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Removing of Organophosphorous Pesticides from Water Using Different Prepared Chitosans Obtained from Shrimp Wastes.

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ABSTRACT

Chitosans have been prepared from shrimp wastes during four processes: demineralization (M), decoloration (C), deproteination (P) and deacetylation (A). Five chitosan samples (I – V) were obtained by change the sequence of the four preparation processes. The varied properties of the samples were determined by measuring: yield, moisture content, ash content, viscosity and surface area. The structure features were examined by FTIR, SEM and XRD techniques. Thermal gravimetric analysis was also carried out. Removal activities of the five samples were tested for pesticides, extensively used in Egypt. Sample I (MPAC) was a well adsorbent for dimethoate and pyrimiphos pesticides sample II (PMCA) for chlorpyrifos and sample V (CMPA) for malathion and diazinone.

Keywords: Shrimp waste, Chitosan, organophosphorous pesticides, waste water

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INTRODUCTION

The enormous use of pesticides by human beings in agriculture as well as in the other spheres of life has added these compounds as pollutants [1,2] . Indiscriminate use of pesticides in agriculture activities lead to contamination of surface as well as ground water accumulation [3]. The pesticides are among the most hazardous for the human beings [1,4] The studies have shown the association between certain types of cancers and suppress the immune system [1,5]. Organ-phosphorous pesticides of high toxicity are widely used in agriculture throughout the world [3,6] . They found extensive application in Egypt for controlling sucking and chewing insects including mosquitos, aphid, turf insects for many floral and vegetable crops and fruits.

Several methods were conventionally used to remove the pesticide pollution from water such as, precipitation, ultra -filtration, reverse osmosis, or electrochemical treatment. All these treatments are found to be either inadequate or expensive [3,7].

Some research activities based on adsorption phenomena are going on to reduce pesticide contamination in water [3] . A large number of low-cost materials including industrial and agricultural wastes have been used in the removal of different pesticides from the aqueous solutions. Activated carbon is one of the widely used material for the removal of organic pollutants [8].

Chitosan is a natural coagulant and its coagulation properties are very effective. Its coagulation and flocculation properties can be used to remove particulate inorganic or organic suspensions, and also dissolved organic substances from polluted water [9,10,11,12]. Chitosan is highly biocompatible and easy biodegradable. High stability, recoverability and reutilization are among of the advantages of chitosan [1] . Chitosan was defined as a linear hetero-polysaccharide composed of randomly distributed β-1,4-linked-D-glucosamine and N-acetyl-glucosamine in vary proportions [13]. Its chemical functional groups OH and NH_2 make it an ideal adsorbent in the treatment of waste water. At low pH the primary amines are protonated and positively charged and chitosan behaves as a water-soluble cationic poly-electrolyte [14]. Chitosan is produced from chitin found in the skeleton of crustaceans, as well as in the exoskeleton of marine zooplanktons. Insects as butterflies and ladybugs, also have chitin in wings. Cell wall of yeast and other fungi also contain this substance [15 , 16].

The industrial-scale production of chitosan involves four steps: demineralization, deproteination, decoloration and deacetylation. They evaluated the various changes in characters of the produced chitosans caused by the changes of the four sequential preparation processes [15] . Researchers reported that chitosan through the amine groups contributes largely in the adsorption of organophosphorous pesticides [1,3] , and of organochlorine pesticides [9].

The present work is focused on: (i) preparation and characterization of five chitosan samples using change the sequential preparation processes and (ii) application of these different chitosan samples on removing of the organophosphorous pesticides: malathion, dimethoate, chlorpyrifos, diazinone, and pyrimiphos-methyl from water.

EXPERIMENTAL

Materials and Methods

Chemicals

The pesticides, malathion, dimethoate, chlorpyrifos, diazinone, and pyrimiphos-methyl were obtained from Sigma-Aldrich Inc. (Germany), Other chemicals and reagents were of analytical grades.

Raw materials

Dried shrimp shell waste was obtained from local market, shells were cleaned from debris, sand and salt crystals according to the standardized conditions described by [17].Shells were washed several times in fresh water, dried at 60 ^{o}C, overnight in a forced air oven and ground to powder with a cutting mill and stored at zero degree for as long as needed.

Preparation of chitosan samples

Preparation was carried out through four processes: demineralization (M) by treating the raw material with 0.7 N HCl at room temperature for 15 min, deproteinization (P) by treating with 1.2 N NaOH for 2.5 hr at 70 – 75 ^{o}C, decoloration (C) by soaking in acetone for 10 min and drying for 2 hr under hood, followed by bleaching with 0.32 % sodium hypochlorite solution for 15 min at ambient temperature and deacetylation (A) by treating the material with 50% NaOH at 15 psi / 121 ^{o}C for 15 min. After each process the solid was filtered off, washed with distilled water to neutral pH and dried in oven at 60 ^{o}C overnight.

