Uptake of 2,4-D and 2,4,5-T

by Chitin and Chitosan

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INTRODUCTION

This project was concerned with the uptake of two acidic pesticides 2,4-D and 2,4,5-T in a flow (or column) system by the polymers chitin and chitosan. Column height and diameter, weight and type of polymer, pH, and flow rate were the variables studied to see how pesticide uptake was effected. Scanning Electron Microscopy/Energy Dispersive Analysis of X-rays (SEM/EDAX) was used for positive identification of the pesticides in the polymers following the uptake measurements.

Chitin, one of the two polymers used, has a chemical structure closely resembling cellulose except that the hydroxy (OH) group on carbon number two of the glucose ring has been replaced by a $NHCOCH_3$ group as shown in Figure 1. Chitosan, a derivative of chitin and the polymer most often used throughout the experiments, is formed by the deacetylation of chitin; that is, removal of the $COCH_3$ group from the NH moiety(1). Chitosan is soluble in some acids and at low pH the amine groups are protonated according to the equilibrium

$$RNH_2 + H^+ \rightleftharpoons RNH_3^+ \qquad [1]$$

The RNH_3^+ groups along the polymer backbone repel one another which causes uncoiling of polymer chains with a subsequent reduction in solution viscosity(2). This structural change may also increase pesticide uptake by the polymer.

Chitin is found in the exoskeleton of a large group of animals known as arthropods which includes insects, crabs, shrimps, spiders, and lobsters. Chitin is the second most abundant polymer in nature and constitutes as much as 50% of the animal's total organic matter(3). Preparation of chitin and chitosan from raw seafood waste is relatively simple. Over 100 million tons of waste is produced annually primarily by shellfish processors. The shellfish industry is growing rapidly today, and the disposal of the processing wastes continues to be a problem(1).

The two acidic pesticides used were 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-T trichlorophenoxy acetic acid) with pk_a values of 2.80 and 2.84, respectively(4). The structures are shown in Figure 2. These two chlorinated compounds are only slightly soluble in water and despite their usefulness against pests, are considered environmental hazards because of their toxicity(5).

These compounds are extremely non-degradable, are synthesized along with unwanted compounds, and often wash off after agricultural application into streams (5,6). 2,4,5-T is suspected of causing cancer, birth defects and miscarriages. A by-product in 2,4,5-T synthesis is 2,3,7,8-tetrachlorodibenzo-p-dioxin and this compound is probably the actual cause of these illnesses (6). The wire services in 1981 carried a report that swimming pools were found contaminated with 2,4-D containing dioxin in San Jose, California.

Pesticides are useful and necessary in a modern society, but if they are not contained within the proper environment and are mistakenly moved to another ecosystem (especially water), problems can and have already occurred. Polychlorinated biphenyls which resemble the structures of 2,4-D and 2,4,5-T are widespread contaminants of the marine environment. They are virtually insoluble in sea water and because of their hydrophobic nature are easily removed from solution by adsorption to particulate matter. These particulates then enter the water ecosystem by sewage, industrial effluents, and runoffs (7). Chlordan and Kepone have been found in Virginia's waters. Chlordan which is thought to be a carcinogen is a persistent pesticide which recently was introduced accidently into a Roanoke water line.

The National Academy of Science estimated that as much as 600 million pounds of these compounds have been dispersed into soil, air, water, and food in the United States (8). Kepone, a highly toxic insecticide, was released into

the James River causing the cessation of commercial fishing for an extended period. If no attempts are made to remove the pesticide, it has been estimated that it would take the ecosystem over 200 years to return to its original condition (9).

The polymers, chitin and chitosan, and the pesticides, 2,4-D and 2,4,5-T, have been studied and characterized separately in some detail, but there has been little research done on the interaction of the two. The polymers are certainly available and quite abundant. The presence of pesticides in water ecosystems will probably continue to be an area of increasing concern. To know then how the polymers take up the pesticides and the conditions which effect this uptake is important. Chitin/chitosan produced from seafood processing wastes may remove pesticides from water and this result could have both industrial and ecological importance.

EXPERIMENTAL

Procedures for Batch Studies - Stock solutions (10×10^{-4} M of 2,4-D and 2,4,5-T were prepared by adding 0.2210 g and 0.2555 g, resp., to distilled water contained in 1 liter volumetric flasks. The flasks were then magnetically stirred at 75-80°C for 2-3 hours on a hot plate. The stock solutions were then diluted to lower concentrations, down to 2×10^{-4} M, and calibration curves were made by measuring the absorbance of each solution of known concentration. Maximum absorbance (or minimum transmittance) was measured using a Hitachi 100-60 spectrophotometer set at a wavelength of 283 nm for 2,4-D and 287 nm for 2,4,5-T. Before each absorbance reading was taken, 100% transmittance (or 0% absorbance) was set at a wavelength 320 nm. Typical calibration plots for 2,4-D and 2,4,5-T are shown in Figure 3.

Fifty ml aliquots of each concentration were then mixed with 0.1 g of

chitosan [Velsicol Chemical Corporation] in 125 ml Erlenmeyer flasks, stoppered, and equilibrated usually for 24 hours at different agitation rates (rpm). After equilibration, changes in the concentration of 2,4-D and 2,4,5-T were interpolated from the appropriate calibration curve. Total uptake of pesticide per unit weight of chitosan was calculated from $V\Delta C/W$ where V is the volume of the pesticide solution (50 ml), ΔC is the change in the pesticide molar concentration and W is the weight of chitosan (0.1 g). Values of $V\Delta C/w$ were plotted versus the final concentration of the pesticide solution.

The polymer samples were observed by using a scanning electron microscope (SEM) before and after equilibration with the pesticide solutions. Pesticide uptake by chitosan was checked by monitoring the chlorine peak characteristic of the pesticide using energy dispersive analysis of X-rays (EDAX).

Procedures for Column Work- The same calibration curves (Figure 3) were used for the column tests. Two columns (5mm and 9mm OD) were packed with different amounts of polymer and the columns were then clamped to a ring stand. Above the column, a 50 ml buret was clamped and a 250 ml separatory funnel was set in a ring above the buret. The buret and separatory funnel allowed control of the flow rate into the column packed with polymer. Beneath the column, test tubes marked off at eight 5 ml or 10 ml portions were set to collect the eluent. The assembled components are diagrammed in Figure 4. To find the moles of pesticide taken up per gram of polymer, the term $V\Delta C/W$ was calculated. V is the volume of pesticide solution passed through the column (10 ml portion or 5 ml portion), ΔC is the change in concentration for each portion collected, and W is the weight of the polymer in the column. To find the total uptake, the values of $V\Delta C/W$ for each portion were summed.

The polymer contained in the column was always washed with 50 ml of

distilled H_2O before addition of any pesticide solution to the column. The last portion of the 50 ml of water was checked for 100% transmission over the wavelength range from 320 to 250 nm. The initial pH of the pesticide solution was read using an Orion 701 digital pH meter standardized with a buffer solution at pH 9. In some column tests, 10 ml of 6×10^{-3} M HNO_3 were added to the previously washed column. After the pesticide solutions were passed through the column, the chitosan was removed and dried for SEM/EDAX analysis. SEM was used again to observe sample morphology and EDAX to confirm pesticide uptake.

