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GRAFT COPOLYMERIZATION OF VINYL MONOMERS ONTO CHITOSAN:III: Graft Copolymerization of Acrylamide onto Chitosan for Antibacterial Activity

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ABSTRACT : The biopolymer chitosan was chemically modified by grafting polyacrylamide in a homogeneous aqueous phase by using ceric ammonium nitrate as the initiator and in the presence of N, N-methylene-bis-acrylamide as a cross linking agent. The graft copolymerization of acrylamide onto chitosan was investigated using ceric ammonium nitrate as the initiator. The effect of initiator concentration, monomer concentration, time and temperature on % G and % GE were studied. The grafted samples were characterized using FTIR, TGA, SEM and XRD methods. From the FTIR data it was ascertained that grafting has occurred considerably. The morphology of the grafted polymer was observed from the SEM picture. The thermal analysis indicated the different stages of degradation of the grafted copolymer. The antibacterial activity of chitosan as well as the grafted samples were investigated using some gram positive and gram negative bacteria. Grafted products improved considerably the antibacterial activity.

Key words: Graft copolymerization, chitosan, AAM (Acrylamide), antibacterial activity

INTRODUCTION

Grafting vinyl monomers onto natural and synthetic polymers is a challenging field of research with unlimited future prospects. During the last four decades Nayak and co-workers have studies 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 peroxydiphophate ions (1-15).

Chitosan (CS) is a biopolymer that has received great attention in a variety of applications because of their biodegradability and biocompatibility [16]. It is derived from chitin, which is the second most abundant biomass on earth next to cellulose. Because of its excellent film-forming property, chitosan can be used effectively as a film-forming material to carry active ingredients such as mineral or vitamin for food packaging applications [17] an hydrophilic or hydrophobic drugs for drug delivery applications [18]. Chitosan is a cationic biopolymer that is bioadhesive, biocompatible and biodegradable. These unique properties make it an attractive carrier for biomedical applications. Of late, chitosan has been widely applied in biomedical fields as a carrier for drug delivery, wound dressing, etc [19]. Since chitosan is already known as a biocompatible, biodegradable and almost nontoxic material, it has been widely used in pharmaceutical research and industry as a carrier for drug delivery and as biomedical material. Orally administered as well as implantable delivery systems containing chitosan as a drug carrier have been prepared to effect sustained release of the drug [20, 21]. Modulation of drug release has been achieved by drug–chitosan complexation involving ionic [22-24] or covalent interactions [25-26]. While the focus for ionic interactions of chitosan involves the amino groups of its glucosamine residues, covalent interactions often involve other sites as well (e.g. the CH2OH moieties) also positively charged chitosan is easy to interact with negative charged glycosaminoglycans in the extracellular matrix.

Therefore, chitosan has prospective applications in many fields such as biomedicine, waste water treatment, functional membranes and flocculation. However, chitosan can only be soluble in few dilute acid solutions, which limits its wide applications. Recently, there has been a growing interest in chemical modification of chitosan to improve its solubility and widen its application.

(27-33) Among various methods, graft copolymerization is most attractive because it is a useful technique for modifying the chemical and physical properties of natural polymers. Chitosan is a very good candidate bearing two types of reactive groups that can be grafted. First, the free amino groups on deacetylated units and secondly, the hydroxyl groups on the C3 and C6 carbons on acetylated or deacetylated units. Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto the chitosan backbone. Recently researchers have also shown that after primary deviation followed by graft modification; chitosan would obtain much improved water solubility and bioactivities such as antibacterial and antioxidant properties (34). Grafting chitosan is a common way to improve chitosan properties such as increasing chelating (35) or complexation properties , bacteriostatic effect or enhancing adsorption properties (37-39). Although the grafting of chitosan modifies its properties, it is possible to maintain some interesting characteristics such as mucoadhesivity (40), biocompatibility (42- 43). Many investigations have been carried out on the graft copolymerization of chitosan in view of preparing polysaccharide- based advanced materials with unique bioactivitiesn and thus widening their applications in biomedicine and environmental fields. A few review articles on the potential applications of chitosan for pharmaceutical, veterinary medicine, biomedical and environmental field have already been reported (44- 51).

