Controlled Release of Biocides in Solid Wood. I. Efficacy Against Brown Rot Wood Decay Fungus (*Gloeophyllum trabeum*)

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ABSTRACT: Nanoparticles containing the fungicides tebuconazole or chlorothalonil were prepared with median diameters of ~ 100–200 nm. The nanoparticle matrices were polyvinylpyridine (PVP), copolymers of PVP and styrene (PVP-*co*-St), and blends of PVP and hyperbranched polyesters (HBPs). The isolated nanoparticles were resuspended in water to yield suspensions able to deliver 0.1, 0.2, 0.4, and 0.8 kg fungicide/m^3 of wood. Southern yellow pine (SYP) was treated with these suspensions using conventional vacuum-pressure treatments. Less concentrated suspensions delivered the nanoparticles quantitatively into the SYP while suspensions capable of delivering up to 0.8 kg of the fungicide (the active ingredient or AI) per cubic meter of SYP delivered 0.4–0.7 kg AI/m^3, depending on the nanoparticle matrix. The SYP lost less than 5% of its mass after 55 days of exposure to *Gloeophyllum trabeum* when the tebuconazole content in the SYP reached 0.2 kg/m^3, and the matrix identity had little effect on the results. The SYP lost between 3 and 6% of its mass after 55 days of exposure to *G. trabeum* when the AI was chlorothalonil at a level of ~ 0.6 kg AI/m^3 of wood and was introduced into SYP in nanoparticles with a PVP/HBP matrix, but it lost ~ 11% of its mass at the same AI levels when the nanoparticle matrix was PVP. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 596–607, 2002

Key words: controlled release; nanoparticles; wood preservatives; tebuconazole; chlorothalonil

INTRODUCTION

The production of wood that is pressure treated with wood preservatives is a major component of the U.S. forest products industry. In 1996 there were 592 million ft^3 of pressure-treated wood produced with gross sales of $3.91 billion.[1] The major product of the industry is southern pine lumber treated with chromated copper arsenate (CCA), accounting for about 50% of the total production volume.[1] Most of this treated lumber is used around the home for decks, fences, landscaping timbers, and so forth. There is concern about the safety and health effects of CCA as a wood treatment.[2] This led to the development and listing of alternative wood preservative systems for lumber with lower perceived risk such as ammoniacal copper quat, copper bis[dimethyldithiocarbamate], ammoniacal copper citrate, and copper azole.[3]

Organic biocides have been developed that are considered to be environmentally benign and not expected to pose the problems associated with CCA-treated lumber. Some biocides (e.g., tebuconazole) are quite soluble in common organic solvents while others (e.g., chlorothalonil) possess only low solubility. The solubility of organic biocides affects which markets are appropriate for the biocide-treated wood product. Biocides with good solubility can be dissolved at high concentrations in a small amount of organic solvent, and that solution can be dispersed in water with appropriate emulsifier to produce an aqueous emulsion. The emulsion may be used in conventional pressure treatments for lumber, and wood treated in such a manner can be used in products such as decking or other household applications where the treated wood will come into contact with humans. Biocides that have little solubility in common solvents can only be conventionally incorporated into wood in a solution of a hydrocarbon oil (e.g., AWPA P9 type A oil[3]) and the resulting organic solution used to treat the wood directly. Although the hydrocarbon oil itself affords additional protection to the wood, the oil has an unpleasant odor and is irritating to human skin. Consequently, wood treated in this manner can only be used in industrial applications such as railroad ties or telephone poles. Therefore, the market for wood treated in this manner is limited.

Several advantages might be realized by incorporating organic biocides into polymeric nanoparticles and introducing the nanoparticles into wood. Because the biocides are dispersed in a solid polymeric nanoparticle that can be suspended in water, any biocide, even

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those with low solubility in organic solvents, can be introduced into wood with conventional water-borne pressure treatment techniques. Therefore, low solubility biocides that until now had limited markets might be used in wood products marketed for household applications. Also, the polymer matrix functions as a storage reservoir for the biocide. Once in the wood, the polymer matrix controls the release rate of the biocide and simultaneously protects the unreleased biocide from exposure to the environment. Because the biocide is afforded some protection from random degradation processes until it is released, this may ultimately increase the long-term protection afforded to the wood.

The detailed preparation of polymeric nanoparticles, which contained tebuconazole (a high solubility fungicide) and chlorothalonil (a low solubility fungicide), was described earlier.[4] The fungicides were efficiently incorporated into three linear polymer matrices; polyvinylpyridine (PVP), PVP-*co*-styrene (PVP-*co*-St), with 10% St, and (PVP-*co*-St) with 30% St, by a simple, one-step preparative method, and incorporated into wood using conventional pressure treatment methods. The biocide release rate was measured in water and was found to increase with the matrix hydrophilicity. The fungicide-containing nanoparticles prepared in that work were effective in protecting southern yellow pine (SYP) against *Gloeophyllum trabeum* (a brown rot fungus). However, in practice, a variety of wood preservatives would be required to be introduced into the wood. Each biocide might require different release rates for optimal efficacy. Therefore, the ability to "tune" the release of individual biocides by controlling the polymer matrix is crucial. Prior work[4] demonstrated that increasing or decreasing the matrix hydrophilicity increased and decreased the biocide release rates respectively. The objectives of the work described here were to investigate the potential to further influence biocide release rates by altering the nanoparticle porosity and to quantify the efficiency of the active ingredient (AI) delivered into the treated wood.

The simplest approach to altering matrix porosity would be to introduce crosslinking into the matrix.[5] However, that would involve synthesis of copolymers containing a crosslinkable monomer. Moreover, the crosslinking efficiency would be difficult to determine, so it would be difficult to increase the matrix porosity in a systematic manner. Relatively low-cost hyperbranched polyesters (HBPs) are commercially available. A hyperbranched polymer is one in which branched monomers are attached in layers around a central core. Because each monomer branches, if a layer consisting of four trifunctional monomers is attached to a tetrafunctional central core, the first "generation" (G) layer possesses 8 functional end groups. If a second generation (G2) is added, the G2 HBP should possess 16 functional end groups, and so on. We thought that these materials could be blended with the matrix polymer and, by increasing the molecular weight through increased G, the porosity would increase systematically, allowing the effect of the porosity on the release rate to be determined. If the porosity was increased, then the ability of water to penetrate the matrix, and thus the AI release rate, would increase. Representations of a G3 HBP and a linear polyester (LPE) control are shown in Figure 1.