RESULTS AND DISCUSSION

Sorption Isotherms for 2,4,5-T and 2,4-D -- Batch test results for 2,4,5-T are shown in Figure 5. The results are consistent with those reported previously by Davar (4). The greater the final concentration of pesticide, the greater the uptake corresponding to larger values of $V\Delta C/W$. Davar's results show the greatest uptake at 300 rpm. The faster the agitation rates, the greater the uptake of pesticide. For 2,4-D uptake, the shape of the sorption isotherms were also similar to earlier work.

Pesticide uptake results from column experiments shown in Figures 6 and 7 confirm that chitosan is an excellent absorbent of the two pesticides 2,4-D and 2,4,5-T. After 280 mls of 10×10^{-4} M solutions of 2,4,5-T were passed through 0.1 g (or 5 mm) of chitosan packed in a 9 mm column, the polymer was still not saturated (Figure 6). Column height (or amount), type of polymer, pesticide concentration, pH, and flow rate all effect uptake.

After determining that chitosan does absorb pesticides significantly, conditions were varied to give the highest pesticide uptake especially for the first 50 mls of pesticide passed through the column. Industrially, one would

want to pass a pesticide solution through a large column packed with polymer and collect purified water. The amount of pesticide needed to fully saturate the polymer would not necessarily be the most significant parameter.

As the weight of polymer increases in the column, more moles of 2,4-D were initially absorbed as shown in Figure 7. After more than one gram of polymer is added, pesticide uptake over the first 50 mls does not increase significantly and the flow rate becomes very low.

The smaller diameter column packed with 0.1 gram of chitosan absorbed more pesticide per gram of chitosan than 0.1 gram of chitosan packed in a 9 mm column. After 50 mls and 250 mls of 10×10^{-4} M solution of 2,4,5-T were passed through a 9 mm column packed with 0.1 gram of chitosan, the number of moles of pesticides absorbed per gram were 30.0×10^{-5} and 175.05×10^{-5} respectively. After 50 and 260 mls of 10×10^{-4} M solution of 2,4,5-T were passed through a 5 mm diameter column with 0.1 gram of chitosan, the number of moles of pesticides absorbed per gram were 43.95×10^{-5} and 172.62×10^{-5} respectively. The height of polymer in the 5 mm column was 20 mm and the height of polymer in the 9 mm column was 5 mm. The longer the chitosan bed, the greater the uptake of pesticides.

Upake of Chitin vs. Chitosan --Of the two polymers used, chitosan takes up more pesticide per unit weight than chitin. A comparison plot is shown in Figure 8. After 50 ml of 2,4,5-T was passed through 0.1 gram of each of the two polymers, 6.5×10^{-5} moles per gram of chitin were absorbed and 26.5×10^{-5} moles per gram of chitosan were absorbed, resp. The flow rate through the chitin bed was faster than through the chitosan bed. The slower the flow rate through the polymers, the greater the uptake of pesticides. Thus, the greater uptake observed for chitosan waw in part due to a flow rate effect.

Crushed crab shells which were decalcified first with $HCl_{(g)}$ were unable to

be used because component(s) in the eluent interferred with the spectrophotometer readings for the pesticide. Another process to clean the shells must be used.

As liquids flowed through the columns packed with the polymers, significant changes in pH were observed. Distilled water with an initial pH of approximately 7.0 was passed through chitosan. The first 10 mls of eluent collected had a pH reading near 8.0. For the next 40 mls collected, the pH began to decrease to 7.0. The initial increase in pH means H^+ ions were being adsorbed by the polymer. The H^+ ions probably protonated the amine function on carbon number two of the glucose ring of chitosan. (see Figure 1)

When the column was packed with chitosan, the pH of the first 10 mls of eluent was significantly greater than the pH of the initial pesticide as noted in Figure 9. The pH then remained nearly constant for the next 40 mls of eluent collected. The pH results for uptake runs with a $2x10^{-4}$ M solution of 2,4-D (initial pH 4.20) using different polymer weights in a 9 mm column are listed in Table I.

When chitin was used, the pH of the first 10 mls of eluent also showed a significant increase compared to the original pesticide solution. However, the pH of the next 40 mls of eluent collected then decreased. The results listed in Table II were obtained with 0.3 grams of chitin using 5.1×10^{-4} M and 10.1×10^{-4} M 2,4-D solutions with initial pH values of 3.58 and 3.25, respectively. The decrease in pH with time probably indicates that chitin contrasted to chitosan was becoming saturated with H^+ ions.

A study was also done at a constant concentration of 10×10^{-4} M solution of 2,4,5-T to see how the initial pH of the pesticide solution (by adding drops of HNO_3 and NaOH) would effect uptake. The pH was adjusted by addition of either dil. $HNO_{3(aq)}$ or dil. $NaOH_{(aq)}$. The original pH was 3.19 at this 10×10^{-4} M concentration and a 9 mm column was packed with 0.1 gram of chitosan for each run made. The results are listed in Table III. The greatest uptake occurred in the

2.86-3.43 pH range. The HNO_3 added dropwise seemed to be increasing the interaction between the polymer and pesticide. Why uptake was not increased below pH of 2.86 with a slower flow rate was questionable. Also at this low pH value, the pesticide species RCOOH and not $RCOO^-$ starts to predominate. The RCOOH does not bind to the functional groups (NH_3^+) of the polymer. Figure 10 depicts the proposed mechanism of reaction (4). When drops of NaOH were added to a 10 x 10^{-4} M pesticide solution to increase the pH to 4.06 the uptake was also greatly reduced.

Pretreatment with HNO_3-- Since HNO_3 added to pesticide dropwise seemed to slow the flow rate down and increase interaction, additional work was done where the chitosan in the column was first saturated with dilute $HNO_{3(aq)}$ before addition of pesticide solution. Dilute solutions of 5-6 x 10^{-5} M $HNO_{3(aq)}$ were used so that the concentration of the pesticides was not altered. If the acid concentration was too large, little pesticide was able to penetrate the column and spectrophotometer readings of pesticide concentration were altered. The amount of HNO_3 added and the time of saturation in the polymer were important.

