

DEVELOPMENT AND APPLICATION OF COLORIMETRIC MICROASSAY FOR DETERMINING BORON- CONTAINING COMPOUNDS

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ABSTRACT

This paper describes the development of a microassay for boron and the application of this microassay for evaluating leachability of boron by post-treatment of southern pine with the calcium precipitating agent NHA (N'-N-naphthaloylhydroxylamine). The microassay method for quantitative estimation of boron content in treated wood and leachates is a microadaptation of the colorimetric azomethine-H method. The primary advantages of this microassay method are that it uses fewer reagents, uses smaller sample volumes, and conserves time for analysis when a large number of tests are required. Wood blocks were treated with boric acid, disodium octaborate tetrahydrate (DOT), and NHA solutions at several concentrations. Boron leaching performance was evaluated on the blocks using AWP A E11-97 standard method. Blocks treated sequentially with DOT and then with 0.5 or 1.0 percent NHA solutions showed ~30% less boron leaching, whereas similar treatment with boric acid and 0.1% NHA solutions did not enhance boron retention compared with the blocks treated with only boric acid. We conclude that precipitation of NHA at higher concentrations decreases or limits boron leaching.

Assay systems modified for simple testing in 96-well microtiter plates allow rapid screening of a large number of test samples and substantially conserve time and reagents, especially when a large number of tests are needed (4,11,18,40). Most commercial microplate readers differentiate colorimetric reactions by transmitted light (absorbance) at a specific wavelength. Either insoluble substrates, used in many assays (2), or the precipitates formed during an assay prevent penetration of light and render measurement by transmitted light inaccurate (18).

Boron wood preservatives are relatively cost-effective chemicals and have minimal toxicity against mammals and

the environment. In addition, boron-based preservatives are colorless, odorless, noncorrosive, and nonflammable (25,29). Several methods have been developed for estimating boron content in wood and leachates based on photometry and titration. Curcumin, carminsäure, crystal violet, carminic acid, and

azomethine-H methods are based on photometry (1,9,33). AWP A A2-98 (1) macroassay method has been used in several studies to measure boron content in wood and leachates (8,13,30,32). In the microassay method we have adapted, each well is read by absorbance, avoiding the time-consuming reading step necessary in AWP A A2-98 standard method (1), which uses a spectrophotometer with cuvettes.

The use of broad-spectrum biocides for wood preservation is being limited because they include components that are toxic to mammals and other non-target organisms in the environment. These concerns have provided incentive to look for new wood preservatives. Ideally, new preservative systems might incorporate a simple, water-soluble chemical moiety, which binds to or precipitates into wood and protects the wood against wood decay fungi and termite attack (26,27). Hydrolyses of wood pectin from ray parenchyma cells and tori of pit membranes has been hypothesized as a necessary step in the colonization of wood by brown-rot and white-rot

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TABLE 1.—Boron retention and percentage boron released from the wood blocks treated with boric acid and NHA solutions. ^a

Boron concentration in solution (%BAE)	Process	NHA concentration in solution (%)	Avg. boron retention (kg/m ³) ^b	Avg. boron released (%) ^c
0.25	Single	None	0.658 (0.03)	95.87 (1.87)
0.125	Combined	0.05	0.480 (0.02)	96.27 (0.18)
0.50	Single	None	1.396 (0.03)	94.14 (1.98)
0.25	Combined	0.05	0.811 (0.02)	93.87 (1.10)
1.00	Single	None	1.797 (0.92)	92.63 (1.12)
0.50	Combined	0.05	0.968 (0.67)	90.04 (2.11)
2.00	Single	None	4.061 (0.10)	90.13 (1.09)
2.00	Sequential	0.1	2.740 (0.10)	89.27 (1.87)

^aValues in parentheses are standard deviations.

^bBased on microassay. Values represent means of two replicates.

^cValues represent means of three replicate sets of six blocks leached.

