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Dynamic light-scattering study of 3-indolbutyric acid polymeric derivative

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This paper describes the dynamics of new slow release formulations of plant growth regulator (PGR) 3-indolbutyric acid (IBA) in aqueous solution. The new PGR formulations are water-soluble copolymers derived from acrylamide and vinyl chloroethyl ether, containing side linkages of IBA, the latter being released after hydrolysis. From the observed photon correlation spectra of the polarized scattered light of the water-soluble PGRs, the distribution of relaxation times is computed by means of a direct Laplace transformation. The results of the light-scattering experiments suggest that the incorporation of the IBA groups does not affect the coil dimensions of the parent polymer. This behaviour is to be contrasted with a similar system containing hydrophobic side groups of 1-naphthylacetic acid that lead to the formation of interpolymeric micellar structures. The hydrodynamic radius R_h which is 42 Å for the parent polymer (PF1), remains approximately the same for the different contents of the IBA polymeric ester considering the relative increase in size due to the increasing numbers of the IBA groups. In alkaline pH, the scattering intensities and apparent hydrodynamic radii display a systematic drop as a function of time due to the loss of the IBA linkages. However, such effects are negligible in neutral pH, in agreement with previous kinetic studies. © 1997 Elsevier Science Ltd. All rights reserved.

(Keywords: plant-growth regulators; light scattering; polymer dimensions)

Introduction

Recently, increasing attention has been focused on various materials which are able to release a bioactive compound at a certain rate[1–3]. The latter is dependent mainly on the character of the system and in particular, the structures of the components. Although the low molecular weight (LMW) plant growth regulators (PGRs) have gained practical relevance, their more widespread use has been limited by a number of factors[4,5]. One of the biological shortcomings of the LMW plant stimulants is the very narrow concentration range in which these compounds are effective. New, more effective and less toxic PGRs involve modification of the LMW bioactive compounds into a controlled release form[6,8,9]. Controlled release systems have been demonstrated to have many benefits over their LMW analogues[10,11]. With respect to the environment the use of controlled release PGRs and other bioactive compounds offers reduced losses and improved efficiency, greater safety towards non-target organisms and wider range of effective concentrations which is convenient for farmers since fewer preparations are required to produce an effective concentration[5,5,7–11].

The polymeric formulations of PGRs are high molecular weight systems in which the bioactive compound PGR unit is attached to the polymeric chain by a hydrolysable chemical bond and are referred to by some authors as 'phytoactive' polymers[4,6]. The type of hydrolysable bond depends upon the reactive groups that the particular low molecular weight bioactive compound structure contains. In addition to linkages with the LMW units, the phytoactive polymers may contain other linkages in order to provide the polymeric system with the appropriate hydrophilicity (e.g. hydroxylic groups, amidic groups, pyrollidonic fragments, ionogenic groups)[4,6–8]. For some applications it may be necessary to decrease the water solubility of the PGR, and this is achieved by its incorporation into an appropriate polymer matrix.

In the present work, static and dynamic light-scattering data from a series of polymeric derivatives of 3-indolbutyric acid (IBA) are reported as a function of concentration. The concentration range from 0.001 to 0.01 g ml^{-1} is well into the dilute regime ($c < c^*$) which is very similar to the range of effective concentrations that are essentially used in the field by farmers. The light-scattering data show changes and trends in the dynamics and scattering intensities. These are discussed in terms of the IBA concentration value in the polymeric form and as a function of concentration and pH in the polymeric water solution and also as a function of time. No allowance was made for 'copolymer heterogeneity in composition'.

Experimental

Preparation of the polymeric IBA derivatives. Esters of IBA with the copolymer of vinyl chloroethyl ether and acrylamide were synthesized by the reaction of the IBA potassium salt with the copolymer solution in DMSO at 100°C for 8 h and by altering the reagent proportions[12]. The content of IBA in the IBA polymeric esters was determined spectrophotometrically. All three polymers (see *Table 1*) have comparable molecular weights with the parent polymer PF1. The hydrolysis of the polymeric ester was studied in neutral (pH = 7) and alkaline (pH = 9) media at various concentrations.

