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SYNTHESIS, CHARACTERIZATION AND OPTIMISATION OF CARBOXYMETHYL CHITOSAN GRAFT GYLCIDYL METHACRYLATE COPOLYMER

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Abstract:
The graft copolymerization of glycidyl methacrylate (GMA) onto the carboxymethyl chitosan (CMCS) has been successfully carried out using ceric ammonium nitrate as a redox initiator in aqueous nitric acid medium. The effect of grafting variables, i.e. concentration of initiator, temperature, monomer (GMA), polymer (CMCS), solvent and reaction time on the grafting parameters (GE (%), GY (%), GP (%) and (%C)) was systematically investigated and optimized for the graft copolymer (CMCS-g-GMA) to achieve a highest percent grafting. From the observed results it was evident that the grafting parameters were increased initially but thereafter it declines with increase in the concentration of initiator, temperature, monomer, polymer, solvent and time. The synthesized graft copolymer was characterized by Fourier Transform Infrared Spectroscopy (FT-IR), and X-ray diffraction (XRD) studies. The FT-IR measurements clearly indicate that the glycidyl methacrylate monomer was effectively grafted onto carboxymethyl chitosan and the XRD studies elucidate the change in the crystallinity of the grafted polymeric samples. The results were investigated.

Key Words: Carboxymethyl Chitosan, Glycidyl Methacrylate, Redox Initiation, Graft Copolymerization & Ceric Ammonium Nitrate

Introduction:

Chitosan is a polycationic hydrogel (poly (1→4) -2-amino-2-deoxy-D-glucan) having many significant biological (biodegradable, biocompatible, bioactive) and chemical properties due to the reactive groups such as hydroxyl and amino groups[1,2]. The degradation products of chitosan are also non-toxic, non-immunogenic and non-carcinogenic[3,4] and hence it has prospective applications in many fields such as waste water treatment, biomedicine, functional membranes and flocculation. However, chitosan is only soluble in few dilute acid solutions, which limits its applications. In order to improve its solubility and widen its applications there has been a growing interest in the chemical modification of chitosan recently[5,6,7]. Derivatization by introducing small functional groups to the chitosan structure, such as alkyl or carboxymethyl groups[8,9] can drastically increase the solubility of chitosan at neutral and alkaline pH values without affecting its cationic character. Substitution with moieties bearing carboxylic groups can yield polymers with polyampholytic properties[10]. Because of its ease of synthesis, ampholytic character and possibilities of ample of applications the carboxymethyl chitosan (CMC) has been widely studied when compared with the other water-soluble chitosan derivatives. Due to its water soluble nature the carboxymethylated chitosan has received more and more attention. Graft copolymerization is a versatile technique used for the modification of structure, shape and size of polymers by the formation of active sites on the polymer backbone. The active sites may be free radicals or ionic chemical groups, which initiate polymerization reaction[11,12]. Among the various methods of modification of polymers, graft copolymerization has been the most commonly used.

Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto the chitosan backbone. Grafting of natural polymers such as water-soluble polysaccharides containing various functional groups is of considerable interest for modification of the polymer structure[13]. So many works were reported on the synthesis of grafted carboxymethyl chitosan copolymers and hence carboxymethyl chitosan was preferable chosen in this work. Recently researchers have also shown that the primary deviation was followed by graft modification [14]. When compared to the other monomers such as acrylic acid, acrylamide, methyl methyl methacrylate, the glycidyl methacrylate (GMA) is an attractive monomer which is having the reactive double bond and an epoxy group in the molecule [15].The epoxy group present in the GMA monomer can form new functional groups by various chemical reactions and hence it was selected in this study for the grafting process. Nayak and coworkers have studied the graft copolymerization of several monomers onto a multitude of natural and synthetic polymers like wool, silk, cellulose, nylon and PET, rubber to enhance their properties using various initiators like hexavalent chromium, quinquevalent vanadium, tetravalent cerium, trivalent manganese, peroxydisulphate and peroxydiphosphate ions [16,17,18,19]. Of all the redox systems investigated the tetravalent ceric ion has received considerable interest because of its high grafting efficiency and very low homopolymer formation and hence in this work the ceric ammonium nitrate was utilized as an initiator.The main aim of the present work was to synthesise and characterize the carboxymethyl chitosan graft

glycidyl methacrylate copolymer using ceric ammonium nitrate as an initiator and in addition in this experiment, we set out to investigate the grafting conditions of GMA onto carboxymethyl chitosan with the aim of optimizing the grafting parameters.

