
Chapter 3

Synthesis of Hydrogel from Template
polymerization of acrylic acid on to modified
chitosan, i.e., 6-amino-6-deoxy-chitosan

3.1 Introduction

Hydrogels are well known as networks of hydrophilic polymers, which can absorb a significant amount of water (>20% of their dry mass) without dissolving or losing their structural integrity^{1,2}. However, the application of swelling behavior of hydrogels is as shown in Fig. 3.1 that, on the left, superporous hydrogel in its (a) dry state and (b) water-swollen state, and on the right, schematic illustration of the transit of superporous hydrogel has been shown.

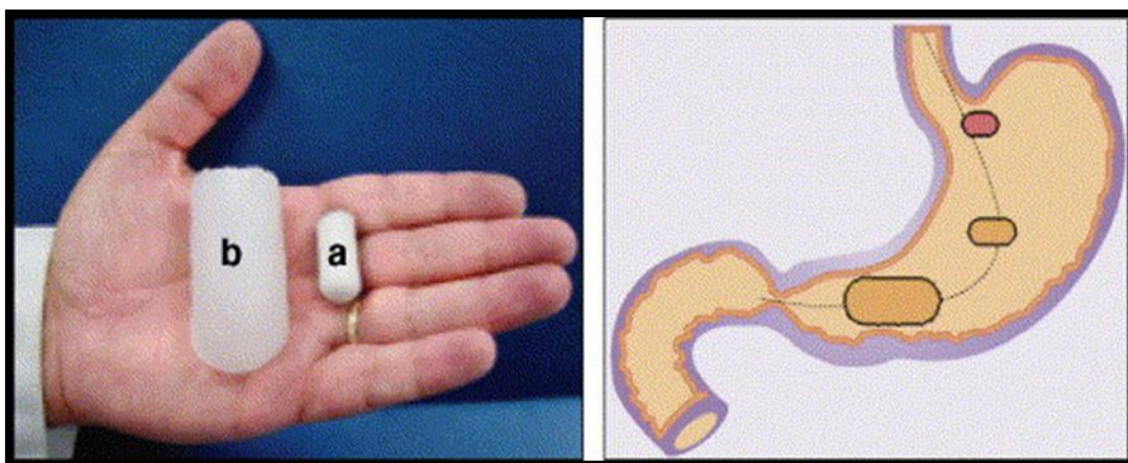


Figure 3.1 Schematic illustration of the transit of superporous hydrogel

Chemically crosslinked hydrogels were developed in the last few decades as carriers for drugs. The controlled drug delivery devices assure a sustained release and targeted effect³. In recent years, the polyacrylic acid (PAA) and its copolymers have been often used as carriers in drug release systems, because of their multifunctional nature, unique properties and good biocompatibility⁴.

Chitosan is a linear polysaccharide composed of β -(1 \rightarrow 4)-linked 2-amino-2-deoxy-*D*-glucopyranose and 2-acetamino-2-deoxy-*D*-glucopyranose (Fig 3.2). It has inter- and intramolecular hydrogen bonds and is water-insoluble due to its rigid crystalline structure⁵. It is also a bio-adsorber with gel forming ability. The superabsorbent resins grafted with chitosan can absorb aqueous solutions up to hundreds of times of their own dry weight⁶ and should have the antibacterial activities also⁷. A variety of graft copolymers of chitosan and vinyl monomers have been synthesized and evaluated as flocculants, ion-exchangers etc. Adsorption of Pb(II) from aqueous solution by using chitosan-g-poly(acrylic acid)/ attapulgit/sodium humate composite hydrogels could also be done⁸. Chitosan- modified poly(acrylonitrile-co-acrylic acid) nanofibrous

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membranes was used for the immobilization of concanavalin A⁹. Chitosan-g-poly(acrylic acid)/ montmorillonite superabsorbent nanocomposite was prepared via in situ intercalative polymerization by using ammonium persulfate as a initiator at 60°C¹⁰. Hollow chitosan/ poly(acrylic acid) nanospheres as drug carriers was prepared by dissolving chitosan in acrylic acid and then at 60°C K₂S₂O₈ was added as a initiator¹¹. Highly enhanced adsorption of lead ions on chitosan granules functionalized with poly(acrylic acid) was also possible¹². Polymorph control of calcium carbonate films in a poly(acrylic acid)–chitosan system depends on the molecular weight of poly(acrylic acid)¹³.

