AMERICAN JOURNAL OF PHARMTECH RESEARCH
**Journal home page:** http://www.ajptr.com/

# Synthesis and Characterization of Chitosan-g-PAMPS Graft Copolymer and its Applications in Drug Delivery of Sodium Diclofenac

**Pratibha Mandal[1], Arti Srivastava[1*], Koushik Nandy[1], Rajesh Kumar[2]**
*1.Department of Chemistry, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.), India-495009*
*2.Department of Chemistry, Banaras Hindu University, Institute of Science, Varanasi, India-221005*

## ABSTRACT

Chitosan-g-AMPS was synthesized using free radical initiators and characterized by FTIR, PXRD, SEM and TGA analysis. The 2-acrylamido-2-methyl-1-propane sulphonic acid (AMPS) concentration has been optimized from 3.5 x $10^{-2}$ to 19.5 x $10^{-2}$ mol $dm^{-3}$ to get maximum grafting of AMPS monomer onto chitosan. The FTIR spectral analysis proves the successful grafting and the PXRD spectra reveals the increase in crystallinity due to grafting of AMPS. SEM images exposed that smooth form of chitosan was changed into porous and fluffy structure after grafting. The cumulative drug diclofenac sodium release was studied in colonic medium. Delivery of drug was 13% and 55% during 4 h of assay in gastro-enteric and colonic system respectively and chitosan was dissolved about 53% and 65% in gastro-enteric and colonic system, respectively. The drug release behavior depends on the pH of medium as well as on the nature of beads, and AMPS grafted chitosan shows slow release of diclofenac sodium.

**Keywords:** chitosan, drug delivery, grafting.

*Corresponding Author Email: reach2arti@yahoo.co.uk
Received 4 August 2017, Accepted 10 August 2017

Please cite this article as: Srivastava A *et al*., Synthesis and Characterization of Chitosan-g-PAMPS Graft Copolymer and its Applications in Drug Delivery of Sodium Diclofenac. American Journal of PharmTech Research 2017.

## INTRODUCTION

In recent years much attention is given on the modification of natural polysaccharides. The development of modified polysaccharides used as a drug binder has concerned large consideration in pharmacology and biochemistry because of their biodegradability, biosafty and sustainability.[1-3] Many reports are available on the functionalization of synthetic polymers using chitosan.[4-6] The N-heterocyclic chitosan derivative based gel are applicable as biomedical candidate.[7] The chitosan and silver oxide based nano composite film have efficiency towards antibacterial activity.[8] It was also reported that the blend of chitosan and poly n-vinyl pyrrolidone with titanium oxide nanoparticles showing the high efficacy towards for pathogenic bacteria and found non toxic towards N1H3T3 and L929 fibroblast cells used in wound care.[9] Chitosan is a hydrophilic biopolymer which is derived by deacetylation of chitin under alkaline hot conditions,[10] which is one of the most abundant natural biodegradable and biocompatible polymer. Chitin is an important constituents of animals especially exoskeletons of crustaceans, mollusks and insects. It can also be obtained from certain cell wall of fungi.[11-13] Recently, it has been reported that some new derivatives of chitosan with lauric acid and capric acid are used in drug delivery and also as antibacterial agent .[14,15]

The chitosan was chemically modified in order to progress its solubility and widen its applications in food and medical.[16-22] Among the various methods of modification, graft copolymerization has been the most widely used method. Grafting of chitosan allows the formation of functional derivatives by covalent binding of a molecules, onto the chitosan backbone. Grafted chitosan is very beneficial for improving the properties such as increasing chelating,[23] adsorption properties,[24] bacteriostatic effect,[25] complexation properties.[26] The grafting of various useful monomers onto chitosan modifies its properties such as mucoadhesivity, biocompatibility,[27,28] and biodegradability.[29] However through chemical modification and purification complex polysaccharides can be used as flocculants, viscosifiers and matrices for sustained controlled drug release [30] and grafting plays a significant role in this regard. Therefore, the synthesis was based on free radical mechanism which is a simpler, highly reproducible and well accepted according to the standards of green chemistry.[31] The grafted polysaccharides is an important medium for controlled release of drugs. Hence, the present study has been conducted for grafting the AMPS onto chitosan by free radical polymerization and making its use for better drug delivery system in colon. In this study, an attempt was made to develop beads of diclofenac sodium using sodium alginate as the

hydrophilic carrier and graft copolymer of chitosan with AMPS as drug release modifiers in various proportions to overcome the drug related adverse effects, improve drug bioavailability.

## MATERIALS AND METHOD

### *Materials*

All the chemicals used in this study were of analytical grade. The chemicals chitosan, AMPS, PMS, sodium alginate were purchased from Sigma Aldrich and calcium chloride, thiourea were procured from Himedia Chemicals. The diclofenac sodium drug used for drug delivery studies was obtained from Ranbaxy Pvt. Ltd., India as a gift sample. Triple distilled water was used for preparing all the solutions.

