Chapter – II

Synthesis of Chs-co-Chi copolymer and Graft Copolymerization of Acrylic Acid and Acrylamide onto the Copolymer by Chemical Method
The present Chapter deals with the synthesis of copolymer from natural polysaccharides, chitin (Chi) and chitosan (Chs), a deacetylated derivative of chitin, and modification of the copolymer so obtained, Chs-co-Chi, by graft copolymerization of hydrophilic water soluble monomers, acrylic acid (AAc) and acrylamide (AAm) by chemical method using ammonium persulfate as radical initiator.

Polymeric carbohydrates belong to the important class of natural biological polymers that are biocompatible and bio-functional and can be used for biomedical applications like biomedicine, tissue engineering, controlled drug delivery, biomedical implants and many others. Variety of naturally occurring polysaccharides, derived from renewable resources, are complex structural carbohydrates (cellulose, carrageenan, chitosan and chitin) and are available for use in various applications including pharmaceutical and biomedical applications because of their inherent biological and chemical properties. Biomaterials can be natural or man-made that accordingly comprises whole or part of a living structure. Synthetic polymers, with wide range of properties and uses are manmade and are produced commercially on a very large scale. Both, natural and synthetic polymers provide ample variety of properties leading to their full exploitation in everyday life and can be produced with a wide range of stiffness, strength, heat resistance, density and even price.

A number of properties of chitosan, such as non-toxicity, biocompatibility, biodegradability, flexibility, physiological inertness etc make it a suitable candidate to be useful in a variety of fields, such as waste water treatment, cosmetics, food applications, pharmaceutics and biomedicine, drug delivery and for delivery of biologically active compounds. Being non-toxic in nature, it is edible and safe for domestic animals. Along with these properties, chitosan is also non-allergenic to living tissues and has antibacterial, antifungal and antitumor activity and can rapidly clot the blood. The cell wall of bacteria is anionic and the amino groups present in chitosan attach to the anionic bacterial cell wall and thus, retard its biosynthesis. Also, chitosan stops the mass transport through the cell wall and leads to the death of the bacteria. Some functional derivatives of chitosan have shown antimicrobial activities against plant pathogens and hence can be used for crop protection. Chitosan and its derivatives have also been used in skin care as skin moisturizer. Because of smoothness and elasticity, chitosan is used in shampoos, hair colors as coloring agent in the form of a solution of chitosan and in gel formation.
Chitin is also a non-toxic, antibacterial, anti-inflammatory, biodegradable and biocompatible natural polymer.\textsuperscript{[10]} It can be easily dissolved to give hydrogels in the presence of calcium chloride dehydrate methanol solvent systems,\textsuperscript{[11]} and therefore, can be used to develop scaffolds and membranes for the biomedical applications like tissue engineering and wound dressing. Therefore, a number of attempts have been made to use chitin in wound dressings and as scaffolds in biomedical fields.\textsuperscript{[12, 13]}

Although natural polymers like chitin and chitosan have been observed as potent candidates for various applications but they have low water absorption capacity and low mechanical strength. Therefore, modification of chitin and chitosan becomes essential to explore their high potentials as materials to be used in various applications. Due to presence of numerous reactive functional moieties on chitosan and chitin, such as, amino, acetamido and primary and secondary hydroxyl groups present on C(2), C(3), and C(6) positions, the chemical modification such as nitration, phosphorylation, sulfonation, xanthation, acylation, hydroxyalkylation, Schiff base formation, alkylation etc of these polymers can be carried out to give derivatized polymers that have a broad spectrum of utility.

Other than the chemical derivatizations, their modification can also be achieved either through cross-linking, copolymerization with natural and synthetic polymers or graft copolymerization methods. As chitosan possess a number of the unique properties, therefore it has been blended and copolymerized with other natural and synthetic polymers imbibing the properties of both the polymers.\textsuperscript{[14, 15]} Specific properties such as hydrophilicity, biocompatibility, flexibility, thermal stability, rigidity etc can be introduced or enhanced in the modified material. The micro particles of chitosan can be formed through emulsion method or through spray drying which can be utilized as a drug carrier.\textsuperscript{[16, 17]} Using cross-linking agents such as glutaraldehyde and divalent anions, the decrease in the rate of drug release and gel formation of chitosan from the chitosan-based systems can be carried out.\textsuperscript{[16, 18]} A complex of chitosan with alginate has been synthesized by Motwani et al.\textsuperscript{[19]} through ionic interactions between carboxyl groups of alginate and amine groups of chitosan and was used as a drug delivery device. It has been observed that in comparison to chitosan or alginate alone, their complex shows better drug release. Similar study has been carried out by Liu et al.\textsuperscript{[20]} which shows that the drug was released in longer duration by using the complex.
Chitosan has amazing affinity towards proteins and resembles collagen protein in many chemical and structural properties. The copolymerization products of chitosan with gelatin have been used for the biological applications because of eminent properties of gelatin to support cell adhesion and relocation and tendency to undergo polyelectrolyte complex formation. Scaffolds made from chitosan-gelatin hydrogels have been used as matrix for cell seeding and other medical uses. Chitosan-gelatin hydrogels have also been employed on a large scale for tissue engineering properties and other biomedical applications. Cheng et al. studied the physical properties and nerve cell affinity of composite films prepared from chitosan and gelatin blends. Chitosan-gelatin copolymer using Schiff base was successfully synthesized and was found to have outstanding bacteriostatic and biocompatibility properties. A number of chitosan based copolymers have been used as drug delivery systems. Both ionic and non-ionic interactions of chitosan with the drugs have been observed due to the amino groups present in its structure and these groups also provide pH-sensitive systems, as they swell under gastric conditions allowing a site-specific release. Chitosan-gelatin sponges have been employed in drug delivery for the controlled release of prednisolone drug. Hari et al. developed chitosan-alginate microparticle system and studied the controlled release of bioactive peptides including insulin.

