Photostability of Abamectin/Zein Microspheres

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Abamectin, a natural product pesticide that degrades rapidly in air and sunlight, has been encapsulated with the protein zein. The result was a mineral oil suspension that included a photostabilized abamectin. After exposure to simulated sunlight, the microsphere suspension provided a several-fold increase in recoverable abamectin residue. The enhanced photostability of the microspheres is due to retarded reaction between oxygen and the diene chromophore of abamectin, in the presence of light. The zein could function as a quencher of singlet oxygen and/or a physical oxygen barrier.

Keywords: *Abamectin; photostability; zein; encapsulation*

The avermectins are a class of macrocyclic lactones that are used widely, in both animal health and agriculture, for control of pests (Campbell, 1989; Dybas, 1989; Lasota and Dybas, 1991). They degrade rapidly in the presence of air and sunlight, and the photodegradation in simulated sunlight of two avermectins in thin films on glass has been studied in detail. Abamectin, which is used mainly as an acaricide, consists of a major component, avermectin B_{1a}, and a minor component, avermectin B_{1b}. The photodegradation of avermectin B_{1a} showed that oxidation of the diene chromophore was an important part of the degradative process (Crouch et al., 1991). Emamectin, which is used mainly as an insecticide, also consists of a major B_{1a} component and a minor B_{1b} component. The study with 4″-(*epi*-methylamino)-4″-deoxyavermectin B_{1a}, i.e., the B_{1a} component of emamectin, showed that the amino group altered the degradative pathways (Feely et al., 1992). However, in both cases, prolonged photodegradation led to loss of the diene chromophore. In the above study with avermectin B_{1a}, some degradants were formed by cis−trans changes in the diene chromophore, while others were formed by the addition of oxygen. The exact mechanisms by which the oxygenated products form are still in doubt, but they are likely similar to those with methoprene. The conjugated diene of methoprene also undergoes rapid geometrical and oxidative changes by several mechanisms (Quistad et al., 1975). In terms of decreasing the rate of abamectin photodegradation, an empirical approach can be used in the absence of exact mechanisms. It was shown earlier that antioxidants and sunscreens were not effective in improving the photostability of avermectin formulations (Peterson et al., 1996). We now report some success with abamectin, by encapsulation in a protein matrix. Methoprene also had been encapsulated in a biodegradable polymer (Jaffe, 1981). The avermectins mainly are ingestion toxicants, so any encapsulated formulation must release the active ingredient in the gut of the target species. It has been demonstrated that the midgut of many insect species contains proteinases (Wolfson and Murdock, 1990). Also, early work with abamectin adsorbed onto wool indicated that the process yielded material with a long residual activity (Bry and Lang, 1984; Bry, 1989). Thus, a formulation matrix of the corn protein zein should provide good bioavailability in insect larvae and, possibly, give a system with greater photostability.

Zein is a protein with unusual solubility properties (Pomes, 1971). It is insoluble in water and hydrocarbons, but it is soluble in some polar organic solvents, e.g., acetic acid. Also, both abamectin and emamectin benzoate are soluble in acetic acid. The encapsulation of abamectin was achieved by a solvent depletion process in a nonaqueous zein emulsion (Demchak, 1995). The process for avermectin/zein microspheres is similar to techniques used in preparing solvent-evaporated microcapsules (Tice and Gilley, 1985). Unlike previous encapsulations with zein (Matsuda et al., 1989), this process does not cross-link the protein.

