*Available online www.jocpr.com*

**Journal of Chemical and Pharmaceutical Research, 2013, 5(5):140-146**

**Research Article**

**ISSN : 0975-7384**
**CODEN(USA) : JCPRC5**

# Chitosan-acryl amide *grafted* polyethylene glycol interpenetrating polymeric network for controlled release studies of Cefotaxime

**Murugesh Shivashankar[*1] Badal Kumar Mandal[1] and Kaliappan Uma[2]**

[1]*Pharmaceutical chemistry division, School of Advanced Sciences, VIT University, Vellore*
[2]*Applied Analysis Division, School of Advanced Sciences, VIT University, Vellore*

---

**ABSTRACT**

*The aim of this present study was to develop a pH sensitive interpenetrating polymeric network (IPN) hydro gels (HG) based on chitosan-acryl amide-grafted-poly ethylene glycol (CTS-AM-PEG) followed by hydrolysis that are cross-linked with glutaraldehyde (GA). Cefotaxime is an antibiotic drug was successfully loaded in these IPNHG's. The HGs formed were characterized by Fourier transform infrared spectroscopy (FTIR) to confirm the grafting and cross linking response, X-ray diffraction (XRD) and differential scanning calorimetric (DSC) to monitor drug encapsulation in the polymer matrix. HG's gels were evaluated for swelling index, drug content, in–vitro release performed in both acidic and basic conditions. The IPN matrix in this study was able to extend the release of Cefotaxime rate more than 11 hours.*

**Key words**: Chitosan, poly ethylene glycol, micro gels, cross-linking, controlled release, Cefotaxime

---

## INTRODUCTION

Currently biocompatible and biodegradable hydro gels find a wide application in the field of medicine, pharmaceuticals, and tissues engineering [1]. This has promoted scientists and technologists to give more attention to develop an effective, useful and biodegradable hydro gels from a sustainable resource [2]. Hydro gel may be chemically stable and degrade and ultimately disintegrates and dissolve [3]. To avoid this fact a controlled cross linking is needed within the hydrogels, accordingly stimuli responsive of hydro gels which swell depending upon the external pH [4].Many structural factors such as concentration of polymeric matrix, pKa of the ionizable group, degree of ionization, cross linking, density, hydrophillicity, influence the degree of swelling of polymers[5].Recently more efforts have recently been focused on targeted drug delivery system in a specific region for sustain release of drug [6].

In recent years, much attention has been given on the development of IPN from natural biodegradable and biocompatible polymeric materials [7, 8].Among these biodegradable chitosan matrix is the best blend with drug system [9]. Chitosan has recently gained approved in United States and Europe for use in bandages and other hemostics agent [10,11]. Chitosan are biopolymers having immense structural possibilities for chemical and mechanical modification to generate novel properties [12, 13]. Graft copolymerization has also been achieve solubility and grafting of polar monomers on to chitosan has been found to give rise to improved solubility [14, 15, 16, 17]. Besides, chitosan is linear polysaccharide has been found to be good chemical entity for synthesizing hydro gels because of its high cross linking ability due to the presence of amino ($-NH_2$) group[18]. The biodegradable, biocompatibility and other unique property of chitosan have been made to use in pharmaceuticals and medicinal

system. A number of methods have been used for the cross linking of chitosan such as chemical cross linking with glutaraldehyde [19].A good number of approaches to improve wet strength of hydro gels and IPN polymeric network of chitosan have been reported by [20, 19]. Cefotaxime is a third-generation Cephalosporin antibiotic it has broad spectrum against Gram positive and Gram-negative bacteria. Which has a biological half-life of 0.8-1.4 h.It is demonstrated one can enhance the short half-life of Cefotaxime by IPN s prepared in this study.

## EXPERIMENTAL SECTION

### Materials

Cefotaxime (USP grade), Chitosan poly (D-glucosamine) purchased from Sigma –Aldrich products of Belgium, acryl amide were purchased from Qualigens Mumbai, India. Poly ethylene glycol 4000, hydrochloric acid, glutaraldehyde, Glacial acetic acid and sodium hydroxide purchased were purchased from Sd Fine Chemicals. All other chemicals used in this work were analytical reagent grade

### Instruments and technique used

**FTIR analysis:** IR Spectra of was carried out using KBr disc technique using JASCO -4100, Japan to identify the chemical structure of the IPN network. Differential Scanning Calorimetry Furthermore to find out the nature of drug in the polymeric network Differential Scanning Calorimetry (DSC) (Model DSC Q 1000 V9.4 Build 287) study was carried out at a scanning rate of 10 °C/min. Scanning Electron Microscope (SEM) Study: The morphology and surface topography of the prepared network was examined by SEM using model (FEI Quanta FEG 200 - High Resolution Scanning Electron Microscope). X-ray diffraction studies (XRD) Studies help to find the crystallinity of drug in the cross-linked network was performed (Brand: Bucker Germany, CuKά radiation, Nickel filter). The XRD patterns of Cefadroxil loaded Chitosan –PEG Hydro gel blend, Placebo Cefadroxil loaded Chitosan –PEG Hydro gel blend, and XRD pattern of pure drug are compared.