### *Method of Graft copolymerization*

The graft copolymer of chitosan with AMPS has been synthesized using the radical initiator via free radical graft copolymeization process.[32-34] Grafting reactions were carried out in 100 ml polymerization flasks by dissolving calculated amount of chitosan in 2% acetic acid solution in triple distilled water. The calculated amount of AMPS and thiourea were added into the reactor and a slow stream of oxygen free nitrogen gas was passed for 30 min. A known amount of deoxygenated potassium peroxy monosulphate solution was added to initiate the reaction. The polymerization reaction was carried out with the continuous flow of oxygen free nitrogen gas at desired temperature. After desired time period the reaction was stopped by letting air into reactor. The graft copolymer was precipitated by pouring the reaction mixture into the water-methanol mixture. The precipitated graft copolymer was filtered and then dried in vacuum to constant weight.

### *Characterization of chitosan and grafted chitosan*

#### *FTIR spectroscopy*

The grafting on polymer backbone was confirmed by FT-IR. The infrared spectra of chitosan and grafted chitosan were recorded in the range from 4000 to 500 $cm^{-1}$ by Perkin Elmer FTIR spectrophotometer. Presence of different functional groups as well as molecular structure of polymer can be obtained from infrared spectroscopy.

#### *X-ray diffraction (XRD) analysis*

X-ray diffractometry analysis of crude and grafted chitosan samples was done in powder form from x-ray diffractometer (JDX 3532, JEOL, Japan). The diffraction angle range of observation was 5-60◦ with a scan step of 0.01◦.

#### *Scanning electron microscope (SEM) analysis*

The surface morphology of beads, chitosan before and after grafting in powder form was evaluated by SEM analysis using scanning electron microscope (Model: JSM5910).

***Preparation of beads***

The beads were prepared according to the technique of ionotropic gelation method. The blend solution contained 2.0% (w/v) sodium alginate and polymer with mass proportions of 1:2, 1:3 and 1:4. Firstly the certain amount of sodium alginate was dissolved in 30 ml of distilled water at 40◦ C under mechanical stirring for 5 min; the certain polymer powder and 2%(w/v) of drug (diclofenac sodium) was added into the solution and mixed homogeneously. The two type of blend solution was prepared one for grafted chitosan and another for ungrafted chitosan under stirring at 40◦ C for 20 min. Lastly the blend solution was dripped through a 21 gauge injection needle into the 100 ml solution of 2% (w/v) calcium chloride solution; spherical and smooth beads were formed under mechanical stirring for 20 min; gently washed the beads with distilled water three times and dried under vacuum at 40◦C.

***In vitro study of Drug release***

The in vitro drug release study of particles was performed through the Hanson SR8 dissolution station, using basket apparatus. In vitro study of a drug release profile provides the most sensitive and reliable information of drug.[35] For the stimulation of gastric and enteric media 900 mL of 0.1 M HCl (pH 1.2) and 0.5 M phosphate buffer (pH 7.4) were used at 37◦C with stirring rate 50 rpm. About 20 mg of diclofenac sodium containing particles were filled in gelatin capsule and placed in basket, 5 mL of sample was withdrawn after appropriate time intervals and drug release was assayed using spectrophotometer at 276 nm. Each experiment was conducted in triplicates. After 2h in gastric medium the basket was removed and placed immediately in 900 mL of the 10 mM phosphate buffer (pH 7.4) after that 5 mL of sample was withdrawn after appropriate time intervals and used for evaluating release profile of diclofenac sodium drug release using spectrophotometer .

## RESULTS AND DISCUSSION

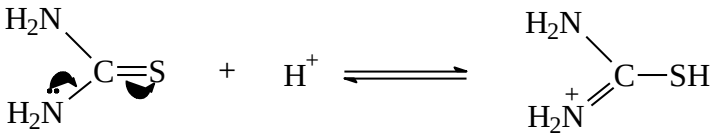
***Graft copolymerization: Synthesis of Chitosan-g-PAMPS***

**Mechanism:**

The system containing chitosan, PMS, AMPS, thiourea and hydrogen ion; the protonated species of thiourea (isothiourea) reacts with potassium peroxymonosulphate to form complex. Subsequently, this complex dissociates, to generate free radicals ($R^{\bullet}$ = $R_1S^{\bullet}$ and $SO_4^{\bullet -}$). These radicals are represented by $R^{\bullet}$, abstract hydrogen atom from the chitosan molecules

producing chitosan macro free radicals ($CO^{\bullet}$). The monomer molecules which are at close proximity of the reaction sites becomes acceptors of the chitosan radicals resulting in chain initiation and thereafter themselves becomes free radical donor to neighboring molecules. In this way grafted chain grows. These grafted chains terminated by coupling to give graft copolymer. The following steps are proposed for mechanism of graft copolymerization of AMPS onto chitosan using PMS/thiourea redox pair.

**Radical Formation**

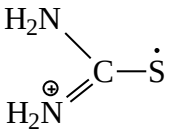


**Thiourea ($R_1S$)** **Isothiourea ($R_1SH$)**

$$R_1SH + HSO_5^- \rightleftharpoons \text{Complex}$$

$$\text{Complex} \Longrightarrow R_1S^{\bullet} + H_2O + SO_4^{\bullet -}$$

where, $R^{\bullet} = R_1S^{\bullet}$ or $SO_4^{\bullet -}$ and $R_1S^{\bullet}$ =



Amidinosulfenyl free radical

**Initiation**

$$COH + R^{\bullet} \longrightarrow CO^{\bullet} + RH$$

$$M + R^{\bullet} \longrightarrow RM^{\bullet}$$

where, COH Chitosan

M Monomer

**Propagation**

$$CO^{\bullet} + M \longrightarrow COM_1^{\bullet}$$

$$COM_1^{\bullet} + M \longrightarrow COM_2^{\bullet}$$

.....