EXPERIMENTAL

Materials

Tebuconazole was supplied by Miles, Inc. (Pittsburgh, PA). Chlorothalonil was supplied by ISK Biosciences (Memphis, TN). The surfactants were from Calgon Company (Pittsburgh, PA). A linear polyester control was prepared as described below. All other reagents, including HBPs, were from Aldrich (Milwaukee, WI). Wood specimens were SYP sapwood (*Pinus* spp.). Fungal tests employed *G. trabeum* (ATCC 11539), a common basidomycete brown rot, wood decay fungus.

Instrumentation

Particle sizing was carried out on a Shimadzu CP-4 particle sizer (centrifugation). Thermal analysis was done with a Shimadzu DSC-50. Molecular weight measurements were made on a Perkin–Elmer 601 size exclusion chromatography (SEC) instrument equipped with Phenomenex phenogel columns and a UV–visible detector. The ^{1}H-NMR was performed on a Varian 200-MHz instrument.

Procedure for preparation of LPE

Glutaric acid (10.000 g/75.068 mmol), 2,2-diethyl-1,3-propanediol (10.510 g/79.501 mmol), and *p*-toluenesulfonic acid (0.0700 g/3.68 $\times$ 10^{-4} mol) were added into a three-necked round-bottom flask equipped with a nitrogen inlet–outlet and a mechanical stirrer. The reaction was stirred and heated to 140°C for 4 h. The reaction solution was then subjected to reduced pressure for 0.5 h to further advance the reaction by removal of residual water. The linear oligomer was characterized by SEC (theoretical M_n 5,419 g/mol; found M_n 5,400 g/mol).

Preparation of linear matrix nanoparticles

The preparation of linear matrix nanoparticles was described earlier.[4] The basic procedure was to dissolve the polymer matrix and the AI (in a 1:1 mass ratio) in a small amount of a water-soluble solvent (methanol

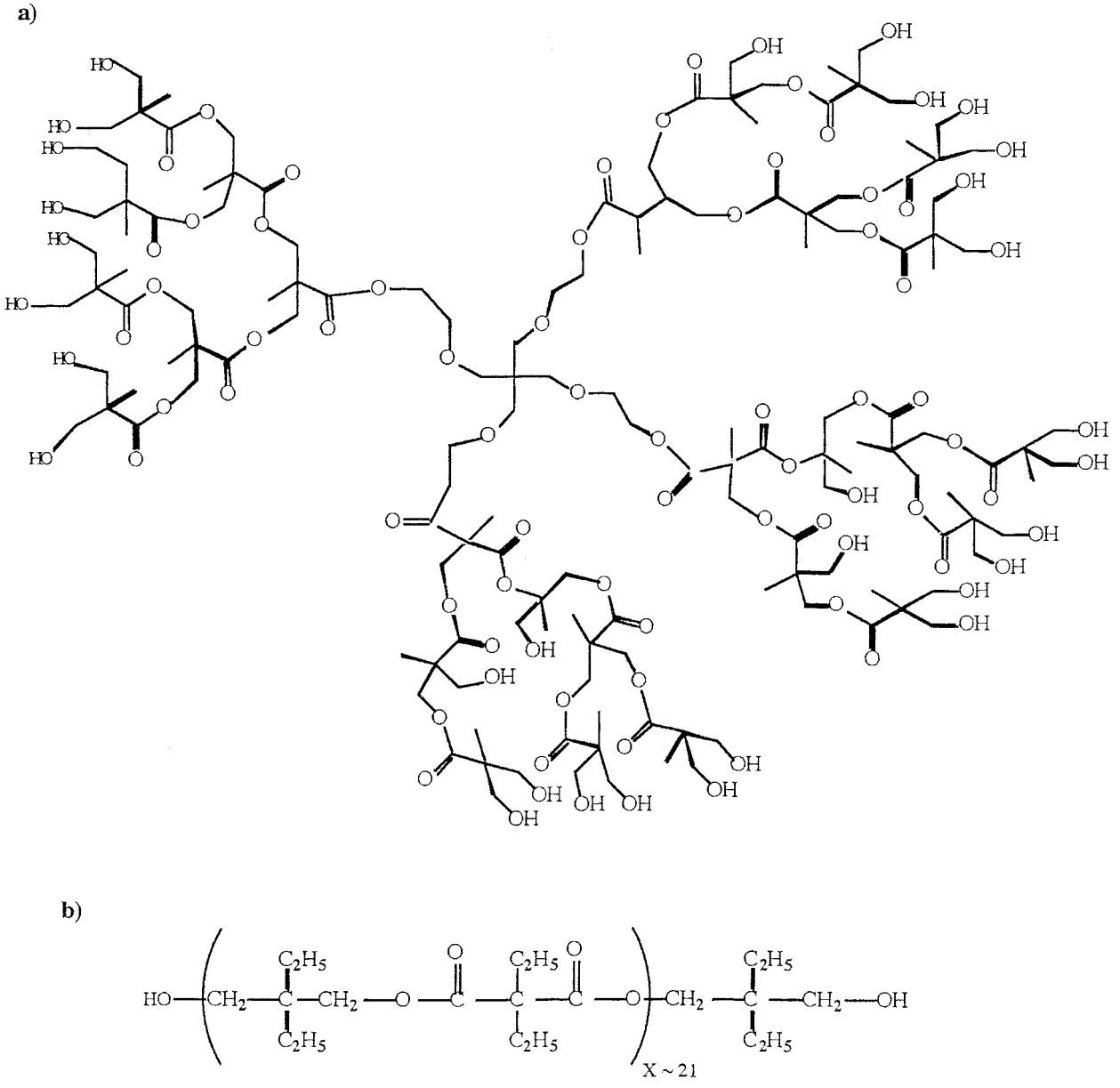

Figure 1 Representative structures for (a) HBP G3 and (b) LPE.

or *N*-methylpyrnolidone) and slowly drip this solution into a stirred mixture of water and surfactant. Nanoparticle yields using this procedure typically ranged from ~ 85 to 95%. Tebuconazole was ~ 99–100% incorporated in the nanoparticles and chlorothalonil appeared to be incorporated into the nanoparticles at ~ 95% of the theoretical value. This corresponds to ~ 45–48% (w/w) of the total nanoparticle mass attributable to the AI.

Preparation of PVP/HBP blend matrix nanoparticles

Solutions of PVP (20 mg in 2 mL of methanol) and HBP (G2, G3, G4, or G5, 20 mg in a minimum amount of acetone) were combined and placed in an addition funnel. The procedure described for linear nanoparticles was then followed. Yield: 75–88%; HBP content (by ^{1}H-NMR): 37–44% (w/w polymer/tebuconazole nanoparticles), 41–46% (w/w polymer/chlorothalonil nanoparticles); AI content: ~ 99–100% tebuconazole and ~ 95% chlorothalonil.

