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UTILIZATION OF CHITIN TO CONTROL PESTICIDE MOBILITY

Charles L. McCormick, Ph.D.
Principal Investigator

and

Kenneth W. Anderson
Graduate Assistant

Department of Polymer Science
University of Southern Mississippi
Hattiesburg, Mississippi 39406-0076

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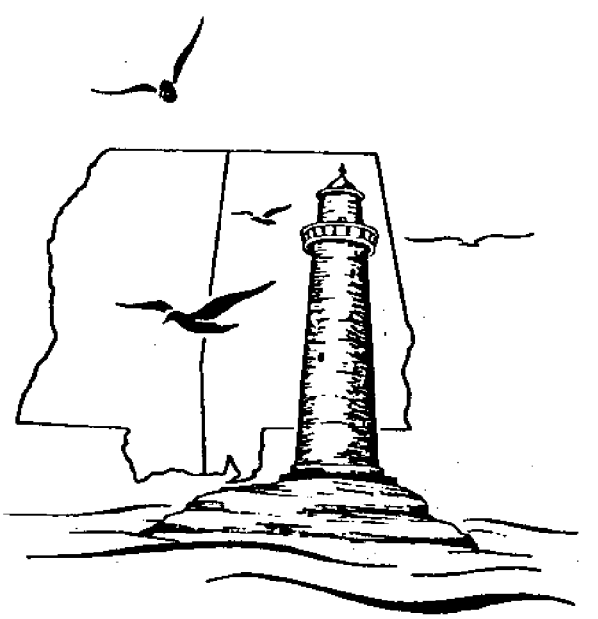

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by

Charles L. McCormick, Ph.D.
Principal Investigator
Professor of Polymer Science
Department of Polymer Science
University of Southern Mississippi
Hattiesburg, Mississippi 39406-0076

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ABSTRACT

Chitin and similar polysaccharides having herbicides chemically attached to the polymer backbone have been prepared and evaluated as potential controlled-release herbicides. Such materials offer promise for increasing pesticide efficiency and reducing mobility of the active agent in the environment. The organic acid herbicides 2,4-dichlorophenoxyacetic acid and 2,2-dichloropropionic acid and the amine herbicide metribuzin have been bound to these polymers through ester and carbamate linkages, respectively, at levels ranging from six to 72 weight percentage herbicide. The esters will release free herbicide at initial rates ranging from 3.5 to 39% in the first two days. The carbamates release metribuzin much more slowly with the highest rate being 4.7% in 40 days. The carbamate is significantly more stable to hydrolysis than the ester. Also, it was generally found that materials containing higher amounts of attached herbicide release more slowly, apparently due to lower hydrophilicity. Systems based on chitin and cellulose showed slower or incomplete release as compared to systems based on water-soluble or water-swellable polymers such as starch, amylose, amylopectin, dextran, and poly(vinyl alcohol). This is apparently due to formation of strong inter-molecular hydrogen bonds at the surface of the polymer particle after removal of surface pendent groups; this process hinders further prenetration and hydrolysis to release the herbicide. New methods for the dissolution and derivatization of chitin and the other polysaccharides have been developed based on a new solvent for these polymers, N,N-dimethylacetamide containing dissolved lithium chloride. New techniques for the characterization and evaluation of polymeric bioactive agents using gel permeation and liquid chromatography have also been demonstrated.

INTRODUCTION

Concern over the potential adverse effects of pesticides on the marine environment has led to efforts to reduce their imput on non-target areas. One factor contributing to this problem is the mobility of the pesticide molecules leading to transport via run-off water. Incorporation of agricultural chemicals into polymeric controlled release (CR) formulations has been recognized as a method to reduce mobility and increase pesticide efficiency [1,2]. One approach involves chemical attachment of the active agent to a macromolecule through a labile linkage; release

is dependent on the breakdown of this bond at the desired rate [3]. The method has been demonstrated with a variety of synthetic polymer systems [3]. Chitin and other marine-derived polysaccharides offer characteristics such as appropriate functionality, biodegradability, and availability which make them excellent candidates for this application. Chitin is a by-product of the shellfish industry and its use in CR formulations would offer a dual benefit to this industry; namely, a high value-added market for this material and protection of the marine environment. A number of potential applications for chitin have been identified [4,5,6] but only a few offer the large volume potential of agrichemical use. Our research has been directed toward the development of methods for the utilization of chitin and similar polymers in chemical combinations with herbicides to produce biodegradable controlled-release systems.

OBJECTIVES

The major objectives of this work were the following:

- preparation of polymeric herbicides by the attachment of amine- and carboxylic acid-functional herbicides to chitin and similar polymers through carbamate and ester linkages;
- characterization of the resulting polymers to determine degree of attachment, purity, and other physical properties; and
- measurement of herbicide release rates under laboratory and simulated field conditions to determine the effects of polymer, herbicide, bond type, degree of attachment, and pH on release.

EXPERIMENTAL

Polymer Preparation

Practical grade chitin was purified by the Rosemann method [7] to insure that it was free of calcium carbonate and proteins. Recovery was 74 to 75%.

Convection oven drying was done at 110°C. Vacuum oven drying was conducted at 45 or 56°C in the presence of P_2O_5. Cellulose and starch were also dried by a solvent-exchange procedure which involved first swelling in water, then repeated washings with fresh absolute methanol followed by the same with DMAC, all in a dry atmosphere. The polymers, still swollen with DMAC, were stored in dessicators. Chitin was dried similarly during the purification steps, but the final solvent, ethyl-ether, was removed under vacuum. This chitin was further dried by

stirring 10 g in 100 ml of DMAC at 80°C for 16 hours, then adding 40 ml of xylene and refluxing two hours into a Dean-Stark trap. The chitin was collected from the slurry and stored wet with solvent in a dessicator over P_2O_5. For the herbicide substitution reactions, poly(vinyl alcohol) (PVA), dextran, and hydroxyethyl cellulose (HEC) were vacuum dried at 40°C; cellulose and starch were dried by the solvent exchange procedure. Different samples of chitin were dried by each technique for use in reactions.

Preparation of 2,4-Dichlorophenoxyacetyl Chloride and 2,2-Dichloropropionyl Chloride

Acid chloride derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,2-dichloropropionic acid (dalapon) were prepared by dropwise addition of 0.80 moles of thionyl chloride in 50 ml of chloroform to 0.50 moles of the organic acid in 150 ml of chloroform at 60°C under nitrogen. The reaction was allowed to continue for twenty-four hours. The resulting crude products were purified by distillation. The 2,4-dichlorophenoxyacetyl chloride was distilled at 147-150°C (130 mm Hg), while 2,2-dichloropropionyl chloride distilled at 155-118°C (760 mm Hg).

Preparation of Metribuzin Chloroformamide

A flask was charged with 400 ml of reagent grade THF and cooled to 0°C. Phosgene was condensed in a jacketed addition funnel at -70°C until 160 ml (224 g, 2.26 mole) was collected; the phosgene was then added to the cold THF with stirring. A solution of 50 g (0.234 mole) of metribuzin in 200 ml THF was added with stirring over a period of 30-45 minutes. The temperature was maintained at 0-3°C throughout the addition, and a fine, white suspension was formed. The suspension was stirred an additional 30 minutes at 0°C after completion of the metribuzin addition. A heating mantle was placed on the flask and the temperature was raised to 42-45°C over one hour and maintained at this point using a relay controller on a thermometer. The solution became clear after 2-3 hours at this temperature; during this time, slight reflux and moderate off-gas evolution were noted. Sufficient vacuum was then applied through the scrubbing system to allow distillation of the excess phosgene/THF/HCL mixture. Distillate temperature slowly climbed from 25 to 40°C as distillation progressed at pot temperatures never exceeding 45°C. The reaction mixture was thus concentrated to about 175 ml. Removal of excess phosgene was judged to be complete when no discernible peak was present at 840 cm^{-1} in the IR. Fresh THF was added and distillation continued if phosgene removal was incomplete. This solution of the chloroformamide was used directly in subsequent reactions.

