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Synthesis and characterization of chitosan nanoparticles as perspective drug delivery carrier: Study of their degradation, antibacterial activity and *in-vitro* blood compatibility behavior

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ABSTRACT

Chitosan-TPP nanoparticles have been prepared using ionotropic crosslinking method. FTIR results show characteristic peaks of functional groups present in the nanoparticles. XRD studies revealed about 44.6 % crystallinity of chitosan-TPP nanoparticle material. SEM and TEM studies show the spherical shape of the particles with 20-90 nm size. Swelling ratio of the particles in PBS and SBF was found to be in the range of 0.55-3.36 and 2.66- 4.19 respectively. % degradation of the material in PBS and in presence of enzyme papain has been calculated 46.6 % and about 80.0 % respectively. *In-vitro* blood protein adsorption for the nanoparticles was found to be in the range of 0.052-0.351 mg/g and % haemolysis was found to be in between 3.31- 42.1 %. Antibacterial activity of the material was checked against strain of E.Coli and Bacillus Cereus and percent inhibition of bacterial growth was found in the range of 11-35 %.

KEYWORDS

Chitosan;
Nanoparticles;
Antibacterial activity;
Degradation;
In-Vitro blood compatibility.

INTRODUCTION

Biopolymer, chitosan is the N-acetylated derivative of chitin. It is of commercial interest due to its high percent of nitrogen (6.89%) compare to synthetically substituted cellulose (1.25%). Chitosan have excellent properties such as biocompatibility, biodegradability, non-toxicity, adsorption properties etc and it has been potentially using in textiles, membranes and medical aids[1]. Chitosan has also been used as material for enzyme immobilization[2]. Its desirable characteristic for immobilizing enzymes include high affinity to proteins, availability of reactive functional groups for direct reaction with enzymes and for chemical modification, hydrophilicity, mechanical stability and rigidity. Chitosan provides ease of preparation in different geometrical configuration that in turns facilities the system with permeability and surface area suitable for a chosen biotransformation. Much attention has been paid to chitosan as a potential polysaccharide resource. It has been suggested that chitosan may be used to inhibit fibroplasia in wound healing and to promote tissue growth and differentiation in tissue culture[3, 4].

Chitosan has three types of reactive functional

groups, an amino group as well as both primary and secondary hydroxyl group at the C-2, C-3 and C-6 positions respectively. This special structure makes it exhibit chelation with various metal ions[5]. It has chemical modification of these groups and the regeneration reaction gives to various novel bio-functional macromolecular products having the original organization or new types of organization. Chitosan is reported to suppress viral infections in various biological systems. Cationic charges of amino groups in chitosan may have additional functions to activate the immune and defense systems in plants and animals[6]. It is a cationic polysaccharide and has gained increasing attention in pharmaceutical field due to its favorable biological properties[7, 8], mucoadhesive properties[9, 10]. Additionally, chitosan micro/nanoparticles can be easily prepared by ionic gelation method using tripolyphosphate (TPP) as precipitating agent[11]. The advantage of gelation method was attributed to the mild condition without applying harmful organic solvent at room temperature in the procedure, and also could efficiently detain the bioactivity of macromolecules (protein, DNA etc) during the encapsulation.

Systems using crosslinking of chitosan with tri polyphosphate (TPP) have been greatly used in controlled drug delivery studies. TPP is a non toxic salt, obtained from triple condensation of PO_4 groups. It acts by increasing the pH and ionic strength of the solution forming gel and promoting ionic interaction between amino groups of chitosan and anionic groups of TPP[12]. In most of the chitosan-TPP systems chitosan is easily biodegradable with gel forming ability at low pH[13]. Moreover chitosan has antacid and antiulcer activities which prevent or weaken drug irritation in the stomach. Also, chitosan matrix formulations appear to float and gradually swell in an acid medium and TPP does not show any negative effect on these properties of chitosan. All these useful features make chitosan an ideal candidate for controlled drug release formulation[14].

Several chitosan dressing materials have been developed commercially for the healing of human and animal wounds as it stimulates the connective tissue formation *in-vitro*[15]. The growth of *E. Coli* was inhibited in the presence of more than 0.025 % chitosan. It also inhibited the growth of *Fusarium Alternaria* and *Helnin – thosporium*[16, 17].

As chitosan has a wide range of applications it may be employed to solve numerous problems in environmental and biomedical engineering. Simultaneously nanoparticles are finding wide spread applications in all fields. The material of choice decides the multifunctional nature of the particles[18, 19]. The present work aimed to synthesize ionic crosslinked nanoparticles of chitosan and TPP and study of their swelling kinetics, enzymatic degradation and antibacterial nature. The nanoparticles thus prepared might be subjected as drug carrier species in near future.

MATERIAL AND METHODS

Materials

Chitosan was obtained from the E. Merck India. glacial acetic acid from E.Merck, India. sodium hydroxide pallets from E-Merck, India. Sodium tripolyphosphate (TPP) by Sigma Chemical Co. (USA). E.Coli (gram (-) ^{ve}) MTCC 118 and Bacillus Cereus (gram (+) ^{ve}) F4810 from Microbiology lab Science College, doubly distilled water. All the chemicals were of analytical grade and no further purification was required.