Measurement of nanoparticle density

A known mass of nanoparticles (1.0000–2.0000 g) and 8.0 mL of silicone oil were placed into a 10.0-mL graduated cylinder and allowed to stand for 0.4 h. The density of the nanoparticles was determined as the

TABLE I
Size and Size Dispersity of Nanoparticles

AI	Polymer matrix	Size range (nm)	Median size (nm)	Mass > 300 nm (%)
Tebuconazole	PVP	30–350	112	14
	PVP-*co*-10% St	50–350	118	18
	PVP-*co*-30% St	50–400	132	20
	PVP/HBP G2	50–800	254	50
	PVP/HBP G3	50–850	209	23
	PVP/HBP G4	50–600	193	30
	PVP/HBP G5	50–800	242	62
	PVP/LPE	50–500	198	22
Chlorothalonil	PVP	50–500	169	14
	PVP-*co*-10% St	50–600	176	22
	PVP-*co*-30% St	50–600	194	35
	PVP/HBP G2	50–800	244	38
	PVP/HBP G3	50–700	231	58
	PVP/HBP G4	50–600	172	34
	PVP/HBP G5	50–800	213	20
	PVP/LPE	50–500	204	—

The nanoparticles with HBP are 50% (w/w) HBP in PVP. The data are cited from Liu et al.[4]

mass of nanoparticles over the change in volume. Density measurements were the average of three measurements and were reproducible, the accuracy being limited by the graduated cylinder (± 0.1 cm^3).

Measurement of AI content in nanoparticles

The AI content of the nanoparticles was determined in several ways. Gravimetric analysis was done by filtering the crude nanoparticle suspension through Whatman #1 filter paper to capture any AI crystals not incorporated within the nanoparticles. This method typically recovered $\sim$ 0–2 and $\sim$ 2–3.5% of the theoretical mass in the PVP/tebuconazole and PVP/chlorothalonil systems, respectively. ^{1}H-NMR was performed on the filtrate of the nanoparticle suspension and the nanoparticles themselves. Immediately after preparation the nanoparticle suspension was filtered through a 50-nm filter and the filtrate was evaluated for the presence of AI (tebuconazole only). A known mass of isolated nanoparticles was dissolved in $CDCl_3$, together with a known amount of methyl ethyl ketone (MEK) as a benchmark, allowing the numerical and mass ratio of MEK to PVP to be determined. Tebuconazole could be determined directly from the ^{1}H-NMR; chlorothalonil has no protons, so the chlorothalonil content was calculated from subtracting the known mass of PVP from the known mass of nanoparticles.

Measurement of nanoparticle size

The size and dispersity of the nanoparticles was measured by particle sizing (Shimadzu CP-4, centrifugal force). The instrument readout provides histograms of all the detected particles. The median particle size and size range reported is based on the middle 80% of the particle mass.

Measurement of AI release rate in water

The AI release rate of an aqueous nanoparticle suspension was measured using gravimetric analysis and is described in detail elsewhere.[4]

General procedure for wood preservation studies

The mass and dimensions of the wood block specimens ($\sim 19 \times 19 \times 19$ mm^3 cubes) were accurately determined. The wood blocks were placed in beakers, covered with a coarse mesh, and weighed down; then a nanoparticle suspension with the desired concentration was poured over the block. The beaker was subjected to a pressure treatment consisting of a partial vacuum of 17.3 kPa for 25 min, followed by pressurization at 790 kPa for 45 min. Specimens were removed and excess liquid was wiped off. The wood blocks were then weighed to determine the mass of the retained suspension, and the undelivered nanoparticles were recovered to further confirm the delivered mass of nanoparticles. The samples were dried overnight (40°C), cut longitudinally into four wafers (two interior and two exterior), and reweighed. The wafers were sterilized in an autoclave for 15 min at 120°C. Using forceps, sterile toothpicks were placed on agar plates that were inoculated with *G. trabeum*. The coded wafer sections were then placed directly on the toothpicks. Untreated wafers were placed in each agar dish as controls to verify fungal activity. The petri dishes were sealed with parafilm and placed in a

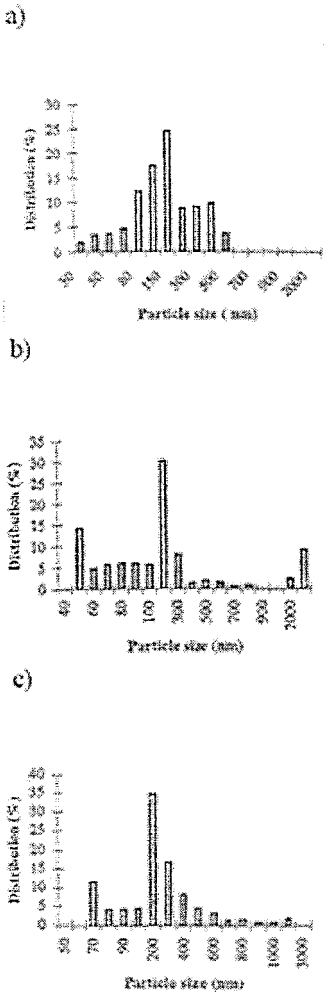

Figure 2 Histograms of nanoparticles containing tebuconazole in (a) PVP, (b) PVP-10% St, and (c) PVP-30% St matrices.

walk-in incubator maintained at 80°F and 80% relative humidity RH. After 55 days of exposure, the wafers were harvested, cleaned, dried, and reweighed to determine the mass loss. The mass loss was measured for interior, exterior, and control wafer sections. Reported weight losses are the average of at least three samples. Nanoparticle "blanks" (nanoparticles containing no AI) were prepared for each system to determine if the polymer matrix and/or surfactant possessed any biological activity.

Measurement of efficiency of nanoparticle and AI delivery into wood

By using the known AI content in the nanoparticles (~ 47–50% w/w nanoparticles), suspensions capable of delivering predetermined amounts of AI (0.1–0.8 kg AI/m^3 wood) were prepared by resuspending freeze-dried nanoparticles in a given volume of water. The theoretical AI in wood was determined assuming the nanoparticles were delivered quantitatively and uniformly into the wood being treated. This assumption, coupled with the measured volume of treated wood, allowed the theoretical AI content to be reported as kilograms of AI per cubic meter of wood.

The actual AI content in the wood was determined by measuring the mass of delivered nanoparticles, subtracting this value from the starting mass of nanoparticles, converting the mass to Kilograms, and multiplying that value by the mass fraction of the AI in the nanoparticles.