TABLE 2.—Boron retention and percentage boron released from the wood blocks treated with DOT and NHA solutions. ^a

Boron concentration in solution (%DAE)	Process	NHA concentration in solution (%)	Avg. boron retention (kg/m ³) ^b	Avg. boron released (%) ^c
3.0	Single	None	3.481 (0.09)	94.27 (1.10)
3.0	Sequential	1.0	3.682 (0.05)	69.43 (1.24)
5.0	Single	None	7.556 (0.11)	98.23 (0.07)
5.0	Sequential	0.5	6.891 (0.10)	88.68 (1.98)
5.0	Sequential	1.0	7.001 (0.09)	81.85 (1.87)
10.0	Single	None	12.602 (0.12)	97.25 (0.45)
10.0	Sequential	0.5	11.807 (0.08)	73.31 (0.53)
10.0	Sequential	1.0	11.687 (0.07)	71.73 (1.12)

^aValues in parentheses are standard deviations.

^bBased on microassay. Values represent means of two replicates per treatment.

^cValues represent means of three replicate sets of six blocks leached.

fungi (15,23). One key to pectin hydrolysis by plant pathogens has been shown to be fungal production of oxalic acid, which lowers pH of the substrate and chelates calcium ions. Production of oxalic acid may serve a similar role during incipient wood decay to that of calcium oxalate. Oxalate crystals have been seen with scanning electron microscopy during both brown-rot and white-rot decay (3,5,10,14,16,24). Therefore, *in situ* precipitation of existing calcium ions in wood may prevent the cascade of biochemical events involved in colonization of wood by fungi, especially hydrolysis of pit membranes. Preliminary studies have shown that brown-rot fungi, white-rot fungi, and termites are selectively inhibited by the water-soluble calcium-precipitating agent NHA (N'-N-naphthaloylhydroxylamine) (19,21,22).

Studies on the inhibition of decay by the selective precipitation of calcium by NHA have demonstrated protection in Southern yellow pine from fungal decay and termite damage at several concentrations in soil-block tests (17,20,22,23). Field tests have also shown that pressure treatment with 1 percent NHA can protect southern yellow pine to the same degree as chromated copper arsenate type C (CCA-C) in environments with high decay hazard indices for 38 months (6,23).

Despite the many advantages of boron wood preservatives, boron by itself does not adequately protect wood in ground contact and exterior applications because of its natural diffusibility and susceptibility to leaching (28,31,36), and pure boron does not fix to the cell wall (34).

To enhance the use of boron compounds as environmentally benign wood preservatives, previous authors have devised several fixation systems to limit or decrease boron leaching (7,8,12,13,34, 35,37,38,39). In this study, we evaluated the effect of NHA on boron leaching via boron precipitation in wood after NHA treatments at several concentrations. Incorporation of easily leachable boron compounds into NHA precipitation may reduce boron leaching by means of precipitation in wood.

MATERIALS AND METHODS

TREATMENT OF WOOD BLOCKS

Wood blocks, 19 by 19 by 19 mm, were cut from sapwood portions of southern yellow pine logs. Before treatment, all wood blocks were conditioned at 20 ± 2°C and 65 ± 5 percent relative humidity (RH) for 2 weeks. The wood blocks were treated with either boric acid (H₃BO₃) (Sigma Chemical Company, St. Louis, Missouri); NHA, or disodium octaborate tetrahydrate (DOT) (Na₂B₈O₁₃ · 4H₂O) (Solubor, U.S. Borax Inc., Valencia, California). There were three different processes for treatment; single, combined, and sequential.

In the single processes, which were done for comparison, 126 wood blocks were treated with either boric acid only or DOT only. The concentration for the single treatments ranged from 0.25 to 2.0 percent for boric acid and from 3 to 10 percent for DOT (Tables 1 and 2).

In the combined process, 60 wood blocks were treated with treatment solutions containing 0.125, 0.25, or 0.5 percent boric acid equivalent (BAE) boric acid and 0.05 percent NHA (Table 1).