Reaction of the Acid Chloride Derivatives with Polysaccharides

The chosen polymer was added to a solution of 5 wt% LiCl in N,N-dimethylacetamide to form a 1 wt% polymer solution. This solution was heated to 110°C under nitrogen, and one or two moles/mole repeating unit of the herbicidal acid chloride was added. The reaction was stirred at 110°C for 2.5 hours. The product polymer was isolated by precipitation in ethanol and purified by washing. These polymers were dried and stored over $CaSO_4$.

Reaction of Metribuzin Chloroformamide with the Polymers

Reaction conditions for the attachment of metribuzin to the polymers are summarized in Table I. The appropriate amount of LiCl was dissolved in DMAC (150 or 250 ml) under N_2 in a three-neck flask equipped with a mechanical stirrer, an addition funnel, and a Friedrich condenser. The corresponding amount of dried polymer was added and dissolved with stirring. Dextran, starch, HEC, and PVA required heating to 150°C for ten minutes to dissolve. These solutions were cooled slowly to ambient temperature. The necessary amount of metribuzin chloroformamide was added as the concentrated solution in THF was obtained from the phosgenation. Amounts were calculated based on a measured yield of 91%. The solution was added dropwise at room temperature with vigorous stirring to prevent high local concentrations of THF which would precipitate the polymer. All reactions remained homogeneous except HEC, which became turbid several hours after the addition. Gas evolution was observed in some cases, particularly for chitin. The mixtures were allowed to stir at ambient temperature for the specified period, then the polymers were isolated by precipitation. The solutions were added dropwise with stirring to a ten-fold volume of the indicated non-solvent. All polymers were fibrous and flocculated in the non-solvents except the PVA samples, which were tacky, and the chitins, which precipitated as beads. The polymers were twice purified by redissolution in the indicated solvent and reprecipitation. The polymers were dried at 40°C in a vacuum oven to constant weight and stored in the dark over $CaSO_4$ at room temperature.

Physical Mixtures of Metribuzin with Chitin and Cellulose

One gram of cellulose was dissolved in 100 ml of DMAC containing 6 wt% of LiCl. One gram of metribuzin was added and dissolved with shaking. Half the solution was precipitated in 500 ml of water in a Waring blender. The fine particles were collected quickly and dried in a vacuum at 45°C. The other half was added dropwise to 500 ml of methanol. The resulting swollen beads were washed twice for three hours with 300 ml of fresh methanol. The beads were collected and dried in vacuum at 45°C. The procedure was repeated using chitin.

Table I

Conditions for Metribuzin Chloroformamide
Reaction with Polymers in DMAC/LiCl

Rxn No.	Polymer	Polymer Conc. (wt%)	LiCl Conc. (wt%)	Mole Ratio (MCF/OH)	Rxn Time (hrs.)	Non-Solvent[a]	Solvent[a]
170-D15	Cellulose	1.0	5.0	2.9	48	1	2
170-25	"	1.5	9.0	2.8	39	1	2
170-D24	Chitin	0.9	4.0	2.8	39	1	3
170-D26	"	1.0	7.0	2.9	24	1	3
170-D28	"	1.0	7.0	4.0	24	1	3
250-3	"	1.0	9.0	2.7	48	1	3
170-49	Dextran	3.3	9.0	0.91	12	1	4
250-4	"	3.3	9.0	1.2	40	5	2
170-27	Starch	1.5	7.0	2.8	24	1	2
250-D4	HEC	2.0	9.0	1.7	42	1	2
170-28	PVA	1.3	-0-	2.3	24	1	2
170-47	"	1.0	-0-	0.92	48	5	4
170-D48	"	3.1	-0-	0.68	17	6	2
250-2	"	2.5	-0-	0.46	24	5	4
250-D2	"	2.5	-0-	0.23	24	1	4

[a]1-MeOH, 2=THF, 3=DMAC/9%LiCl, 4=DMAC, 5-EtOAc, 6=toluene

Characterization

The degree of substitution was determined by elemental analysis of chlorine, nitrogen, and/or sulfur content and, in some cases, by quantitative infrared and gel permeation chromatographic (GPC) methods reported elsewhere [8]. The GPC method involved integration of the ultraviolet detector trace for the metribuzin chromophore in the polymer peak. The amount of unattached herbicide was determined similarly from analysis of the GPC chromatogram. Unattached metribuzin was also determined by dissolving the polymer in an organic solvent, dialyzing the polymer solution against the same solvent, and analyzing the dialysate for metribuzin by liquid chromatography[8].

Release Studies

A measured quantity (25-50 mg) of sample having known particle size range (100-200 or 20-100 mesh) was placed in a 15 cm length of dialysis tubing (Spectrapor #2, 10 mm flat width). The tube was filled with one to two ml of filtered aqueous buffer and sealed. This tube was rinsed with distilled water and placed in a glass vial (2.5 cm dia. x 10 cm) filled with the same buffer. The total amount of buffer used was determined by weighing. The vial was sealed with a Teflon-backed silicone rubber septum, wrapped with black vinyl tape, and agitated by end-over-end rotation at room temperature. This rotation, combined with the fact that the tubing was distended with solution and was long enough to prevent inversion in the vial, served to agitate the particles very efficiently. Twenty-five microliter aliquots were removed periodically from the buffer outside the tubing and analyzed using reverse-phase liquid chromatography (RPLC) to determine the amount of herbicide released from the particles. For samples in pH 10 buffer, one ml aliquots were withdrawn and diluted with 1.0 ml of the pH 6 buffer to prevent damage to the column. Subsequent determinations were compensated accordingly. The compositions of the buffer solutions are given in Table II. Sodium azide was used to prevent the growth of microorganisms.

Table II

Compositions of Buffers Used in Release Studies

PH	Buffer Salt	NaOH	NaN_3
4	0.05 M KHP[a]	-0-	0.0015M
6	0.05 M KH_2PO_4	0.006M	"
7	"	0.029M	"
8	"	0.047M	"
10	0.025 M $NaHCO_3$	0.011M	"

[a]Potassium hydrogen phthalate

Chromatography studies were conducted with a Waters Model 2000A solvent delivery system and a Waters Model U6K injector. A Waters Model 440 fixed-wavelength detector at 254 nm and a Perkin-Elmer LC75 variable wavelength detector operating at 294 nm were used for detection. Both detectors used air as reference with offset background cancelling.

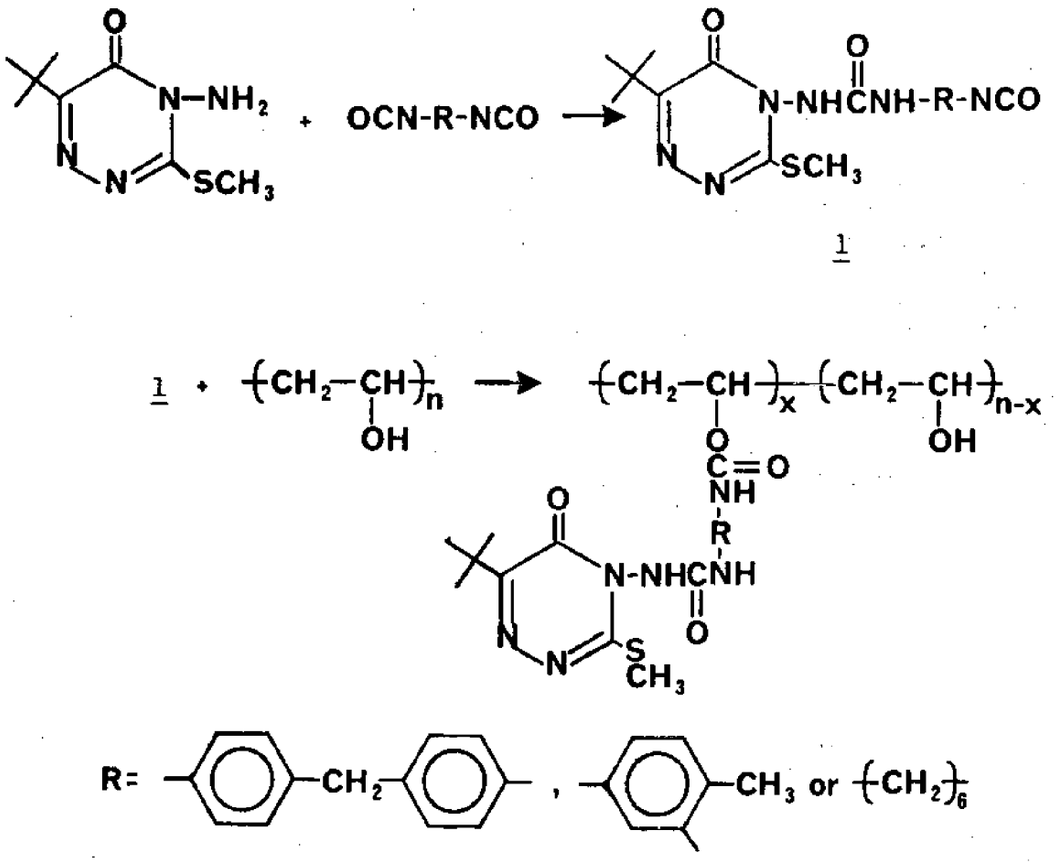
The mobile phase was 44 wt% acetonitrile and 56 wt% of an aqueous pH 7 buffer solution comprised of 0.05 M KH_2PO_4 and 0.0291 M NaOH. The column was a Waters μ-Bondapak C-18 (3.9 mm i.d. x 30 cm). A precolumn filter (5 micron) and a guard column packed with 37-50 micron C-18 Corasil (Waters) were used to protect the analytical column. The flow rate was 1.5 ml/min. Recorder chart speed was 1.0 in/min. Injection volume was no larger than 25 microliters. The concentration of the desired component was determined by comparison of its peak height to a linear calibration generated by two to four equal-volume injections of each of four concentrations of the component in the same solvent media. The mobile phase could be regenerated by distillation of the acetonitrile/water azeotrope, followed by addition of water and buffer salts according to the following ratios: 400 ml azeotrope/101 ml 0.1 M NaOH/193 ml 0.18 M KH_2PO_4.