Simultaneous static and dynamic light scattering. The experimental autocorrelation function was measured with an apparatus equipped with an argon ion laser (Coherent

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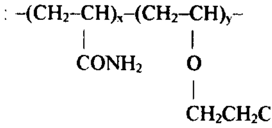
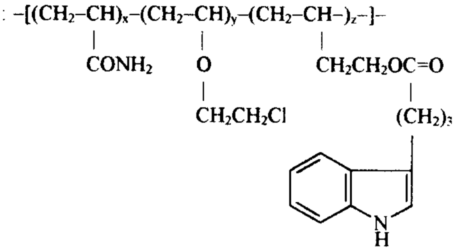
Table 1 Molecular characteristics of the polymeric derivatives. Type A is the copolymer of acrylamide with vinyl chloroethyl ether with $x = 92$ mol% and $y = 8$ mol%. Type B is the copolymer of acrylamide with vinyl chloroethyl ether and vinyl 3-indolbutyric acid ether with $z = 0.9$, 3.16 and 5.9 mol% ($x = 92$ mol% and $y + z = 8$ *mol%*)

Polymeric form	$\bar{M}_w$	Type of molecular structure	IBA linkage content (mol%)	IBA linkage content (wt%)
PF1	18000	A	0	0
PIBA1	19000	B	0.9	2.3
PIBA2	22000	B	3.16	8.0
PIBA3	24000	B	5.9	14.1

A :

$-(CH_2-\underset{CONH_2}{CH})_x-(CH_2-\underset{\underset{CH_2CH_2Cl}{O}}{CH})_y-$

B :


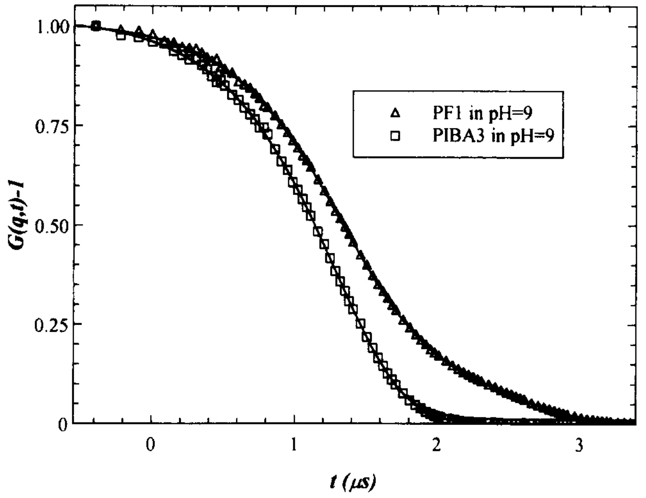


Figure 1 Experimental correlation functions for PF1 ($c = 0.0118$ g ml^{-1}) and PIBA3 ($c = 0.0096$ g ml^{-1}) in alkaline conditions (pH = 9)

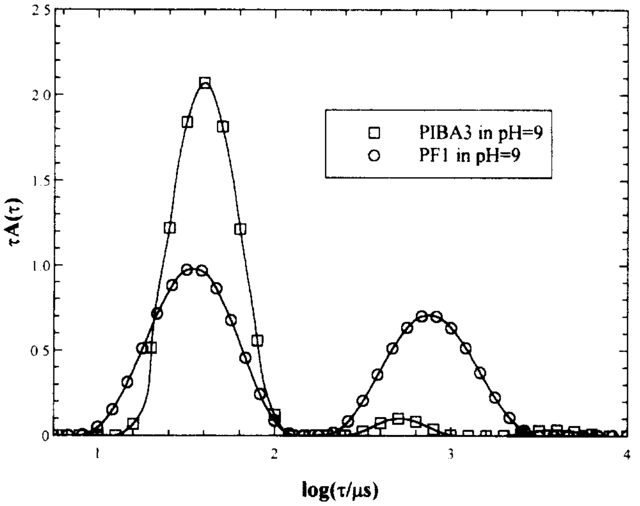


Figure 2 The distributions for the experimental correlation functions of *Figure 1* using REPES

Radiation Model Innova 304) operating at a wavelength of 488 nm. The incident and scattered beams were polarized with Glan and Glan–Thompson polarizers with extinction coefficients better than 10^{-6} and 10^{-7}, respectively. An ALV-5000 multibit, multi-τ full digital correlator was used that covered a broad dynamic range of about 10 decades.