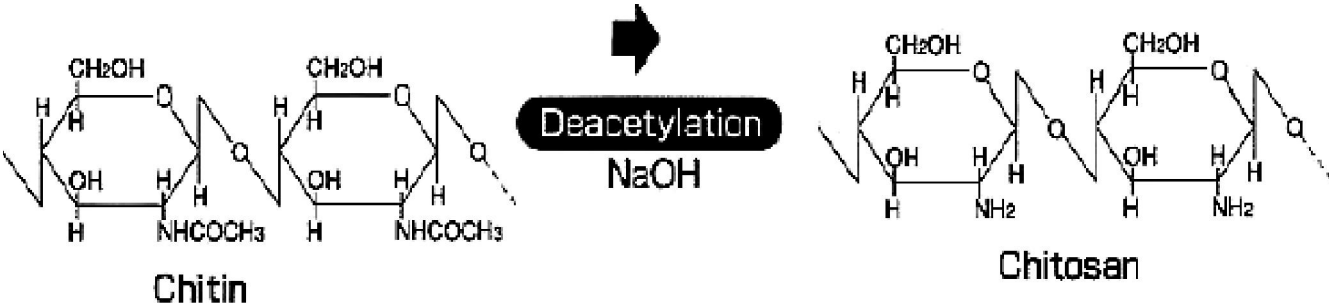

Figure 1 : Reaction scheme showing the deacetylation process of chitin.

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Method

Deacetylation of chitin into chitosan

Chitin was refined twice by dissolving it in dilute HOAc (acetic acid) solution. The solution was filtered and chitosan was precipitated with aqueous sodium hydroxide. The precipitate was dried in vacuum at room temperature[20]. The degree of deacetylation was about 85% as determined by pH-metric titrimetry[21]. Brief process of deacetylation of chitin is shown in following reaction scheme given in Figure 1.

Preparation of chitosan – tpp nanoparticles

Chitosan-TPP nanoparticles have been prepared by the slight modification in the methods already available and reported in literature[22-24]. Reaction scheme for the preparation of chitosan- TPP particles is shown in Figure 2.

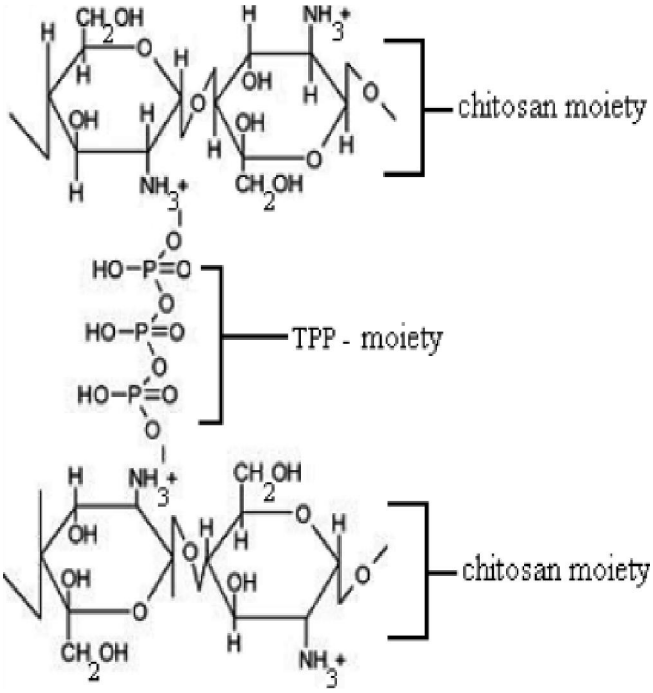

Figure 2 : Figure showing the crosslinking of chitosan with TPP.

Characterization

Fourier tansform infrared spectroscopy (ftir) - studies

IR spectra of the powdered specimens were recorded on a FTIR-8400S, Shimadzu spectrophotometer.

X-rays diffraction (xrd) - studies

For phase identification, X-ray diffraction (XRD) analysis was carried out using Cu Ka radiation (k = 1.5406 A °) in the XRD apparatus (Philips PW 1820) powder diffractometer.

Electron microscopy studies

An insight into of the morphology of the prepared nanoparticles was achieved by using SEM instrument (STEREO SCAN, 430, Lecica SEM, USA). Simultaneously TEM studies were performed on a TEM apparatus (MORGAGNI 268) to ensure the nanometer size of the chitosan-TPP particles.

Swelling studies

The extent of swelling was determined by a conventional gravimetric procedure as reported in literature[25]. In a typical experiment, preweighed chitosan-TPP particles were allowed to swell in PBS (phosphate buffer saline) for a predetermined time period (up to equilibrium swelling), thereafter the particles were taken out from the solution and then gently pressed in-between the two filter papers to remove excess of water and finally the particles were weighed using a sensitive balance (Denver instrument APX - 203). The swelling ratio was calculated by the following Equation (1).

$$\text{Swelling ratio} = \frac{\text{Weight of swollen particles}}{\text{Weight of dry particles}} = \frac{(Sw)}{(Sd)} \quad (1)$$

Degradation studies

Degradation in PBS

In a simple experiment preweighed amount of nanoparticles were immersed in 10 mL of PBS at 37°C for the predetermined time period. After equilibrium swelling was achieved the particles were taken out from the PBS solution and then pressed between the two filter papers gently to remove excess amount of water and the weight of swollen particles was measured on a sensitive balance (Denver Instrument APX-203) at an appropriate time interval. The medium was changed daily to a fresh one and the swollen particles were put into the vial after weight measurements. The weight loss of the particles was recorded and the percent degradation was calculated using following formula given in Equation 2.

$$\%\text{Degradation} = \frac{Wo - Wd}{Wo} \times 100 \quad (2)$$

Where Wo is the weight of swollen particle at equilibrium swelling and Wd is the weight of the swollen particles after degradation.