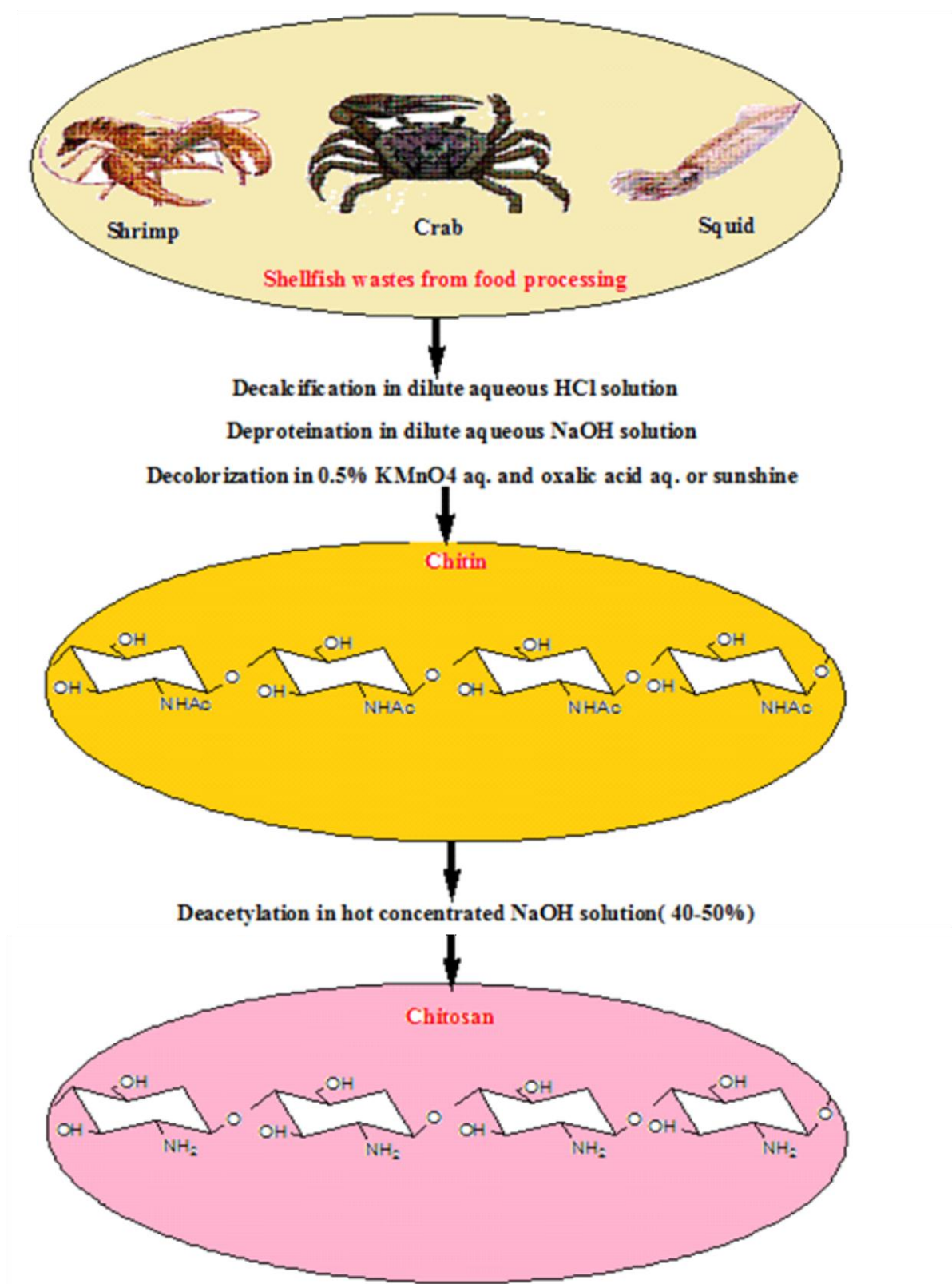


Figure 3.2 Brief introductions of chitin & chitosan

Chitosan and PAA were blended in different weight ratios and the resulting membranes were post treated to enable the formation of the polyelectrolyte complex

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which could be used as proton exchange membranes for fuel cells¹⁴. Information on the mechanism formation process for chitosan-poly(acrylic acid) complexes could also be used for microencapsulation purpose as well¹⁵. The pH-induced changes in the fabrication of multilayers of poly (acrylic acid) and chitosan were useful for a drug storage and delivery system¹⁶.

The graft copolymerization of acrylic acid on chitosan is done with several initiators such as, ammonium cerous sulfate under microwave irradiation at 60°C¹⁷, photoinitiated¹⁸, ceric ammonium nitrate at 70°C¹⁹⁻²¹, and ammonium/ potassium persulfate at 60°C²². But all these reactions have been done in diluted acids as a solvent because as we have discussed above chitosan is soluble in a acidic medium only. However, chitosan-related applications are limited by its insolubility at neutral or high pH values.

With a view to enhance the solubility of chitosan, a water soluble diamino derivative of chitosan i.e., 6-amino-6-deoxy chitosan (6a6dC), were prepared where the hydroxyl group on the C6 was converted to the amine group. This modification led to the enhanced solubility of chitosan²³. So, the present work focuses on the synthesis of a novel hydrogel, 6a6dC-g-PAA, through graft copolymerization of acrylic acid on water soluble derivative of chitosan (6a6dC) by using the new redox initiator system, hydrogen peroxide/L-ascorbic acid, which is possible at room temperature and in aqueous medium only, in contrast to the aqueous acetic acid, formic acid etc. in the conventional methods.

3.2 Experimental

3.2.1 Materials

Chitosan was purchased from Sigma Aldrich. The degree of deacetylation (DDA) of the commercial chitosan (i.e., 72 %) used in this study was determined by potentiometric titration method²³. The viscosity average molecular weight was calculated to be 7.1×10^4 using the Mark-Houwink equation ($K = 0.119$, $a = 0.59$). Other chemicals of AR grade such as phthalic anhydride, dimethyl formamide, p-toluene sulfonyl chloride, sodium azide, triphenylphosphene (TPP), hydrazine monohydrate, hydrogen peroxide and L-ascorbic acid were purchased from Merck India Ltd. and used as received. Acrylic acid monomer was similarly purchased and purified by passing through alumina column and subsequent drying under molecular sieves.

3.2.2 Instruments and measurements

Fourier Transform Infrared (FTIR) spectroscopic measurements were recorded on a Shimadzu 8400S Fourier Transform Infrared Spectrometer by KBr pellet method. Thermogravimetric analyses (TGA) were done on a Shimadzu TGA 50 instrument with a heating rate of 10 °C/min in the mixed atmosphere of nitrogen and oxygen in the ratio 60/40.

Scanning electron microscopy (SEM) images were obtained on an Oxford JSM-5610LV equipped with a charge-coupled device camera, operating at an accelerating voltage of 100 kV. The specimens for SEM were prepared by directly dropping the dispersion of corresponding products onto the carbon-coated copper grids.