In the boric acid sequential process, 25 wood blocks were first treated with 2.0 percent boric acid solution, then dried at 60°C for 1 day, and then conditioned at 20 ± 2°C and 65 ± 5 percent RH for 2 weeks. After the conditioning period, the blocks were treated with 0.1 percent NHA solution (Table 1). In the DOT sequential process, 70 wood blocks were first treated with 3.0, 5.0, or 10.0 percent BAE DOT solutions, then dried at 60°C for 1 day, and then conditioned at 20 ± 2°C and 65 ± 5 percent RH for 2 weeks. After conditioning, the blocks were treated with 0.5 or 1.0 percent NHA solutions (Table 2).

In all processes, the pressure cycle consisted of a 30-minute vacuum (88

kPa absolute pressure) followed by a 2-hour pressure period (only 1 hr. for DOT treatments) (880 kPa) in a treatment cylinder. After all treatments, samples were blotted dry and reweighed to determine uptake boron and NHA retention. All treated blocks were then reconditioned at $20 \pm 2^\circ\text{C}$ and 65 ± 5 percent RH for 2 weeks before the leaching tests.

LEACHING TESTS

The leaching procedures were similar to AWP Standard Method B11-97 (1) for the blocks. After the conditioning period, three replicate sets of six blocks for each boric acid treatment and two replicate sets of six blocks for each DOT treatment were removed from the conditioning room and reweighed. Leaching sets of six blocks were chosen based on uptake boron retention in the blocks. Each set of six blocks was placed into a 500-mL bottle, submerged in 300 mL of deionized water, and subjected to a vacuum to impregnate the blocks with deionized water. The sample bottles were subjected to mild agitation, and the water was replaced after hours, 1 day, 2 days, and every 2 days thereafter for a total of 14 days. Leachates were collected after each water replacement and were analyzed for boron content.

MICROASSAY FOR ESTIMATION OF BORON CONTENT IN LEACHATES AND WOOD

The sample preparation was similar to the AWP A2-98 standard method (1). The wood blocks were ground to pass through a 40-mesh screen (0.42-mm openings) in the Wiley Mill and oven-dried; 1.5 g of ground wood was weighed to the nearest 0.001 g and placed into a 250-mL flask. For each treatment group, two blocks were ground and analyzed. Then 100 mL of deionized water were added to the flask containing the ground wood. The flask was placed in a water bath at 90° to 95°C for 60 minutes with agitation every 15 minutes. After being cooled, the contents in the flask were filtered through Whatman #4 filter paper, rinsed three times with 20 mL of hot deionized water, and diluted to 200 mL in a volumetric flask.

For microassay determination of boron in wood and leachates, buffer solution was prepared by dissolving 25 g ammonium acetate in 40 mL deionized water and adding 1.5 g disodium ethylenediaminetetraacetic acid (EDTA) and

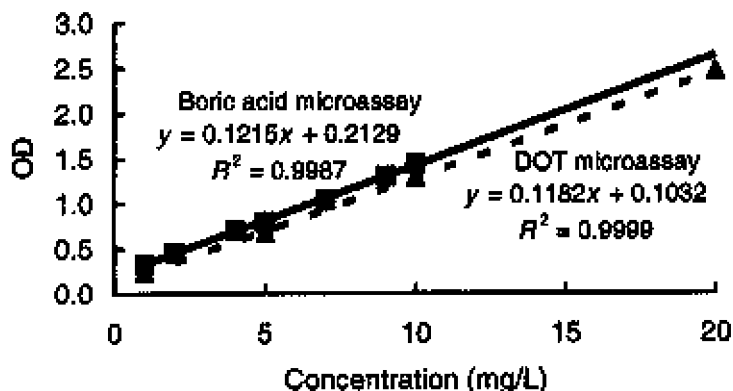


Figure 1. —The relationship between concentrations of standard boric acid and DOT solutions and optical density (OD) of the solutions measured by microassay.