Optimization of reaction conditions and characterization of graft copolymers of chitosan with methyl acrylate was carried out by Pati and Nayak. The graft copolymers were found to have a good antimicrobial and antifungal properties. The block copolymerization of mono methoxy poly(ethylene glycol) macromer onto chitosan, using KPS to yield thermo sensitive hydrogel was carried out by Ganji and Abdekhodaie. The gelation behaviour of the hydrogel makes it a promising and attractive material for biomedical applications. Comb-type ampholyte copolymers of chitosan as flocculants with double electrical behaviour was prepared by polymerizing chitosan, acrylamide and sodium carboxymethyl cellulose together using ammonium per sulfate as the initiator in aqueous medium. Polyaniline was chemically grafted onto chitosan by using ammonium peroxydisulfate initiator in presence of 1M HCl to give us a conducting polymer. The conductivity of the copolymer was enhanced by grafting polyaniline onto chitosan electrochemically polymerized on the surface of Pt disk. Atom transfer radical polymerization (ATRP) method was used to synthesize chitosan-g-poly(oligoethylene glycol methacrylate) copolymer. Graft copolymerization of AAc
onto chitosan was carried out using ceric ions by Don et al. Swelling behaviour of graft copolymer was observed under different pH and acrylic acid concentration. Grafting of acrylonitrile and methyl acrylate onto chitin was carried out by chemical method using ceric (IV) as redox initiator in aqueous medium. Tanodekaew et al. prepared graft copolymer of chitin with poly(AAc) with hydrogel characteristics for wound dressing applications. Radiation-induced grafting of styrene onto chitin and chitosan was carried out as a function of absorbed dose, solvent and oxygen. Graft copolymerization of AAc and AAm onto chitin using Ce as initiator was carried out by Kurita et al. A hybrid material of natural polysaccharide and a synthetic polymer was prepared by grafting styrene onto mercapto-chitin. Under optimum conditions, maximum grafting percentage (97.0%) was achieved indicating high efficiency of the mercapto-chitin as an initiator for the polymerization of styrene.

In view of the above, it was thought worthwhile to synthesize a copolymer of chitosan and chitin and use it as a base polymer for grafting of hydrophilic monomers, acrylic acid and acrylamide by chemical method using ammonium persulfate as radical initiator and sodium bicarbonate as foaming agent.

2.1. EXPERIMENTAL

2.1.1. Materials and Method:
Chitosan (Himedia, Mumbai, India), chitin (purified powder lab. reagent, S.D. Fine Mumbai), sodium bicarbonate (NaHCO₃) (S.D. Fine, Mumbai), a foaming agent and ammonium persulfate (APS) (S.D. Fine, Mumbai), a free radical initiator, were used as received. Monomers, acrylic acid (AAc) and acrylamide (AAm) were received from Merck, India and were used as received. Distilled water was used throughout the study as the reaction medium.

2.1.2. Synthesis of Chitosan-Chitin, Chs-co-Chi, copolymer:
Known weights of chitosan (0.3g-0.7g) and chitin (0.3g-0.7g) were suspended in water (2.5- 4.5 mL), taken in a test tube. The mixture was stirred to prepare a thick dispersion and to it was added the radical initiator, APS (0.73 × 10⁻¹-3.65 × 10⁻¹ mol/L) and NaHCO₃ (5.95 × 10⁻²-13.89 × 10⁻² mol/L), used as a foaming agent. Thick dispersion so obtained was heated for different time periods (2-6 h) at a constant temperature (50-
90°C). After the stipulated time, the product, chitosan (Chs)-chitin (Chi) copolymer, Chs-co-Chi, received as a monolith, was removed from the test tube, washed thoroughly with water and dried to a constant weight.

2.1.3. Graft copolymerization of Acrylic acid and Acrylamide onto Chs-co-Chi copolymer: Synthesis of (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) graft copolymers:

Graft copolymerization of AAc and AAm onto Chs-co-Chi copolymer has been carried out by chemical method using APS as the radical initiator.

A known weight of Chs-co-Chi copolymer base, in the form of the monolith, was taken in a test tube containing water (2.5-4.5 mL) and to it was added a definite amount of APS (0.73 x 10^{-1} - 3.65 x 10^{-1} mol/L), monomer, AAc, (4.86 x 10^{-1} - 43.76 x 10^{-1} mol/L)/AAm (2.34 x 10^{-1} - 11.72 x 10^{-1} mol/L). The reaction mixture was placed in a bath maintained at a constant temperature (50-90°C) and the reaction was allowed to proceed for different time periods (2-6 h). After the stipulated reaction time, the product was removed from the test tube, washed thoroughly with water to remove any homopolymer, poly(AAc)/poly(AAm) formed during the reaction. The graft copolymer, thus, obtained was dried and weighed to a constant weight.

2.1.4. Swelling behaviour:

Swelling behaviour of pristine copolymer, Chs-co-Chi and each of the AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), prepared under different reaction conditions, has been studied as a function of time at room temperature.

2.1.5. Swelling studies of pristine and grafted Chs-co-Chi copolymers in water:

Accurately weighed samples of pristine copolymer, Chs-co-Chi and AAc and AAm grafted copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) were separately immersed in beakers containing 30 mL of distilled water. The beakers were covered and placed undisturbed at room temperature. The sample was removed from the beaker after every half an hour and the adhered water was immediately removed by blotting the sample between the folds of filter paper and weighed. Increase in weight due
to water sorption was measured till it became constant and percentage of swelling was calculated as follows:

\[
\text{Percentage of swelling} = \frac{W_s - W_d}{W_d} \times 100
\]

Where \( W_s \) and \( W_d \) are the weights of swollen and original dried samples respectively.

2.1.6. Swelling studies of pristine and grafted Chs-co-Chi copolymers in buffer solutions:

Dried and weighed sample, (0.100 g) of pristine Chs-co-Chi copolymer and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), were separately immersed in 30 mL of citrate buffer of pH 2.8 and 30 mL of phosphate buffer of pH 7.2 in separate beakers. The beakers were covered and kept undisturbed at 37°C. The swelling percentage was measured by the procedure described above.

2.1.7. Preparation of citrate buffer:

Citrate buffer solution (citric acid and tri sodium citrate dehydrate) of pH 2.8 was prepared by adding 48.8 mL of citric acid (0.1 M) and 1.2 mL of tri sodium citrate dehydrate (0.1 M) solutions, each prepared in distilled water. The resulting solutions were diluted with distilled water to make total volume as 100 mL and the pH was adjusted with the addition of dilute HCl/ dilute NaOH solution.

2.1.8. Preparation of phosphate buffer:

Phosphate buffer solution (potassium monophosphate and potassium diphosphate) of pH 7.2 was prepared by mixing 0.1 M solution of potassium dihydrogen phosphate to 0.1 M solution of potassium hydrogen phosphate, each prepared in distilled water, till the pH reaches 7.2 and becomes stable.

2.1.9. Swelling studies of pristine and grafted Chs-co-Chi copolymers in salt solutions:

Dried and weighed samples, (0.100 g) of pristine Chs-co-Chi copolymer and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), were separately immersed in a known amount (30 mL) of 0.9% NaCl
solution in separate beakers. The beakers were covered and kept undisturbed at 37°C. Percentage of swelling was evaluated according to the method described above.

2.2. Characterization:

Pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), have been characterized by Fourier transform infrared (FTIR), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), X-ray diffraction (XRD) and particle size measurements.

2.2.1. Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectra of the pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), were obtained by Nicolet-5700 Spectrophotometer.

2.2.2. Scanning Electron Microscopy (SEM):

SEM analysis of pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), was obtained using JEOL JSM-6100 scanning microscope. Electron micrographs of the sample were recorded at different magnifications.