## METHODS

### Preparation of Poly ethylene glycol grafted acryl amide

Poly ethylene glycol grafted acryl amide prepared as per reported method [19] accordingly, known amount of PEG was dissolved in water on heating at 60-65 $^{o}$C and treated with acryl amide under nitrogen atmosphere, to this solution potassium per sulfate was introduced in a small quantity under constant stirring at a temperature 65 $^{o}$C for about 5 hr. The obtained product was precipitated in methanol and then with water-methanol (1:1 v/v). The final product was filtered and kept in a vaccum for drying at 60 $^{o}$C. The resultant product to make acidic, 1 wt% solution of polymer blend was heated in a hot plate at 60 $^{o}$C and stochiometric amount of sodium hydroxide was added and then mixture was stirred with magnetic stirrer at 60 $^{o}$C for 5 hr to complete the hydrolysis of the polymer blend then 2N hydrochloric acid was added with a solution of methanol. The obtained products were precipitated in methanol and washed with a solution of methanol, filter and vacuum dried at 60 $^{o}$C and stored in an airtight desiccators for further use.

### Preparation of drug loaded polymer blend

About 1 g of polymer and required amount of drug were dissolved in 2% acetic acid solution resulting solution were mixed with liquid paraffin added slowly by gentle heating and stirred with a magnetic stirrer on a hot plate. Until a complete dispersion of the drug in the polymer solution was obtained [18]. A portion of drug loaded polymer solution was added drop wise in to a solution of methanol. The obtained IPN network formed were removed from methanol and were repeatedly washed with distilled water to remove any un-reacted materials. In order to know, the drug release characteristic of different formulation with varying drug concentration and chitosan with polymeric blend concentration were prepared and studied (Table 1).

**Table 1 Different formulation with varying drug concentration**

| Formulation Code | Drugloading (%) | Encapsulation efficiency (%) | Mean Particle size* |
|---|---|---|---|
| CS-100 | 100 | 86.9±0.8 | 76±0.5 |
| CS-PEG-100 | 100 | 85.06±0.1 | 103±0.4 |
| CS-g co-polymer 100 | 100 | 89.28±0.4 | 134±0.1 |
| CS-grafted co-polymer100(H) | 100 | 93.01±0.9 | 163±0.4 |

***(*mean± SD)**

**Characterization of drug loaded polymer blend**
The synthesized polymeric blend was evaluated for their physical properties like water content, surface pH and SEM analysis. The formation of grafted blend was confirmed by FTIR spectral analysis, X-ray diffraction (XRD) and differential scanning calorimetric (DSC) to monitor drug encapsulation in the polymer matrix.

**Water content and swelling ratio of the drug loaded polymer blend**
All the prepared IPN network in the form of beads were tested by placing each in 30mL of distilled water and incubated at an appropriate time interval (30 minutes), beads were taken out and excess water was removed from the polymeric blend using filter paper and blend were weighed immediately using Electronic weighing balance of accuracy 0.00001mg (SHIMADZU Japan). The swelling of HG's was expressed as swelling ratio using equation 1,

$S= (W_1-W_o)/W_o$

Where, $W_1$ is the weight of the sample at time t, $W_0$ is the weight of dry sample

**Determination of drug content**
The concentration of drug in all the formulation was determined by UV-visible-NIR Spectrophotometer 670 V (JASCO, USA). Formulation of a sample was weighed accurately and quantatively transferred in to a 100 mL volumetric flask and diluted up to the mark using distilled water. This mixture was stirred for overnight to allow the total release of the drug from the IPN polymeric network. After filtration the filtrate was assayed using spectrophotometer at the wavelength of maximum absorbance at 253nm. Assay was performed in triplicate. The drug content (DC, %) and encapsulation efficiency (EE, %) were calculated in equation 2 and 3

$DC = (Md/Mm)100$ …… (1)

where Md is mass of drug in the microsphere and Mm is the mass of microsphere.

