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Synthesis and characterization of chitosan based graft copolymers for drug release applications

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ABSTRACT

Graft copolymerization technique is used to enhance the application spectrum of chitosan in controlled drug release applications. Optimum grafting reaction conditions were evaluated for grafting of acrylic acid onto chitosan by using potassium persulphate as free radical initiator in aqueous solution. Glycidyl methacrylate was grafted for five different concentrations using the optimum grafting conditions evaluated for acrylic acid alone onto chitosan. Characterization techniques like FTIR, DSC, XRD and SEM were used to give chemico-physical evidences of grafting. The swelling behaviour of chitosan and graft copolymers were studied as a function of pH for defining their end use in the controlled release of anti-inflammatory drug, Diclofenac sodium.

Keywords: Graft copolymerization; swelling, Diclofenac sodium.

INTRODUCTION

In the past years, a huge amount of work has been done to explore the potential of biopolymers like cellulose, guar gum, dextrin, chitosan and their modified versions in number of biomedical applications like drug delivery, tissue culture, gene therapy etc. These biopolymers provide a safe, sustainable, controlled and target-specific way of drug delivery. Chitosan is a biopolymer obtained by the alkaline deacetylation of chitin [1]. It exhibits excellent biological properties such as biodegradation in the human body, and immunological, antibacterial, and wound-healing activity. Chitosan is also a good candidate as a support material in the gene delivery, cell culture and tissue engineering. However, the practical use of chitosan has been limited to the unmodified forms. For its utilization in frontline technologies, graft copolymerization [2] onto chitosan is key point, which introduces desired properties on the biopolymer and enlarges its spectrum of its applications by choosing various types of side chains.

Literature reveals that in the past years a good amount of work has been done on grafting of various types of vinyl monomers using various initiator systems onto chitosan and its derivatives. ceric ion [3], potassium persulfate [4], or γ-irradiation[5-7] as initiator. Graft-polymerization of 2-hydroxyethyl acrylate onto chitosan using ammonium persulfate [8] as an initiator was carried out in an aqueous solution by Grigoriy A. Mun and et al whereas M.H. Casimiro and co-workers [9] reported grafting of 2-hydroxyethyl methacrylate onto chitosan by using chemical initiation, photo-induction and gamma radiation-induced polymerisation, under heterogeneous conditions. Ge Huacai and co-workers [10] grafted acrylic acid onto chitosan by using the ceric ion as an initiator in the presence of N,N'-methylenebisacrylamide as a crosslinker under microwave irradiation. Ammonium persulfate initiated homogeneous graft copolymerization of (N,N-dimethylamino)ethyl methacrylate [11] onto N-carboxyethylchitosan (CECTS) and methacrylic acid [12] onto Carboxymethyl chitosan CMCTS is also reported in aqueous solution. Thermo- and pH-sensitive hydrogels were prepared by Hong Cai and et al [13] by graft copolymerization of chitosan and N-isopropylacrylamide via γ-radiation. Graft copolymerization of chitosan with hydroxyethyl

methacrylate using azobisisobutronitrile (AIBN) [14], methyl methacrylate using Fenton's reagent as redox initiator [15], dimethylamino ethyl methacrylate, and N, N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl) ammonium using ceric(IV) salt as redox initiator [16-17] and N-isopropylacrylamide by γ-irradiation [18] method have been reported in the literature. Chitosan and its graft copolymers were reported for control release of variety of drugs like ofloxacin [19], amoxicillin [20], cefadroxil [21], 5-fluorouracil [22], acyclovir [23], clozapine [24] and diclofenac sodium[25].

In the present work, we have synthesized graft copolymers of chitosan (Ch) with acrylic acid (AAc) by free radical initiation method. Glycidyl methacrylate (GMA) was grafted as comonomer for five different concentration terms along with AAc at the optimum grafting reaction conditions evaluated for AAc alone. Chitosan and graft copolymers were studied for drug delivery of Diclofenac sodium.

EXPERIMENTAL SECTION

Materials

Chitosan (Sisco Research Laboratories Pvt. Ltd. Mumbai, India), AAc, (Merck, Germany), GMA (Sisco Research Laboratories Pvt. Ltd. Mumbai, India) and potassium persulphate (KPS) (Ranbaxy, SAS Nagar, India) were used as received. All the chemicals used were of analytical grade.

Graft Copolymerization

KPS is used as free radical initiator for obtaining optimum grafting reaction conditions for grafting of AAc onto 1g chitosan. Nature of solvent, amount of solvent, concentration of initiator and monomer, reaction time and reaction temperature are the various parameters which were optimized. One reaction parameter was varied for a set of reaction keeping other reaction parameters constant (Table 1). The parameter which gave the maximum percent grafting (P_g) was selected for next step. At optimum grafting reaction conditions binary monomer mixture of AAc and GMA were grafted over five concentrations of the GMA.