2.2.3. X-Ray Diffraction Studies (XRD):

The X-ray diffraction patterns of pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), were recorded on a Philips PANALYTICAL X’PERT PRO X-ray powder diffraction using Cu Kα (λ = 1.54060 Å) radiation.

2.2.4. Measurement of particle size:

Average Crystallize Size (D) in the sample was calculated from peak widths Δ2Θ_{1/2} in the characteristic diffraction peaks using the Debye-Scherrer’s equation:

\[ D = \frac{0.89 \lambda}{(\Delta 2\theta_{1/2}) \cos \theta_b} \]

where 2Θ_b is the position of a peak in the diffractogram from a specific crystallographic plane (hkl).
2.2.5. Particle size analysis:

Particle size analysis of pristine Chitosan, Chitin, pristine copolymer, Chs-co-Chi and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), were performed on Navowavetrac Particle Size Analyzer (Metrohm, US).

2.2.6. Thermogravimetric Analysis (TGA):

Thermogravimetric analysis of pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), was carried out on Thermal Analyzer (LINSEIS, L81-11) by taking 5.0 mg of each sample into silica crucible for TG-DTA analysis in air at a heating rate of 10°C per min with temperature range of 55 to 700°C.

2.3. Results and Discussion:

2.3.1. Synthesis of Chitosan-Chitin, Chs-co-Chi Copolymer:

Ammonium persulfate (APS), a well known radical initiator, is used for polymerization and graft copolymerization reactions. Persulfate ions cleave homolytically to give sulfate ion radicals, which can further, in the presence of water, yield hydroxyl radicals. Both sulfate ion radicals and hydroxyl radicals are active to generate active sites on the polymer backbones through abstraction process. The active free radical sites on the polymer backbones offers sites for grafting. In the present case, active sites are generated on the chitosan and chitin polymer backbones and the radicals generated on these polymers, combine to yield the Chitosan-chitin copolymer, Chs-co-Chi.

![Chs-co-Chi Copolymer](image-url)
Based on this, following are different steps involved in the synthesis of the copolymer (Scheme I).

\[
S_2O_8^{2-} \rightarrow 2SO_4^{2-}
\]

\[
SO_4^{2-} + H_2O \rightarrow HSO_4^- + \cdot OH
\]

Chitosan: \( R=\text{NH}_2 \), Chitin: \( R=\text{NHCOCH}_3 \)

**Scheme I: Mechanism of copolymerization of Chitosan and Chitin**

The generation of free radicals on chitin and chitosan using APS as the radical initiator, both in the presence and absence of inert atmosphere (N₂ gas), has been reported in literature while studying graft copolymerization of vinyl monomers onto these polymers. \cite{51-53} Sodium bicarbonate, as a foaming agent along with APS as radical initiator helps to give a copolymer with good porosity. \cite{54}

The synthesis of the copolymer, Chs-co-Chi, was studied as a function of various reaction parameters and the optimization of these parameters has been based with respect
to the swelling percentage of each of the copolymer, prepared as a function of the variable studied, as a function of time.

2.3.1.1. Effect of chitosan and chitin ratio:

The effect of variation of ratio of chitosan and chitin on the formation of the copolymer and on percent swelling of the copolymer has been studied as a function of time and the results are presented in Fig. 2.1. The total amount of the polymer substrates was fixed at 1.0 g and the amount of chitosan and chitin was varied. It is observed from the figure that percent swelling of the copolymer increases with time in all the cases while varying the weight ratio of the two polymers in the mixture reaches maximum and then becomes constant. This is due to the reason that the copolymer is obtained with porous and flexible structure that allows the diffusion of water into the copolymer bulk. It is further observed that percent swelling of the mixture containing 0.7: 0.3 and 0.5: 0.5 (wt/wt) ratio of Chs to Chi go almost parallel till 150 min beyond which percent swelling for 1: 1 mixture shows better swelling with maximum (145.20%) in 300 min. Increase in the amount of chitin to chitosan (0.7: 0.3) shows lower swelling percent with slow rate through out because of the reason that chitin has more of acetamido groups which have little affinity for water in comparison to chitosan with more free amino groups that interacts with water. The copolymer prepared using higher amount of chitin was poor in strength and was unstable affecting swelling behaviour.

![Figure 2.1 Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of Chs/Chi ratio](image)

**Fig. 2.1 Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of Chs/Chi ratio**

Reaction Conditions: [APS] = 1.46 × 10⁻¹ mol/L, [NaHCO₃] = 7.93 × 10⁻³ mol/L, H₂O = 3 mL, reaction temperature = 70°C, reaction time = 4 h
2.3.1.2. Effect of [APS]:

The synthesis of the copolymer has been studied as a function of the initiator concentration and its effect on the swelling properties of the copolymer has also been evaluated and the results are presented Fig. 2.2. It is observed from the figure that swelling percent in each case increases with time, reaches maximum and then becomes constant thereafter. Maximum swelling (145.20% in 300 min) is observed for the sample prepared by using $1.46 \times 10^{-1}$ mol/L of APS beyond which it decreases. At higher [APS], the excess hydroxyl radicals generate more radicals on the polymer backbone and thus increase the cross-linking density and hence swelling properties. It has been reported that as the cross-linking density increases, swelling properties are affected due to inhibition to the diffusion of water. At higher [APS], degradation of chitosan has been reported in literature which affects the copolymer structure and hence swelling characteristics.

![Graph](image)

**Fig. 2.2** Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of [APS]

Reaction Conditions: Chs = 0.500 g, Chi = 0.500 g, [NaHCO₃] = $7.94 \times 10^{-2}$ mol/L, H₂O = 3 mL, reaction temperature = 70°C, reaction time = 4 h

2.3.1.3. Effect of [NaHCO₃]:

Fig. 2.3 demonstrates the effect of concentration of NaHCO₃, used as a foaming agent, on the synthesis and swelling properties of Chs-co-Chi copolymer. It is observed from the figure that increase in swelling percent of each of the copolymers, prepared at different concentrations of NaHCO₃, does not change much with time but steadily increases with low rate. However, swelling increases with increasing [NaHCO₃]. In each case after attaining maximum value, percent swelling becomes constant. Maximum swelling
percentage (319% in 180 min) is obtained for the sample prepared at $[\text{NaHCO}_3] = 11.90 \times 10^{-2}$ mol/L. Higher swelling percentage at higher bicarbonate concentration is due to the increase in porosity of the copolymer making availability of increased space volume helping better diffusion of water and increased holding capacity through interaction between the copolymer functionalities and water molecules.