RESULTS AND DISCUSSION

Initial PVA Model Systems

Since no methods existed for the preparation and characterization of the desired polymeric herbicides, early efforts utilized poly(vinyl alcohol) (PVA) as a model for the polysaccharides. Use of PVA allowed derivatization reactions to be conducted in homogeneous solutions and simplified characterization. The chemistry of the first systems prepared is shown in Scheme 1. The herbicide metribuzin was coupled to PVA using various diisocyanate bridging compounds in a method which has been previously reported [9]. Mobility of the polymeric herbicide was greatly reduced, relative to the free herbicide, in soil thin layer chromatographic studies [9,10]. Initial rates of herbicide release into water ranged from 42%/day to 1%/day. The initial rates increased with increasing length of the bridging group and decreasing degree of substitution. The data were very similar in soil release studies. A bioassay technique demonstrated that the materials were effective for weed control up to 134 days and showed selectivity between the weed species and soybeans [10]. These results indicated the concept was promising but the activity could have been due in part to unattached perticide. The polymers were crosslinked and therefore difficult to purify and characterize.

Scheme 1. Reaction of an Isocyanate Derivative of Metribuzin with Poly(Vinyl Alcohol)

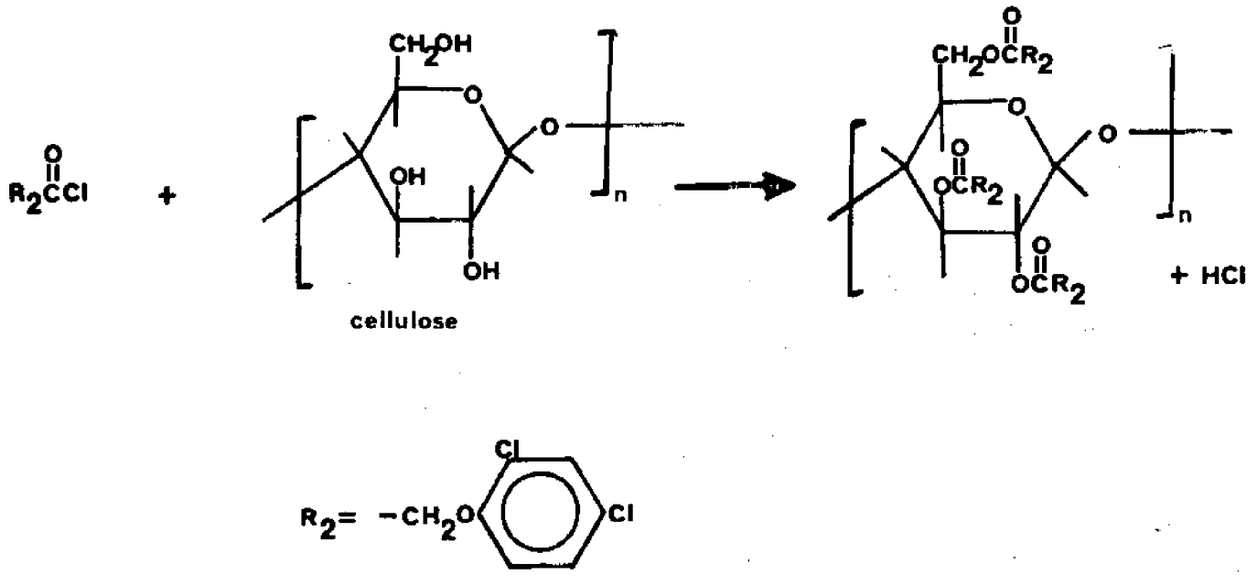


Acid-Functional Herbicide Systems

Heterogeneous Reactions with 2,4-Dichlorophenoxyacetyl Chloride

A subsequent study involved attachment of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) to various polysaccharides through a direct ester linkage. The acid chloride of 2,4-D was reacted with the hydroxyl group of the polysaccharide as shown in Scheme 2. However, no aprotic solvents were available for chitin and cellulose which would allow this reaction. Therefore, these reactions were conducted heterogeneously in mixtures of pyridine and DMF. It was hoped that as substitution proceeded, the polymers would dissolve and produce soluble products. Solubilization, however, did not occur and only low degrees of substitution were obtained. Homogeneous reactions would be required to obtain the soluble, uniformly substituted polymeric herbicides needed for a well-characterized study.

Scheme 2. Reaction of the Acid Chloride of 2,4-D with Cellulose



Development of Homogeneous Reaction Conditions

F. A. Rutherford [11] and P. R. Austin [12] reported the use of solutions of LiCl in N,N-dimethylacetamide (DMAC) to dissolve chitin for preparation of films and fibers of regenerated chitin. We then utilized this solvent for acid chloride and isocyanate reactions on chitin. Later DMAC/LiCl was found to dissolve cellulose, amylose, amylopectin, and dextran. We demonstrated the utility of this solvent for homogeneous derivatization of these polymers with the acid chlorides of 2,4-D and dalapon [13,14,15] and with model isocyanates [16,17]. This discovery of homogeneous reaction conditions was the key breakthrough for preparation of the desired polymeric herbicides. This solvent is also potentially applicable to the preparation of a number of other derivatives of chitin including ethers [18].

The reaction of chitin with the acid chloride derivatives of 2,4-dichlorophenoxyacetic acid and 2,2-dichloropropionic acid is illustrated in Scheme 3. The product polymeric herbicide contains the active agent bound through the ester linkage. These materials were prepared as described in the Experimental Section. The product structure is idealized since every hydroxyl group on every repeating unit is not necessarily

substituted with an ester group. The average number of herbicide molecules bound to the polymer per repeating unit is referred to as the degree of substitution (DS). For chitin, the maximum DS is two, due to the acetylamino group. For all the other polysaccharides used in this study, the maximum possible DS is three. The DS may be varied by changing the reaction conditions or the amount of acid chloride used. Attachment of the acids to the polymers was confirmed by the appearance of the ester carbonyl absorption at 1725 and 1740 cm^{-1} in the infrared spectra, respectively, for 2,4-D and dalapon derivatives. The degree of substitution of herbicide onto the polysaccharides was determined from elemental analysis for chlorine. The DS values and the weight percent of available herbicide in the polymeric products are shown in Table III for the various polymer/herbicide combinations. Degrees of substitution were intentionally kept below 1.5 to retain some hydrophilicity from unsubstituted hydroxyl groups. Earlier work had indicated that PVA fully substituted with 2,4-D was hydrophobic enough to completely preclude hydrolysis [19], while partially substituted samples were hydrolyzable [20]. Additionally, the weight percentage of available herbicide increases only slowly with increasing substitution above DS values of 1.5.