Enzymatic degradation

In a very simple experiment instead of PBS, preweighed amount of the particles were immersed in 10 mL of deionized water containing enzyme, papain (from papya latex, crude, at concentration of 10 mg/mL.) at 37 ^{o} C for a predetermined period (0-14 days). The degradation experiment was performed as described in section 3.7.1. It has been reported in literature that enzyme papain was found to be responsible for chitosan degradation[27].

***In-Vitro* blood compatibility**

Nanoparticles are the type of hydrogels that are three-dimensional crosslinked polymeric structures, which can swell in an aqueous environment. Over the past 35 years, these materials have been extremely useful in biomedical and pharmaceutical applications mainly due to their biocompatibility, as well as their high water content and rubbery nature which are similar to natural tissues[28]. A biomaterial is a substance used in medical devices for contact with the living body for the intended method of application and for the intended time period. To acquire biocompatibility, the materials used in medical applications must meet certain regulatory requirements. The surface of biomaterials is believed to play an important role in determining biocompatibility. For materials that come into contact with blood, the formation of clot is the most undesirable but frequently occurring event that restricts the clinical acceptance of material to be used as biomaterial. Therefore, certain test procedures have been developed and they need to be employed to judge the haemofriendly nature of materials before their *in-vivo* use. The two in-vitro blood compatibility tests are [1] Protein Adsorption [2] Percent Haemolysis.

Anti bacterial activity

Indicator bacteria and inoculum preparation

Strain of E.coli (gram (-) ^{ve}) MTCC 118 and Bacillus Cereus (gram (+) ^{ve}) F4810 was obtained from the culture collection maintained in Microbiology Department of Science College. The cultures were maintained at 6^{o}C on BHI (brain- heart in fusion) agar slants (Himedia, Mumbai, India) and subcultured at 15 day intervals. Before use, the culture was successively propagated twice in BHI broth at 37^{o} C. Cell suspension of the culture, individually, were prepared from 20 h – hold BHI culture broth with appropriate dilution in 0.85 % saline, giving individual counts of $10^{2} – 10^{-6}$ CFU. mL^{-1} (where CFU stands for colony forming unit).

Bacterial growth inhibitory activity

Antibacterial activity of native chitosan and chitosan nanoparticles was studied against indicator bacterial strains, individually, in nutrient broth following the method of[31]. Bacterial activity was calculated using Equation (3) given below.

$$[(C-T)/C] \times 100 \quad (3)$$

Where in C and T are colony numbers in the control and chitosan sample plates respectively[32].

RESULTS AND DISCUSSION

FT-IR

FTIR spectra of TPP, chitosan and chitosan nanoparticles were analyzed. Figure 3(a) representing the FTIR- spectra of native TPP where measurements showed distinct ν3 (at around 1000-1100 cm-1) and ν4 (at around 610 cm-1) phosphate group[33, 34]. Figure 3(b) showing the FTIR – spectra of native chitosan which reveals the characteristic band at 3449 cm $^{-1}$ which is attributed to $–NH_2$ stretching vibration and the band for amide I at 1655 cm^{-1}. In crosslinked chitosan (Figure 3c) peak at 1655 cm $^{-1}$ disappears and two new peaks at 1645-1554 cm $^{-1}$ appears. The crosslinked chitosan also showed peak for P=O at 1155 cm $^{-1}$[35, 36]. The disappearance the peak could be attributed to the linkage between phosphoric and ammonium ions in chitosan-TPP particles[37] which also confirm the crosslinking reaction scheme given in Figure 1. Peak at 1635 cm $^{-1}$ for – CONH and 1060 cm $^{-1}$ for C-O-C has been obtained that confirms the presence of chitosan phase in nanoparticles. Also characteristic peaks are present at 2934 cm $^{-1}$ and 2850 cm $^{-1}$ for asymmetric and symmetric stretching of methylene (-CH_2-) groups, respectively for chitosan. On the other hand, the bands around at 1544 and 1620 cm $^{-1}$ are for N-H stretching can be observed for native and

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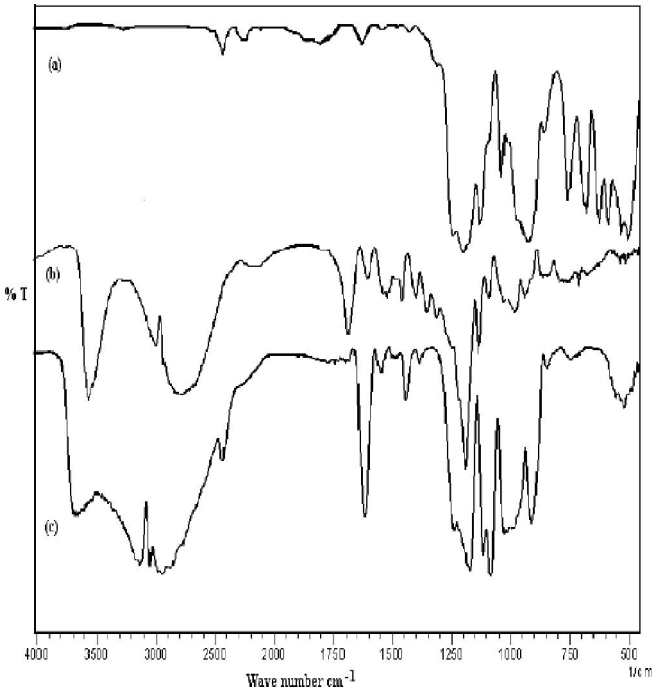

Figure 3 : (a) FTIR – spectra of native TPP. (b) FTIR – spectra of native chitosan. (c) FTIR – spectra of chitosan-TPP nanoparticles.

crosslinked chitosan with varying intensities. In nanoparticles peak appears at 1217 cm $^{-1}$ is probably due to stretching vibration of P=O[38].

