Carbohydrate Polymers 86 (2011) 58–64

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Efficient one-pot synthesis and loading of self-assembled amphiphilic chitosan nanoparticles for low-leaching wood preservation

Xiaochu Ding[a,*], Dana L. Richter[b], Laurent M. Matuana[c], P.A. Heiden[a,*]

[a] Department of Chemistry, Michigan Technological University, Houghton, MI 49931, USA
[b] School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA
[c] School of Packaging, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

Article history:
Received 30 December 2010
Received in revised form 24 March 2011
Accepted 1 April 2011
Available online 9 April 2011

Keywords:
Self-assembled amphiphilic nanoparticles
Chitosan
Fungicide leach
Wood-preservation
Controlled release
Chitosan-g-PMMA nanoparticle
Amphiphilic nanoparticle
Core–shell structure
Tebuconazole
Biocide leach

ABSTRACT

A simple, one-step and one-pot method was used to synthesize amphiphilic self-assembling chitosan-g-PMMA nanoparticles (~100 nm diameter by SEM, but ~150–200 nm in water by DLS), containing ~25–28 wt.% (~82–93% capture efficiency) of the fungicide tebuconazole. The matrix composition was selected to be environmentally low impact, while the nanoparticle preparation conditions were designed to ensure the nanoparticles were sufficiently small to be able to penetrate the pit pairs of solid wood. These nanoparticles were delivered into southern pine sapwood blocks at target fungicide retentions of 0.2, 0.4 and 0.8 kg tebuconazole/m^3 wood. SEM analysis of a 19 mm × 19 mm × 455 mm nanoparticle-treated wooden stake confirmed penetration throughout the interior of the treated stake. Leaching studies confirmed that biocide introduced into sapwood via nanoparticle carriers leached only about 9% as much fungicide as solution-treated controls, while soil jar tests showed the nanoparticle-treated wood blocks effectively protected the wood from biological decay when tested against *G. trabeum*, a brown rot fungus.

1. Introduction

Amphiphilic block and graft copolymers are routinely used to prepare self-assembling core–shell nanoparticles. Such copolymers are readily prepared from synthetic polymers, biopolymers, or combinations of both (Kakizawa et al., 2010; Craparo et al., 2010; Kuskov et al., 2010; Kamps et al., 2010; Leonardis, Mannina, Diociaiuti, & Masci, 2010; Jiang, Wu, He, & Nie, 2010). Polysaccharides and proteins are well suited for preparing grafted amphiphilic copolymer nanoparticles because of the large number of polymerizable groups they possess along their backbone.

Chitosan is possibly the most commonly selected polysaccharide for grafted amphiphilic copolymer nanoparticles. Its repeat units bear either a primary amine group or an amide group if that unit is not hydrolyzed. The large number of amine groups can be utilized in either grafting-to or grafting-from reactions. A recent review describes the synthesis, properties, and uses of many different chitosan amphiphiles (Aranaz, Harris, & Heras, 2010; Lao, Zhang, Xu, & Jiang, 2010). One "grafting from" reaction uses these amines to react with peroxides under mild conditions yielding amine radicals that can efficiently react with hydrophobic monomers to form amphiphilic core–shell nanoparticles (Salma et al., 2010).

Chitosan's biocompatibility, biodegradability, and antimicrobial activity are why it is employed in many biomedical and cosmetic applications. For example, chitosan and its derivatives exert antifungal activity on *Saprolegnia parasitica*, *Aureobasidium pullulans* and other pathogenic fungi (Muzzarelli, Muzzarelli, Tarsi, Miliani, & Cartolari, 2001). One recent publication describes amphiphilic chitosan micelles for the controlled release of rotenone (Lao et al., 2010). However, that used a multi-step synthesis and the encapsulation efficiency was low. Another report described controlled release of agrochemicals from chitosan (Boehm, Martinon, Zerrouk, Rump, & Fessi, 2003), but those were microparticles. While high-value applications still account for most nanoparticle uses, improvements in technology as well as consumer trends are allowing nanoparticle uses to penetrate some commodity areas and this trend is growing.

We had previously studied controlled release nanoparticles in solid wood, prepared using synthetic polymers under dilute conditions in a solvent-displacement route (Liu, Laks, & Heiden, 2002a,b,c). The nanoparticle-treated wood was tested against both

* Corresponding authors. Tel.: +1 906 487 3452; fax: +1 906 487 2061.
E-mail addresses: xding@mtu.edu (X. Ding), paheiden@mtu.edu (P.A. Heiden).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.04.002

a brown and white rot fungus, and biological efficacy compared well with the solution-treated controls. At that time no work was done to test the biocide leaching.

The rationale for employing controlled release nanoparticles to deliver organic biocides into solid wood was the hypothesis that a controlled biocide release would maintain an effective protection of the solid wood and might also reduce biocide leaching into the environment. The significance of reducing biocide leaching from treated wood is that when biocide leaches from wood it leaves the wood less well-protected from biological attack, and the leached biocide can have detrimental effects on the environment into which it is released. If less biocide is lost to the environment, then potentially the wood could be effectively protected with less biocide, which is beneficial to both the environment and to the cost of the preserved wood. In this application solid nanoparticles are favored over liquid micelles, which are more easily delivered into wood than solid particles, because of the desire to control the biocide release and to avoid the use of surfactant. The amphiphilic design is preferred because this allows a hydrophobic core composition to be used to manipulate the release rate while the shell can be hydrophilic to give a stable suspension in water. The nanometer size, preferably below 150 nm, is required because of the anatomy of solid wood, where the small size is required to penetrate the pit pairs to enter into the wood interior.