3.2.3 Synthesis of 6-amino-6-deoxy-chitosan

6-amino-6-deoxy-chitosan was synthesized according to the literature method²⁴⁻²⁶ and is shown in Scheme 3.1. Initially 8.3g (5.6mmol) phthalic anhydride was added to 60ml of dimethyl formamide and then 3.0g chitosan was added to the solution and magnetically stirred for 8h at 120°C. The phthaloyl chitosan (1) precipitate was obtained by adding cold water, the precipitate was then washed with methanol for 1h, filtered, and dried at 40°C. In the second step^{15, 16} of the reaction, 4.0g of phthaloyl

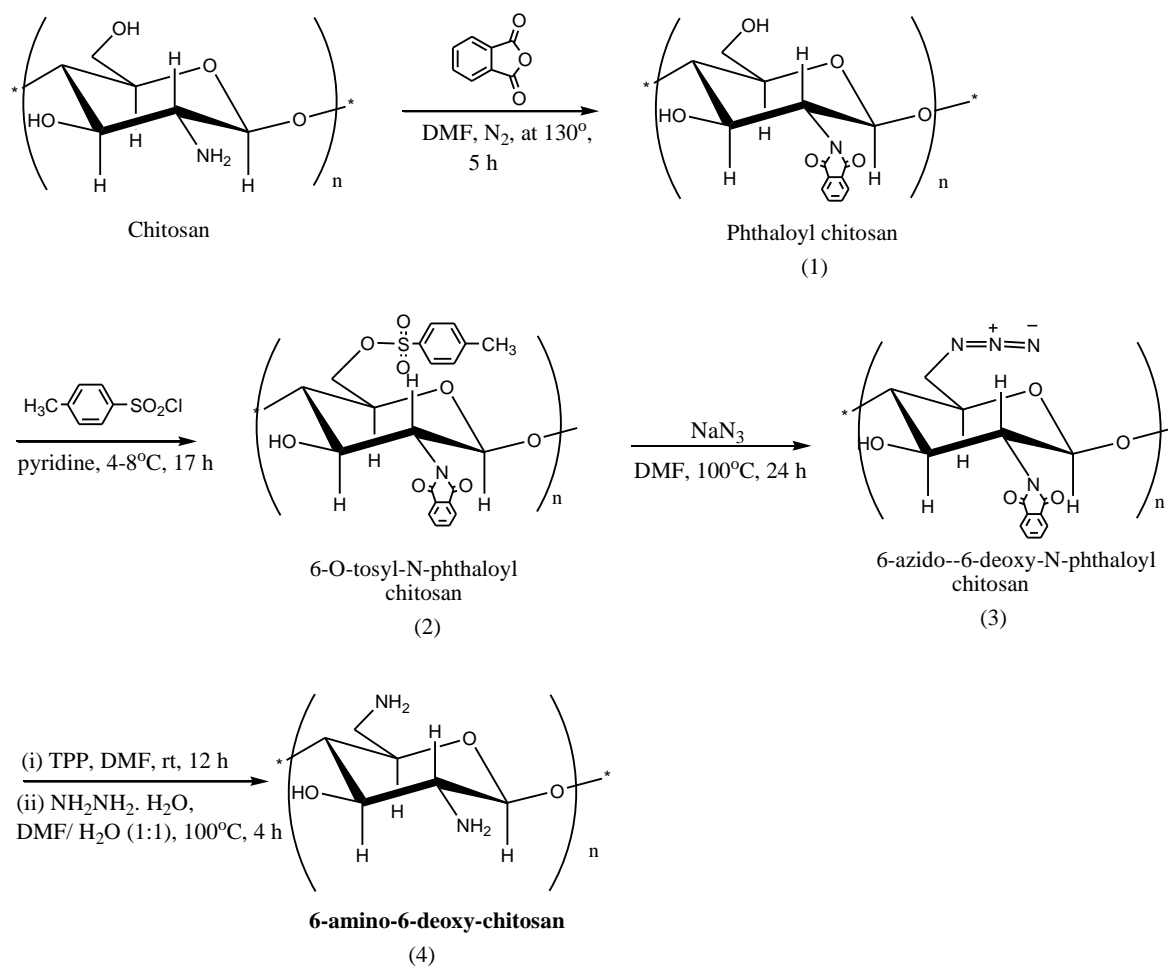
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chitosan was dissolved in 80ml pyridine and 26.0g p-toluene sulfonyl chloride was added to the solution. To obtain a viscous solution, the mixture was stirred at room temperature for 17h. In order to precipitate the obtained product (2), 1000ml of cold water were added and the precipitate was washed with 200ml ethanol and 200ml of diethyl ether, respectively, and dried at room temperature.

Now, in the third step, sodium azide (926 mg, 14.2 mmol) was added to a solution of 2 (500 mg, 1.42 mmol of sugar unit) in DMF (50 ml), and the mixture was stirred at 80 °C for 4 h under nitrogen. The mixture was filtered through cotton to remove the salts and the filtrate was poured into ethanol (500 m). The resultant precipitate was collected by centrifugation and washed with EtOH–water, then acetone. After drying under a reduced pressure at 40 °C, 6-azido-6-deoxy-N-phthaloyl-chitosan (3) was obtained as a light brown powder (388 mg, 86% yield).

In the last step, TPP (496mg, 1.89mmol) was added to a solution of 6-azido-6-deoxy-N-phthaloyl chitosan (200mg, 0.63mmol of sugar unit, d.s. azido 0.95) in DMF (20ml), and the reaction solution was stirred at room temperature for 12h under N₂. The reaction mixture was then treated with 4 M aqueous hydrazine monohydrate (20 ml) and stirred at 100 °C for 4 h. Following evaporation of the water, the suspended reaction mixture was poured into ethanol (180 ml). The resultant precipitate formed was collected by centrifugation (104 rpm, 7 min, three times) and washed with ethanol. The precipitate was dissolved in neutral water and purified by ultrafiltration using an ultrafilter with a molecular weight cut-off of 1 kDa. The product (4) was obtained by lyophilization as an ivory-colored amorphous material (76 mg, 75% yield). This obtained product was characterized by FT-IR, TGA, SEM & EDX analysis data.