MATERIALS AND METHODS

Encapsulation. Abamectin (Merck & Co., Inc.) contained >90% B_{1a} and <10% B_{1b}. It was recrystallized from 1-hexanol to remove excess water from the wet-cake technical. Zein (Freeman Industries), acetic acid (Fisher, ACS grade), and U.S.P. mineral oil (Witco, Protol grade) were used as supplied. Lecithin (Central Soya, Centolex P grade) was fractionated by a process similar to the one in U.S. Patent 5,436,355. Amberlite IRA-93 (Sigma, 16−50 mesh, 250 g), a weakly basic ion-exchange resin, was washed consecutively with 2-propanol and Protol. It was packed into a 75 × 300 mm glass column (Ace Glass) that contained a 310 μm screen. The dispersed phase was a solution of zein and abamectin in acetic acid; the continuous phase was a solution of lecithin in Protol that was saturated with acetic acid. The dispersed phase contained ca. 2% abamectin and 20% zein, whereas the continuous phase contained 10−16% lecithin. The former was emulsified into the latter using a 1-L stirred kettle and homogenizer (IKA LR-A 1000). The 5−10% emulsion was diluted with Protol (1:1) for 0.5−2 h and then was pumped through the ion-exchange column to remove acetic acid from the continuous phase. Finally, the system was filtered through a plastic frit with a pore size of 70 μm. The resulting microspheres were characterized by HPLC analyses, prior to an evaluation of photostability.

Analytical Instrumentation and Methodology. The HPLC system consisted of the following components: Spectroflow 400 pump (Perkin-Elmer), Hewlett-Packard 1040A diode array detector (DAD), and Vectra 486/66U computer with version A.02 software, Sedex 55 (Sedere) evaporative, light-scattering detector (ELSD), Alcott autosampler (Model 728), and Valco valve. The columns (4.6 × 250 mm) were either Hypersil ODS (Keystone Scientific, 3 μm) or Zorbax Rx-

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Table 1. Properties of Abamectin/Zein Microspheres

batch size (L)	ca. 1	% encapsulation	35
particle size (μm)	<70	abamectin concn (mg/g)	0.25
residual acetic acid (%)	<1	yield (%), based on zein	ca. 50

Sil (Mac-Mod). Eluent from the DAD was pumped into the ELSD, to obtain both a UV and a mass chromatogram for each sample. The HPLC grade solvents, ethylene glycol, cyclohexane (C_6H_{12}), 1-chlorobutane (BuCl), and 2-propanol (IPA), were obtained from Aldrich. Ethanolamine and HPLC grade methanol were obtained from Aldrich and EM Science, respectively. The centrifuge was a Sorvall SS-3; the 10 mL tubes were made of Teflon. The solar simulator was a Kratos Model LH 153 that was focused on a rotating support (MacConnell et al., 1989; Peterson et al., 1996). The unit has a 1000-W xenon lamp, filtered to provide an air mass 1 spectrum. The glass dishes were 15 cm in diameter. The abamectin ($B_{1a} + B_{1b}$) in each phase was quantitated by either reversed-phase (RP) or normal-phase (NP) HPLC. A 2.5-g aliquot of Protol suspension was diluted with 5 mL of BuCl to dissolve unencapsulated abamectin. The system was centrifuged for 15 min at 5000 rpm; the supernatant was saved, and the solids were sonicated with 5 mL of C_6H_{12} to remove mineral oil. After centrifugation, the supernatants were combined and the solids were again sonicated with C_6H_{12}. After centrifugation, the composite supernatant was diluted with BuCl for NP-HPLC. Residual C_6H_{12} was evaporated from the solids, and the weighed solids were sonicated in 3 mL of ethylene glycol. The solution of abamectin and zein was diluted with methanol for RP-HPLC. Acetic acid in the system was titrated using 0.1 N NaOH, with 0.01% thymolphthalein in 10% aqueous ethanol as indicator. For photostability, abamectin/zein microspheres or the commercial EC formulation (18 g/L) were diluted to 36 mg/L with C_6H_{12}. In previous work (MacConnell et al., 1989), this concentration gave a rapid degradation curve for the commercial EC formulation. A 3-mL aliquot from each system was applied to several dishes and allowed to evaporate for 1 h before exposure in the solar simulator. The dish residues were processed in the following way. A dish that contained the EC formulation was rinsed with methanol (2 × 10 mL). The solution was taken to dryness in a rotary evaporator, and the residue was redissolved in 3 mL of methanol for RP-HPLC. A dish that contained microspheres was rinsed with 10 mL of BuCl and then 10 mL of C_6H_{12}. The combined rinses were centrifuged while the dish was soaked with 20 mL of IPA/water (9:1 v/v). The supernatants were evaporated to near dryness, and the residue was dissolved in 3 mL of BuCl for NP-HPLC. The IPA/water rinse was used to dissolve the centrifuged solids, the solution was evaporated to dryness, and the residue was dissolved in 3 mL of IPA/water for RP-HPLC. The HPLC conditions were as follows. In RP-HPLC, the mobile phase was methanol/water/ethanolamine (90:10:0.02 v/v/v), flowing at 0.4−0.5 mL/min. (Ethanolamine is used to elute emamectin benzoate.) The column was at room temperature, and the detector was at 245 nm, the absorbance maximum of the diene chromophore. In NP-HPLC, the mobile phase was 1.0 M methanol in BuCl, flowing at 0.6 mL/min. The column temperature was 30 °C, and the detector was at 254 nm, for better baseline stability. The B_{1a} and B_{1b} components are resolved in RP-HPLC, and the ELSD showed no interference from zein. The two components coelute under the NP-HPLC conditions, and the ELSD showed no interference from Protol. The quantitation was completed using external standards. The RP-HPLC analyses in the photostability work were quantitated for the B_{1a} component and then corrected for the B_{1b} component, based on original assays. The B_{1b} component was not detected at some low recoveries after prolonged exposure in the solar simulator.