Separation of Homopolymers/Copolymers

Homopolymers were separated from graft copolymers by solvent extraction method using water as solvent for poly(AAc), until constant weight of graft copolymer was obtained. Copolymers or homopolymer of AAc with GMA were removed by using different solvent systems of equal solvent compositions viz: water-ethyl methyl ketone for poly(AAc-*co*-GMA) and ethyl methyl ketone for poly(GMA). The percent grafting (P_g) and percent grafting efficiency (%*GE*) were calculated which can be expressed as [26]:

$$P_g = \frac{\text{Weight of graft copolymer - weight of polymer backbone}}{\text{Weight of polymer backbone}} \times 100$$

$$\%\, GE = \frac{\text{Weight of graft copolymer - weight of polymer backbone}}{\text{Weight of monomer charged}} \times 100$$

Characterization of Graft Copolymers

Characterization of chitosan and its graft copolymers were done by FTIR, XRD, SEM, TGA/DSC and swelling studies. FTIR spectra of chitosan and its graft copolymers were taken in KBr pellets by using Thermo Nicolet (Model 6700) spectrometer. X-ray Diffraction studies were carried out using X'Pert PRO (PAN analytical, Netherlands), Rigaku Rota Flex operating with Cu Kα radiation, 45kV, 40mA and equipped with a graphite monochromator. Scanning Electron Micrographs were taken on Jeol, JSM-6100 at an accelerating voltage of 20 kV. Thermal analysis was done on Shimadzu DTG-60; simultaneous TG/DT model. Swelling studies were carried out on chitosan and graft copolymers at different pH. 25mg of the copolymer was taken in 10.00mL of water**.** Percent swelling (P_s) was calculated by the following expression [27].

$$P_s = \frac{\text{Weight of the swollen polymer - weight of dry polymer}}{\text{Weight of dry polymer}} \times 100$$

Drug release

Chitosan and its graft copolymers with maximum P_g were selected for drug release study at variable conditions of pH. Sorption of diclofenac sodium (DS) onto chitosan and graft copolymers were done by equilibration method. Readings of drug solution were taken at 276 nm using Thermo Evolution 300 model UV–vis spectrophotometer.

Drug solutions from 2 µg/mL to 100 µg/mL concentrations were prepared and a standard curve was plotted between absorption values and concentration of drug solution. 25 mg of polymeric samples were dipped for 24 hrs in 10 mL solution of concentration 100 µg/mL. Absorption values of the filtrate were taken to know the percentage uptake of drug by polymeric samples. Then drug loaded polymeric samples were put in 10 mL buffer solution of pH 2.2, 7.0 and 9.4 to study the release pattern after regular interval of time. *Wolform Mathematica 7* software is used to calculate values of concentration of drug release from corresponding absorption values of drug solution from standard curve. Buffer solution of pH 2.2 was prepared by mixing 50 mL of 0.2 M KCl and 7.8 mL of 0.2 M HCl. Another buffer solution of pH 7.0 was prepared by dissolving buffer tablet in 100mL water and buffer solution 9.4 was prepared by mixing 100 mL 0.025 M $Na_2B_4O_7.10H_2O$ (borax) and 12.4 mL of 0.1 M NaOH.

Table 1: Effect of reaction conditions on graft copolymerization of AAc onto chitosan[a]

Sr. No.	Solvent	Amount of Solvent (mL)	Conc. of Monomer [AAc] moles/L×10^{-2}	Conc. of Initiator [KPS] moles/L × 10^{-2}	Time (h)	Temp (ºC)	Percent Grafting (P_g)	Grafting Efficiency %*GE*
1	H_2O	10.0	145.85	10.0	1.0	70	45.5	42.33
2	Benzene	,,	,,	,,	,,	,,	17.8	16.56
3	Dioxane	,,	,,	,,	,,	,,	12.9	12.00
4	CCl_4	,,	,,	,,	,,	,,	19.3	17.95
5	Acetone	,,	,,	,,	,,	,,	5.5	5.12
6	H_2O	5.0	292.17	20.0	,,	,,	146.7	139.6
7	,,	10.0	145.85	10.0	,,	,,	99.8	94.96
8	,,	15.0	97.23	6.66	,,	,,	130.0	123.69
9	,,	20.0	72.93	5.0	,,	,,	133.5	127.02
10	,,	25.0	58.34	4.0	,,	,,	118.1	112.37
11	,,	20.0	36.46	5.0	,,	,,	50.6	96.20
12	,,	,,	72.93	,,	,,	,,	133.5	127.02
13	,,	,,	109.39	,,	,,	,,	181.0	114.85
14	,,	,,	145.85	,,	,,	,,	157.2	69.74
15	,,	,,	182.31	,,	,,	,,	146.6	59.82
16	,,	,,	109.39	2.5	,,	,,	155.8	98.86
17	,,	,,	,,	5.0	,,	,,	181.0	114.85
18	,,	,,	,,	7.5	1.0	70	190.5	120.88
19	,,	,,	,,	10.0	,,	,,	160.3	101.71
20	,,	,,	,,	12.5	,,	,,	131.0	83.12
21	,,	,,	,,	7.5	1.0	,,	190.5	120.88
22	,,	,,	,,	,,	1.5	,,	103.6	65.74
23	,,	,,	,,	,,	2.0	,,	102.8	65.23
24	,,	,,	,,	,,	2.5	,,	103.5	65.67
25	,,	,,	,,	,,	3.0	,,	59.0	37.44
26	,,	,,	,,	,,	1.0	60	162.2	102.91
27	**,,**	**,,**	**,,**	**,,**	**1.0**	**70**	**190.5**	**120.88**
28	,,	,,	,,	,,	1.0	80	80.3	50.95
29	,,	,,	,,	,,	,,	90	59.7	37.88
30	,,	,,	,,	,,	,,	100	48.3	30.65