The results of columns of chitosan pretreated with HNO_3 were significantly different from column work with no HNO_3 pre-treatment. The results are summarized in Figure 11. The first 50 mls showed more moles of pesticide absorbed. A steady rise toward saturation occurred also. Without HNO_3 pre-treatment, less pesticide was absorbed and the rise toward saturation was not as sharp. The pH changes with HNO_3 treatment. The pH results using a 10 x 10^{-4} M solution of 2,4-D with an initial pH of 3.00 are summarized in Table IV. The 9 mm column was packed with four different weights of chitosan. Chitosan seems to saturate faster although there is initially more uptake for the first 50 ml with HNO_3 than without HNO_3. Perhaps the NH_2 groups were first protonated with

H^+ from HNO_3 by the equilibrium [1]. The pesticide species $RCOO^-$, which predominates above pH of 2.80 (its pK_a), may then react with the protonated amine in the following reaction:

$$R'NH_3^{\oplus} + RCOO^- \rightleftharpoons R'NH_3^{\oplus}\,{}^{\ominus}OOCR \; . \qquad [2]$$

This is the proposed mechanism of interaction. Without HNO_3 added to the polymer, this mechanism of reaction would depend on a proton and the $RCOO^-$ species from the pesticide dissolved in H_2O.

On a comparison basis, it appears that uptake was greater in the column experiments than with batch tests for 0.1 gram of chitosan. Comparative uptake results are listed in Table V.

SEM/EDAX Results from Batch and Column Work.-- An SEM photomicrograph of chitosan from the batch test after 8 hours of equilibration with a 10×10^{-4} M solution of 2,4-D shows the general characteristic features of the original polymer. The EDAX spectrum of the polymer shows a strong Cl peak, confirming pesticide uptake.

From the column experiment, a sample of chitosan washed with 50 mls of H_2O from a 9 mm column showed characteristic features. Using EDAX, this sample showed a large calcium peak which was not present in the batch test. This calcium and possibly phosphorous washing off with the pesticide solutions as they passed over the polymer could have caused small variations in absorption readings throughout this experiment. In the batch work, longer equilibration times (8 hours versus 30-60 minutes) probably were sufficient to wash chitosan free of calcium. Two other samples from columns saturated with HNO_3 and then exposed to pesticide solution showed larger chlorine peaks with smaller calcium peaks using EDAX. Surface features of the polymers before and after reacting with pesticides were not significantly different as seen by SEM.

CONCLUSIONS

1. Results from batch tests agreed with earlier results and the faster the rate of agitation, the greater the pesticide uptake. Also the longer the equilibration time, the greater the uptake.

2. Chitosan was an excellent absorbent of 2,4-D and 2,4,5-T.

3. The amount of pesticide uptake was increased by

   a) a slower flow rate.

   b) a greater change in pH between the original pesticide and eluent.

   c) decreasing the column diameter.

   d) increasing the weight of the polymer.

4. Chitosan shows more pesticide uptake than chitin.

5. Pretreatment of the polymer with HNO_3 increases the interaction time between the pesticide and polymers and increases uptake initially.

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TABLE I

VALUES OF pH FOR CHITOSAN COLUMN ELUENT

Eluent Fraction collected		pH Values Weight of Chitosan		
		0.2g	0.5g	1g
1st	10 ml	7.63	7.60	7.82
2nd	10 ml	7.89	8.30	8.73
3rd	10 ml	7.88	8.57	8.85
4th	10 ml	7.89	8.65	8.84
5th	10 ml	7.63	8.71	--

TABLE II

VALUES OF pH FOR CHITIN COLUMN ELUENT

Eluent Fraction Collected		pH Values Concentration 2,4-D	
		$5x10^{-4}M$	$10x10^{-4}M$
1st	10 ml	7.54	7.0
2nd	10 ml	7.38	6.7
3rd	10 ml	7.19	6.3
4th	10 ml	6.99	5.25
5th	10 ml	6.91	4.62

TABLE III

UPTAKE OF 2,4,5-T BY CHITOSAN AT DIFFERENT pH VALUES

pH	Uptake (moles/gram)(x 10^{-5})	Flow Rate (ml/min)
2.10	3.05	1.1
2.59	14.90	1.23
2.86	30.50	1.88
3.19	30.0	2.34
3.43	12.3	6.36
3.73	7.1	3.68
4.06	4.9	3.50

TABLE IV

VALUES OF pH FOR ACID-PRETREATED CHITOSAN COLUMN ELUENT

Eluent Fraction Collected		pH Values — Weight of Chitosan			
		0.1	0.3g	0.5g	1.0g
1st	10 ml	5.07	7.10	7.23	8.82
2nd	10 ml	4.86	7.00	7.31	8.85
3rd	10 ml	4.73	6.90	7.26	8.81
4th	10 ml	4.27	6.67	7.21	8.82
5th	10 ml	3.92	5.59	7.20	8.81

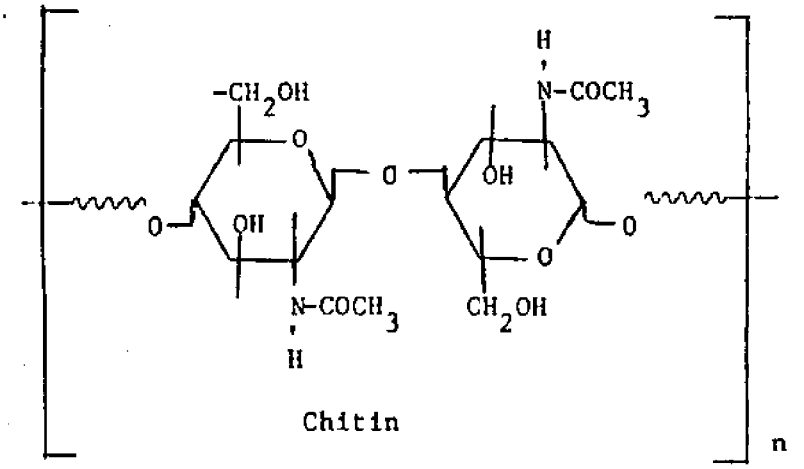
TABLE V

COMPARISON OF BATCH AND COLUMN UPTAKE OF 2,4-D BY CHITOSAN

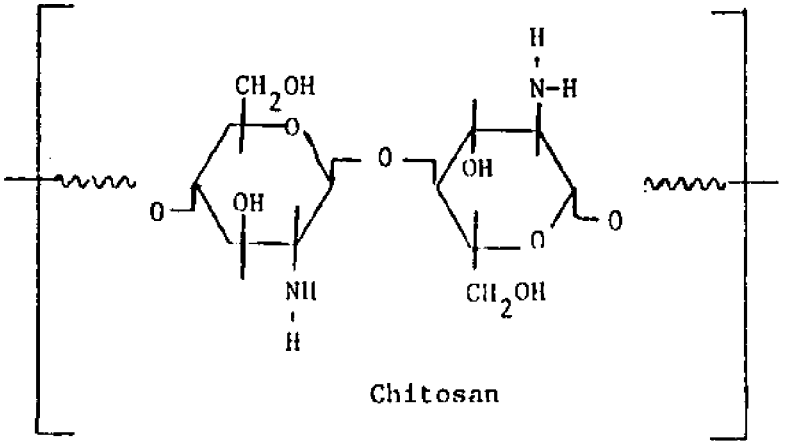
Procedure	Concentration (M)	$V\Delta C/W$ (moles/g)
Batch	10×10^{-4}	1.3×10^{-4}
Column	10×10^{-4}	2.1×10^{-4}
Batch	5×10^{-4}	1×10^{-5}
Column	5×10^{-4}	10.4×10^{-4}
Batch	2×20^{-4}	0
Column	2×10^{-4}	3×10^{-6}