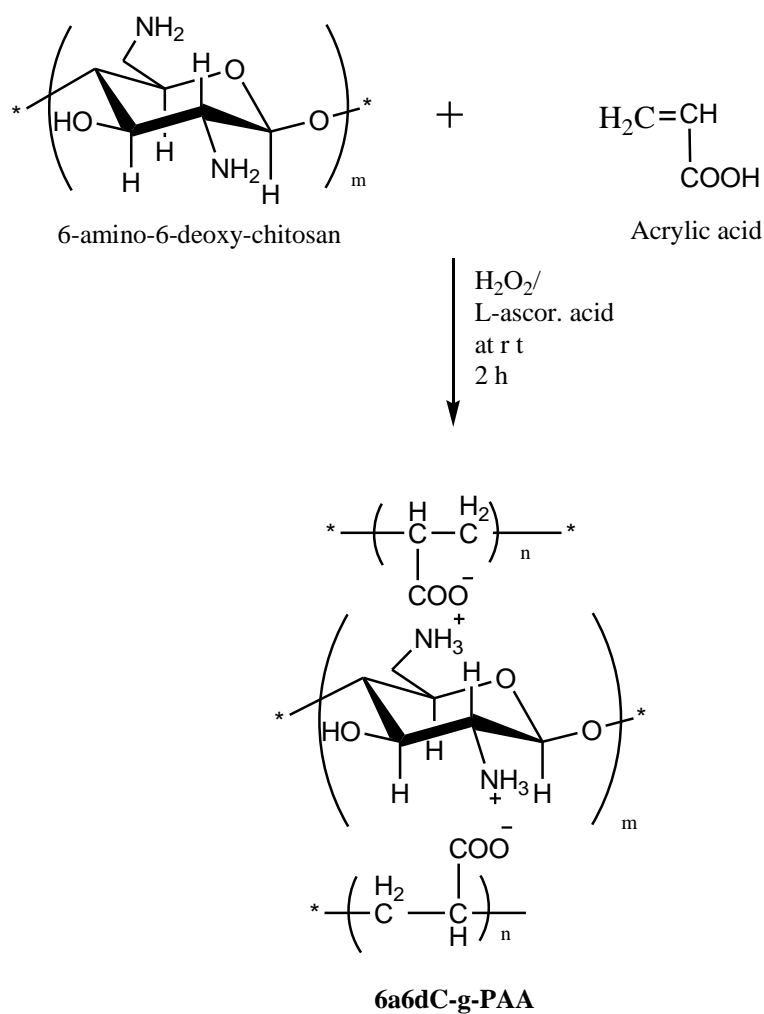
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Scheme 3.1 Preparation of 6-amino-6-deoxy-chitosan

3.2.4 Graft Copolymerization of acrylic acid on 6-amino-6-deoxy-chitosan

The graft copolymerization of acrylic acid on di-amino derivative of chitosan, 6-amino-6-deoxy chitosan is shown in Scheme 3.2. A certain amount of (1gm) 6-amino-6-deoxy-chitosan (6a6dC) and L-ascorbic acid (5% of monomer) was separately dissolved in 50 ml of water as a solvent was taken in a three-neck reactor equipped with nitrogen purging with continuous stirring. At room temperature simultaneously 10ml of acrylic acid and initiator, 30% hydrogen peroxide (5% of monomer), were added dropwise. After stirring for 2h a gel was obtained which on cooling appeared as a opalescent suspension. This was dried in a vaccum oven at 60°C and characterized by FT-IR, TGA & SEM. Similar procedure was followed for the synthesis of chitosan-g-PAA & PAA also for comparison.