Data analysis. The dynamic light-scattering experimental correlation functions were treated in the homodyne limit. The measured intensity autocorrelation function $G(q,t)$ is related to the desired normalized field correlation function $g(q,t)$ [where $q = (4\pi n/\lambda)\sin(\theta/2)$ is the scattering vector, n is the refractive index of the bulk polymer, θ is the scattering angle and λ the laser wavelength] by: $G(q,t) = A[1 + f\,|ag(q,t)|^2]$ where f is the instrumental factor, calculated by means of a standard, a is the fraction of the total scattered intensity associated with density fluctuations with correlation times longer than 10^{-6} s and A is the baseline. Two types of analyses have been performed for the $g(q,t)$ correlation functions. First, we used the sum of two Kohlrausch–Williams–Watts (KWW) functions,

$$ag(q,t) = A_f\exp\left[-(t/\tau_f)^{\beta_f}\right] + A_s\exp\left[-(t/\tau_s)^{\beta_s}\right] \quad (1)$$

with parameters $\{A_f, \tau_f$ and $\beta_f\}$ and $\{A_s, \tau_s$ and $\beta_s\}$ that give the contrast, relaxation time and shape of the fast and slow processes, respectively. *Figure 1* shows experimental correlation functions for the parent polymer PF1 and the PIBA3 polymeric ester in pH = 9. The results of the fitting of the experimental correlation functions to the Kohlrausch–Williams–Watts (KWW) function give β_f values between 0.82 and 0.95 suggesting that the non-exponential shape of the experimental correlation functions is caused by a distribution of molecular sizes and hence of molecular weights. This is not surprising since these samples exhibit the considerable polydispersity that is usually found in thermally polymerized polymers. A second type of analysis which does not assume any functional form for the correlation functions was also made using the inverse Laplace transform (ILT) of the time correlation functions with the REPES algorithm[12], which minimizes the sum of the squared differences between the experimental and calculated intensity–intensity autocorrelation functions $g_2(t)$ using non-linear programming.

$$ag(q,t) = \int_0^\infty A(\tau)\exp(-t/\tau)\,\mathrm{d}\tau = \int_0^\infty \tau A(\tau)\exp(-t/\tau)\,\mathrm{d}\ln\tau \tag{2}$$

Thus, relaxation time distributions are given in the form of $\tau A(\tau)$ *versus* $\log\tau$ plots. Relaxation rates are obtained from the moments of the peaks in the relaxation time distribution. *Figure 2* shows the distributions of the experimental correlation functions in *Figure 1* using REPES. The weight average molecular weight was calculated for each peak in the relaxation time distribution. The relative intensities of the two peaks (see *Figure 2* for example) were obtained from the output of the REPES algorithm (see below) and applied to the total average measured intensity over the run in the evaluation of the reduced scattered intensity, Kc/R_θ where K is the optical constant ($K = 4\pi n_0^2(\mathrm{d}n/\mathrm{d}c)^2/N_A\lambda^4$) with n_0 the solvent refractive index, dn/dc the refractive index increment and R_θ the Rayleigh ratio as obtained by calibration measurements. Measurements were made at an angle of 90°, after it had been established that there was no angle dependence of the reduced scattered intensity.