Five shrimp chitosan samples labeled MPAC (I); MPCA (II); PMCA (III); MCPA (IV) and CMPA (V) were prepared by changing the four sequential preparation processes.

Characterization

Deacetylation degree (DD)

It was determined for the five chitosan samples by the potentiometric titration method described by Brous – signac, reported by [18] .Chitosan solution in a known excess of HCl was titrated with 0.1 M NaOH solution, a curve with two inflexion points was obtained. The degree of deacetylation was determined through equation: % NH_2 = 16.1 ($V_2 - V_0$) x M_b / W, where V_0 and V_2 are the base volumes referred to first and second inflexion points, respectively, in ml, M_b is the base molarity in g / mol, W is the original weight of the polymer in g.

Viscosity measurements

It was determined with a Brookfield viscometer (Brookfield Engineering Laboratories). The chitosan sample was dissolved in 1% acetic acid and the measurement was carried out using a No. 5 spindle at 50 rpm on solution at 25 ^{o}C with values reported in centipoises (cPs) units.

Surface area

It was calculated according to the method of [19] .depending on the amount of adsorbed methylene blue on a certain quantity of chitosan sample. The adsorption was determined spectrophotometrically by CECCIL Ce 7400 UV- vis instrument at λ= 660 nm. The surface area was calculated using the equation: $A_s = G\ N_{Av}\ Ø\ 10^{-20} /\ M\ M_w$ where A_s is the chitosan surface area in m^2/g, G is the amount of adsorbed methylene blue (g), N_{Av} is the Avogadro's number (6.02×10^{23}), Ø is the methylene blue molecular cross section (197.2 Å), M_w is the molecular weight of methylene blue (319) and M is the mass of the adsorbent (g).

Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)

They were measured using the method of [20] .The procedure was carried out by weighing a centrifuge tube containing 0.5 g sample, adding 10 ml of water or corn oil and mixing on a vortex mixer for one minute. The contents were left at ambient temperature for 30 min then centrifuged at 3200 rpm for 30 min. The supernatant was decanted, the tube was weighed again and the percentage of the bound water or fat was calculated.

FTIR Spectroscopy

The Fourier transform infrared (FT-IR) spectral studies were performed with NICOLET IS 10 instrument. The samples were mixed uniformly with potassium bromide and the obtained discs were scanned in the range of 400 – 4000 cm^{-1}.

Scanning Electron Microscope (SEM)

The prepared chitosan samples were examined by a JEOL JSM- 4510, scanning electron microscope from Japan. All the samples were coated with gold before SEM testing.

X- ray Diffraction (XRD)

The XRD patterns were recorded on a Philips PW 3050 / 10 model. The analysis was applied to detect the crystallinity of the prepared samples. The relative crystallinity of the polymers was calculated by dividing the area of the crystalline peaks by the total area under the curve.

Thermal Analysis

Thermo-gravimetric analysis (TGA) of the prepared chitosan samples were carried out in a nitrogen atmosphere using TGA. SDT-Q600 SIMULTANOUS (DSC – TGA), USA with a heating rate of 10 ^{o}C / min. The flow rate of nitrogen was adjusted to 20 cm^3 / min.

General procedure for the removal of pesticides

The five prepared chitosan samples were tried to determine their efficiency to remove organophosphorous pesticides from artificial waste water by the column elution technique. A chitosan sample (1g) was loaded into a glass column (30 cm x 1.8 cm) and washed with 25 ml distilled water. Water sample (500 ml) cotaining 14.68, 31.95, 16,16, 13.61 and 14.31 mg of malathion, dimethoate, chlorpyrifos, diazinone, and pyrimiphos-methyl respectively was passed through the column at flow rate 5 ml / min. The elute (100 ml) was extracted several times with dichloromethane and the extract was dried with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The final residue was dissolved in ethyl acetate for GC analysis to determine the concentration of the pesticides after adsorption.

The assay was performed on gas liquid chromatograph equipped with electron capture detector GC – FPD. Gas chromatographic analysis of organophospohorous pesticides was conducted on a PAS- 1701 (Agilent, Folsom, CA) fused silica capillary column of 30 m length, 0.32 mm id., o.25 µm film thickness. The oven temperature was programmed from an initial temperature 180 (2 min hold) to 240 ^{o}C at a rate of 10 ^{o}C / min and was maintained to 5 min. Injector and detector temperatures were maintained at 240 and 260 ^{o}C respectively. Nitrogen was used as a carrier at flow rate of 3 ml /min Hydrogen and air flow rate were 75 and 100 ml / min respectively. Peak was identified by comparison of sample retention time value with those of the corresponding of pure standard compound.

Statistical Analysis

Each value represents the mean of the three independent experiments performed in duplicates, with average deviations of < 5%.