Materials and Methods:

Chitosan was kind gift from Indian Sea foods Cochin, Kerala. The glycidyl methacrylate (GMA) monomer was supplied by Aldrich. The initiator ceric ammonium nitrate and ethanol was obtained from Nice Chemicals Pvt. Ltd, Cochin .The chloro acetic acid and Isopropanol was purchased from Lo Ba chemie Pvt. Ltd, Mumbai. All the reagents used to prepare the solutions were of analytical reagent grade.

Preparation of Carboxymethyl Chitosan (CMCS):

Carboxymethyl chitosan was prepared by method as suggested by Mark Soucek and his coworkers[20]. About 10g of chitosan, 10g of sodium hydroxide, 50ml of isopropanol and 50 ml of water taken in a beaker was allowed to swell and alkalize at 50^{o}C for 1 h. A required amount of monochloroacetic acid (15 g) dissolved in isopropanol (20 ml) was then added into the reaction mixture drop-wise for 30 min and reacted for 4 h at the same temperature. The reaction was then stopped by adding 250 ml of 70% ethyl alcohol. The obtained solid was filtered and rinsed in 70-90% ethyl alcohol, and vacuum dried at room temperature. The product was Na salt CC (Na-CC). This obtained Na-CC salt was suspended in 100ml of 80% ethyl alcohol solution followed by 10ml of 37% hydrochloric acid solution was added and then stirred well for 30 min. The final solid of carboxymethyl chitosan obtained was then filtered, rinsed in 70- 90% ethyl alcohol and vacuum dried.

Preparation of Carboxymethyl Chitosan Graft Glycidyl Methacrylate Copolymer (CMCS-G-GMA):

About 0.5 g of CMCS was dissolved in 30 ml of water with constant stirring. GMA (0.5g) dissolved in minimum amount of ethanol was then added to that solution. To initiate the polymerization process in the above reaction mixture, the initiator ceric ammonium nitrate (CAN) (0.5 g of CAN in 10 ml of 1N nitric acid) was added. The mixture was heated to 70˚C and simultaneously the solution was stirred using magnetic stirrer. This solution was then poured into excess of NaOH solution to precipitate the graft copolymer. It was then filtered, dried and weighed.

Grafting Parameters:

The graft copolymerization of glycidyl methacrylate onto carboxy methyl chitosan was carried out using ceric ammonium nitrate as an initiator. The grafting parameters including the grafting efficiency, grafting yield, grafting percentage and % monomer conversion were systematically evaluated as function of various initiator concentrations, temperature, monomer concentration, carboxymethyl chitosan concentration, solvent and reaction time. The percent of grafting, % monomer conversion, grafting efficiency and grafting yield were calculated as follows

$$GE(\%) = \frac{\text{Wt. of graft copolymer}}{\text{Wt. of graft copolymer + Wt. of homopolymer}} \times 100$$

$$GY(\%) = \frac{\text{Wt of graft copolymer – Wt of ungrafted polymer}}{\text{Wt of ungrafted polymer}} \times 100$$

$$GP(\%) = \frac{\text{Weight of graft copolymer}}{\text{Weight of substrate}} \times 100$$

$$\%C = \frac{\text{Weight of graft copolymer}}{\text{Weight of monomer}} \times 100$$

W_i – weight of ungrafted copolymer, W_g – weight of graft copolymer.

Characterization:

FT-IR Spectral Analysis: The FT-IR spectra of CMCS-g-GMA copolymer was recorded by Fourier transform infra-red spectrophotometer (FT-IR) using the Perkin Elmer 200 FT-IR spectrophotometer, in the wavenumber range of 500-4000 cm^{-1} during 64 scans, with 2 cm^{-1} resolution at 25°C

X-Ray Diffraction Analysis: X-Ray diffraction patterns of the grafted copolymeric samples were carried out by scattering SHIMADZU XD-D1 diffractometer using Ni filter, Cu Kα radiation source (λ=0.154nm), set a scan rate = 10˚/min. The voltage and the current used were of 40kV and 30 mA.