FIGURE 1

STRUCTURES OF CHITIN AND CHITOSAN



Chitin



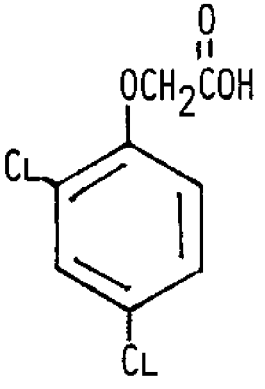
Chitosan

FIGURE 2

PESTICIDES

2,4-D

2,4-DICHLOROPHENOXY ACETIC ACID

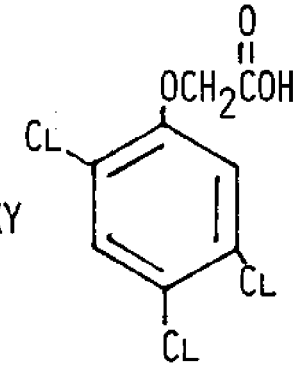


pK_A = 2.80

M.W. = 221.0 G/MOL

2,4,5-T

2,4,5-TRICHLOROPHENOXY ACETIC ACID



pK_A = 2.84

M.W. = 255.5 G/MOL

FIGURE 3

CALIBRATION PLOTS FOR PESTICIDES

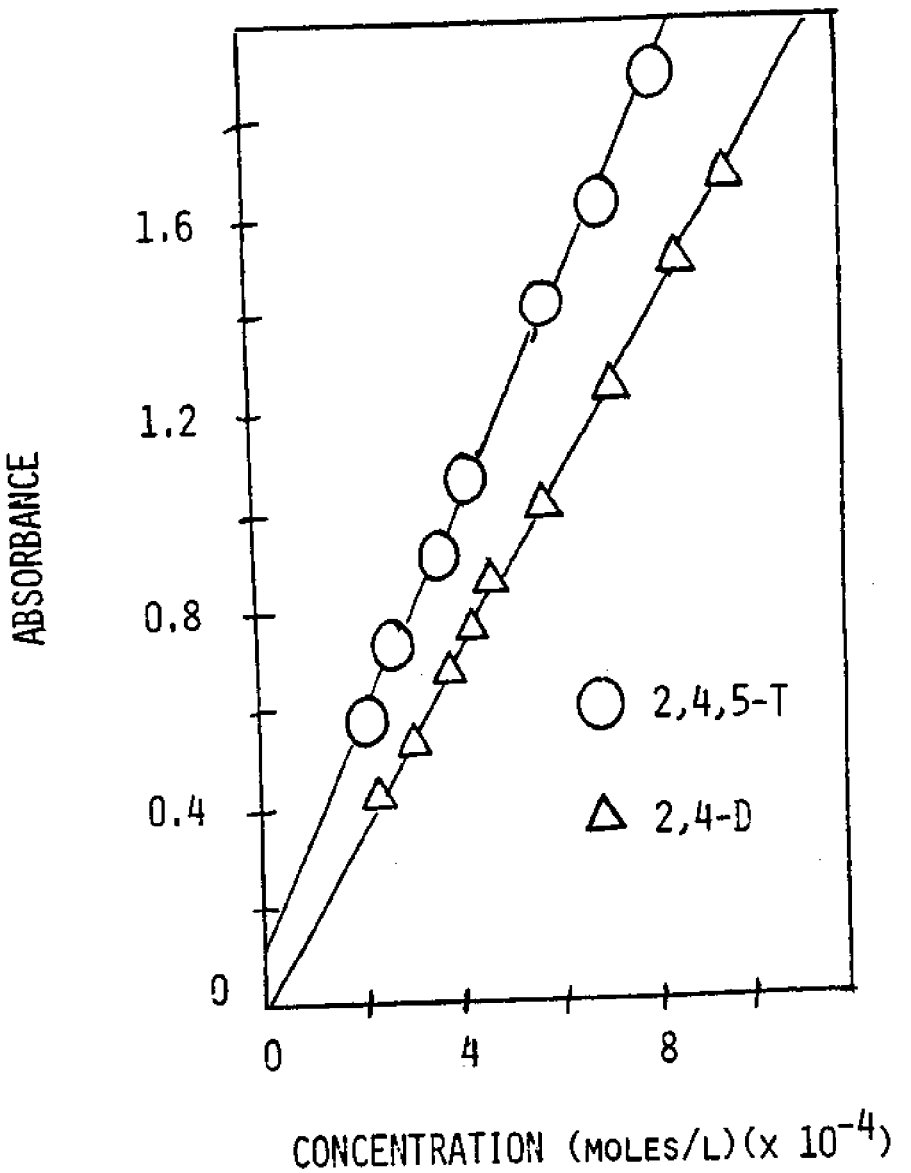


FIGURE 4

COLUMN SET-UP

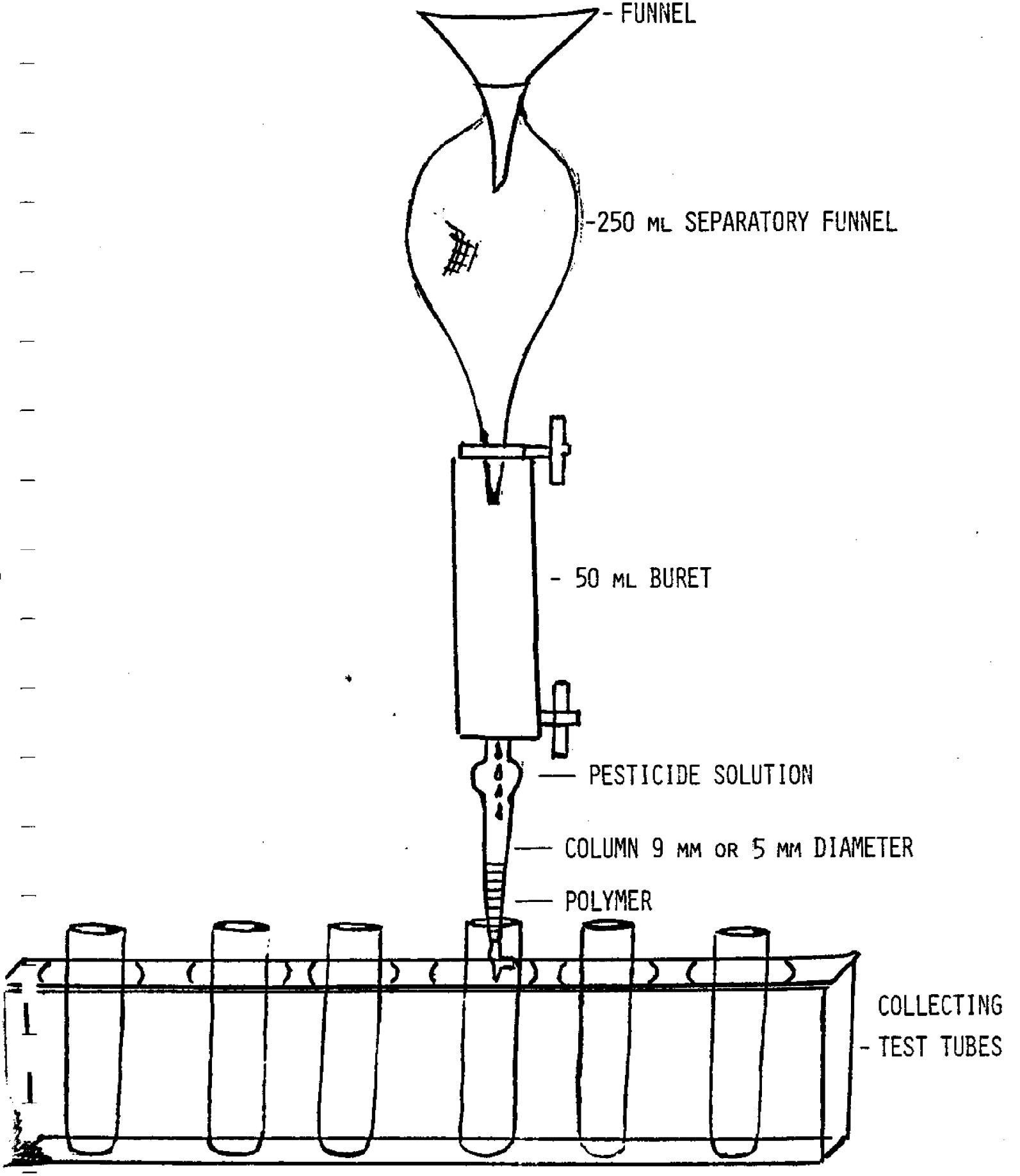


FIGURE 5

SORPTION ISOTHERM FOR 2,4,5-T ON CHITOSAN

FROM A BATCH TEST

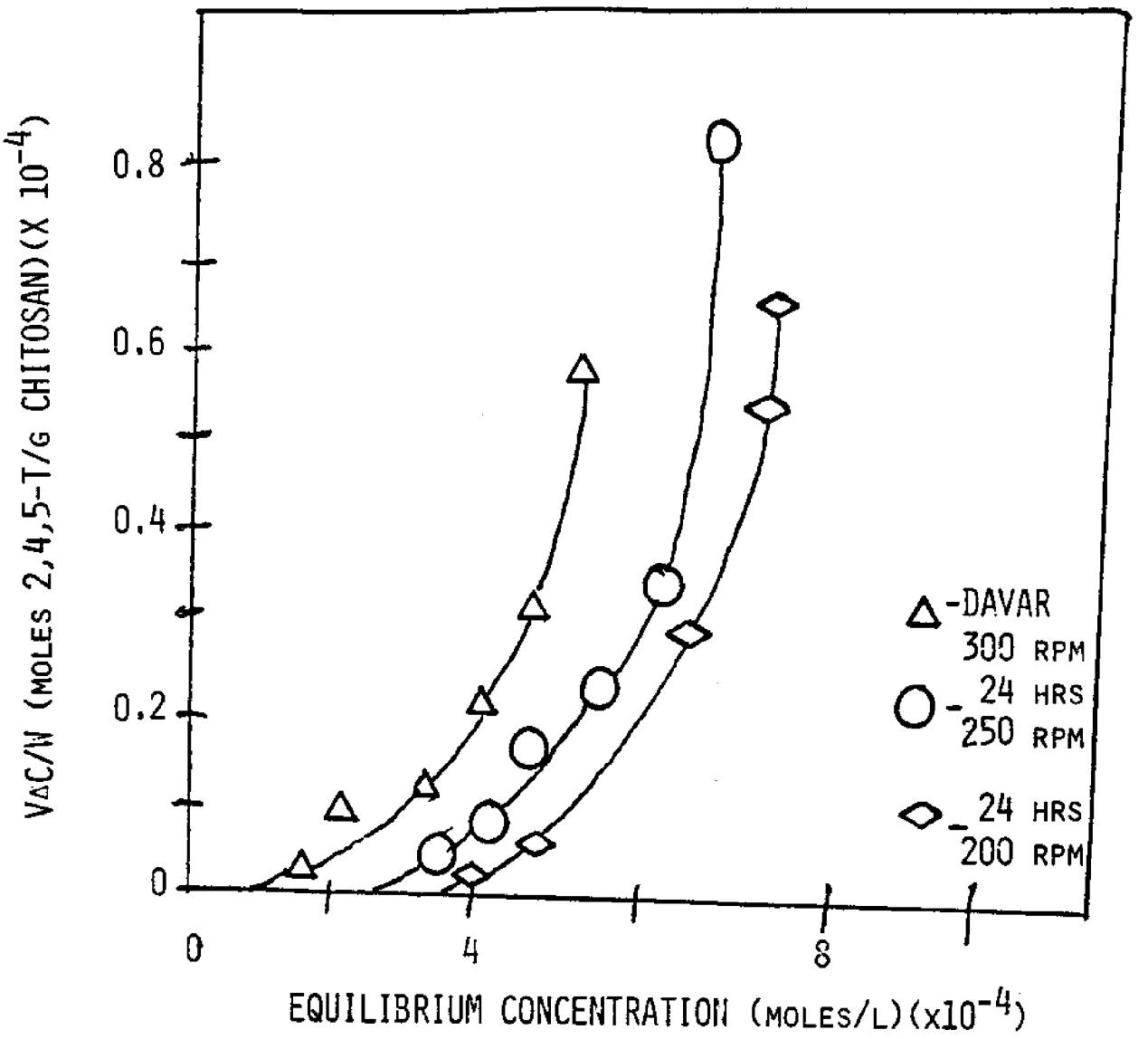


FIGURE 6

UPTAKE OF 2,4,5-T BY CHITOSAN