EXPERIMENTAL

Chitosan (degree of deacetylation >90%) was form India Sea Food, Kerala, India and used without further purification, acrylamide (AAM) monomer (>99%, fluka) was purified from inhibitor by vacuum distillation at 30 c and kept at 0 c until used, ceric amino nitrate (CAN) was used as obtained. All reagents used were of analytical grade.

Copolymerization Process

A mixture of 1 g of dried chitosan and 75 mL distilled water was stirred magnetically under N2 atmosphere and then was treated with a predetermined amount of CAN in 25 mL of 250 acetic acid solution for 15 min to facilitate free radical formation on chitosan. This treatment was followed with drop wise addition of AN monomer and the polymerization proceeded at 25° C for 120 min unless stated elsewhere. After120 min is over, the graft copolymer precipitated was washed with several times with warm distilled water and warm 5% acetic acid solution to remove excess alkali, excess chitosan and homopolymer. Trace amounts of excess homopolymer can be removed by warm 5% acetic acid solution and can be checked by precipitation with methanol, which was followed by FTIR spectra. This process is repeated until no precipitation of homopolymer was observed, then the copolymer precipitate was dried to constant weight. This percentage grafting efficiency and add – on can be calculated from the relations

FTIR Analysis

A Nicolet (avator – 360, USA) FTIR spectrometer in the range of 4000-400 cm was used to record the IR spectra for grafted and ungrafted chitosan in the form of KBr pellet.

X-Ray Diffraction (XRD)

The X- ray diffraction studies were performed using a Philips-Holland diffractometer (model PW 1729) with copper as target material in an X- ray tube under the operational conditions 30KV , 40 mA and wavelength between 1.54060 and 1.54438 a. The samples were scanned between 3oand 100.

Thermogravimetric Analysis

Thermogravimetric analysis TGA was carried out using Shimadzu TGA- 50 Japan under N2 atmosphere at a heating rate of 10 c/min

Antibacterial Activity Test

The antibacterial tests were carried out by standard discagar diffusion method by using 6 mm diameters discs prepared from Whatman-4 filter paper, and measuring the inhibition zones (mm**).**

RESULTS AND DISCUSSION

Gravimetric Estimation

The grafting process was followed by gravimetry. The increase in weight of the grafted chitosan over the weight of the neat chitosan indicated the grafting of AAM (acrylamide) onto chitosan to the grafted backbone were calculated according to the following method.

% G = (W2- W1)/W1/ W3 x 100
% E = (W2-W1)/W2 x 100
Where W1, W2 and W3 represent the weight of the original chitosan, grafted chitosan and monomer respectively.

Infra-red Spectra of the Grafted Chitosan: Proof of Grafting

Graft copolymer based on chitosan has been synthesized by grafting acrylamide onto chitosan the polysaccharide molecule in aqueous medium using ceric ammonium nitrate as the initiator. The grafting was confirmed by comparing the IR spectra of chitosan (Fig. 1(a)) with that of the grafted product (Fig. 1(b)).

It has been seen that the amidecarbonyl absorption band from grafted chains appears at 1649 cm-1. The band is located at 1670 cm-1 for acrylamide homopolymer. On the other hand, the most typical absorption bands of chitosan situated at 1558 cm-1 and 1661 cm-1 corresponding to amide – I and amide – II bands respectively, are not clearly visible since they are hidden by strong carbonyl absorption band of PAAM in this spectrum region. However the CHI amide – I absorption can be observed as a shoulder at 1540 cm-1. The shift of the carbonyl absorption band of PAAM amide – I band of chitosan to lower frequencies could be due to inter – and/or intramolecular interacting though hydrogen bonding. Naturally, the insolubility of PAAM grafted CHI samples, inspite of containing a large number of amide groups, is produced by the cross linking.