RESULTS AND DISCUSSION

Five chitosan samples (I – V) were prepared from shrimp shell waste through change the sequence of the four preparation processes: demenralization (M), decoloration (C), deproteinization (P), and deacetylation (A). Values of yield of each of them, as percentage of dry weight of the obtained chitosan compared with the dry weight of raw material, are presented in Table 1. The close yield results observed in this study may be due to keeping the deproteinization and deminerlization steps always prior the deacetylation, which keep the chitin polymer intact and prevent the breakdown of polymer chain.

Moisture and ash contents were determined according the standard method [21], and presented in Table 1. Generally, no significant differences were noticed in the moisture contents of the samples, ranged from 6.03 to 6.98 %. According to [22] , commercial chitosan products contain less than 10 % moisture content. The ash contents in chitosan is an important parameter, some residual ash of chitosan may affect their solubility, consequently contributing to lower viscosity. The sample II has 0.74 % ash content and it should be considered as a high quality grade chitosan.

Degree of deacetylation (DD)

The degree of deacetylation of the obtained chitosan samples ranged from 80.86 to 86.93 % with an average of 82.73 %, table 1. Our results are quite similar to those of [23], who reported DD of chitosan ranged

from 56 % to 99 % with an average of 80 %. [24] , reported that deacetylation value are dependant on the type of analytical method employed, sample preparation, type of instrument used, and other conditions. It was noticed an increase in DD if the deproteinization process was done directly prior deacetylation at the end of preparation (samples IV and V).

Table 1: Yield, moisture content, ash content and degree of deacetylation (DD) of chitosans produced by different preparation processing sequences.

Chitosan Sample	Yield %	Moisture content %	Ash content %	DD %
MPAC (I)	16.05^{a}	6.45 ^{a}	0.94^{ab}	80.86^{e}
MPCA (II)	14.17^{b}	6.53 ^{a}	0.74^{b}	83.45^{c}
PMCA (III)	13.63^{b}	6.98 ^{a}	1.10^{a}	82.08^{d}
MCPA (IV)	15.66^{a}	6.18^{a}	1.14^{a}	84.65^{b}
CMPA (V)	16.1^{a}	6.03 ^{a}	0.97^{ab}	86.93^{a}

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.01$), while the same letters indicate non significant variation.

Viscosity

The viscosity of chitosan solution reported in the literature generally ranged from 60 to 780 cp, [25] . This ranges of viscosities have been also observed by [26] with five commercially available chitosanes. Sample V showed the highest viscosity (212 cp), while samples I and II showed the lowest value (41 cp), Table 2. Generally, there are some factors affecting viscosity during the production of chitosan such as the degree of deacetylation, molecular weight, concentration, ionic strength, pH, temperature and others. [27] stated that it is not desirable to bleach the material at any stage since bleaching considerably reduces the viscosity of the final chitosan products. In our study deproteinization should be done after decoloration to obtain high viscosity.

Table 2: Viscosity, surface area , water binding capacity (WBC) and fat binding capacity (FBC) of chitosans produced by different preparation processing sequences.

Chitosan Sample	Viscosity cp.	Surface area m^2/g	WBC	FBC
MPAC (I)	41^{d}	50.79^{a}	586^{c}	455^{d}
MPCA (II)	41^{d}	54.36^{a}	687^{b}	647^{b}
PMCA (III)	67^{c}	28.07^{d}	691^{b}	539^{c}
MCPA (IV)	155^{b}	48.19^{b}	837^{a}	759^{a}
CMPA (V)	212^{a}	39.84^{c}	815 ^{a}	692^{ab}

Each value is the mean ± SD. Means have different superscript letters indicate significant variation at ($P \leq 0.01$), while the same letters indicate non significant variation.

Surface area

The surface area of the prepared chitosan samples ranged from 28.07 to 54.36 m^2 /g with an average of 44.26 m^2 /g, table 2. The results indicated that when the demineralization was done prior to the other steps, the prepared chitosan had the highest surface area, may be due to smaller particle size. Deproteinization before demineralization led to decreased surface area.