.....

$$COM_{n-1}^{\bullet} + M \longrightarrow COM_n^{\bullet}$$

$$RM_1^{\bullet} + M \longrightarrow RM_2^{\bullet}$$

.....

.....

$$RM^{\bullet}_{n-1} + M \longrightarrow RM^{\bullet}_{n}$$

**Termination**

$$COM^{\bullet}_{n} + COM^{\bullet}_{n} \rightarrow \text{Graft copolymer}$$

$$COM^{\bullet}_{n} + RM^{\bullet}_{n} \rightarrow \text{Graft copolymer}$$

**Determination of grafting Characteristics**

The grafted co-polymer was characterized according to Fanta's definition .[36]

$$\text{Grafting ratio (\%G)} = \frac{\text{weight of grafted polymer}}{\text{Weight of substrate}} \times 100$$

$$\text{Grafting Efficiency (\%E)} = \frac{\text{weight of grafted polymer}}{\text{weight of polymer formed}} \times 100$$

$$\text{Add on (\%A)} = \frac{\text{weight of synthetic polymer}}{\text{weight of grafted copolymer}} \times 100$$

$$\text{Conversion (\%C)} = \frac{\text{weight of polymer formed}}{\text{weight of monomer charged}} \times 100$$

$$\text{Homopolymer (\%H)} = 100 - \% \text{ Grafting efficiency}$$

**Determination of optimum reaction condition**

To determine the optimum reaction condition for the grafting of AMPS onto chitosan using PMS /thiourea redox pair, graft copolymerization reaction was carried out under different conditions of reaction time, temperature, concentration of PMS, thiourea, chitosan and AMPS. The optimum temperature and time for maximum %grafting ratio was found to be 45°C and 150 min. respectively. The variation of AMPS concentration was given in details in the Table 1.

***Effect of 2-acrylamido-2-methyl-1-propane sulphonic acid (AMPS) concentration***

The effect of AMPS on graft copolymerization has been studied by varying the concentration of AMPS from $3.5\times10^{-2}$ to $11.5\times10^{-2}$ mol $dm^{-3}$ and results are given in Table 1. It has been observed that the %G, %C and %A increase on increasing the concentration of AMPS from $3.5\times10^{-2}$ to $19.5\times10^{-2}$ mol $dm^{-3}$ and this increment might be due to the greater availability of monomer molecule at the close proximity of polymer backbone. The monomer molecules, which are at the immediate vicinity of the reaction sites becomes acceptor of Chitosan radicals resulting in chain initiation and thereafter it becomes free radical donor to the neighbouring molecule (monomer) leading to lowering the termination.

**Table 1: Effect of AMPS Concentration**

[H+] = $3.0\times10^{-3}$ mol $dm^{-3}$ [Chitosan] = 1.0 g $dm^{-3}$

[PMS] = $8.0\times10^{-3}$ mol $dm^{-3}$ [TU] = $5.0\times10^{-3}$ mol $dm^{-3}$

Temp. = 45°C Time = 150 min

| S. No. | [AMPS ] × $10^2$ mol $dm^{-3}$ | %G | %A | %C |
|---|---|---|---|---|
| 1 | 3.5 | 143.3 | 57.8 | 16.6 |
| 2 | 7.5 | 170.0 | 62.9 | 20.4 |
| 3 | 11.5 | 194.6 | 65.9 | 21.7 |
| 4 | 15.5 | 225.0 | 69.0 | 43.7 |
| 5 | 19.5 | 245.0 | 71.4 | 35.8 |

***Characterization of chitosan –g-PAMPS graft copolymer***

***FTIR spectral analysis***

FTIR spectra of the chitosan and chitosan-g-PAMPS are shown in Fig. 1 line A and line B, respectively. In the spectrum of chitosan, the OH stretching frequency occurred with broad peak at 3421 cm-1 due to strong hydrogen bonding with moisture, while in chitosan-g-PAMPS no absorption is observed for –OH stretching (line B). The peak at 2928 (line A) and 2920 cm-1 (line B) are due to C-H stretching vibration of chitosan and grafted chitosan, respectively. Two bands appeared in line B at 1660 and 1549 cm−1, due to C=O stretching (amide-I band) and N–H bending (amide-II band), respectively, which indicates the grafting of AMPS onto chitosan. In the line A, two bands were observed at 1422 $cm^{-1}$ and 1383 $cm^{-1}$, due to bending vibration of methyl and methylene group of chitosan backbone, the absorption occurred in the range of 1680 to 1480 $cm^{-1}$ due to carbonyl group of secondary amide (1644 $cm^{-1}$ ) and protonated amine group (1595 $cm^{-1}$) (line A). The band appeared at 1322 $cm^{-1}$ (line A) confirms the presence of glycosidic linkage (C–O–C) of chitosan, while O-H bending vibration occurred at 1253 $cm^{-1}$. The two peaks observed at 1398 and 1206 $cm^{-1}$ (line B) are due to asymmetric and symmetric stretching of S=O group of AMPS, respectively, while the strong stretching absorption in the region of 1042 to 882 $cm^{-1}$ represent S–O–C group of AMPS, indicating the grafting of MAPS onto chitosan (line B). The appearance of additional peaks at 1660, 1549, 1398, 1206, 1042 to 882 cm−1 in graft copolymer (line B) indicates the successful grafting of AMPS onto chitosan.

