Antifungal Effect of Carbendazim Supported on Poly(ethylene-*co*-vinyl alcohol) and Epoxy Resin

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ABSTRACT: Ethylene-vinyl alcohol copolymers (EVOH) were prepared by the conventional saponification of poly(ethylene-*co*-vinyl acetate) using a solution of potassium hydroxide in ethanol. An organic fungicide, consisting of a 2-benzimidazole carbamoyl (CBZ) group supported on EVOH (EVOH-CBZ), was prepared by the transesterification reaction of methyl 2-benzimidazole cabamate (carbendazim) with EVOH. The antifungal activity of the synthesized polymers was examined by the halo zone test against *Aspergillus fumigatus* and *Penicillium pinophilum*. The synthesized EVOH-CBZ complex showed a strong antifungal activity. The bound CBZ units were susceptible to hydrolysis. CBZ bonded to an epoxy resin precursor, diglycidyl ether of bisphenol A (DGEBA-CBZ), retained its antifungal activity, which was somewhat weaker in comparison with that of EVOH-CBZ. When the DGEBA-CBZ complex was crosslinked by isophoronediamine, the antifungal activity disappeared almost completely, indicating that it is necessary for the CBZ units to release from their polymer supports to have the antifungal effects. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 728–736, 2001

Key words: antifungal effect; carbendazim; poly(ethylene-*co*-vinyl alcohol); epoxy resin

INTRODUCTION

Contamination by microorganisms invites serious problems to polymeric materials for sanitary, biomedical, and alimentary applications. One possible way to avoid microbial contamination is to develop materials that possess antimicrobial activities.[1]

Pentachlorophenol, a well known biocide, was chemically anchored to polymers by copolymerizing pentachlorophenol methacrylate with methyl methacrylate.[2] Pittman copolymerized pentachlorophenyl acrylate with both vinyl acetate (VAc) and ethyl acrylate.[3] Pittman's copolymers significantly retarded or prevented growth of *Aspergillus* sp., *Pseudomonas* sp., *Alternaria* sp., or *Aureobasidium pullulans*.

Nonaka and coworkers introduced a phenolic hydroxy moiety by treating amine-functionalized resins with *p*-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, and 3,4,5-trihydroxybenzoic acid.[4] The antibacterial activities increased in the order of the increasing number of hydroxyl groups.

Ikeda and Tazuke evaluated the antibacterial activities of trialkyl-vinyl benzyl ammonium chloride monomers and their polymers by the conventional spread plate method and the viable counting method.[5] The compounds with dodecyl chains exhibited particularly high activity. They also

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found that the polymers were more active than the corresponding monomers, which was possibily due to their favored adsorption onto the bacterial cell surface and the cytoplasmic membrane with subsequent disruption of its integrity.

Polycationic biocides with phosphonium salt were immobilized on a polypropylene surface through surface photografting to show high antibacterial activity. The bacterial cells in contact with the immobilized polycationic biocides were observed by SEM and were found to be significantly shrunken and deformed.[6]

Sun et al. modified polystyrene by chemically attaching hydantoin derivatives[7] and a chlorinated triazinedione moiety.[8] These polymeric biocides are water insoluble so that toxicological evaluation would not be required when used for disinfecting potable water.

N-Halamine polymeric disinfectants were synthesized and tested for efficacy in inactivating bacteria.[9] Polymeric *N*-halamine has the advantage of needing short contact time to kill microorganisms and its biocidal activity can be regenerated once exhausted by simply flowing an aqueous solution of free halogen through it. *N*-Halamine precursor monomers were copolymerized with other monomers in water with the aid of a surfactant to produce latexes, which could be used in numerous coating applications.[10]

Oh and colleagues synthesized 2,4,4′-trichloro-2′-acryloyloxydiphenyl ether,[13] its homopolymer, and its copolymer with styrene. The bactericidal activities decreased in the order of monomer > homopolymer > copolymer.[11]

Methyl 2-benzimidazolecarbamate (carbendazim), which has been used since the 1960s as various pesticides, has relatively low toxicity (rat LD_{50} = 6400 mg/kg). Recently it was discovered that carbendazim inhibits the growth of fungi very efficiently.[12]

In this study 2-benzimidazole carbamoyl (CBZ)[17] was bonded to poly(ethylene-*co*-vinyl alcohol) (EVOH), and its fungicidal effects were investigated. The release rate of CBZ units was examined during an accelerated hydrolysis test. The CBZ was anchored to a bisphenol A type epoxy precursor (diglycidyl ether of bisphenol A, DGEBA) to reduce the hydrolysis of CBZ units. The precursor could be used for various coating applications when crosslinked by curing agents. The cured epoxy resin was subjected to the antifungal test against *Penicillium pinophilum*.