Scheme 3. Reactions of Acid Chloride Derivatives of 2,4-D and Dalapon with Chitin

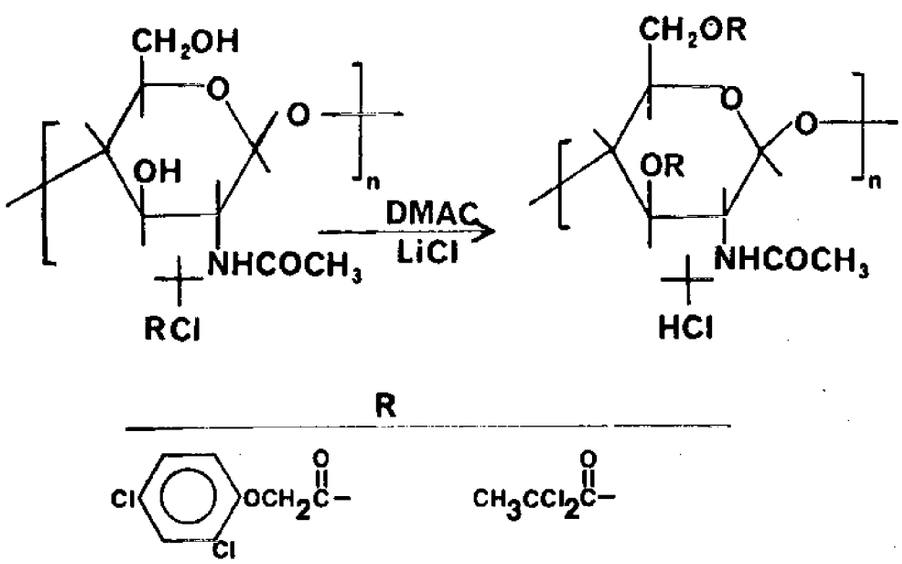


Table III

Polymeric Herbicides Containing Attached 2,4-D and Dalapon

Polymer	Herbicide	DS	wt% Herbicide
Chitin	2,4-D	0.73	46
Cellulose	"	0.65	49
Amylose	"	0.65	49
Amylopectin	"	1.0	61
Dextran	"	0.98	60
Starch	"	1.1	62
Chitin	Dalapon	0.75	36
Cellulose	"	0.41	27
Amylose	"	1.3	57
Amylopectin	"	0.97	49
Dextran	"	0.97	49
Starch	"	1.2	54

Release of 2,4-D from the Polymeric Ester Derivatives

Hydrolytic release of the herbicide from these systems was measured by placing the polymer particles in water at room temperature and periodically determining the concentration of free 2,4-D in the aqueous phase by liquid chromatography. The percentages of available herbicide released at various time intervals are shown in Table IV. Several of the systems show an initial "burst" of released herbicide, which would indicate that they contained residual unattached 2,4-D which had not been removed in purification. The polymers based on the water-insoluble chitin and cellulose showed little release after this initial stage, while the systems based on the water-swellable polymers continued to release. This seems to indicate that after removal of pesticide at or near the particle surface, the underlying groups are not accessible for hydrolysis in the chitin and cellulose systems. This phenomenom may be due to formation of strong intermolecular hydrogen bonds between chains near the surface after removal of the pendent groups. This morphology then restricts or prevents the diffusion of water into the particle or of herbicide from the particle. In the water-swellable systems, pendent groups underlying the particle surface continually become accessible, and the hydrolytic release can proceed. In light of studies which showed that all of the unattached pesticide is not necessarily released immediately, efforts were made to optimize the release rates from these systems.

Table IV

Release of 2,4-D from Polysaccharides Containing the Pendently Bound Herbicide

Polymer	DS	Percentage of Available Herbicide Released			
		0.1 hr	8 hr	1 day	2 days
Chitin	0.39	5.5	5.9	4.9	3.5
Cellulose	1.9	12.7	14.8	16.2	10.1
Starch	1.3	0.7	1.7	3.5	6.4
Amylose	1.2	2.3	3.7	5.7	12.9
Amylopectin	1.2	9.3	10.7	20.0	39.0
Dextran	1.4	4.8	3.4	7.3	4.2

Metribuzin-Polysaccharide Chemical Combinations

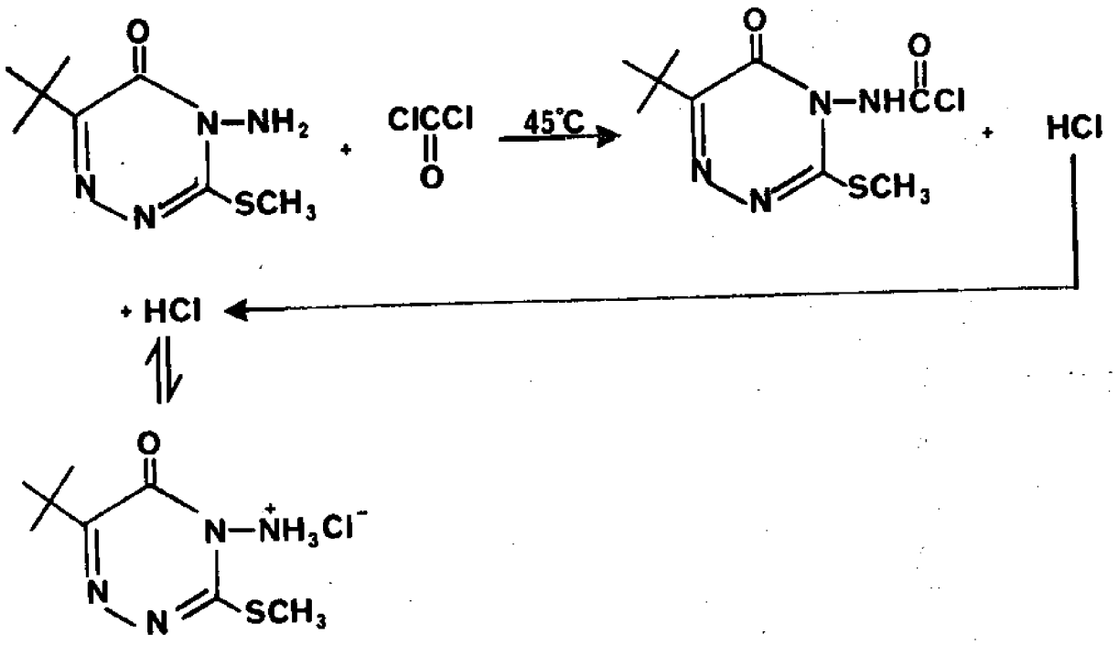
Based on the PVA-metribuzin systems in the original model studies, the direct attachment of metribuzin to chitin and the other polysaccharides was investigated. The carbamate linkage was chosen, since it can be easily formed by the reaction of an isocyanate derivative of an amine with the hydroxyl groups of the polymer. The feasibility of this reaction in the DMAC/LiCl solvent had been previously demonstrated using model isocyanates with polysaccharides [16].

Preparation of Metribuzin Chloroformamide

The isocyanate derivative of metribuzin could not be produced in reasonable yields by the reaction of metribuzin and phosgene [8]. However, this reaction gave yields up to 91% of the chloroformamide derivative, which is the intermediate in conversion of amine to isocyanate. The reaction is illustrated in Scheme 4. A temperature of 45°C and ten moles of phosgene per mole of amine were used. The reaction must have excess phosgene present until all of the metribuzin hydrochloride has been converted back to free amine through equilibrium deprotonation and the amine has been trapped by reaction with phosgene to form the chloroformamide. This product was relatively stable and underwent little dehydrochlorination to form isocyanate at 45°C. No other significant side reactions were detected during synthesis. The IR absorption of the chloroformamide carbonyl group showed no significant change with time at this temperature.