RESULTS AND DISCUSSION

Polymeric nanoparticles were prepared that contained ~ 50% AI (chlorothalonil or tebuconazole) and 50% polymer matrix (PVP, PVP-*co*-St, or a blend of PVP/HBP). The effect of the nanoparticle size and matrix composition was studied for the delivery efficiency into wood and for the biological efficacy of the AI-containing nanoparticles in wood exposed to fungal attack by *G. trabeum*.

Efficiency of AI incorporation in nanoparticles.

Gravimetric analysis and ^{1}H-NMR analysis indicated near quantitative incorporation of the AIs into the linear polymer matrices. Gravimetric analysis showed that the presence of HBP in the blended PVP/HBP nanoparticles did not have a significant effect on the AI content in the nanoparticles, but this was not confirmed by ^{1}H-NMR because of considerable overlap of the bands of the PVP and tebuconazole. The high efficiency of the incorporation is attributed to the fact that the nanoparticles are rapidly formed by the precipitation method of preparation in which an organic solution containing matrix and AI is dripped into a water/surfactant mixture. Once the organic solution is dripped into the water, the water-soluble solvent "flashes" into the water, precipitating the polymer and entrapping the AI within the polymer phase.

Effect of matrix composition on nanoparticle size

The objective of the first phase of this research was to prepare nanoparticles with median diameters below 250 nm. This diameter was targeted because the effective pit-pore diameters of the common pine species loblolly pine (*P. taeda*) and longleaf pine (*P. palustris*) have been measured in the range of 6,400–10,000,[6] 160–1,800,[6] 500,[7] and 400 nm,[8] depending on the test method used and wood dimension over which the measurements were taken. Other wood species have smaller pores, but pine is the most commonly preserved wood species used in the United States; thus, success with this wood species is required. A median nanoparticle diameter of 250 nm or less should ensure adequate ability to penetrate into the wood.

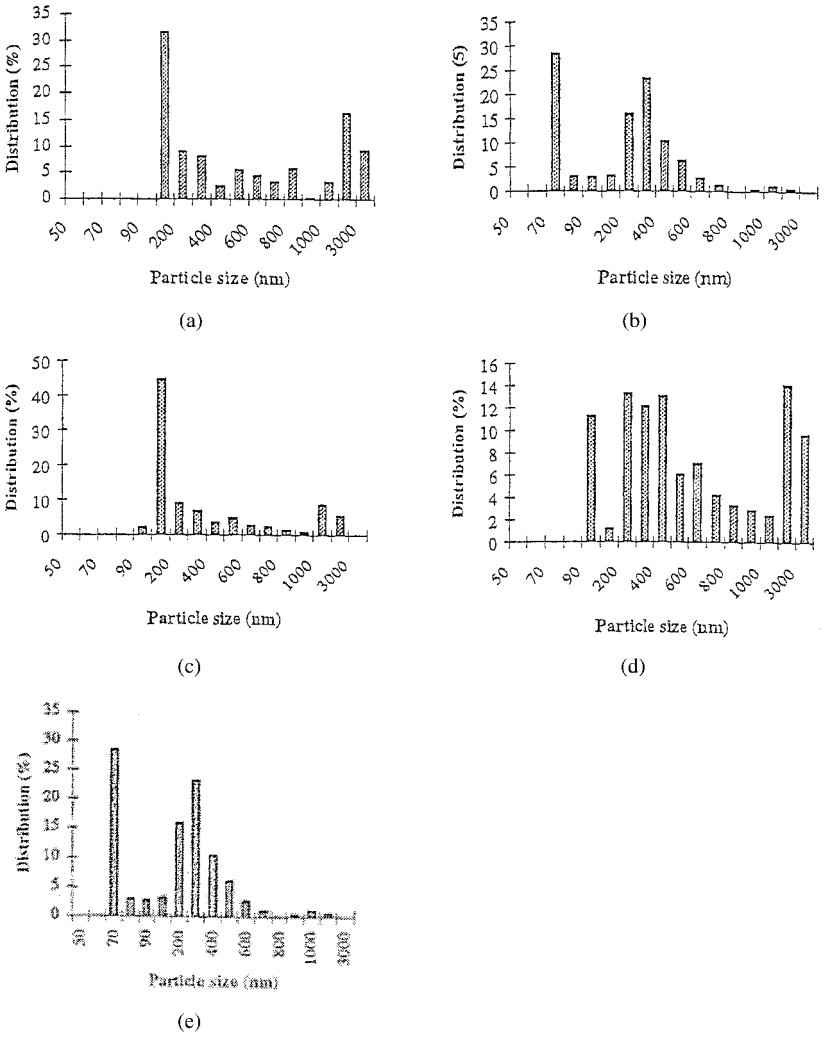

Figure 3 Histograms of nanoparticles containing tebuconazole in (a) PVP/HBP G2, (b) PVP/HBP G3, (c) PVP/HBP G4, (d) PVP/HBP G5, and (e) PVP/LPE matrices.

The method employed to prepare the nanoparticles resulted in a median diameter below the 250-nm target for all polymers and polymer blends studied, but some matrix-dependent trends were evident. First, increasing the matrix hydrophobicity broadens the particle size distribution and increases the median particle diameter as evidenced from the differences in particle size distribution as the St content increased in the PVP and PVP-*co*-St nanoparticles (Table I). Also, chlorothalonil-containing nanoparticles typically had slightly greater median diameters than tebuconazole-containing nanoparticles with the same matrix, which might also be due to the greater hydrophobicity of this AI relative to tebuconazole. When PVP/HBP matrices were prepared, the median diameter increased relative to the all-linear PVP matrix (Table I), and this is also attributed to increased hydrophobicity. However, the identity of the AI appeared to have little effect on the median nanoparticle diameter for the PVP/HBP blended nanoparticles. The generation of the HBP used in the blend had a complex affect. As the generation of the HBP increased in PVP/HBP blended matrices there was a slow but steady decrease in the median diameter of the nanoparticles until PVP/HBP G5. The median diameter of the PVP/HBP G5 nanoparticles was greater than that of the PVP/HBP G4. The reason for this is not understood.