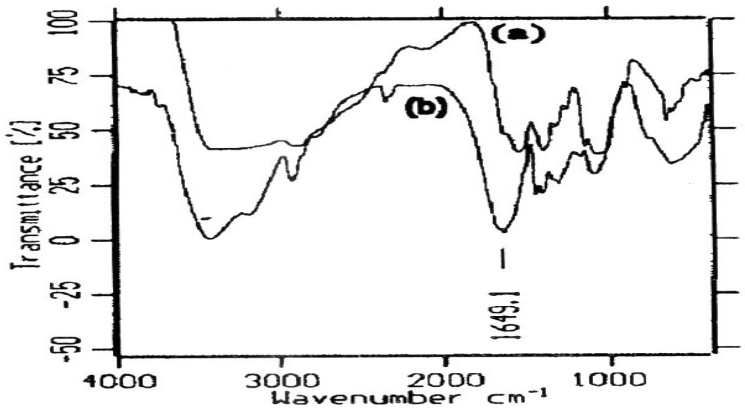


Fig 1; FTIR Spectra of Chitosan(a) and Chitosan-g-AAM(b)

Effect of Initiator Concentration

Table 1 depicts the effect of the concentration of initiator Ce+4 on grafting AAM (acrylamide) onto chitosan. By keeping constant all the variables, the amount of CAN used was varied. It was observed that with increasing the initiator concentration the percentage of grating as well as the grafting efficiency increases in the initial stages and with further increase of initiator the graft percentage as well as the grafting efficiency decreases dramatically. This may be due to the fact that with high concentration of the initiator more and more homopolymers are formed thereby decreasing both G% and GE%.

Table 1. The effect of initiator concentration on grafting Chitosan = 1 gm, [AAM] = 3.00 gm, Temp = 25° C, Time = 180 min.

CAN (mol) [Ce+4] × 103(M)	%G	% E
3.94	50.62	34.91
5.79	109.30	74.14
7.58	43.07	25.24
9.30	44.44	29.65

Effect of Monomer Concentration

The effect of monomer concentration on the grafting of AAM onto chitosan is shown in Table - 2.

Table - 2 Effect of monomer concentration on grafting of AAM onto chitosan Chitosan = 1 gm, [CAN] = 3.90 $\times 10^{-3}$M, Temp = 25° C, Time = 180 min. in 100mL

AAM (g)	%G	%E
0.50	-	-
1.00	3.01	1.49
2.00	69.33	68.63
3.00	10.50	7.35

It can be seen from the results of Table - 2 that as the monomer concentration increases the %G and %E increases at the initial stages and with further increase of monomer concentration it decreases. This is mainly due to the formation of homopolymer of AAM at the higher concentration. These homopolymers successfully hinders the rate of penetration of monomer molecules to chitosan macroradical

Effect of Temperature Concentration

Table - 3 : Effect of temperature on grafting of AAM onto chitosan Chitosan = 1 gm., [CAN] = 3.90 × 10-3M, Time = 180 min.

, resulting in the low percentage of grafting. Further as the monomer concentration increases, the rate of homopolymerization increases decreasing the formation graft copolymer.

Temperature	% G	% E
25	10.50	7.35
35	54.41	36.82
42	4.14	2.81
52	-	-

It can be seen from the Table - 3 that grafting increases with increase of temperature upto 35° C and with further increase of temperature it decreases. This may be due to the fact that at higher temperature the chain transfer reaction takes place between the macroradicals causing to decreases of the grafting process. Further at higher temperature there is the possibility of oxidation of the chitosan moiety since ceric ion has a very high oxidation potential and it may attack the chitosan moiety forming the oxidation products thereby decreasing the grafting percentage.

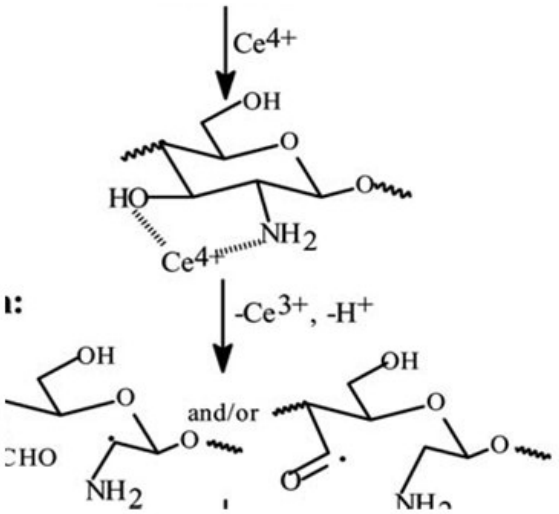
Effect of Time Concentration

Table 4 Effect of time on the graft copolymerization of AAM onto chitosan Chitosan = 1.5 gm., [AAM] = 3.00 g, [CAN]= 3.90 × 10^{-3}M, Temp = 25° C