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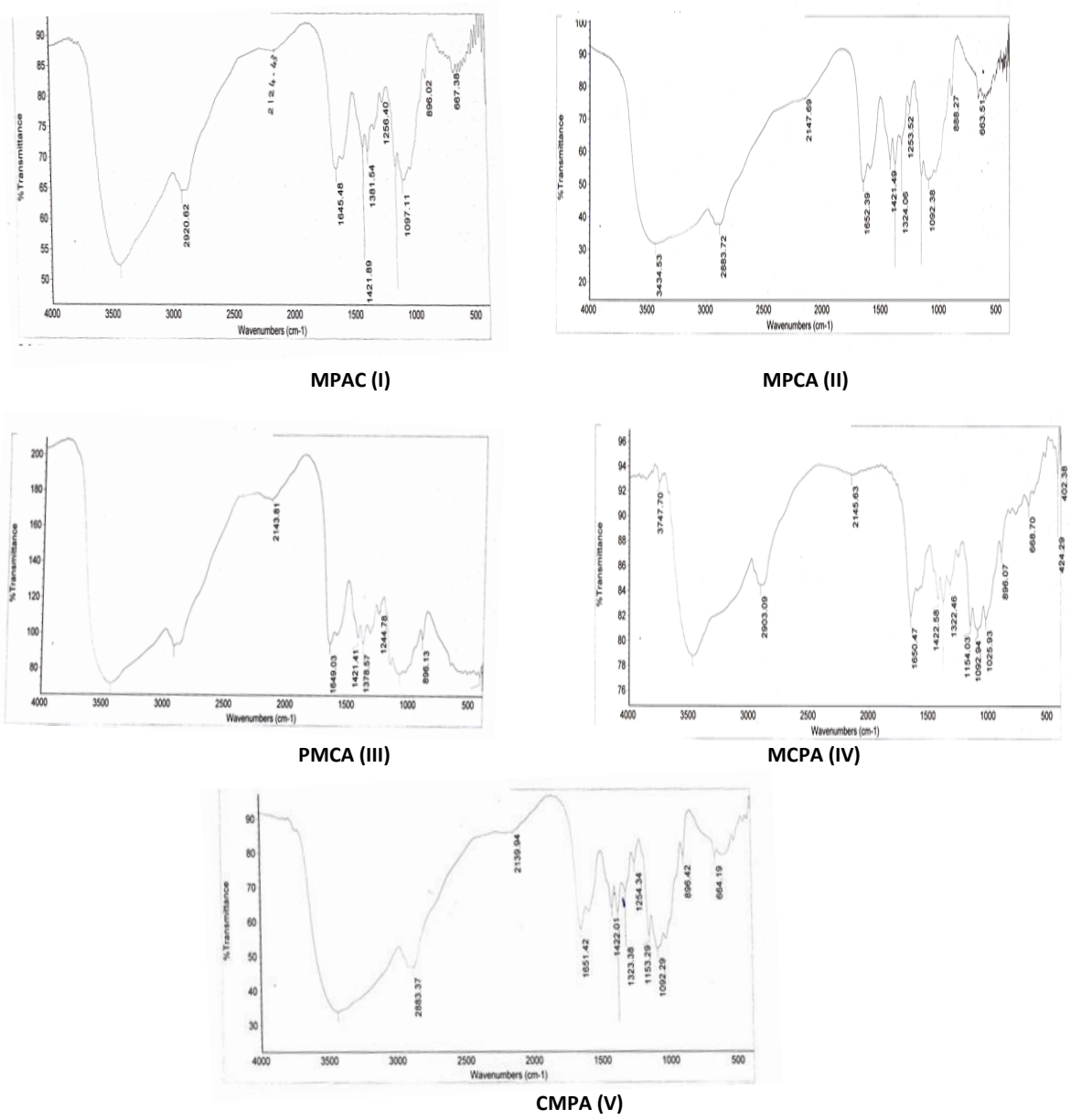
Water Binding Capacity (WBC) and Fat Binding Capacity (FBC)

The water binding capacity of the prepared chitosan samples ranged from 586 to 837 % ,table 2 . These values were in agreement with those reported by **[**28] **,** where WBC for chitosans ranged from 581 to 1150 % with an average of 702 %.

Fat binding capacity differed among the chitosan samples, ranging from 455 to 759 % table 2. Increasing viscosity is probably the main factor to increase both WBC and FBC.

FTIR analysis

The FTIR spectra of the prepared chitosan samples show the absorption bands at between 3438 and 3290 cm^{-1} which are characteristics of hydroxyl and amine groups, respectively. The absorption bands that appear at between 2920 and 2883 cm^{-1} are due to the alkyl chains. The amide carbonyl bands appear at the regions around 1650 cm^{-1} and around 1380 cm^{-1}. The strong bands at the range of 1075 and 1092 cm^{-1} correspond the C – O bond, which are the characteristic peaks for polysaccharides.



MPAC (I)

MPCA (II)

PMCA (III)

MCPA (IV)

CMPA (V)

Figure 1: FTIR of chitosans produced by different preparation processing sequences.