Our recent study supported the hypothesis of reduced biocide leach using gelatin-g-PMMA nanoparticles (Salma et al., 2010), but ungrafted gelatin complicated analysis of the data, and the gelatin may have promoted biological decay within the wood. The purpose of this paper is to prove the hypothesis that decreased biocide leach occurs by use of controlled-release nanoparticles, to quantify the decrease in biocide leaching, to show that wood preservation is not decreased, and to confirm nanoparticle penetration into wood interior on larger wood specimens than those used in standard soil jar studies.

2. Experimental part

2.1. Materials

Chitosan (~70% deacetylation) from crab shell was donated by Cochin University of Science and Technology (Cochin, India). The tebuconazole fungicide was donated by Lanxess Corporation (Pittsburg, PA). Methyl methacrylate (MMA, 99.0%) and 2-hydroxyethylmethacrylate (HEMA, 97%) were from Sigma Aldrich (Milwaukee, USA) and were distilled prior to use. Ammonium persulfate (APS, 95%) and benzophenone (99%) were from Mallinckrodt Chemical Works (St. Louis, USA) and Sigma Aldrich, and were used as received. Fungal tests used *Gloeophyllum trabeum* (ATCC 11539), a basidomycete brown rot wood decay fungus. Wood blocks were 19 mm × 19 mm × 19 mm, unless otherwise noted, and were cut from southern pine sapwood in the MTU School of Forest Resources and Environmental Science.

2.2. Preparation of nanoparticles

Nanoparticles were prepared using a modification of the methods reported (Li, Zhu, & Harris, 2003; Li, Zhu, Sunintaboon, & Harris, 2002; Qian, Cui, Ding, Tang, & Yin, 2006). Briefly, chitosan was dissolved in deionized water containing acetic acid (0.67 g acetic acid/g chitosan). For example, 0.5 g chitosan was dissolved in 0.43 wt.% AcOH to give 75 mL of solution at 50 °C. The reaction solution was purged with nitrogen gas for 0.5 h prior to the addition of MMA (MMA to Chitosan 2:1, w/w). The total concentration of chitosan and MMA ranged from 1 to 5% (w/w). Tebuconazole (30 wt.% based on the combined mass of chitosan and MMA) was dissolved in about 5 mL of acetone and then added dropwise to the reaction solution under magnetic stirring. After mixing for 10 min, the reaction temperature was increased to 70 °C. Ammonium persulfate (0.037%, w/v), dissolved in a small amount of deionized water, was then added to initiate the grafting reaction. The reaction was kept at 70 °C with magnetic stirring at 400 rpm for 24 h. This procedure was also used to prepare nanoparticles without tebuconazole by skipping the tebuconazole addition step. The yield of chitosan-g-PMMA nanoparticles without tebuconazole ranged from ~94 to 99% with a grafting efficiency of ~74–87%. The particle size of as-made nanoparticles was determined (in aqueous suspension) by Dynamic Light Scattering (Coulter NP4 Plus, Beckman Coulter, Fullerton, CA) and by SEM (Shimadzu QP5050A, Shimadzu Corporation, Germany) using freeze-dried nanoparticles.

2.3. Nanoparticle composition

Nanoparticle composition (without tebuconazole) was determined by gravimetric analysis. The as-made nanoparticle suspension was collected, gently heated to evaporate most solvent, and then vacuum dried at 50 °C for 48 h. The dried nanoparticles were extracted with 3 × 15 mL of chloroform to separate any PMMA homopolymer, and then 3 × 20 mL of warm deionized water containing 0.67 g acetic acid to separate any ungrafted chitosan. The extracts were vacuum dried at 50 °C for 48 h to obtain the mass of PMMA and ungrafted chitosan. The residual mass was chitosan-g-PMMA.

2.4. Tebuconazole content in nanoparticles

An aliquot of the tebuconazole-containing nanoparticle suspension was weighed, gently heated to remove most solvent, and then vacuum dried at 50 °C for 48 h to get the initial mass of tebuconazole-containing nanoparticles. The tebuconazole component was extracted from the dried nanoparticles using 3 × 15 mL of ethanol, and the combined extracts were then vacuum dried at 40 °C to get the tebuconazole mass. The solid extracts were confirmed to be pure tebuconazole by ^{1}H NMR. The calculation of the actual content of tebuconazole in nanoparticle suspension and the needed amount of the as-made nanoparticle suspension to treat wood blocks by pressure-treatment is shown in Eqs. (1) and (2):

$$\text{Tebuconazole}\,(\%) = \frac{\text{Mass}\,(\text{Teb.})}{\text{Mass}\,(\text{suspension})} \times 100\% \quad (1)$$

$$\text{Mass}\ (\text{suspension}) = \frac{\text{Retention}\ (\text{Target}) \times V\,(\text{woodblocks})}{\text{Tebuconazole}\ (\%)} \quad (2)$$

2.5. Delivery efficiency into wood blocks

Wood blocks were submerged in alcohol for 24 h to remove some soluble extractives and wood sawdust near the surface pores, which would interfere with GC–MS analysis and gravimetric analysis. The wood blocks were treated with nanoparticle suspension in accordance with procedures given as the standard method in Wood Pressure Treatment (AWPA E11-97). The quantity of as-made nanoparticle suspension needed to deliver target retentions of 0.2, 0.4 or 0.8 kg tebuconazole/m^3 wood, assuming quantitative delivery, was taken and diluted to 90 mL. The 90 mL volume was required to sufficiently cover 6 wood blocks having dimensions of 19 mm × 19 mm × 19 mm when these blocks were placed in a beaker. The wood blocks were covered with a plastic mesh and aluminum blocks to keep them submerged throughout the treatment process. The beaker was then transferred into the pressure cylinder and subjected to a pressure treatment consisting of a partial vacuum of less than 25 mm Hg for 0.5 h, followed by pressurization to