Results and Discussion:

The optimization of the various parameters such as concentration of initiator, temperature, concentration of monomer, concentration of carboxymethyl chitosan, solvent and and reaction was studied for graft copolymerization of glycidyl methacrylate onto carboxymethyl chitosan and the obtained results were presented below

The Effect of Initiator Concentration:

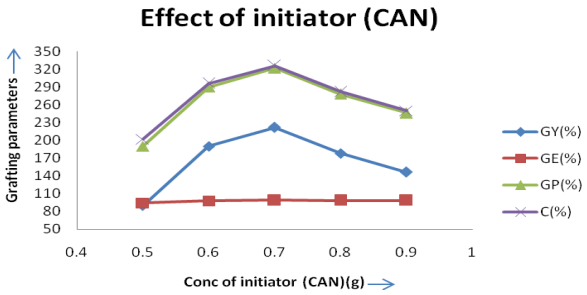

Figure 1: Effect of initiator concentration on the grafting parameters

Figure 1 shows the effect of ceric ammonium nitrate (initiator) concentration on the grafting parameters of CMCS-g-GMA copolymer. The effect of initiator on the synthetic grafting parameters has been investigated in the range of 0.5 g to 0.9 g by keeping the other variables such as temperature, monomer, polymer, solvent and reaction time as constant. The results presented in the Figure 1 indicate that the grafting parameters was found to increase with increase of CAN concentration up to 0.7g and then decreases with increase of CAN concentration beyond the limit. Initially the increased CAN concentration leads to the formation of more free radical sites on the carboxy methyl chitosan backbone and thereby the grafting parameters were increased. When the initiator concentration is increased beyond a certain limit (>0.7g) the initiator might interact with the monomer molecules producing homopolymer, thereby decreasing graft yield. In other words we can say that when the concentration of the initiator exceeds a certain value, increased free radical concentration results in serious homopolymerisation and hence lowers the graft copolymerization[21,22].

The Effect of Temperature:

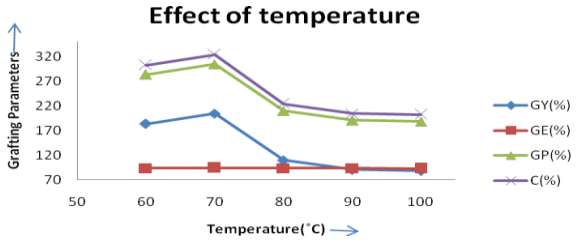

Figure 2: Effect of temperature on the grafting parameters

The grafting reactions were carried out at different temperatures (60°C- 100°C) by keeping all other variables as constant [23].The effect of temperature on the grafting parameters was shown in Figure 2. In the case of CMCS-g-PGMA, with the increase in temperature from 60°C to 70°C the grafting parameters shows an increase at first, reaches a maximum at 70°C and thereafter it declines. The maximum %GY and %GE obtained at 70°C was due to the generation of increased number of free radicals formed on the carboxymethyl chitosan backbone which increased the propagation of the graft copolymerization onto the substrate. The decrease in the grafting parameters at higher temperature was due to the radical termination which leads to the formation of homopolymer more rapidly.

The Effect of Monomer Concentration:

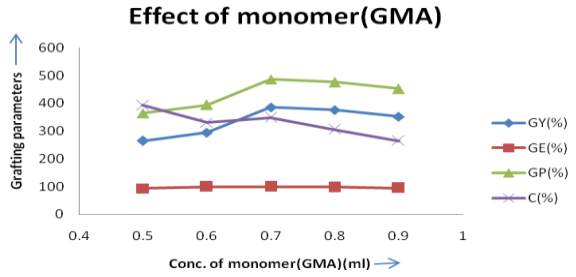

Figure 3 : Effect of monomer concentration on the grafting parameters

Figure 3 represents the effect of monomer concentration on the grafting of GMA onto carboxymethyl chitosan. The grafting reactions was done at various monomer concentration (0.5ml - 0.9 ml) by keeping the CMCS concentration, initiator concentration , solvent, temperature and time as constant. From the results presented in the Figure 3, it was observed that the grafting parameter initially increases with increase in monomer concentration and with further increase of monomer concentration it shows a decrease. The increase in the rate of grafting with the increase in monomer concentration was obviously owing to the greater availability of monomer in the proximity of CMCS macroradicals. The observed decrease at the higher