EXPERIMENTAL

Materials

The ethylene VAc copolymer (EVAc) was used as received from Scientific Polymer Products (Sp^2). Aluminum isopropoxide (Aldrich) was dried in a desiccator under a vacuum over 24 h. Dimethyl sulfoxide (DMSO, Aldrich) was distilled under nitrogen at reduced pressure. Carbendazim (Aldrich) was recrystallized from toluene. The bisphenol A type epoxy resin YD-128 (DGEBA, epoxide equivalent weight 184–190 g/equiv, viscosity 11,500–13,500 cps) and isophoronediamine (IPDA) were obtained from Kukdo Chemical (Seoul) and used without further purification. Other chemical compounds were reagent grade and were used as received.

Preparation of EVOH

Poly(ethylene-*co*-VAc) (EVAc) was dissolved in 200 mL of 0.5*M* KOH in ethanol solution. Then the mixture was refluxed with stirring for the predetermined time, precipitated by excess distilled water, filtrated, washed with distilled water, and dried under a vacuum.

Synthesis of EVOH-CBZ

The mixture of 5 g of saponified EVAc (EVOH) and 60 mL of DMSO was stirred for 30 min at 60°C, and then 2 g of carbendazim was added into the above solution. The solution was heated to 115°C and then stirred for 150 min in the presence of 0.2 g of $Al(O\text{-}iPr)_3$. The resulting solution was precipitated into excess distilled water. The precipitate was filtered, washed with distilled water and ethanol, and dried under a vacuum to constant weight. To remove the unreacted carbendazim, the synthesized polymer was stirred in distilled water overnight.

Characterization of EVOH-CBZ

The EVOH-CBZ was analyzed at 110°C with a 250-MHz NMR spectrometer (Bruker AC-250) using DMSO-d_6 as a solvent. The thermal characteristics of EVOH-CBZ were examined with differential scanning calorimetry (DSC, Perkin–Elmer DSC 7). The first DSC thermogram was obtained by scanning to 200°C at a heating rate of 20°C/min. The sample was maintained at 200°C for 2 min and cooled to 30°C at the rate of 5°C/min. The second thermogram was carried out by reheating the sample to 200°C at 20°C/min. The

dynamic mechanical properties were measured with a Rheovibron (DDV-II, Toyo Baldwin Co.). The dimension of the sample was 35 × 5 × 0.2 mm and the properties were measured in the range between −80 and 100°C.

Antifungal Activity Test of EVOH-CBZ

The antifungal activity was evaluated by the halo(inhibition) zone test.[18–22] The fungi used in this study were *Aspergillus fumigatus* and *P. pinophilum*. Cultures of *A. fumigatus* (IFO 30870) and *P. pinophilum* were prepared by incubation at 28°C in potato dextrose agar (PDA) for 72 h. By diluting with 10 mL of sterile distilled water, a culture containing about 10^3–10^5 cells/mL was prepared for each strain and used for the antifungal tests. The sterile Petri dish containing PDA was inoculated by this culture. Fungicide was dissolved in DMSO (0.9 wt %). Twenty microliters of the solution was placed on a 10-mm diameter paper disk. The paper disk was sterilized by UV for 1 h and was placed in the center of the inoculated Petri dish. The agar dish was then incubated at 28°C for 72 h. The diameter of the inhibition zone was measured.

Hydrolysis Experiment of EVOH-CBZ

The sample of the film type was prepared using a hot press. The dimension of the sample was 10 × 10 × 0.2 mm. The sample was put in phosphate buffer solution (pH 7.0) maintained at 70°C. The CBZ content was measured by ^{1}H-NMR spectra recorded at 110°C on a Bruker AC-250 FTNMR spectrometer.

