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Synthesis and Characterization of *N*-methylenephenyl Phosphonic Chitosan

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Chitosan is a natural based polymer obtained by alkaline deacetylation of chitin, exhibiting excellent properties such as non-toxicity, biocompatibility and biodegradability. *N*-Methylenephenyl phosphonic chitosan (NMPPC) is synthesized from chitosan by reacting with phenyl phosphonic acid using formaldehyde. The NMPPC was characterized by FTIR, ^{31}P-NMR, X-ray diffraction, scanning electron microscopy, thermogravimeteric analysis and solubility studies. A significant decrease of molecular weight was observed in the NMPPC. The TGA studies suggested that NMPPC has less thermal stability than chitosan. The X-ray diffraction analysis showed that NMPPC was amorphous in nature. The solubility property of the polymer was improved after the incorporation of a phenyl phosphonic group.

Keywords: NMPPC; chitosan; ^{31}P-NMR; thermal properties; X-ray diffraction

1 Introduction

Chitosan, the fully or partially deacetylated form of chitin, the principal component of living organisms such as fungi and crustaceans, contains 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose groups. This polymer is known to be non-toxic as well as being enzymatically biodegradable. Much attention has been paid to its biomedical, ecological, and industrial application in the past decades. Chitosan and its derivatives have been reported to be useful biomedical applications such as wound healing and dressings, drug delivery agents, anti-cholesterolemic agents, blood anti-coagulants, anti-tumor agents, and immunoadjuvants (1). Chemical modification of chitosan to generate new bifunctional materials is of prime interest because the modification would not change the fundamental skeleton of chitosan, would keep the original physicochemical and biochemical properties and finally would bring new properties depending on the nature of the group introduced. Several techniques to obtain phosphate derivatives of chitosan have been proposed due to interesting biological and chemical properties of such compounds, in addition, it could exhibit bactericidal (2) and metal chelating properties (3). Introduction of groups such as phosphonic acid or phosphanate onto chitosan by reacting phosphorylating agent onto the amino groups are known to increase the chelating properties (4–6) of chitosan and could modify its solubility properties.

Phosphorylation of hydroxyl functions of chitosan to give phosphonate has been studied according to two main methods. On one hand, the reaction is carried out between chitosan hydroxyl functions and phosphorous pentoxide functions in the presence of methane sulphonic acid (7–10). On the other hand, the chitosan, the chitosan hydroxyl functions are reacted with phosphoric acid in the presence of urea (11). The use of such derivatives concerns mainly biomedical (9, 11), metal chelating fields (7–10) and drug delivery (12). Phosphate derivatives of chitosan may also be obtained by interpolymer linkage of chitosan with tripolyphosphate or polyphosphate (13–15). Few works deal with the introduction of α-aminomethylphosphonic acid functions onto chitosan using the Kabachnik-Fields reactions (16, 17) in the spite of the interest of such groups (4–6). Previously, the phosphorylation of chitin was carried out and reported by the P_2O_5/CH_3SO_3H or H_3PO_4/Urea/DMF method (9). By using strong methanesulphonic acid, the purification of the polymer was very difficult. From our method, it is very easy to purify the polymer.

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The present paper describes the synthesis, characterization and thermal properties of novel *N*-methylenephenyl phosphonic chitosan (NMPPC) for the purpose of creating finely designed biomedical materials.