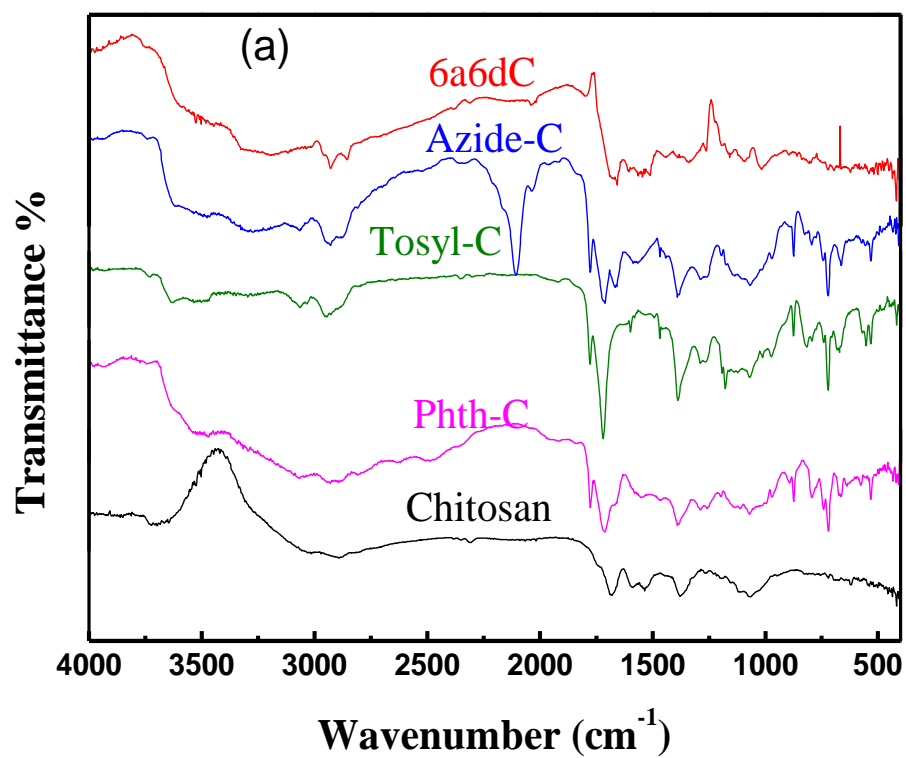


Scheme 3.2 Graft copolymerization of 6-amino-6-deoxy-chitosan with acrylic acid

3.3 Result and discussion

3.3.1 FT-IR spectra

The structural confirmation of 6-amino-6-deoxy-chitosan (6a6dC) and its graft copolymer (6a6dC-g-PAA) were characterized by FTIR spectroscopy. In Fig. 3.3(a) the IR spectrum of the chitosan, a strong broad C-O stretching band appears at 1050cm^{-1} was get reduced in 6a6dC spectrum confirms the absence of primary hydroxyl group in 6a6dC. Thus, it confirms the structure of 6a6dC. For 6a6dC-g-PAA, Fig. 3.3(b), the intensities of amide band I at 1629 cm^{-1} and amide band II at 1559 cm^{-1} , which can be observed clearly in 6a6dC, decrease dramatically, and two new absorption bands at 1747 and 1628 cm^{-1} , which can be assigned to the absorption peaks of the carboxyl groups of PAA (the absorption peak of carboxyl groups in pure PAA appears at 1750 cm^{-1}), and the NH_3^+ absorption of 6a6dC, respectively, are observed. The broad peaks appeared at 2500 and 1900 cm^{-1} also confirmed the presence of $-\text{NH}_3^+$ in 6a6dC-g-PAA. Furthermore, the absorption peaks at 1532 and 1414 cm^{-1} could be assigned to asymmetric and symmetric stretching vibrations of COO^- anions groups. These results indicate that the carboxylic groups of PAA are dissociated into COO^- groups which complex with protonated amino groups of 6a6dC through electrostatic interaction to form the polyelectrolyte complex during the polymerization procedure.



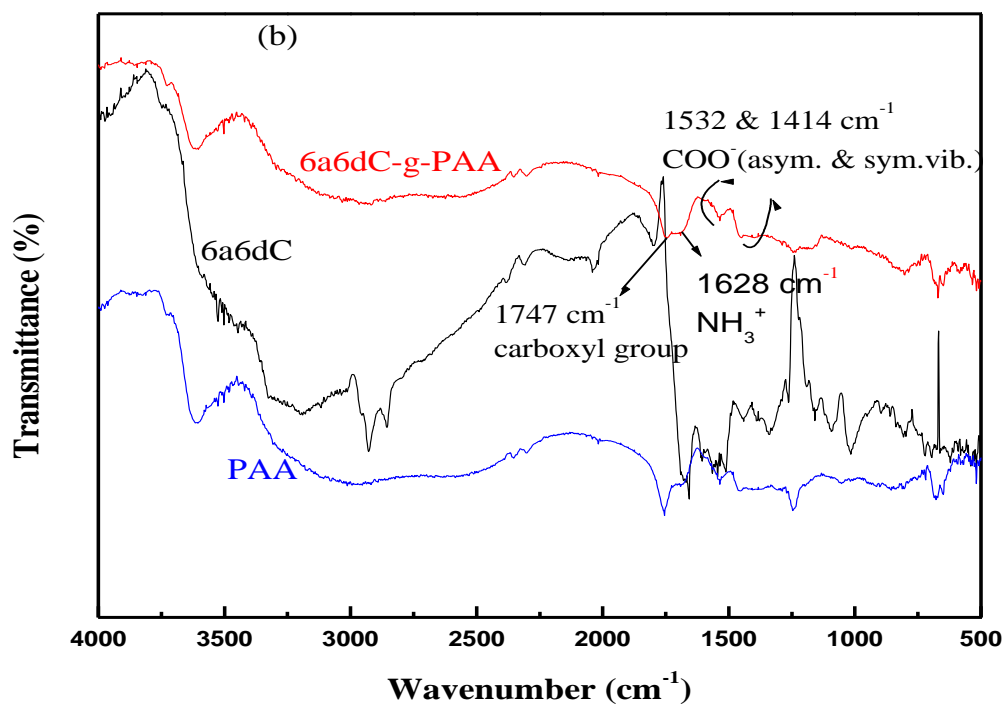


Figure 3.3 FT-IR spectra of (a) Chitosan to 6a6dC and (b) 6a6dC, PAA & 6a6dC-g-PAA

3.3.2 Thermal Analysis

The thermal stability and degradation behavior of chitosan, 6-amino-6-deoxy-chitosan (6a6dC) and poly (acrylic acid) grafted 6a6dC were evaluated by TGA under nitrogen atmosphere. TGA analysis also confirmed the grafting (Fig. 3.4). TGA of chitosan shows a weight loss in two stages. The first stage begins at about 50-280 °C for the chitosan materials with weight loss of 10 % due to loss of residual or physically adsorbed water on membrane surfaces. The second stage of weight loss starts at 350 °C and that continues up to 528°C during which there was 44 % weight loss due to the degradation of chitosan. The TGA of the grafted product has three stages of distinct weight loss. The first stage ranges between 250-300 °C with 48 % of the adsorbed and bound water weight loss. The second stage of weight loss starts at 300 °C and that continues up to 460°C during which there is 35 % of weight loss due to the degradation of chitosan at ungrafted part of the 6a6dC-g-PAA. There is 5 % weight loss in the third stage from 500 to 587°C that contributes to the decomposition of poly (acrylic acid) grafted on 6a6dC-g-PAA. So, after comparison it shows that 6a6dC-g-PAA is thermally more stable than chitosan & its di-amino derivative, 6a6dC.

