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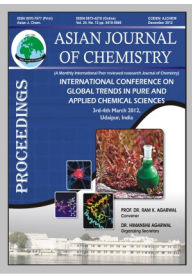

Synthesis and Characterization of Chitosan Based Interpenetrating Network Micro Gel for Controlled Release of Cephalexin†

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The aim of this study is to prepare pH-sensitive interpenetrating network micro gels based on chitosan and its grafted copolymer, hydrolyzed grafted copolymer (that are cross linked with glutaraldehyde by precipitation and cross linking methods) for the controlled release of cephalexin as model drug. The networks were characterized by Fourier transformed infrared spectroscopy, SEM, X-ray diffraction studies and swelling studies. All physical chemical characteristic are performed. The *in vitro* release is performed using phosphate buffer media (pH 2 and pH 7.4) and the release rate was controlled by tuning buffer pH. The results indicated that the networks could be potentially useful for controlled drug delivery. The extended release rates were observed from the conventional dosage release study which was more than 12 h.

Key Words: Chitosan, Network micro gel, Controlled release, Cephalexin.

INTRODUCTION

From the beginning of the pharmaceutical era, the oral route has always dominated over any other routes of drug delivery. This can be accredited to the numerous advantages of the oral route such as ease of administration of drug, patient compliance, economical production methods, easy approvals from regulatory bodies and so on. Despite being the most superior route of administration, it is not always possible to deliver every therapeutic agent through the oral route[1,2]. The major problem faced while delivering a therapeutic agent through the oral route is poor oral bioavailability due to incomplete and/or erratic absorption through the gastrointestinal tract (GIT), degradation of the drug or drug carriers due to varying pH of the stomach and enzymatic degradation of many proteins and peptide drugs[3,4]. Hence, the importance of different drug delivery matrices is realized to improve the oral absorption and bioavailability by improving the stability of the therapeutic agent within the harsh condition of the gastrointestinal tract. Among different drug delivery systems (DDS) available, the biodegradable[5,6] chitosan is a cationic polysaccharide known for its good film forming ability. It is obtained from waste chitin and as such can be considered a material from renewable resources. Amino groups attached to its backbone enable chitosan to become positively charged in acidic medium. Therefore, chitosan can serve as a biomaterial for pH-responsive hydro gel applications. However, these hydro gels generally lack mechanical stability unless they are cross linked and/or reinforced by suitable compounds[7]. In present study, a novel pH sensitive interpenetrating polymeric network micro gels based on chitosan, acrylamide-grafted-poly ethylene glycol followed by hydrolysis that are cross-linked with glutaraldehyde, was used in the controlled release of cephalexin. Cephalexin is an antibiotic which has short biological half life 3 to 4 h. Cephalexin is a water soluble antibiotic prepared from semi synthetic cephalosporin antibiotic with antimicrobial activity is similar to that of cephaloridine or cephalothin, but somewhat less potent. It is effective against both gram-positive and gram-negative organisms having molecular mass 347.389 with chemical formula $C_{16}H_{17}N_3O_4S$ and its biological half life as 1 h. To the best of our knowledge no work has been done so for by using this drug. The present work deals with the *in vitro* release of loaded formulation with micro spheres on interpenetrating polymeric network micro gel.

EXPERIMENTAL

Cephalexin USP grade, chitosan poly (D-glucosamine) purchased from Sigma-Aldrich product of China, acrylamide were purchased from Qualigens Mumbai, India. Poly ethylene

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TABLE-1
RESULT OF ENCAPSULATION EFFICIENCY (%), MEAN PARTICLE SIZE OF MICROSPHERE IN DIFFERENT EXTERNAL pH MEDIA

Sample code	Drug loading (%)	Encapsulation efficiency (%)	Mean particle size (mean± SD)	pH 2	pH 7.4
C-50	50	71.42 ± 0.68	53 ± 0.2	218	190
C-100	100	86.9 ± 0.08	76.3 ± 0.5	218	190
C-PEG-50	50	81.96 ± 0.04	81.96 ± 0.04	251	268
C-PEG-100	100	85.06 ± 0.01	103 ± 0.14	251	268
C-grafted co-polymer 50	50	83.33 ± 0.23	110 ± 0.24	258	273
C-grafted co-polymer 100	100	89.28 ± 0.45	134 ± 0.16	258	273
C-grafted co-polymer 50 (Hydrolysis)	50	91.04 ± 0.16	148 ± 0.45	251	293
C-grafted co-polymer 100 (Hydrolysis)	100	93.01 ± 0.98	163 ± 0.14	251	293

glycol 4000, hydrochloric acid, glutaraldehyde, acetic acid and sodium hydroxide were purchased from SD Fine Chemicals. All other chemicals used in this work were analytical reagent grade.