100 psi for 1 h. Specimens were removed, and the remaining suspension was transferred into a pre-weighed aluminum dish and heated to dryness to determine the nanoparticle mass that was not absorbed by wood. Because some extractives are absorbed into the suspension during the wood treatment, a measurable residue also results from treating wood "blanks" with water. Therefore the measured mass from wood "blanks" is used to adjust the undelivered mass from the nanoparticle-treated wood specimens. Therefore the delivery efficiency percentage is calculated as:

$$\%\,\text{Delivery} = \left[\frac{\text{dry nanoparticle mass in wood} - \text{average mass loss from blanks}}{\text{initial dry nanoparticle mass}}\right] \times 100 \quad (3)$$

2.6. Leaching tests

The leaching test was performed according to The American Wood Preservers Association Standard (AWPA E11-97) [American Wood-Preservers Association Book of Standards, 1998]. The leachate was collected in a beaker and heated at 80 °C to remove water, and then acetone was added to the dried leachate. The beaker was sealed for 3 h to completely extract the tebuconazole from the leachate. Then the acetone solution was transferred to a volumetric flask and a known quantity of benzophenone was added to the solution for use as an internal standard to allow a quantitative analysis by GC–MS.

2.7. GC–MS analysis

A Gas chromatograph/mass spectrometer (Shimadzu 5050A, Shimadzu Corporation, Germany) equipped with a programmed-temperature vaporizer was used to measure the amount of leached tebuconazole collected as described. MS with electron-impact (EI) ionization (electron energy 70 eV) was performed in selected ion monitoring (SIM) mode. The injection temperature and volume were 280 °C and 1 μL, which was 100% delivered into the chromatography column under a flow rate of 1 mL He/min as the carrier gas. The oven temperature, initially at 50 °C, was raised to 100 °C and held for 3 min to remove solvent. Then the MS detector began to identify the analyte while ramping the temperature at 10 °C/min, and finally holding for 5 min at 325 °C to remove possible residues in column. The quantitative analysis was based on the peak areas from the mass chromatograph. The internal response factor (IRF) was firstly identified by a standard solution with a known amount of tebuconazole and benzophenone. The IRF was calculated according to Eq. (4):

$$\text{IRF} = \frac{\text{area (benzophenone)} \times \text{amount (tebuconazole)}}{\text{amount (benzophenone)} \times \text{area (tebuconazole)}} \quad (4)$$

After determining the IRF, the leached tebuconazole amount could be calculated based on the internal standard method according to Eq. (5).

$$\text{Amount (tebuconazole)} = \frac{\text{area (tebuconazole)} \times \text{amount (benzophenone)} \times \text{IRF}}{\text{area (benzophenone)}} \times V\,(\text{solution}) \quad (5)$$

2.8. Soil jar decay test

Treated southern pine wood blocks (19 mm × 19 mm × 19 mm) were dried at 40 °C for 24 h. The mass was measured to ±0.005 g. All blocks were exposed to the brown rot fungus, *Gloeophyllum trabeum* ATCC 11539 for 12 weeks. Decay testing was done using American Wood Preservers Association (AWPA) testing method E-10-07, "Standard Method of Testing Wood Preservatives by Laboratory Soil Block Cultures".

3. Results and discussion

3.1. Study of reaction conditions

Nanoparticle diameter typically shows a strong dependency on the concentration of the medium in which they are made regardless of if they are made by reaction, solvent-displacement or precipitation. To be used as controlled release devices in solid wood nanoparticle diameter below 150 nm was desired for good penetration into the wood interior. Consequently, the first study tested the effect of the reaction concentration on nanoparticle diameter (Fig. 1). Because the nanoparticles will be introduced into the wood in aqueous medium, the diameter of the water-swollen nanoprticles will affect their ability to penetrate the wood interior. Fig. 1 shows the particle size of the water-swollen nanoparticles, measured by DLS of chitosan-g-PMMA nanoparticles (designated chitosan-g-2PMMA, indicating the polymer matrix was made using a mass ratio of 2 parts of MMA to 1 part chitosan) after 24 h of reaction at concentrations, based on polymer solids. The polymer solids concentration ranged from 1.5 up to 5 wt.% in acidic H_2O (0.43 wt.% AcOH). The results show that a concentration of 2 wt.% or less is required to afford nanoparticles with a water-swollen diameter below 200 nm. The chitosan-g-2PMMA water-swollen diameter was ~167 ± 56 nm and typically increased by ~20–30 nm when prepared with biocide. Therefore, 2 wt.% chitosan-g-2PMMA in deionized water was used as the maximum acceptable concentration for these studies, although the diameter was larger than desired (Scheme 1).