$EE = (D_L/T_L)100$ …… (2)

where $D_L$ is actual drug loading and $T_L$ is theoretical drug loading.

***In vitro* release study**
The *in vitro* drug release studies were performed using (USP paddle single stage digital) apparatuses) 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 periodically and drug was assayed using a spectrophotometer.

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

## RESULTS AND DISCUSSION

**FT-IR Studies for drug –polymer compatibility**
This study was carried out to confirm the grafting of acryl amide as well as hydro gel. In addition, FTIR spectra of alone PEG, PEG-grafted copolymer and PEG-grafted Copolymer (Hydrolyzed) were shown in Figure 1. A broad band appearing at 3435cm$^{-1}$ corresponds to associated –OH stretching vibrations of the hydroxyl group of grafted copolymer. A new peak appeared at 3251cm$^{-1}$ and the related peak at 1630cm$^{-1}$ corresponds to –NH bending vibrations of the primary amides of acryl amide. A relatively high intense peak at 292cm$^{-1}$, 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 1500cm$^{-1}$ and 1450cm$^{-1}$ which are due to antisymmetric vibrations of -COOH groups.

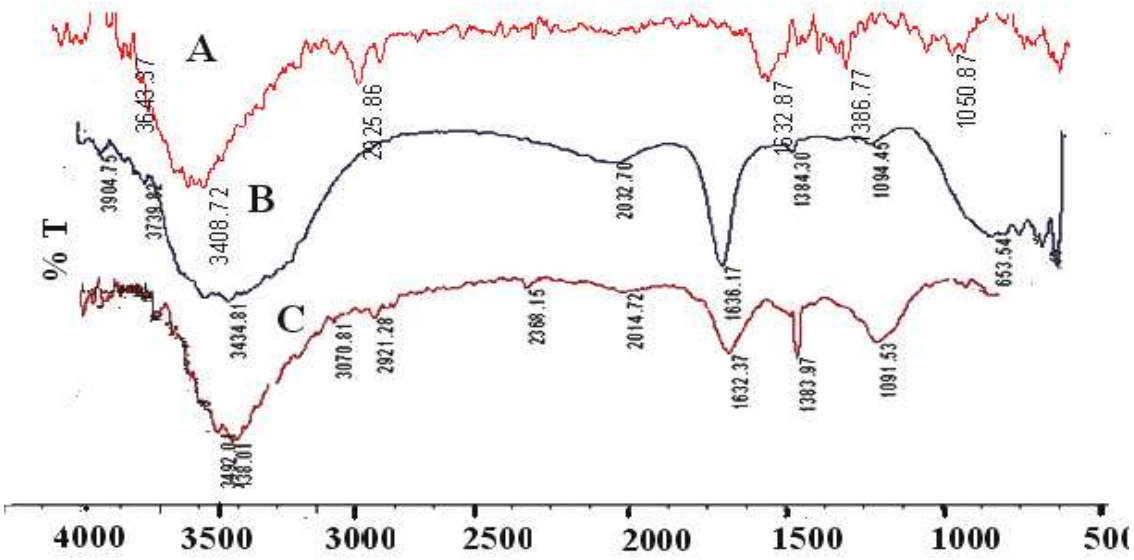


**Figure 1: FTIR spectra a) Hydrolyzed poly ethylene–g-acryl amide (Green) b) Poly ethylene glycol–g-acryl amide (red) c) Poly ethylene glycol (violet).**