The most significant difference in the size of the nanoparticles prepared using all-linear matrices and the HBP-containing matrix blends was the mass percentage of nanoparticles having a diameter greater than 300 nm. The PVP and PVP-*co*-St matrices generally had less than 20% by mass of nanoparticles with diameters greater than 300 nm, while the HBP-containing nanoparticles typically had $\sim 30-60\%$ by mass of nanoparticles with diameters in excess of 300 nm. Histograms of the tebuconazole-containing nanoparticles using the all-linear matrices are shown in Figure 2, and histograms using blends of PVP/HBP and a PVP/LPE control are shown in Figure 3. Histograms

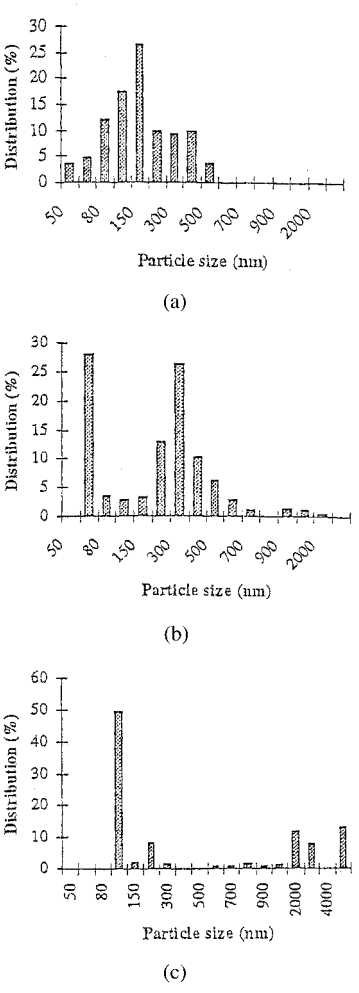

Figure 4 Histograms of nanoparticles containing chlorothalonil in (a) PVP, (b) PVPy-10% St, and (c) PVP-30% St matrices.

of the chlorothalonil-containing nanoparticles using the all-linear matrices are also shown in Figure 4, and chlorothalonil-containing blends of PVP/HBP are shown in Figure 5. It can be seen that the nanoparticles prepared with the PVP and PVP-*co*-St linear matrices populate the smaller diameter region, while a significant population of larger nanoparticles is present in the blended PVP/HBP nanoparticles. Although most of the nanoparticles are of a size that will penetrate the wood, the mass fraction of nanoparticles from the PVP/HBP matrices that are unable to penetrate into the wood is significant, decreasing the cost effectiveness of the HBP-containing nanoparticles. However, the purpose of using the HBPs is to introduce porosity into the nanoparticles in a systematic manner to determine if controlling nanoparticle porosity is a useful method to modulate the release rate of the AI.

The reason for the increased size of the PVP/HBP nanoparticles appears to be due largely to the hydrophobicity increase rather than porosity differences because the median size of the PVP/LPE nanoparticles is similar to that of the PVP/HBP. The branching of the HBP does appear to have some effect on the distribution because the PVP/LPE had ~ 20% of its mass in particles greater than 300 nm, while the PVP/HBP nanoparticles tended to have larger numbers of larger nanoparticles. However, there was no significant difference measured in the density of the PVP/HBP nanoparticles (Tables II, III). Also, the lack of a consistent trend in the percentage of nanoparticles greater than 300 nm suggests the possibility that some of the largest particles that were detected and represented in the histograms might be dust contamination.

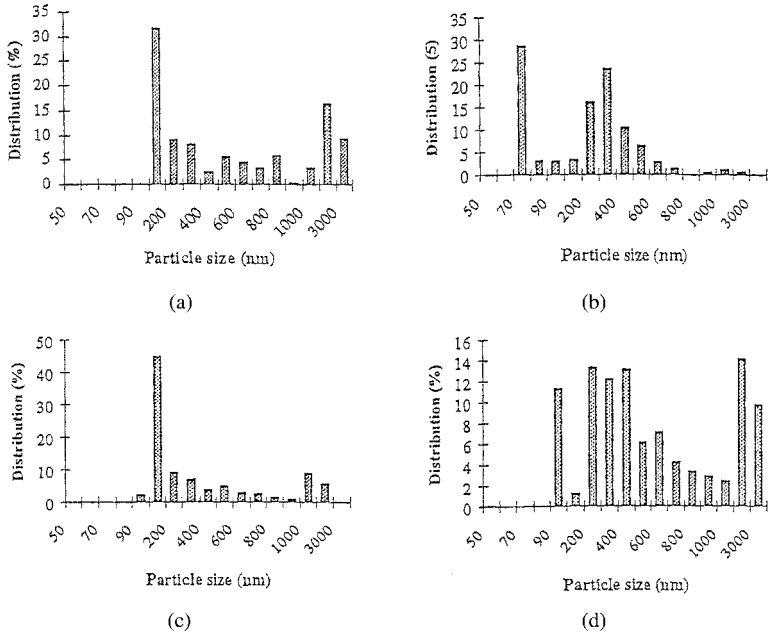

Figure 5. Histograms of nanoparticles containing chlorothalonil in (a) PVP/HBP G2, (b) PVP/HBP G3, (c) PVP/HBP G4, and (d) PVP/HBP G5 matrices.

TABLE II
Measurement of Density of Control Substances

Substance	Reported density[a] (g/cm^3)	Measured density ($g/cm^3 \pm 0.1$)
Tebuconazole	1.4	1.4
Chlorothalonil	1.8	1.8
HBP G2	1.300	—
HBP G3	1.300	—
HBP G4	1.300	1.3
HBP G5	1.300	—

[a] Data reported by the respective suppliers.

A porous nanoparticle would be expected to have a lower density than less porous nanoparticles, because the polymer matrices were denser than water or air; but there was no definite evidence that the PVP/HBP nanoparticles were porous. Therefore, the results show the size and size distribution had a clear effect on the matrix polarity but did not confirm that HBP affected the porosity.

Aqueous release rates of PVP/HBP nanoparticles

The results of the aqueous release studies for the all-linear matrices were presented and discussed in an earlier article[4] but are summarized here to facilitate comparison. The release was diffusion controlled and the most hydrophilic matrix, PVP, released the AI the fastest. The release rate decreased as the St content increased. Tebuconazole is more water soluble that chlorothalonil and its release rate was faster than chlorothalonil, although the chlorothalonil release showed an initial burst that was attributed to desorption of chlorothalonil from the surface of the nanoparticles followed by a very slow, diffusion-controlled release.

The decrease in the diffusion rate with decreased temperature was studied at 30, 25, and 10°C. The data are shown in Figure 6. The release rates were initially nearly identical, but after 20 days an ~ 20% decrease in the release of tebuconazole from PVP nanoparticles was found between 30 and 10°C.