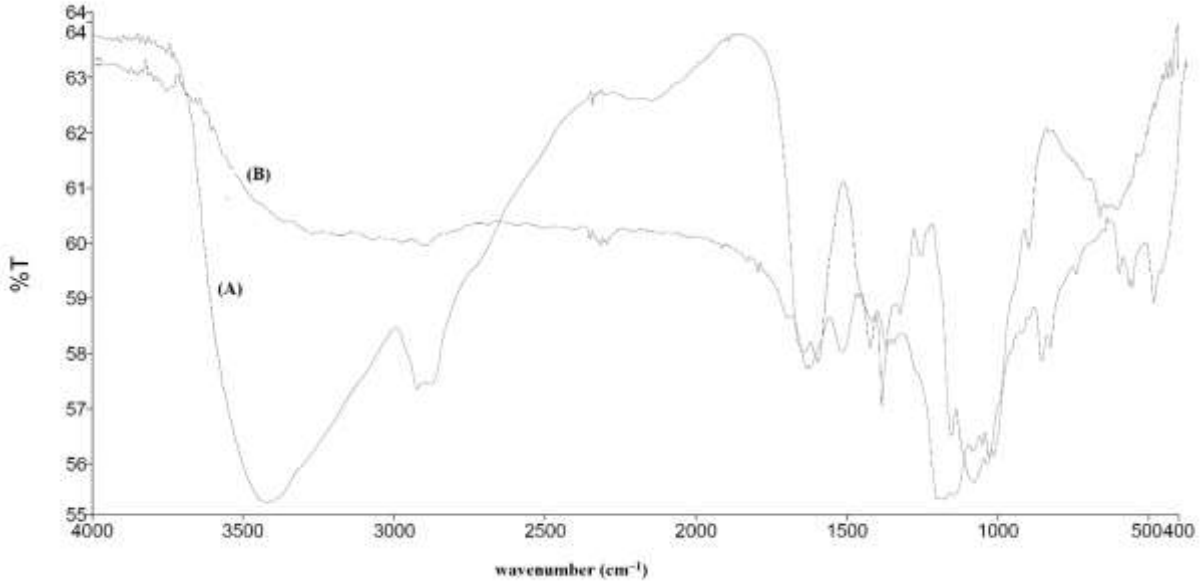



**Figure 1: FTIR Spectra of (A) chitosan and (B) chitosan-g-PAMPS**

***X-ray diffraction (XRD) analysis***

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. Chitosan is a partially crystalline natural polymer, crystallinity is mainly formed due to the accumulation of linear chains in the structure. After comparing the XRD spectra of chitosan and graft copolymer of chitosan with AMPS (Figure 2), shows improvement in crystallinity, which clearly indicates that the chitosan-g-PAMPS are more crystalline polymer than chitosan polymer.

