; ●, 4-amino 2,2-6,6-piperidinooxy; △, unlabelled PAA + 2,2-6,6-piperidinooxy

RESULTS

The purification of PAA by dialysis was less efficient than the same procedures with non-ionic water-soluble polymers. This may have been due to an interaction between unreacted (*I*) with the polymer, or to blocking of the dialysis bag by the polymer. If the former were the case, the molecular motion motion of the label would be altered by the binding to the polymer, though the changes in linewidth caused by this binding would be small. Thus the ratio of the height of the low field line to that of the high field line was measured for (*I*) in the presence of unlabelled PAA as a function of pH, and the results are shown in *Figure 3*. The concentration of (*I*) was 2 x 10^{-4} mol/dm^3. The height ratios are shown for pure (*I*) in the absence of PAA as well as for 2,2- 6,6 piperidinooxy (tempo) in the presence and absence of PAA. The results show a reaction between (*I*) and PAA, having a maximum at about pH 8 and associated with the amine group of (*I*) and the polymer. The pK of the amine label[8] (*I*) is 9.2, and the negative charge on PAA decreases below pH of 8. Thus the electrostatic interaction between (*I*) and PAA would be a maximum between pH 8–9. Further support for this comes from increasing the ionic strength of the PAA – (*I*) system at pH 8. The height ratio reduced to 1.08 on addition of 0.3 mol/dm^3 NaCl, indicating weakening of the electrostatic interaction with increasing ionic strength.

As the dialysis at low pH improved the efficiency of the dialysis, the stability of the labelled polymer at high and low pH as a function of time was studied. It was found that at pH values between 6 and 12, the intensity of the labelled polymer was unaltered. At lower pH values the intensity decreased, but was restored to the original value when the pH was increased to 6. After 10 days at pH of 2, the intensity of the labelled polymer was reduced by about 5% (comparisons made after restoring pH to 6). This suggests that prolonged dialysis at pH lower than 3 should be avoided. The stability of the amine label (*I*) and tempo under acid and alkaline conditions was measured as a function of time. After 30 days at pH 11, the intensity of both decreased by less than 5% whereas at pH 2, the intensity of tempo had decreased by 20% and that of the amine label by about 7%.

The shape of the spectrum of the labelled polymer changed on lowering the pH, as shown in *Figure 1*. The linewidth showed no significant change with decreasing pH, though at pH 2 the intensities measured from the height x (linewidth)^2 were about 40% of those from the integrated intensities, using a Nicolet 1074 computer for signal integration. This might be explained by the spectrum at low pH consisting of 2 spectra, one being the normal solution spectrum and the other arising from a more immobile.component. This immobile component may arise from aggregation of the polymer at low pH, though confirmation of this requires further study.

The molecular weight of both polymers, measured before and after labelling, was increased by amounts consistent with the added label, indicating that no significant cross-linking had occurred.

Thus, using Woodward's Reagent K, polyacids can be spin-labelled, though care is necessary to ensure that efficient dialysis removes unreacted spin label.

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