![Figure 2.3](image)

**Fig. 2.3** Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of $[\text{NaHCO}_3]$

Reaction Conditions: Chs = 0.500 g, Chi = 0.500 g, $[\text{APS}] = 1.46 \times 10^{-1}$ mol/L, $\text{H}_2\text{O} = 3$ mL, reaction temperature = 70°C, reaction time = 4 h

**2.3.1.4. Effect of amount of water:**

The reaction medium has an important role in the synthesis of the copolymer as the amount of water ascertains the formation of copolymer which can hold its physical structure obtained as a monolith. Therefore, the synthesis and percentage of swelling of Chs-co-Chi copolymer has been studied as a function of amount of water and the results are presented in Fig. 2.4. It is observed from the figure that percentage of swelling of each of the copolymer sample, prepared by using varying amount of water, increases with swelling time, reaches maximum and then decreases. Maximum percentage of swelling (319% in 180 min) is obtained for the sample, prepared by using an optimum amount of water (3 mL) beyond which it decreases. Amount lower than the optimum may not be sufficient to completely homogenize the two polymers while the amount higher than the optimum seems not facilitating maximum interaction between the two
polymers due to higher solvation of each of the individual polymer, thus affecting the formation of the copolymer with higher volume content of each of the polymer that impinge on the swelling properties.

Fig. 2.4 Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of amount of water

Reaction Conditions: Chs = 0.500 g, Chi = 0.500 g, [APS] = 1.46 × 10⁻¹ mol/L, [NaHCO₃] = 11.90 × 10⁻² mol/L, reaction temperature =70°C, reaction time = 4 h

2.3.1.5. Effect of reaction temperature:

The effect of reaction temperature on the synthesis and percentage of swelling of Chs-co-Chi copolymer has been studied and the results are presented in Fig. 2.5. It is observed from the figure that percent swelling of the copolymer samples, prepared at lower temperatures (50 & 60°C), does not show much variation with time while the samples prepared at higher temperatures show gradual increase in percent swelling with time. Maximum percentage of swelling (319% in 180 min) is obtained for the sample prepared at an optimum temperature of 70°C. Reactions carried out at lower temperatures do not completely generate active radicals from the initiator and the polymer substrates that affect the formation of the copolymer and hence the swelling behaviour of the copolymer.
Fig. 2.5 Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of reaction temperature

Reaction Conditions: Chs = 0.500 g, Chi = 0.500 g, [APS] = 1.46 × 10⁻¹ mol/L, [NaHCO₃] = 11.90 × 10⁻² mol/L, H₂O = 3 mL, reaction time = 4 h

2.3.1.6. Effect of reaction time:

The effect of time of reaction for the synthesis of the copolymer, Chs-co-Chi, and thence on percentage of swelling was studied and the results are presented in Fig. 2.6. Percentage of swelling of all the samples is found to increase gradually with slow rate with both time of reaction and time of swelling. Maximum swelling (319% in 180 min) is obtained for the copolymer, prepared in 4 h of reaction time beyond which it decreases.

Fig. 2.6 Percent swelling of Chs-co-Chi copolymer as a function of time: Effect of reaction Time

Reaction Conditions: Chs = 0.500 g, Chi = 0.500 g, [APS] = 1.46 × 10⁻¹ mol/L, [NaHCO₃] = 11.90 × 10⁻² mol/L, H₂O = 3 mL, reaction temperature =70°C
2.3.2. Graft Copolymerization of Acrylic acid and Acrylamide onto Chs-co-Chi copolymer:

Once the copolymer, Chs-co-Chi is formed, it is graft copolymerized with acrylic acid and acrylamide using APS as radical initiator.

Both sulphate ion radical and hydroxyl radical generate active sites on the copolymer and also initiates the monomer to give growing polymeric chains. The interaction between the two give the AAc/ AAm grafted Chs-co-Chi copolymer, (Chs-co-Chi)-g-poly(AAc)/ (Chs-co-Chi)-g-poly(AAm). Based on this, the mechanism of grafting of AAc and AAm onto Chs-co-Chi is proposed as follows (Scheme II):

\[
\begin{align*}
(\text{Chs-co-Chi})^\cdot +\text{SO}_4^- &\rightarrow (\text{Chs-co-Chi})^\cdot \\
(\text{Chs-co-Chi})^\cdot +\text{OH} &\rightarrow (\text{Chs-co-Chi})^\cdot \\
\text{M} +\text{OH} &\rightarrow \text{HO-M}^\cdot +\text{M} \\
(\text{Chs-co-Chi})^\cdot +\text{HO-M}^\cdot &\rightarrow (\text{Chs-co-Chi})^- +\text{M} \\
\text{HO-M}^\cdot +\text{M} &\rightarrow \text{HO-M}_2\cdot \\
\end{align*}
\]

Scheme II: Mechanism of grafting of AAc and AAm onto Chs-co-Chi copolymer

Graft copolymerization of AAc and AAm onto Chs-co-Chi copolymer has been carried out under various reaction conditions that seem to affect graft copolymerization reactions. The optimization of the reaction conditions have been established on the basis of the swelling behaviour of each of the product in water as a function of time at room temperature. The results are explained in the light of the mechanism.

2.3.2.1. Effect of monomer concentration:

The concentration of monomer plays a vital role in optimization of the graft copolymer to exhibit best possible swelling behaviour. Therefore, the effect of the concentration of AAc and AAm on the swelling behaviour of the respective graft copolymers, (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) has been investigated and the results are presented in Figs. 2.7a & 2.7b respectively. It is observed from Fig. 2.7a that percent swelling of all the samples, prepared with varying concentration of the monomer, AAc,
increases with time and [AAc], reaches maximum and then decreases to finally become constant. Maximum swelling percentage (271.43% in 60 min) is observed for the sample prepared by using $34.03 \times 10^{-1}$ mol/L of AAc. At higher [AAc], homo polymerization becomes the preferred process leading to lower graft level thus affecting swelling percentage. From Fig. 2.7b, it is observed that in case of grafting of AAm, percentage of swelling increases gradually with higher rate, reaches maximum and becomes constant after experiencing a slight decrease. Maximum swelling percentage (140% in 240 min) is observed for the graft copolymer, prepared by using lower concentration of AAm ($4.69 \times 10^{-1}$ mol/L). Further increase in the concentration of AAm, beyond the optimum, leads to the decrease in percentage of swelling.

From the above results it is, thus, observed that percent swelling in the AAc grafted sample decreases to 271.43% from that of 319% swelling of the Chs-co-Chi copolymer but the time of swelling also decreases from 180 min required for copolymer swelling to just 60 min for swelling of the graft copolymer which compensates for not to that extent lower percent swelling of the graft copolymer. However, in case of AAm grafted copolymer, maximum percent swelling decreases to 140% from 319% of the pristine copolymer and also with increase in swelling time from 180 min to 240 min. The decrease may be attributed to the reason that the amide groups have lower tendency to interact with water to enter into H-bonding as compared to the carboxylic acid moieties of the poly(AAc) chains. Therefore, lower percent swelling in higher swelling time is observed for the AAm grafted copolymer as compared to the AAc grafted Chs-co-Chi copolymer.