concentration of monomer is mainly due to the formation of homopolymer of GMA[24]. These homopolymers successfully hinders the rate of penetration of monomer molecules to carboxymethyl chitosan macro radical

The Effect of Polymer (CMCS) Concentration:

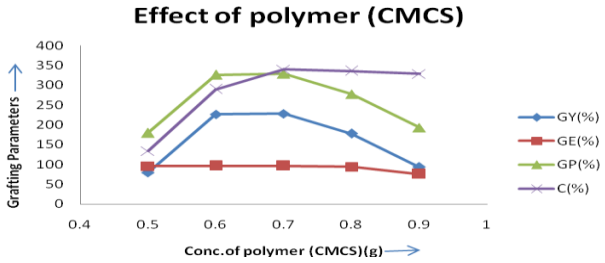

Figure 4: Effect of polymer concentration on the grafting parameters

The effect of concentration of carboxymethyl chitosan on the grafting parameters was studied within the range 0.5 g - 0.9 g for CMCS-g-GMA at constant initiator, monomer concentration, solvent, temperature and time. The observed results were presented in Figure 4.The results presented in the figure-4 indicate that the grafting parameters increased with the increase in CMCS concentration and reached a maximum at CMCS = 0.7 g for CMCS -g-GMA and thereafter decreases [25]. There exists an optimum CMCS concentration at which the percent of grafting reaches maximum. Initially the increase in backbone concentration can make more monomers and initiators easily approachable to the surface of CMCS. So they produce more grafted side chains which cause grafting efficiency to increase. At higher concentrations of the monomer the gel effect was more pronounced. The formation of a large amount of gel increases the viscosity of the reaction and so the grafted polymeric chain is restricted [26]. As a result, the attack on the actives sites of CMCS backbone is decreased and homopolymer is formed. The homo polymerization of GMA being relatively much faster than the grafting of GMA onto CMCS and hence this will result in the decrease in the grafting parameters [27].

The Effect of Solvent:

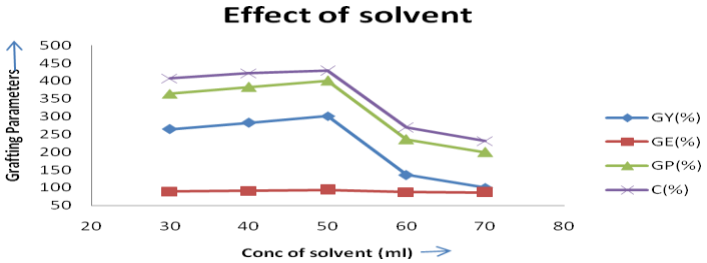

Figure 5: Effect of solvent on the grafting parameters

The effect of solvent on the grafting yield, grafting efficiency, grafting percentage and percentage monomer conversion was studied in the range of 30ml to 70ml for CMCS-g-GMA at constant initiator, temperature, monomer, and time. The results were presented in Figure 5.The observed results indicate that the GY(%), GE(%), GP(%), C(%) was increased with the increase in concentration of the solvent at first and thereafter the grafting yield, decreases with increase in the amount of solvent. This decrease probably occurs due to the dilution of reaction medium. As a result of this dilution the monomer radical concentration per unit volume decreases and hence the grafting parameters shows a decrease.