Preparation of blend

Synthesis of polyethylene glycol grafted-acrylamide: Polyethylene glycol was dissolved in water on heating at 60-65 ºC and treating with acrylamide under nitrogen gas atmosphere[8]. Potassium persulfate was introduced in a small quantity under continuous stirring at 65 ºC for 5 h. The obtained product was precipitated in methanol and then washed with water:methanol (1:1 v/v). The final product was filtered and kept in vacuum for drying at 60 ºC. In order to make the grafted polymer to undergo hydrolysis[8], about 2 wt % solution of PEG-grafted acrylamide was heated in a hot plate at 60 ºC. 50 mL of equimolar concentration of sodium hydroxide solution was added and the mixture was stirred with magnetic stirrer at 60 ºC for 5 h, to complete the hydrolysis of the prepared polymer blend. Then 2N hydrochloric acid was added to make just acidic. The obtained products were precipitated in methanol and washed with a solution of methanol, filtered and vacuum dried at 60 ºC.

Drug loading: In order to load the drug[9], approximately 2 g of polymer blend obtained from the above step was dissolved in a required amount of cephalexin. Polymer mix with 2 % acetic acid was dispersed with equal quantities of light liquid paraffin stirred at high speed and the resulting water-oil emulation was stabilized by addition of 1 % Tween 80 solutions. Afterword aqueous phase of emulation was hardened to form micro gel, which was cross linked with glutaraldehyde. The obtained micro gel was filtered and washed with hexane and water to remove paraffin oil, acid, water, excess of glutaraldehyde. In order to know the drug release characteristic 8 formulations with varying drug concentrations and chitosan with polymeric blend concentrations were prepared and studied.

Swelling studies: Swelling studies were performed with different drug loaded formulations on micro gel[9]. In all the cases the standard deviation were less than 2.5 %.

***In vitro* studies:** The *in vitro* drug release studies were performed using (USP paddle single stage digital apparatus) using 900 mL of phosphate buffer (pH 2 and pH 7.4) as dissolution medium. The release rate was studied for all formulations. At fixed time intervals, aliquots were withdrawn and drug was assayed using a spectrophotometer (Make Jasco UV-Visible NIR Spectrophotometer Model-V-670PC).

Assay of drug and entrapment efficiency: Entrapment assay and drug assay were carried out as per reported procedure[9]. Results were summarized in Table-1.

Fourier transform infrared spectroscopy (JASCO-4100, Japan) analysis was carried out to identify the chemical structure of the prepared network. Furthermore to find out the nature of drug in the polymeric network differential scanning calorimetry (Model DSC Q 1000 V9.4 Build 287) study was carried out at a scanning rate of 10 ºC min. X-ray diffraction analysis was performed (Brand: Bucker Germany, CuK_α radiation, Nickel filter). Morphology of polymeric network was examined by scanning electron microscopy (SEM) (FEI Quanta FEG 200-high resolution scanning electron microscope).

RESULTS AND DISCUSSION

FTIR studies: This study was carried out to confirm the grafting of acrylamide as well as micro gel. In addition, FTIR spectra of plain PEG, PEG-g-copolymer and PEG-g-copolymer (hydrolyzed) are presented (Fig. 1). A broad band appearing at 3435 cm^{-1} corresponds to associated -OH stretching vibrations of the hydroxyl group of grafted copolymer. A new peak appeared at 3251 cm^{-1} and the related peak at 1630 cm^{-1} corresponds to -NH bending vibrations of the primary amides of acrylamide. A relatively high intense peak at 2923 cm^{-1} (Fig. 1), which is characteristic to aliphatic -CH stretching vibrations in the grafted copolymer, confirms the completion of reaction. In the spectra of hydrolyzed PEG-grafted copolymer, the shoulder peak disappeared but two new peaks appeared around 1500 cm^{-1} and 1450 cm^{-1}, which are due to antisymmetric vibrations of -COOH groups.

