Tropical Journal of Pharmaceutical Research April 2013; 12 (2): 155-161
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v12i2.4

Original Research Article

Design and Evaluation of Chitosan-Based Novel pH-Sensitive Drug Carrier for Sustained Release of Cefixime

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Received: 12 December 2012 *Revised accepted:* 13 February 2013

Abstract

Purpose: *To formulate and evaluate pH-sensitive controlled release cefixime microspheres based on crosslinked chitosan and acryl amide-grafted-poly ethylene glycol*
Method: *pH sensitive interpenetrating network (IPN) cefixime microspheres based on chitosan, its grafted copolymer, and hydrolyzed grafted copolymer were prepared by precipitation and crosslinking methods. The formulations were characterized by Fourier transform infrared spectroscopy (FTIR),X-ray diffraction(XRD),Differential scanning calorimetry (DSC), Scanning electron microscopy(SEM),Raman spectroscopy for particle size analysis. Swelling ratio, Effect of drug loading on encapsulation efficiency was investigated. FTIR, SEM, DSC, X-ray diffraction XRD, swelling studies were investigated for controlled drug release of drug at pH 2 and pH 7.4.*
Results: *The microspheres were spherical in shape with a narrow particle size distribution (56 – 124 µm) and encapsulation efficiency of up to 93 %. The % swelling ratio for blend microspheres of* C-PEG are higher than that of plain chitosan in both pH. *In both the pH media release was much faster in plain Chitosan microspheres. For instance, only 83% drug was released at 11.5 h for* C-grafted copolymer 50 *in pH 7.4 media compared to 41% drug released in pH 2 media for the same time whereas 80% drug was released at 11.5 h for* C-grafted copolymer 50 (hydrolyzed) in pH 7.4 media *compared to 38% drug released in pH 2. Drug release followed Higuchi release kinetics. FTIR, DSC, XRD data indicate that there was no interaction between the drug and the polymers used.*
Conclusion: *It is evident from this study that chitosan microspheres could be further developed to serve as an effective biodegradable carrier for controlled release of cefixime.*

Keywords: *Chitosan, Polyethylene glycol, Cross-linking, Controlled release, Cefixime.*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Chitosan is the generic name of a linear random copolymer of β-(1-4) linked –D-glucosamine whose molecular structure comprises a linear backbone linked through glycosidic bonds. The basic amine groups of this polysaccharide are protonated and thus positively charged in most physiological fluids. Chitosan is mostly hydrophilic and the percentage of acetylated monomers and their distribution in the chains has a critical effect on its solubility and conformation in aqueous media. Hence, chitosan exhibits pH-dependent behavior in solution and some interesting biopharmaceutical properties such as muco-adhesiveness, ability to open, and epithelial tight junction. These characteristics of chitosan have attracted many scientists working in the biomedical field, particularly drug delivery [1-4]. Due to its unique polymeric cationic

character, chitosan-based gels and films have been extensively examined in the pharmaceutical industry for its potential in the development of drug delivery system [5].

In recent years, much attention has been given to the development of interpenetrating polymeric network (IPN) from natural biodegradable and biocompatible polymeric materials [6]. Chitosan is biopolymer with immense structural possibilities for chemical and mechanical modification to generate novel properties [7]. Among many methods of modifying the original structures of polymers, graft copolymerization is an easier method, which makes the derived polymer as attractive biomaterials in controlled released application [8]. Grafting of polar monomers onto chitosan by Graft copolymerization improves solubility which enhances its adsorption capacity [8,9].