The Effect of Reaction Time:

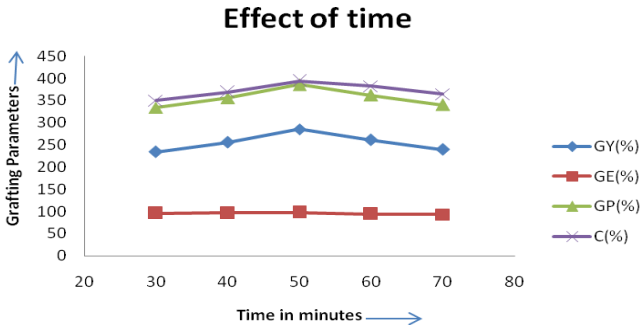

Figure 6: Effect of reaction time on the grafting parameters

The effect of reaction time on the grafting reaction of GMA onto CMCS was shown in Figure 6. It can be seen from Figure 6 that the grafting parameters increase sharply with increase in reaction time from 30 minutes to 50 minutes. The graft copolymerization rate increases to a maximum value of 50 minutes. With an

increase in reaction time, the free radicals have more time for reaction and therefore results in higher level of grafting. After some time, all the initiators and monomers are used up. Thus no further change in grafting level was observed with increasing reaction time (>50 min). The decrease in grafting may be due to the induced decomposition of the initiator leading to decrease in the concentration of the initiator and hence decrease in active radicals required to generate active sites on polymeric backbone. In other words we can say that the leveling off of grafting parameters is perhaps, a direct consequence of depletion of monomer available for grafting [28].

Fourier Transform IR Spectroscopy:

Fourier transform spectroscopy helps in identifying the functional groups present in the prepared polymeric samples. The FT-IR spectral details of pure chitosan, carboxymethyl chitosan and CMCS-g-GMA copolymer was represented in Figures-7(a)-7(c).

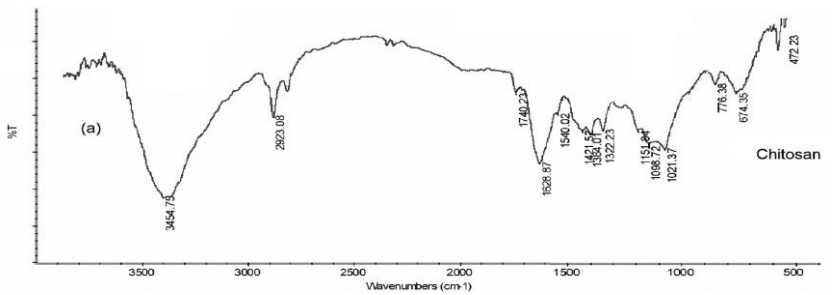

Figure 7(a): FT-IR spectrum of pure chitosan

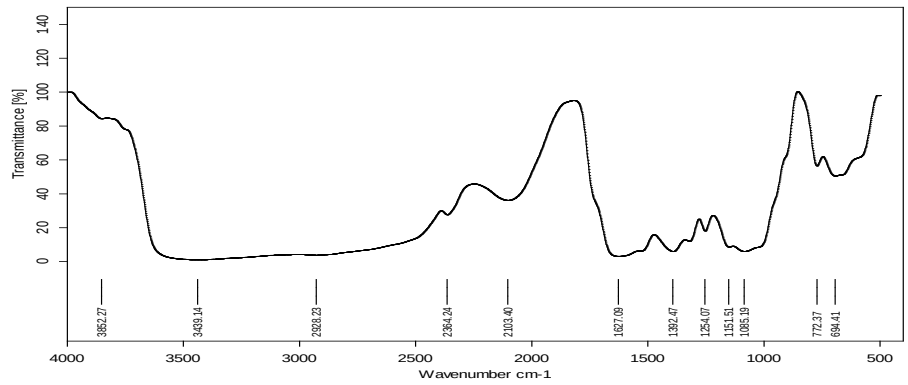

Figure 7(b): FT-IR spectrum of carboxymethyl chitosan (CMCS)