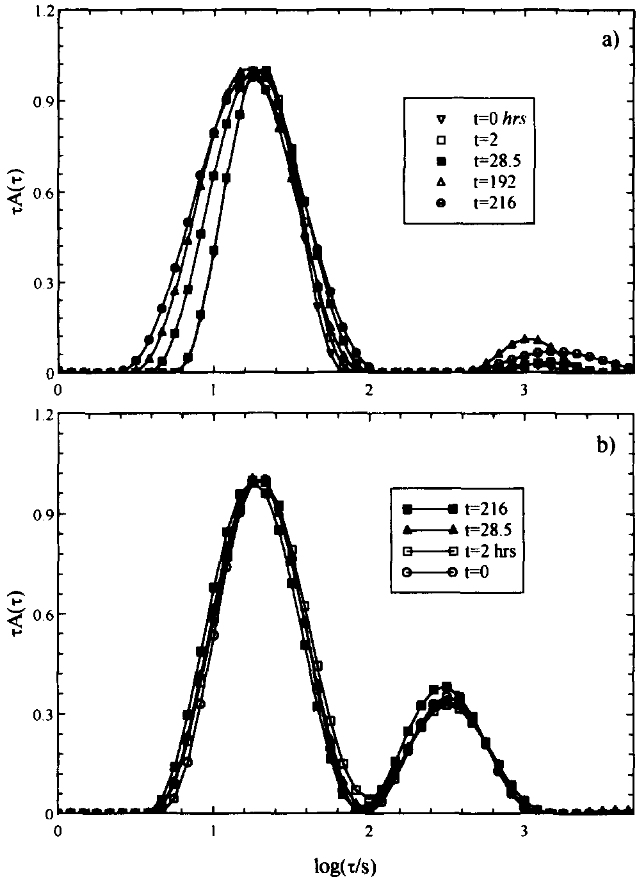


Figure 3 (a) Shift of the fast mode to shorter times after hydrolysis for PIBA3 in pH = 9. (b) The fast mode for PIBA3 (c = 0.0096 g ml^{-1}) in pH = 9 as a function of time

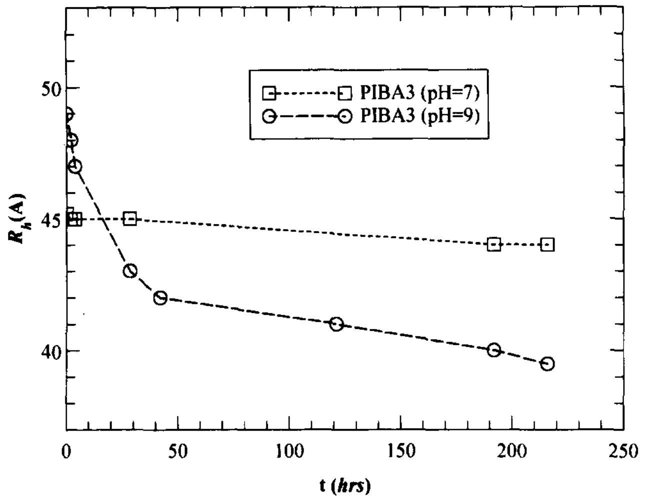


Figure 4 The changes in the apparent hydrodynamic radius of the PIBA3 polymeric ester during hydrolysis as a function of time in pH = 7 and 9

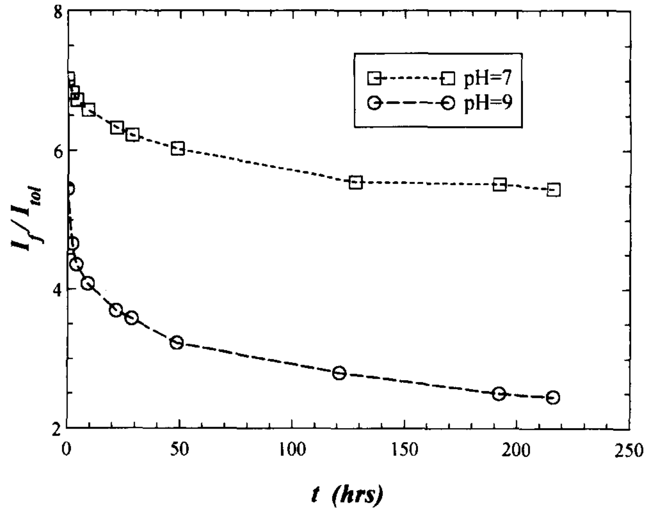


Figure 5 The drop in scattering intensity of the fast process (I_f) divided by the pure toluene intensity (I_{tol}) I_f/I_{tol} for PIBA3 (c = 0.0096 g ml^{-1}) as a function of time in pH = 7 and 9