The particle size can be decreased by increasing the protonation of the chitosan amine groups, which is accomplished by increasing the acetic acid from 0.67 g to 2.0 g/g chitosan. At either concentration of acetic acid the diameter of the nanoparticle can be further decreased by sonication. For example, the final diameter of chitosan-g-2PMMA nanoparticles made at 0.67 g of acetic acid per gram of chitosan resulted in decreasing the diameter from 167 ± 56 nm to 132 ± 48 nm. Interestingly, the nanoparticle diameter was not significantly altered by the ratio of the MMA to chitosan in the range we studied (1, 2 or 3 parts MMA to 1 part chitosan). The nanoparitcles with 1, 2, and 3 parts of MMA to 1 part of chitosan (indicated by chitosan-g-PMMA, chitosan-g-2PMMA, and chitosan-

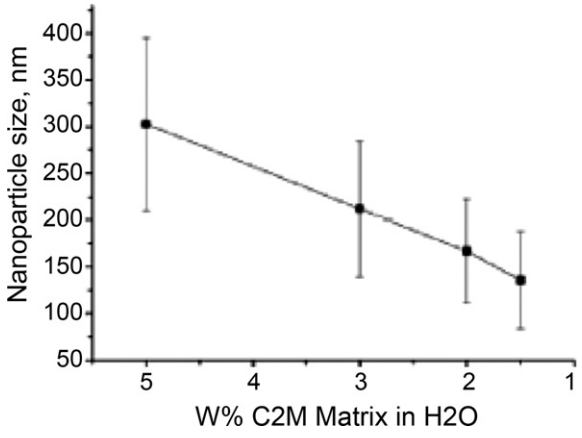
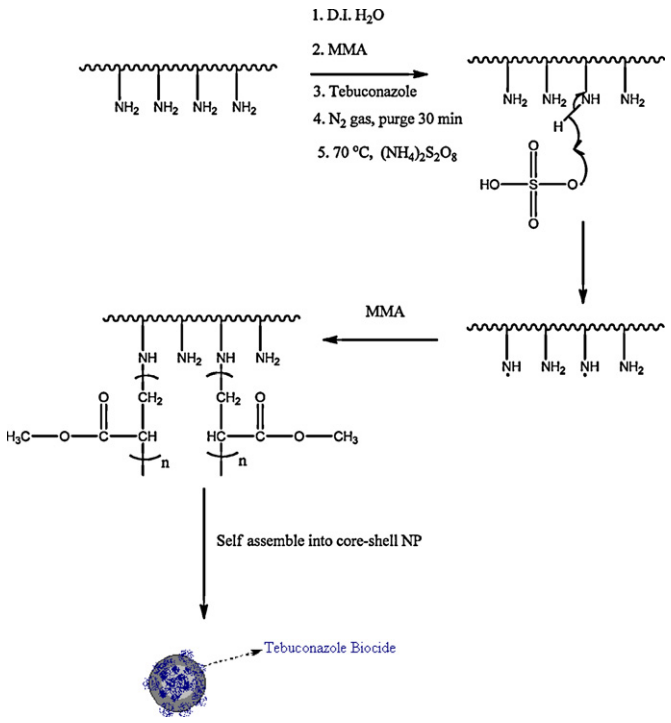


Fig. 1. Particle size (by DLS) of chitosan-g-2PMMA nanoparticles as a function of reaction concentration. In this figure, chitosan-g-2PMMA is designated as C2M.



Scheme 1. Synthetic route producing self-assembled chitosan-g-PMMA nanoparticles.

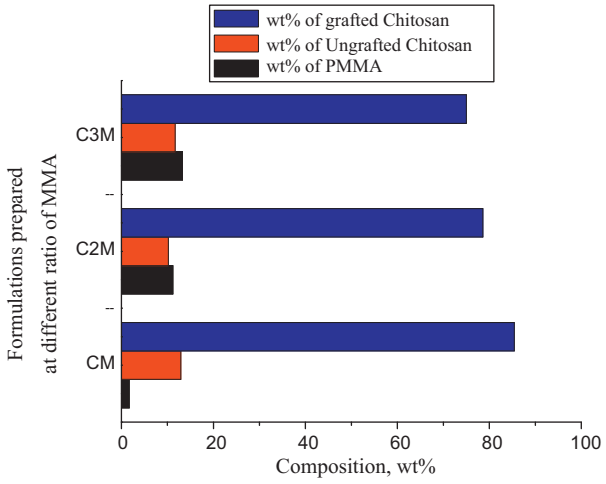


Fig. 2. Composition of chitosan-g-PMMA, chitosan-g-2PMMA, and chitosan-g-3PMMA nanoparticles (142 ± 60, 167 ± 56 and 166 ± 51 nm respectively when prepared at 2 wt.% in 75 mL deionized H_2O with 0.43 wt.% AcOH). In figure chitosan-g-PMMA, chitosan-g-2PMMA, and chitosan-g-3PMMA are designated as CM, C2M, and C3M respectively.

g-3PMMA respectively), prepared at 2 wt.% increased by ~22 nm as MMA content increased from 1:1 to 3:1 with respect to chitosan.

Interestingly, changing the ratio of MMA to chitosan does not significantly affect particle size, though it does increase the quantity of PMMA homopolymer produced, as shown in Fig. 2. The increase in the extent of homopolymerization is coincident with a slight decrease in the extent of grafting to chitosan. The amount of ungrafted chitosan is relatively constant. The ungrafted chitosan is probably the major contributor to the water-swollen diameter of the nanoparticles, which are typically ~50–100 nm larger than the diameter observed by SEM.

3.2. Characterization of fungicide-containing nanoparticles

In the second study the amount of tebuconazole introduced into the nanoparticles and the effect of the tebuconazole on nanoparticle diameter were determined. In this work we arbitrarily selected a target tebuconazole quantity to be 30% of the final nanoparticle mass. Table 1 shows that the tebuconazole is incorporated at ~93% yield. This high incorporation efficiency of the water-insoluble biocide supports the conclusion that this polymerization does not follow an emulsion polymerization route, but proceeds in a micellar route, leading to a final solid nanoparticle. The final suspension, after sonication, affords water-swollen chitosan-g-2PMMA-tebuconazole nanoparticles of ~150 nm.