Several reagents have been used for the crosslinking of chitosan, including glutardehyde, genipin, ethylene glycol, diglycidylether and diisavanate [9,10]. Although the grafting of chitosan modifies its properties, it is possible to retain some interesting characteristics such as mucoadhesivity, biocompatibility and biodegradability [11-12]. Besides, chitosan being a linear polysaccharide has been found to be a good chemical entity for preparing microspheres because of its high crosslinking ability due to the presence of amino ($-NH_2$) groups. A good number of approaches to improve the wet strength of hydrogels and IPN polymeric network of chitosan have been reported elsewhere [13].
Cefixime is an oral third generation cephalosporin antibiotic. It possess anti-bactericidal activity and used in the treatment of gonorrhea, tonsillitis and pharyngitis. Its biological half-life is of 3 -4 h. Cefixime has p^{ka} value of 2.5 and being a weak acid is insoluble in water after its oral administration which results into the poor bioavailability. Around 40-50% is excreted through renal and biliary route. In order to improve the therapeutic effect of the drug by increasing its bioavailability the IPNs of chitosan with acrylamide-grafted –PEG and hydrolysized acrylamide-grafted PEG microspheres was synthesized for controlled release of Cefixime in both acidic and basic media.

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 IPN microspheres.

EXPERIMENTAL

Materials

Cefixime USP was obtained as a gift from Kosher Pharmaceuticals, India. High molecular weight chitosan poly(D-glucosamine) was purchased from Sigma–Aldrich, Belgium; acryl amide was purchased from Qualigens Mumbai, India; while polyethylene glycol 4000, hydrochloric acid, glutaradehyde, acetic acid and sodium hydroxide were purchased from SD Fine Chemicals, India. All other chemicals used in this work were of analytical reagent grade

Synthesis of polyethylene glycol grafted–acryl amide

Polyethylene glycol 4000 was dissolved in water at 60 - 65 ^oC and treated with acryl amide under nitrogen gas atmosphere followed by addition of trace quantity of potassium persulfate under continuous stirring at a temperature of 65 ^oC for 5 h [14]. The resulting product was washed with water: methanol (1:1 v/v), filtered and kept in vacuum for drying at 60 ^oC. About 2 wt % (100ml) solution of PEG-grafted acryl amide was hydrolyzed using 50 ml of equimolar concentration of sodium hydroxide solution at 60 ^oC for 5 h. The final product was filtered and dried under vacuum at 60^oC.

Drug loading

Approximately 2 g of the polymer blend obtained above was dissolved in 2 % acetic acid containing a required amount of Cefixime (100mg) and was dispersed with equal quantities of light liquid paraffin stirred at high speed and the resulting water-oil emulsion was stabilized by addition of 1 % Tween 80 solution. Thereafter, the aqueous phase of the emulsion was hardened to form a microgel. Glutaraldehyde (5ml) was added drop-wise to the emulsion and stirred for 30 min at room temperature to stabilize the prepared microsphere. The mixture was then left to cool at 10-15^oC for 30 min. The microspheres were collected by filtration using Whatman-41 filter paper (pore size: 25µm). Finally, the microspheres were washed with 30ml of hexane and water to remove paraffin oil, acid, water and excess glutaraldehyde. In order to determine the drug release characteristics of the microgels, the formulation was prepared by varying amount of drug and blend ratio of chitosan with PEG, PEG-grafted copolymer as well as hydrolyzed PEG-grafted copolymer matrices. Drug concentration with different

Table 1: Encapsulation efficiency and equilibrium swelling of Cefixime formulation

Formulation code	Drug loading (%)	Encapsulation efficiency (%)*	Particle size (µm)*	(%) Swelling pH 2	pH 7.4
C-50	50	76.42±0.2	56 ± 0.2	196	185
C-100	100	79.02±0.8	76.3±0.5	201	190
C-PEG-50	50	83.96±0.4	89.9±0.4	226	245
C-PEG-100	100	85.06±0.1	103±0.4	239	258
C-grafted copolymer 50	50	87.03±0.3	105±0.2	248	265
C-grafted copolymer 100	100	89.28±0.5	113±0.2	256	270
C-grafted copolymer 50 (hydrolyzed)	50	91.04±0.2	119±0.5	261	273
C-grafted copolymer-100 (hydrolyzed)	100	93.01±0.9	124±0.1	271	283
C-grafted copolymer-100 (hydrolyzed)-(2.5GA)	100	90.4±0.5	122±0.1	253	294
C-grafted copolymer-100 (hydrolyzed)-(5.0G A)	100	85.9±0.2	114±0.1	247	292
C-grafted copolymer-100 (hydrolyzed)-(7.5GA)	100	78.9±0.2	104±0.11	243	289