[a]*Chitosan = 1g*

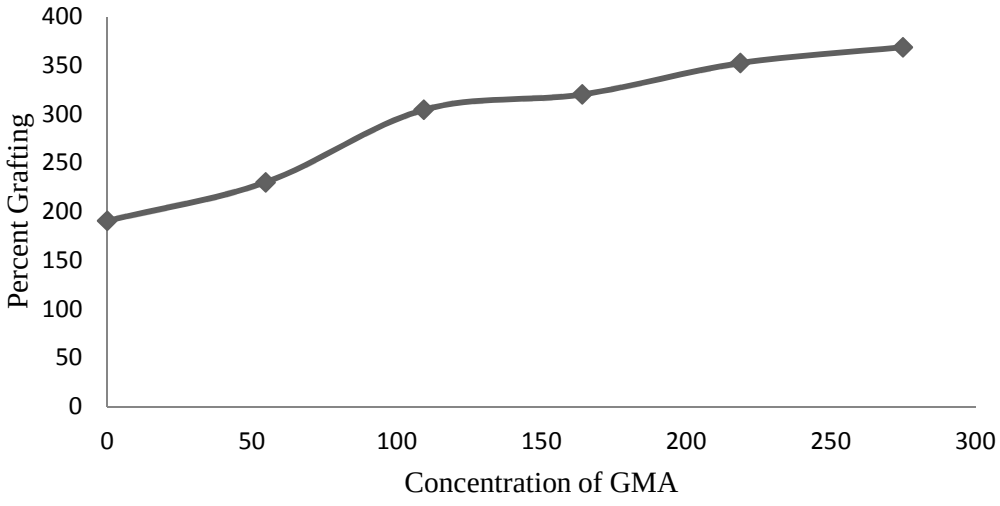

Figure 1: Grafting parameters of binary monomer mixture of GMA with AAc onto chitosan

RESULTS AND DISCUSSION

Effects of various reaction parameters on P_g and %*GE* of AAc onto chitosan have been investigated and results are discussed.

Effect of nature and amount of solvent system

Effects of nature of solvents such as acetone, benzene, dioxane, CCl_4 and water on P_g and %*GE* were studied in 10.0mL of these solvents keeping other reaction conditions constant. The order of P_g in these solvents can be presented as acetone < dioxane < benzene < carbon tetrachloride < water (Table 1). Maximum P_g (45.5) as well as %GE (42.33) was observed in water. It may be because of solubility of monomer and initiator in water. KPS easily undergo dissociation in water to give $SO_4^{-\bullet}$ radicals which generate radical sites on chitosan and AAc for graft copolymerization.

Then amount of water was varied from 5.0 mL to 25.0 mL. Continuous increase in P_g has been observed upto 20.0 mL, optimum value of P_g (133.5) and %*GE* (127.02) were reported at 20.00mL water (Table 1). With further increase in amount of solvent decrease in P_g and %*GE* is observed it may be due to accessibility of monomeric reacting species to enhances the chances of homopolymer formation.

Effect of monomer and initiator concentration

The concentration of monomer (AAc) was varied from 36.46×10^{-2} mol/L to 182.31×10^{-2} mol/L at optimum solvent composition (20mL). Optimum P_g of 181 and 114.85 %*GE* was recorded at 109.39×10^{-2} mol/L of AAc concentration (Table 1). But with further increase in concentration of AAc decrease in P_g was observed. This trend is expected as at higher monomer concentrations chances of termination of the growing polymeric radicals to form homopolymer increases.

KPS concentration was increased from 2.50×10^{-2} mol/L to 12.50×10^{-2} mol/L and maximum P_g of 190.5 and maximum %*GE*, 120.87 were observed at 7.50×10^{-2} mol/L (Table 1). At higher initiator concentrations it generate many more growing chains of AAc at same time and this increases the chances formation of homopolymer by mutual termination of growing polymeric chains. This results in decrease in graft yields.

Effect of reaction time and temperature

Reaction time was varied from 1.0 h to 3.0 h. But maximum P_g of 190.5 and maximum %*GE*, 120.87 were reported at 1.0 h reaction time (Table 1) at 70˚C temperature, 7.50×10^{-2} mol/L of KPS and 109.39×10^{-2} mol/L of AAc. Decrease in P_g was reported with further increase in reaction time. This may be explained that it may be due to some chain transfer reaction along with some other side chain reactions which finally results in homo-polymerization.

Temperature plays role in acceleration of decomposition of initiator, enhanced mobility and diffusion of monomer molecules to the polymer backbone. Temperature was varied from 60˚ to 100˚C at the evaluated optimum reaction parameters discussed above. Grafting at different temperatures were observed to follow the order 60˚ < 70˚ > 80˚ > 90˚ > 100˚C (Table 1). Increase in temperature beyond 70˚C may accelerate various hydrogen abstraction and chain transfer reactions and lead to a decrease in P_g.