Time (min)	% G	% E
30	2.24	1.53
60	3.02	2.05
90	3.84	2.61
150	3.80	2.58

The effect of the reaction time on the percentage of grafting and grafting efficiency is shown in Table – 4. It can be seen from the Table – 4 that the grafting increases with increase of time upto 90 minutes and thereafter decreases and levels off. The rapid increase of grafting upto 120 minutes may be due to rapid increases initiation forming more and more macro radicals enabling the increase of grafting. But with further increase of time there might be get effect hindering the approach of the macro radicals which might decrease grafting process.

Chitosan Oxidation



Oxidation of Chitosan

X-Ray Diffraction

The X-Ray diffraction spectra of pure chitosan(a) and chitosan-*g*-AAM) were analyzed as shown in the Fig. 2. The spectrum of chitosan is more convex than that of copolymer. The crystallinity of ungrafted and grafted chitosan was calculated as 54.6% and 38.4%, respectively. This indicated that the incorporation of AAM had impaired the crystallinity of chitosan, which may provide some useful information.

Scanning Electron Microscopy

Fig. 3 shows the SEM micrographs of chitosan(a) and chitosan/PAAM(b) blend. It provides direct evidence that phase separation occurred in chitosan/PAAM blend. This sample has a distinct two-phase morphology, i.e., a continuous PAAM phase with a dispersed chitosan phase indicates poor interfacial adhesion between terpolyamide and chitosan phases. It is well known that chitosan has the good ability of degradation. Therefore, PAAM interfered with chitosan can improve its biodegradability.

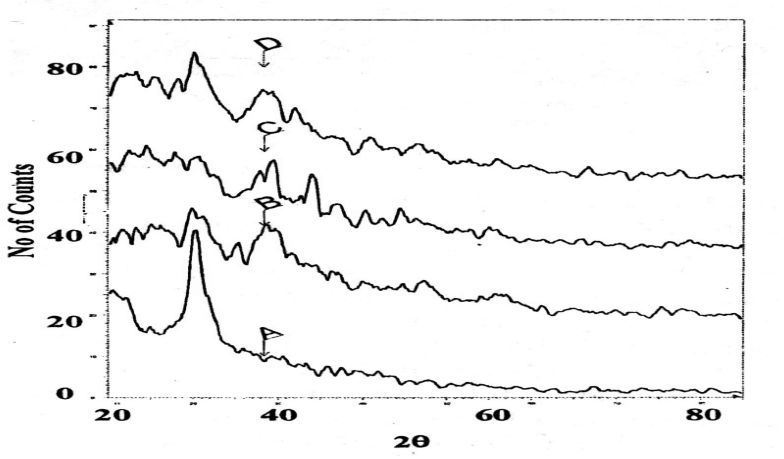


Fig. 2 : XRD of pure chitosan(a) and chitosan-g-AAM graft copolymer(b)

Fig. 3 : SEM of chitosan(a) and chitosan PAAM graft copolymer(b)

TGA Studies

The TGA of pure chitosan and chitosan-g-AAM is given in fig 4.