XRD

XRD spectra of native chitosan (Figure 4a) showed two prominent crystalline peaks at 10° (2°θ) and 20° (2°θ). In the case of crosslinked chitosan in Figure 4(b) there was a significant decrease in intensity of characteristic peaks of chitosan, which was in agreement with the study reported by Wan et al[39].

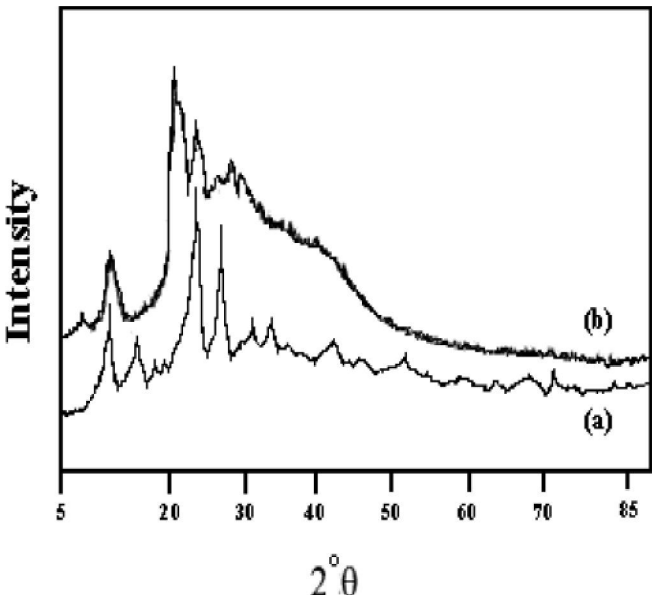

Figure 4 : (a) X-ray diffraction spectra of native chitosan. (b) X-ray diffraction spectra of. chitosan-TPP nanoparticles

The distinct difference in the diffraction patterns of chitosan and crosslinked chitosan could be attributed to modification in the arrangement of the molecules in crystal lattice. In chitosan crosslinked with TPP at 10° (2oθ) and 20° (2°θ) are suppressed might be due to amophization. Physical and mechanical properties of the polymers are profoundly dependent on the degree of crystallinity. All the X-ray diffraction methods reported in literature for calculating the crystallinity in polymer are based on the following assumptions:

(1). The scattering capability of crystallite is equal to that in amorphous with the same mass.

(2). The intensity of the X-rays scattered from a specimen is approximately equal to the sum of that from the crystalline and amorphous in specimen.

Percent crystallinity has been calculated for the chitosan and chitosan- TPP nanoparticles using the expression given in literature[40]. The numerical formula to calculate % crystallinity (%X) has been given in the following Equation (5),

$$\% \text{ Crystallinity} = (Ic / Ia + K) \times 100 \quad (4)$$

Where Ic and Ia are the integrated intensities of crystalline and amorphous peaks respectively, K is is a constant taken as unity[41]. Areas of the peaks were determined by the "cut and weight method". The relation between integrated intensities and area of crystalline and amorphous peaks has been evaluated from the literature[42]. It has been found that the % crystallinity of the chitosan and chitosan-TPP nanoparticles was calculated to be about 61.18 % and 44.6 % respectively. The net decrease in the crystalline nature of the chitosan nanoparticles as compared to the native chitosan is may be due do the formation of more amorphous regions within the polymer chains after crosslinking.

Determination of the crystallinity of the nanoparticles is one of the important features to decide its applicability as drug carrier device.

Electron microscopy studies

The result of scanning electron microscopy has been shown in Figure 5(a) that clearly reveals the spherical geometry of the nanoparticles. Spherical geometry of

nanoparticles promotes consistent release of loaded drug when they will be applied as drug delivery carriers. Figure 5(b) showed the particle size in the range of 20-90 nm when chitosan-TPP nanoparticles were subjected to transmission electron microscopy measurements.