The FTIR spectrum of plain chitosan showed two peaks around 901 cm^{-1} and 1210 cm^{-1} corresponding to saccharine structure (Fig. 2). The observed sharp peaks at 1350 cm^{-1}

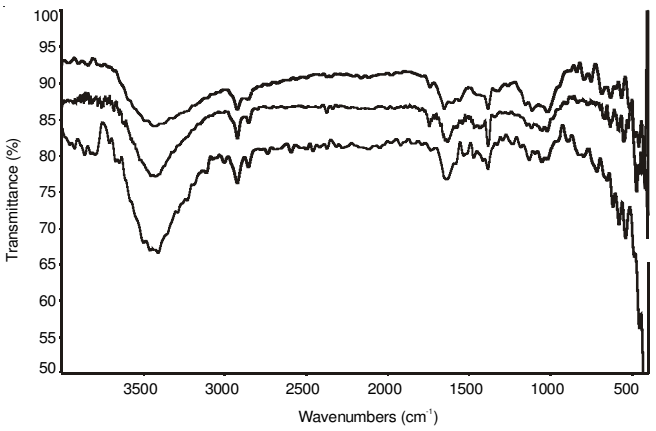

Fig. 1. FTIR spectra a) poly ethylene glycol, b) poly ethylene glycol-g-acrylamide c) hydrolyzed poly ethylene-g-acrylamide

and 1501 cm^{-1} are assigned to CH_3 group. A broad band appearing around 1100 cm^{-1} indicates the C-O stretching vibration of chitosan. Another band appearing around at 3480 cm^{-1} is due to amine N-H symmetric stretching vibration. A new peak appeared at 1600 cm^{-1} due to imine bonds (-C-N) as a result of cross linking reaction between amino groups in chitosan and aldehydic group in the glutardehyde (Fig. 2).

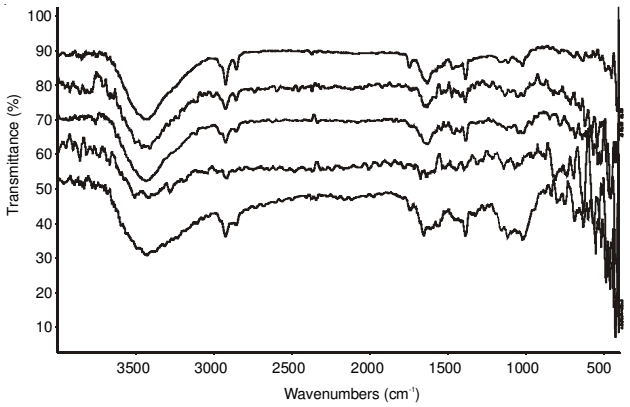

Fig. 2. FTIR spectra of a) pure chitosan, b) cross linked chitosan, c) chitosan blend with poly ethylene, d) chitosan blend with poly ethylene-g-acrylamide, e) chitosan blend with hydrolyzed poly ethylene-g-acrylamide

In curve 3 blends of chitosan with poly ethylene glycol, ether linkage observed at 1150 cm^{-1}, in curve 4, two distinguishing peaks at 1420 cm^{-1} and 1575 cm^{-1} in curve 5, chitosan hydrolyzed complex observed around 1590-1575 cm^{-1} due to NH_3.

XRD studies: XRD helps to trace the crystallinity of the drug after the encapsulation in the cross linked micro gels. Cephalexin has shown characteristics intense peaks at 10º and 30º due to crystalline nature of the drug, however peaks for plain drug were not seen in the drug loaded matrix complex, placebo micro gels and drug loaded micro gels which indicate that encapsulated drug is amorphous (Fig. 3).

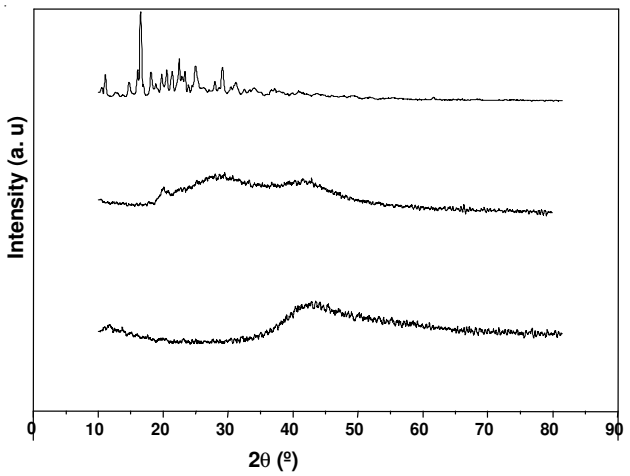

Fig. 3. X-RD diffractions of cephalexin, cephalexin loaded chitosan -PEG micro gel blend, placebo cephalexin loaded chitosan-PEG micro gel blend