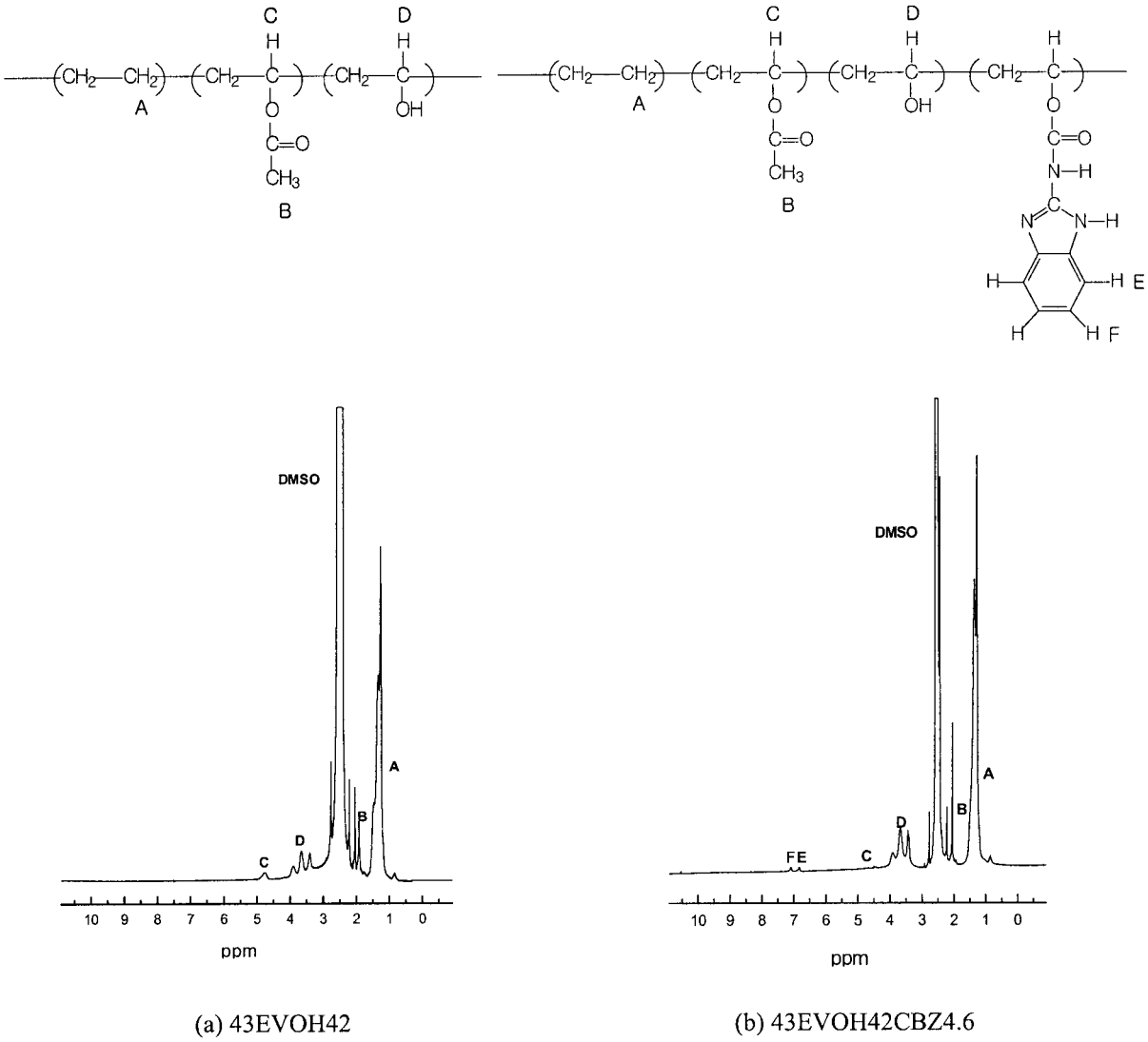


Figure 1 ^{1}H-NMR spectra of (a) 43EVOH42 and (b) 43EVOH42CBZ4.6.

Table I Degree of Hydrolysis of EVOH Series and CBZ Content

	Composition Ratio (mol %)				
Sample Code	Ethylene	Vinyl Acetate	Vinyl Alcohol	Degree of Hydrolysis (%)	CBZ Content (mol %)
43EVAc	57.0	43.0	0.0	0	—
43EVOH28	57.0	15.0	28.0	65	—
43EVOH40	57.0	3.0	40.0	93	—
43EVOH42	57.0	1.0	42.0	98	—
43EVOH28CBZ2.1	—	—	—	—	2.1
43EVOH40CBZ4.3	—	—	—	—	4.3
43EVOH42CBZ4.6	—	—	—	—	4.6
61EVAc	39.0	61.0	0.0	0	—
61EVOH43	39.0	18.0	43.0	70	—
61EVOH51	39.0	10.0	51.0	84	—
61EVOH54	39.0	7.0	54.0	89	—
61EVOH51CBZ3.8	—	—	—	—	3.8
61EVOH54CBZ5.9	—	—	—	—	5.9

Synthesis of Precursor (DGEBA-CBZ)

The precursor was prepared by reacting the epoxy with carbendazim at 160°C for 30 min after perfect mixing at 60°C. The absence of the unreacted carbendazim in the synthesized precursor was confirmed by thin layer chromatography (TLC). The content of CBZ in the DGEBA was determined from ^{1}H-NMR spectra measured at room temperature with a Bruker AC-250 250-MHz NMR spectrometer using $CDCl_3$ as the solvent.

Crosslinking of DGEBA-CBZ

The DGEBA-CBZ (precursor) was dissolved in chloroform and mixed with IPDA. The solution was cast on a glass plate and then cured at 100°C for 3 h.

