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New polymeric systems with controlled release action: a light scattering investigation

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Abstract

New controlled release water soluble formulations of sorbic (2,4-hexadienoic) acid (SA) were prepared and their inhibitory activity on mycelium growth of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* was evaluated. The new products are epoxidized polymers of polyvinylpyrrolidone (PVP) containing covalently bonded SA (polymeric esters of SA) and complexes of PVP with hydrogen bonded SA, characterized by controlled release of SA. It was shown that the polymeric complexes of SA with PVP were more effective fungicidal agents than SA polymeric esters. In all cases the activity of polymeric derivatives (esters and complexes) was increasing by lowering the molecular weight of the polymeric carriers. We have performed static and dynamic light scattering measurements in a series of PVP/SA water soluble polymeric derivatives. The light scattering data suggest that the polymer structure and/or solution conformation may be important determinants of the inhibitory effect (bioactivity).

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1. Introduction

The problem of food contamination by fungi and their toxic metabolites is quite common. One way to deal with this problem is to use organic acids with a small carbon chain like sorbic acid (SA) or propionic acid as food preservatives. They are known to prevent fungal growth and spore germination and reduce mycotoxin biosynthesis by inhibiting the biological pathways responsible for mycotoxin production and release [1–3]. SA is of special interest. It possesses low toxicity (LD_{50} = 7.36 g/kg after oral administration in rat) and it is widely used in different kinds of products (baked goods, beverages, fresh fruits and vegetables, ...) for taste improvement, pH balance and as a preservative against bacterial and fungal contamina-

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tion. It is also used against bacterial and fungal contamination and for possible prevention of mycotoxin biosynthesis [4–6].

Our aim is to investigate the activity of polymeric derivatives of SA with controlled release action, on the mycelial growth of *Fusarium oxysporum* f.sp. *cucumerinum* (*Fusarium oxysporum* is a common mould that contaminates tomato and potato plants, wheat and corn) [7,8]. It is very important to understand the solution behaviour with dynamic light scattering [9–12].

2. Experimental

Polymeric carriers for the covalent immobilisation of SA (PVP containing epoxy groups) were prepared by the reaction of PVP with chloroacetamide in the presence of Na ethylate in ethanol [13]. Polymeric esters of SA were prepared by the reaction of acid with PVP containing epoxy groups in dry DMF at 90 °C for 4 h. Polymeric esters were precipitated in ethanol, washed by ethanol and dried in vacuum. The content of SA was determined spectrophotometrically at 254 nm.

The SA complexes prepared, contained SA attached to the polymer via a hydrogen bond. One gram of SA in 50 ml of water and 40 g of PVP (Mw = 10 kDa or Mw = 40 kDa) in 100 ml of water or 18 g of PVP (Mw = 360 kDa) in 100 ml of water respectively were prepared separately. Both solutions were heated until completely dissolved. The above solutions were mixed and the temperature was adjusted to 100 °C. The reaction led to a clear solution after lowering the temperature, which is an indication that all SA was attached to the polymer. The molecular characteristics of the polymeric esters and complexes with PVP are collected in Tables 1 and 2.

Table 1
Molecular weight, content of epoxide groups in epoxidized PVP and content of SA in polymeric esters

Polymeric sample	Molecular weight (kDa)	Content of epoxide group (mol%)	Content of SA	
			(mass %)	(mol%)
Polymeric ester 1	10	11.2	4.6	5.1
Polymeric ester 2	40	10.1	4.5	4.9
Polymeric ester 3	360	7.2	3.2	5.1

Table 2
Molecular weight and content (w/w %) of SA in polymeric complexes

Polymeric sample	w/w % in SA	SA:PVP	Molecular weight of PVP (kDa)
Complex 1	2.5	1:40	10
Complex 2	2.5	1:40	40
Complex 3	2.5	1:40	360

