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Dynamic light scattering study of 1-naphthylacetic acid polymeric derivative, a novel plant hormone

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This paper describes the dynamics of new slow release formulations of plant growth regulator (PGR) 1-naphthylacetic acid (NAA) in aqueous solution. The new PGR formulations are water-soluble copolymers derived from acrylamide and vinyl chloroethyl ether, containing side linkages of NAA 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 hydrophobic naphthyl group dramatically affects the conformation of the parent polymer. The interactions between the hydrophobic NAA groups lead to the formation of interpolymeric micellar structures. The hydrodynamic radius R_h which is 42 Å for the parent polymer (PF1), increases to 70 Å for the NAA polymeric ester with 1.94% mol content (PF2) and becomes 82 Å by increasing the amount of the hydrophobic substituent to 3.54 mol.% content (PF3). The scattering intensities and apparent hydrodynamic radii display a systematic drop as a function of time due to the loss of the NAA linkages and R_h becomes 58 Å for PF2 and 68 Å for PF3, 116 h after the preparation of the water polymeric solutions. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: light scattering; polymeric derivatives; plant hormones)

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. The response is characterized by a sharp rise and drop in biological activity, moving on to a negative response with a further increase in the dose of the hormone[6,7].

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 safety to those handling the products. Moreover, the modified PGRs are characterized by a wider range of effective concentrations which is convenient for farmers since fewer preparations are required to produce an effective concentration[5–11].

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 plant growth regulator units may either be introduced directly onto the polymer chain, attached to the terminal linkages of the chain, or attached to the side groups of the main chain. The latter method of linking the regulator units to the polymer has been shown to be the most promising since, in this case, both the content of the bioactive compound in the polymer and also the properties of the overall polymeric system may be regulated during synthesis. The regulator units may be either directly attached to the side polymer linkage or through a spacer[12,13]. The hydrolysable bond between the polymeric chain and the PGR units may be ionic, covalent, or of coordination character. The functional moieties most often used for covalent bonding are esters, amides, urea, etc. 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

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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 1-naphthylacetic acid (NAA) 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 ($c < c^*$). When the hydrophobic NAA groups are incorporated in the parent polymer, the polymer molecular dimensions change abruptly followed by aggregation of individual polymer chains. The light scattering data show changes and trends in the dynamics and scattering intensities. These are discussed in terms of the NAA concentration value in the polymeric form and as a function of concentration in the polymeric water solution and also as a function of time.

EXPERIMENTAL

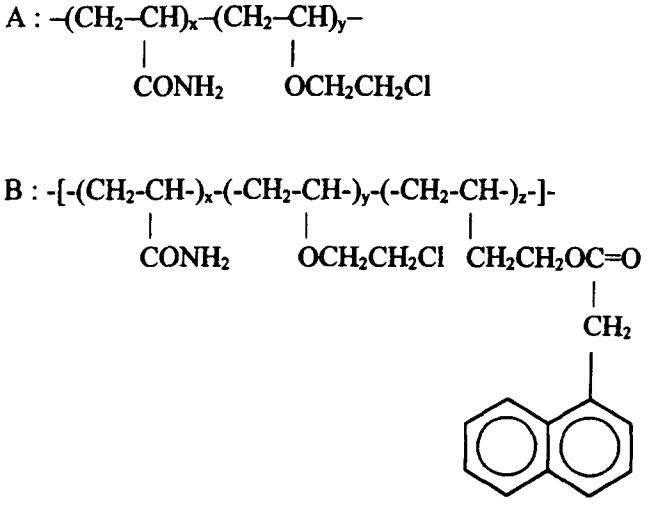
Preparation of the polymeric NAA derivatives

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

Table 1 Molecular characteristics of the polymeric derivatives

Polymeric form	$\bar{M}_w$	Type of molecular structure	NAA linkage content (mol.%)
PF1	18 000	A	0
PF2	19 000	B	1.94
PF3	21 000	B	3.54