*SD = standard deviation; *mean ± SD (n = 3); GA = glutaraldehyde crosslinked; C = chitosan.*
Drug concentrations used were 50 mg and 100 mg in each formulation.

polymeric blend and their formulation codes are given in Table 1.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of Cefixime and drug-loaded microspheres were recorded on an FTIR system (JASCO-4100, Japan) using KBr disc with a scanning range of 4000 - 400 cm^{-1} in order to assess structural changes that could have occurred in the drug due to drug-polymer interaction following microsphere formation.

Evaluation of water content and swelling ratio of microspheres

The microsphere formulations (beads) (~ 50 mg) were placed in 50 mL distilled water and at 30 min intervals, beads were taken out and excess water was removed using filter paper and weighed immediately on an electronic weighing balance (Model AUY220, Shimadzu, Japan). Swelling ratio was expressed as % weight gain.

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

X-ray diffraction (XRD) studies (Bruker D8 Advance diffractometer, Germany, CuKά radiation, Nickel filter) were performed to determine crystalline patterns of the drug in the cross-linked polymer network. The XRD patterns of pure cefixime, drug-loaded chitosan microspheres matrix and blank microspheres were recorded and compared.

Differential scanning calorimetry (DSC)

DSC thermograms of the pure drug and IPN microsphere were recorded with a differential scanning calorimeter (DSC Q1000V9.4 Build 287) from 0-400 ^{o}C at a heating rate of 10°C/min.

Particle size

The particles size of the microsphere was measured by Raman spectrometer (Lesser 536 Aglitron USA) equipped with camera-microscope system. The test sample was spread flat on a glass microscope slide and the experiment was performed at room temperature at a magnification of 100x200 µm slit width and exposure time of 5 sec. Triplicate tests were carried out for all samples.

Scanning electron microscopy (SEM) studies

The surface and cross sectional morphologies of the microspheres were examined using high resolution scanning electron microscopy (FEI Quanta FEG 200).

D**rug release studies**

In vitro drug release studies were performed using USP paddle single stage digital apparatus with 900 ml of phosphate buffer (pH 7.4) and HCl/KCl buffer (pH 2) as dissolution medium. At predetermined time intervals, 5 ml samples each were withdrawn from the medium at various time intervals and the medium replenished immediately with the same volume of fresh dissolution medium to maintain sink conditions. The amount of dissolved drug in the sample

taken was measured (after filtration through Whatman-41 filter paper (pore size: 25µm) spectrophotometrically (model-V-670PC, Jasco UV-visible-NIR spectrophotometer) at a λ_{max} of 288 nm.

Moreover all the four release kinetics model namely, zero order kinetics, first order kinetics, Korsenmeyer Peppas model and Higuchi model were applied to Data obtained from *in vitro* release studies. It was observed that zero order kinetics model showed a r^2 value of 0.7453 (~0.7182-0.8012); first order kinetics model with r^2 value of 0.8660 (~ 0.8120-0.8710); Higuchi model with r^2 value of 0.990 (~ 0.980-.0.990) and Korsenmeyer peppas model with r^2 value of 0.9215 (~ 0.9210-0.9219). The assessment of the release kinetics revealed that drug release from the chitosan microspheres followed Higuchi model. The mechanism of drug release from microspheres was diffusion controlled. Data obtained from *in vitro* release studies were fitted to Higuchi model (Q = Kt½) to find out the mechanism of drug release from the chitosan microsphere. The tests were carried out in triplicate .

Determination of drug content

A sample (100 mg) of the formulation was weighed accurately, transferred quantatively into a 100 mL volumetric flask and diluted up to mark using distilled water. The mixture was stirred overnight to allow total release of the drug from the microspheres. After filtration through Whatman-41 filter paper (poresize:25µm) the filtrate was assayed spectrophotometrically at 264 nm.