RESULTS AND DISCUSSION

CBZ supported on EVOH

The EVOH was prepared by alcoholysis of EVAc containing 43 and 61 mol % VAc units in 0.5*M* KOH ethanol solution. The degree of saponification was controlled by means of the alcoholysis reaction time. The composition of the resulting EVOH was determined from ^{1}H-NMR spectra, as shown in Figure 1, by measuring the intensities of the methylene protons appearing in the range of 0.9–1.8 ppm, those of methine protons in the 4.4–4.8 ppm range, and those of methyl protons of the residual acetate groups in the 2.1–2.0 ppm range. The results are summarized in Table I, where *x*EVOH*y* indicates EVOH with *y* mol % VAc units was synthesized from EVAc containing *x* mol % VAc units.

CBZ was bound to EVOH through the transesterification reaction. The CBZ content in the EVOH-CBZ complex was determined by ^{1}H-NMR in Figure 1. Peaks at 6.8 and 7.2 ppm correspond to the protons in the benzimidazole ring. The peak at 10.5 ppm is ascribed to —NH in the carbamate and to that in the benzimidazole ring. The absence of unreacted cabendazim in the EVOH-CBZ was confirmed by the fact that the peak at 3.7 ppm corresponding to methyl protons of carbendazim disappeared in Figure 1.

Thermal properties measured by DSC are summarized in Table II. Melting behavior was not

Table II Thermal Properties of EVOH Measured by DSC

Sample Code	T_m	T_c	T_g
43EVAc	—	—	−22.6
43EVOH28	131.2	115.7	33.9
43EVOH40	136.6	116.5	39.3
43EVOH42	136.8	120.1	42.7
43EVOH28CBZ2.1	128.5	113.5	38.9
43EVOH40CBZ4.3	106.3	80.3	44.3
43EVOH42CBZ4.6	104.1	80.8	50.8
61EVAc	—	—	1.1
61EVOH43	160.9	142.6	52.1
61EVOH51	162.7	143.4	54.9
61EVOH54	164.9	146.1	55.9
61EVOH51CBZ3.8	135.5	111.5	61.1
61EVOH54CBZ5.9	130.6	104.4	63.6