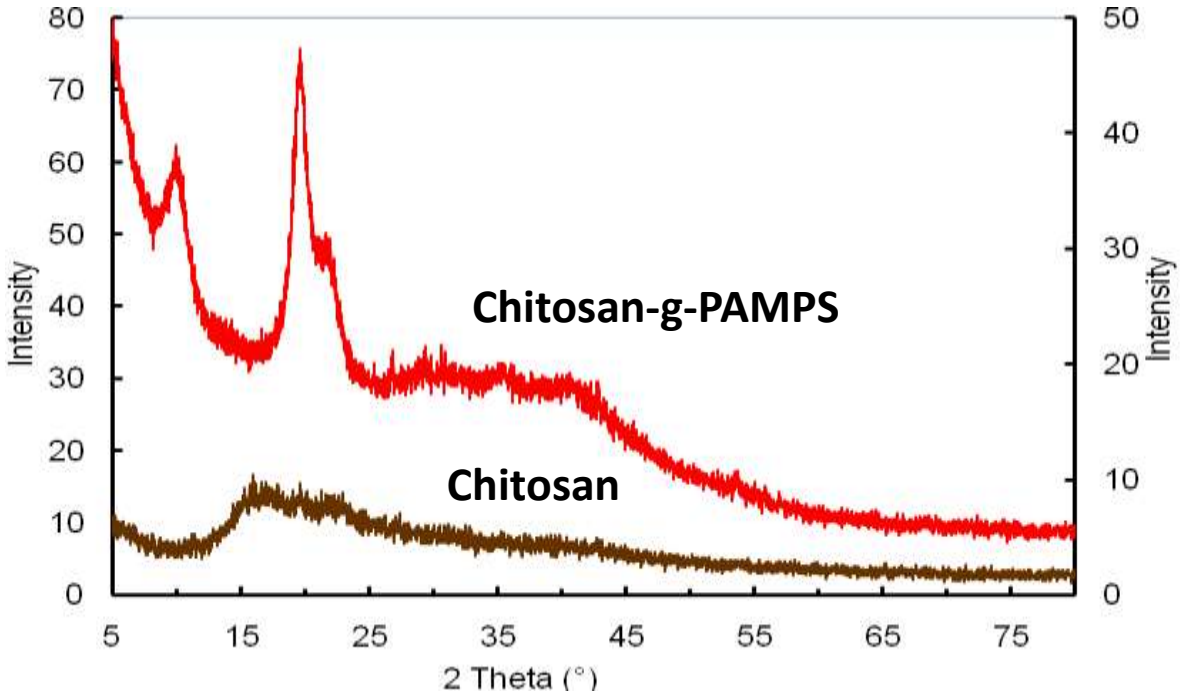



**Figure 2: X- ray diffraction of chitosan and chitosan-g- PAMPS**

***Scanning electron microscope (SEM) analysis***

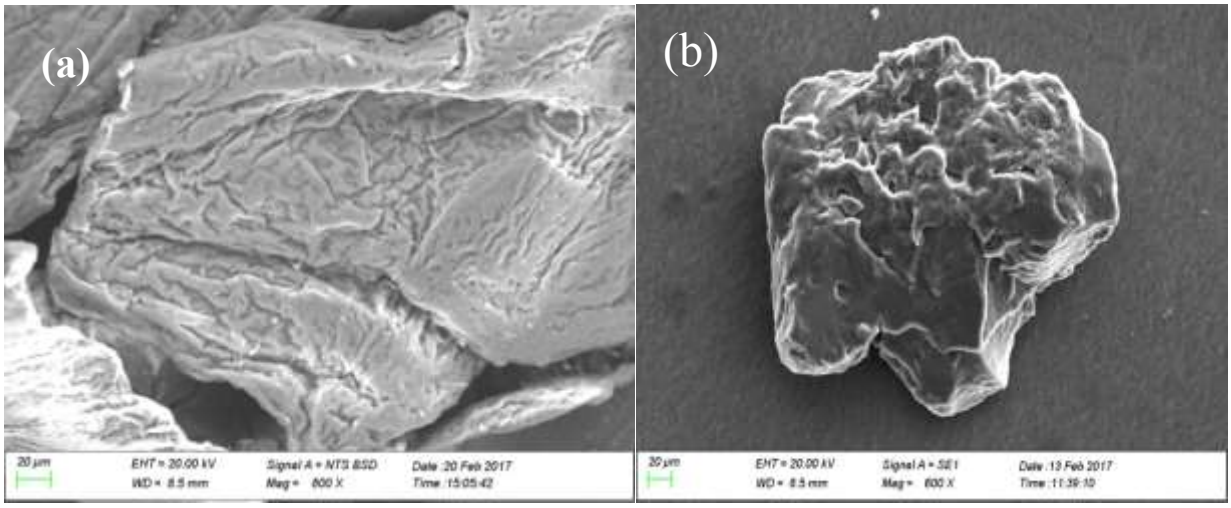
***Chitosan and chitosan-g-PAMPS:***

In order to understand the graft copolymerization, SEM photographs of crude and grafted chitosan are shown in Figure 6a and 6b respectively. Chitosan usually exists in a globular form and the particles of native chitosan before grafting are small with rough surface morphology . SEM analysis of grafted chitosan showed less roughness as compared to crude chitosan which may be due to removal of impurities. There was a specific distinction in the structure of the synthesized grafted chitosan and ungrafted chitosan. The surface of grafted products clearly indicate the morphological changes drafted by grafting chitosan which has been changed to porous and fluffy structure from nonporous form.

**Beads:**

The scanning electron micrographs of dry beads are shown in figure 3cand 3d.

The surface morphology of beads formed using chitosan and five different graft copolymers of chitosan was studied. The SEM picture of two samples of graft copolymers a and b are shown in figure 3c and 3d respectively. The surface morphology of the beads shows that beads are almost spherical with non porous smooth surface in both the sample of sodium alginate beads with graft copolymer.