Thus, grafting of hydrophilic monomer improves the rate of water absorption giving comparative maximum percent swelling in small interval of time. However, the decrease in swelling upon grafting is considered due the reason that the introduction of the polymeric chains as grafts onto the copolymer and the polar carboxylic and amide groups of the grafted poly(AAc)/ poly(AAm) chains are also intra-molecular H-bonded that reduces the porosity of the copolymer affecting the diffusion of water and hence its interaction with the functional moieties of the copolymer.
Fig. 2.7a Percent swelling of (Chs-co-Chi)-g-poly(AAc) as a function of time: Effect of [AAc]

Reaction Conditions: Chs-co-Chi = 1.0 g, [APS] = 1.46 x 10⁻¹ mol/L, reaction temperature = 70°C, reaction time = 4 h

Fig. 2.7b Percent swelling of (Chs-co-Chi)-g-poly(AAm) as a function of time: Effect of [AAm]

Reaction Conditions: Chs-co-Chi = 1.0 g, [APS] = 1.46 x 10⁻¹ mol/L, reaction temperature = 70°C, reaction time = 4 h

2.3.2.2. Effect of [APS]:
When the effect of [APS] on the synthesis of graft copolymers, with monomers, AAc and AAm, and on swelling behaviour of the respective graft copolymers was studied, it
is observed from Fig. 2.8a that swelling capacity of the graft copolymer with monomer AAc, prepared by using varied concentration of APS, increases slowly with time, reaches maximum and becomes constant. Maximum swelling percentage (271.43% in 60 min) is observed for the graft copolymer, (Chs-co-Chi)-g-poly(AAc) sample, prepared at [APS] = 1.46 × 10⁻¹ mol/L. In case of grafting of AAm, the swelling percentage of each of the graft copolymer, (Chs-co-Chi)-g-poly(AAm), also increases gradually with higher rate, becomes constant after attaining maximum value. Maximum swelling percentage (140% in 240 min) was observed for the sample prepared at the same concentration of APS (1.46 × 10⁻¹ mol/L).

Further increase in [APS], beyond the optimum, percent swelling experiences small decrease in both the cases, before attaining the constant value. The decrease in swelling may be attributed to an increase in chain termination reactions via bimolecular collision leading to self cross-linking of the polymers. Preferred homopolymer formation at higher [APS] also leads lower swelling of the graft copolymer. It is also true for chitosan that persulfates results in the cleavage of polymer chains that leads to decrease in percent swelling. [58-60]

![Graph showing percent swelling as a function of time for different APS concentrations.](image)

**Fig.2.8a.** Percent swelling of (Chs-co-Chi)-g-poly(AAc) as a function of time: Effect of [APS]

Reaction Conditions: Chs-co-Chi = 1.0 g, [AAc] = 34.03 × 10⁻¹ mol/L, reaction temperature = 70°C, reaction time = 4 h
Fig. 2.8b Percent swelling of (Chs-co-Chi)-g-poly(AAm) as a function of time: Effect of [APS]

Reaction Conditions: Chs-co-Chi = 1.0 g, [AAm] = 4.69 × 10⁻¹ mol/L, reaction temperature = 70°C, reaction time = 4 h

2.3.2.3. Effect of reaction temperature:

Fig. 2.9a and Fig. 2.9b respectively represent the percentage of swelling of (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) graft copolymer samples, prepared as a function of temperature. It is observed from the figures that swelling percentage of each of the AAc and AAm grafted copolymer samples, prepared at different temperatures, increases with time gives maximum, decreases slightly and then becomes constant. Maximum percentage swelling, (271.43% in 60 min) and (140% in 240 min) respectively, is obtained for the (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) grafted samples, prepared at an optimum temperature of 70°C. Grafting reactions carried out at lower temperatures does not completely generate active radicals from the initiator and hence on the copolymer thus affecting grafting reaction and swelling capacity of the graft copolymer. At temperatures higher than optimum leads to the preferential homopolymer (poly(AAc)/ poly(AAm) which being soluble in water, the reaction medium, increases the viscosity of the reaction restricting the mobility of the growing polymeric chains to the active sites of the copolymer affecting the grafting reaction and hence swelling characteristics.
Fig.2.9a. Percent swelling of (Chs-co-Chi)-g-poly(AAc) as a function of time: Effect of reaction temperature

Reaction Conditions: Chs-co-Chi = 1.0 g, [AAc] = $3.403 \times 10^{-1}$ mol/L, [APS] = $1.46 \times 10^{-1}$ mol/L, reaction time = 4 h

Fig.2.9b. Percent swelling of (Chs-co-Chi)-g-poly(AAm) as a function of time: Effect of reaction temperature

Reaction Conditions: Chs-co-Chi = 1.0 g, [AAm] = $4.69 \times 10^{-1}$ mol/L, [APS] = $1.46 \times 10^{-1}$ mol/L, reaction time = 4 h

2.3.2.4. Effect of reaction time:

The effect of reaction time on grafting of AAc and AAm onto Chs-co-Chi copolymer and swelling behaviour of the respective graft copolymers has also been studied and the
results are presented in Fig. 2.10a & 2.10b respectively. On basis of percentage of swelling of the graft copolymers prepared as a function of time, it is observed from the Fig. 2.10a that maximum swelling percentage (271.43% in 60 min) is observed for (Chs-co-Chi)-g-poly(AAc), while (Chs-co-Chi)-g-poly(AAm) graft copolymer shows (Fig. 2.10b) maximum percent swelling (140% in 240 min) for the samples where grafting reaction is carried out for 4 h.

Lower swelling percentage observed for the graft copolymer samples, prepared in 2 h and 3 h of reaction time, may be attributed to the reason that the reaction time is insufficient for completely activating the copolymer, Chs-co-Chi, and the monomers and hence the formation of the graft copolymer which affects the swelling properties. Increase in the time of reaction, beyond the optimum, leads to chain scission reactions through backbiting processes, thus affecting the copolymer structures and hence swelling characteristics of the copolymer.\footnote{61, 62}

![Graph](image)

**Fig.2.10a.** Percent swelling of (Chs-co-Chi)-g-poly(AAc) as a function of time: Effect of reaction Time

Reaction Conditions: Chs-co-Chi = 1.0 g, [AAc] = 34.03 × 10^{-1} \text{ mol/L}, [APS] = 1.46 \times 10^{-1} \text{ mol/L}, reaction temperature = 70^\circ \text{C}
2.3.3. Effect of pH on swelling behaviour of pristine and grafted Chs-co-Chi copolymers:

The effect of the medium on swelling behaviour of the copolymer establishes its importance as a material for biomedical applications. Therefore, swelling equilibrium of pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm), has been studied in acidic (pH 2.8) and basic (pH 7.2) media at 37°C and the results are presented in Figs. 2.11a and Figs. 2.11b respectively.