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 1-naphthylacetic acid ether with z = 1.94 and 3.54 mol.% (x = 92 mol.% and $y + z$ = 8 mol.%)



Dynamic light scattering

The experimental autocorrelation function was measured with an apparatus equipped with an argon ion laser (Coherent 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:

$$G(q,t) = A[1 + f|ag(q,t)|^2] \quad (1)$$

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,

$$ag(q,t) - A_f \exp[-(t/\tau_f)^{\beta_f}] + A_s \exp[-(t/\tau_s)^{\beta_s}] \quad (2)$$

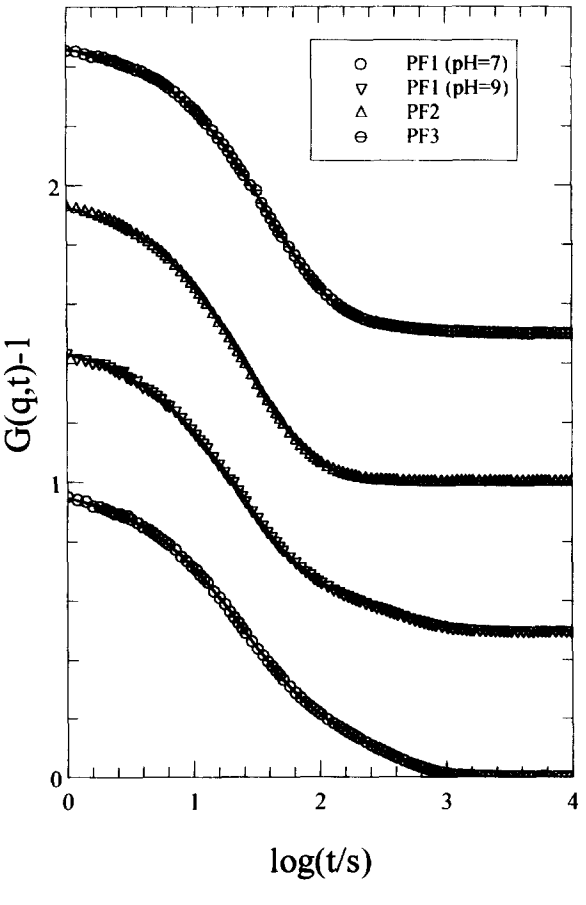


Figure 1 Experimental correlation functions for PF1 in neutral and alkaline conditions, PF2 and PF3

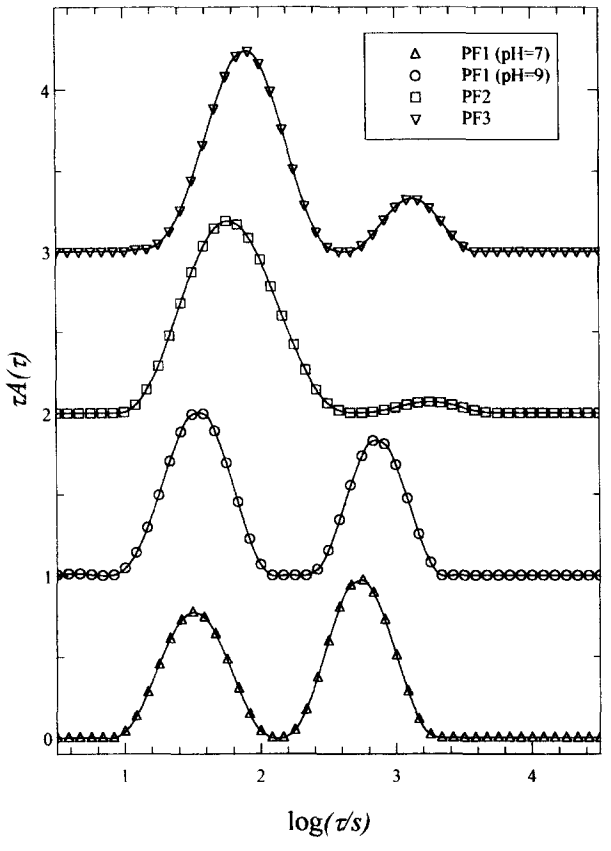


Figure 2 The evolution of the distributions for the experimental correlation functions of *Figure 1* using REPES