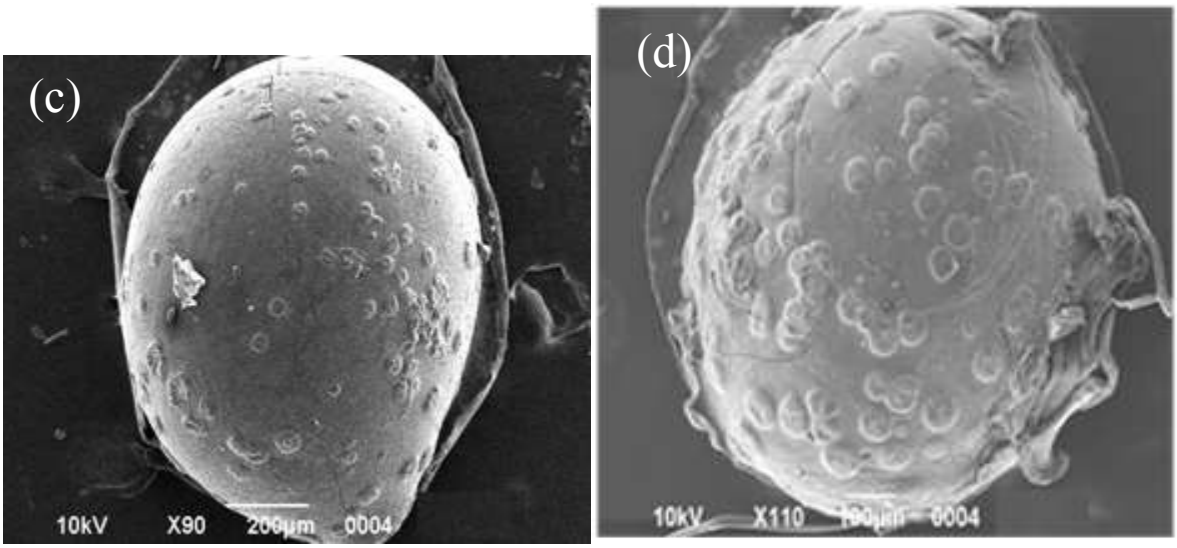



**Figure.3: Scanning electron micrographs of (a) Chitosan and (b) Chitosan-g-AMPS (c) beads of diclofenac sodium using chitosan-g-PAMPS (sample a) and (d) beads of diclofenac sodium using chitosan-g-PAMPS (sample b)**

***In vitro drug release studies:***

For this study diclofenac sodium drug which is a non-steroidal anti-inflammatory agent used in long-term therapy for rheumatoid arthritis. It is poorly soluble in water and acidic pH but is rapidly soluble in alkaline pH. From the formulations of chitosan-g-PAMPS with sodium diclofenac, chitosan was taken as a reference and we compared drug release profile in colonic and gastro interic medium (Figure 4 and 5). The particles of the formulations containing chitosan-g-PAMPS demonstrated slow drug release in gastro-interic system in comparison to colon, 6% of drug was then dissolved from chitosan-g-AMPS b, 5% from chitosan-g-AMPS c, 4% from chitosan-g-AMPS d, 3% from chitosan-g-AMPS e, however; a slight release (2%) of drug was released by chitosan-g-AMPS f . Strong control over drug release was presented by chitosan-g-AMPS f where 23% drug released was observed for chitosan matrix ( Fig 4 and 5). In colonic system during first hour assay, dissolution was 30% for chitosan-g-AMPS b, 28% for chitosan-g-AMPS c, 26% for chitosan-g-AMPS d, 24% for chitosan-g-AMPS e and 22% for chitosan-g-AMPS f. The samples containing AMPS grafted chitosan have released more drugs in colonic medium than in gastric medium with a more effective release control. Delivery of drug was 13% and 55% during 4 h of assay in gastro-enteric and colonic system respectively and chitosan was dissolved about 53% and 65% in gastro-enteric and colonic system, respectively. The slowest release of drug might be due to the binding of chitosan-g-PAMPS which resulted in less swelling of the formulation particles. The chitosan-g-PAMPS f: sodium diclofenac was proved to be the best formulations because it

demonstrates stronger control over the drug release. From these drug release profiles, it is apparent that grafting plays a vital role because drug release is more sustained with chitosan-g-PAMPS f having highest grafting percentage. Due to increased grafting, number of graft chains increased and as a result of this more effective intermingling of chains occurs that does not allow the rapid erosion rate of formulation. Hence slower rate of drug release of enclosed drug has been observed.