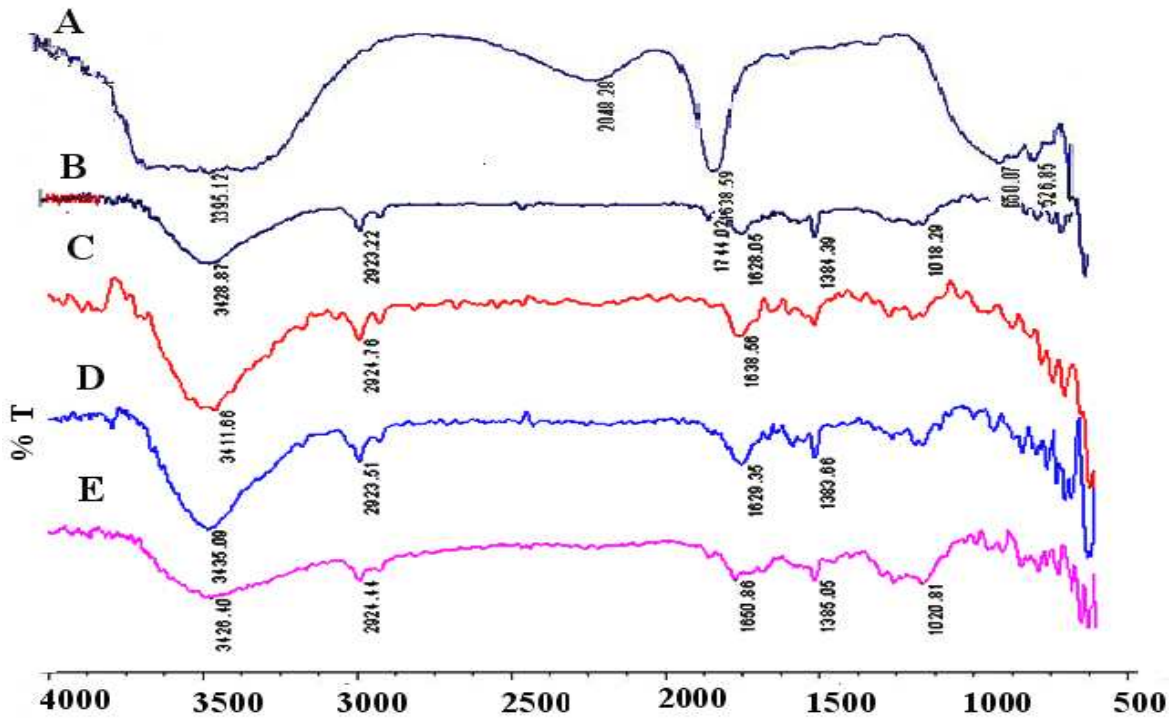


**Figure 2: FTIR spectra of A) chitosan blend with hydrolyzed poly ethylene-g-acryl amide, B) Cross linked chitosan, C) Chitosan blend with poly ethylene, D) Chitosan blend with poly ethylene-g-acryl amide, E) pure chitosan**

The FTIR spectrum of plain chitosan showed two peaks around 901cm$^{-1}$ and 1210cm$^{-1}$ corresponding to saccharine structure (Figure 2). The observed sharp peaks at 1350 cm$^{-1}$ and 1501cm$^{-1}$ are assigned to -$CH_3$ group. A broad band appearing around 1100cm$^{-1}$ indicates the C-O stretching vibration of chitosan. Another band appearing around at

3480cm$^{-1}$ is due to amine N-H symmetric stretching vibration. A new peak appeared at 1600cm$^{-1}$ due to imine bonds (-C-N) as a result of cross linking reaction between amino groups in chitosan and aldehydic group in the glutaraldehyde (Figure 2). In spectra (c), blends of chitosan with poly ethylene glycol, ether linkage observed at 1150cm$^{-1}$, In spectra (d), two distinguishing peaks at 1420cm$^{-1}$ and 1575cm$^{-1}$ In spectra (e), chitosan hydrolyzed complex observed around 1575-1590cm$^{-1}$ due to -$NH_2$.

**XRD studies**

XRD helps to find out the crystallinity of the drug after the encapsulation in the cross linked hydro gel. Cefatoxime has shown characteristics intense peaks at 10$^o$ and 30$^o$ due to the crystalline nature of the drug, however, peaks for the plain drug were not seen for the drug loaded microspheres complex due to the encapsulation of drug in the IPN network, and same applied to the placebo microspheres, thus indicates that encapsulated drug is amorphous in nature are shown in Figure 3.

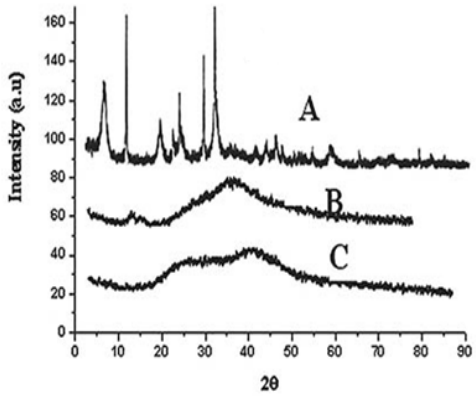


**Figure 3: XRD diffraction of (a) pure drug (b) Drug loaded Chitosan grafted PEG hydro gel Cefatoxime (c) Placebo Chitosan grafted PEG microsphere**