Grafting of binary monomers

Binary monomer mixtures of AAc with GMA were grafted onto chitosan at the optimum grafting conditions evaluated above. GMA concentration were varied in 0.5, 1.0, 1.5, 2.0 and 2.5 molar ratios with respect to molar concentration of AAc (Table 2) keeping other reaction conditions constant [Solvent H_2O (20mL), KPS (7.50×10^{-2} mol/L), GMA (109.39×10^{-2} mol/L), reaction time (1h) and reaction temperature (70˚C)]. Continuous increase in P_g and %*GE* was observed with increase in GMA concentrations. Maximum P_g with AAc-*co*-GMA binary monomers was observed as 368.

Characterization of chitosan and graft copolymers

Characterization of chitosan and its graft copolymers was done to give important information of grafted polymers and structural differences between backbone and graft copolymers.

FTIR analysis

Chitosan has both amino and hydroxyl functional groups in it, a broad peak is observed at $3413.7cm^{-1}$ (due to N–H and O–H stretching) in FTIR of chitosan (Fig. 2a), this broad band is due to hydrogen bonding of $–NH_2$ and –OH groups. Peak due to C–H stretching vibrations is observed at $2922.7cm^{-1}$. Several peaks ranging from 1654.9, 1637.7, 1560.5 and $1320.2cm^{-1}$ were observed which are characteristic of chitin and chitosan moieties. A prominent peak due to C–N stretching is observed at $1382.7cm^{-1}$. A peak at $1067.7cm^{-1}$ is due to the O–H bending vibration of

primary alcoholic group of chitosan. Two peaks around 897.7 and 1155.0cm^{-1} are corresponding to saccharide structure (C–O stretching vibration). FTIR spectrum of chitosan-*g*-poly(AAc) shows (Fig. 2b) peaks at 1698.2 due to C=O stretching vibrations of carboxylic acid group and at 1406.2cm^{-1} due to coupled vibration of C−O stretching and O−H in plane bending which is characteristics of carboxylic acids. Another characteristics peaks are observed at 1168cm^{-1} (due to C−O stretching) and at 1019.5cm^{-1} (due to O−H in plane bending). FTIR of Ch-*g*-poly(AAc-*co*-GMA) (Fig. 2b) shows prominent peaks at 1735.6cm^{-1} and (C=O stretching) while another distinguished and prominent peak at 1268.1cm^{-1} (symmetrical stretching for epoxide ring), 906.4 and 847.2cm^{-1} (due to asymmetric stretching of epoxide ring), which are characteristic of GMA.