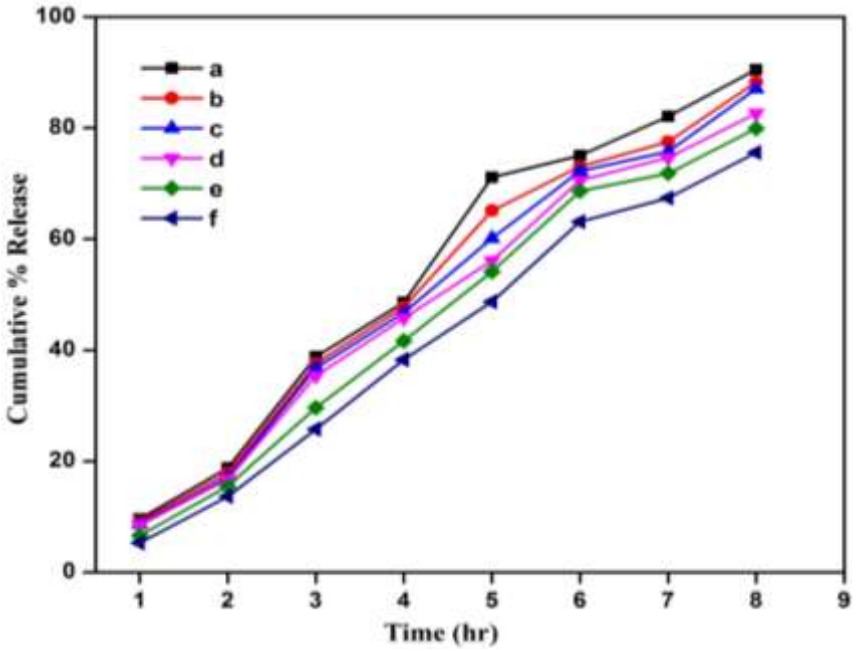



**Figure 4: *In vitro* release of diclofenac sodium drug in colonic medium**

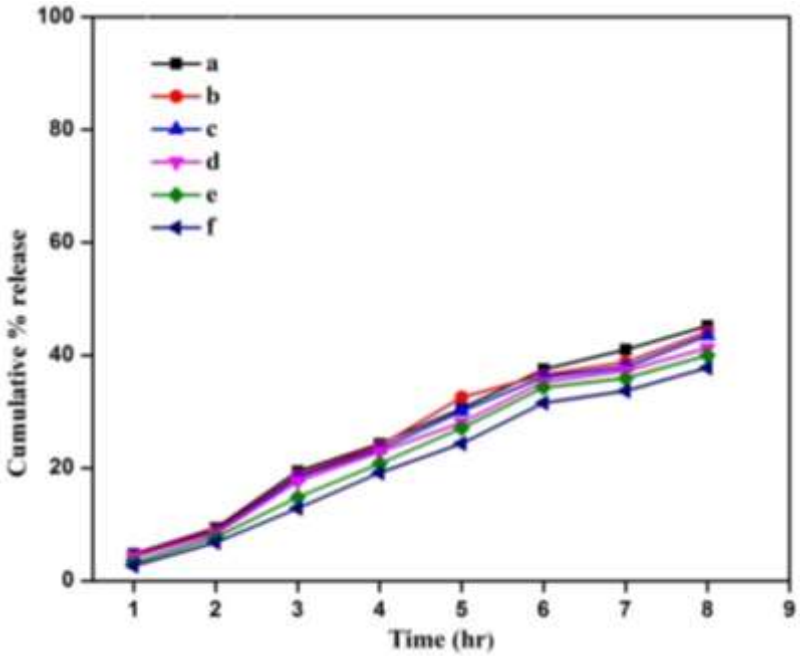



**Figure 5: *In vitro* release of diclofenac sodium drug in gastric medium**

The diclofenac sodium beads is a three-dimensional network composed of chitosan -g-PAMPS and sodium alginate in which chitosan -g-PAMPS is used as carriers for the slow-release of sodium diclofenac drug. It has been observed that with increasing the pendent chain of PAMPS on to chitosan the release of drug becomes slow because pendent chain may give the longer path for the migration of diclofenac sodium from graft copolymer matrices to the buffer solution in comparison to chitosan matrices.

## CONCLUSION

In this study chitosan-g-PAMPS graft copolymer was prepared using free radical initiator for drug delivery. No cytotoxicity was recorded by the chitosan and its graft copolymer. The *in vitro* drug release showed that the grafted copolymer to the diclofenac sodium resulted in higher control drug released in different pH media. In colonic medium drug release was high as compare to gastric medium, however comparatively high drug release was observed in basic medium in colonic system.

## ACKNOWLEDGMENT

The authors thank the STIC Cochin University, Cochin, India for providing spectral and analytical facilities and UGC, New Delhi, India for financial support in the form of research project No- 42-387/2013(SR) are gratefully acknowledged.

## REFERENCES:

1. D. V. Prajapati, K. J. Girish, G. M. Naresh and P.R. Narayan, Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydr. Polym*, 2013; 92: 1685-1699.
2. H. Mirhosseini and B. T. Amid, A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Res. Int*, 2012; 46: 387-398.
3. G. Dodi, D. Hritcu and I.M. Popa, Carboxymethylation of guar gum synthesis and characterization, *Cell. Chem Technol*, 2011; 45: 171-176.
4. A.J.M. Al-Karawi, Z.H.J. Al-Qaisi, H.I. Abdullah, A.M.A. Al-Mokaram and D.T.A. Al-Heetimi, Synthesis, characterization of acrylamide grafted chitosan and its use in removal of copper(II) ions from water. *Carbohydr. Polym*, 2011; 83: 495-500.
5. D. Pathania, D. Gupta, N.C. Kothiyal, G. Sharma, G.E. Eldesoky and Mu. Naushad, Preparation of a novel chitosan-g-poly(acrylamide)/Zn nanocomposite hydrogel and its applications for controlled drug delivery of ofloxacin. *Int. J. Biol. Macromol*, 2016; 84: 340-348.

6. Z. Yuan, Y.Ye, F. Gao, H. Yuan, M. Lan, K. Lou and W. Wang, Chitosan-graft-cyclodextrin nanoparticles as a carrier for controlled drug release. *Int. J. of Pharma*; 446: 191-198.
7. S. Kumar, J. Dutta, and P.K. Dutta, Preparation and characterization of N-heterocyclic chitosan derivative based gels for biomedical applications. *Inter. J. Bio. Macro*, 2009; 45 (4): 330-337.
8. S. Tripathi, G.K. Mehrotra and P.K. Dutta, Chitosan-silver oxide nanocomposite film: Preparation and antimicrobial activity. *Bull. Mater. Sci*, 2011; 34(1): 29-35.
9. D. Archana, J. Dutta, and P.K. Dutta, Evaluation of chitosan nano dressing for wound healing characterization, in vitro and in vivo studies. *Inter. J. Bio. Macro*, 2013; 57: 193-203.
10. A.G. Chmielewski, Chitosan and radiation chemistry. *Radiat. Phys. Chem*, 2010; 79: 272-275.
11. P.K. Dutta, J. Dutta, M.C. Chattopadhyaya and V.S. Tripathi, Chitin and chitosan: noval biomaterials waiting for future developments. *J. Polym. Mater*, 2004; 21(3): 321-333.
12. P.K. Dutta, J. Dutta, and V.S. Tripathi, Chitin and Chitosan: Chemistry, properties and applications, *J. Sci. Ind. Res*, 2004; 63: 20-31.
13. P.K. Dutta, M.N.V. Ravikumar and J. Dutta, Chitin and chitosan for versatile applications. *J. Macromol. Sci., PartC*; 2002: 42 (3): 307-354
14. N. Sinha, B. K. Singh and P.K. Dutta, Preparation and characterization of chitosan-lauric acid derivative for antibacterial activity and drug delivery study. *J. polym. Materi*; 2016: 33(3): 479-490.
15. N. Sinha and P.K. Dutta, Preparation and characterization of chitosan-capric acid derivative for drug delivery system. *Asian Chi. J*, 2016; 12(1): 19-26.
16. Singh, S.S. Narvi, P.K. Dutta and N.D. Pandey, External stimuli response on a novel chitosan hydrogel crosslinked with formaldehyde. *Bull. Mater. Sci*, 2006; 29(3): 233-238.
17. J. Singh, P.K. Dutta, J. Dutta, A.J. Hunt, D.J. Macquarrie and J.H. Clark, Preparation and properties of highly soluble chitosan-l-glutamic acid aerogel derivative. *Carbohydr. Polym*, 2009; 76 (2): 188-195.
18. P.K. Dutta, S. Tripathi, G.K. Mehrotra and J. Dutta, Perspectives for chitosan based antimicrobial films in food applications. *Food Chem*, 2009; 114 (4): 1173-1182.