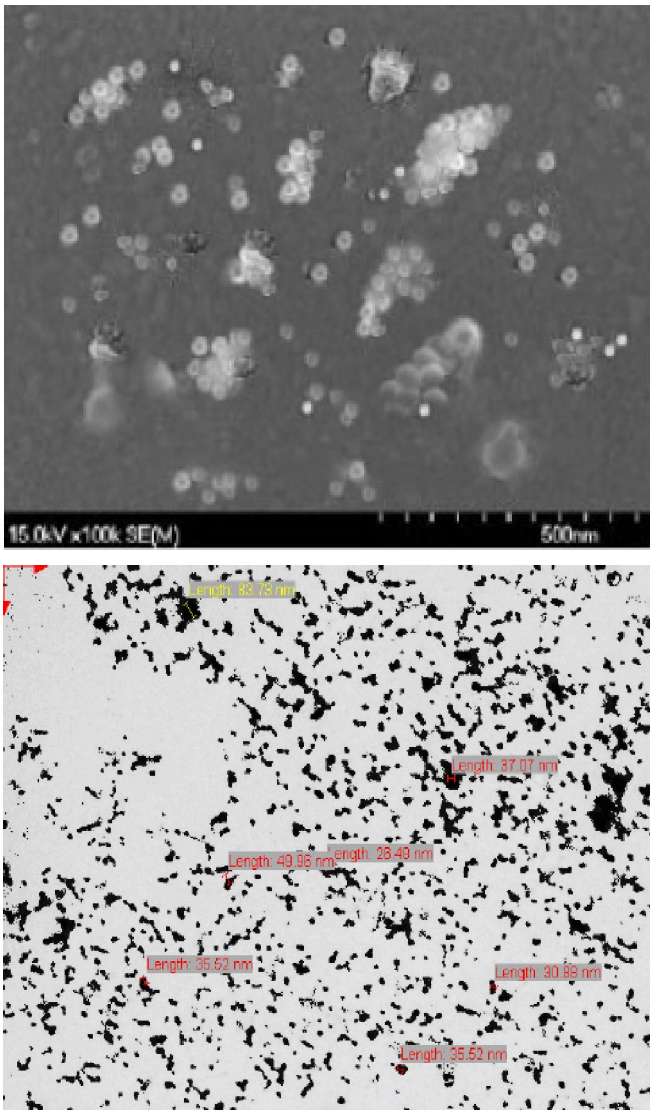

Figure 5 : (a) SEM image of chitosan-TPP nanoparticles. (b) TEM image of chitosan-TPP nanoparticles showing size of particles.

Swelling studies

PBS (phosphate buffer saline) provides ideal conditions as blood for in-vitro swelling, release experiments resembling with blood pH 7.4. The swelling results of chitosan-TPP nanoparticles in PBS are shown Figure 6. Chitosan with pka of 6.3 is polycationic when dissolved in acid and presents $-NH_3^+$ sites. Sodium tripolyphosphate ($Na_5P_3O_{10}$) dissolved in water dissociates to give both hydroxyl and phosphoric ions. The crosslinking of the chitosan would be dependent on availability of the cationic sites and negatively charged species.

Figure 6(a) shows the effect of chitosan concentration on the swelling ratio of nanoparticles in PBS. In the present study as the amount of chitosan was varied from 0.5 g to 2.0g with definite amount of TPP (3.0 g) the extent of swelling increases from 0.5- 1.5 g. It may be due to the fact that up to this concentration quite porous hydrogel network is formed within the chitosan-TPP nanoparticles that permits solvent

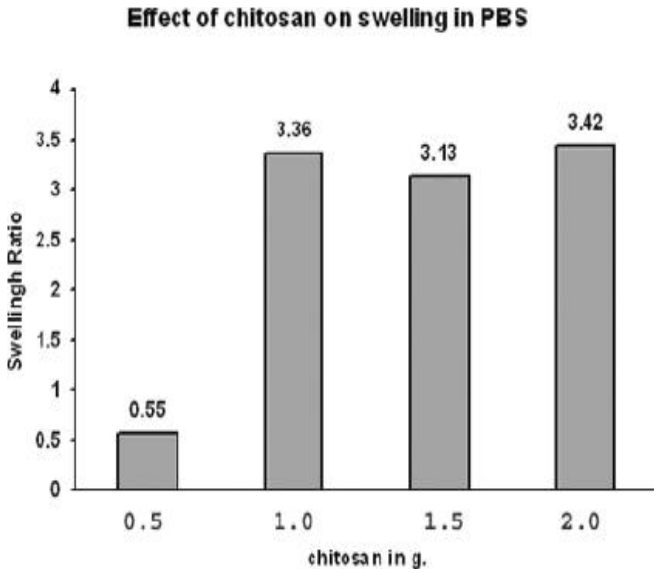

Figure 6(a) : Graph showing the effect of chitosan concentration on swelling ratio of chitosan-TPP nanoparticles in PBS.

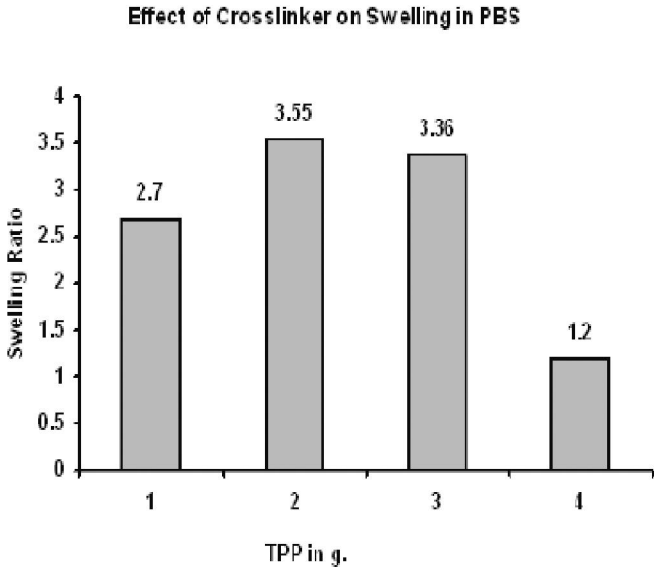

Figure 6(b) : Graph showing the effect of TPP concentration on swelling ratio of chitosan-TPP nanoparticles in PBS.

molecules enter into the nanoparticles network easily. Since chitosan-TPP particles have been prepared either by deprotonation or by ionic interaction thus the mutual interaction between the groups of polymer chain and that of solvent would affect the swelling a lot. Till 1.5 g of chitosan concentration polymer chains of nanoparticles are quite flexible for the entry of solvent molecules and the system shows increase in the swelling ratio. At 2.0 g of chitosan concentration, swelling ratio decreases. Although the chitosan is a hydrophilic polymer but its greater amount (2.0 g) results in the formation of more dense polymer hydrogel system and does not allow the passage of solvent molecules within the nanoparticles feasibly. An important fact has also been observed that for higher concentrations of chitosan, formation of nanoparticles become difficult with definite amount of TPP and water. At higher concentrations of chitosan a gel like deposition was obtained which was strictly due to the increase in the viscosity of the reaction mixture. Enhanced viscosity of the reaction system results in the decrease in the agitation speed that required forming particles of nano range.