The chloroformamide structure was confirmed by IR, NMR, and mass spectroscopy as well as wet chemical tests. Attempted conversion of the chloroformamide to the isocyanate produced the di-metribuzin urea analog [8]. However, it was recognized that the chloroformamide would react with the polymers to produce the same carbamate product as the isocyanate and this approach was investigated.

Scheme 4. Preparation of the Chloroformamide Derivative of Metribuzin



Polymer Drying and Solubility

The presence of water in chitin and other polymers thwarted initial efforts to attach the herbicide. Metribuzin chloroformamide was consumed by reaction with water before substitution could occur. This process was evidenced by evolution of carbon dioxide, identified by IR spectroscopy. Efficient methods of polymer drying were needed which would still allow the polymers to dissolve in DMAC/LiCl. Table V summarizes results for several drying methods in terms of amounts of water removed and remaining, along with solubility characteristics in DMAC/LiCl before and after drying.

In general, oven drying removed the largest fraction of water. However, the polymers became much more difficult to solvate, probably due to compaction of the morphology and formation of more interchain hydrogen bonds [21]. Solvent exchange methods actually improved solubility character by swelling the polymer particles. For starch, solvent exchange was required before the polymer would dissolve at all. However, this process removed less water.

Table V

Comparison of Drying Methods

Method	Polymer	g H_2O removed[a] / 100g sample	g H_2O remaining[b] / 100g sample	Solubility in DMAC/LiCl: Before drying	Solubility in DMAC/LiCl: After drying
Convection Oven					
100°C	Cellulose	7.3	-	fair	poor
Vacuum Oven					
56°C	Cellulose	9.7	- (2.5)[c]	fair	poor
(P_2O_5)	Chitin	10.9	- (4.2)[c]	good	poor
	Starch	14.4	- (3.4)[c]	insoluble	insoluble
45°C	Dextran	12.0	-	fair	fair
(P_2O_5)	HEC	5.4	-	fair	fair
Solvent Exchange					
(H_2O/	Cellulose	8.5	1.3 (3.7)[d]	fair	excellent
MeOH/	Starch	13.9	3.0 (3.9)[d]	insoluble	fair
DMAC)	Chitin	5.9	1.6 (9.2)[d]	good	excellent
Azeotropic Distillation					
Xylene	Chitin	6.0	- (9.1)[d]	good	fair
DMAC/Xylene	Chitin	7.3	0.4 (7.8)[d]	good	good

[a]determined gravimetrically or by KF titration on solvent, [b]determined by KF titration of solid polymer in MeOH, [c]calculated from elemental analysis, [d]calculated assuming oven method + elemental analysis = total water

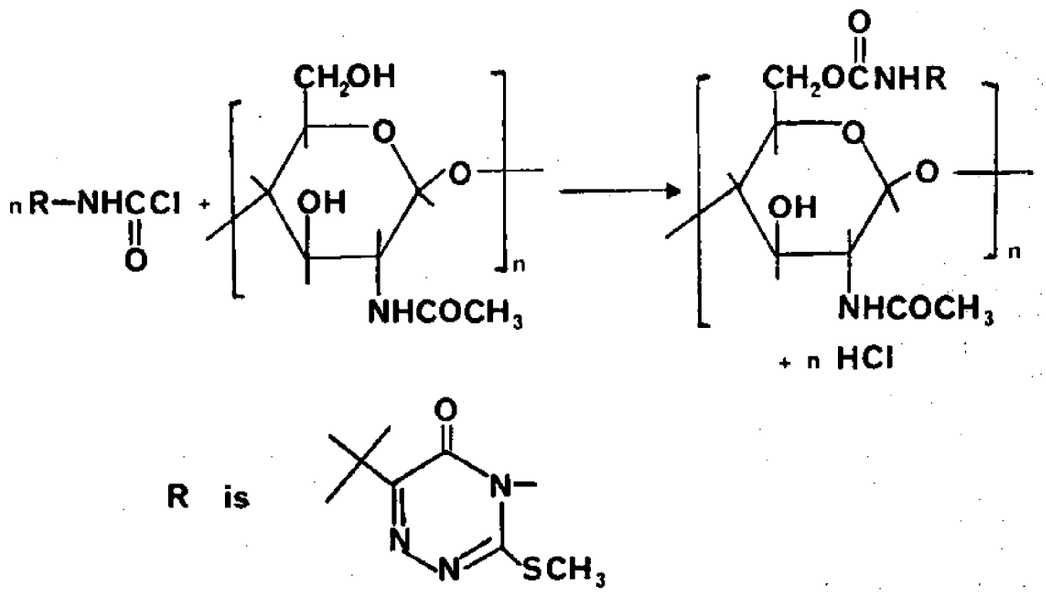
Values of remaining water as determined by Karl Fischer titration were probably low in all cases since the titration was heterogeneous and water may have been trapped within the particles. This disparity is evidenced by comparing the apparent total water contents determined by various methods. For example, solvent exchanged chitin gave a total of 8.6%, which is less than the 10.9% value obtained from oven drying alone. Therefore, titration values are questionable, and remaining water contents can only be estimated until a more reliable method is found to determine water in the polymers after drying. Attempts were made to titrate in homogeneous solutions of DMAC/LiCl, but the Karl Fischer reagent was unreactive in this solvent medium. Conductive iodine remained even in the presence of large excesses of water.

Total water contents for cellulose, starch, and chitin were approximated by the sum of the oven drying value and the value calculated from elemental analysis. These totals were 12.2, 17.8, and 15.1%, respectively. Estimates of remaining water in other samples were then based on the amount of water removed, and these estimates are shown in parentheses following the titration values in Table V. Comparisons show water was more efficiently removed by solvent exchange from cellulose and starch than chitin. Agreement between the estimated value and the titration was better for the former two polymers. These facts imply more swelling and accessibility for cellulose and starch. The distillation methods were slightly better for drying chitin, but most of the additional water removed was accessible after solvent exchange as well. Significant amounts of water still remained in chitin. This water must be in non-accessible regions, such as the crystalline lattice, or tightly bound through a mechanism such as hydrogen bonding.

Reaction of Metribuzin Chloroformamide with the Polymers

The reaction of metribuzin chloroformamide with chitin is illustrated in Scheme 5. As mentioned previously, the same carbamate linkage and product can be produced using the isocyanate derivative of metribuzin. The reactions were complete after 12 hours at most, at room temperature. No chloroformamide was detected by IR after this time even when molar ratios of chloroformamide to hydroxyl greater than one were used. This indicated that hydrolysis or other side reactions were also occurring. Evolution of CO_2 confirmed hydrolysis, and significantly larger amounts of gas were evolved from the reactions with chitin than the other polymers. Recovery of purified polymer from these reactions ranged from 34-95%, and considerable loss or fractionation occurred during reprecipitation of some samples.

Scheme 5. Reaction of Chitin with the Chloroformamide Derivative of Metribuzin



Proof of Attachment

Initial proof of herbicide attachment was provided by IR analysis of the isolated polymers after purification. The following indicative IR absorption frequencies (in cm^{-1}, with assignments) were readily apparent: 3550 (broad O-H), 3250 (secondary N-H), 1770 (carbamate carbonyl), 1710 (metribuzin carbonyl), 1530 (triazine ring), and 1040 (backbone C-O).

Resonances corresponding to backbone and metribuzin carbon atoms were observed in the carbon-13 NMR spectrum of each sample. However, resonances for the backbone carbons were very broad and could not be accurately integrated under any spectral conditions used. Therefore, the degree of substitution could not be determined using this technique. Peak broadening was probably due to long relaxation times and splitting brought about by partial substitution.

Attachment of herbicide to the macromolecule was also confirmed by strong UV absorption at 294 nm due to the polymer during gel permeation chromatography studies which denoted presence of the metribuzin chromophore. The area of the polymer peak was used to determine degree of substitution.

Effect of Reaction Conditions on Degree of Substitution

The degrees of substitution (DS) calculated from elemental analysis are shown in Table VI along with pertinent reaction conditions. Since all reactions were run at least 12 hours and were complete after that time, reaction time had no effect on DS. The LiCl concentration also had no significant influence on DS.