Statistical analysis

Statistical analysis was performed using Origin 7.0 software. All the tests were run in triplicate and the data were analyzed by one-way ANOVA for drug release. Statistical significance was set at $p < 0.05$.

RESULTS

FTIR spectra

The FTIR spectra of plain PEG, PEG-grafted copolymer and hydrolyzed PEG-grafted copolymer are shown in Figure 1. The broad band appearing at 3495.09 cm^{-1} corresponds to the associated –OH stretching vibrations of the hydroxyl group of the grafted copolymer (Fig 1a-A). A new peak appeared at 3071.24 cm^{-1} and the related peak at 1632.35cm^{-1} corresponds to –NH bending vibrations of the primary amides of acryl amide. In the spectra of hydrolyzed PEG-grafted copolymer, the shoulder peak (at 856 cm^{-1}) disappeared but two new peaks appeared around 1330 cm^{-1} and 1091cm^{-1}, respecrively, which are due to the anti-symmetric vibrations of -COOH groups.

The FTIR spectrum of plain chitosan showed two peaks around 836 and 1020 cm^{-1} corresponding to saccharin structure in the spectra (Fig 1b-A). The observed sharp peaks at 1385 and 1650 cm^{-1} are assigned to -CH_3 group (Fig 1b-B). Due to increase in peak intensity at ~1594 cm^{-1}, there are two distinguishing peaks at ~1398 and ~ 1475cm^{-1}. The spectrum of chitosan blend with acrylamide grafted with PEG hydrolyzed complex was observed at around 1638 cm^{-1} and corresponds to –NH_3^+ group due to protonation of the primary amino group in the presence of the carboxylic group of the hydrolyzed polymer (Fig 1b-C). The peak at ~1428 cm^{-1} confirms the presence of carboxylic acid in the polymer.

Swelling of microgels

In plain chitosan microspheres in pH 2 media, % equilibrium swelling of microspheres is higher than the value of it in pH 7.4. Swelling increased from 185-190% in pH 7.4 media compared to swelling in pH 2 media which increased about 196-201%. The % equilibrium swelling for blend microsphere of chitosan and PEG are higher than plain chitosan microspheres, formulations of C-PEG-50 to C-PEG-100. The % equilibrium swelling of C-grafted copolymer 50 to C-grafted copolymer 100 increased to 245-258% in pH 7.4 and 226-239% in pH 2 respectively. The % equilibrium swelling of formulations containing C-grafted copolymer 50 (hydrolyzed) to C-grafted copolymer 100 (hydrolyzed) increased to 273-283% in pH 7.4 and to 261-271% in pH 2.

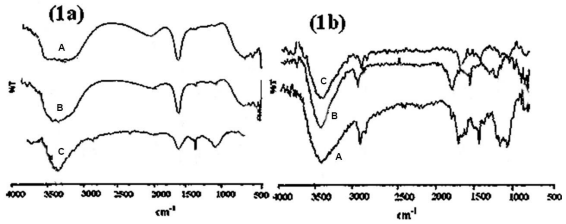

Fig 1: FTIR spectra of hydrolyzed PEG-g–copolymer (1a A), PEG-g–copolymer (1a B), plain PEG ((1a C) pure chitosan (1b A), chitosan/acrylamide grafted with PEG (1b B), and chitosan/acrylamide grafted with PEG after hydrolysis (1b C)

X-ray diffraction (XRD)

XRD aids in assessing changes in the crystallinity of a drug after formulation or processing. As indicated in Fig 2 b, cefixime

showed characteristic intense peaks at 10 and 30 ^{o} due to its crystalline nature. However, these peaks were not seen in the drug-loaded matrix complex, placebo microgels and drug-loaded microgels which indicates that the encapsulated drug became amorphous.

Differential scanning calorimetry (DSC) thermograms

DSC is a useful tool that could assist in determining if any changes have taken place in the drug itself and the drug matrix. Figure 2b-A indicates that the thermogram of the pure drug exhibited an endothermic peak at 202 ^{o}C, corresponding to its melting point, but this peak was absent from the thermogram of the drug-loaded microspheres which suggests that most of the drug was well dispersed within the matrix.