19. S. Tripathi, G.K. Mehrotra, and P.K. Dutta, Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. Inter. *J. Bio. Macro*, 2009; 45 (4): 372-376.
20. S. Tripathi, G.K. Mehrotra, P.K. Dutta, Carbohydr. Polym.,Preparation and physicochemical evalution of chitosan/poly(vinyl alcohol)/pectin ternary film for food-packaging applications. 2010; 79: 711-716.
21. D. Archana, J. Dutta, and P.K. Dutta, Evaluation of chitosan nano dressing for wound healing: Characterization, in vitro and in vivo studies. *Inter. J. Bio. Macro*, 2013; 57: 193-203.
22. D. Archana, B.K. Singh, J. Dutta, and P.K. Dutta, In vivo evaluation of chitosan-PVP-titanium dioxide nanocomposite as wound dressing material. *Carbohydr. Polym*, 2013; 95 (1): 530-539.
23. Z. Yang and Y. Yuan, Studies on the synthesis and properties of hydroxyl azacrown ether-grafted chitosan. *J. Appl. Polym. Sci*, 2001; 82: 1838-184.
24. M. Thanou, J.C. Verhoef and H.E. Junginger, Oral drug absorption enhancement by chitosan and its derivatives. *Adv. Drug Deliv*, 2001; 52: 117- 126.
25. B.O. Jung, C.H. Kim, K.S. Choi, Y.M. Lee and J.J. Kim, Preparation of amphiphilic chitosan and their antimicrobial activities. *J. Appl. Polym.* Sci, 1999; 72: 1713-1719.
26. S. Chen and Y. Wang, Study on β-cyclodextrin grafting with chitosan and slow release of its inclusion complex with radioactive iodine. *J. Polym. Sci*, 2001; 82: 2414-2421
27. R.A.R. Tasker, B.J. Connell, S.J. Ross and C.M. Elson, Development of an injectable sustained-release formulation of morphine: anti nociceptive properties in rats. *Lab.* Anim, 1998; 32: 270-275.
28. K. Ono, Y. Saito, H. Yura, K. Ishikawa, A. Kurita, T. Akaike and M. Ishihara, Photocrosslinkable chitosan as a biological adhesive. *J. Biomed. Mater. Res.*, 2000; 49: 289-295.
29. D.K. Singh and A.R. Ray, Characterization of grafted chitosan films. *Carbohydr. Polym*, 1998; 36: 251-255.
30. S. Pal, Carboxymethyl Guar:Its synthesis and Macromolecular characterization. *J. Appl. Polym. Sci*, 2009; 111: 2630-2636.
31. E.I. Nep and B.I. Conway, Polysaccharide gum matrix tablets for oral controlled delivery of cimetidine. *J. Pharm. Sci. Res. 2*, 2010; 11: 708-716.

32. J. Tripathy, D.K. Mishra, M. Yadav and K. Behari, Synthesis, characterization and applications of graft copolymer (Chitosan-g-N,N-dimethylacrylamide). *Carbohydr. Polym*, 2010; 79(1): 40-46.
33. Srivastava, D.K. Mishra and K. Behari, Graft copolymerization of N-vinyl-2-pyrrolidone onto chitosan: Synthesis, characterization and study of physicochemical properties. *Carbohydr. Polym*, 2010; 80(3): 790-798.
34. D.K. Mishra, J. Tripathy, A. Srivastava, M.M. Mishra and K. Behari, Graft copolymer (chitosan-g-N-vinyl formamide): Synthesis and study of its properties like swelling, metal ion uptake and flocculation. *Carbohydr. Polym*, 2008; 74(3): 632-639.
35. Y. Xu, C. Zhan, L. Fan, L. Wang and H. Zheng, Preparation of dual cross-linked alginate–chitosan blend gel beads and in vitro controlled release in oral site-specific drug delivery system. *Inter. J. Pharma*, 2007; 336: 329-337.
36. Fanta, G.F. In: Block and copolymerization, Ceresa, R. J., Ed.; Wiley Intersciences: New York, NY, 1973; p 1