One important advantage of using this radical grafting approach to amphiphilic copolymer nanoparticles is that it is easy to "tune" the core composition from hydrophobic (only MMA) to increasingly hydrophilic by increasing the HEMA content. Even small changes in core hydrophilicity can alter the biocide release rate, which is diffusion controlled. Here the core composition was changed slightly by replacing 2 wt.% of MMA monomer with HEMA monomer, giving chitosan-g-2PMMA-co-HEMA nanoparticles. The results, shown in Table 1, show the water-swollen chitosan-g-2PMMA-co-HEMA-tebuconazole nanoparticles have a larger diameter than that found for chitosan-g-2PMMA-tebuconazole nanoparticles (189 ± 74 compared to 149 ± 57 nm). However, SEM found no significant difference in the average diameter of these nanoparticles. The tebuconazole incorporation efficiency remained high, but was less than that obtained for chitosan-g-2PMMA nanoparticles, down from ~93% to ~82%. This indicates that increasing the hydrophilicity of the system compromises the efficiency of the micelle "capture" of tebuconazole. Again, the high entrapment efficiency shows that this polymerization proceeds by a micellar route (Kreuter, 1978) rather than a typical emulsion route. That is, the biocide was present within a chitosan-stabilized micelle together with the acrylic monomer(s) at the start of the polymerization. Possibly the HEMA content allowed more tebuconazole to be retained in the shell and this was lost during nanoparticle isolation.

4. Wood leaching and wood preservation

4.1. Nanoparticle delivery into wood blocks

Aqueous suspension of tebuconazole-containing chitosan-g-2PMMA and chitosan-g-2PMMA-co-HEMA nanoparticles (chitosan-g-2PMMA-TEB and chitosan-g-2PMMA-co-HEMA-TEB) were prepared and used to pressure-treat southern pine sapwood blocks according to the standard method of wood pressure treatment (AWPA E11-97). The delivered nanoparticle mass was determined by measuring the undelivered mass, and the retention of tebuconazole in the wood blocks was calculated from the absorbed nanoparticle mass, as described in the Section 2. The results are shown in Table 2.

Table 1
Nanoparticle size and tebuconazole content in chitosan-g-2PMMA and chitosan-g-2PMMA-co-HEMA nanoparticles.

Sample	NP size (nm) (DLS/SEM)	NPs (g)	Tebuconazole (g)	Tebuconazole (wt.%) (Theo./actual)
Chitosan-g-2PMMA-30% TEB	149 ± 57/100~150	0.3500	0.0972	30.0/27.8
Chitosan-g-2PMMA-co-HEMA-30% TEB	189 ± 74/100~150	0.5100	0.1257	30.0/24.6

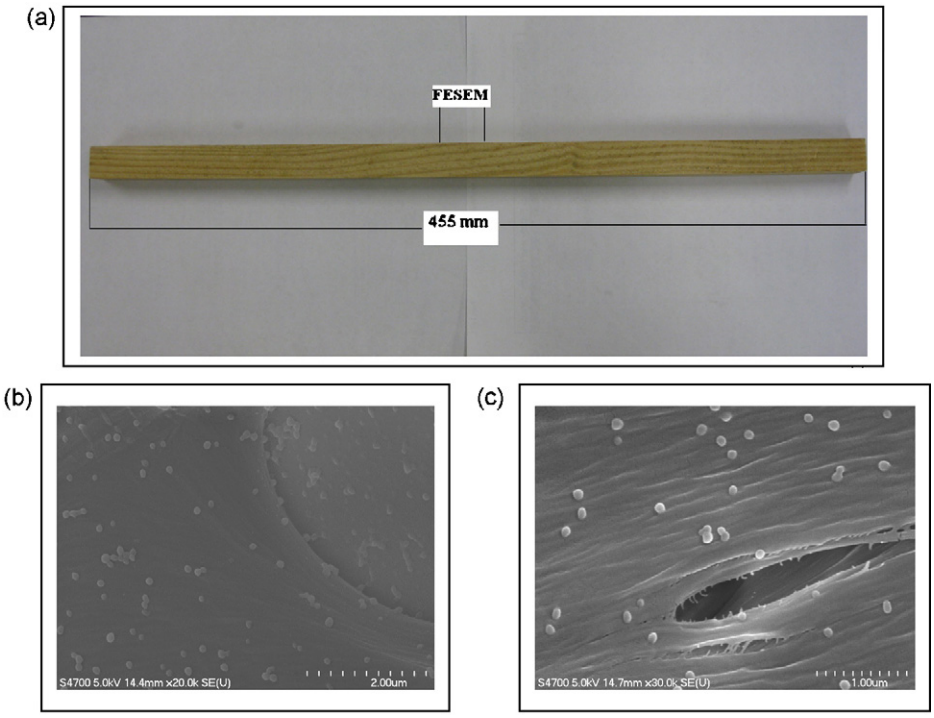


Fig. 3. A wooden stake after nanoparticle treatment is shown, with a central section indicated (a, top); after the indicated central section is cut, it is then specimens suitable for FESEM are taken from two locations, one deep in the interior (b, bottom left) and from near the surface (c, bottom right).

Previous studies with nanoparticle-treated southern pine sapwood (Salma et al., 2010; Liu et al., 2002a,b,c) showed good biological efficacy and good penetration, but treatment was limited to 19 mm × 19 mm × 19 mm blocks. One of the objectives of this work was to further prove good penetration of wood, using large wooden "field" stakes (19 mm × 19 mm × 455 mm). Several stakes were treated and sectioned to verify penetration of the wood interior achieved even on larger wooden parts.