It is observed from the figures that swelling percentage of pristine copolymer, Chs-co-Chi and AAc and AAm grafted Chs-co-Chi copolymers increases with time reaches maximum and becomes constant in both the pH media. Percentage of swelling of pristine copolymer, Chs-co-Chi, decreases in both low (2.8) and high (7.2) pH media with maximum swelling (182.86%) achieved in 1230 min and 264.52% in 330 min respectively as compared to that observed in water (319% in 180 min). This indicates functional groups of the copolymer are affected by the change in the pH of the medium. In the medium of low pH (2.8) the hydroxyl, amino and the acetamido groups of chitosan and chitin are protonated thus preventing interaction with water \(^{[63]}\) while in medium with little higher pH (7.2) than water, the functional groups of the copolymer and some
of the acetamido groups that are hydrolyzed to carboxylic group remain intramolecularly associated through H-bonding, thus, preventing the diffusion and interaction with water leading to lower swelling percentage.

However, when the copolymer is grafted with hydrophilic monomer, AAc and AAm, swelling properties of the copolymer changes and gives higher swelling percentage in both pH media as compared to the pristine copolymer but lower than that observed for respective graft copolymers in water. Maximum swelling percentage (190.32% in 1230 min) in pH 2.8 medium, observed for AAc grafted Chs-co-Chi copolymer, is higher than the pristine copolymer in the same medium but is lower than that observed in water (271.43% in 60 min). This is due to reason that the pendant carboxylic groups of the grafted poly(AAc) chains are intramolecularly H-bonded and in addition are protonated in the acidic medium thus preventing the diffusion and interaction with water. The pKa of carboxylic groups is 4.57, therefore, at lower pH (acidic) the poly(AAc) chains remain interwind and collapse to prevent diffusion of the solvent thereby lowering of swelling percentage.  

When effect of higher pH (7.2) is studied on swelling behaviour of (Chs-co-Chi)-g-poly(AAc), it is observed from Fig. 2.11b that swelling percentage initially increases with time and then becomes almost constant giving maximum percent swelling (286.21% in 330 min) higher than that observed in lower pH and pristine copolymer as well as that observed in water (271.43% in 60 min). Increase in swelling in medium of higher pH as compared to lower pH is due to reason that functional groups remain unprotonated and freely interact with water. Increase in swelling in higher pH (7.2) has also been observed for poly(AAc-co-AAm) hydrogels with higher AAc content. Few of the carboxylic groups exist as anion that also helps in increasing swelling percentage. The increase in swelling is not much high (from 271.43% to 286.21% in higher swelling time). This can be due to the reason that since the change in pH is very low (0.2), the H-bonded structure of the graft copolymer is not disturbed and this slows down the diffusion of water thereby giving maximum swelling in higher swelling time.

When swelling properties of AAm grafted Chs-co-Chi copolymer in lower pH (2.8) and higher pH (7.2) is studied, it is observed that in pH (2.8) medium, the graft copolymer gives higher swelling percentage (244.74% in 1230 min) as compared to that of the pristine copolymer (182.86% in 1230 min) and AAc grafted copolymer (190.32% in 1230 min).
1230 min) and higher than that observed in water. This is attributed to the reason that the pendant amide group of the grafted poly(AAm) are hydrolyzed to carboxylic group thereby increasing swelling percentage. In the basic medium (pH 7.2), the graft copolymer gives lower swelling percentage (121.74% in 120 min) as compared to that of the pristine copolymer and AAc grafted copolymer and that observed in water (140% in 240 min). Similar observations with higher percentage of swelling of poly(AAm) (1014-716% in pH 3.0) in comparison to (973-754%) in higher pH 7.4 were made by Thakur et al.\cite{64}

Fig. 2.11 (a) Percent swelling of Chs-co-Chi copolymer, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm): Effect of pH 2.8 buffer

Fig. 2.11 (b) Percent swelling of Chs-co-Chi copolymer, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm): Effect of pH 7.2
2.3.4. pH sensitivity and pulsative behaviour:

The pulsative behaviour of pristine copolymer, Chs-co-Chi, and AAc and AAm grafted Chs-co-Chi copolymers as a function of swelling in different pH (2.8 and 7.2) media has been studied at 37°C for exhibiting reproducible swelling-deswelling cycles. Swelling measurements were made after every 15 min in the medium of each pH up to 120 min before switching to the next pH. The studies were continued till 720 min and the results are presented in Fig. 2.11c.

Percentage of swelling of pristine copolymer, Chs-co-Chi, increases with increasing time of swelling in pH 7.2 medium giving maximum 103.23% within 120 min. The sample was then shifted to the medium of pH 2.8. It is observed from the figure that percentage of swelling starts decreasing and reaches the minimum value 61.29% in next 120 min. This cycle was repeated with shifting the sample to pH 7.2 where again increase in swelling is observed giving maximum swelling 148.39% in 120 min. As the process of switching to alternate pH continues, it is observed that the change in swelling percent is not high and sharp when the sample is next shifted to lower pH 2.8. Non-responsiveness of the sample towards swelling while shifting to different pH in the later stages is attributed to the reason that alternate shifting in acidic and basic media involves protonation and deprotonation of the functional groups of chitosan (NH2/ OH) and chitin (NH2/ NHCOCH3/ OH) which at later stages become saturated and behave indifferently.

Similar trend is observed in case of (Chs-co-Chi)-g-poly(AAc) but (Chs-co-Chi)-g-poly(AAm) with continuous increase in swelling in medium of pH 7.2 and a continuous decrease in medium with lower pH till 720 min, AAc grafted copolymer giving higher swelling percentage in comparison to pristine and AAm grafted copolymer. In addition to the functional groups of the base copolymer the pendant carboxylic groups of grafted poly(AAc) and amide groups of grafted poly(AAm) chains behave differently in acidic and basic media and hence are sensitive towards pH change of the medium.

This sharp swelling and deswelling behaviour at different pH value makes the system highly pH responsive and suitable for tailoring pulsative (on-off switching) drug delivery devices/ systems.
2.3.5. Effect of counter ion on swelling behaviour of pristine and grafted Chs-co-Chi copolymers:

The extent of swelling of the copolymer in any swelling medium is dependent on the nature of the external solution i.e. charge number and ionic strength and the nature of the polymer i.e. the presence of the functional groups present on the polymer, the extent of cross-linking and the presence of salt also affects the swelling properties of the copolymers. In order to investigate the utility of pristine copolymer, Chs-co-Chi and the AAc and AAm grafted Chs-co-Chi copolymers in biomedical applications, the effect of counter ion on the swelling behaviour of the copolymer has been investigated in aqueous solution of NaCl (0.9%) at 37°C and the results are presented in Fig. 2.11d.