2 Experimental

2.1 Materials

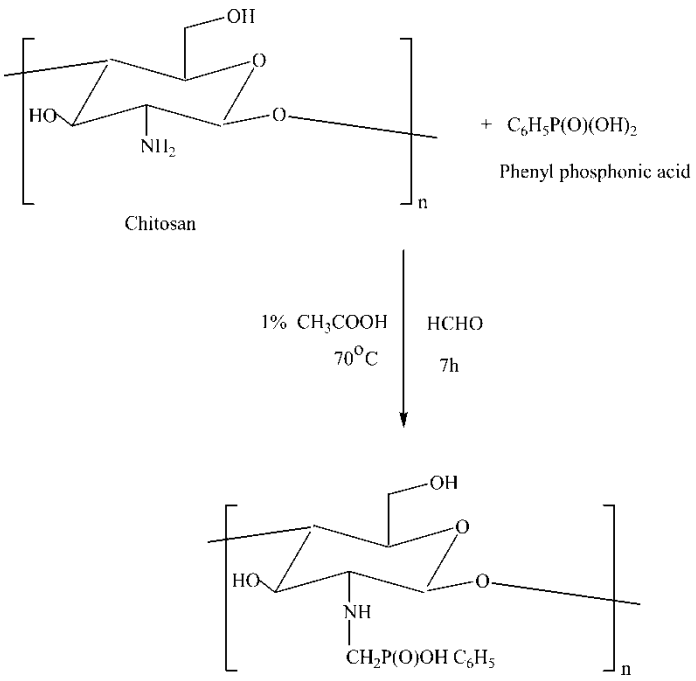
Chitosan (M_w − 48000, deacetylation degree 75–85%), was received from Aldrich. Phenyl phosphonic acid and formaldehyde (37%) were received from Alfa Aesar Company. All other materials used were of analytical grade.

2.2 Synthesis of NMPPC

Chitosan solution 2% (w/v) in glacial acetic acid 1% (v/v) was prepared. One part (by weight) of chitosan was used and one part of phenyl phosphonic acid (by weight) dissolved in water was added dropwise with continuous stirring for 2 h. Then the reaction temperature was raised to 80°C. One part of formaldehyde (37% by weight) was added to this reaction mixture dropwise for 1 h with reflux. Then the reaction was allowed to remain for 6 h at the same temperature. The obtained product was dialyzed against demineralized water for 48 h or until the pH of water was raised to 6.8 in dialysis tubing with a cut-off value of 2500 Da. Finally, the product was subjected to freeze-drying. The product was dried in avacuum oven at room temperature for 48 h. The synthesis of NMPPC is shown in Scheme 1.

2.3 Characterization Techniques

The IR spectra of the polymers were recorded in a Perkin-Elmer FT-IR 2000 series spectrophotometer at room temperature using the KBr pellet method. The ^{31}P-NMR spectra of the polymers were recorded with a JEOL JMN-GSX-400 MHz spectrometer in D_2O using 85% H_3PO_4 as a reference standard. The molecular weight of the polymer was determined by using GPC Hittachi L-7490 chromatography. Thermogravimetric analysis (TGA) was performed with a SII TG-DTA 6200 thermal analyzer using 2 mg of the sample at a heating rate of 10°C/min in nitrogen. The surface morphology of samples was analyzed by scanning electron microscopy (SEM) using a JEOL JSM-6700 microscope. X-ray diffractions were recorded according to a powder method with a Mac Science M_3X (Model No. 1030) diffractometer using CuKα radiation. The solubility of the polymers was tested in various polar and non-polar solvents by taking 10 mg of polymers in 2 mL of different solvents in a closed test tube and set aside for one day. The solubility of the polymers was noted after 24 h. The phosphorous content was determined spectrometrically by the Kjeldhal method (18, 19). The degree of substitution was calculated as previously reported (20).



Sch. 1. Synthesis of NMPPC.

3 Results and Discussion

3.1 Synthesis of NMPPC

NMPPC was synthesized by using phenyl phosphonic acid and chitosan with formaldehyde. The introduction of the phosphonic acid function in the chitosan macromolecule via the Moedritzer and Irani (20) reaction was followed in the preparation of NMPPC. When the temperature of the reaction was increased to above 70°C, the yield of the product decreased. The yield of the NMPPC was 73%. The molecular weight of the NMPCC is 18500. The molecular weight of the NMPPC was decreased to 59% compared to chitosan molecular weight. The degree of substitution was found to be 0.72. It confirms the phosphorylation occurs onto chitosan. The solubility data of chitosan and NMPPC in different solvents are shown in Table 1. The incorporation of phenyl phosphonic group was increased the solubility properties of the polymer.