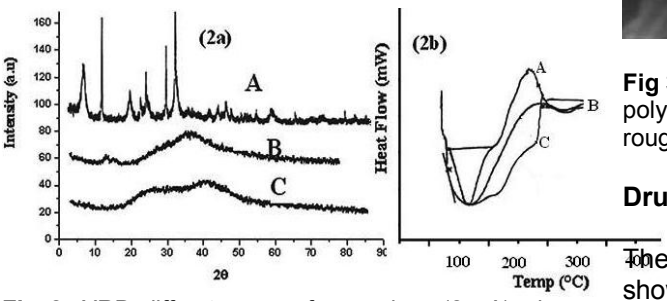

Fig 2: XRD diffractogram of pure drug (2a A), drug-loaded chitosan microspheres (2a B), and blank microspheres (2a C); DSC thermograms of pure drug (2b A), drug-loaded microspheres (2b B), and blank microspheres (2b C)

Particle size

The size of the microsphere was dependent on the amount of drug loaded, extents of drug loading and degree of crosslinking. All the microspheres were spherical with diameter ranging from 56 to 124 µm. The size of the microspheres increased from 103 to 124 µm for 100 % drug-loaded chitosan-grafted copolymer 100 (hydrolyzed).

Drug content and entrapment efficiency

In developing an effective formulation, it is important to achieve high encapsulation efficiency (EE). In the present case, % EE data show a dependence on % drug loading, For instance, formulation containing high amount of drug exhibited higher value of EE and also % EE is higher for chitosan microspheres of blends of hydrolyzed grafted copolymer as compared to other formulations. The effect of cross linking was studied by adding different amount of glutaraldehyde ranging from 2.5 mL to 7.5 mL. It was observed that increasing amount of glutardehyde in the microspheres decreased %EE (Table 1).

Scanning electron micrographs (SEM)

SEM microgram indicates that the microspheres were fairly spherical in shape and that polymeric material surrounded the microspheres (Fig 3A). The surface was, however, rough and rugged (Fig 3B). The type and blend of polymeric used in microsphere formulation did alter surface properties.

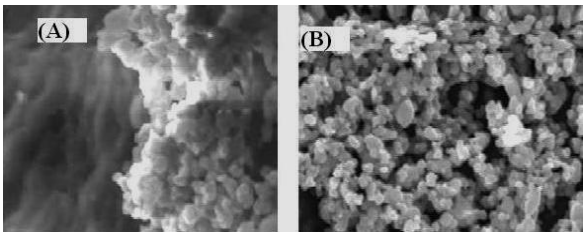

Fig 3: SEM images of microspheres showing (A) polymeric materials around microspheres and (B) roughness of microsphere surface

Drug release

The release data for the various formulations are shown in Figure 4. In both the pH media release was much faster in plain chitosan microspheres. For instance, only 83% drug was released at 11.5 h for C-grafted copolymer 50 in pH 7.4 media compared to 41% drug released in pH 2 media for the same time whereas 80% drug was released at 11.5 h for C-grafted copolymer 50 (hydrolyzed) in pH 7.4 media compared to 38% drug released in pH 2. On subjecting the drug release data to Higuchi model ($Q = Kt^{1/2}$) in order to ascertain the drug release mechanism, a linear relationship was observed with a regression coefficient close to 1 ($r^2 = 0.990$).