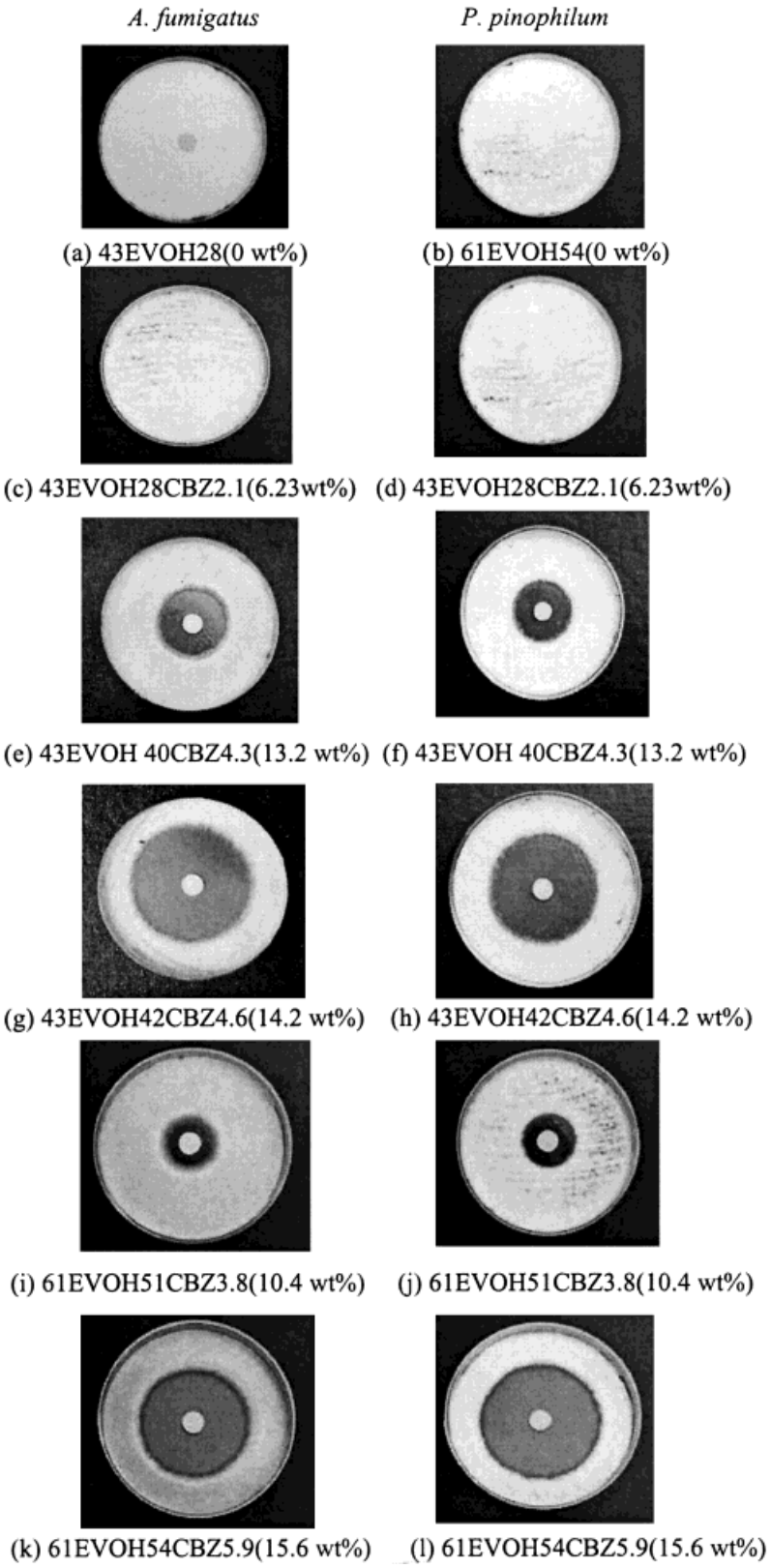


Figure 2 Photographs of the halo zone test. The numbers in the parentheses are the CBZ concentration.

seen in EVAc. The glass-transition temperature (T_g) of the EVAc increased from −22.6 to 1.1°C as the VAc content increased from 43 to 61 mol %. When EVAc was saponified, the resulting EVOH became semicrystalline to show melting behavior. The melting (T_m) and crystallization (T_c) temperatures both rose as the degree of saponification was raised. The T_c measured during cooling at 5°C/min from the melt state increased with the vinyl alcohol content, indicating that more highly saponified EVOH crystallized more easily.

It is interesting to note that EVOH from EVAc containing 61 mol % VAc units showed higher T_m, T_c, and T_g at a comparable level of vinyl alcohol content than EVOH originated from EVAc comprising 43 mol % VAc units. Admitting that VAc units are randomly distributed in EVAc, the above results imply that the saponification was not carried out homogeneously. That is to say, the vinyl alcohol units were unevenly distributed in the EVOH so that crystallization took place preferentially where vinyl alcohol units were locally concentrated.[22–24] The T_m, which was not very dependent on the degree of saponification of EVOH when made from the identical EVAc, supported the above hypothesis. The VAc units in 43EVAc were more sparsely distributed than those in 61EVAc. Hence, the vinyl alcohol units in 43EVOH were more randomly distributed than those in 61EVOH to show a lower melting temperature.

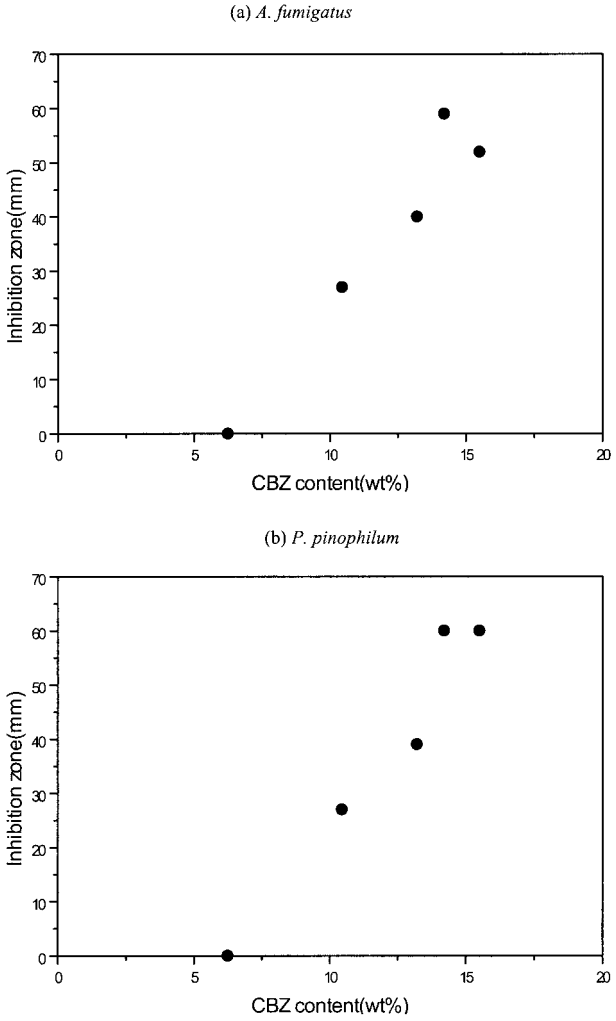


Figure 3 A plot of the inhibition zone as a function of the CBZ content.