Fig. 3a shows an image of a treated field stake. Despite nanoparticle aggregates reducing delivery efficiency, good penetration of the wood stake was achieved as shown by FESEM images (Fig. 3b and c) showing interior sections. Fig. 3b shows a region deep in the interior of the wood stake while Fig. 3c shows a section nearer the surface.

4.2. Leaching from wood blocks

The primary purpose of this work was to prove the hypothesis that use of controlled-release biocide-containing nanoparticles will reduce biocide leaching without compromising wood preservation. Wood blocks were treated to give theoretical biocide-retentions of 0.2, 0.4, and 0.8 kg tebuconazole/m^3 sapwood. Fig. 4a and b shows the measured biocide leach from solution-treated tebuconazole controls comparing with leach from wood treated with chitosan-g-2PMMA-TEB and chitosan-g-2PMMA-co-HEMA-TEB nanoparticles at theoretical loadings of 0.4 and 0.8 kg/m^3 wood respectively. In both cases the solution-treated control leached significantly more tebuconazole than the nanoparticle-treated wood, and the chitosan-g-2PMMA-co-HEMA-TEB nanoparticle-treated wood leached more tebuconazole than the chitosan-g-2PMMA-TEB nanoparticle-treated wood.

Based on the cumulative leach the chitosan-g-2PMMA-co-HEMA-TEB treated wood typically leached about two to three times the amount of tebuconazole as the chitosan-g-2PMMA-TEB treated wood. Because the retentions of the chitosan-g-2PMMA-co-HEMA-TEB and chitosan-g-2PMMA-TEB treated sapwood were similar the leaching data are comparable with each other, but not with the solution-treated specimens. Therefore, Fig. 4c re-plots the leach data for TEB-solution treated wood with the nanoparticle-treated wood, where the actual retentions are all close to 0.4 kg/m^3. Fig. 4c plots the leach from a TEB-solution treated wood with an actual tebuconazole content of 0.4 kg/m^3, on the same axes as the leach from chitosan-g-2PMMA-co-HEMA-TEB and chitosan-g-2PMMA-TEB nanoparticle-treated wood systems with actual retentions of 0.45 and 0.44 kg/m^3 respectively. The solution-treated control releases ~2300 μg of tebuconazole compared to ~200 μg for the chitosan-g-2PMMA-TEB treated wood. This means that the chitosan-g-2PMMA-TEB treated-wood leached less than 9% of the amount of biocide compared to the solution control, and, as the next section shows, did not sacrifice wood preservation based on results from a soil jar test.

Table 2
Nanoparticle delivery and tebuconazole retention in wood blocks.

Sample	Target retention (Kg Teb/m^3)	Delivery efficiency (%)	Actual retention (Kg/m^3)
Chitosan-g-2PMMA-TEB	0.20	66.1	0.13
	0.40	59.9	0.24
	0.80	54.7	0.44
Chitosan-g-2PMMA-co-HEMA-TEB.	0.20	68.5	0.14
	0.40	55.7	0.22
	0.80	56.6	0.45
TEB Control	0.20	100	0.20
	0.40	100	0.40
	0.80	99.4	0.795

4.3. Wood preservation efficacy by soil jar decay test

Soil jar decay tests were performed on already-leached wood blocks treated with chitosan-g-2PMMA-TEB and chitosan-