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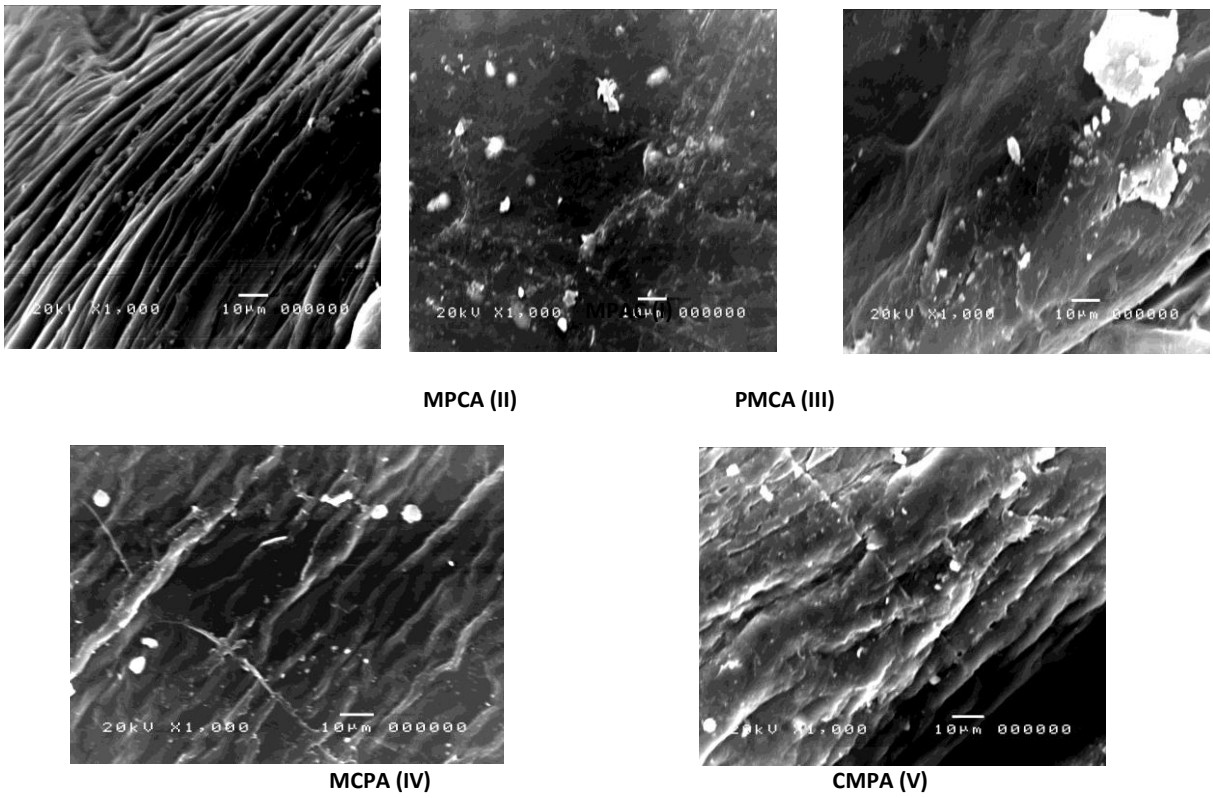
SEM analysis

The topographical features of the five prepared chitosan samples were examined using scanning Electron Microscope (SEM). SEM micrographs were presented in Figure 2. These topographical changes can be attributed to the changes of the sequential processes of the preparation. Sample V showed rough surface more than the other samples. Decoloration after deacetylation in sample (I) leads to weaken the internal forces between polysaccharide chains including hydrogen bonding to give a surface having separated polymer chains.



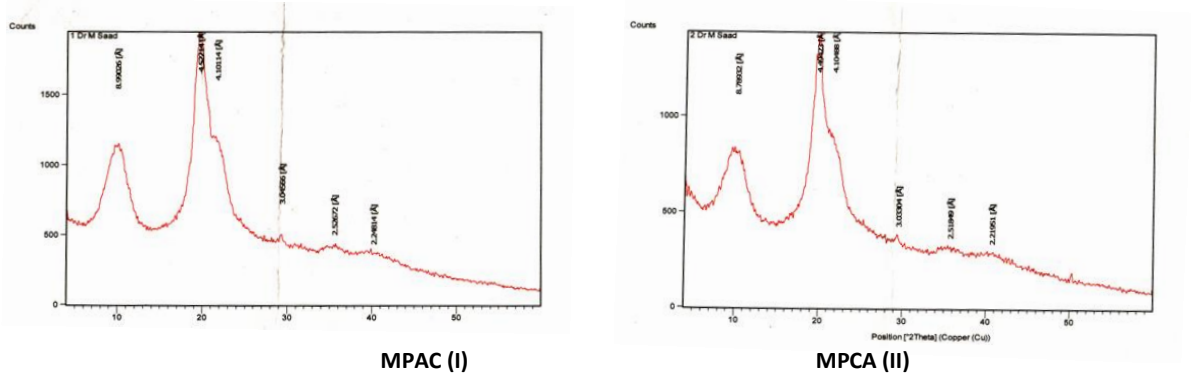
MPCA (II) **PMCA (III)**

MCPA (IV) **CMPA (V)**

Figure 2: SEM of chitosans produced by different preparation processing sequences.