3.2 Characterizations

3.2.1 FT-IR

The FTIR spectrum of chitosan (Figure 1a) showed a broad-OH stretching absorption band between 3450 and 3100 cm^{-1} and the aliphatic C-H stretching between 2990 and 2850 cm^{-1}. As the -OH stretching band and the aliphatic C-H stretching band were appeared as a broad band from 3450 and 2850 cm^{-1} in the spectrum, another major absorption band between 1220 and 1020 cm^{-1} represented the free primary amino group (-NH_2) at C_2 position. The peak at 1647 cm^{-1} represented the acetylated amino group of

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Table 1. Solubility data of chitosan and NMPPC

Solvent	Chitosan	NMPPC
H_2O	Insoluble	Soluble (slowly)
NaOH (1%)	Insoluble	Low viscosity gel
HCl (1%)	Swelling	Soluble (slowly)
Acetic acid (1%)	Soluble	Soluble (slowly)
Dimethyl acetamide	Swelling	Swelling
Dimethylformamide	Swelling	Swelling
Dimethylsulfoxide	Insoluble	Insoluble
Pyridine	Swelling	Low viscosity gel
Acetone	Insoluble	Insoluble
Ethanol	Insoluble	Insoluble
Dichloromethane	Insoluble	Swelling (slightly)

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chitin, which indicated that the sample is not fully deacetylated. The peak at 1384 cm^{-1} represents the -C-O stretching of the primary alcoholic group (-CH_2-OH). The FTIR spectrum of NMPPC (Figure 1b) shows a broad peak at 3407 cm^{-1}, which represents P-OH and -NH groups. The peaks at 3058 and 693 cm^{-1} are due to the aromatic C-H stretching group, peaks at 1631 and 1595 cm^{-1} are due to the -$NHCH_2$ group, and the peaks at 1094 and 568 cm^{-1} are due to the P-OH group. The adsorption peak at 1154 cm^{-1} is due to P-O stretching. The above peaks confirmed the structure of NMPPC.

3.2.2 ^{31}P-NMR

^{31}P-NMR of the NMPP polymers shows a peak at $\delta = 0.93$ ppm, indicating the PO_4 functionalities (9). The ^{31}P-NMR spectrum confirms the successful incorporation of the phosphorous group into the polymer. The ^{31}P-NMR spectrum of NMPPC is shown in Figure 2.

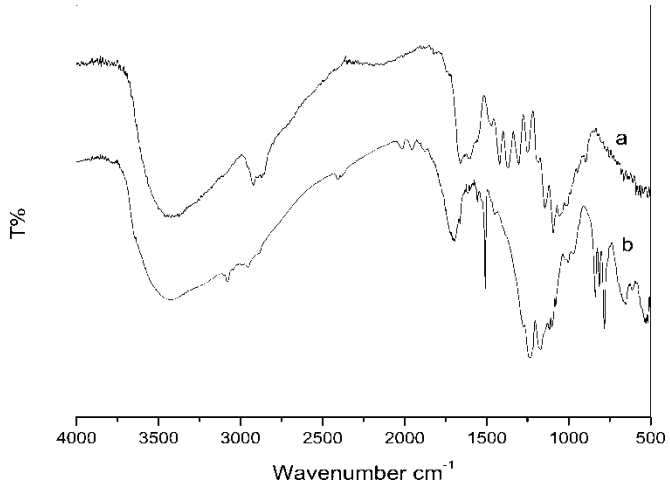

Fig. 1. FTIR Spectra of (a) Chitosan and (b) NMPPC.