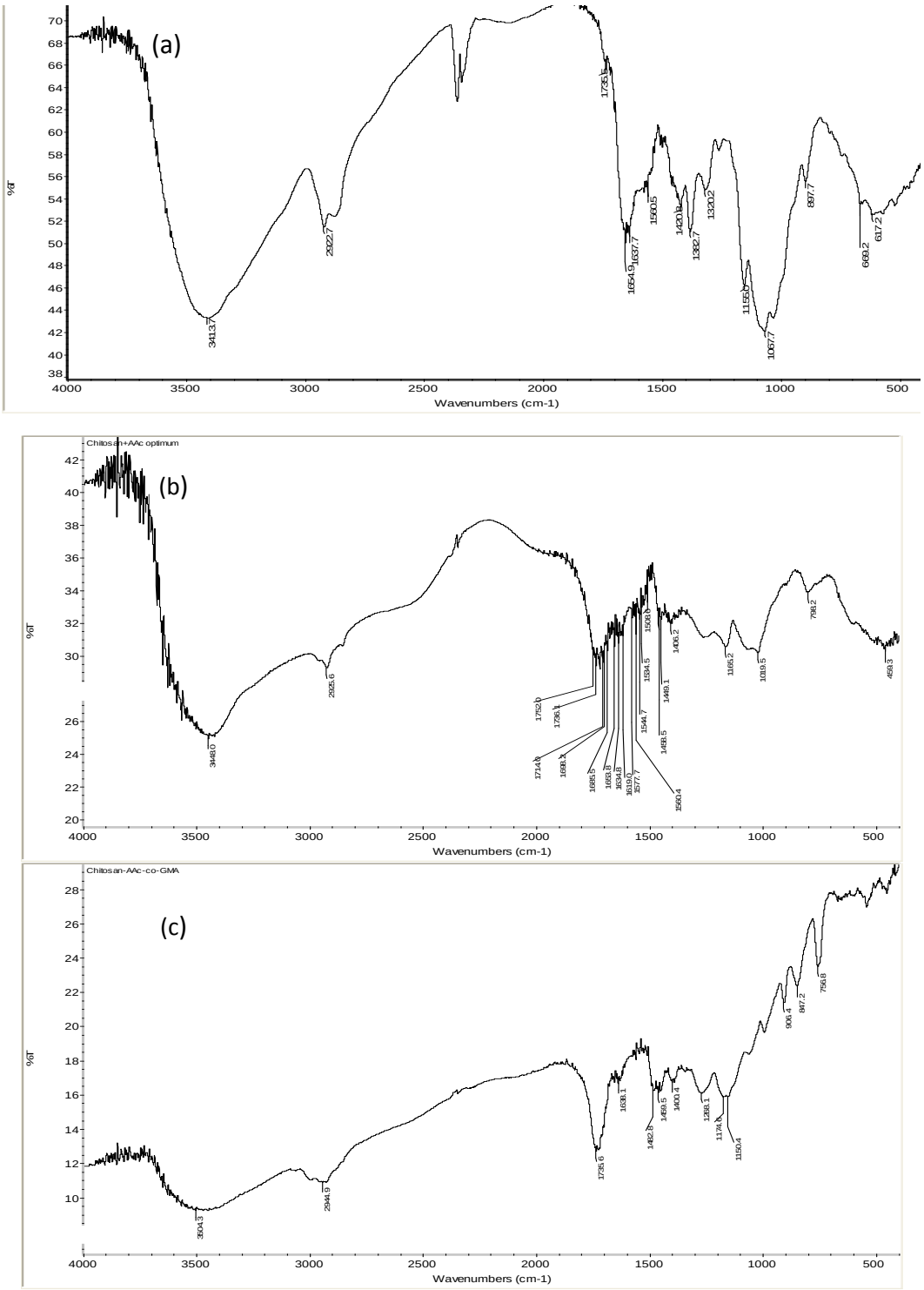

Figure 2: FTIR of (a) Chitosan, (b) Ch-*g*-poly(AAc), (c) Ch-*g*-poly (AAc-*co*-GMA)

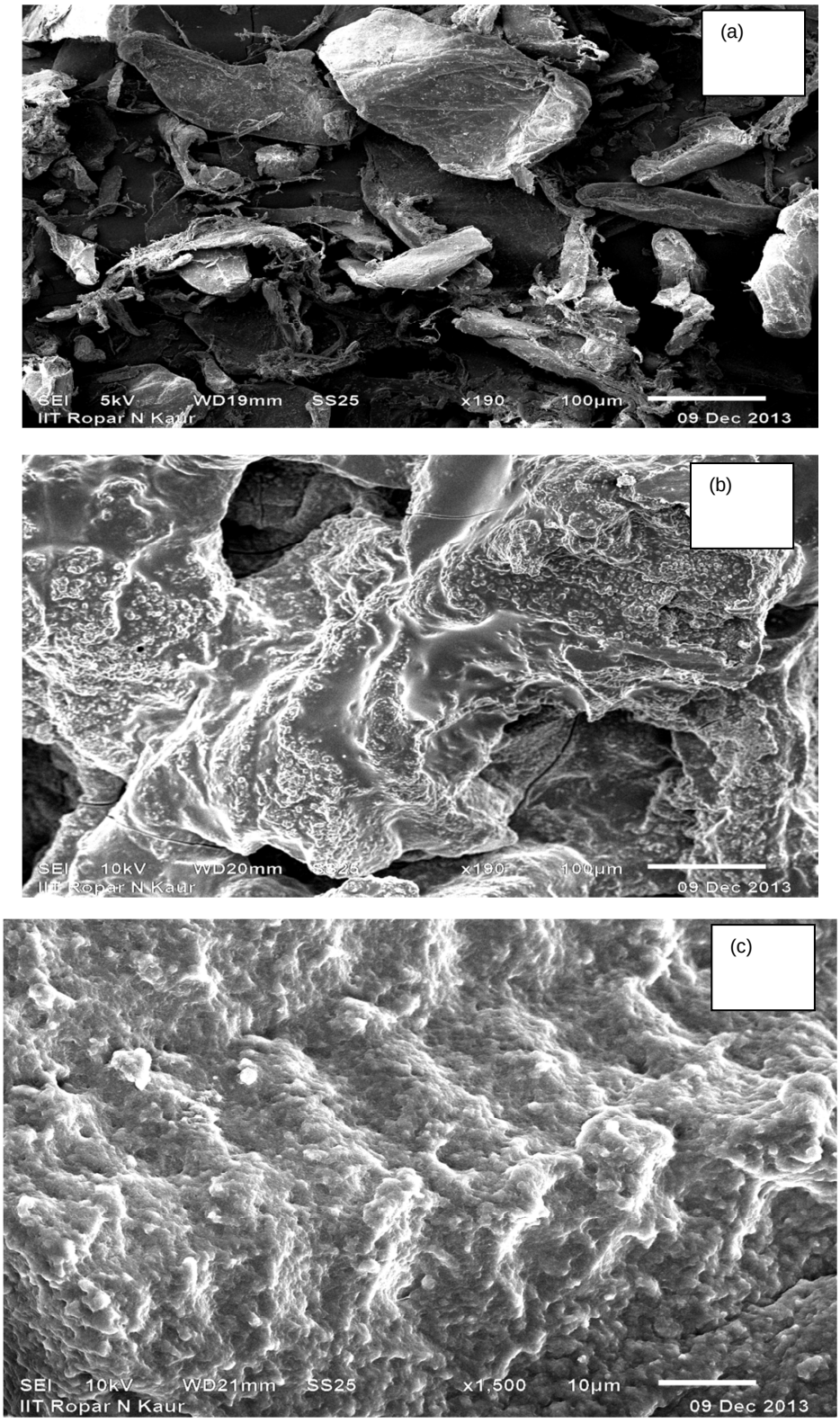

Figure 3: SEM of (a) Chitosan, (b) Ch-*g*-polyAAc (c) Ch-*g*-poly (AAc-*co*-GMA)