X-ray diffraction

In Fig. 3, the X-ray diffraction patterns of the five prepared chitosan samples are illustrated. All chitosan samples show strong reflections at 2θ around 9.8 – 10.1° and 2θ of 19.5 – 22°. The band at 9.9° corresponds to a *d* spacing of 8.92 Å and is due to the incorporation of bound water molecules into the crystal lattice [29] .The reflection at 2θ 19.4 – 20° corresponds to a *d* spacing of about 4.41 Å [30] . X–ray diffraction (XRD) analysis was applied to detect the crystallinity of the prepared chitosan samples. Relative mass crystallinity of the prepared samples are listed in Table 3.



MPAC (I) **MPCA (II)**

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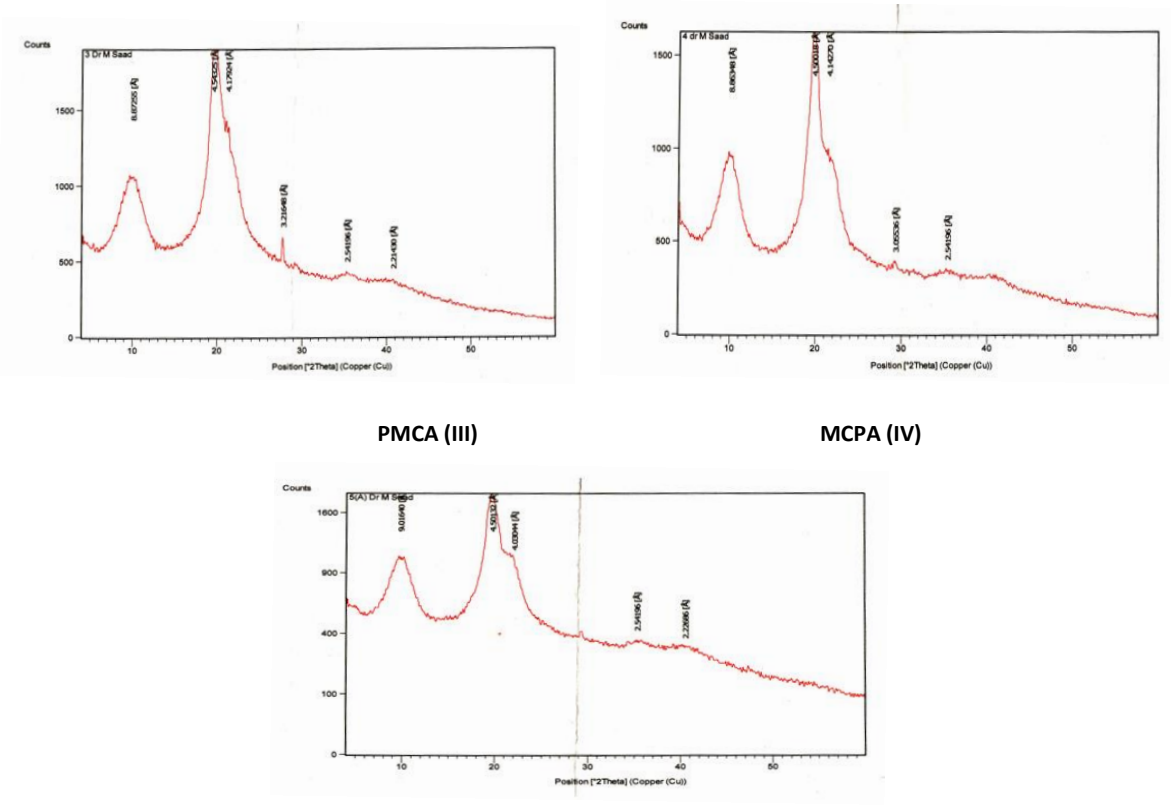


PMCA (III) **MCPA (IV)**

CMPA (V)

Figure 3: X-ray of chitosans produced by different preparation processing sequences

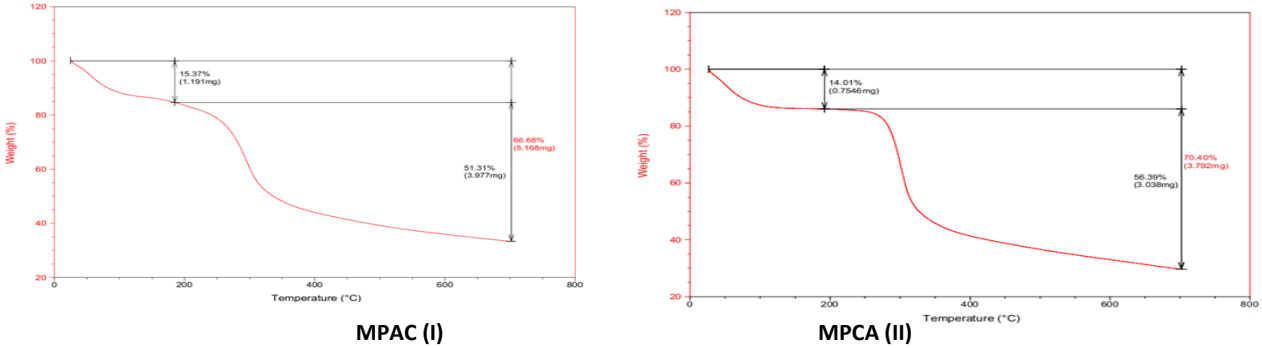
Table 3: Crystallinity of chitosans produced by different preparation processing sequences.