IN A COLUMN TEST

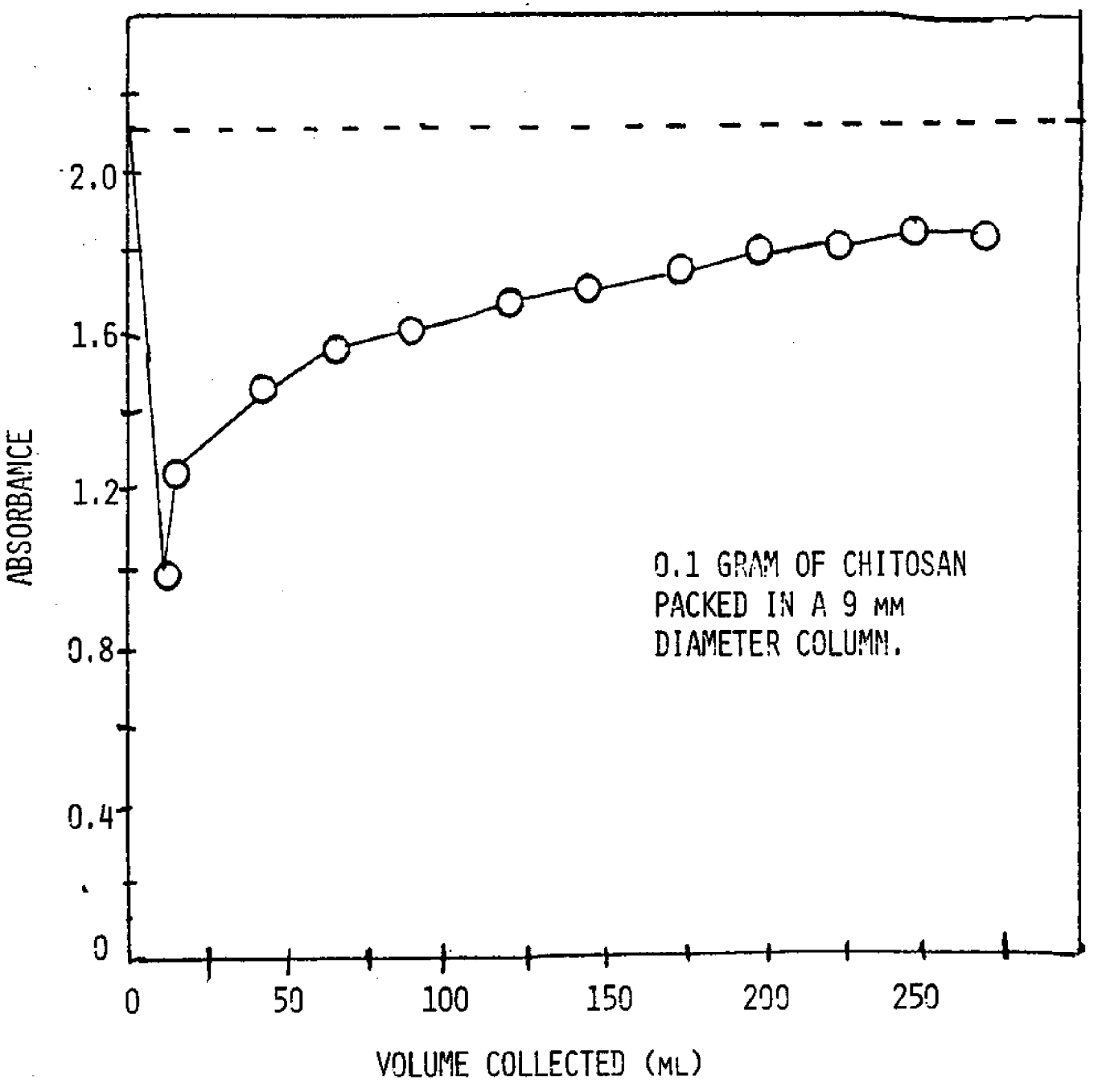


FIGURE 7
UPTAKE OF 2,4-D USING

DIFFERENT AMOUNTS OF CHITOSAN

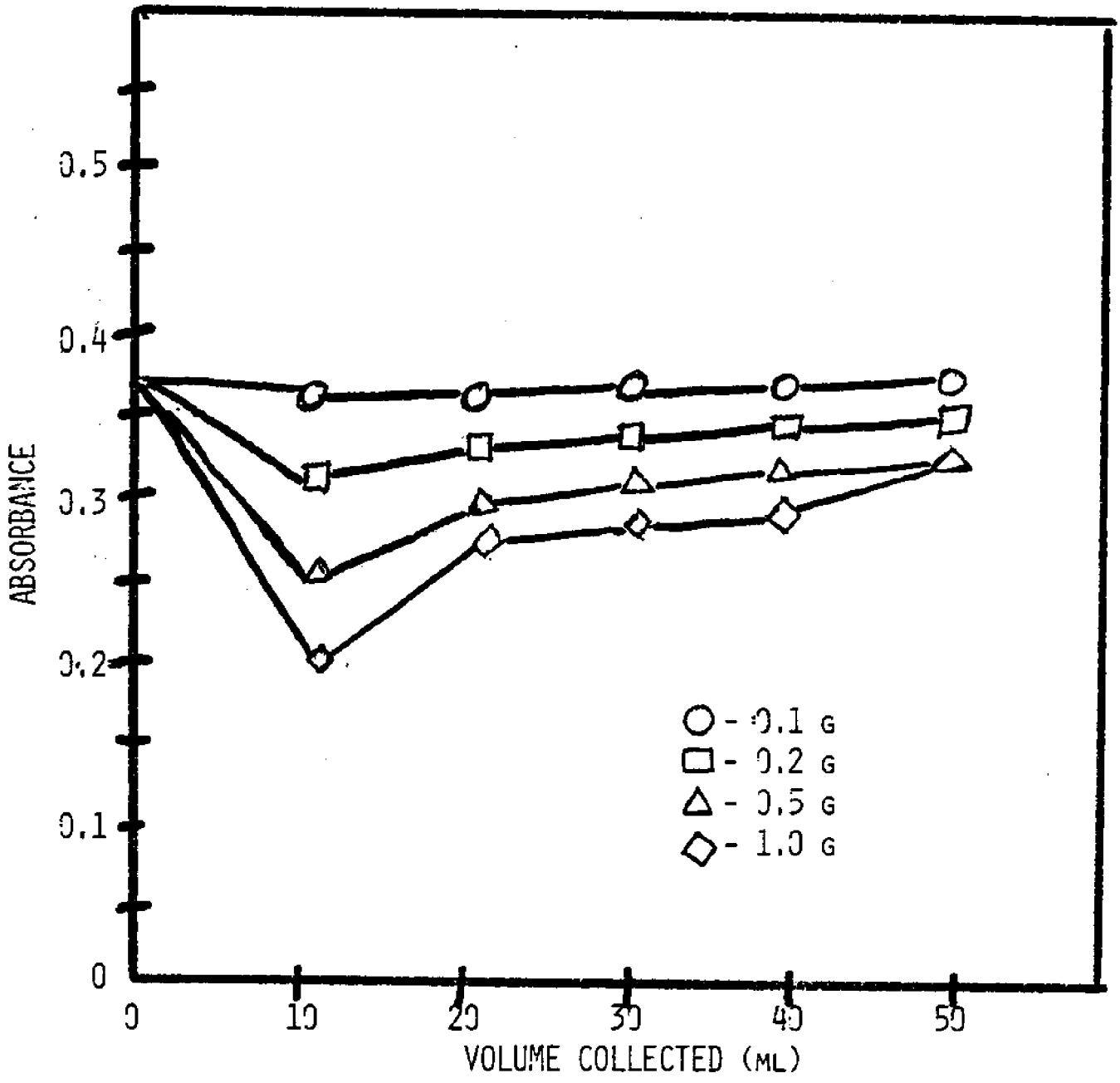


FIGURE 8

UPTAKE OF 2,4,5-T BY

CHITIN AND CHITOSAN

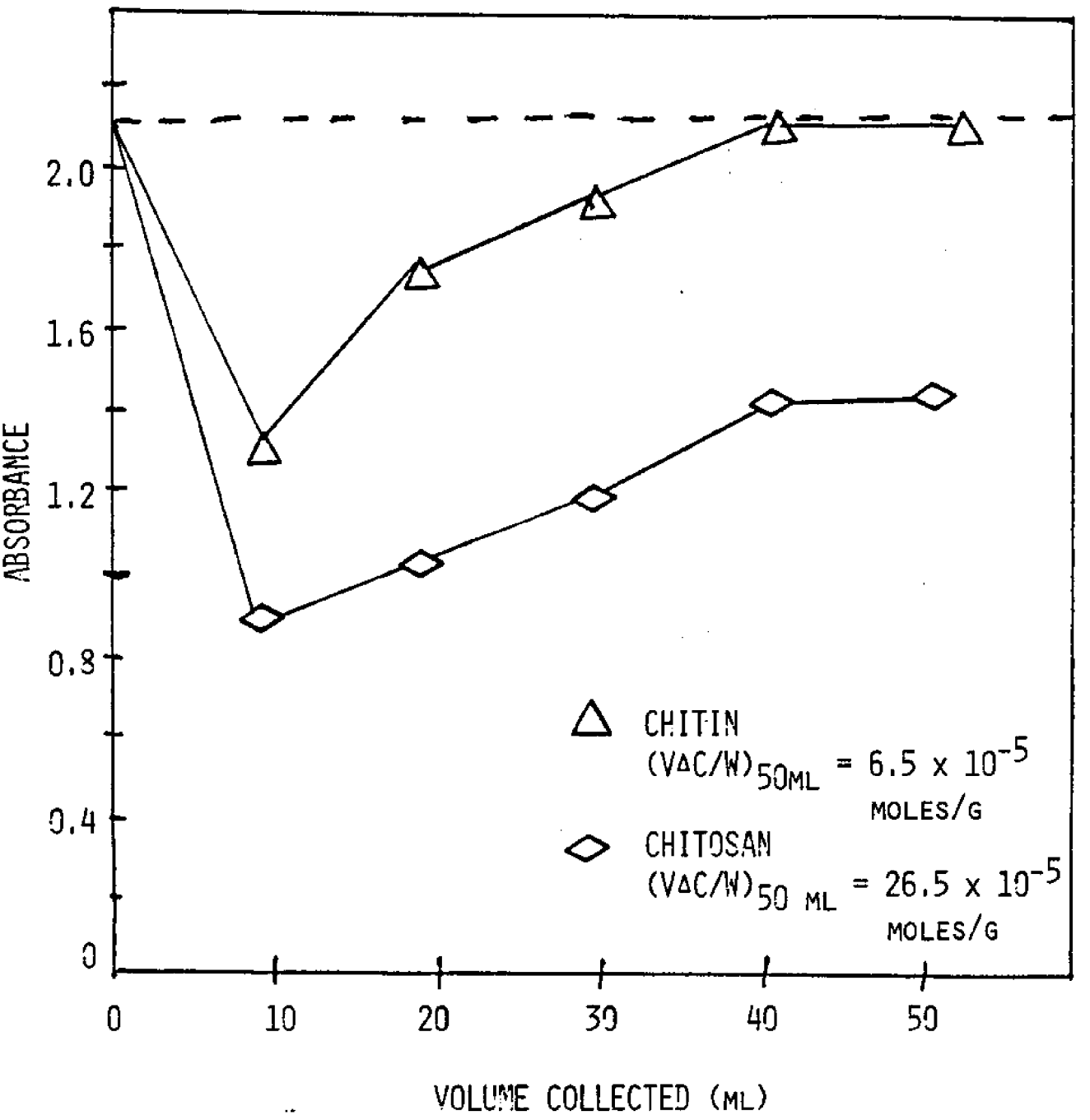


FIGURE 9

pH OF 2,4,5-T CONTAINING ELUENTS