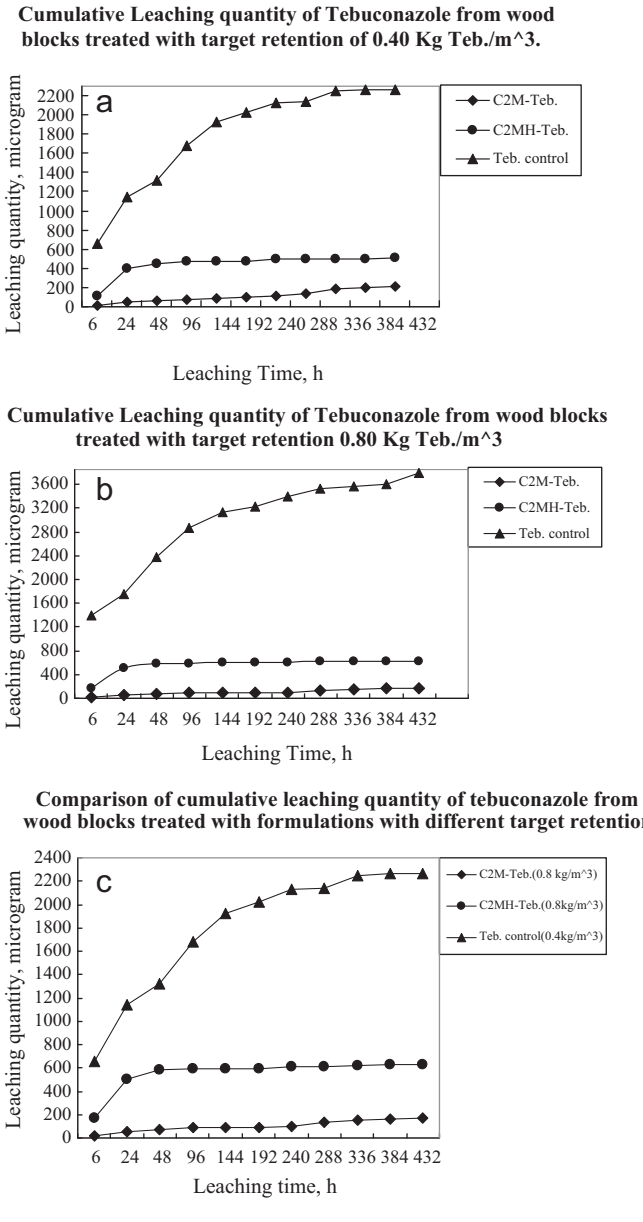


Fig. 4. Cumulative leaching quantity of a.i. from wood blocks treated with TEB solution control, chitosan-g-2PMMA-TEB and chitosan-g-2PMMA-co-HEMA-TEB nanoparticle formulations. In figure, chitosan-g-2PMMA is abbreviated as C2M and chitosan-g-2PMMA-co-HEMA is abbreviated as C2MH.

g-2PMMA-co-HEMA-TEB nanoparticles and a solution treated tebuconazole control, according to the AWPA E-10 standard method (see Section 2.8). The decay test results are shown in Fig. 5.

The weight loss of the chitosan-g-2PMMA controls (wood treated with chitosan-g-2PMMA nanoparticles that contain no biocide) may be slightly greater than the untreated pine controls when the nanoparticle "blanks" are introduced at higher levels. If this difference is real then chitosan, like gelatin, at least after it is modified by MMA, may slightly promote fungal decay by *G. trabeum*, but the standard deviations are high, so the trend is not definitive. The weight loss for the tebuconazole-containing nanoparticle treated specimens show no significant difference in the preservation efficacy at any retention with any formulation except the chitosan-g-2PMMA-co-HEMA-TEB treated wood at a theoretical retention of 0.2 kg/m^3, but with actual retention of 0.14 kg/m^3. Therefore, the tebuconazole introduced in controlled-release nanoparticles retain efficacy against *G. trabeum*, and does

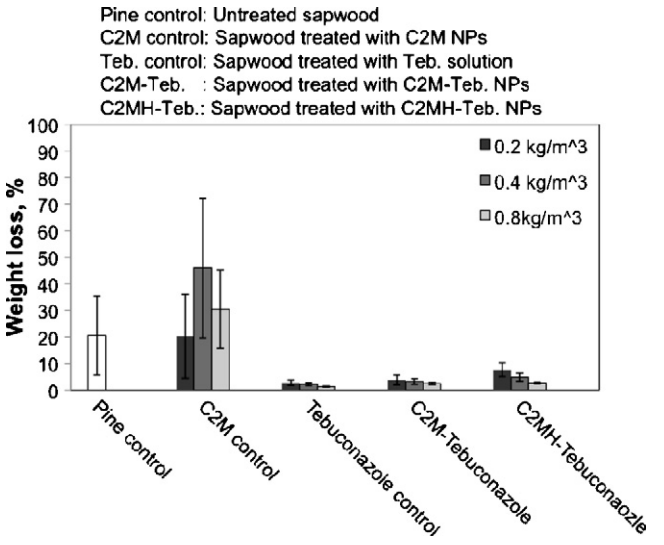


Fig. 5. Weight loss of leached southern pine sapwood from soil jar decay tests. Theoretical retentions were 0.2, 0.4, and 0.8 kg tebuconazole/m^3 wood. For 0.2 kg/m^3 wood actual tebuconazole retentions were 0.2, 0.13, and 0.14 kg/m^3 for TEB, chitosan-g-2PMMA-TEB, and chitosan-g-2PMMA-co-HEMA-TEB respectively. For 0.4 kg/m^3 wood actual tebuconazole retentions were 0.4, 0.24, and 0.22 kg/m^3 for TEB, chitosan-g-2PMMA-TEB, and chitosan-g-2PMMA-co-HEMA-TEB respectively. For 0.8 kg/m^3 wood actual tebuconazole retentions were 0.795, 0.44, and 0.45 kg/m^3 for TEB, chitosan-g-2PMMA-TEB, and chitosan-g-2PMMA-co-HEMA-TEB respectively. (In figure, chitosan-g-2PMMA is abbreviated as C2M and C2MH indicates the chitosan-g-2PMMA-co-HEMA).

so with substantially less leach than tebuconazole introduced in solution.

5. Conclusion

A simple, efficient, and versatile one-step route was used to prepare self-assembling and biocide-containing core–shell nanoparticles from chitosan grafted with acrylic monomers. The method gives high biocide capture efficiency and a high yield of nanoparticles (~150 nm) that can be delivered into southern pine sapwood. SEM investigation of nanoparticle-treated field stakes showed nanoparticles penetrated throughout the wood interior. The biocide-containing nanoparticles, chitosan-g-2PMMA-TEB, captured 93% of the available biocide during their preparation, effectively protected sapwood from biological attack, and showed only ~9% of the biocide leach as wood blocks treated with a tebuconazole solution. Using this method the nanoparticles' core composition can be manipulated by replacing portions of MMA with other acrylic monomers during the nanoparticle synthesis. This allows the core composition to be appropriately "tuned" for other biocides. When just 2% of the MMA was replaced with HEMA the nanoparticles possessed a more hydrophilic core, resulting in slightly larger nanoparticles. The wood blocks treated with these nanoparticles leached much more biocide than the chitosan-g-2PMMA nanoparticle-treated wood, but it was still only ~26% of the biocide leached by the solution-treated tebuconazole control wood blocks. This shows that very small changes in the core-composition can effectively tune biocide release and minimize biocide leaching. The results demonstrate this method can efficiently incorporate the biocide, significantly reduce biocide leach without compromising wood preservation, and the core-composition is easily altered to allow the nanoparticles to be "tuned" appropriately for other organic biocides.