In Figure 6(b) shows the effect of crosslinker concentration on the swelling ratio of chitosan-TPP particles in PBS, as the amount of crosslinker increases in the feed mixture the swelling ratio decreases. It has been seen that for the low concentrations of TPP (1.0 and 2.0 g) yield of nanoparticles was very low with poor quality of product material. For 4 g. of TPP concentration the swelling ratio was found to be very less due to the greater amount of crosslinking which may results in the formation of more compact polymer network and restricts the feasible transport of solvent molecules within nanoparticles. For 3.0 g of TPP concentration in the feed mixture fair level of swelling ratio was achieved with good quality and yield of nanoparticles.

Beside all above discussion it is worthy to mention here that in the swelling phenomenon of chitosan-TPP nanoparticles, many volume and phase transitions were induced independently by pH and ionic strength changes. However the swelling results that have been reported in the present study, had helped the authors to decide control set of nanoparticles with fine quality, good yield and desirable swelling properties so that they can be used as good drug delivery vehicles in future.

Degradation studies

Degradation is an important factor for the material to be biocompatible. The percent degradation results of the control set of chitosan-TPP nanoparticles (1 g chitosan, 3 g TPP) have been shown in the Figure 7 (with white hollow columns). The results clearly indicate that in the absence of any enzyme (only in PBS medium) particles show maximum 46.6 % degradation till 14 th day. The results are quite satisfactory and reveal the self degradation tendency of chitosan-TPP nanoparticles. It is obvious that the material which will come in contact with body environment will also be in contact with different enzymes present inside the animal body. As, enzymes are specific for their substrates they selectively affect the substrates that come in contact with them. The results of enzymatic degradation for the control set of chitosan –TPP particles have been shown in Figure 7 (with black solid columns). The results show the maximum degradation of the particles in the presence of enzyme papain is about 80 % that is almost double of the degradation results that found in PBS environment only[43].

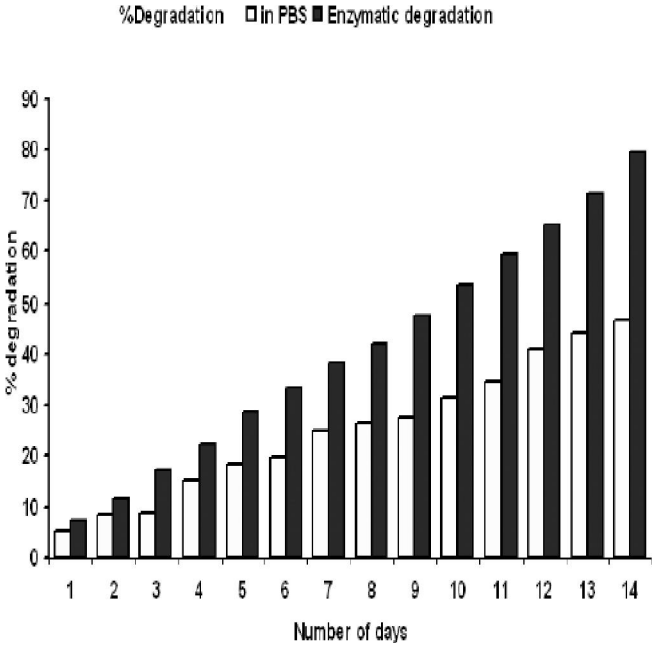

Figure 7 : Graph showing results of % degradation of chitosan-TPP (1.0 g chitosan, 3.0 g TPP) in PBS and in presence of enzyme papain.

In-Vitro blood compatibility

Results for the BSA (bovine serum albumin) adsorption and percent haemolysis for chitosan-TPP

nanoparticles have been shown in the TABLE 1. It has been observed that the optimum protein adsorption was obtained for the nanoparticle system containing 1g chitosan and 3g TPP with optimum haemolysis. It may be possible that for greater amount of chitosan (2.0 g) and crosslinker (4.0 g TPP) the swelling capacity of the system is lesser and so the blood compatibility. Since the swelling properties of the system directly affect the blood compatible behavior of the material. It has been reported that the systems having good water content able to achieve reversible protein adsorption onto the material surface and inturns reduce the chances of blood coagulation[44, 45]. The control set of chitosan- TPP nanoparticles (chitosan 1.0 g, TPP 3.0 g) showed fair level of blood compatibility.

TABLE 1 : Results of *in-vitro* blood compatibility of different compositions of chitosan-TPP nanoparticles.

S.No.	chitosan (g)	TPP (g)	BSA adsorption mg/g	% Haemolysis
1	0.5	3.0	0.241	15.2
2	1.0	3.0	0.052	17.5
3	1.5	3.0	0.171	18.7
4	2.0	3.0	0.135	28.9
5	1.0	1.0	0.092	3.31
6	1.0	2.0	0.172	32.7
7	1.0	4.0	0.351	42.1

Also the protein adsorption and percent haemolysis for 1g chitosan and 1 g TPP containing system is 0.092 mg/g and 3.3 % respectively that may be attributed to the lesser amount of crosslinking with greater swelling. It is the well known fact that greater amount of crosslinking enhances the compactness of the system to reduce water absorption capacity simultaneously it also reduces the smoothness of the polymer system. Increased roughness of the polymer surface causes destructive interactions among the material surface and blood components and thus results reduced blood compatibility. It is also evident from the results that the system having lesser amount of polymer and crosslinker content showing fair level of blood compatibility though the yield and quality of the material has been reported to be found excellent in case of control set only.