with parameters $\{A_f, \tau_f \text{ and } \beta_f\}$ and $\{A_s, \tau_s \text{ and } \beta_s\}$ that give the contrast, relaxation time and shape of the fast and slow processes, respectively. Typical polarized intensity–intensity correlation functions $g^{(2)}(t)$ for a scattering angle of 90° are shown in *Figure 1* for the parent polymer PF1 in neutral and alkaline media (pH 9) and for the NAA polymeric ester with 1.94 (PF2) and 3.54 mol.% content (PF3) in pH 9.

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[14], which minimizes the sum of the squared differences between the experimental and calculated intensity–intensity autocorrelation functions $g_2(t)$ using nonlinear programming.

$$ag(q,t) = \int_0^\infty A(\tau) \exp(-t/\tau)\, d\tau$$

$$= \int_0^\infty \tau A(\tau) \exp(-t/\tau)\, d \ln \tau \quad (3)$$

Thus, relaxation time distributions are given in the form of $\tau A(\tau)$ versus log τ plots. Relaxation rates are obtained from the moments of the peaks in the relaxation time distribution. The evolution of these distributions for the experimental correlation functions displayed in *Figure 1* is shown in *Figure 2*. The ILT result shows a double peak structure.

RESULTS AND DISCUSSION

Static light scattering

Measurements of the scattered intensity were made on solutions as a function of scattering angle and as a function of concentration. *Figure 3* shows static light scattering data of the reduced scattered intensity Kc/R_θ at 25°C of the parent polymer where K is the optical constant ($K = 4\pi n_0^2 (dn/dc)^2 / N_A \lambda^4$) with n_0 the solvent refractive index, dn/dc the refractive index increment and R_θ is the Rayleigh ratio as obtained by calibration measurements. The molar mass of the parent polymer in dilute solution is $M_w = 1.8 \times 10^4$ with the second virial coefficient $A_2 = 1.02 \times 10^{-5}$ ml mol g^{-2}.

Dynamic light scattering

The results of the fitting of the experimental correlation functions to the Kohlrausch–Williams–Watts (KWW) function give $\beta_f = 0.92 \pm 0.03$ and $\beta_s = 0.68 \pm 0.05$ for PF2 and PF3 strongly suggesting that the nonexponential 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 very important characteristic of the present polymeric formulations is their ability to release gradually the LMW

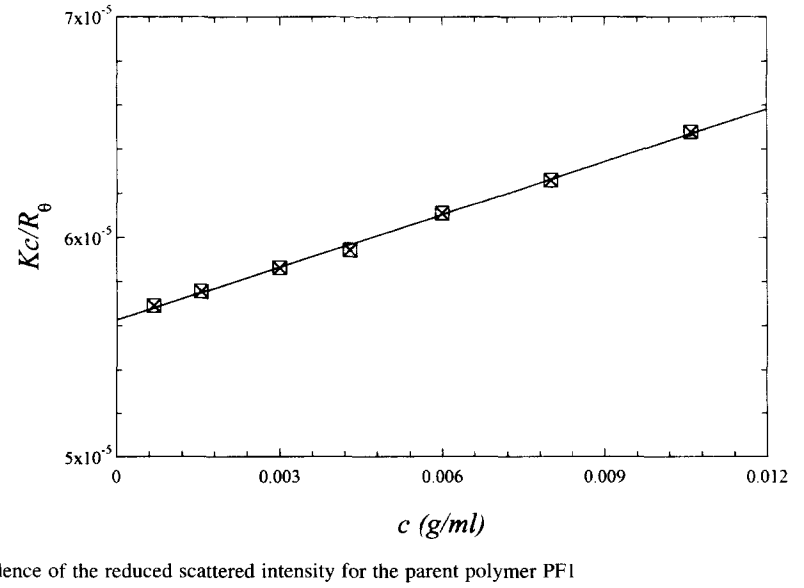


Figure 3 The angular dependence of the reduced scattered intensity for the parent polymer PF1