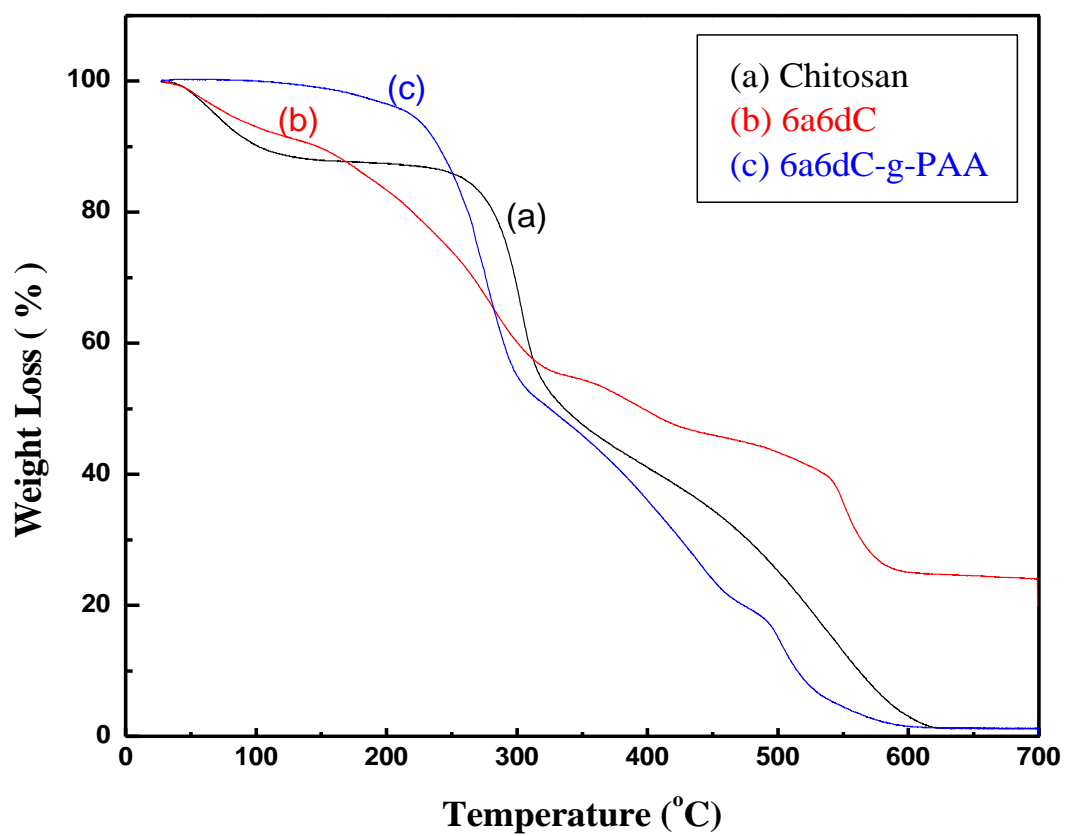


Figure 3.4 TGA of chitosan, 6a6dC and 6a6dC-g-PAA.

3.3.3 Measurement of water absorbancy

This 6a6dC-PAA can absorb water upto 140 times their own weight which was measured by dispersing the powdered superabsorbent resin (0.5g) in distilled water (100ml) for at least 2h at room temperature to reach swelling equilibrium, which resulted in the absorption of water inside the network of the resin and the formation of swelled sample. The residual water was removed by filtrating with 0.1mm whattmen filter paper & until water ceased to dropped. The weight of the resin containing absorbed water was measured after draining for 1h, 2h, 3h, 4h, 5h, 10h, 20h, 30h then upto equilibrium weight of water absorbed (Fig.3.5). Then water absorbancy was calculated in the form of water uptake percentage according to the following equation:

$$\text{Water Uptake (\%)} = (W' - W) \times 100 / W = 13910.8 \%$$

(At equilibrium weight of water absorbed which shows about 140 times of its own weight.)

Where W and W' are the weight of the dry and swollen superabsorbent resin, respectively.

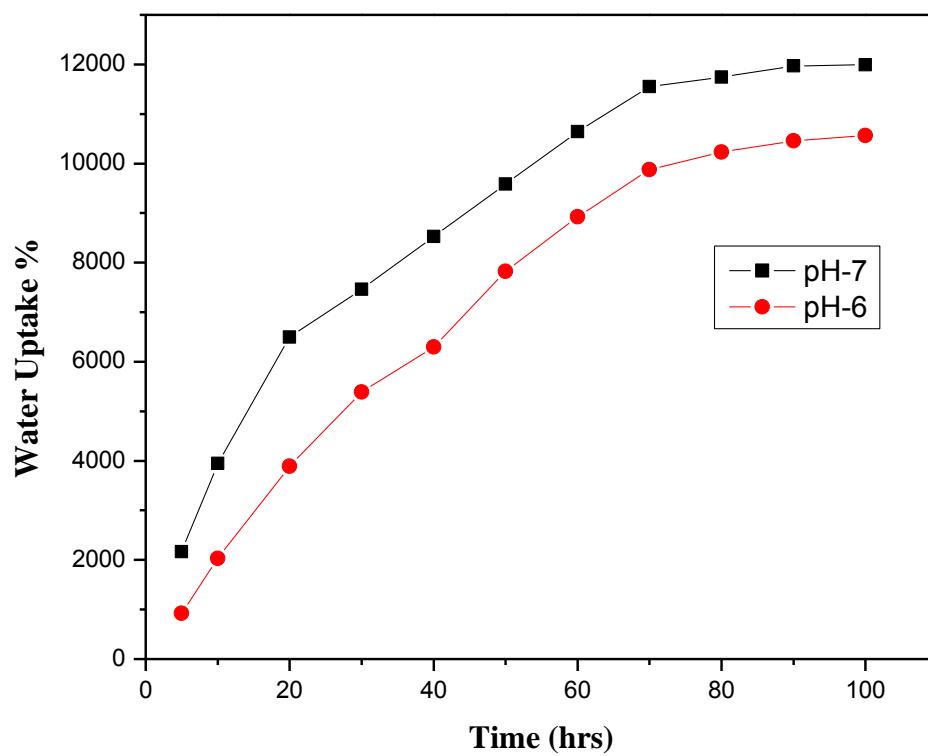


Figure 3.5 Water uptake (%) capacity of 6a6dC-g-PAA

3.3.4 Swelling behavior

The swelling behavior depends on the pH value of the solution²⁷. In order to investigate the effect of pH values on swelling behavior of 6a6dC-g-PAA, were immersed in buffer solution with different pH values. The swelling ratio can be calculated by using the following formula,

$$\text{Swelling Ratio} = (W' - W) / W$$

where W and W' are the weight of the dry and swollen superabsorbent resin, respectively.

Results of these experiments showed that these hydrogel were swell maximum at neutral pH as compared to acidic and basic medium. Therefore, the copolymer sample at pH 7 has the higher swelling ratio than those at pH 4 or pH 9, as shown in Fig. 3.6. From these results, it could be seen that these hydrogel are pH-sensitive, which would be good for drug carrier application.