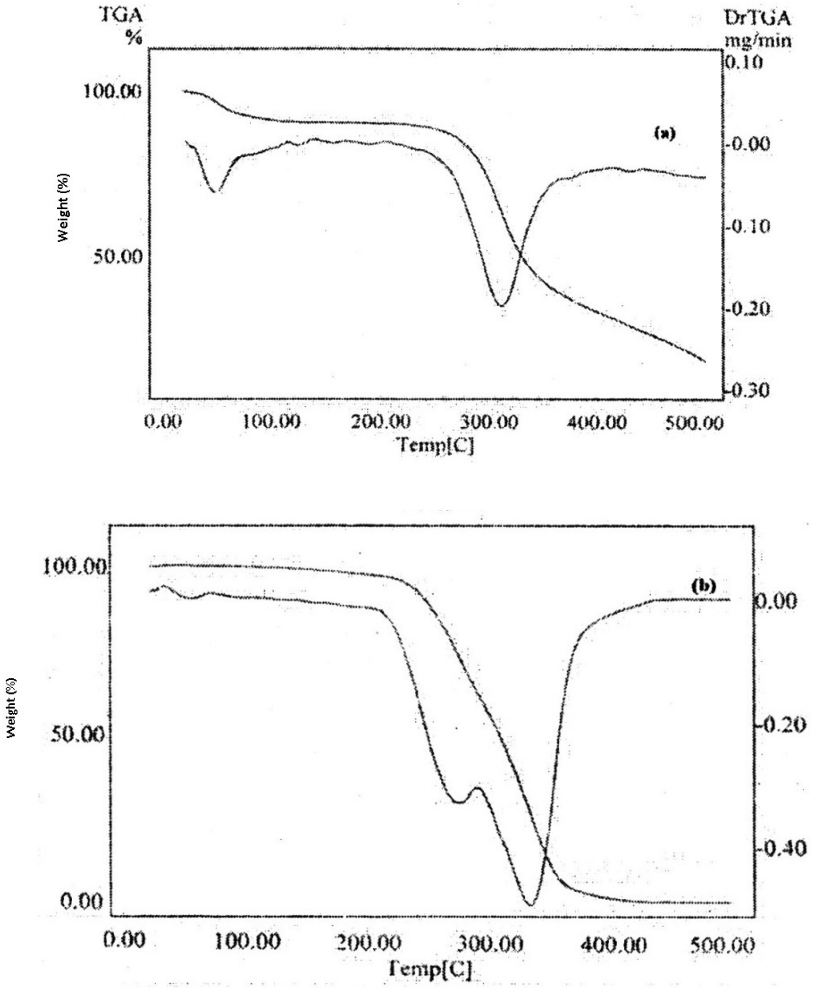


Fig. 4 : TGA of chitosan (a) TGA of chitosan-g-AAM graft copolymer(b)

It can be noticed that both chitosan and PAAM decomposes above 200° C and as a result of that grafted chitosan decomposes at similar temperature range. Pure chitosan shows three decomposition stages with the major weight loss of 50.8% takes place in the range of 200° – 365° C at T_{max} = 305° C derived from derivatogram, whereas PAAM shows two stages of decomposition with major weight loss of 89% in the range of 200° - 365° C at T_{max} = 315° C. The grafted chitosan contains two well identified stages, the first is the range of 200° - 290° C T_{max} = 274° C and the second is at the range of 290° - 500° C at T_{max} = 316° C. The peak is clearly due to PAAM chains grafted to chitosan whereas the first peak refers to chitosan in the copolymer. The Tmax of chitosan have appeared to be at 305° C in the pure form and at 274° C in the grafted form. The difference in degradation temperature confirms that upon grafting of AAM onto chitosan, some chemical changes in the structure of chitosan takes place.

Mechanism of Grafting

The following mechanism has been suggested for the graft copolymerization of AAM (Acrylamide) onto chitosan initiated by ceric ion. Similar mechanism has also been postulated by various workers for grafting vinyl monomers onto cellulose, starch and other polysachharide initiated by ceric ion (1-10)

Production of free radical: Oxidation

CS + Ce+4 _____ Complex _____ CS . + Ce^{+3}+ H^{+} (1)

Initiation

CS. + M . _____ CSM.

Ce+4 + M Complex M..+Ce+3 + H+

Propagation

CS M . + M _____ CSM1 .

CSM1. + nM _____ CS(M)n+1 .

M.+ + nM _____ (M)n+1.