Antibacterial activity assessment

Since chitosan is only soluble in acidic media, the precipitation of chitosan solution in acetic acid occurred upon addition to bacterial suspension, while chitosan nanoparticles could be well distributed in bacterial suspension after a slight shock for a nice dispersion. Bacteria can adhere to the surface of chitosan and chitosan nanoparticles significantly in short time thus chitosan and chitosan nanoparticles exhibit anti-bacterial activity. According to the literature[46, 47], chitosan possess antimicrobial activity against a number of gram-negative and gram-positive bacteria.

In TABLE 2 anti- bacterial activity of chitosan nanoparticles was compared with that of chitosan. TABLE shows the MIC and MBC of chitosan and chitosan nanoparticles against strain of E.coli (gram (-) ^{ve}) MTCC 118 and Bacillus Cereus (gram (+) ^{ve}) F4810. According to the data, the antibacterial activity of chitosan nanoparticles is significantly higher than that of chitosan.

TABLE 2 : Growth inhibitory effect of native chitosan and chitosan-TPP nanoparticles (control set = 1 g chitosan, 3 g TPP) towards E.Coli (10^4 CFU. mL^{-1}), B. cereus (10^6 CFU. mL^{-1})

S.No.	compound	Concentration in mg.	% Inhibition of indicator bacterium	
			E.Coli	B. Cereus
1	Native chitosan	0.05	09	12
2		0.07	11	19
3		0.09	16	17
4	Chitosan-TPP nanoparticles	0.05	11	17
5		0.06	19	10
6		0.07	25	32
7		0.08	16	22
8		0.09	35	19

The study solving the initial purpose of authors to check antibacterial activity of the prepared material towards gram positive & negative bacterial strains. The results of antibacterial activity found to be quite encouraging with maximum 32% and 35 % of zone inhibition for the E.Coli and B.Cereus bacterial strains respectively.

CONCLUSION

Nanosized chitosan-TPP particles have been pre-

pared by the ionotropic crosslinking. The FTIR- studies reveals the presence of characteristic packs of native materials such as chitosan and TPP. The disappearance of –NH- str. peak of chitosan was found in chitosan-TPP particles clearly shows the crosslinking of chitosan with TPP. XRD studies show the characteristic peaks of chitosan phase at $10^{o}\theta$ and $20^{o}\theta$ for native chitosan and chitosan-TPP nanoparticles. The suppression of XRD peaks in nanoparticles reveals the decrease in crystallinity of chitosan moiety in the nanoparticles. The % crystallinity of native chitosan and chitosan-TPP nanoparticles was calculated as 61.18 and 44.6 % respectively. SEM and TEM studies show spherical shape of nanoparticles with size in between 20-90 nm.

Swelling of the nanoparticles was checked in PBS and also in SBF. The results that have been found are quite satisfactory. The nanoparticles with composition 1g chitosan and 3 g TPP show optimum swelling while greater amount of chitosan (2.0g and more) results in viscous reaction mixture that restricts the formation of nanoparticles. Higher amount of crosslinker (4.0 g TPP) showed poor swelling ratio that is not desirable for the material to be subjected as drug delivery system.

Degradation is an obvious and essential phenomenon for the biomaterials. The % degradation of the material was checked in PBS and in the presence of enzyme papain. It was found that the chitosan –TPP particles show self degradation about 46.6 % in 14 days in PBS. The results of % degradation in presence of enzyme were calculated about 80 % in 14 days. The results of *in-vitro* blood compatibility showed protein adsorption about 0.052 mg/g and % haemolysis was about 17.5 % for the control set. In-*vitro* blood protein adsorption and % haemolysis for the chitosan-TPP nanoparticles having composition 1g chitosan and 1 g TPP were found to be 0.092 mg/g and 3.31 % respectively. The results of blood compatibility tests *in-vitro* are quite satisfactory. The material was subjected to check its resistance against the bacterial *strains* of E.Coli (gram (-) ^{ve}) MTCC 118 and Bacillus Cereus (gram (+) ^{ve}) F4810 and it was found that the chitosan- TPP nanoparticles are more effective against the described bacterial strains as compare to the native chitosan.