5 mL glacial acetic acid according to AWP A2-98 method (1). Azomethine-H solution was prepared by solving 0.5 g ascorbic acid and 0.225 g azomethine-H in 50 mL deionized water. In a 96-well microplate, 50 μL of sample was placed in each well of the polystyrene plate with an Eppendorf micropipet (Middleton, Wisconsin). After a 10-second gentle agitation of the microplate, 50 μL of buffer solution was added to each well of the plate. Fifty μL of azomethine-H solution were then placed in each well after a second shaking step. The microplate was covered with a polystyrene cover, and optical density (OD) measurements were read following a 35-minute incubation at 25°C . Colorimetric measurements were read at 420 nm with an MRX Microplate Reader (Dynex Technologies Inc., Chantilly, Virginia). A standard curve was prepared using standard boron solution at concentrations of 10, 9, 7, 5, 4, 2, and 1 mg/L boron and 20, 10, 5, and 1 mg/L boron for boric acid and DOT treatments, respectively. The boron concentration of the unknowns was determined from the standard curve. The results were expressed as percentage of boron released, relative to the amounts retained in the unleached wood blocks. For each 96-well plate analyzed by OD measurements, one standard curve was prepared and used for estimation of boron content.

RESULTS AND DISCUSSION

Figure 1 shows standard curves for standard boron solutions determined by microassay. This microassay for boron determination in wood and leachates is a sensitive, rapid method and also can be

effectively substituted for the AWP A2-98 standard method (1) for boron determination in wood, thus conserving reagents and test time.

When wood blocks were treated with boric acid and NHA using the combined process, boron preservative retention in all cases was lower than that of wood blocks treated with boron alone in the single processes (Table 1). However, the starting solutions in the combined process contained half as much boron as the corresponding boron-only treatments. In the sequential process, the starting solution contained the same amount of boron (2% BAE) as the boron-only treatment, yet less boron was still retained compared with the boron-only treatment. Thus, if the starting solution contained equal concentrations of boron, the boron retention in the combined process may have been higher. Boron retention in the DOT-treated wood blocks (5% and 10% BAE DOT) in the sequential processes was less than in the boron-only treatments (single process). These results may reflect depletion of surface boron into the NHA solution during the second treatment in the sequential process. Because of less waiting time in the second treatment (1 hr.) and higher NHA concentration in the DOT-NHA sequential process, boron losses might be less because of more NHA precipitation with boron in wood compared with the boric acid-NHA sequential process. When boric acid was combined with NHA, the treatment solutions were diluted in half, lowering the retention of boron (Table 1).