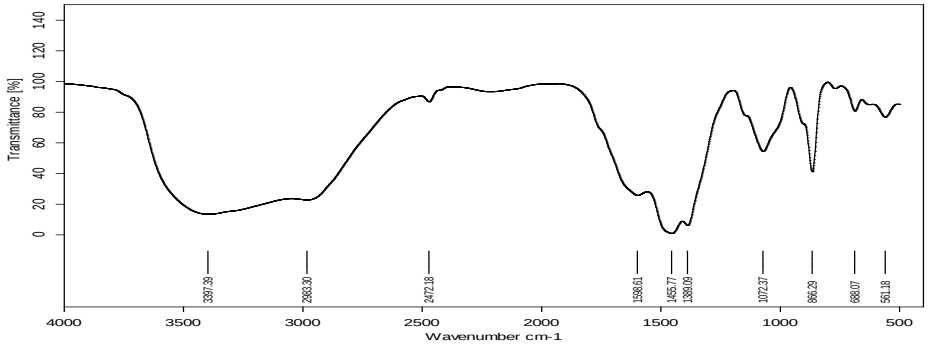

Figure 7(c): FT-IR spectrum of CMCS-g-GMA copolymer

The FT-IR spectral details of pure chitosan (Figure 7(a)) showed the prominent peaks at various wavenumbers such as 3454.75 cm^{-1}, 2923.08 cm^{-1}, 1628.87 cm^{-1} and so on. For the pure chitosan molecule, the hydroxyl (OH) peaks can be assigned at 3454.75 cm^{-1} and alkyl C–H stretching vibration were identified as doublets at 2923.08/2830.05 cm^{-1} respectively [29,30]. Strong peaks were observed at 1628.87cm^{-1}, 1540.02 cm^{-1} and 1421.52 cm^{-1} showing the presence of C=O stretching (amide-I band) [31], N-H bending and C-H deformation. The absorption bands observed at 1384.01 cm^{-1}, 1322.23 cm^{-1}, 1151.84 cm^{-1}, 1098.72 cm^{-1} and 1021.37 cm^{-1} were due to the presence of OH in plane bending in alcohols, twisting and wagging in CH_2 group, C-O stretching in secondary alcohols, C-O-C linkage and C-C stretching respectively. Figure 7(b) shows the FTIR spectral details of pure carboxymethyl chitosan. The FT-IR spectra of the carboxy methyl chitosan shows an absorption peak at 3439.27 cm^{-1} corresponding to the inter molecular H bonded NH stretching in secondary amines[32]. The peak observed at 2928.23 cm^{-1},1627.09 cm^{-1} and 1392.47cm^{-1} indicate the presence of

asymmetrical C-H stretching in CH_2, amide II (N-H bending in amide) and C-O-H bending in acids. On comparing the FT-IR spectra of pure chitosan with carboxy methyl chitosan it was observed the broadened of peak appeared at around 3500 cm^{-1} indicate the presence of OH stretching in acids in the case of FT-IR spectra of carboxymethyl chitosan and the appearance of this new peak suggest that carboxymethyl chitosan is formed from chitosan effectively.

The FT-IR spectral details of carboxymethyl chitosan graft glycidyl methacrylate copolymer was shown in Figure 7(c). The prominent peaks observed at 3397.39cm^{-1} indicate the OH- stretching in alcohols. The peak obtained at 2983.3cm^{-1} was attributed to the asymmetrical CH stretching in CH_3. On comparing the FT-IR spectra of carboxymethyl chitosan graft glycidyl methacrylate copolymer with pure chitosan, it was observed that the NH_2 bond associated band at around 1550 cm^{-1}, which can be ascribed to the characteristic peak of primary amine N-H vibration deformation obtained in the case of pure chitosan was disappeared. This indicates that the amino group present in the pure chitosan was involved in the grafting reaction. While comparing the FT-IR spectra of CMCS-g-GMA with carboxymethyl chitosan it was observed that an additional band obtained at 1165.74 cm^{-1} in case of carboxymethyl chitosan graft glycidyl methacrylate copolymer corresponds to the C-(C=O)-O stretching in esters. This obtained peak confirms that the grafting had taken place effectively between the carboxymethyl chitosan and glycidyl methacrylate.

X-Ray Diffraction Analysis (XRD):

XRD analysis were carried out to determine the nature of materials whether the material is amorphous or crystalline. X-ray diffraction patterns of various samples were measured to investigate the change of crystalline nature of carboxymethyl chitosan after polymerization. The XRD spectral details of pure chitosan, carboxymethyl chitosan and carboxymethyl chitosan graft glycidyl methacrylate polymer was represented in Table 1.