Chitosan sample	DMPAC (I)	DMPCA (II)	DPMCA (III)	DMCPA(IV)	DCMPA (V)
% Crystallinity	40.12	75.00	47.87	14.25	54.11

* Crystallinity percent was calculated by dividing the area of the crystalline peaks by the total area under the curve

Thermal analysis

Thermal gravimetric analysis (TGA) curves of prepared chitosan samples are shown in Fig. 4. Two endothermic peaks are observed. The first peak appears around 90-99 °C corresponds to loss of water. The second peak appears at 291.47 °C (0,01397 °C min/mg) , 294.89 °C (0.03502 °C min/mg) , 290.43 °C (0.03306 °C min/mg) , 293.67 °C (0.01298 °C min/mg) , 297.18 °C (0.04129 °C min/mg) for samples from I to V respectively. The mass decrease in this step is caused by strong decomposition of the polymer, including dehydration of the saccharide rings, depolymerisation and decomposition of the acetylated and deacetylated units. This results showed that sample V is the most thermally stable and the rate of its mass decrease needs higher temperature.



MPAC (I) **MPCA (II)**

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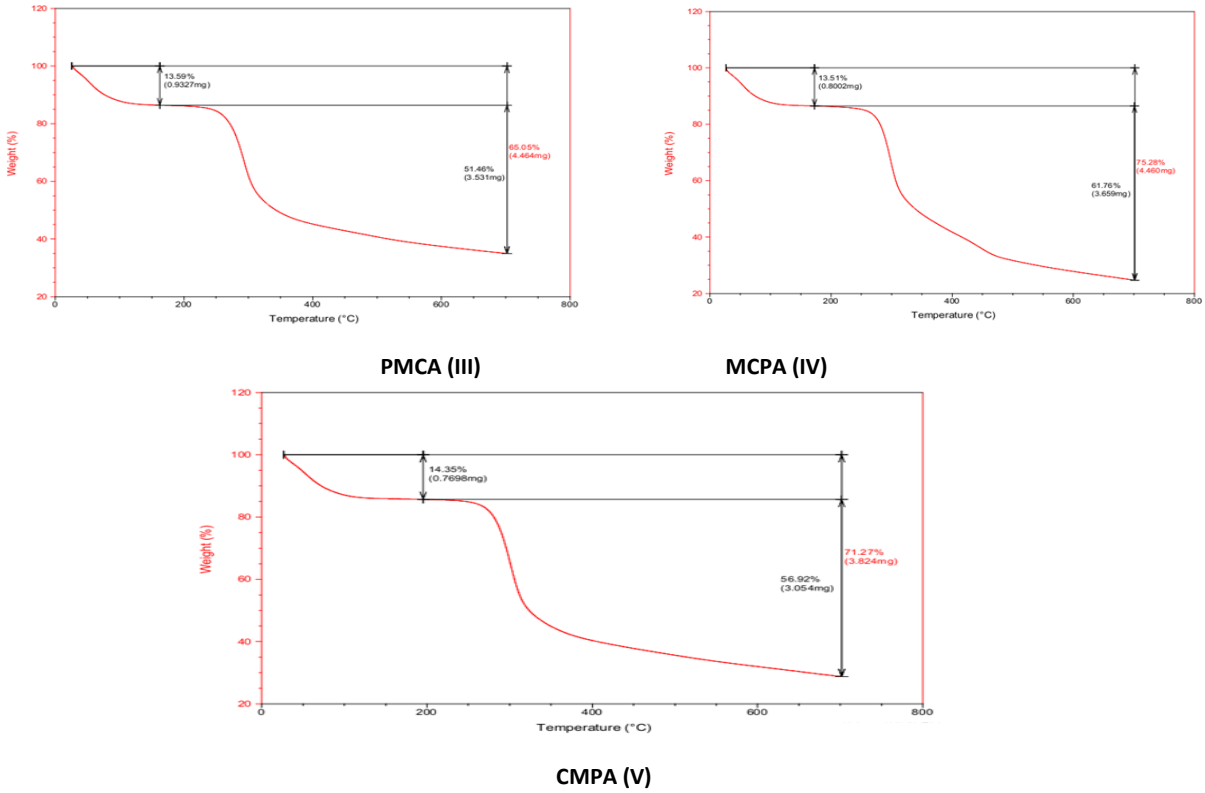

Figure 4: TGA of chitosans produced by different preparation processing sequences.