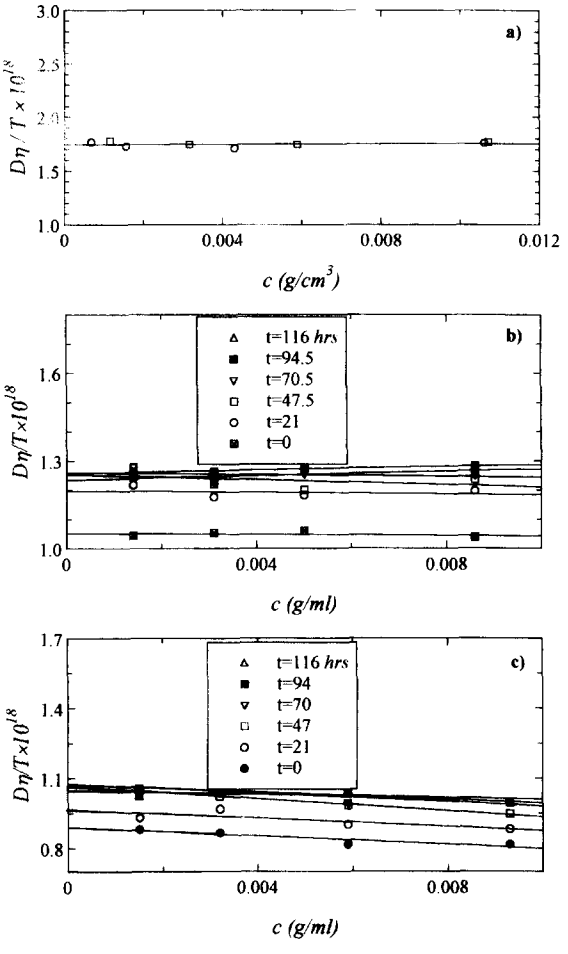


Figure 4 Reduced diffusion coefficients as a function of concentration and time for (*a*) the parent polymer PF1, (*b*) PF2 and (*c*) PF3

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 in which the quite big molecules of regulators are gradually discharged, 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:

$$R_h = \frac{kT}{6\pi\eta_0 D} \tag{4}$$

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 (4) are apparent hydrodynamic radii. *Figure 4a–c* shows data for the reduced diffusion coefficients ($D\eta_0/T$) of the three samples as a function of concentration and also as a function of time. The reduced diffusion coefficients for polymers PF2 and PF3 increase as a function of time suggesting more subtle effects. The extrapolated quantity $(D\eta/T)_{c \rightarrow 0}$ is used in equation (4) to obtain the hydrodynamic radius R_h which is 42 Å for the parent polymer, 70 Å for PF2 and 82 Å for PF3.

Figure 5 shows the evolution of the ILTs of the experimental correlation functions with time. The distribution of decay rates shows two resolved peaks. There is a clear shift of the faster peak to shorter times as time progresses. This is obviously a result of the hydrolysis of the polymeric ester that loses its NAA linkages progressively, and therefore the size of the polymeric aggregates decreases steadily as a function of time. The shift to faster dynamics is followed by a concomitant drop in scattering intensity displayed in *Figure 6* for PF2 and PF3 that is also a consequence of the loss of the bioactive compound.

These polymers which are characterized by the bulky hydrophobic NAA aromatic groups clustered toward the center of the coil and the hydrophilic polar groups located in the aqueous boundary have been reported[15–19] to exist in dilute solution as 'hypercoils'. In this work the results of the light scattering experiments suggest that the incorporation of the hydrophobic naphthyl group dramatically affects the