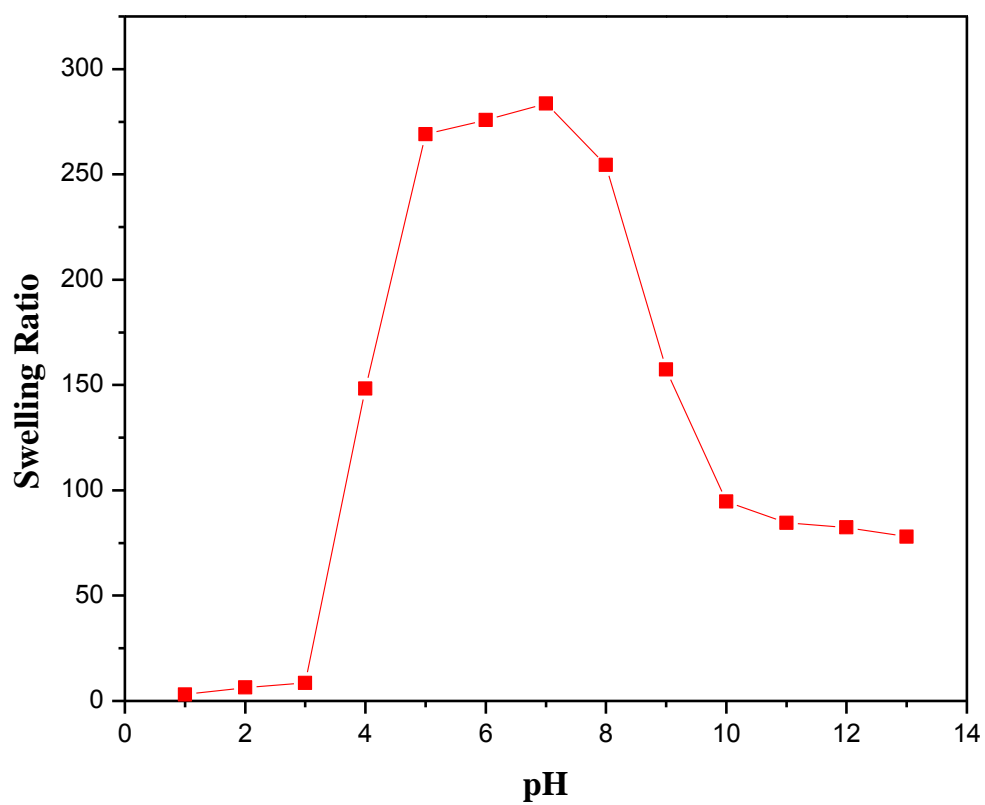


Figure 3.6 Water absorption capacity at different pH range

3.3.5 SEM & EDX analysis

The significant differences in the surface morphology of the chitosan, C-g-PAA, 6a6dC and 6a6dC-g-PAA, shown below in Fig. 3.7, indicate grafting of poly(acrylic acid) onto the chitosan & 6a6dC polymer. The SEM micrograph of chitosan matrix show rather a smooth surface as shown in [Fig. 3.7(a)] when compared to agglomerated surface [Fig. 3.7(b)] of C-g-PAA. This can be attributed to the fact that PAA upon grafting formed agglomeration onto the surface of chitosan. Whereas in case of 6a6dC and its grafted form [Fig. 3.7(c) & 3.7(d)], 6a6dC shows a rough surface and grafted form shows a very clear pores on its surface which can also give proof for its water absorbance ability.