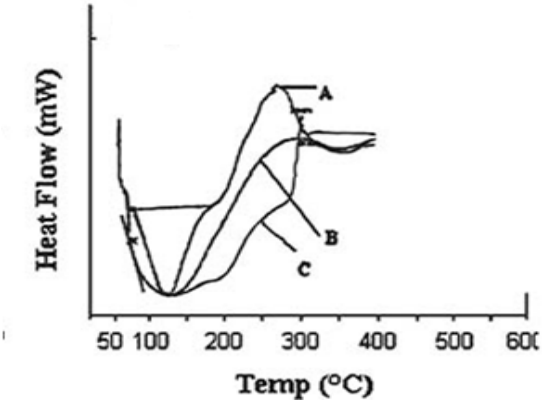


**Figure 4: DSC Curve of A) Cefatoxime, B) Cefatoxime containing hydro gel C) placebo hydro gel.**

**DSC Studies**

DSC thermograms of chitosan, formulated microspheres and pure drug are shown in Figure 4. The thermograms of chitosan showed a broad peak at 109 $^o$C which was attributed to loss of water due to evaporation of absorbed water and no degradation was observed for chitosan in thermograms, which would normally occur at 257 $^o$C Cefatoxime thermograms showed a broad beak at 255 $^o$C which corresponds to its melting point which is usually in the range of 255 – 256 $^o$C. This peak was absent in the thermograms for the drug-loaded chitosan microspheres, however, a peak appeared at 239 $^o$C.

**SEM studies**

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

**Water content and swelling ratio of the drug loaded polymer blend**

Chitosan hydro gel is ionized hydro gel accordingly their swelling nature purely depends on chemical structure and pH of the medium. The amount of cross linking agent added to the polymeric matrix at equilibrium increases in concentration of functional group present in the polymeric net work and decrease in the extent of cross linking observed. The strength and water preservation efficiency of hydro gel is depends on amount of cross linking agent used because the chitosan molecules can be transformed in to polymeric network structure by the addition of cross linking agent so water molecules can be preserved in this net work. If amount of cross linking is less, this will greatly affecting the strength as well water preservation efficiency of hydro gel was low. If excessive amount of cross linking agent was used resulting in the decrease of network volume for water preservation efficiency. The amount of cross linking should be moderate to get highest water preservation efficiency. In this report swelling was performed in triplicate and in all the studies, the standard deviation was found to be 2.88%.

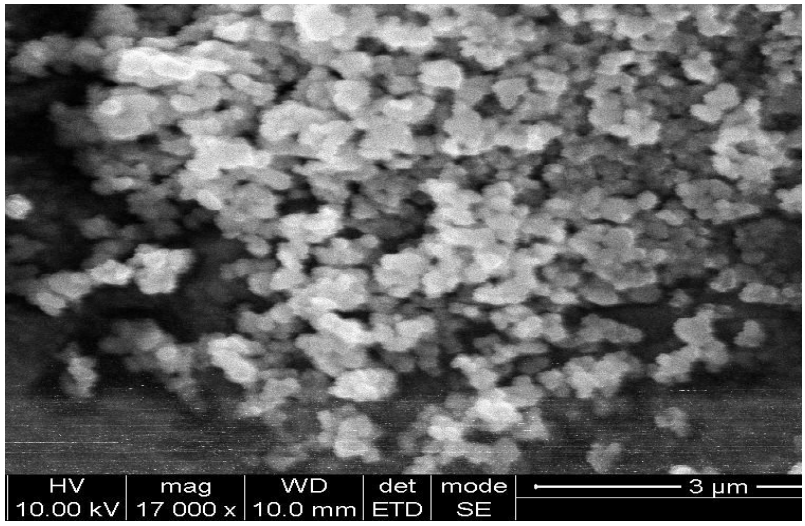


**Figure 5: SEM Picture of drug loaded hydro gel**

**Drug content uninformative**

The drug content uniformity among the different loaded polymeric net work was observed in the range of 86.6% to 93%, The result indicate that the process employed in this work to prepare hydro gel is capable of producing hydro gel with uniform drug contents with minimum variation, Thus the results showed that all the formulation having drug content uniformity within the acceptable range.

***In Vitro drug release***

Cefatoxime 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 about 40% within the same time period (Figure 6). 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.