3. Results

The experimental autocorrelation function was measured with an apparatus equipped with a diode laser operating at a wavelength of 532 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 multi-bit, multi-τ full digital correlator was used that covered a broad dynamic range of about 10 decades. 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 solvent, 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 concentration fluctuations with correlation times longer than 10^{-6} s and A is the baseline. Typical polarized intensity–intensity correlation functions for a scattering angle of 90° are shown in Fig. 1 for complexes 2 and 3. Relaxation time distributions are obtained by using the inverse Laplace transform (ILT) of the time correlation functions with the REPES algorithm [14] that minimizes the sum of the squared differences between the experimental

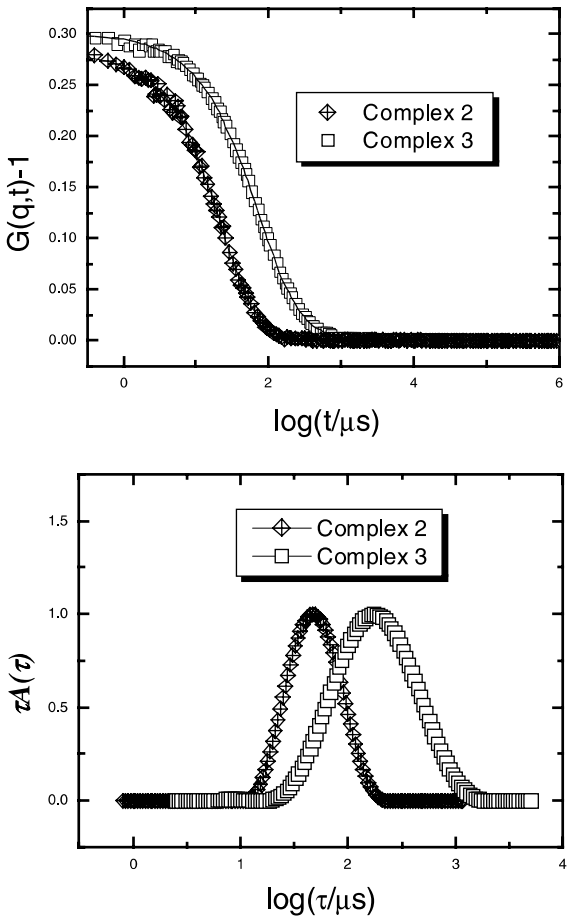

Fig. 1. Experimental correlation functions for polymeric complexes 2 and 3 with molecular weights of 40 and 360 kDa respectively and the ILTs of the experimental correlation functions.

and calculated intensity–intensity autocorrelation functions $g_2(t)$ using non-linear programming.

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

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 and displayed in Fig. 1. The ILT result shows a single peak structure. 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:

$$R_h = \frac{kT}{6\pi\eta_0 D}, \quad (3)$$

where η_0 is the solvent shear viscosity and k_B is the Boltzmann constant. As this equation is valid in the limit of $c \to 0$ the values calculated from Eq. (2) are apparent hydrodynamic radii.

4. Discussion

The dosage-mycelial growth inhibition curves of SA and its complexes were linear (Fig. 2). The ED_{50} (dosage for 50% inhibition of mycelial growth) value of complex 1 was 1089 ppm, of complex 2 was 1478 ppm and of complex 3 was 2169 ppm, while the ED_{50} value of SA was 5592 ppm. PVP without SA had no effect on mycelial growth of *F. oxysporum* f.sp. *radicis-cucumerinum*. The slope of the curves decreased with increasing molecular weight of the complex, while the smallest value was observed with SA. The inhibitory effect of polymeric esters and complexes of SA on mycelial growth of *F. oxysporum* f.sp. *radicis-cucumerinum* was increased in a manner dependent on the molecular weight of PVP (Fig. 2). The lower the molecular weight the higher the inhibition. This is probably due to the easier release of SA from PVP with a lower molecular weight, which consequently acts upon the fungus in a higher concentration. It has been shown that in a polymeric system the bioactivity is attributed to the release of the bioactive substance (e.g. SA) from the polymer. The hydrolysis rate of the bioactive ligand from the system is associated with the entire expression of bioactivity [15].