Differential scanning calorimetry studies: Differential scanning calorimetry is used widely for examining polymers' composition. Melting points and glass transition temperatures for most polymers are available from standard compilations and the method can show possible polymer degradation by the lowering of the expected melting point (T_m). T_m depends on the molecular weight of the polymer. The percentage crystallinity of a polymer can be found from the crystallization peak of the differential scanning calorimetry graph. Cephalexin exhibits a sharp endothermic peak at 188.6 ºC, but cephalexin-loaded beads did not show any peaks (Fig. 4). It suggests that most of the drug is dispersed in the polymeric matrix.

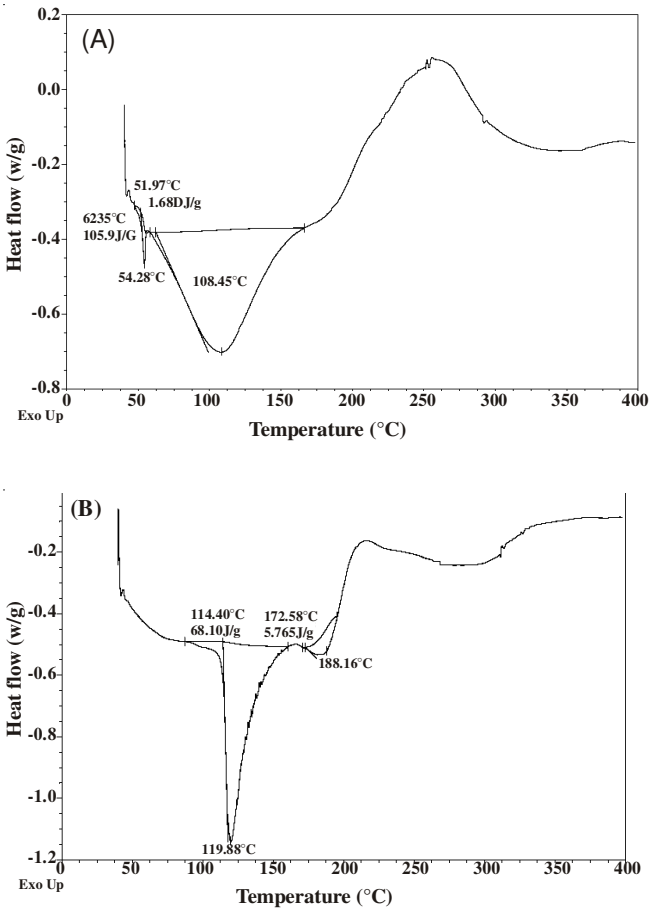

Fig. 4. (A) Differential scanning calorimetry curve of cephalexin, -loaded bead; (B) Differential scanning calorimetry curve of cephalexin

Scanning electron microscope studies: Surface morphology of polymeric complex was studied under a scanning electron microscope. The micro gels are spherical and polymeric materials are seen around the micro gels. Blending of micro gels with polymeric materials has no effect on surface properties (Fig. 5).

Swelling studies: Normally swelling study is used to study drug release characteristics of micro gels. Micro gels are hydrophilic in nature and swells considerably in phosphate buffer solution. pH-dependent study suggests that swelling ratio at pH 7.4 was higher than that of micro gels at pH 6 and almost all chitosan was in its ionized form *i.e.*, forms COO^- groups which would form intermolecular H-Bonding with OH^- groups within the network during swelling. The polymeric complex has been expanded more in size at pH 7.4 compared to pH 2.