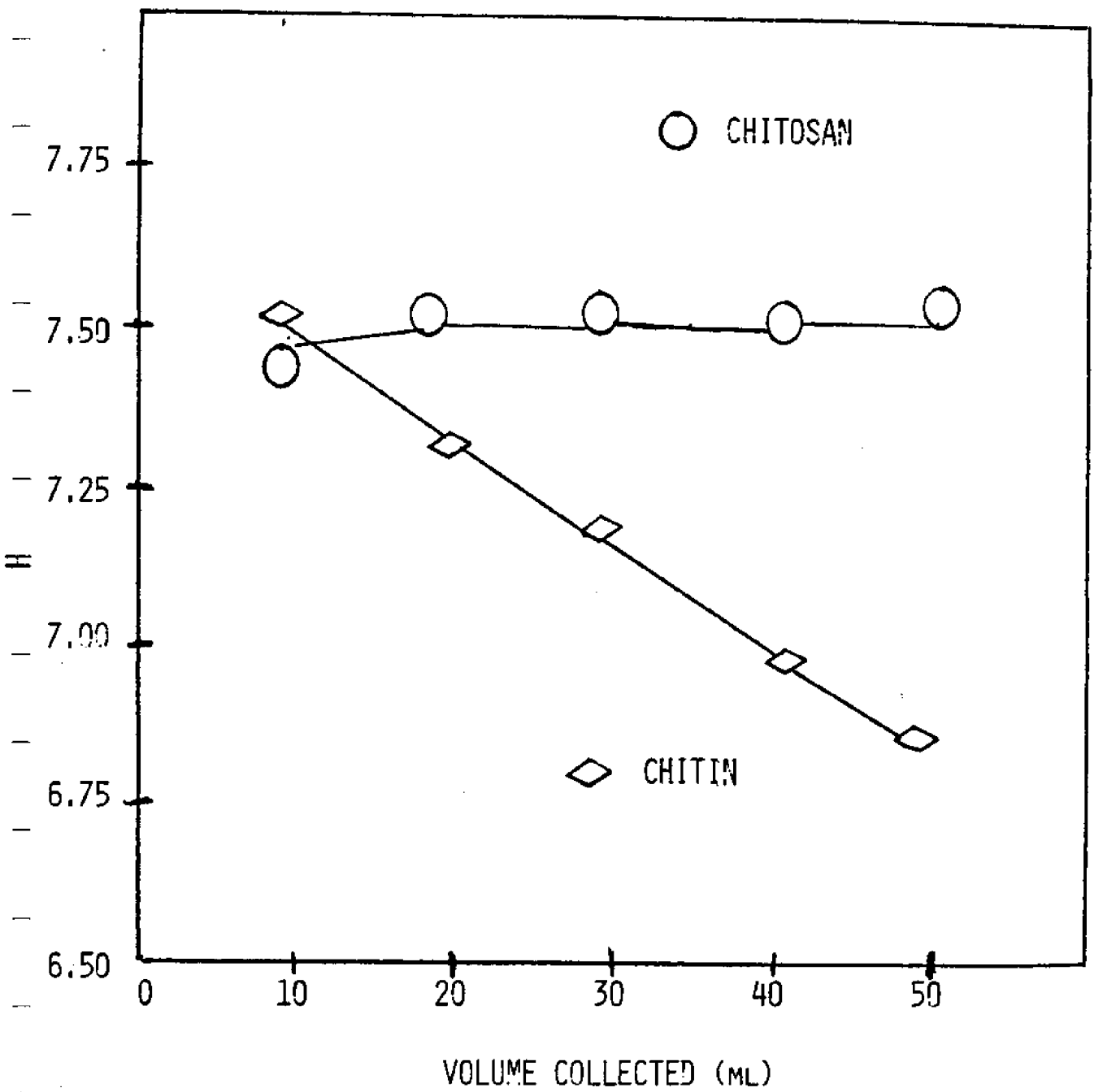
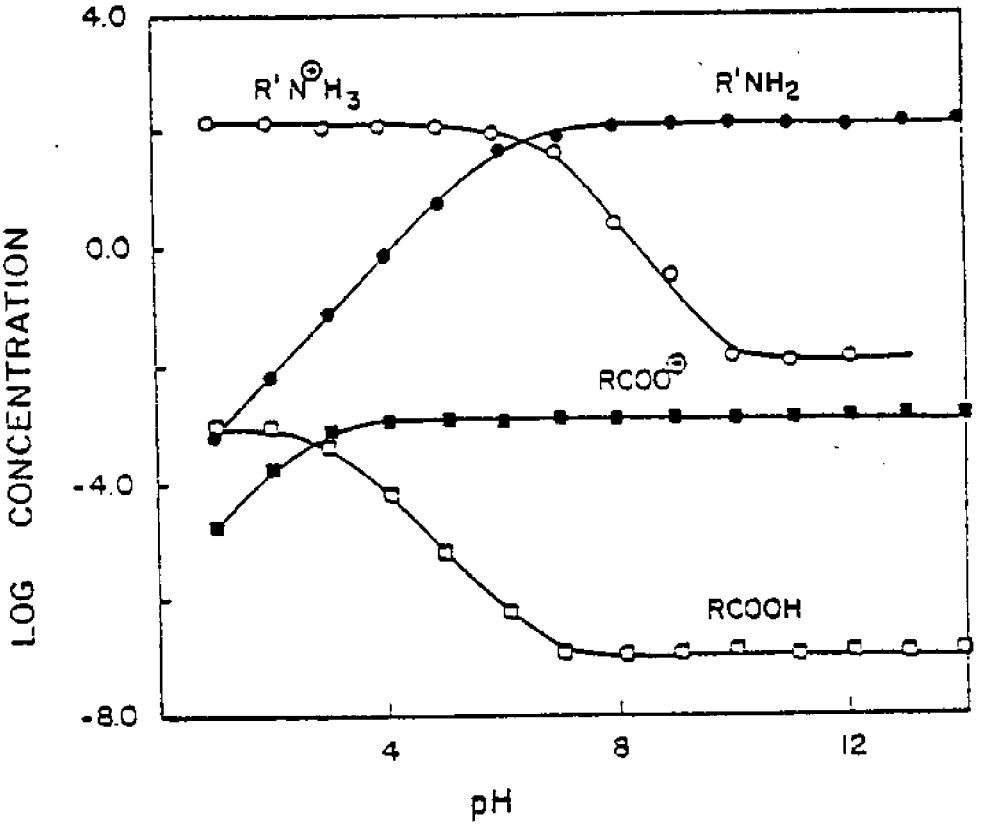


FIGURE 10

WHAT IS THE MECHANISM OF THE REACTION?



$$RNH_2 + H^{\oplus} \rightleftharpoons RNH_3^{\oplus} \qquad [1]$$

$$R'COO^{\ominus} + RNH_3^{\oplus} \rightleftharpoons R'COO^{\ominus}NH_3^{\oplus}R \qquad [2]$$

FIGURE 10