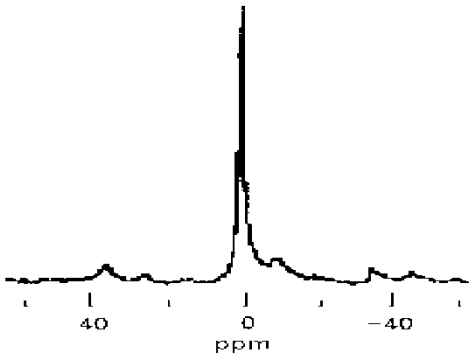

Fig. 2. ^{31}P-NMR spectrum of NMPPC.

3.3 SEM Studies

The SEM pictures of both chitosan and NMPPC are shown in Figure 3. The chitosan has smooth surface morphology. But the NMPPC showed very porous and rough morphology. This may be due to the incorporation of the phenyl phosphonic group. It shows a relatively homogeneous aspect with a tightly packed structure. The surface morphology studies confirmed the chemical modification of chitosan.

3.4 Thermal Properties

Figure 4 shows the thermograms of chitosan and NMPPC. TGA of chitosan (4a) shows a weight loss in two stages. The first stage ranges between 10 and 100°C, this corresponds to the loss of adsorbed and bound water. The second stage of weight loss starts at 210°C and continues up to 360°C due to the degradation of chitosan. However, the TGA of the NMPCC (4b) is different. The latter also has a two stage of weight loss between 10 and 550°C. The first stage of weight loss starts between 10 and 195°C and another weight loss between 195 and 310°C and then degrades very quickly, similar to the original chitosan. This may be due to the phosphorylation. The phosphorylation degraded the crystalline structure of chitosan. The TGA studies suggested that NMPCC has poor thermal stability.

3.5 X-ray Diffraction

Figure 5 shows the XRD patterns of chitosan and NMPPC. The incorporation of phenyl phosphonic group into chitosan sharply decreased the crystalline structure of the polymer. Chitosan shows a diffraction peak at about 20°, which corresponds to crystal forms (21). But NMPPC is not showing any sharp diffraction peaks due to the presence of phenyl group in the polymer backbone. The introduction of substituents into polysaccharide structures should disrupt the crystalline structure of chitosan, especially by the loss of the hydrogen bonding (22). After phosphorylation onto chitosan, the original crystallinity of chitosan was destroyed.

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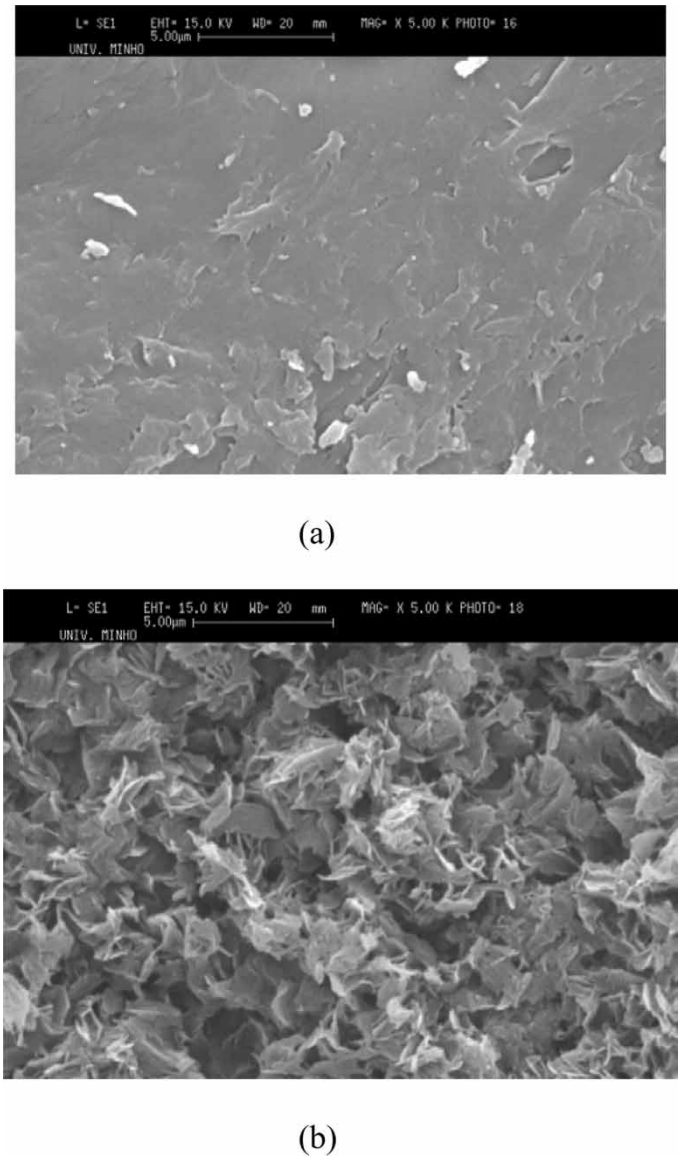