RESULTS AND DISCUSSION

The properties of the abamectin/zein microspheres (MS) used in the photostability work are shown in Table 1. The percent encapsulation is derived from two analyses. The RPHPLC analysis defines the encapsulated fraction (EF), and the NP-HPLC analysis defines the nonencapsulated fraction (NEF). The abamectin/zein MS that were described in U.S. Patent 5,436,355 were prepared in very low yield, without using an ion-exchange resin. Also, using the procedures described here, the photostability results with those MS were obtained only with an EF. Since then, many process changes have been made to increase the yield, so it was unknown if a system of MS-EF plus MS-NEF still would show enhanced photostability. The recoveries of abamectin in the photostability study are shown in Table 2. After MS-EF is divided by the percent encapsulation, these MS-EF data agree well with those in U.S. Patent 5,436,355. Also, even with 35% encapsulation and a lower, 5-day recovery for the MS, the MS total residue after 4 days was greater than the EC residue after 1 day. Despite process changes, results from two batches of zein MS made years apart showed enhanced abamectin photostability. Enhanced photostability of abamectin MS results from retarded reaction(s) of the diene. Crouch et al. (1991) showed that photodegradation of avermectin B_{1a} led to formation of the 8a-oxo and 8a-OH isomers, which form by reaction of the diene with oxygen. Also, Feely et al. (1992) showed that in the photodegradation of the B_{1a} component of emamectin some degradants form as a likely result of quenching of singlet oxygen by the 4″-*epi*-methylamino moiety. These degradants also form prior to loss of the diene. Zein MS could retard reaction(s) of the abamectin diene by either chemical or physical means. Chemically, zein could act as a quencher for singlet oxygen (Michaeli and Feitelson, 1994). Physically, the MS could function as an oxygen barrier. Both zein and the encapsulation process contribute to a system that is probably impermeable. Films of zein have a low permeability to oxygen (Aydt et al., 1991; Beck et al., 1996), and encapsulation with a solvent evaporation process gives relatively nonporous MS (Pavanetto et al., 1992).

Table 2. Percent Recovery of Abamectin with Time

time, exposure	EC	MS-EF	MS-NEF	MS total
initial, none	100	35	65	100
1 day, solar simulator	17	23	34	57
4 days, solar simulator	0	15	13	28
5 days, foil-covered	89	29	49	78
3 days, solar simulator (U.S. Pat. 5,436,355)	1.5	40		
4 days, solar simulator (U.S. Pat. 5,436,355)	0.6	38		

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LITERATURE CITED

Aydt, T. P.; Weller, C. L.; Testin, R. F. Mechanical and barrier properties of edible corn and wheat protein films. *Trans. ASAE* **1991**, *34*, 207−211.