Table VI

Effect of Reaction Conditions on Degree of Substitution

Rxn No.	Polymer	Polymer Conc. (wt%)	Drying Method[a]	Mole Ratio (MCF/OH)	DS
170-D15	Cellulose	1.0	1	2.9	1.6
170-25	"	1.5	1	2.8	1.9
170-D24	Chitin	0.9	1	2.8	0.01
170-D26	"	1.0	1	2.9	0.06
170-D28	"	1.0	1	4.0	0.23
250-3	"	1.0	2	2.7	0.39
170-49	Dextran	3.3	3	0.91	0.05
250-4	"	3.3	4	1.2	1.4
170-27	Starch	1.5	1	2.8	1.3
250-D4	HEC	2.0	4	1.7	2.0
170-28	PVA	1.3	4	2.3	0.75
170-47	"	1.0	4	0.92	0.24
170-D48	"	3.1	4	0.68	0.60
250-2	"	2.5	4	0.46	0.25
250-D2	"	2.5	4	0.23	0.06

[a] 1 = solvent exchange, 2 = azeotropic distillation (DMAC/Xylene), 3 = no drying, 4 = vacuum/45°C

As expected, the degree of substitution increased using higher molar ratios of chloroformamide to hydroxyl functionality. In most cases, excess chloroformamide was used to obtain the highest possible DS. Values of DS approached but never exceeded 2.0 for the tri-functional polysaccharides. For cellulose, a limiting value of 2.0 may indicate that after substitution at the more reactive hydroxyls on C-2 and C-6, reaction at the C-3 hydroxyl may be hindered. Lower molar ratios were used with PVA to prepare samples having lower DS and, consequently, higher hydrophilicity. Substitution could be controlled by adjusting the molar ratios.

Residual water had a significant effect on the attainable DS. Much of the chloroformamide was consumed by water, as shown by comparison of DS to the molar ratio combined with the fact that no excess chloroformamide was left. Efficiency was particularly low for chitin, which is in agreement with the higher values of residual water in chitin calculated in Table V. Drying chitin by azeotropic distillation allowed a higher DS at a lower molar ratio.

Higher polymer concentrations increased both the attainable DS at a given mole ratio and the efficiency of chloroformamide utilization. With PVA, for example, an equivalent DS was obtained using only one-half the molar ratio when the polymer concentration was increased from 1.0 to 2.5 wt%. The highest efficiency (88%) was obtained at the highest polymer concentration (3.1 wt%).

Physical Mixtures of Chitin and Cellulose with Metribuzin

Chitin and cellulose containing physically entrapped metribuzin were prepared using a precipitation technique described in the Experimental Section. These materials were prepared for comparison with samples having pendently attached herbicide in order to determine the effects of the matrix on release. Amounts of metribuzin incorporated were calculated from elemental analysis. For chitin and cellulose solutions precipitated in water, the weight fractions of metribuzin in the resulting mixtures were 0.16 and 0.14, respectively. Precipitations in methanol gave corresponding values of 0.003 and 0.007. Precipitation behavior of the two polymers was very similar. The latter values confirmed that methanol precipitation is effective for removing unattached material. The results for precipitation in water show that the limited solubility of metribuzin in water allows retention of herbicide in the polymer matrix; however, only 12-15% of the available metribuzin was incorporated in the polymers. High local concentrations of DMAC around the particles during precipitation assisted in removal of the herbicide.

Determination of Degree of Substitution

The degrees of substitution of metribuzin on the polymers were calculated from elemental analysis, as well as measured by quantitative IR and GPC methods [8]. The results of these determinations are summarized in Table VII. None of the chitin samples were soluble in solvents which would allow use of either the IR or the GPC method; their DS values from elemental analysis are given in Table VI.

Generally, the three methods showed fair correlation and each has its advantages. Elemental analysis is the most accurate and precise method of the three; however, it cannot differentiate between attached and unattached herbicide, so purity must be assumed. The IR method is rapid and simple; GPC is somewhat more complex and can only be used for samples which are soluble in appropriate solvents. The GPC method

was successful in separating the unattached herbicide and small-molecule impurities from the polymer; therefore, independent quantitative analyses were possible. Representative chromatograms are shown in Figure 1.

Table VII

Comparison of Degrees of Substitution
Determined Using Various Methods

Rxn No.	Polymer	Degree of Substitution by		
		EA	IR	GPC
170-D15	Cellulose	1.6	1.8	-
170-25	"	1.9	1.9	1.3
170-49	Dextran	0.05	0.09	-
250-4	"	1.4	1.6	1.4
170-27	Starch	1.3	1.3[a]	-
250-D4	HEC	2.0	2.3	1.9
170-28	PVA	0.75	0.75[a]	0.5
170-47	"	0.24	0.6	-
170-D48	"	0.60	0.7	-
250-2	"	0.25	0.7	-
250-D2	"	0.06	0.2	-

[a]standard

A bulk UV technique [8] was used to determine the DS of sample 170-D15, a cellulose-based system. The DS obtained on five replicates was 2.5 ± 0.3. Availability of accurate standards is a limitation of IR, GPC, and UV methods. Nonetheless, these three methods, together with elemental analysis, complement each other and represent alternatives when a particular choice is not feasible.

Determination of Unattached Herbicide

The amounts of residual, unattached metribuzin contained in the purified polymers were determined using dialysis/LC and GPC [8]. The results are shown in Table VIII. The low values for free herbicide indicate purification by reprecipitation was efficient.

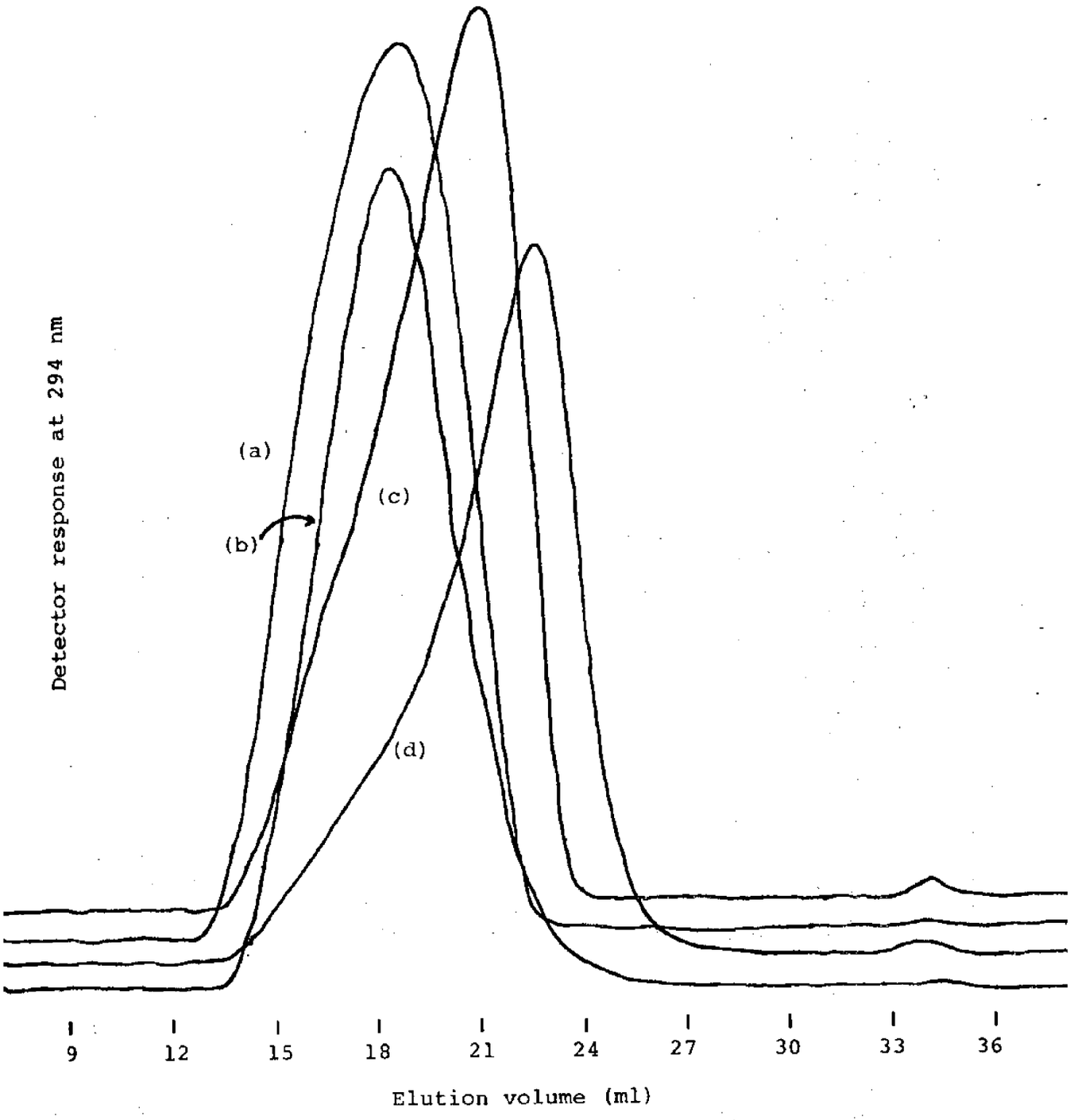


Figure 1. Gel Permeation Chromatograms for PVA 170-28 (a), HEC 250-D4 (b) Cellulose 170-25 (c), and Dextran 250-4 (d)