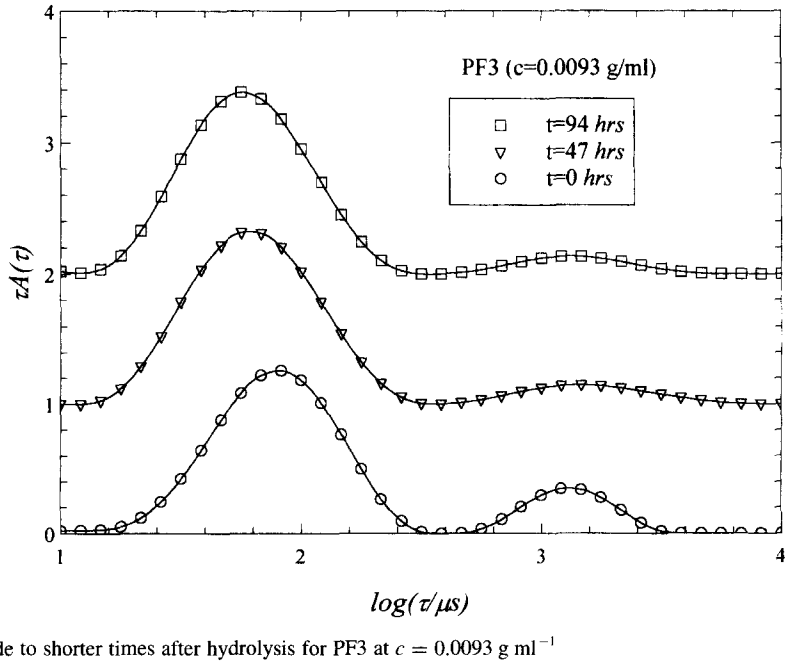


Figure 5 Shift of the fast mode to shorter times after hydrolysis for PF3 at $c = 0.0093$ g ml^{-1}

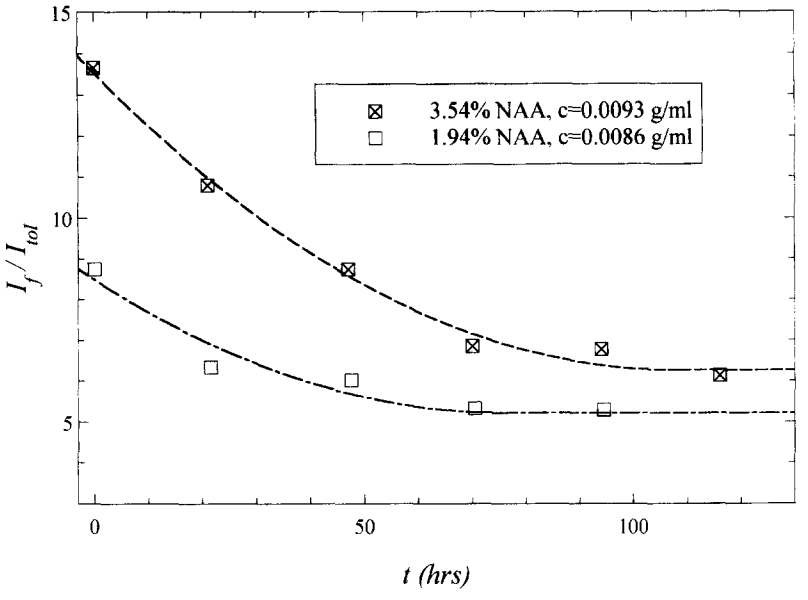


Figure 6 Drop in scattering intensity of the fast process (I_f) divided by the pure toluene intensity (I_{tol})I_f/I_{tol} for PF2 and PF3 as a function of time

conformation of the parent polymer. The hydrodynamic radius R_h which is 42 Å for the parent polymer PF1, increases to 70 Å for PF2. By increasing even more the amount of the hydrophobic substituent in PF3 the hydrodynamic radius becomes 82 Å. This suggests that the large increase in the hydrodynamic size is caused by aggregation of individual chain molecules to form multimolecular clusters. It is proposed, therefore, that under these conditions the polymers form an interior region containing a high concentration of the hydrophobic aromatic NAA groups surrounded by an outer region of the hydrophilic acrylamide groups.