Termination

CS (M)n+1 .+ Ce_____ CS(M) n+1 + Ce+3 (Graft Copolymer)

(M) n+1. + Ce+4 _____ (M)n+1 + Ce+3 (Homopolymer)

Chain Transfer

CS Mn. + Ce 4+ ____ CSMn + Ce+3 + H+

CSMn. +M _____ CSMn + M.

Antibacterial Activity Tests

The antibacterial tests were carried out by standard disc agar diffusion method by using 6 mm diameters discs prepared from Whatman-4 filter paper, and measuring the inhibition zones (mm).

Table 5: Antibacterial Activity of Chitosan and Grafted Chitosan nhibition zone tests for chitosan and AAM grafted polymer

	Inhibition Zones			
	Gram –positive		**Gram -negative**	
	P.aeruginosa	*E.coli*	*B. subtilis*	*S.aureus*
Chitosan	6	6	8	8
25 % Graft	11	10	13	12
55 % Graft	12	13	15	14
70 % Graft	14	15	17	18

The study has been carried out to compare the antibacterial activity of grafted chitosan film samples with that of chitosan. The study was carried out against *P. aeruginosa, E. coli*, *B. subtilis* and *S. aureus* using the inhibition zone method. The results are shown in Table 5. It was observed that grafting of AAM improved the antibacterial activity of chitosan. While the inhibition zone diameter for chitosan film ranged between 6 and 8 mm against indicated bacteria, the inhibition zone increased up to 17 mm (against B. subtilis) by grafting.. Grafted samples showed an increasing antibacterial activity as the degree of grafting increased for all of gram-negative and gram-positive bacterias; a minimum of 2 mm increase was observed consistently when the grafting percentage increased from 55. to 70 %. Average film weight (thickness) also effected the degree of antimicrobial activity of both chitosan and grafted chitosan samples.

CONCLUSION

Chitosan possesses the desired properties for safe use in biomedicine, pharmacology and waste water treatment and in many other applications. However, due to its insolubility in neutral and basic aqueous media, its application is restricted. Graft copolymerization is a most attractive technique, which is used to improve chitosan's solubility and widen its applications. Moreover, graft copolymerization is used to attach various functional groups and to control hydrophobic, cationic and anionic properties of grafted chitosan. Chitosan has been grafted with AAM initiated by tetravalent ceric ion. The effect of monomer, initiator, time and temperature on graft yield have been reported. With increasing the concentration of the monomer as well as the initiator the graft yield decreases due to the formation of homolpolymer formation. This is obvious since ceric ion is a very good oxidizing agent with very high oxidation potential and it interact with AAM forming the homopolymer rather than the graft copolymer at higher concentrations. The evidence of grafting has been ascertained from the FTIR spectra and the morphology has been noted from the SEM figure. Grafting products show improved antibacterial activity. The activity increases with increasing percent grafting and film thickness.

REFERENCES

1. P.L Nayak, Grafting of Vinyl Monomers onto Wool Fibers.,(1996) J.Macromol.Sci-Rev.Macromol,Chem. C14(2),193
2. P.L.Nayak, Grafting of vinyl monomers onto nylon. (1979), J.Macromol. Sci-Rev. Macromol, Chem. C17(2),267
3. P.L.Nayak, Vinyl and graft copolymerization initiated by potassium peroxydiphosphate (1985) , J.Macromol. Sci-Rev.Macromol, Chem. Physics (25),2,157
4. P.L.Nayak, S.Lenka and N.C.Pati (1978)Grafting vinyl monomers onto wool fibers:I:Garfting copolymerization of methyl methacrylate onto wool usingV(v)- thiourea redox system,J.Appl.Polym.Sci,22,3301
5. P.L.Nayak, R.K.Samal and M.C.Nayak (1979) Garfting of vinyl monomers onto Nylon-6:I:Graft co-polymerization of acryl amide onto Nylon-6Mn(III) as initiator Angew.Macromol-Chem.A,13,261
6. P.L.Nayak, M.K.Mishra and A.K.Tripathy (1981)Grafting vinyl monomer onto polyster fibres:I:Graft copolymerization of methyl methacrylate onto PET using hexavalent chromium ,Ion,J.Appl.Polymer .Sci.,26,2109
7. A.K.Pradhan, N.C.Pati and P.L.NayakGrafting vinyl monomers onto polyster fibers:III:graft copolymerization of methyl methaacrylate onto PET using KMnO4-oxalic acid redox system,,J .Appl.Polym.Sc 27,1903(1982).
8. S.Lenka and P.L.Nayak, (1982).Grafting of vinyl monomers onto polyster fibers:IV :Graft copolymerization of ethyl methacrylate on PET using Acetylacetonate complex of Mn(III), Co(III) and Fe(III),, J. Appl. Polymer Science 19, 987
9. S.Lenka, P.L.Nayak and M.R.Dash (1983)Photo induced graft copolymerization: VI: Graft copolymerization of methyl methacrylate onto cellulose in presence of pyridine- bromine charge- transfer complex as initiator, S. lenka, P.L. Nayak and M.R. Dash, polym, photochemistry, 3, 109.