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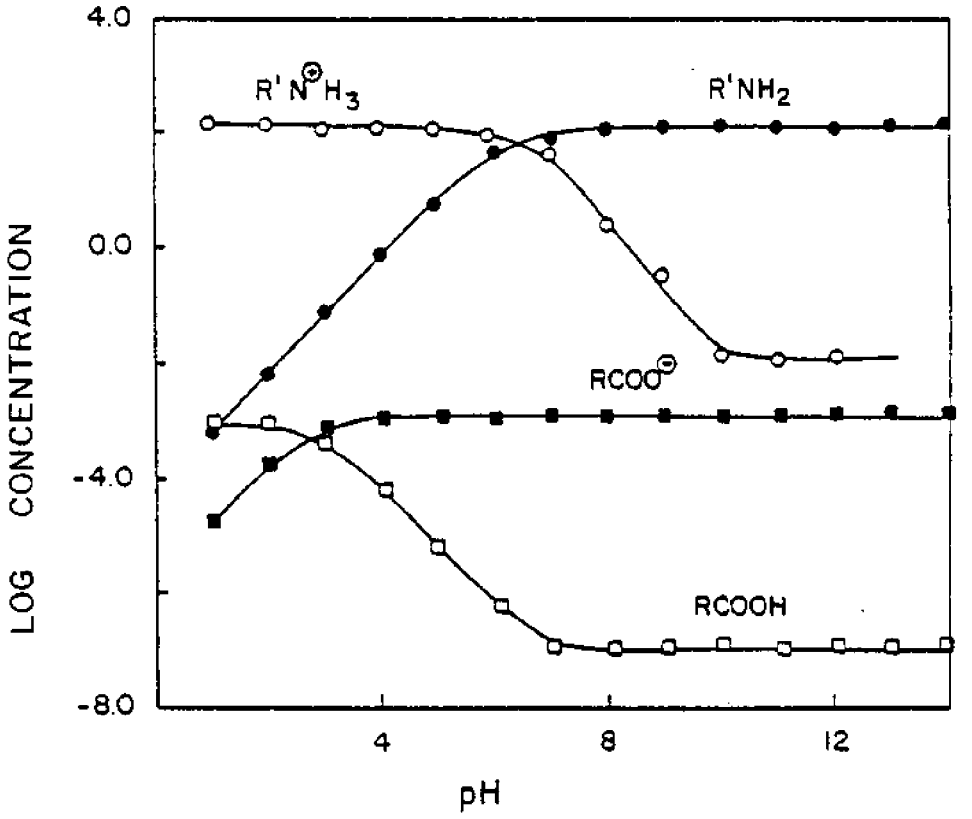

$$RNH_2 + H^{\oplus} \rightleftharpoons RNH_3^{\oplus} \qquad [1]$$

$$R'COO^{\ominus} + RNH_3^{\oplus} \rightleftharpoons R'COO^{\ominus}NH_3R^{\oplus} \qquad [2]$$

FIGURE 11

UPTAKE WITH AND WITHOUT

PRE-ACID TREATMENT