Beck, M. I.; Tomka, I.; Waysek, E. Physico-chemical characterization of zein as a film coating polymer. A direct comparison with ethyl cellulose. *Int. J. Pharm.* **1996**, *141*, 137−150.

Bry, R. E. Avermectin B1a as a long-term protectant of woolen fabric. *J. Entomol. Sci.* **1989**, *24*, 214−217.

Bry, R. E.; Lang, J. H. Avermectin B1a: effectiveness against three species of fabric insects. *J. Ga. Entomol. Soc.* **1984**, *19*, 524−532.

Campbell, W. C., Ed. *Ivermectin and Abamectin*; Springer-Verlag: New York, 1989.

Crouch, L. S.; Feely, W. F.; Arison, B. H.; VandenHeuvel, W. J. A.; Colwell, L. F.; Stearns, R. A.; Kline, W. F.; Wislocki, P. G. Photodegradation of avermectin B1a thin films on glass. *J. Agric. Food Chem*. **1991**, *39*, 1310−1319.

Demchak, R. J. Process for making avermectin/zein compositions. U.S. Pat. 5 436 355, 1995.

Dybas, R. A.; Hilton, N. J.; Babu, J. R.; Preiser, F. A.; Dolce, G. J. Novel second generation avermectin insecticides and miticides for crop protection. In *Novel Microbial Products for Medicine and Agriculture*; Demain, A. L., Ed.; Elsevier: Amsterdam, 1989; pp 203−212.

Feely, W. F.; Crouch, L. S.; Arison, B. H.; VandenHeuvel, W. J. A.; Colwell, L. F.; Wislocki, P. G. Photodegradation of 4″-(epimethylamino)-4″-deoxyavermectin B1a thin films on glass. *J. Agric. Food Chem*. **1992**, *40*, 691−696.

Jaffe, H. Microencapsulation process. U.S. Pat. 4 272 398, 1981.

Lasota, J. A.; Dybas, R. A. Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annu. Rev. Entomol*. **1991**, *36*, 91−117.

MacConnell, J. G.; Demchak, R. J.; Preiser, F. A.; Dybas, R. A. Relative stability, toxicity, and penetrability of abamectin and its 8,9-oxide. *J. Agric. Food Chem*. **1989**, *37*, 1498−1501.

Matsuda, Y.; Suzuki, T.; Sato, E.; Sato, M.; Koizumi, S.; Unno, K.; Kato, T.; Nakai, K. Novel preparation of zein microspheres conjugated with PS-K available for cancer immunotherapy. *Chem. Pharm. Bull*. **1989**, *37* (3), 757−759.

Michaeli, A.; Feitelson, J. Reactivity of singlet oxygen toward amino acids and peptides. *Photochem. Photobiol.* **1994**, *59*, 284−289.

Pavanetto, F.; Conti, B.; Genta, I.; Giunchedi, P. Solvent evaporation, solvent extraction and spray drying for polylactide microspheres preparation. *Int. J. Pharm*. **1992**, *84*, 151−159.

Peterson, R. F.; Alverez, M. D.; Mookerjee, P. K. Photolytic degradation of formulated avermectins: effect of *p*-aminobenzoic acid (PABA). *Pesticide Formulations and Application Systems*, 15th ed.; ASTM STP 1268; American Society for Testing and Materials: Philadelphia, PA, **1996**; pp 88−96.

Pomes, A. F. In *Encyclopedia of Polymer Science and Technology*; Bikales, N. M., Ed.; Wiley: New York, 1971; Vol. 15, pp 125−132.

Quistad, G. B.; Staiger, L. E.; Schooley, D. A. Environmental degradation of the insect growth regulator methoprene (isopropyl(2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate). III. Photodecomposition. *J. Agric. Food Chem*. **1975**, *23*, 299−303.

Tice, T. R.; Gilley, R. M. Preparation of injectable controlled-release microcapsules by a solvent-evaporation process. *J. Controlled Release* **1985**, *2*, 343−352.

Wolfson, J. L.; Murdock, L. L. Diversity in digestive proteinase activity among insects. *J. Chem. Ecol.* **1990**, *16*, 1089−1102.

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