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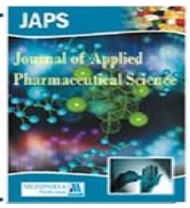

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Chitosan/guargum-g-acrylamide semi IPN microspheres for controlled release studies of 5-Fluorouracil

E. Chandra Sekhar, K.S.V. Krishna Rao and R. Ramesh Raju

E. Chandra Sekhar and R. Ramesh Raju
Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P. India.

K.S.V. Krishna Rao
Department of Chemistry, Yogivemana University, Kadapa, A.P., India.

For Correspondence
R. Ramesh Raju
Email: *rrraju1@gmail.com*
Phone: 0863-2346578,
Fax : 0863-2293378.

ABSTRACT

Chitosan and guargum-gt-acrylamide (CH-GG-g-AAm) semi interpenetrating microspheres (semi IPNMs) were prepared by water-in-oil (w/o) emulsion cross linking method using glutaraldehyde as a crosslinker. 5-fluorouracil (5-FU) is an anticancer drug was successfully loaded in these semi IPNMs. X-ray diffraction (XRD) and differential scanning calorimetric (DSC) examined the crystalline nature of drug after encapsulation into semi IPNMs. Scanning electron microscopy (SEM) shows the formation of semi IPNMs is spherical with size around 200 □m. The encapsulation efficiency of 5-FU was achieved 58%. *In-vitro* release studies were performed basic (pH 7.4) buffer medium. The release patterns depend on graft polymer composition, effect of cross linker and drug content in the polymer matrices. *In vitro* release studies indicated the release of 5-FU more than 12 hours.

Key words: Semi IPNMs, Chitosan, Graft co-Polymer, Anticancer Drug, In-vitro release studies.

INTRODUCTION

Carbohydrate polymers based Interpenetrating networks (IPN's) are extensively used in pharmaceutical applications (Rao et al., 2006). Based on arrangement patterns of polymeric chains the IPN's are classified into novel IPN's, simultaneous IPN's, sequential IPN's and polymeric semi IPN's. Among them the semi IPN is blending of two polymers where only one polymer is cross linked in presence of another to produce a mixture of fine morphology. Semi IPN's of natural and synthetic polymers has been found to be useful in enhancing the release of short half-lived drugs under physiological conditions. In order to achieve this, the properties of natural and synthetic polymers have been modified by grafting, blending and other means (Rokhade et al., 2006; Rokhade et al., 2007; Isiklan, 2006). Grafting of vinyl monomers on to natural polymers such as chitosan has been widely accepted. The semi IPN's of chitosan grafted acryl monomers blending with natural polymers are important for controlled release of drugs due to it exhibits swelling change in response to external stimuli such as pH and temperature. Chitosan is a polymer of natural origin, which is composed of repeating units of N-acetylglucosamine and D-glucosamine, being obtained from the deacetylation of chitin, the main component of the exoskeleton of crustaceans (Kurita, 2006; Muzzarelli, 1985; Singh and Ray, 2000). This polysaccharide possesses structural characteristics similar to those displayed by glycosaminoglycans (GAGs), which are an important component of connective tissues and owing to that feature, chitosan has been investigated for a range of biomedical applications, such as wound healing, tissue engineering, dentistry, and orthopaedics (Singh and Ray, 2000). This polymer presents well-documented favourable biological properties such as biocompatibility,

and low toxicity, (Dornish et al., 1997; Hirano et al., 1988) and it also displays mucoadhesive properties, (Lehr et al., 1992) rendering this molecule very attractive for drug delivery applications. In general native polysaccharides may not be suitable in controlled delivery systems due to their substantial swelling and rapid enzymatic degradation in biological fluids. However mechanical strength of Chitosan is poor and it is therefore necessasery to modify the Chitosan to improve its mechanical properties and chemical stability, etc. So graft copolymerization of acrylic monomers on to polysaccharide backbone exhibits hydrophobicity and steric bulkiness, which considerably protects the matrix and carbohydrate backbone to retard the drug release.