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REFERENCES

[1] N.V.Majeti, R.Kumar; React.Funct.Polym., **46**, 1 **(2000)**.
[2] W.S.Adriano, E.H.C.Filho, J.A.Silva, R.L.C.Giordano, L.R.B.Goncalves; Brazilian J.of Chemi.Engin., **22**, 529 **(2005)**.
[3] M.Nidhin, R.Indumathy, K.J.Sreeram, B.U.Nair; Bull.Mater.Sci., **31**, 93 **(2008)**.
[4] A.M.Delorino, S.P.Cresidio; World Appl.Sciences Journal (Special Issue for Environment), **5**, 98 **(2009)**.
[5] F.Boukhlifi, A.Bencheikh; Ann.Chim.Sci.Mater., **25**, 153 **(2000)**.
[6] X.M.Zhao, X.P.She, W.Yu, X.M.Liang, Y.G.Du; J.Plant Pathology, **89**, 55 **(2007)**.
[7] M.George, T.E.Abraham; J.Control Release, **114**, 1 **(2006)**.
[8] Q.Gan, T.Wang, C.Cochrane, P.McCarron; Colloids Surfaces B., **44**, 65 **(2005)**.
[9] P.He, S.S.Davis, L.Llum; Int.J.Pharm., **166**, 75 **(1998)**.
[10] A.B.Schnurch, C.Humenberger, C.Valenta; Int.J.Pharm., **165**, 217 **(1998)**.
[11] A.Berthold, K.Cremer, J.Kreuter; J.Control Release, **39**, 17 **(1996)**.
[12] F.L.Mi, H.W.Sung, S.S.Shyu; J.Appl.Poly.Sci., **81**, 1700 **(2001)**.
[13] C.K.Gupta, N.V.Majeti, R.Kumar; Polym.Reviews, **40**, 273 **(2000)**.
[14] D.Thacharodi, K.P.Rao; Biomaterials, **16**, 45 **(1995)**.
[15] C.P.Paul, W.Sharma; Trends Biomater Artif.Organs, **18**, 18 **(2004)**.
[16] S.Hirano, C.G.Gebelein, C.E.Carraher, Jr (Eds.), International Biotechnological Polymers, Technomic, Lancaster, 189 **(1995)**.
[17] J.Young, S.K.Kim; J.Agric.Food Chem., **54**, 6629 **(2006)**.

[18] M.Check, F.L.Sung, H.W.Shyu; J.Appl.Poly.Sci., **81**, 1700 **(2001)**.

[19] Z.Yong, et al.; Recent Patents on Biomedical Engineering, **1**, 34 **(2008)**.

[20] H.Y.Jiang, X.Ding, Y.Ge, H.Yuan, Y.Yang; Biomaterials, **23**, 3193 **(2002)**.

[21] Avadi et al.; Iran Polym.J., **13**, 431 **(2004)**.

[22] Y.B.Fei, F.Q.Li, J.H.Hu, J.Y.Liu, Y.Z.Zhao; Pharm.Care Res., **8**, 119 **(2008)**.

[23] Z.X.Tang, L.E.Shi; Biotechnol & Biotechnol EQ., **21(2)**, 223-228 **(2007)**.

[24] Y.W.Wuli, Y.Changchun, W.J.Hu, S.Fu; J.of Pharmaceutics, **295**, 235 **(2005)**.

[25] A.K.Bajpai, R.Sainy; J.of Mater Sci.Mater.Med., **17**, 49 **(2006)**.

[26] P.Li, K.Nakanishi, T.Kokubo, K.D.Groot; X.Biomaterials, **14**, 963 **(1993)**.

[27] K.V.H.Prashanth, R.N.Tharanathan; Trends Food Sci.& Technol., **18**, 117 **(2007)**.

[28] N.A.Peppas, P.Bures, W.Leobandung, H.Ichikawa; Europ.J.Pharma.Biopharm., **50**, 27 **(2000)**.

[29] A.K.Bajpai, D.D.Mishra; J.Mater.Sci.Mater.Med., **15**, 583 **(2004)**.

[30] R.Saini, A.K.Bajpai; Polym.Int., **54**, 1233 **(2005)**.

[31] C.S.Chen, W.Y.Lian, G.J.Tsai; J.Food Prot., **61**, 1124 **(1998)**.

[32] Y.J.Jeon, P.J.Park, S.K.Kim; Carcohy.Polym., **44**, 71 **(2001)**.

[33] R.Viitala, J.Simola, T.Peltola, H. Rahiala, M.Linden, M.Langlet, J.B.Rosenholm; J.Biomed.Mater.Res., **54**, 109 **(2001)**.

[34] J.S.Oh, J.H.An, S.O.Lee, Y.H.Yun, B.A.Kang, S.B.Kim, K.S.Hwang; Metals and Mater.Int., **8**, 459 **(2002)**.

[35] N.A.Peppas, P.Bures, W.Leobandung, H.Ichikawa; Eur.J.Pharm.Biopharm., **50**, 27 **(2000)**.

[36] Y.Xu, Y.Du; Int.J.Pharm., **250**, 215 **(2003)**.

[37] X.Wang, J.Ma, Y.Wang, B.He; Biomaterials., **22**, 2247 **(2001)**.

[38] L.F.Qi, Z.R.Xu, X.Jiang, C.H.Hu, X.F.Zou; Carbohyd.Res., **33916**, 2693 **(2004)**.

[39] S.T.Lee, F.L.Mi, Y.J.Shen, S.S.Shyu, Polym., **42**, 1879 **(2001)**.

[40] Y.Wan, C.Kam, B.Peppley, V.T.Bui; Turk.J.Chem., **24**, 177 **(2000)**.

[41] J.E.Johnson; J.Appl.Polym.Sci., **2**, 205 **(1959)**.

[42] Y.Ning; Chienes J.Polym.Sci., **7**, 315 **(1989)**.

[43] M.Yamazaki; Fiber and Polymer Science, Raleigh.NC., **3**, 21 **(2007)**.

[44] S.H.Ajili, N.G.Ebrahimi, M.T.Khorasani; Iran Polym.J., **1**, 179 **(2003)**.

[45] A.K.Bjpai; Polym.Int., **56**, 231 **(2007)**.

[46] K.Ueno, T.Yamaguchi, N.Sakairi, N.Nishi, S.Tokura; Adv.Chitin.Sci., **2**, 156 **(1997)**.

[47] Y.J.Jeon, P.J.Park, S.K.Kim; Carbohydr.Polym., **44**, 71 **(2001)**.