Table VIII

Unattached Metribuzin in Polymers

Rxn No.	Polymer	DS	% unattached	
			Dialysis/LC[a]	GPC
170-25	Cellulose	1.9	0.21 ± 0.04	0.4
250-4	Dextran	1.4	0.09 ± 0.04	0.4
170-27	Starch	1.3	0.12 ± 0.03	-
250-D4	HEC	2.0	0.1 ± 0.1	0.2
170-28	PVA	0.75	0.04 ± 0.03	0.1
170-D48	"	0.60	1.16 ± 0.06	-
250-2	"	0.25	0.1 ± 0.1	-
250-D2	"	0.06	0.1 ± 0.3	-

[a]95% confidence limits

Molecular Size Characterization

The molecular weights and intrinsic viscosities of these polysaccharides with attached metribuzin are shown in Table IX. Molecular weights of the unsubstituted polymers were determined as noted and values for the substituted materials were calculated from the DS values. For some samples, estimates of the effective spherical diameter of molecules eluting at the peak elution volume in GPC were also made by comparison to polystyrene standards using the Southern calibration method [8,22].

The intrinsic viscosity of each polymer drops significantly with herbicide attachment indicating a decrease in solvating power of the solvent for the resulting polymer. This decrease more than offsets the expected increase usually observed for increased molecular weight. Poor solvation may be a secondary contributor to the difficulty in obtaining complete substitution of herbicide on every hydroxyl group.

The solubility of unmodified chitin in the DMAC/LiCl solvent also allowed direct light scattering characterization. The molecular weight of the chitin used was determined to be 2.1 million [8] which is in reasonable agreement with values obtained by Rutherford and Austin from viscometry [23].

Release of Attached Metribuzin from the Polymers

The percentages of metribuzin released at ambient temperature during the indicated period at various pH values are shown in Tables X and XI for

Table IX

Molecular Weight and Molecular Size Data

Rxn No.	Polymer	DS	MW[a]x10^{-6} (daltons)	$[\eta]_o$[b] (dl/g)	Solvent[c]	Diameter[d] (Å)
-	Cellulose	-0-	0.5[e]	1.6 ±0.02	1	-
170-D15	"	1.6	1.7	0.60±0.04	2	-
170-25	"	1.9	1.9	0.50±0.03	2	347
-	Chitin	-0-	2.1[e]	8.7 ±0.1	1	-
170-D28	"	0.23	2.6	1.51±0.02	1	-
250-3	"	0.39	3.0	1.59±0.01	1	-
-	Dextran	-0-	0.070[f]	-	-	-
250-4	"	1.4	0.22	0.60±0.02	2	252
250-D4	HEC	2.0	-	1.27±0.04	2	634
-	PVA	-0-	0.086[f]	2.77±0.08	3	-
170-28	"	0.75	0.44	1.18±0.06	3	592
170-D48	"	0.60	0.37	1.25±0.01	3	-
250-2	"	0.25	0.20	1.33±0.01	3	-
250-D2	"	0.06	0.11	2.09±0.02	3	-

[a]calculated except as noted; [b]at 30°C, 95% confidence limits; [c]for viscosity: 1=DMAC/6%LiCl, 2=THF, 3=DMAC; [d]by GPC in THF; [e]from light scattering; [f]manufacturers data

Table X

Percentages of Release for Polysaccharide Systems

Rxn No.	Polymer	DS	pH	Percent released[a]	Time (days)	Percent unattached[a,b]
170-D15	Cellulose	1.6	7	1.04±0.05	218	-
170-25	"	1.9	4	ND[c]	41	0.21±0.04
"	"		7	ND	41	"
"	"		10	0.05±0.05	47	"
170-D26	Chitin	0.06	7	0.3 ±0.3	29	-
170-D28	"	0.23	4	ND	40	-
"	"		7	0.04±0.05	40	-
"	"		10	0.1 ±0.1	46	-
250-3	"	.39	7	0.04±0.03	47	-
170-49	Dextran	0.05	4	0.1 ±0.2	19	-
"	"		7	0.1 ±0.3	19	-
"	"		10	0.8 ±0.2	24	-
250-4	"	1.4	4	0.03±0.04	29	0.09±0.04
"	"		7	0.02±0.03	29	"
"	"		10	0.14±0.02	34	"
170-27	Starch	1.3	4	ND	40	0.12±0.03
"	"		7	0.01±0.02	41	"
"	"		10	0.07±0.04	47	"
250-D4	HEC	2.0	4	0.01±0.03	29	0.01±0.1
"	"		7	0.02±0.04	29	"
"	"		10	0.05±0.06	29	"

[a] 95% confidence limits; [b] by dialysis/LC; [c] none detected

Table XI

Percentages of Release for PVA Systems

Rxn No.	DS	pH	Percent released[a]	Time (days)	Percent unattached[a,b]
170-28	0.75	4	ND[c]	40	0.04±0.03
"		7	0.01±0.03	40	"
"	"	10	ND	40	"
170-47	0.24	7	0.05±0.05	29	-
170-D48	0.60	4	0.02±0.02	40	1.16±0.06
"	"	7	0.01±0.02	40	"
"	"	10	0.01±0.05	40	"
250-2	0.25	4	0.01±0.03	29	0.1 ±0.1
"	"	7	0.03±0.05	29	"
"	"	10	1.79±0.06	40	"
250-D2	0.06	4	0.04±0.06	16	0.1 ±0.3
"	"	7	1.15±0.04	40	"
"	"	10	4.7 ±0.2	40	"

[a]95% confidence limits; [b]by dialysis/LC; [c]none detected

the polysaccharides and PVA, respectively. The percentage of the available metribuzin which was unattached is also shown. In most cases extremely slow hydrolysis of the carbamate linkage was observed. The release profiles for these samples showed varying rates of appearance of these small concentrations.

The systems which showed definite evidence of slow hydrolysis were cellulose 170-D15, dextran 170-49, and the low substitution PVA samples 250-2 and 250-D2. Cellulose was considerably slower, with only 1% released after 218 days. The level after 57 days was very similar to that for 170-25 at 47 days, indicating no significant difference in the amount of unattached herbicide between the two. The dextran sample at pH 10 showed increasing hydrolysis; the metribuzin did not appear until the third sampling, then increased slowly. The sample was soluble in the buffer, so unattached material would have appeared immediately. The PVA samples showed significantly more metribuzin release.

The pH had a marked effect on release rate for the PVA samples. Figure 2 shows profiles at three pH values for sample 250-D2. The data indicate basic conditions favor faster hydrolysis of the carbamate, as expected.

The DS also influenced the rate of release for PVA, as shown in Figure 3 and Table XI. Higher rates at lower DS indicate greater accessibility to the bonds for hydrolysis, probably due to high hydrophilicity. Low hydrophilicity is probably the major factor underlying the low degrees of herbicide release from the highly substituted samples; the pH effect cannot overcome the DS effect in these cases. Therefore, the concept of attaching as much herbicide to the polymer as possible is counter-productive for two reasons. First, the release rate will be reduced; and, secondly, above certain levels, significant increases in DS produce very small increases in weight fraction of herbicide in the resulting polymer.