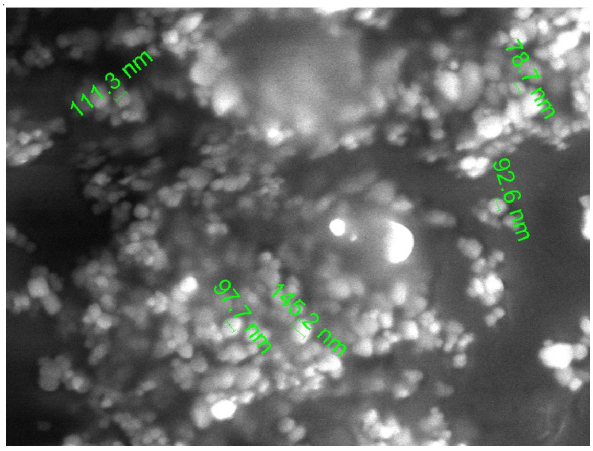

Fig. 5. SEM micrograph of micro gel

Particle size analysis: The particle size of micro gels was carried out by an optical microscope and results are provided in Table-1. This result suggests that the size of micro gels depends on the amount of drug loaded and all the micro gels are in spherical shapes with diameters ranging from 53 to 153 µm.

Encapsulation efficiency: It is observed that percentage encapsulation increased steadily with increasing drug loading from 50 % to 100 % in the polymeric matrix. In case of the grafted micro gels of chitosan with hydrolyzed copolymer the percentage of drug loading was lesser in pH 2 media than that in pH 7.4 due to the complexation of amine group of chitosan and the acidic group of hydrolyzed grafted co-polymer. This results in lower encapsulation in pH 2 media.

***In vitro* drug release:** Cephalexin release from the polymeric complex was evaluated by USP dissolution study. In both pH media drug release was found to be much faster in the case of plain chitosan than the polymeric matrix in pH 2. The release of drug is almost complete within 8-12 h in pH 2 whereas in pH 7.4 release of drug is aboutt 40 % within the same time period (Figs. 6 and 7). This may be due to blending of different polymer with chitosan and release rate of blended micro gels get much delayed in comparison with plain chitosan. Thus drug release depends upon the nature of the polymer matrix as well pH media.

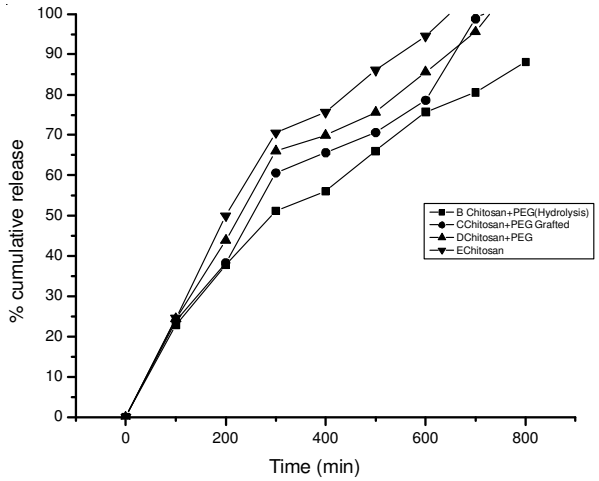

Fig. 6. % Cumulative release of drug *vs.* time for chitosan-grafted PEG (hydrolysis) encapsulated with different amount of drug in pH 7.4

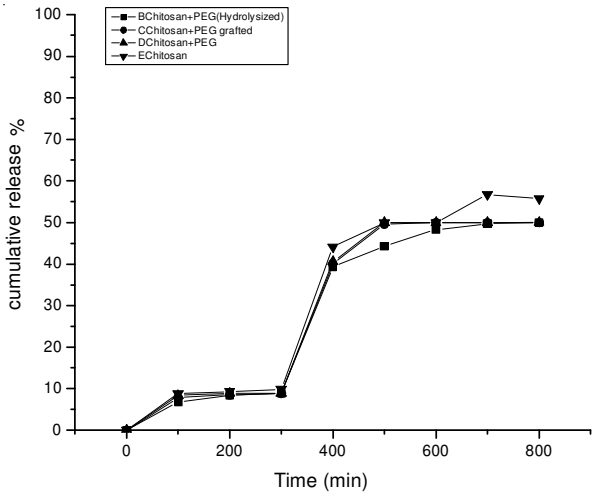

Fig. 7. % Cumulative release of drug *vs.* time for chitosan-grafted PEG (hydrolysis) encapsulated with different amount of drug in pH 2

Conclusion

The hydrophilic nature of polyacrylamide modified poly ethylene glycol was used to synthesize the micro gels blended with chitosan. The blend micro gels showed favourable controlled releases *i.e.*, release rate was more than 12 h. This blended micro gels could be used for controlled release of cephalexin. Further research works warrant its practical applications *in vivo*.

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