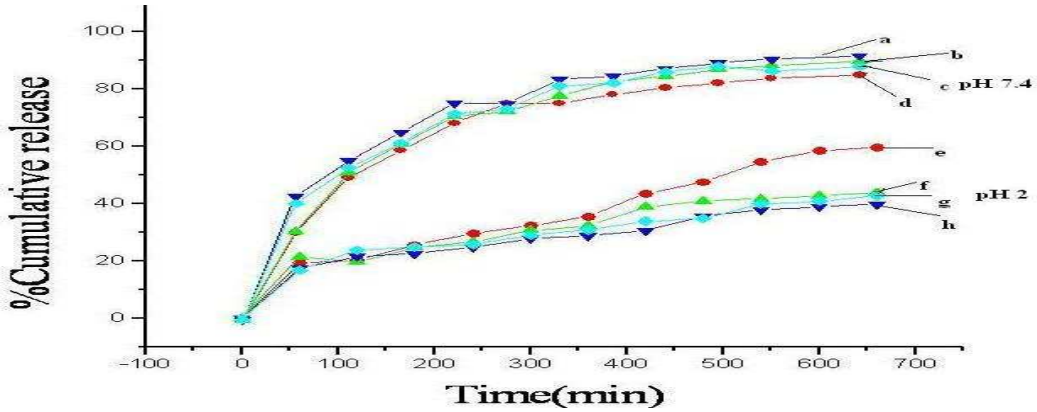


**Figure 6: In vitro drug release profile of % cumulative drug release Vs time for different drug formulation at pH 7.4 and pH 2.0.**

## CONCLUSION

The hydrophilic nature of polyacrylamide modified poly ethylene glycol was used to synthesis the micro gels blended with chitosan. The blend micro gels showed favorable controlled releases i.e., release rate was more than 11 h. This blended micro gels could be used for controlled release of Cefatoxime.further research works warrant its practical applications in vivo.

**Acknowledgements**
Authors great full to VIT University Vellore, Tamil nadu, India for providing facility to carry out this research

**REFERENCES**

[1] KN Plunkett; KL Berkowski; JS Moor, *Biomacromolecules*, **2005**, 6(2), 632-637.
[2] Y Guan; L Shao; J Liu; KD Yao, *J.Appl.Polym.Sci*., **1996**, 61, 23-25.
[3] AS Hoffman, *Adv.Drug Delivery Rev.*, **2002**, *54*(17.1), 3-12.
[4] Y Qui; K Park, *Adv.Drug Delivery Rev.*, **2001**, 53, 321-329.
[5] AA Askadskii, *Polym.Sci*., **1990**, 32(10), 2061-2069.
[6] C Elvira; JF Mano; JS Roman; RL Reis, *Biomaterials*, **2002**, 23, 1955-1966.
[7] KSV Krishna Rao; K Madhusudana Rao; PV Nagendra Kumar; II-Doo Chung, **2010**,*Iranian Polymer Journal*, 2010, 19(4), 265-276.
[8] GK Buddy; JK Sara; H Jose; SG Fermin; SJ Andrew, *Journal of emergency medicine*, **2008**, 15(1), 74-81.
[9] CKS Pillai; WilliPual; S Chandra,*Polymer Science*, **2009**, 34, 641-678.
[10] MJ Zohuriaan-Mehr, *Iran polym J*, **2005**, 14, 235-258.
[11] N Gorochovceva; R Makusuka, *Eur Polym J*, **2004**, 40,685-691.
[12] R Jayakumar; M Prabaharan; Raiser; JF Mans, *Carbohydrate polym*, **2005**, 62,142-215.
[13] DW Jenkins; SM Hudson, *Chem.Rev.*, **2001**, 101(11), 3245-3273.
[14] MNV Ravi Kumar,*Poly*, **2000**, 46, 1-7.
[15] A Singh; SS Narvi; PK Dutta; ND Pandey, *Bull.Mater.Sci.*, **2006**, 29,233-238.
[16] KS Krishna Rao; B VijayaKumarNaidu; MCS Subha; M Sairam; TM Aminabhavi, 2006, *Carbohydrate Polymer*, **2006**, 66,333-344.
[17] SG Kumber; AR Kulkarni; KS Soppimath; TM Aminabahvi; WE Rudzinski, *Eur.J. Pharmaceut. Biopharmaceut.*,**2001**, 51,127-133.
[18] Aysu Yurdasiper; Ferhan Sevgi, *J.Chem.Pharm.Res*., **2010**, 2(3),704-721.
[19] Y Vimalkumara; AB Gupta; Rajkumar; SY Jaideep; Brajeshkumar, *J.Chem.Pharm.Res.*,**2010**,2(5),418-432.
[20]TADervan; U Riaz;BS Kumar;Md.Shohel Rana, *J.Chem.Pharm.Res*.,**2012**,4(3),1573-1579.