Acknowledgements

This work was funded by the United States Environmental Protection Agency Science to Achieve Results (STAR) Program, Grant Number GR832371. The authors thank Dr. Peter Laks, Mr. Glen Larkin, and Mr. Erik Keranen, at MTU School of Forest Resources and Environmental Science, for preparing and pressure treating the wood blocks and Mr. Owen Mills, Department of Materials Science and Engineering at MTU, and Mr. Ning Chen (Labmate), for the SEM images.

References

American Wood-Preservers Association Book of Standards (1998). Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Culture E10-91. Branbury, Texas, E10-91. 375–385.

Aranaz, I., Harris, R., & Heras, A. (2010). Chitosan amphiphilic derivatives. Chemistry and applications. *Current Organic Chemistry*, *14*, 308–330.

Boehm, A. L., Martinon, I., Zerrouk, R., Rump, E., & Fessi, H. (2003). Nanoprecipitation technique for the encapsulation of agrochemical active ingredients. *Journal of Microencapsulation*, *20*(4), 433–441.

Craparo, E. F., Teresi, G., Ognibene, M. C., Casaletto, M. P., Bondı', M. L., & Cavallaro, G. (2010). Nanoparticles based on novel amphiphilic polyaspartamide copolymers. *Journal of Nanoparticle Research*, *12*, 2629–2644.

Jiang, M., Wu, Y., He, Y., & Nie, J. (2010). Synthesis and characterization of an amphiphilic hyperbranchedpoly(amine-ester)-co-D,L-lactide (HPAE-co-PLA) copolymers and their nanoparticles for protein drug delivery. *Journal of Applied Polymer Science*, *117*(2), 1156–1167.

Kakizawa, Y., Nishio, R., Hirano, T., Koshi, Y., Nukiwa, M., Koiwa, M., Michizoe, J., & Ida, N. (2010). Controlled release of protein drugs from newly developed amphiphilic polymer-based microparticles composed of nanoparticles. *Journal of Controlled Release*, *142*, 8–13.

Kamps, A. C., Sanchez-Gaytan, B. L., Hickey, R. J., Clarke, N., Fryd, M., & Park, S. J. (2010). Nanoparticle-directed self-assembly of amphiphilic block copolymers. *Langmuir*, *26*(17), 14345–14350.

Kreuter, J. (1978). Nanoparticles and nanocapsules—New dosage forms in the nanometer size range. *Pharmaceutica Acta Helvetiae*, *53*(2).

Kuskov, A. N., Voskresenskaya, A., Goryachaya, A. V., Shtilman, M. I., Spandidos, D. A., Rizos, A. K., et al. (2010). Amphiphilic poly-N-vinylpyrrolidone nanoparticles as carriers for non-steroidal anti-inflammatory drugs: Characterization and in vitro controlled release of indomethacin. *International Journal of Molecular Medicine*, *26*(1), 85–94.

Lao, B., Zhang, Z., Xu, H. H., & Jiang, G. (2010). Novel amphiphilic chitosan derivatives: Synthesis, characterization and micellar solubilization of rotenone. *Carbohydrate Polymers*, *82*, 1136–1142.

Leonardis, P. D., Mannina, L., Diociaiuti, M., & Masci, G. (2010). Atom transfer radical polymerization synthesis and association properties of amphiphilic pullulan copolymers grafted with poly(methyl methacrylate). *Polymer International*, *59*(6), 759–765.

Li, P., Zhu, J., Harris, F.W. (2003), Amphiphilic core–shell latexes, United States Patent, 6,573,313.

Li, P., Zhu, J., Sunintaboon, P., & Harris, F. W. (2002). New route to amphiphilic core–shell polymer nanospheres: graft copolymerization of methyl methacrylate from water-soluble polymer chains containing amino groups. *Langmuir*, *18*(22), 8641–8646.

Liu, Y., Laks, P., & Heiden, P. (2002a). Controlled release of biocides in solid wood. Part 1. Efficacy against *Gloeophyllum trabeum*, a brown rot wood decay fungus. *Journal of Applied Polymer Science*, *86*, 596.

Liu, Y., Laks, P., & Heiden, P. (2002b). Controlled release of biocides in solid wood. Part 2. Efficacy against trametesversicolor and *Gloeophyllum trabeum* wood decay fungi. *Journal of Applied Polymer Science*, *86*, 608.

Liu, Y., Laks, P., & Heiden, P. (2002c). Controlled release of biocides in solid wood. Part 3. Preparation and characterization of surfactant-free nanoparticles. *Journal of Applied Polymer Science*, *86*, 615.

Muzzarelli, R. A. A., Muzzarelli, C., Tarsi, R., Miliani, M., & Cartolari, M. (2001). Fungi static activity of modified chitosans against *Saprolegnia parasitica*. *Biomacromolecules*, *2*, 165–169.

Qian, F., Cui, F., Ding, J., Tang, C., & Yin, C. (2006). Chitosan graft copolymer nanoparticles for oral protein drug delivery: Preparation and characterization. *Biomacromolecules*, *7*, 2722–2727.

Salma, U., Chen, N., Richter, D. L., Filson, P. B., Dawson-Andoh, B., Matuana, L., & Heiden, P. A. (2010). Amphiphilic core/shell nanoparticles to reduce biocide leaching from treated wood, 1 – leaching and biological efficacy. *Macromolecular Materials and Engineering*, *295*(5), 442–450.