Samples	**2 theta**
Chitosan	10˚, 20˚
Carboxymethyl chitosan	31.5^0,45.2^0,56.2^0,65.9^0,75^0
CMCS-g-GMA copolymer	32.5^0,$33.6^0$$37.0^0$

The XRD spectrum of pure chitosan was shown in the Figure 8(a). The X-ray diffractogram of chitosan had a semi crystalline nature with two main diffraction peaks at around 2θ = 10º and 20º. The semi crystalline nature of pure chitosan was confirmed from the appearance of the diffraction peak centered at diffraction angle 2θ-10° and sharp diffraction peak at 20° which are indicative of high degree of crystalline morphology [33]. The chitosan molecule easily form the crystalline regions and this may be due to the presence of plenty of -OH and -NH_2 groups in the chitosan structure, which could form stronger inter and intramolecular hydrogen bonds[34]. Figure 8(b) and 8(c) represents the shows the XRD spectral details of carboxymethyl chitosan and CMCS-g-GMA. Carboxymethyl chitosan shows peaks at various 2θ values such as 31˚, 45˚, 56˚, 65˚, 75˚ and carboxymethyl chitosan graft glycidyl methacrylate polymer also shows peaks at various 2θ values such as 32.5˚,33.6°, 37.0˚. From the no of peaks observed it was concluded that the carboxymethyl chitosan graft glycidyl methacrylate sample and the carboxymethyl chitosan has more number of crystalline forms when compared to the pure chitosan.

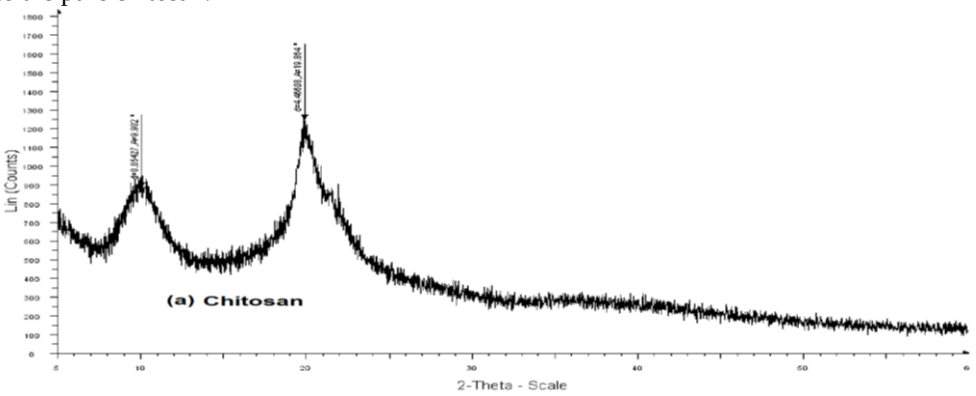

Figure 8(a): X-ray diffractogram of pure chitosan

The degree of crystallinity of a sample measures the ratio of the crystalline part to the amorphous part [35]. The degree of crystallinity can be expressed as

$$X_c(\%) = A_c / A_c + A_a \times 100$$

where X_c= degree of crystallinity, A_c=crystallized area on the X-ray diffraction, A_a=amorphous area on the X-ray diffraction Based on the major peak observed the degree of crystallinity values are calculated. The degree of crystallinity(%) values observed for the CMCS-g-GMA prepared under the various conditions are represented in the Table 2

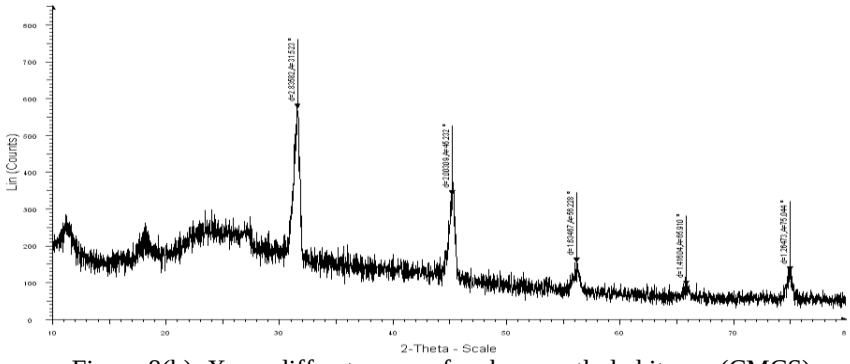

Figure 8(b): X-ray diffractogram of carboxymethyl chitosan (CMCS)