Natural gums are biodegradable and nontoxic, which hydrate and swell on contact with aqueous media, and these have been used for the preparation of dosage form (Nakano and Ogata, 2006). Guar gum is galactomannan, obtained from the ground endosperm of the guar plant, Cyamopsis tetragonolobus. It has been investigated as controlled release carrier and regarded as nontoxic and non-irritant material (Gohel et al., 1999; Krishnaiah et al., 2002; Patra et al., 2004).

In the present study to improve mechanical properties and chemical stability guar gum grafted poly acrylamide can be added to chitosan. 5-Fluorouracil is an antimetabolic drug, used extensively in cancer chemotherapy (Matsuyama et al., 1997; Heidelberger, 1982; Waxman et al., 1982; Sommadossi et al., 1982; Einmahl et al., 1999; Fournier et al., 2004) and is an antimetabolite, which is used to prevent the subsequent scarring following trabeculectomy and to improve the prognosis for long-term retinal reattachment. 5-Fluorouracil is an acidic, water soluble (Ermis and Yuksel, 1999) hydrophilic drug and is an antineoplastic agent of extensive use in clinical chemotherapy for the treatment of solid tumours. It has been widely used in drug administration due to its large number of secondary effects that accompany its conventional administration. 5-FU was successfully loaded into semi IPN microspheres composed chitosan/GG-g-AAm. The resulting microspheres are capable of being to control the release of 5-FU more than 12 hours.

EXPERIMENTAL

Materials and methods

Chitosan with 300kDa molecular weight and a deacetylation percentage of ~ 95% was supplied by Primex Enterprises Company (Iceland) and light liquid paraffin oil was purchased from S.D. fine chemicals, Mumbai, India. Guar gum (S.D. Fine Chem. Mumbai, India) was purified by repeated washings with methanol and dried. Cericammonium nitrate (CAN), Glutarldehyde (GA, 25% w/v) solution and Methanol were AR grade samples (SD Fine Chemicals). 5-FU was purchased from Himedia, Mumbai, India.

Synthesis of G.G-g-AAm

Guar gum grafted acrylamide was prepared by 2 gms of GG was weighed and dissolved in water by stirring overnight. To these solution 0.105 mol acrylamide and 5.47 X 10^{-4}ceric ammonium nitrate were added and stirred well. This reaction mixture is polymerized under nitrogen atmosphere for 6 h at 70^{o}C. This polymerized polymer was cooled and extracted by precipitating the polymer in acetone and precipitated polymer was dried under vacuum for 24 h.

Preparation of semi IPN micro spheres

Guar gum-g-AAm and Chitosan Semi interpenetrating network (semi IPN) microspheres have been prepared a different weight ratio of chitosan and GG-g-AAm was dissolved in the water of certain concentration and left overnight. The two polymer solutions were mixed and stirred well for proper mixing which lead to miscible polymer solution. A known amount of the 5-fluorouracil was dissolved above polymer solution. The drug loaded blend polymer solution was emulsified into liquid paraffin to form water-in-oil (w/o) emulsion technique (Kurkuri and Aminabhavi, 2004) at 400 rpm using REMI Motors (vasai, India) high-speed stirrer for 30 min in a separate 500 mL beaker containing 100 mL of light liquid paraffin oil, 2 % (w/v) of Tween-80, 1 mL of 0.1 M HCl and the required amount of GA is added. The microspheres formed were filtered, washed repeatedly with hexane and water to remove the oil as well as excess amount of surfactant and the unreacted GA. These microspheres were dried under vacuum at 40^{0}C and stored in desiccators before further analysis. The microspheres were characterized by Differential scanning calorimetry (DSC), X-ray diffractometry (X-RD). The release of 5-FU from these microspheres was studied in 7.4 pH media. Microspheres network consists of chitosan blended with graft polymer of GG-g-AAm. In this system chitosan swells more compared to GG-g-AAm and this is attributed to the diffuse in controlled release of the drug through the surface of microspheres.