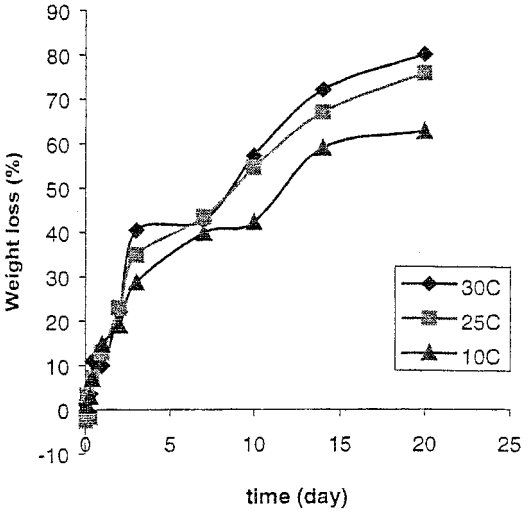

Figure 6. The effect of temperature on the release of tebuconazole from PVP.

The effect of the porosity on the AI release rate was to be investigated by using HBP of different generations. It was found that as the generation of the HBP used in the nanoparticle matrix blend increased, the AI release rate also increased. However, this was attributed at least in part to an increase in the porosity of the nanoparticle through hydrolysis of the ester bonds rather than the HBP architecture. This is because the AI release rate in the PVP/HBP nanoparticles with G4 and G5 HBPs actually showed a release rate increase with time and because the gravimetric analysis was done using a 50-nm filter, it is unlikely that any hydrolyzed ester moieties passed through the filter to contribute to the measured mass and artificially increase the numbers. Therefore, the porosity increase promoted AI release, but we were unsuccessful in trying to promote a systematic change in porosity to allow us to compare the relative effect with the hydrophilicity changes.

The HBP architecture did have an additional effect in promoting AI release because a LPE control with a

TABLE III
Measurement of Density of PVP/HBP Blends

Nanoparticle matrix	AI	Density ($g/cm^3 \pm 0.1$)	AI	Density ($g/cm^3 \pm 0.1$)
PVP	None	1.1	None	1.1
PVP	Tebuconazole	1.3	Chlorothalonil	1.5
PVP/HBP G2	Tebuconazole	1.3	Chlorothalonil	1.6
PVP/HBP G3	Tebuconazole	1.3	Chlorothalonil	1.6
PVP/HBP G4	Tebuconazole	1.3	Chlorothalonil	1.5
PVP/HBP G5	Tebuconazole	1.2	Chlorothalonil	1.5
PVP-*co*-10% St	None	1.2	None	1.1
PVP-*co*-30% St	None	1.3	None	1.3
PVP-*co*-10% St	Tebuconazole	1.3	Chlorothalonil	1.5
PVP-*co*-30% St	Tebuconazole	1.4	Chlorothalonil	1.7

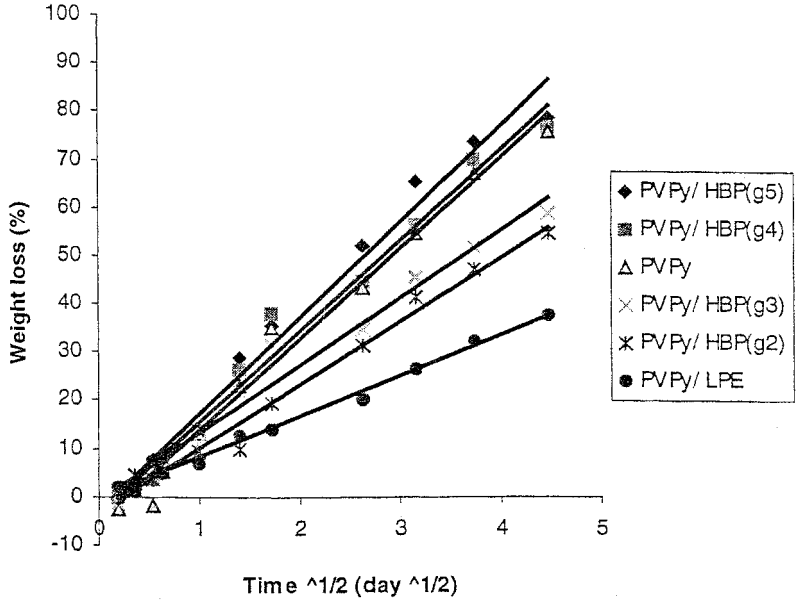

Figure 7. The release of tebuconazole from HBP-containing nanoparticles.

similar repeat unit structure and a molecular weight that was similar to that of the G3 HBP had a significantly slower AI release than either PVP/HBP G2 or PVP/HBP G3. This architecture effect is indirectly attributed to polarity changes due to the greater number of hydroxyl end groups associated with the HBP.

The AI release data are shown for PVP/HBP nanoparticles containing tebuconazole in Figure 7 and for PVP/HBP nanoparticles containing chlorothalonil in Figure 8. The release profile from pure PVP is also shown for comparison. It can be seen that tebuconazole releasing from PVP is similar to that of the PVP/HBP G4, but the chlorothalonil from PVP is different. It was pointed out in an earlier publication[4] that in PVP and PVP-*co*-St some chlorothalonil is present in crystalline form (confirmed by DSC) and it is thought that the crystalline chlorothalonil is present on or near the surface and quickly desorbs. DSC confirmed that in PVP/HBP and PVP/LPE the chlorothalonil is entirely solvated, and so the AI is released only by a diffusion process.

The effect of the different matrices on AI release rates is summarized in Table IV using relative release rates. The AI release rate was assigned a value of 1.0 for each AI in PVP. As the hydrophobicity was increased in linear matrices by using PVP-*co*-St and increasing the styrene content from 10 to 30%, the AI release rate slowed. The relative release rates for the

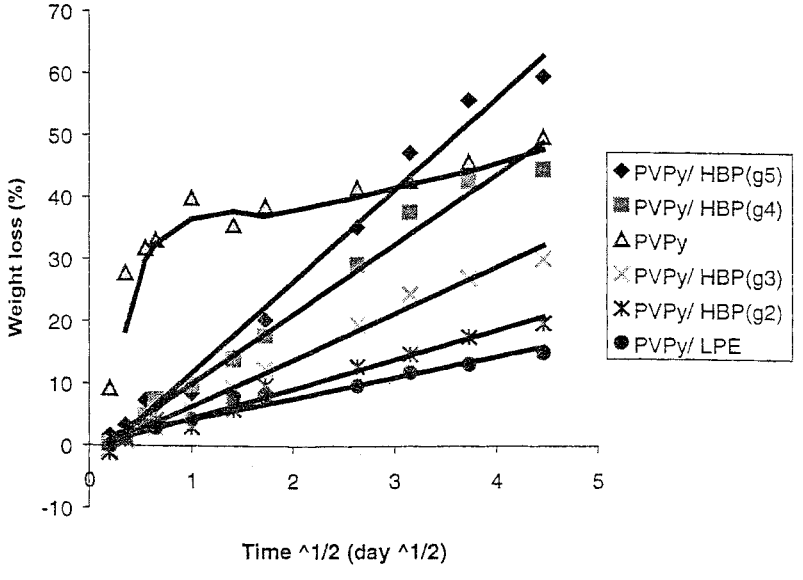

Figure 8. The release of chlorothalonil from HBP-containing nanoparticles.