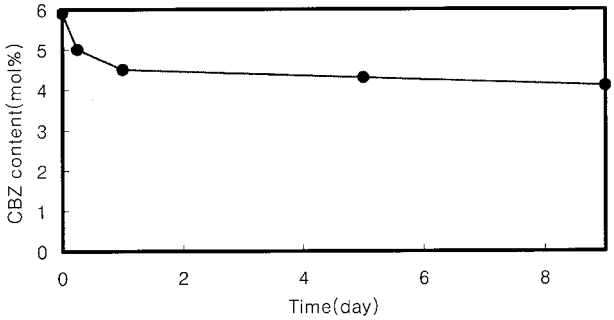


Figure 4 The content of CBZ (mol %) in 61EVOH54CBZ5.9 after hydrolysis at 70°C as measured by ^{1}H-NMR.

As CBZ units replaced the hydroxyl groups in EVOH-CBZ, the T_m and T_c decreased because of the crystallization-repressing action of the bulky CBZ units. The T_g increased as a result of the incorporation of CBZ units because of the bulkiness of the CBZ units and the possible interaction among —NH, —OH, and carbonyl groups that restrained the segmental motion of the backbone chain.

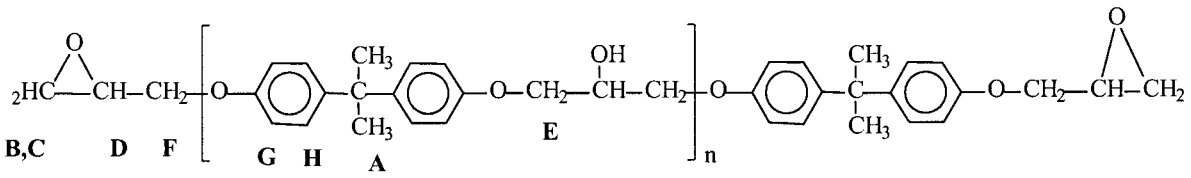
Carbendazim preserves its antifungal activity when the methyl ester group is replaced by other substituents.[25] Figure 2 presents the halo zone test results. It can be seen that the inhibition zone around the circular EVOH-CBZ specimens was enlarged because of the increasing content of the anchored CBZ.

Figure 3 plots the inhibition zone diameter as a function of the CBZ concentration. Inhibition zone diameter is known to increase in proportion to the logarithmic concentration of biocides.[16] Hence, the inhibition zone diameter is expected to increase and level off as the concentration of the biocides increases.

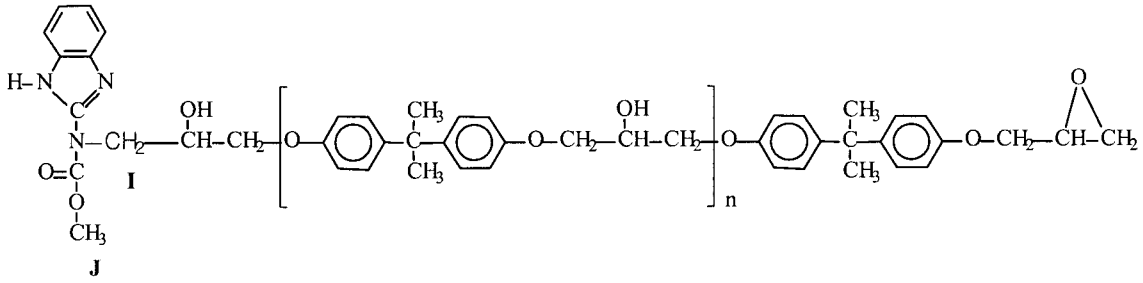
The results in Figure 3 show that the inhibition zone diameter seems to level off above a CBZ content of 14 wt %.