Swelling studies

Dynamic swelling of GG-g-AAm blended with chitosan microspheres were prepared using three different concentrations of cross-linker as well as three different drug loadings were studied in water by mass uptake measurements with time. Swelling experiments performed in 7.4 pH buffer solution. To perform swelling experiments, microspheres were soaked in buffer solution 7.4 pH, several of them were removed from the swelling bottles at different time intervals and blotted carefully with tissue papers (without pressing hard) to remove the surface-adhered buffer solution. The microspheres were then weighed (w_1) on an electronic microbalance (ADAM AFP-210L England accurate to ± 0.0001 g). The microspheres were then dried to a constant weight (w_2) in an oven maintained at 40^{0}C for 5 hours. Swelling experiments were repeated thrice for each sample and average values were used in data analysis. The standard deviations (S.D.) in all cases were < 5 %. The weight % water uptake was calculated as:

$$\% \text{ Water uptake} = \left(\frac{\text{Wt of swollen } \textit{Microspheres } (w_1) - \text{Wt of dry } \textit{Microspheres } (w_2)}{\text{Wt of dry } \textit{Microspheres } (w_2)} \right) \times 100 \quad (1)$$

Estimation of drug loading and Encapsulation Efficiency

The drug loaded microspheres (10 mg) were pulverized and incubated in 10 ml of 0.02M phosphate buffer (pH = 7.4) at room temperature for 24 h. The suspension was agitated with agitate mortar and filtered through filter paper. The drug solution was assayed spectrophotometrically for 5-FU content at the wavelength of 270nm. The results of % of drug loading and encapsulation efficiency were calculated using following equations.

$$\% \text{ Drug loading} = \left(\frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}} \right) \text{x} 100 \quad \text{--- (2)}$$

$$\% \text{ Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}} \right) \text{x} 100 \quad \text{--- (3)}$$

In-vitro Release study

Dissolution was carried out using Tablet dissolution tester (Lab India, Mumbai, India) equipped with eight baskets. Dissolution rates were measured at 37± 0.5°C at constant speed of 100 rpm. Drug release from the microspheres was studied in 7.4 pH phosphate buffer solutions. At regular intervals of time, sample aliquots were withdrawn and analyzed using UV spectrophotometer (Lab India, Mumbai, India) at the fixed λ_{max} value of 270 nm. After each sample collection, the same amount of fresh release medium at the same temperature was added to the release medium to maintain the sink condition. All measurements were carried out in triplicate, and values were plotted with standard deviation errors.

CHARACTE RIZATION TECHNIQUES

Differential Scanning Calorimetric (DSC) studies

Differential scanning calorimetric (DSC) curves were recorded on a TA instruments (Model: STA, Q_{600} USA). The sample was weighed between 10 to 12mg. The samples were heated from 50° to 400° C at heating rate of 10° C/min in nitrogen atmosphere (flow rate 100 mL/min).

X-Ray Diffractions (X-RD) studies

X-RD measurement of plain drug, drug-loaded microspheres and plain microspheres were recorded using a Rigaku Geiger flex Diffractometry (Tokyo, Japan) equipped with Ni-filtered Cu Kα radiation (λ=1.548A^0). The dried microspheres of uniform thickness were mounted on sample holder, and the patterns were recorded in the range 0 to 500 at the speed of 50/min.

Particle size and scanning electron microscopic (SEM) studies

To determine the particle size and size distribution, ~ 100 - 200 microspheres were taken on a glass slide and their sizes were measured using an optical microscope under regular polarized light. SEM micrographs of microspheres were obtained under high resolution (Mag 300X5kv) Using JOEL MODEL JSM 840A, scanning electron microscope (SEM), equipped with phoenix energy dispersive analysis of X-ray (EDAX).

RESULTS AND DISCUSSIONS

Differential Scanning Calorimetric study

Differential scanning calorimetric (DSC) curves for 5-FU loaded microspheres and pure 5-FU drug are shown in Figure 1. The drug 5-FU, exhibit sharp peak at 287° C due to polymorphism and melting. However, this peak is not appeared in the curve of 5-FU loaded microspheres, suggesting that most of the drug was uniformly dispersed in polymer matrices at molecular level.