Results and discussion

A very important characteristic of the present polymeric formulations is their ability to gradually release the LMW bioactive compound by hydrolytic cleavage of the bond between the bioactive substance residues and the polymeric chain. The examination of the dynamic processes that are experimentally manifested in solution proved to be most interesting. Two hydrodynamic modes are observed, a fast and a slow one. The relaxation rate of the fast mode is proportional to the square of the scattering vector q characteristic of diffusional dynamics. From the position of the peaks one can obtain the translational diffusion coefficient D and hence the equivalent hydrodynamic radius R_h using the Stokes–Einstein equation:

$$R_h = \frac{kT}{6\pi\eta_0 D} \tag{3}$$

where η_0 is the solvent shear viscosity and k_B is the Boltzmann constant. As this equation is valid in the limit of $c \rightarrow 0$ the values calculated from equation (3) are apparent hydrodynamic radii. The extrapolated quantity $(D\eta/T)_{c\rightarrow 0}$ is used in equation (3) to obtain the hydrodynamic radius R_h which is 42 Å for the parent polymer, 43 Å for PIBA1, 44 Å for PIBA2 and 47 Å for PIBA3. The normalized R_h values as $(R_h)^2/\bar{M}_w$ are closely similar indicating that the size is close to invariant. Considering the complex nature of polymer conformations the R_h which is obtained here represents a useful quantity for polymer dimensions.

Figure 3 shows the evolution of the ILTs of the experimental correlation functions with time in alkaline and neutral pH. The distribution of decay rates shows two resolved peaks. There is a clear shift of the faster peak to shorter times [*Figure 3*(a)] as time progresses for the case of the alkaline solution. This is obviously a result of the hydrolysis of the polymeric ester that loses its IBA linkages progressively, and therefore the size of the polymeric derivative decreases steadily as a function of time. Chemical data concerning the hydrolysis of the polymeric ester of IBA are not yet available, however extensive hydrolysis studies[6,9,13] have been performed for polymeric esters of other bioactives (e.g. 1-naphthylacetic acid, 2,4-dichlorophenoxyacetic acid) using the same polymeric carrier PF1. *Figure 4* displays the changes in the hydrodynamic radius R_h during hydrolysis in alkaline and neutral environment. Apparently, the R_h decreases as hydrolysis progresses in alkaline pH; however, there are no perceptible changes in neutral pH. The shift to faster dynamics is followed by a concomitant drop in scattering intensity displayed in *Figure 5* for PIBA3 in neutral and alkaline pH that is also a consequence of the loss of the bioactive compound. There is an obvious difference in the decay rates of the polymeric regulator in the two pH environments.

In this work the results of the light-scattering experiments suggest that the incorporation of the IBA groups does not affect the conformation of the parent polymer. This behaviour is to be contrasted with the results of a recent study[1] in which the same parent polymer contained side linkages of 1-naphthylacetic acid (NAA). In that study a large increase in the hydrodynamic size was observed that was caused primarily by the aggregation of the individual chain molecules to form multimolecular clusters. The different behaviour is caused by the smaller degree of aromatic character of the IBA linkages as compared to the increased hydrophobic character of the NAA side groups, since the IBA linkages have moderately polar character.

The slower aggregate peaks are well defined, suggesting that they have a discrete size. The large values of R_h for the slow mode indicate that this mode may be attributed to micellar polymeric components of larger size, both for the parent polymer and the three IBA polymers. The slowest component in the spectrum of the parent polymer has a radius of 600 Å, while the cluster components in the three IBA polymers are comparatively larger with a radius of about 1000 Å. It should be emphasized that although the aggregate species dominate the scattered intensity due to their heavy weighting, they constitute a very low number fraction. In fact, the weight concentration of the structures responsible for the slow mode is very small, less than 0.3%.

Conclusion

The investigation of the dynamics of the new slow release formulations of the plant growth regulator 3-indolbutyric acid in aqueous solution was very promising. When IBA groups are incorporated in the parent polymer the polymer molecular dimensions retain their previous values. The hydrodynamic radii and scattering intensities display a systematic drop as a function of time, apparently due to the loss of the IBA linkages during hydrolysis in alkaline pH. The obtained data concerning the properties of the macromolecular structures in solution lead the way toward a deeper understanding of the complex molecular phenomena occurring in solutions of hydrophobically modified polymers.

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