10. Grafting Vinyl monomers onto chemically modified wool fibres: XI: Graft copolymerization of methyl methacrylate onto reduced wool fiber using Acetylacetonate complex of Manganese (III), S. Sasmal, G. Sahu and P.L. Nayak. J. Macromolecular Science, A20 (2), 153 (1983).
11. P.L.Nayak, S.Lenka, and A.P.Das((1985).Grafting of vinyl monomers onto natural rubber: V: Graft copolymerization of methyl methacrylate onto natural rubber using Pottasium peroxydisulphate, P.L. Nayak, S. Lenka and A.P. Das, J, Polymer Science, Polymer Chemistry, E10,23, 1615
12. P.L.Nayak,(1991) Photo Induced Graft Copolymerzation onto selected Fibers: J.Macromol.Sci- Rev- Macromol.Chem.Phy.31(1), 91
13. P.L.Nayak, S.Lenka and N.C.Pati, (1979)Grafting Vinyl Monomers onto Silk Fibers V: Graft Copolymerization of Methyl methacrylate onto Sil Fibers using Potassium Permanganate as Intiator, J.Macromol. ScienceChem, A13, 1157
14. P.L.Nayak, N.C. Pati and A.K.Pradhan(1981) Grafting Vinyl Monomers onto Nylon-6: III: Graft Copolymerization of Methyl methacrylate on Nylon-6 using Fe(III)- Thiourea Redox System, J.Poly,mer. Sci, Polym.Chem.Ed. 19, 831
15. P.L.Nayak, and S.Lenka,(1982)Photo-Induced Graft Copolymerization IV: Photo induced Graft Coploymerization of Methyl Methacrylate using N-bromo succinamide as the initiator, J.Appl. Polymmer.Sci, 27, 3625
16. P.L.Nayak and S.Lenka, (1981)Grafting Vinyl Monomers onto CelluloseII: Graft-Copolymerization of Methyl Methacrylate onto Cellulose using V(V)-Thiourea Redox System, J.Polym.Sci.Polm. Chem Ed., 19, 1561
17. Debasish Sahoo a, Sarmila Sahoo a, Priyanka Mohanty a, S. Sasmal band P. L. Nayak (2009) Chitosan: a New Versatile Bio-polymer forVarious ApplicationsDesigned Monomers and Polymers 12, 377–404
18. Muzzarelli, R.A.A.(2005) Adv Polym Sci. , 186, 151–209.
19. Senel S, Ikinci G, Kas S(2000). Int J Pharm. 193,197-203.
20. Shu, X.Z.; Zhu, K.J. Int.(2000) J. Pharm. 201, 51–58.
21. Muzzarelli, R.A.A. Chitin(1977); Pergamom: Oxford
22. Vasudev, S.C.; Chandy. T; Sharma, C.P. Biomaterials (1997), 18,375–81.
23. Park, I.K.; Kim T.H.; Park, Y.H.; Shin, B.A.; Choi, E.S.; Chowdhury, E.H. J Control Release. 2001, 76,349–62.
24. Agnihotri, S.A.; Aminabhavi, T.M. Control Release. 2004, 96,245–59.
25. Shiraishi, S.; Imai, T.; Otagiri, M.J Control Rel. 1993, 25:217-225.25.
26. Imai, T.;Shiraishi, S.; Saito, H.; Otagiri M.Int J Pharm. 1991, 67,11-20.
27. Sabnis, S.S.; Rege, P.; Block, L.H. Pharm Dev Technol. 1997, 2,243-255.
28. Sugimoto, M., Morimoto, M., & Sashiwa, H. (1998). Carbohydrate, Polymers, 36(1), 49–59.
29. Kurita, K. (2001). Progress in Polymer Science, 26(9), 1921–1971.
30. Sashiwa, H., & Shigemasa, Y. (1999). Carbohydrate Polymers, 39(2), 127–138.
31. Terada, N., Morimoto, M., Saimoto, H., Okamato, Y., Minami, S., & Shigemasa, Y. (1999). Chemistry Letters, 28(12), 1285–1286.
32. Alexandrova, V., Obukhova, G. V., Dominina, N. S., & Topchiev, D. A. (1999). Macromolecular Symposia, 144(2), 413–418.
33. Sridhari, T. R., & Dutta, P. K. (2000). Indian Journal of Chemical Technology, 7(1), 198–204.
34. Heras, A., Rodriguez, N. M., & Ramos, V. M. (2001). Carbohydrate Polymers, 44(1), 1–8.
35. Xie, W. M., Xu, P. X., Wang, W., & Lu, Q. (2001). Bioorganic & Medicine Chemical Letters, 11, 1699.
36. Yang, S., Tirmizi, S. A., Burns, A., Barney, A. A., & Risen, W. M. (1989). Synthetic Metals, 32(2), 191–200.
37. Chung, T. W., Lu, Y. F., Wang, S. S., Lin, Y. S., & Chu, S. H. (2002). Biomaterials, 23(24), 4803–4809.