Scanning electron microscopy

Scanning electron micrographs (SEM) of ungrafted chitosan and grafted copolymers are presented in Fig. 3. The SEM of ungrafted chitosan shows that particles are small having rough surface morphology (Fig. 3a). Surface of grafted samples clearly shows the deposits of the graft copolymers brought about by grafting as compared to the continuous surface of the backbone (Fig. 3b-3c).

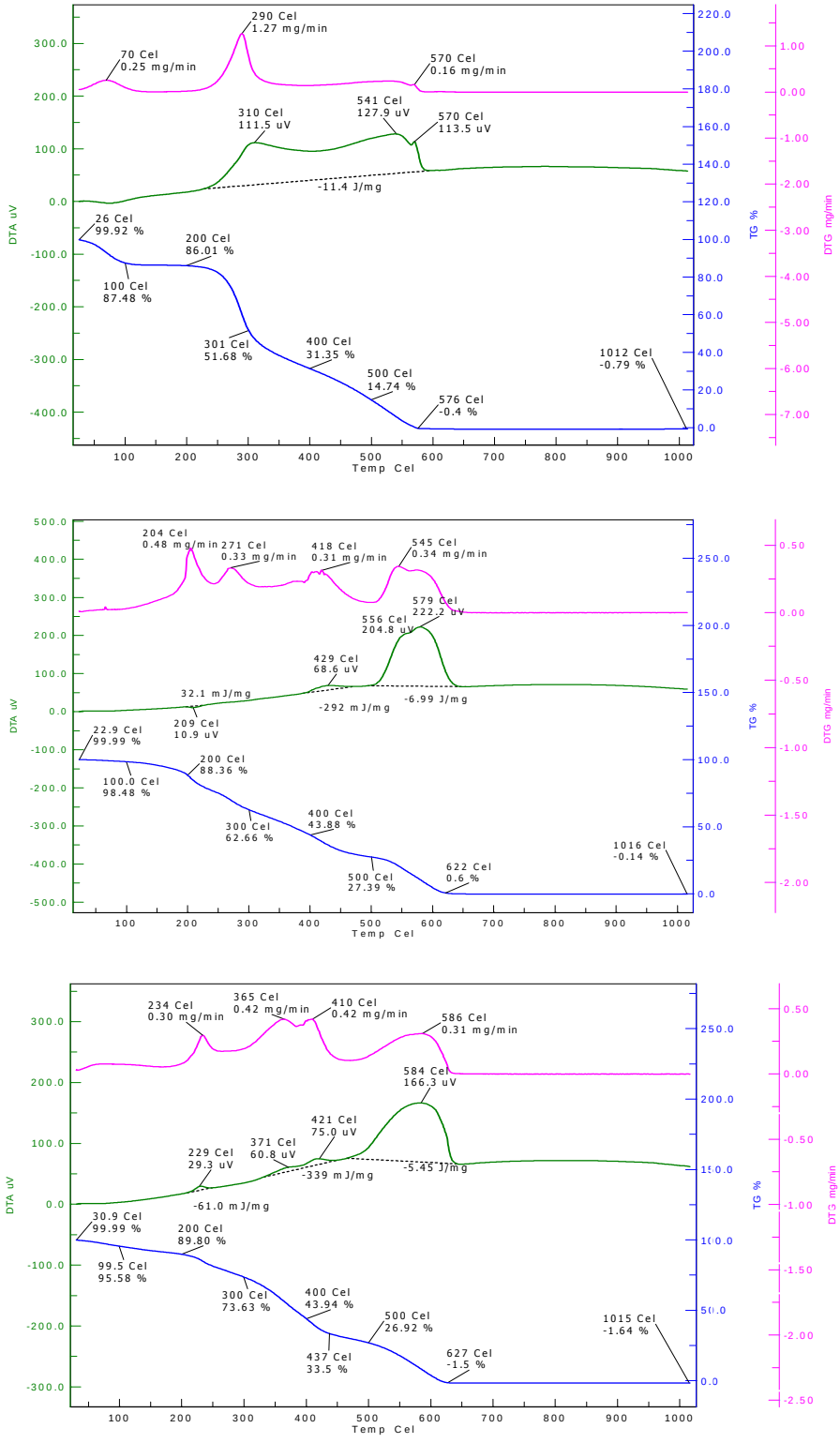

Figure 4: TGA of (a) Chitosan, (b) Ch-*g*-polyAAc, (c) Ch-*g*-poly (AAc-*co*-GMA)