Fig. 3. Surface morphology of (a) Chitosan and (b) NMPPC.

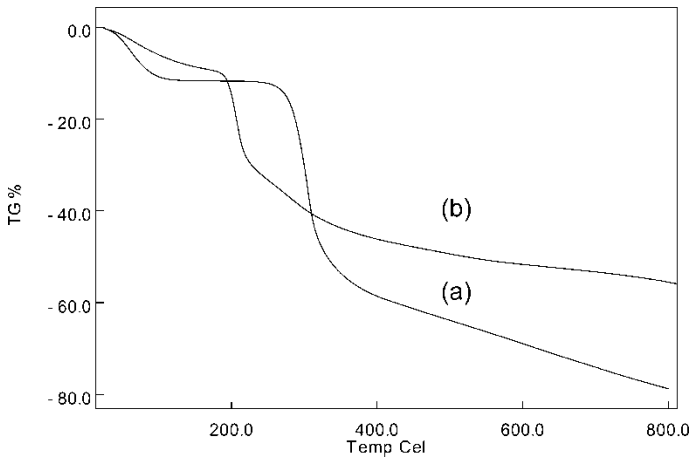

Fig. 4. Thermogram of (a) Chitosan and (b) NMPPC.

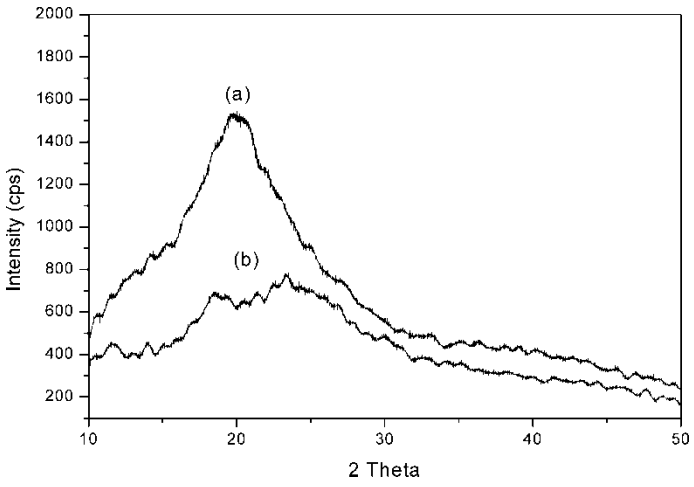

Fig. 5. X-ray diffraction pattern of (a) Chitosan and (b) NMPCC.

4 Conclusions

A new type of NMPPC was prepared by reacting chitosan with phenyl phosphonic acid in the presence of formaldehyde. The prepared NMPPC was characterized by FT-IR, ^{31}P-NMR, XRD, solubility and TGA. The FT-IR and ^{31}P-NMR confirmed the structure of the NMPPC. The solubility of the polymer was improved after incorporating the phosphorous group. XRD studies showed NMPPC was amorphous in nature. The molecular weight of NMPCC was found to be low when compared to the control chitosan. NMPPC is a new type of biomaterial, and is useful for several biomedical applications.

5 Acknowledgement

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