The comparison between the apparent hydrodynamic radius of pure PVP and PVP/SA complexes as a function of the molecular weight (Fig. 3) indicates that the presence of SA causes a significant decrease in the size of the PVP/SA complex.

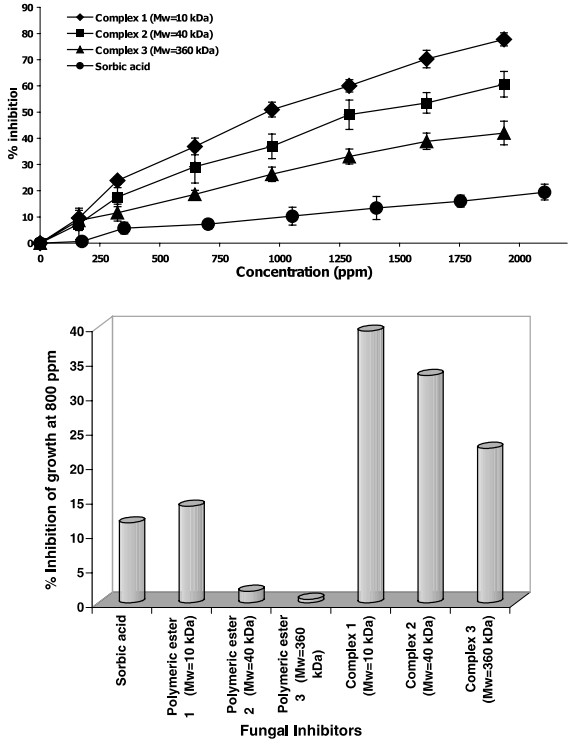

Fig. 2. Inhibitory effect of SA and the polymeric derivatives. On vertical bars indicate standard errors of the mean on mycelium growth of *F. oxysporum* f.sp. *radicis-cucumerinum* at 27 °C for six days, pH = 6.4 at 800 ppm. Inhibition of water solutions of SA and its PVP–SA complexes on mycelium growth of *F. oxysporum* f.sp. *radicis-cucumerinum* at 27 °C, pH = 6.4.

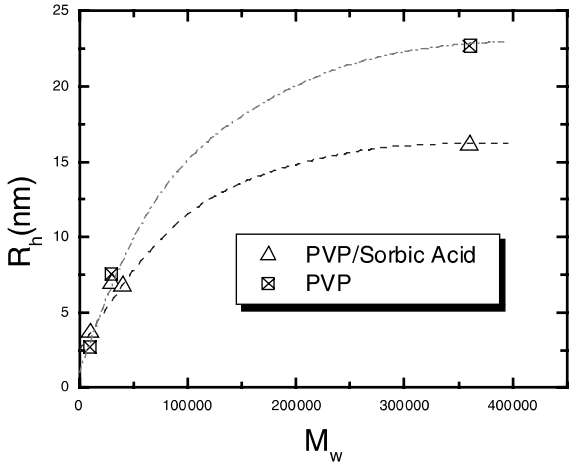

Fig. 3. The apparent hydrodynamic radius as a function of molecular weight for PVP and PVP/SA complexes.

The higher inhibitory effect of SA complexes than that of polymeric esters on mycelial growth of *F. oxysporum* f.sp. *radicis-cucumerinum* may be attributed to the structure of the hydrogen bonding in the SA complex with PVP, which is weaker than the SA–PVP covalent bond in the polymeric esters.

5. Conclusions

Polymeric formulations of SA exerted an inhibitory effect on the mycelial growth of *Fusarium oxysporum* f.sp. *Cucumerinum*. The bioactivity was higher with the lower molecular weight polymers. All complexes showed a higher inhibitory effect than SA in water. The light scattering data indicate that the polymer structure and/or solution conformation may be important determinants of the inhibitory effect. Controlled release formulations of these systems are new promising products due to their low toxicity wide range of effective concentrations for applications and ability to regulate lyophilicity.

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