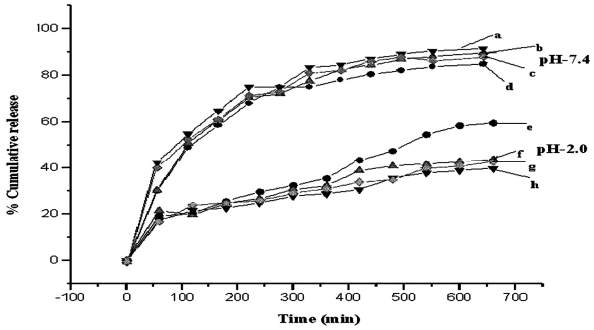

Fig. 4: % Cumulative drug release of microsphere formulations loaded with 50 % drug at pH 2 and 7.4 ***Key:*** For pH 7.4, a = C-50, b = C-PEG-50, c = C-grafted copolymer 50, d = C-grafted copolymer 50 (hydrolyzed); while for pH 2.0, e = C-50, f = C-PEG-50, g = C-grafted copolymer 50, h = C-grafted copolymer 50 (hydrolyzed)

Drug release

The release data for the various formulations are shown in Figure 4. At both pH (2.0 and 7.4), drug release was considerably faster non-crosslinked chitosan microspheres than from crosslinked microspheres, with 89.7 and 53.4 % release in 11.5 h from C-50 at pH 2.0 and 7.4, respectively. On the other hand, 35 % drug was released in 11.5 h for C-grafted copolymer 50 (hydrolyzed) at pH 7.4. On subjecting the release data to the Higuchi model in order to ascertain the release mechanism, a linear relationship was observed with a regression coefficient of 0.990.

DISCUSSION

Chitosan exhibits a pH-sensitive behavior as a weak polybase due to the large number of amino groups on its chain. It dissolves easily at low pH but is insoluble at high pH. [16].The mechanism of pH-sensitive swelling involves protonation of the amine groups in chitosan at low pH conditions. This protonation leads to chain repulation, diffusion of proton and counter ions together with water inside gel and dissociation of secondary interaction. This property helps in the delivery of drugs to the stomach and chitosan has been widely investigated as a delivery matrix. [17,18].

The carboxylic groups of acryl amide moieties are hardly ionized and PEG chains are unable to interact with non-ionized carboxylic groups by hydrogen bonding. As a result, copolymers present a collapsed conformation; on the other hand, as the pH value approaches to the pKa value and above, carboxylic acid ionization leads to the breakage of the hydrogen bonds and the number of ionized units increases dramatically. The ionization of the carboxylic groups results in a repulsive interaction between the PEG chains and acrylic acid molecules at high pH.

In case of the swelling-controlled mechanism when diffusion of a drug is significantly faster than hydrogel distention, swelling is considered to be controlling the release behavior [19]. Micro gels are hydrophilic in nature and swell 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. Almost all chitosan was in its ionized form i.e., -COOH forms COO^- groups which could 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 2.0.

Increase in the amount of glutaraldehyde incorporated in the matrix lowered encapsulation efficience due to reduced free volume space available in the polymeric matrix as the microsphere became more rigid due to higher crosslink density and complexation.

XRD and DSC results indicate that there were no interactions between drug, chitosan and other ingredients used in preparing the microspheres. Thus, it suggests that the prepared microsphere would be stable.

Sustained drug release over a period of 12 h was achieved by crosslinking chitosan with the release data fitting well to the Higuchi model. It suggests that the drug released was controlled by diffusion mechanism. Uptake of water significantly hindered and slowed down drug diffusion due to strong electrostatic interactions between the polymeric matrix and the drug. Chitosan is deprotonated at pH 7.4, leading to disruption of the interaction between drug and polymeric matrix. The carboxylic groups of chitosan become gradually ionized at pH 7.4 which favors microsphere drug diffusion from the microsphere into the surrounding medium [15]. Thus, the collapsed polymeric matrix played an important role in hindering drug diffusion, effectively contributing to sustained release at pH 7.4. This can be attributed to complexation of the amino group of chitosan at this pH, while at pH 2; the complex might have been deformed.

CONCLUSION

The hydrophilic nature of polyacrylamide-modified PEG has been successfully utilized to develop crosslinked chitosan microspheres. The resulting pH-sensitive interpenetrating network of the microsphere matrix facilitated sustained release of cefixime. Further work, including *in vivo* evaluation, is however, required to ascertain the suitability of the procedure used on a large scale.

ACKNOWLEDGEMENT

The authors wish to thank VIT University, Vellore, Tamil Nadu, India for providing the facilities for this research.