It is observed from figure that percentage of swelling of each of the copolymers increases with time giving maximum and becomes constant thereafter. It is further observed that percent swelling of pristine copolymer and AAc grafted copolymer shows a sharp increase in first 60 min beyond which the increase in swelling is constant with slow rate giving maximum 90% and 106.90% in just 150 min respectively. However, in case of AAm grafted copolymer a regular increase in swelling percentage with little higher rate is observed giving maximum (115.15% in 150 min).

Decrease in percentage of swelling in pristine and graft copolymers in comparison to water is attributed to the “charge screening effect” of the additional cations. [65, 66] This
effect leads to decrease in ionic repulsions resulting in lowering of osmotic pressure due to the difference in the mobile ion concentration between hydrogel and aqueous phase.\textsuperscript{[67]}

![Graph showing swelling percentage over time for different copolymers](image)

**Fig. 2.11d** Percent swelling of Chs-co-Chi copolymer, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) copolymers: Effect of salt solution

2.4. Characterization of pristine and AAc and AAm grafted Chs-co-Chi copolymer:

2.4.1. FTIR spectroscopy:

IR spectra of pristine copolymer, Chs-co-Chi, and (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) graft copolymers are presented in Figs. 2.12.

IR spectrum of Chs-co-Chi (Fig. 2.12a) shows a broad and strong band ranging between 3700-3200 cm\(^{-1}\) due to \(v(O-H)\) stretching and extension vibration of \(v(N-H)\) of chitosan,\textsuperscript{[68]} a small peak at 3100 cm\(^{-1}\) due to \(v(N-H)\) stretching of secondary amide group of chitin, small peaks in region between 2960.25 and 2891.25 cm\(^{-1}\) due to \(v(C-H)\) stretching (asymmetric & symmetric respectively), peaks at 1626 cm\(^{-1}\) due to \(v(C=O)\) stretching of the acetylated amide group. In addition to this a peak at 896.28 cm\(^{-1}\) is observe due to the peroxide linkage of the copolymer confirming successful synthesis of the copolymer.

In the IR spectrum of (Chs-co-Chi)-g-poly(AAc) (Fig. 2.12 b), in addition to the exiting peaks of the copolymer, peaks at 1720 cm\(^{-1}\) and 1597 cm\(^{-1}\) are observed due to \(v(C=O)\) symmetric and asymmetric stretching respectively and a peak at 1261 cm\(^{-1}\) due to \(v(C-O)\)
stretching coupled with \( \nu_{(O-H)} \) in plane bending of carboxylic groups of the grafted poly(AAc) chains is also observed.

In case of (Chs-co-Chi)-g-poly(AAm), the IR spectrum (Fig. 2.12 c), shows additional peaks at 1632 cm\(^{-1}\) and 1575 cm\(^{-1}\) due to \( \nu_{(C=O-NH)} \) of amide I and amide II and a peak at 1401 cm\(^{-1}\) due to \( \nu_{(C-O-C)} \) stretching while 1114.43 cm\(^{-1}\) peak is related to the amide group.\(^{[69]}\)

The presence of additional peaks due to grafted polymeric chain confirms the formation of the grafting of acrylic acid and acrylamide onto the copolymer.

Fig.2.12. FTIR Spectra of (a) Chs-co-Chi copolymer, (b) (Chs-co-Chi)-g-poly(AAc) and (c) (Chs-co-Chi)-g-poly(AAm)

2.4.2. Scanning Electron Microscopy:

Surface topology and homogeneity of Chitosan, Chitin, pristine copolymer, Chs-co-Chi and (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) graft copolymers was
studied by Scanning Electron Microscopy and the SEMs are presented in Figs. 2.13 (a-e respectively) at different magnifications.

Fig. 2.13a and Fig. 2.13b present scanning micrographs of chitosan and chitin depicting smooth surface with straps and shrinkage of the Chitosan [70] while rough and thick surface morphology (50X) with microfibrillar crystalline structure observed at higher magnification (3000X) for chitin. [71] Interaction between chitosan and chitin to yield the copolymer, Chs-co-Chi, changes the surface morphology of both the individual polymers. The copolymer exhibits a thick dense but porous structure with small cavities distributed on the entire surface of copolymeric matrix (Fig. 2.13 c).

Upon grafting of AAc and AAm onto Chs-co-Chi copolymer, change in surface topology and morphology of the copolymer is observed. The surface of copolymer becomes heterogeneous due to the anchored grafted polymer (Fig. 2.13 d and 2.13c). The porosity of the graft copolymers can be well observed due to the presence of pores on the surface of the polymers.

Fig. 2.13a SEM of Pure Chitosan  
Fig. 2.13b SEM of Pure Chitin

![Fig. 2.13c SEM of Chs-co-Chi copolymer at different magnifications](image)
2.4.3. X-Ray Diffraction Studies:

X-ray diffraction studies of Chitosan, Chitin, pristine copolymer Chs-co-Chi, (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) have been carried out and the respective diffractograms are presented in Figs. 2.14 a-e respectively. The XRD pattern of chitosan (Fig. 2.14 a) shows peaks at 11.7° and 20.2° on 2θ scale with very low intensities (50 & 70 respectively) indicating amorphous nature of the polymer. XRD pattern of chitin (Fig.13,b), on the other hand, shows two strong peaks at 9° and 19° on the 2θ scale with respective intensities at >1000 and >1500 indicating semi-crystalline nature of the polymer.

When the two are reacted to give the copolymer, the structural behaviour of the two polymers changes, XRD patterns of the copolymer Chs-co-Chi experiences a shift in the peak. A strong peak at 19.79° with an intensity at 250 counts and the peaks due to chitin
shift to higher values (26.19° and 29.28° respectively) with lower intensities indicating that semi crystalline structure of the copolymer.

Upon grafting of the AAc and AAm, it is observed that the peak area decreases with increase in intensities and sharp peaks appear at 19.47° (370 counts) and 19.44° (570 counts) respectively and small peaks at 26.32° (200 counts) and 29.47° (170 counts) in AAc grafted Chs-co-Chi and at 26.11 (210 counts) and 29.44 (179 counts) in AAm grafted Chs-co-Chi on 2θ scale and the peak area decreases in comparison to pristine copolymer indicating a shift to crystalline structure. This is due to the reason that the grafted chains of poly(AAc) and poly(AAm) hold the structure.

Change in the structural behaviour upon copolymerization of the two polymers and graft copolymerization suggests successful synthesis of the pristine Chs-co-Chi copolymer and AAc and AAm grafted Chs-co-Chi copolymers.