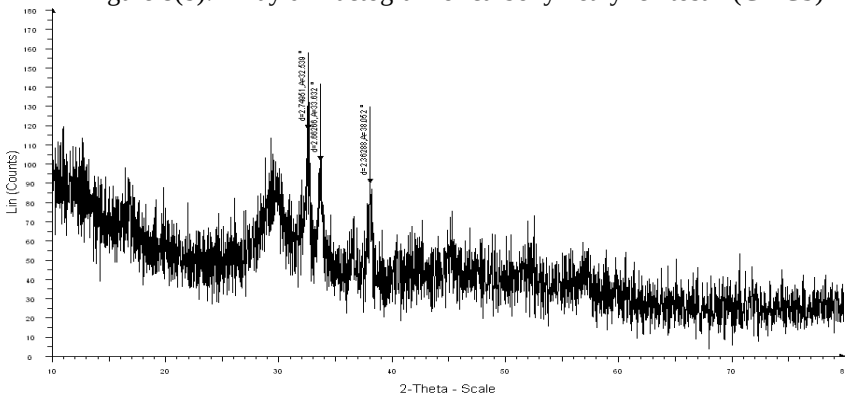

Figure 8(c): X-ray diffractogram of CMCS-g-GMA copolymer

Samples	Degree of Crystallinity
Chitosan	7.18
Carboxymethyl chitosan	5.67
CMCS-g-GMA copolymer	5.20

From the results presented in the Table 2, it was observed that eventhough the carboxymethyl chitosan with carboxymethylchitosan graft glycidyl methacrylate shows more number of crystalline forms based on the calculated lower percentage degree of crystallinity values it was identified that both carboxymethyl chitosan and graft copolymer of carboxymethyl chitosan has highly amorphous nature. On comparing the XRD spectral details of pure chitosan, carboxymethyl chitosan with carboxymethylchitosan graft glycidyl methacrylate it was observed that there were some differences of peak height, width and position between them. CS consisted of one major peaks at 2θ = 20◦. Compared with pure CS, CMCS and CMCS-g-GMA, CS showed a relatively narrow peak at 2θ = 20◦ whereas the CMCS had a relatively broader peak at 2θ = 20◦. In diffraction spectrum of CMCS-g-GMA, the peak at 2θ =20◦ became even broader and it became amorphous. It is well-known that the width of X-ray diffraction peak is related to the size of crystallite, the broadened peak usually results from small crystallites. Hence, in this reaction, the carboxymethylation reaction first took place preferentially in the amorphous region and then proceeded very moderately from the edge to the inside of the crystalline region and with further reaction with GMA, the crystalline structure was destroyed and the crystallinity disappeared.

Conclusion:

The present work was aimed to prepare and characterize graft copolymer of a carboxymethyl chitosan with the glycidyl methacrylate monomer. The synthetic conditions were systematically optimized by studying the effective factors including concentration of the initiator, temperature, monomer GMA, substrate CMCS, solvent and reaction time. The optimum conditions were represented as follows: Initiator conc: 0.7g; Temperature: 70˚C; Monomer conc: 0.7ml; Polymer conc: 0.7g; Solvent: 50ml and Time: 50 mins. The graft copolymers of carboxymethyl chitosan thus prepared was characterized by FTIR- spectral studies. The proof of grafting was evident from the appearance of new peaks due to ester in case of CMCS-g-GMA copolymer from the FT-IR results and the change in crystallinity of the carboxymethyl chitosan due to polymerization was confirmed with the help of comparison of the results of XRD of carboxymethyl chitosan and X- ray diffraction patterns of graft copolymer of carboxymethyl chitosan with glycidyl methacrylate (CMCS-g-CMA). This type of work could encourage the synthesis of new grafted polymers, where some functionality is required, for specific applications.

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