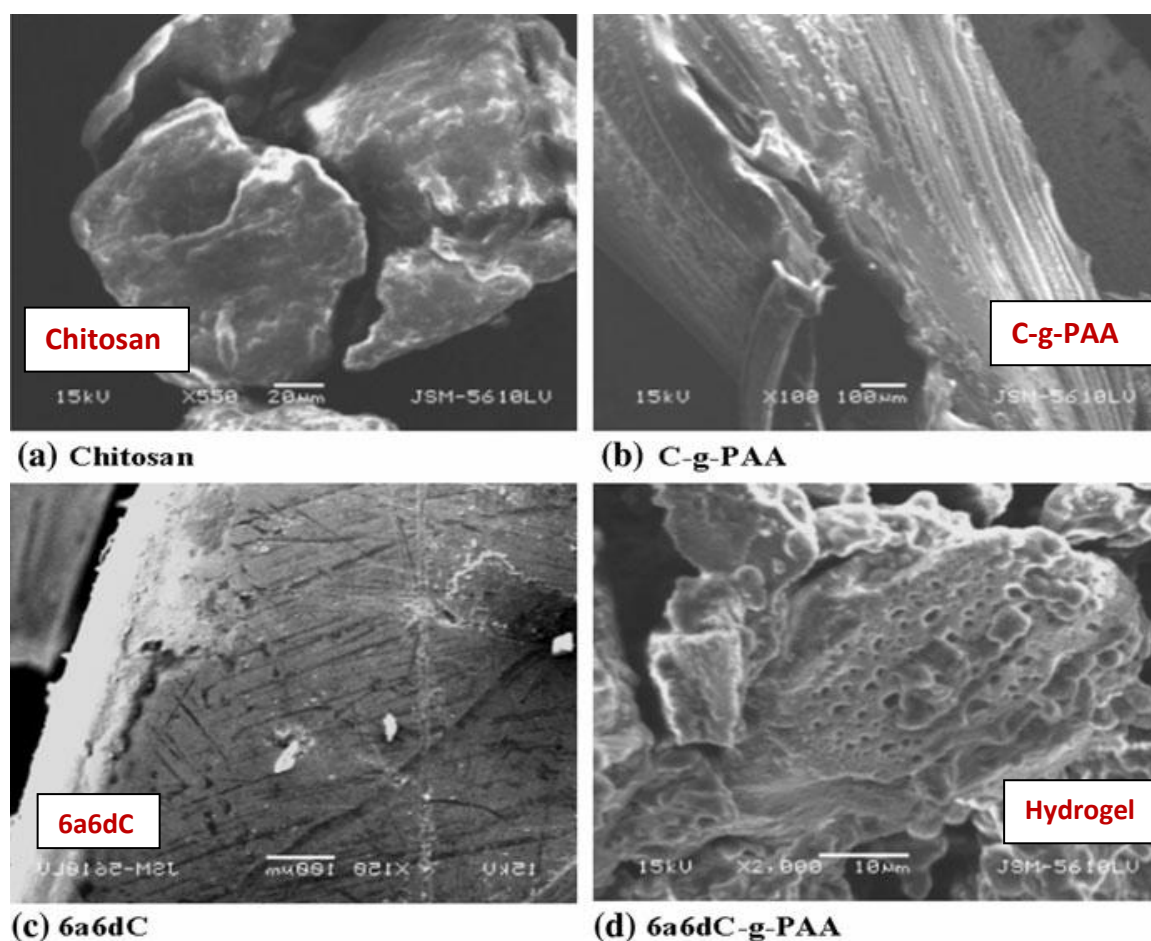
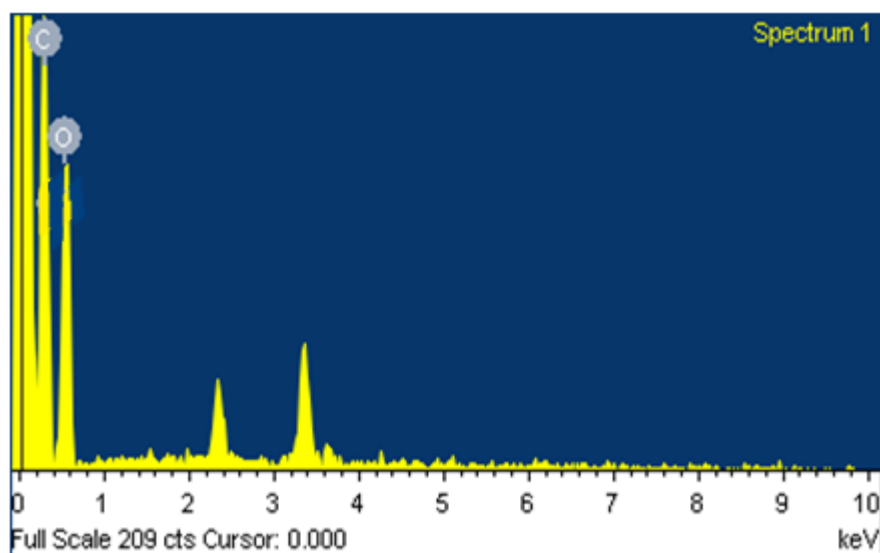


Figure 3.7 SEM analysis of (a) Chitosan, (b) C-g-PAA, (c) 6a6dC & (d) 6a6dC-g-PAA

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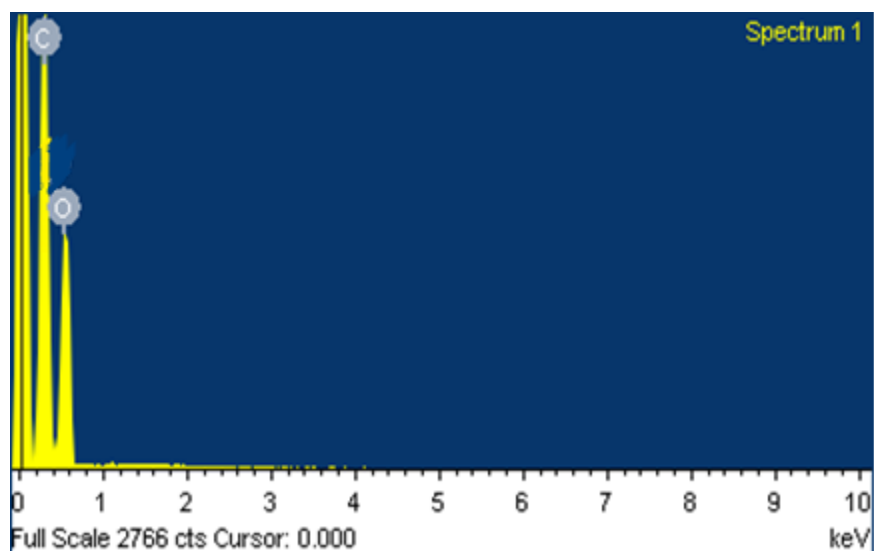
The EDX spectra [Fig. 3.8(a) chitosan & 3.8(b) 6a6dC] confirm the presence of nitrogen % which gets doubled in 6a6dC by the functionalizations of primary OH to NH₂ group via series of reaction such as tosylation of phthaloyl chitosan, then azidation followed by reduction.

(a) CHITOSAN



Element	Weight %	Atomic %
C K	47.61	54.41
O K	47.11	40.41
N & H K	5.28	5.18
Totals	100.00	

(b) 6a6dC



Element	Weight%	Atomic%
C K	47.61	54.41
O K	42.00	35.03
N & H K	10.39	10.56
Totals	100.00	

Figure 3.8 EDX analysis of (a) Chitosan & (b) 6-amino-6-deoxychitosan

3.4 Conclusion

A water soluble derivative of chitosan (i.e., diamino derivative of chitosan), 6-amino-6-deoxy-chitosan, was graft copolymerized with acrylic acid in aqueous medium (in contrast to aq. acetic acid) using hydrogen peroxide/L-ascorbic acid (instead of KPS) as a redox initiator system to give hydrogel, 6a6dC-g-PAA. The new initiator system provided the reaction at room temperature. It was found to be thermally more stable than chitosan and 6-amino-6-deoxy-chitosan. The gel shows a higher swelling ratio at pH 7 and has higher water absorbancy capacity up to 140 times its own weight. Below this pH non-neutralized carboxyl groups disfavor water uptake where as above this pH non-protonated amino groups disfavor water uptake. The prepared chitosan network exhibits pH-dependent swelling characteristics and has potential application in drug delivery and biomaterials preparation.

3.5 References

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