Thermal analysis

Thermogram of Chitosan show single stage degradation. From 26˚ to 200˚C only 14% weight loss is recorded and that can be because of moisture. From 200−301˚C sharp loss in weight (34.33%) is observed, but then slow decomposition till 576˚C (Fig. 4a). DTG of chitosan shows only one prominent exothermic peak at 290˚C. Thermogram of Ch-*g*-poly(AAc) shows increase in initial decomposition temperature (IDT) and grafted sample is stable upto 100˚C. Maximum weight loss of 60.97% from 200−500˚C range (Fig. 4b). DTG of Ch-*g*-poly(AAc)

shows exothermic peaks 204, 271, 418 and 545˚C, that may be because of decomposition of carboxylic functional group of AAc. Thermogram of Ch-*g*-poly(AAc-*co*-GMA) shows increase in IDT as compare to chitosan and left more residue at 500˚C than chitosan. DTG of Ch-*g*-poly(AAc-*co*-GMA) shows exothermic peaks 218, 377 and 630˚C.

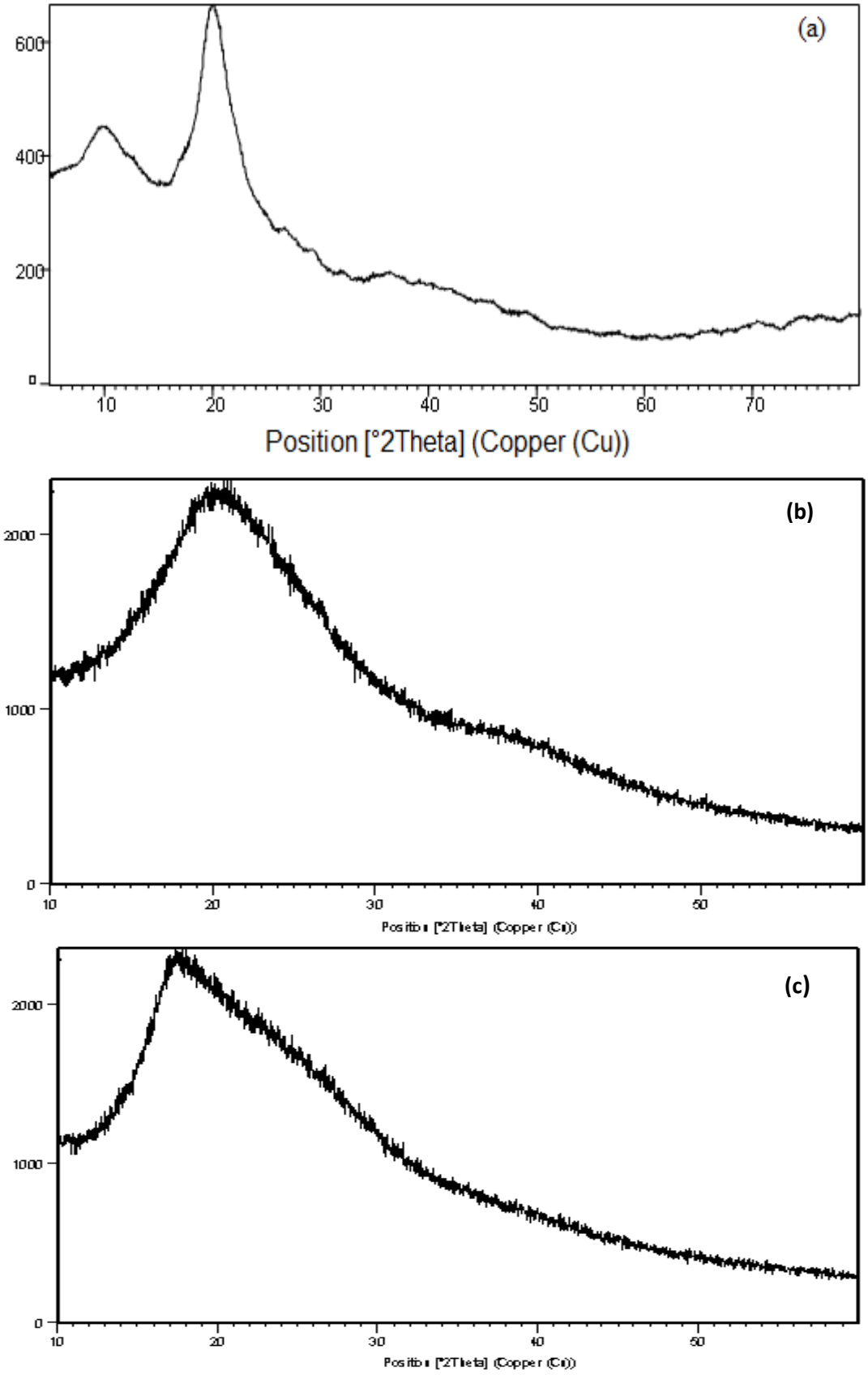

Figure 5: XRD of (a) Chitosan, (b) Ch-*g*-poly AAc, (c) Ch-*g*-poly (AAc-*co*-GMA)