Incorporation of NHA into the boric acid treatment solution had no effect on

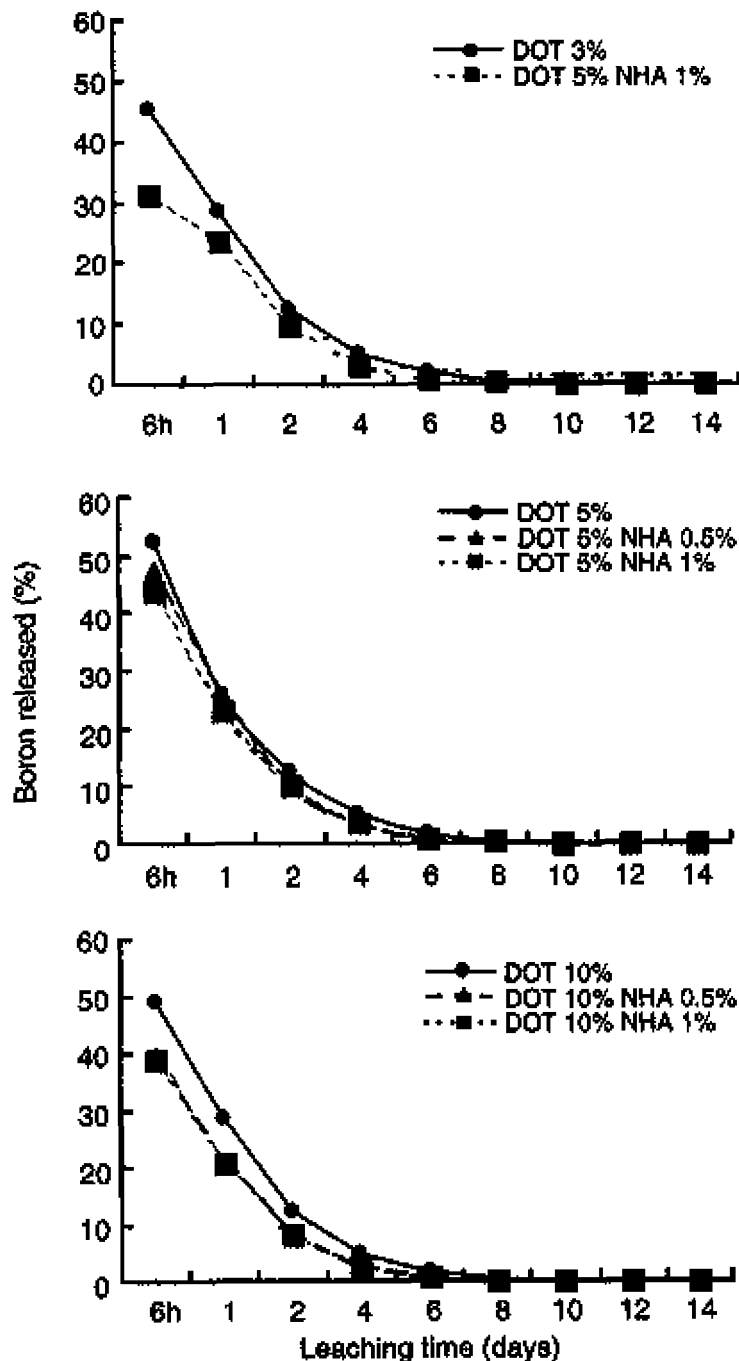


Figure 2. — Percentage of released boron from DOT- and NHA-treated wood blocks at several concentrations.

the resistance of boron to leaching (Table 1). In the blocks treated in the combined processes, released boron content after a 14-day leaching course was as much as in the blocks treated in the single processes. However, blocks treated with DOT, then dried and retreated with 0.5 or 1.0 percent NHA (sequential processes) showed less boron leaching than blocks treated with only DOT solutions

(single processes) (Fig. 2; Table 2). After a 14-day leaching, the average percentage boron remaining in wood blocks treated with 3.0 and 10.0 percent BAE DOT and 1.0 percent NHA was about 30 percent in both cases compared with blocks treated with only DOT solution ($\leq 5.73\%$). The results also show that the higher NHA concentration in sequential processes caused less boron to

be released from the wood blocks. The addition of NHA solutions at concentrations of 0.1, 0.5, and 1.0 percent did not completely inhibit boron leaching from wood. The majority of boron released from the wood blocks during the 14-day leaching course occurred during the first 6-hours and 1-day leaching periods in all treatments (Fig. 2). The reason for this effect is not clear. However, it may indicate that the boron precipitation products in wood are not completely insoluble complexes.

CONCLUSIONS

The boron macroassay method utilizes large amounts of reagents and individual absorbance readings in a spectrophotometer. This is the first report of a microassay procedure that allows accurate detection of boron in wood and leachates. The development of a boron microassay allows researchers to estimate boron concentration with small amounts of reagents, leachates, and filtrates. In addition, using a microassay method, 96 samples can be evaluated at once in a matter of minutes. In addition to conserving time and reagent, serial dilution can be performed on the plates to readily estimate boron concentration in leachates or filtrates that exceed the detection limit of the microplate reader. Replicate samples can also be analyzed for statistical significance.

Preliminary results showed that treatment with boric acid plus 0.05 percent NHA solutions did not prevent boron leaching from wood blocks compared with boron-only solutions. However, treatment with DOT and 0.5 or 1.0 percent NHA solutions in sequential processes reduced boron leaching by 10 to 25 percent compared with DOT-only solutions. Precipitation of boron in wood via NHA treatment seems to have the potential to reduce boron leachability. In addition, the existence of boron and NHA in wood together would be expected to have an additive, and possibly a synergistic, effect against wood-degrading organisms. Further research on precipitation of boron in wood by NHA solutions at several concentrations and on fungal resistance of boron- and NHA-treated wood is currently in progress to attain a better understanding of this relationship.

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