![Graphs](a.png)  ![Graphs](b.png)  ![Graphs](c.png)  ![Graphs](d.png)
2.4.4. Particle Size Distribution: Crystallite size and particle size

Distribution of the crystallite size and particle size of the pristine copolymer, Chs-co-Chi and the graft copolymers, (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) has been determined from the XRD data applying Scherer’s equation to FWHM and particle size analyzer (Figs 2.15 a-c respectively) and results are presented in the Table 2.1. From the Table, it is observed that the pristine copolymer and the graft copolymers have nano structures. The change in the particle size is due to the reason that particle may consists of different number crystallites thus increasing that size.

Table 2.1 Comparison of size particles in XRD and particle size analyzer (nm)

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>Chs-co-Chi</th>
<th>(Chs-co-Chi)-g-poly(AAc)</th>
<th>(Chs-co-Chi)-g-poly(AAm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRD</td>
<td>2.33 nm</td>
<td>3.20 nm</td>
<td>3.03 nm</td>
</tr>
<tr>
<td>Particle Size</td>
<td>3360 nm</td>
<td>1.01 nm</td>
<td>1778 nm</td>
</tr>
</tbody>
</table>
Fig. 2.15 Particle size distribution of (a) Chs-co-Chi copolymer, (b) (Chs-co-Chi)-g-poly(AAc) and (c) (Chs-co-Chi)-g-poly(AAm)

2.4.5. Thermogravimetric analysis:

Thermogravimetric analysis (TGA) of pristine copolymer Chs-co-Chi and (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) graft copolymers was carried out in air at a heating rate of 10°C/min and the respective primary thermograms are presented in Figs. 2.16 (a–c). The initial decomposition temperature, (IDT), final decomposition temperature, (FDT) and decomposition temperature (DT) at every 10% weight loss are presented in Table 2.2. It is observed from the Fig. 2.16a that the pristine copolymer, Chs-co-Chi, and the graft copolymers (Chs-co-Chi)-g-poly(AAc), (Chs-co-Chi)-g-poly(AAm) show single stage of decomposition.

The first stage of decomposition of pristine copolymer begins from 210°C, the initial decomposition temperature. The decomposition continues with further rise in
temperature till 550°C from where begins the final decomposition which continues leaving 11.43% of residue. The temperature difference between each decomposition temperature at every 10% wt. loss, although increases and decreases with rising temperature, indicating variable rate of decomposition, is high signifying slow rate of decomposition. Comparing the TG analysis of the copolymer with individual polymers, chitin and chitosan, \(^{[72]}\) it is observed that the thermal degradation of both chitin and chitosan undergo fast rate of decomposition with small temperature difference between DT at each 10% wt. loss. Thus, when the two polymers are copolymerized to (Chs-co-Chi), the thermal properties are improved.

In the DTA curve, four endothermic peaks at 315°C (27.5μV), 395°C (19.38 μV), 505°C (13.12 μV) and 530°C (16.9 μV) are observed. All the peaks corroborate the decomposition depicted in the thermogram.

The copolymer upon grafting with acrylic acid undergoes a change in the thermal behaviour. The decomposition occurs in a single stage with initial decomposition beginning at lower temperature (204.6°C) in comparison to the pristine copolymer (210°C) due to the early decomposition of the pendent carboxylic groups of the grafted poly(AAc) chains. The decomposition continues with rising temperatures till 541.82°C from where begins the final decomposition that continues to leave 2.86% residue. The DT values at each 10% wt. loss are although lower than those of the copolymer but the temperature difference between DT at every 10% wt. loss is much higher and consistent indicating a slow and gradual rate of decomposition. The lower values of the graft copolymer are due the reason that grafting has structural changes that affects the thermal properties.

The DTA curve also corroborates the decomposition observed in the TG analysis. The endothermic peaks at 342.5°C (21.67 μV), 460°C (18.85 μV) and 510°C (20 μV) are observed.

When the copolymer is grafted with acrylamide the decomposition occurs in a single stage with initial decomposition beginning at lower temperature (204.35°C) in comparison to the pristine copolymer (210°C) and is same as AAc grafted copolymer. The decomposition continues with rising temperatures till 554.35°C where begins the final decomposition leaving 7.53% residue. The DT values at each 10% wt. loss are
lower than those of the pristine copolymer till 30% decomposition beyond which it increases. The temperature difference between DT values at each 10% weight loss is also much higher and consistent indicating a slow and gradual rate of decomposition.

The DTA curve also corroborates the decomposition observed in the TG analysis. The endothermic peaks at 339.13°C (26.62 μV) and an exothermic peak at 476.09°C (23.08 μV) are observed.

From the thermal data, it is thus, observed that the polymers in the form of copolymer have better thermal behaviour in comparison to the individual polymers. Upon grafting with AAc and AAm, the thermal behaviour improves due to high temperature difference between DT values at each 10% weight loss indicating a slow rate of decomposition. The obtention of residue, although lower than pristine copolymer, also indicates thermal stability.
Fig. 2.16 Primary Thermograms of (a) Chs-co-Chi copolymer (b) (Chs-co-Chi)-g-poly(AAc) (c) (Chs-co-Chi)-g-poly(AAm)

Table 2.2. Thermogravimetric Data of Chs-co-Chi copolymer, (Chs-co-Chi)-g-poly(AAc) and (Chs-co-Chi)-g-poly(AAm) graft copolymers

<table>
<thead>
<tr>
<th>Sample</th>
<th>IDT (°C)</th>
<th>FDT (°C)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>% Residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chs-co-Chi</td>
<td>210</td>
<td>550</td>
<td>232.5</td>
<td>275.0</td>
<td>292.5</td>
<td>307.5</td>
<td>340.0</td>
<td>395.0</td>
<td>445.0</td>
<td>497.5</td>
<td>---</td>
<td>11.43</td>
</tr>
<tr>
<td>(Chs-co-Chi)-g-poly(AAc)</td>
<td>204.55</td>
<td>541.82</td>
<td>206.82</td>
<td>252.97</td>
<td>286.36</td>
<td>291.10</td>
<td>338.62</td>
<td>381.80</td>
<td>431.80</td>
<td>474.97</td>
<td>556.78</td>
<td>2.86</td>
</tr>
<tr>
<td>(Chs-co-Chi)-g-poly(AAm)</td>
<td>204.35</td>
<td>554.35</td>
<td>223.91</td>
<td>266.52</td>
<td>293.48</td>
<td>316.52</td>
<td>352.17</td>
<td>417.39</td>
<td>463.48</td>
<td>500.00</td>
<td>545.65</td>
<td>7.53</td>
</tr>
</tbody>
</table>

Conclusion:

Chitin, a natural aminoacetyl polysaccharide, has been successfully copolymerized with chitosan, deacetylated product of chitin, through chemical method using ammonium persulfate as radical initiator. The pristine copolymer, Chs-co-Chi, has been modified by graft copolymerization of hydrophilic monomers, acrylic acid and acrylamide to get a product with improved thermal and swelling properties.
References