The polymer type also has an effect on release rate. For example, dextran sample 170-49 was fully soluble but released more slowly than PVA sample 250-D2 at pH 10. Since the bond stabilities would be expected to be very similar, the dextran backbone may hinder the hydrolysis reaction. This effect is apparently even more pronounced for the polysaccharides having 1,4-linkages between repeating units.

The polymer may also affect release through a morphological change as pendent groups are removed. Such a change might be reformation of intermolecular hydrogen bonds after herbicide removal into a more highly-ordered, compacted structure resulting in no further penetration by water or herbicide molecules. This consideration might explain the extremely low amounts of metribuzin released from the chitins. At these low levels of DS, considerable hydrophilicity should remain and allow hydrolysis, albeit slow. This phenomenon was also observed for physical mixtures of herbicide in chitin.

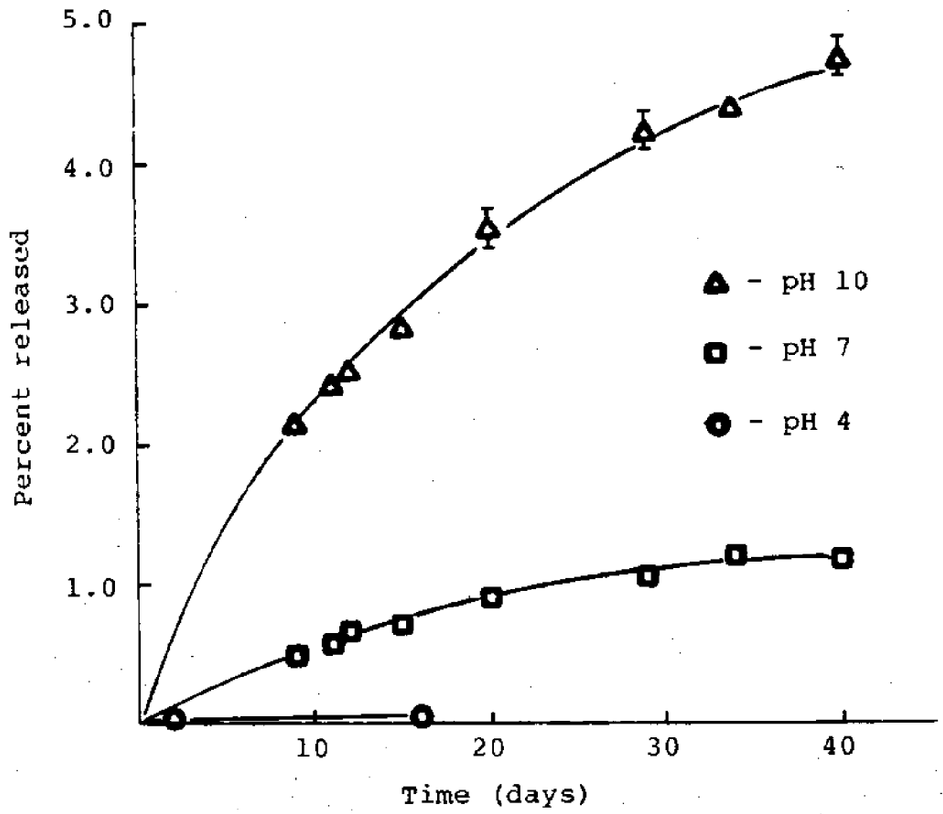


Figure 2. Apparent Metribuzin Release Rates from Sample P5 at Three pH Values

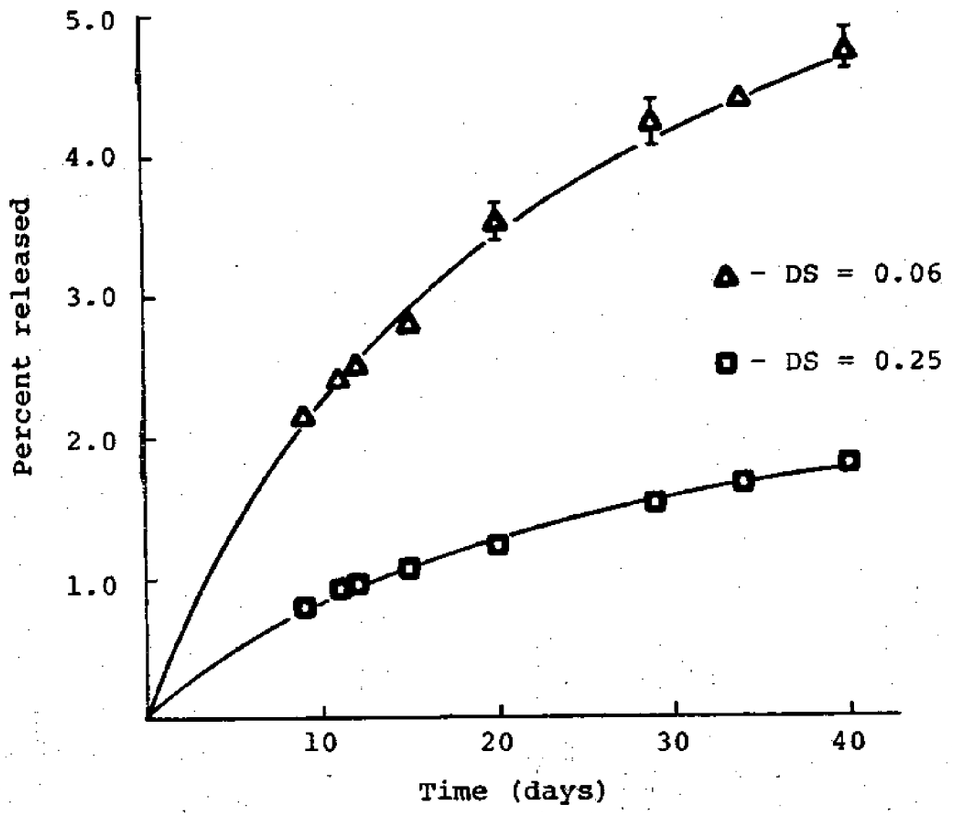


Figure 3. Apparent Metribuzin Release Rates from Samples P5 (DS = 0.06) and Sample P4 (DS = 0.25)

Release of Entrapped Metribuzin from Chitin and Cellulose

Chitin and cellulose containing physically entrapped metribuzin released only eight to ten and seven to eight percent of the available herbicide, respectively. All release occurred during the first four to eight hours after immersion in the buffers; pH had no significant effect on the release. These observations support the concept that a morphological change upon leaching of a portion of the material may close the surface of the particles to further release of herbicide, as discussed earlier. The implications for bound systems using polymers which do not swell in water are that slow or incomplete release may occur even using very labile bonds. These facts indicate the need to utilize water-soluble or water-swellable polymers for CR applications.

The results of our studies of metribuzin directly attached to chitin and cellulose indicate that hydrolysis alone would not yield desirable rates of herbicide release within 90-150 days. More hydrophilic polymers with low degrees of substitution and those with spacer groups to pendent herbicides give faster release rates. However, preliminary greenhouse results indicate that soil microorganisms may have a synergestic effect in releasing herbicide from polysaccharides probably due to degradation of the polymer backbone. Effects of amylose and cellulose enzymes on levels of herbicide release from starches and cellulose are presently under study.

SUMMARY

Systems in which herbicides are covalently bound to chitin and other marine-derived polysaccharides have been successfully prepared and characterized. These materials will release herbicide at rates which are strongly dependent on both the polymer and bond types. Fastest systems are esters based on water-soluble polymers while the slowest are carbamates based on water-insoluble polymers such as chitin and cellulose having high levels of attached herbicide.

One of the major benefits derived from this research is the development of reaction conditions and synthetic routes for the homogeneous derivatization of chitin, cellulose, and starch. These methods are potentially applicable to the preparation of a variety of useful derivatives of these polymers. Additionally new characterization methods for these polymers have been demonstrated. Mobility control of pesticides using chitin-based controlled release systems has been demonstrated although rates of hydrolytic release are low. The effects of soil microorganisms on enhancing release rates and biodegradability are presently under study.

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