Pesticide removal

Removing of the pesticides, malathion, dimethoate, clorpyrifos, diazinone and pyrimiphos- methyl by the five prepared chitosan samples was determined with gas chromatography. The procedure was described in the experimental part. A gas chromatogram (Fig. 5) defines the retention times of each pesticide when tested in a mixture.

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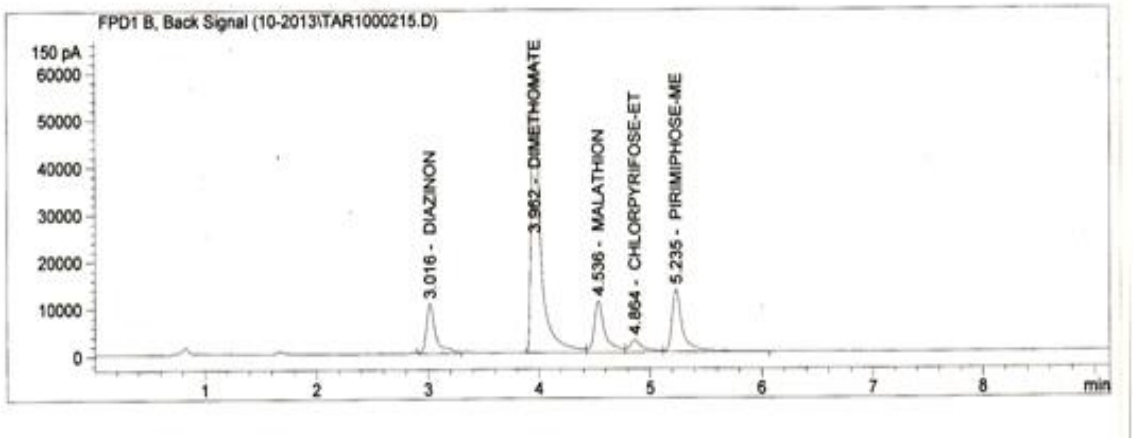

Figure 5: Retention times of organophosphorous pesticides tested in a mixture

Adsorption is a process highly dependent on the chemical structure of the pesticide [1]. Amine groups of chitosan are mainly responsible for chemical interaction between the pesticide and the polymer. Electrostatic and dipole interaction may play an important role in the enhancement of the adsorpitivity [3].

Table 4 shows the removing capacities of the chitosan samples (I – V) for the tested pesticides. The results indicated that: i) chlorpyrifos was maximally adsorbed on chitosan sample II having the largest surface area (54 m^2 / mg) and crystallinity percent, this may be attributed to the presence of several chlorine atoms on the structure which need more electrostatic attraction from the wide area of the polymer. ii) Malathion and diazinone were well adsorbed on chitosan sample V having high viscosity and an elevated degree of deacetylation. These pesticides have relatively low net negative charge due to the presence of several withdrawing groups in their structures. Due to high concentration of amine groups of chitosan and high molecular weight causing high viscosity, chitosan sample V take the advantage to remove malathion and diazinone in a good quality. iii) Dimethoate and pyrimiphos- methyl contains several methyl groups in their structures enhancing their net negative charges due to hyperconjugation and electron repelling properties. Chitosan sample I, with medium characters, was the best polymer to adsorb these two pesticides.

Chitoisan sample IV, prepared through decoloration prior deproteinization and deacetylation showed bad removing propertied may be due to low crystallinity percent.

Table 4: The removing capacities of the chitosan samples (I – V) for the tested Pesticides

Pesticides	Chitosan samples removal percent %				
	MPAC (I)	MPCA (II)	PMCA (III)	MCPA (IV)	CMPA (V)
diazinone	40.41	37.50	39.23	18.66	43.63
dimethoate	39.18	24.51	20.84	0.00	24.94
Malathion	48.91	41.85	48.68	36.44	54.90
Chlorpyrifose-ethyl	77.72	79.76	67.38	70.48	73.36
Pyrimiphose-methyl	37.52	28.86	28.49	6.21	33.05

CONCLUSION

Change of sequence of the four preparation processes lead to obtain five different chitosan samples (I – V) with different properties. Characterization was carried out by using FTIR, SEM, XRD and TGA analysis. Yield, moisture content, ash content viscosity and surface area were also determined. The adsorptivity of the polymer is highly dependent on the chemical structure of the pesticide. Sample II having large surface area was convenient for removing chlorpyrifos. Sample V ,with high deacetylation degree and high viscosity was suitable to remove malathion and diazinone, while sample I adsorbed dimethoate and pyrimiphos properly

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