Table 2: Swelling study of chitosan and graft copolymers[b] at room temperature

Sr. No.	Polymer	P_g	*p*H	Percent Swelling (P_s)			
				2 h	4 h	6 h	24 h
1.	Chitosan	00.0	2.0	258	268	214	256
2.	Ch-*g*-polyAAc	190.5	2.0	80	120	228	246
3.	Ch-*g*-poly (AAc-*co*-GMA)	368.6	2.0	112	124	108	96
4.	Chitosan	00.0	7.0	392	240	220	268
5.	Ch-*g*-polyAAc	190.5	7.0	116	144	188	200
6.	Ch-*g*-poly (AAc-*co*-GMA)	368.6	7.0	160	168	172	180
7.	Chitosan	00.0	9.4	342	376	352	312
8.	Ch-*g*-polyAAc	190.5	9.4	164	208	336	748
9.	Ch-*g*-poly (AAc-*co*-GMA)	368.6	9.4	232	340	486	544

[b]*Graft copolymer = 25mg, Amount of water = 10 mL*

Table 3: Drug release behaviour of graft copolymers[c] at different pH as a function of time

Sr. No	Polymeric Sample	% Drug upload	pH	% of drug released						
				1h	2h	3h	4h	5h	6h	24h
1	Chitosan	11.86	2.2	16.78	13.49	10.40	11.31	14.15	14.25	14.92
2	Ch-*g*-polyAAc	55.47	2.2	0.0	0.0	0.0	3.32	8.82	8.82	15.38
3	Ch-*g*-poly (AAc-*co*-GMA)	62.16	2.2	0.0	2.24	2.82	7.88	12.95	12.68	13.22
4	Chitosan	11.86	7.0	88.03	90.22	91.42	90.84	92.24	93.32	92.56
5	Ch-*g*-polyAAc	55.47	9.4	56.84	77.73	80.41	83.69	87.57	87.85	96.82
6	Ch-*g*-poly (AAc-*co*-GMA)	62.16	7.0	18.55	19.89	20.56	23.89	25.49	28.42	36.16
7	Chitosan	11.86	9.4	15.32	16.62	18.52	17.92	20.14	24.38	24.36
8	Ch-*g*-polyAAc	55.47	7.0	35.66	48.49	63.12	70.27	79.22	85.48	95.92
9	Ch-*g*-poly (AAc-*co*-GMA)	62.16	9.4	28.59	37.23	40.43	43.63	48.70	48.70	55.10

[c]*Graft copolymer = 25mg, Amount of pH solution = 10 mL*

XRD analysis

X-ray diffraction data collection was recorded in the range of 2θ = 5 to 80° with a step size of 0.0170°. XRD curve of ungrafted chitosan shows peaks at 2θ positions at 10 and 20 with high relative intensities due to crystalline region (Fig. 5a), but in XRD curve of grafted samples no such sharp peaks are observed (Fig. 5b-5c) which confirms the increase in amorphous nature in polymer because of grafting.

Swelling study

Drug release application of polymeric samples are directly related with swelling studies. Swelling depends upon the pH and extent of interaction between solvent molecules and polymer chains. Swelling results of chitosan and its graft copolymers are presented in Table 2. The pH was varied as 2.2, 7.0 and 9.4. It is clear from table 2, that chitosan and its graft copolymers showed higher swellings in alkaline medium at pH 9.4. Time taken for maximum swelling for chitosan was 4h whereas for Ch-*g*-poly(AAc) and Ch-*g*-poly(AAc-*co*-GMA) was 24h. Th reason for swelling in alkaline medium may be due to the fact that graft copolymers in alkaline medium undergo reactions like formation of sodium salt of acrylic acid and ring opening of epoxide ring of GMA.

Drug release study

Sorption of diclofenac sodium (DS) onto polymeric samples was done by equilibration method from its solution of concentration 100 µg/mL. DS is an acidic drug so it can form more hydrogen bonds grafted samples and hence percentage of drug loaded in graft copolymers was better as compared to chitosan. Percent drug release w.r.t. drug loaded in polymeric sample was studied in buffer solutions of pH 2.2 , 7.4 and 9.4 as function of time (table 3). At 2.2 pH, the cumulative release ratio of DS from the chitosan and graft copolymers is very low even after 24 h. But when the pH of the medium was changed to 7.0 the drug release rate become fast. Chitosan release maximum drug within one hour. Best results at pH 7.0 are shown by Ch-g-poly(AAc). But when the drug release pattern was studied at pH 9.4, Ch-g-poly(AAC) and Ch-g-poly(AAc-co-AAm) showed very good results for sustained release of drug. This may be due to the fact that these graft copolymers swell better in alkaline medium than acidic and neutral medium and drug released from polymeric samples easily.

CONCLUSION

The chitosan was modified by grafting of AAc alone and binary monomer mixture AAc with GMA. The graft copolymers have been characterized physio-chemically by a variety of characterization techniques, confirming that vinyl monomers have been grafted onto chitosan backbone. Graft copolymers were studied to swell in aqueous media with different pH, and then these grafted samples were investigated for the sustainable release of anti-inflammatory drug diclofenac sodium as a function of time and pH and found that polymeric samples showed best results for sustained drug release at pH 9.4.

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