TABLE IV
Relative Aqueous Release Rates for Tebuconazole and Chlorothalonil

Matrix	Tebuconazole	Chlorothalonil
PVP	1.0	1.0
PVP-*co*-10% St	0.85	0.80
PVP-*co*-30% St	0.55	0.72
PVP/LPE	0.50	0.32
PVP/HBP G2	0.70	0.40
PVP/HBP G3	0.71	0.61
PVP/HBP G4	1.01	0.89
PVP/HBP G5	1.04	1.20

tebuconazole-containing nanoparticles reflect a decrease from decreased polarity in the linear PVP-*co*-St copolymers and a decrease in the PVP/LPE blended nanoparticles. The release rates from the PVP/HBP blended nanoparticles move toward that of the PVP nanoparticles as the HBP generation is increased. This might be due to a combination of polarity and porosity, although it is not clear if the porosity is due partly or entirely to ester hydrolysis.

In the linear matrices, the decrease in the AI release rate was less for the chlorothalonil than for the tebuconazole. This is thought to be attributable to the fact that the chlorothalonil solubility is higher in the less polar St-containing matrices than in PVP, although some chlorothalonil is still present in crystalline form in these matrices. However, the chlorothalonil is completely soluble in the LPE- and HBP-containing matrices; thus, only diffusion-controlled release occurred and the decrease in the chlorothalonil release rate was significantly greater than the tebuconazole release rate, except in the G5 HBP. Therefore, the data may have some inconsistencies because of differences in the percentage of crystalline chlorothalonil present in the linear matrix nanoparticles, as well as differences in the particle size and distribution of sizes.

Biological efficacy of biocide-containing nanoparticles in wood

Biological studies were done using nanoparticle-treated wood blocks that were cut into wafers and placed in agar dishes, which had been inoculated with brown rot wood decay fungus. The wafers were harvested after 50 days and the weight losses measured. The methods are described in detail in a prior article.[4]

Effect of nanoparticle matrix on antifungal efficacy in wood

Control studies were done using nanoparticle "blanks" where SYP was pressure treated with nanoparticles that contained no AI to determine if the matrix polymer and/or the surfactant had any biological activity. Additionally, every agar plate used throughout this project contained at least one untreated SYP wood control. Untreated wood controls consistently lost 17% of their mass (±1%) by this test method. The results of the control studies are given in Table V. Essentially no difference in weight loss was observed between untreated SYP controls and SYP wood containing PVP, so it was concluded that PVP and the surfactant neither promoted nor retarded fungal attack. The SYP treated with PVP/HBP nanoparticle blanks may have had some biological efficacy. This wood only lost ~ 11% of its mass while typically 17% mass loss was found for our wood controls. The relative error associated with any fungal study can be high, so this difference is not sufficient to conclude that the matrix had biological efficacy; but the study was not repeated, and so the possibility of some small efficacy associated with this system was not eliminated.

TABLE V
Weight Loss of SYP after 55 days of Exposure to Brown Rot Fungus

Polymer matrix	AI	Ave. wt loss (%) (SD)
Untreated control	—	17 (3)
PVP	0	17 (3)
PVP/HBP (G2–G5)[a]	0	10–13 (3)

[a] The range was 10–13%.

Efficacy of AI-containing nanoparticles against *G. trabeum*

The ability of chlorothalonil and tebuconazole to protect wood against *G. trabeum* was studied in different matrices by exposing nanoparticle-treated SYP wafers to *G. trabeum* for 50 days.[4,9] The data show the theoretical amount of the AI that would have been introduced had the nanoparticles been delivered into the SYP quantitatively and the actual amount of AI introduced into the wood, which was determined by collecting the undelivered nanoparticles and calculating the amount of AI contained within the undelivered nanoparticles.

TABLE VI
***Gloeophyllum trabeum* Resistance of SYP Treated with Tebuconazole in PVP Nanoparticles**

AI Loading (kg/m^3)	AI delivered (kg/m^3)	Weight loss (%)		
		Exterior section	Interior section	Average
Control	—	17	17	17 ± 3
0.05	—	12	17	15 ± 2
0.1	0.1	6	8	7 ± 1
0.2	0.2	3	7	5 ± 2
0.4	0.4	2	6	4 ± 2
0.8	0.6	1	2	2 ± 1

TABLE VII
***Gloeophyllum trabeum* Resistance of SYP Treated with Tebuconazole in PVP/HBP Nanoparticles**

AI loading (kg/m^3)	Weight loss (%) in PVP-HBP matrices				
	PVP	PVP-HBP G2	PVP-HBP G3	PVP-HBP G4	PVP-HBP G5
Control	17 ± 4	10 ± 3	10 ± 3	13 ± 3	10 ± 3
0.1	7 ± 1	6.1 ± 0.7	5.8 ± 0.7	6.5 ± 0.9	6 ± 1
0.2	5 ± 2	4.2 ± 0.6	5 ± 1	5 ± 1	3 ± 1
0.4	4 ± 2	3.1 ± 0.3	3 ± 1	4.9 ± 0.3	3 ± 3[a]
0.5	—	1.7 ± 0.5	0.5 ± 0.5	0.3 ± 0.5	0.6 ± 0.6
0.8	2 ± 1[b]	2.8 ± 0.7[c]	3.1 ± 0.5[b]	3 ± 1	2.7 ± 0.1[b]

[a] The AI delivered into SYP was 0.3 kg/m^3.
[b] The AI delivered into SYP was 0.7 kg/m^3.
[c] The AI delivered into SYP was 0.6 kg/m^3.

Table VI shows the mass loss of SYP treated with tebuconazole in PVP nanoparticles after 50 days of exposure to *G. trabeum*. These data show two significant facts. First, SYP is probably the easiest wood species to treat using a nanoparticle method because of the relatively large pit-pore size compared with other wood species. Yet, even with this wood species, the nanoparticles are delivered quantitatively only up to a suspension loading that is able to deliver up to 0.4 kg AI/m^3; and when the suspension is sufficient to deliver 0.8 kg AI/m^3; only 0.6 kg AI/m^3 is actually delivered. Second, a mass loss of less than ~ 5% is generally considered to be negligible in fungal studies and it is generally thought that tebuconazole must be used at a level of ~ 2 kg AI/m^3 of wood for it to be well protected in the field[10]; however, here an AI level in SYP of only 0.2 kg/m^3, which is well below the usual target loading for AI, was sufficient to bring the mass loss to under 5% in this study.