38. Jung, B. O., Kim, C. H., Choi, K. S., Lee, Y. M., & Kim, J. J. (1999). Journal of Applied Polymer Science, 72(13), 1713–1719.
39. Kotze, A. R., Lueßen, H. L., De Leeuw, B. J., De Boer, A. G., Verhoef, J. C., & Junginger, H. E. (1997). Pharmaceutical Research, 14(9), 1197–1202.
40. Thanou, M., Verhoef, J. C., & Junginger, H. E. (2001). Advanced Drug Delivery Reviews, 52(2), 117–126.
41. Hoffman, A. S., Chen, G., Wu, X., Ding, Z., Kabra, B., Randeri, K., et al. (1997). Polymer Preparations, 38(2), 524–525.
42. Tasker, R. A., Connell, B. J., Ross, S. J., & Elson, C. M. (1998). Laboratory Animals, 32(2), 270–275.
43. Ono, K., Saito, Y., Yura, H., & Ishikawa, K. (2000). Journal of Biomedical Material Research, 49(2), 289–295.
44. Singh, V., Tripathi, D. N., Tiwari, A., & Sanghi, R. (2005). Journal of Applied Polymer Science, 95(4), 820–825.
45. Suh, J. K. F., & Matthew, H. W. T. (2000). Biomaterials, 21(24), 2589–2598.
46. Ravi Kumar, M. N. V., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chemical Reviews, 104(12), 6017–6084.
47. Ravi kumar, M. N. V. (2000). Reactive and Functional Polymers, 46(1), 1–27.
48. Berger, J., Reist, M., Mayer, J. M., Belt, O., & Gurny, R. (2004). European Journal of Pharamaceutics and Biopharmaceutics, 57(1), 35–52.
49. Senel, S., & McClure, S. J. (2004). Advanced Drug Delivery Reviews, 56(10), 1467–1480.
50. Verma, A. J., Deshpande, S. V., & Kennedy, J. K. (2004). Carbohydrate Polymers, 55(1), 77–93.
51. Guibal, E., Sweeney, N. V. O., Vincent, T., & Tobin, J. M. (2002). Reactive and Functional Polymers, 50(2), 149–163.
52. Prabaharan, M., & Mano, J. F. (2005). Drug Delivery, 12(1), 41–57.