Figure 7 shows the changes in the apparent hydrodynamic radius R_{ha} of the two NAA polymeric esters during hydrolysis as a function of concentration at different times. The hydrodynamic radius is decreasing steadily and it acquires the value of 58 Å for PF2 and 68 Å for PF3 116 h after the preparation of the samples. Due to the apparent loss of the bulky bioactive residues, the R_{ha} of the aggregates in solution is slowly decreasing as time progresses. As the hydrophobic core is losing its NAA substituents, the hydrophobic interactions inherent within the polymer are decreasing with a concomitant change in the conformation adopted by the respective polymer coils, that now become expanded. Therefore the aggregates lose gradually their stability in solution and begin to dissolve breaking into smaller aggregates. Therefore, the occurrence of interpolymeric aggregation would be restrained as hydrolysis proceeds and, finally, after infinite time the hydrodynamic radius of the fully hydrolyzed polymer will become comparable to the R_h of the parent polymer. Indeed the current values of the R_h corroborate the above expected result.

The large values of R_{ha} 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 two NAA polymers. The slower aggregate peaks are well defined, suggesting that they have a discrete size. The slowest component in the spectrum of the parent polymer has a radius of 600 Å, while the cluster component in the two NAA polymers PF2 and PF3 are comparatively larger with a radius of 1000–3000 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%.

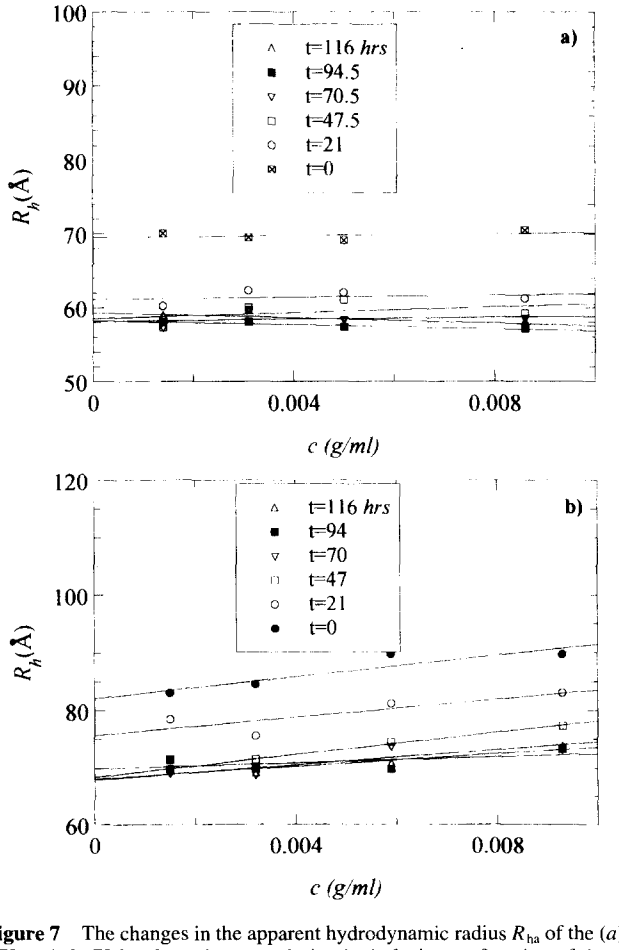


Figure 7 The changes in the apparent hydrodynamic radius R_{ha} of the (*a*) PF2 and (*b*) PF3 polymeric esters during hydrolysis as a function of time

CONCLUSION

The investigation of the dynamics of the new slow release formulations of the plant growth regulator 1-naphthylacetic acid in aqueous solution was very promising. When hydrophobic NAA groups are incorporated in the parent polymer the polymer molecular dimensions change abruptly followed by aggregation of individual polymer chains due mainly to the intermolecular interaction between the hydrophobic groups. The hydrodynamic radii and scattering intensities display a systematic drop as a function of time apparently due to the loss of the NAA linkages during hydrolysis. 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 and will further contribute to the design of desirable formulations with optimal properties to be applied in agrotechnology.

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