Biological efficacy was found at similarly low AI loadings when the nanoparticle matrix was PVP/HBP (Table VII). Here the mass loss is summarized as the average mass loss for the treated wood, rather than giving the data for both interior and exterior sections of the treated wood block. The interior sections of the treated wood blocks consistently lost slightly greater mass than the exterior sections. These data show essentially the same level of protection afforded to the SYP as the PVP matrix, even though the PVP/G2 and G3 nanoparticles released the AI significantly more slowly than the PVP nanoparticles. This suggests the AI release rate is already greater than that required for biological efficacy. However, it cannot be overlooked that there is a possibility that the PVP/HBP matrix itself may be contributing to the protection of the SYP from fungal attack.

Chlorothalonil has not been used to treat wood previously, except as a solution in organic oils such as P9 that also have biological efficacy, so no precise threshold level for chlorothalonil in wood is known, although it is thought to be considerably higher than the 2 kg/m^3 often given for tebuconazole.[9] However, we found biological efficacy for chlorothalonil at levels well below 2 kg/m^3 of SYP when introduced into the wood in polymer nanoparticles. When introduced into SYP in PVP (theoretical loading of 0.5 kg chlorothalonil/m^3), no efficacy was observed; but when introduced into SYP in the slower releasing PVP-*co*-St (10 and 30% St), some efficacy was evident (Table VIII). The reason for the observed efficacy with the slower releasing matrices is not known. Possibly the matrix polarity of the nanoparticles might affect not only the release rate but also the manner in which the nanoparticles interact with the wood structure, but there is no evidence to support this.

Chlorothalonil shows significant efficacy against brown rot when it is delivered into SYP in the PVP/HBP nanoparticles (Table IX). From the control studies with nanoparticle blanks there is again some indication that the PVP/HBP matrices themselves might have some efficacy. In general, although there are minor differences in the measured mass losses with the HBP generation, the differences are not significant and the efficacy of chlorothalonil in PVP/HBP is essentially independent of the HBP generation, despite differences in the AI release rate. The significance of these data is that significant biological efficacy against fungal attack is seen at a very low AI loading level in SYP. At chlorothalonil levels as low as 0.2 kg/m^3, SYP, some protection from fungal attack is afforded to the wood. Once the chlorothalonil loading within the wood reaches 0.6–0.7 kg/m^3, the mass loss is essentially at background levels.

TABLE VIII
***Gloeophyllum trabeum* Resistance of SYP with Chlorothalonil in PVP and PVP-*co*-St Nanoparticles**

Matrix (0.5 kg AI/m^3)	Weight loss (%)		
	Exterior section	Interior section	Average
0	17	17	17
PVP	17	15	16
PVP-*co*-10%St	6	6	6
PVP-*co*-30%St	9	8	9

The target loading was 0.5 kg/m^3 wood. The actual delivery was not measured.

TABLE IX
***Gloeophyllum trabeum* Resistance of SYP Treated with Chlorothalonil in PVP/HBP Nanoparticles**

AI loading (kg/m^3)	Matrix				
	PVP	PVP-HBP G2	PVP-HBP G3	PVP-HBP G4	PVP-HBP G5
Control	17 ± 3	—	—	—	—
0	17	10	10	13	10
0.1	16	9 ± 4	9.6 ± 0.7	8 ± 1	7 ± 4
0.2	15	6.8 ± 0.3	5 ± 1	7 ± 3	5 ± 5
0.4	13[a]	7 ± 1[a]	5 ± 2	7 ± 4	4 ± 1
0.8	11[b]	5 ± 2[b]	5.2 ± 0.2[b]	6 ± 1[c]	3 ± 2[b]

[a] The actual AI delivery in wood was 0.3 kg/m^3.
[b] The actual AI delivery in wood was 0.6 kg/m^3.
[c] The actual AI delivery in wood was 0.7 kg/m^3.

Such significant levels of biological efficacy with such low levels of biocide were unexpected and are not understood. Collectively, the data suggest two assumptions. First, no real correlation between the AI release rate and biological efficacy was found, which indicates the release rate exceeds what is required to protect the wood. Second, significant levels of biological efficacy are seen at very low levels of AI loading in wood, even with matrices where there is clearly no biological protection afforded by the polymer matrix itself. This shows that introducing the biocide in a polymer matrix has advantages and either helps to deliver the biocide to the most susceptible parts of the wood or significant amounts of biocide are lost to the environment when not introduced in a polymer carrier, and biocide is not lost by this method.

CONCLUSIONS

A versatile method for introducing organic biocides into solid wood is under development. Two fungicides, tebuconazole and chlorothalonil, were incorporated into polymer nanoparticles with nearly equal ease. Therefore, this method is highly advantageous for biocides such as chlorothalonil, which previously could only be used to treat wood with the use of toxic oils as solvents, which would prevent that wood from being used in household applications.

The biocides were easily incorporated into different polymer matrices: PVP, PVP-*co*-St, and blends of PVP/HBP. The median particle diameter increased as the matrix hydrophobicity increased, but the nanoparticles were sufficiently small so that they could be introduced into SYP quantitatively at lower suspension concentrations and at ~ 75–85% efficiency at the highest levels attempted (0.8 kg AI/m^3 wood).

HBP was used with the intent of systematically increasing the nanoparticle porosity to determine if the porosity was an effective method to increase the AI release rate, but the data did not support this effect. The AI release rates increased as the HBP generation increased; however, this might have been due to ester hydrolysis and not because the HBP architecture contributed to the porosity. Density measurements also failed to confirm that the HBP generation promoted porosity in the nanoparticles.

The data conclusively showed that decreasing the hydrophilicity and the temperature decreased the AI release rate. Biological studies using the brown rot fungus *G. trabeum* failed to find that the release rate influenced the protection afforded to SYP, which suggested even the most hydrophobic matrices used in this work were releasing AI at a faster rate than required to protect the SYP. The biological studies also showed wood treated with AI-containing nanoparticles was protected against fungal attack at very low levels of AI incorporation. Using this method, mass losses of less than 5% were found with AI loading levels as low as 0.2 kg tebuconazole/m^3 SYP and as low as 0.4 kg chlorothalonil/m^3 SYP. No evidence was found that the linear polymer matrices contributed to the biological efficacy, but the blended matrices (PVP/HBP) may have contributed to the observed resistance to fungal attack.

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