REFERENCES

1. *Singh A, Narvi SS, Dutta PK, Pandey ND. External stimuli response on a novel chitosan hydro gel cross linked with formaldehyde. Bull Mater Sci 2006; 29: 233-238.*
2. *Dutta PK, Dutta J, Tripathi VS. Chitin and chitosan properties and application. J Sci Ind India 2004; 63: 20-31.*

3. Shivashankar M, Srujana S, Mandal BK. A Review on Chitosan Based Nanoparticles as a new ocular drug delivery. Pharmacologyonline 2011; 2: 208-221.
4. Shivashankar M, Mandal BK, Yerappagari Y, Preveen V. Chitosan–Based hydro gel for the drug delivery system. Int Res J Pharm 2011; 2: 1-6.
5. Yao RD, Peng T, Goosen MFA, Min JM, He YY. pH-sensitivity of hydro gel based on complex forming chitosan:polyether interpenetrating polymer network. J Appl Polym Sci 1993; 48: 343-354.
6. Elvira C, Mano JF, Roman JS, Reis RL. Starch-based biodegradable hydro gels with potential biomedical applications as drug delivery system. Biomaterials 2002; 23: 1955-1966.
7. Pillai CKS, WilliPual, CS. Chitin and chitosan polymer. Progress in polymer. Science 2009; 34: 641-678.
8. Soppimath KS, Aminabhavi TM. Water transport and drug release study from crosslinked polyacrylamide-grafted-guargum hydrogel microspheres for the controlled release application. European Journal of Pharmaceutics and Biopharmaceutics 2002; 53:87-98.
9. Devi DA, Smitha B, Sridhar S, Aminabhavi TM, Pervaporationseparation of isopropanol/water mixtures through crosslinked chitosan membranes. J Membr Sci 2005; 262: 91-99.
10. Monteiro OAC, Airoldi C. Some studies of crosslinking chitosan-glutaraldehyde interaction in a homogeneous system. Int J Biol Macromol 1999; 26: 119-128.
11. Hoffman AS, Chen G, Wu X, Ding Z, Kabra B, Randeri K, Schiller L, Ron E, Peppas NA, Brazel C. Graft copolymers of PEO-PPO-PEO triblock polyether on bioadhesive polymer backbones,: synthesis and properties. Polymer preprints (American Chemical Society, Division of Polymer Chemistry) 1997; 38: 524-525.
12. Tasker RA, Connell BJ, Ross SJ, Elson CM. Development of an injectable sustained-release formulation of morphine:antinociceptive properties in rats. Lab Anim 1998; 32: 270-275.
13. Ono K, Saito Y, Yura H, Ishikawa K, Kurita A, Akaike T, Ishihara M. Photo-crosslinkable chitosan as a biological adhesive. J Biomed Mat Res 2000; 49: 289-295.
14. Krishna KSV, Naidu BVK, Subha MCS, Sairam M, Aminabhavi TM. Novelchitosan-based pH-sensitive interpenetrating network microgels for the controlled release of cefadroxil. Carbohyd Polymers 2006; 66: 333-344.
15. Li G, Guo L, Chang X, Yang M. Thermo-sensitive Chitosan based semi-IPN hydrogels for high loading and sustained release of anionic drugs. Int J Biol Macromol 2012; 50: 899-904.
16. Narayan B, Jonathan G, Miqin Z. Chitosan-based hydrogels for controlled localized drug delivery. Adv Drug Deliv Rev 2010; 62: 83-99.
17. Risbud MV, Hardikar AA, Bhat SV, Bhonde RR. pH-sensitive freeze-dried chitosan-polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. J Control Release 2000; 68: 23-30.
18. Patel VR, Amiji MM. Preparation and characterization of freeze-dried chitosan-poly(ethylene oxide) hydrogels for site-specific antibiotic delivery in the stomach. Pharm Res 1996; 13: 588-593.
19. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcelluose (HPMC). Adv Drug Deliv Rev 2001; 48: 139-157.