Figure 4 shows the CBZ content in 61EVOH54CBZ5.9 film (10 × 10 × 0.2 mm) after

(a) DGEBA



(b) DGEBA-CBZ



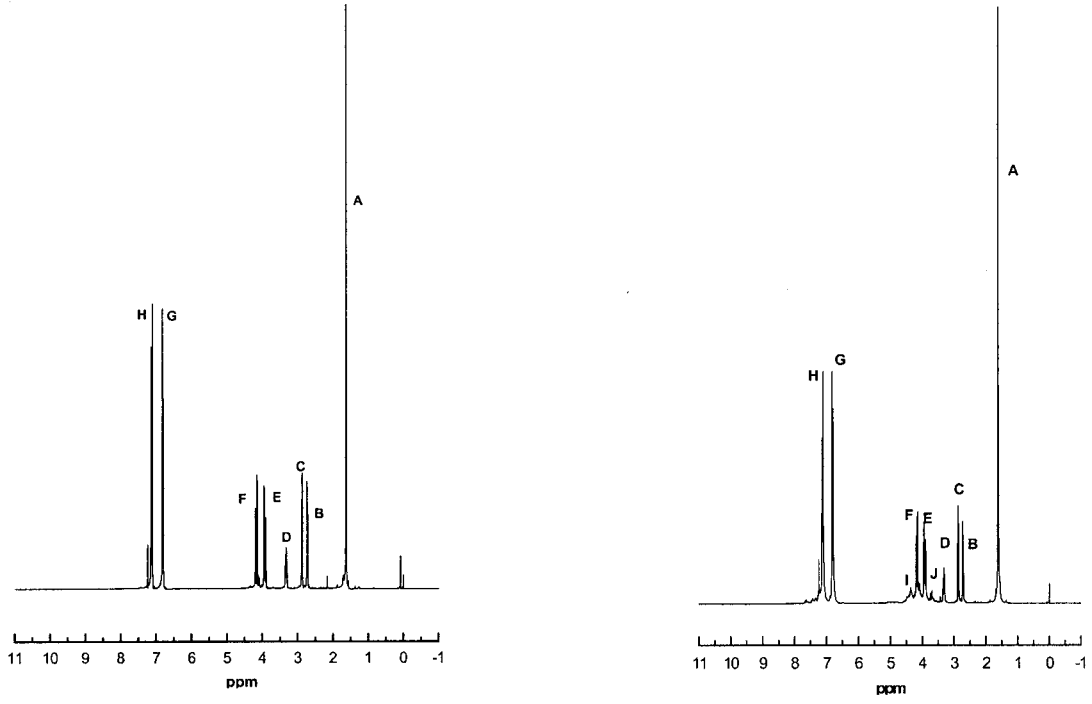


Figure 5 ^{1}H-NMR spectra of (a) DGEBA and (b) DGEBA-CBZ.

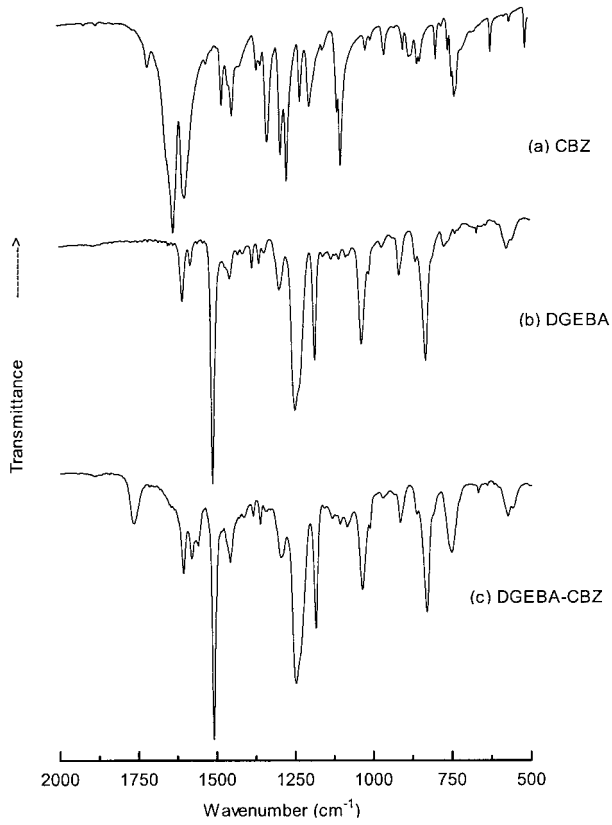


Figure 6 FTIR Spectra of (a) carbendazim, (b) DGEBA, and (c) DGEBA-CBZ.

hydrolysis at 70°C, indicating that CBZ units were released from the film due to the hydrolysis.

The CBZ content in the film (measured by ^{1}H-NMR) decreased sharply in the initial stage of the hydrolysis.

Some fungal mycelia were observed to grow on the film surface when the film, which was subjected to hydrolysis for 7 h, was placed into and incubated amid PDA medium inoculated with the fungal spore suspension. This was because the actual content of CBZ on the surface of the film must be lower than that depicted in Figure 4, which was the average content of CBZ in the whole film.

Most of the polymeric biocides studied so far have acryloyl or other structures from which biocidal groups could be split off, and the service life of the polymeric biocides depends on the release rate of the biocidal functional groups.

CBZ Supported on Epoxy Resins

A mixture of DGEBA and carbendazim (4:1 w/w) was heated at 160°C for 30 min. ^{1}H-NMR spectra of the resulting DGEBA-CBZ are shown in Figure 5. In Figure 5(b) new peaks appear at 3.7–3.8 and 4.3–4.4 ppm that are due to the incorporation of CBZ units when compared with Figure 5(a), which corresponds to DGEBA. The peaks at 3.7–3.8 ppm are assigned to methyl protons of the CBZ units and the peaks at 4.3–4.4 peaks are methylene protons formed by ring opening of the oxirane groups. Protons in the oxirane ring appearing at 2.7, 2.9, and 3.35 ppm evidence that some oxirane groups survived the reaction between DGEBA and carbendazim.

FTIR spectra of carbendazim, DGEBA, and DGEBA-CBZ are shown in Figure 6. Carbonyl groups of carbamate in CBZ at 1630 cm^{-1} disappeared and instead a new peak at 1760 cm^{-1} appeared in the spectra of DGEBA-CBZ.

The IR peak shifts were thought to be due to the change in the chemical structure around the carbonyl linkage of carbamate during the oxirane ring opening reaction, indicating that not only the secondary amine groups in the benzimidazole ring but also the amide groups of carbamate, which was supposed to be less reactive than the former, participated in the reaction between DGEBA and carbendazim. Zhong and Guo[15] also observed that peaks for amide carbonyl groups shifted from 1630 to 1726 cm^{-1} as nylon 6 reacted with DGEBA.

The IR peak at 915 cm^{-1} originating from oxirane groups confirms that some of the oxirane groups in DGEBA remained after the reaction of DGEBA with carbendazim.

Figure 7 exhibits the halo zone test results for DGEBA-CBZ against *P. pinophilum*. As expected, the inhibition zone diameter increased with increasing CBZ concentration. However, when compared with the results shown in Figure 3, the antifungal activity of DGEBA-CBZ was less pronounced than that of EVOH-CBZ.

The absence of unreacted CBZ was confirmed by developing DGEBA-CBZ on TLC as shown in

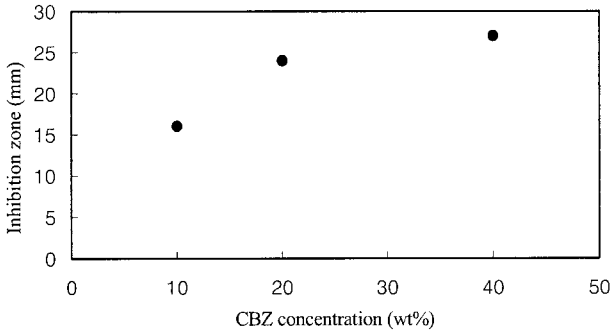


Figure 7 A plot of the inhibition zone for *Penicillium pinophilum* as a function of the CBZ concentration in DGEBA-CBZ.

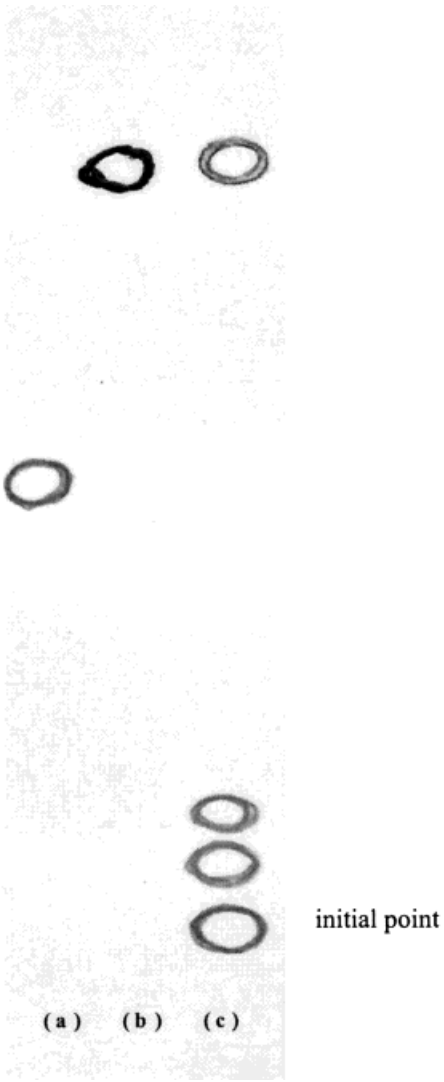


Figure 8 Thin layer chromatography of (a) cabendazim, (b) DGEBA, and (c) DGEBA-CBZ.

Figure 8, which also demonstrates that unreacted DGEBA and differently structured DGEBA-CBZ complexes exist.

The DGEBA-CBZ complex was cured with IPDA and then the residual precursor and IPDA were removed by overnight Soxhlet extraction with ethanol.

The epoxy resin thus produced was insoluble and cleavage of CBZ units from the epoxy matrix should be much slower than that from EVOH-CBZ. Therefore, the antifungal activity could not be tested by the halo zone test for which diffusion of molecules having biocidal effects is absolutely needed. Hence, the antifungal activity of the epoxy resin was tested by observing whether fungal mycellia infect and bury the surface of the epoxy resin sheet placed in a nutrient rich media inoculated with fungal spores. Unfortunately the cured DGEBA-CBZ complex sheet did not show any appreciable antifungal activity, indicating that release of CBZ units is needed to exhibit antifungal activity. Therefore, it cannot be said that CBZ supported on polymers will be of much use, because large weight percentages are required for activity and because they could not be used for long-term applications where water exposure would occur.

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