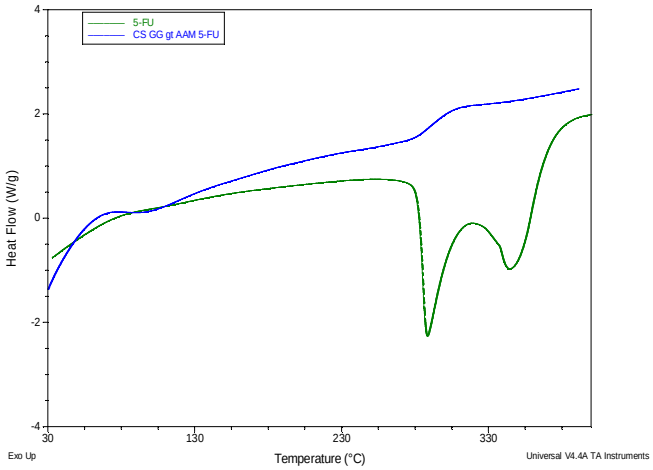
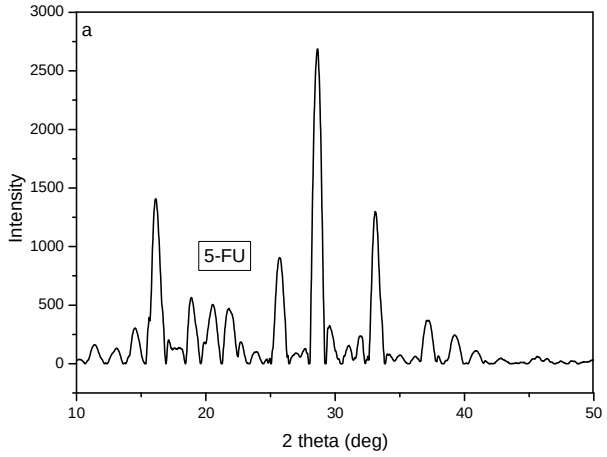


Fig 1: DSC curve of 5-FU and 5-FU loaded semi IPN 1 microspheres.

X-Ray Diffraction (X-RD) studies

X-RD study helps to find the crystallinity of drug in the IPNMs. X-ray diffraction analysis of pure 5-FU, plain semi IPN microspheres, and 5-FU loaded semi IPN microspheres are shown in Figure 2. The most intensive peaks of 5-FU are observed at 2θ of 17°, 29°, and 32° suggesting its crystalline nature. But, these peaks are not found in drug loaded semi IPN, indicating that the drug is dispersed at molecular level in the polymer matrix.



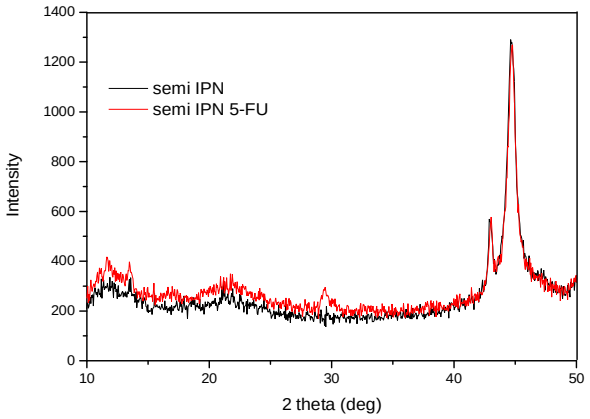


Fig 2: XRD curves of 5-FU, semi IPN 1 and 5-FU loaded semi IPN 1.

Particle Size and Scanning Electron Microscopic (SEM) studies

The results of particle size of microspheres were in the range 100-180 µm. The variations of particle size with GA content and polymer compositions are shown in table 1. As GA content increases the average size of microspheres decreases. This was due to the increased resistance to the water diffusing out from the microspheres during the microsphere formation. A similar observation was reported by Kulkarni et al. from their drug delivery studies (Anita et al., 2010). The % of polymer composition also affected the size of microspheres. As % of graft polymer increases the average size of microspheres increased. This may be due to higher viscosity of the internal phase, which might have rendered higher resistance to the shearing of emulsion, thereby increasing the microspheres size.

The purpose of SEM study is to obtain a topographical characterization of microspheres figure 3 shows the SEM micrographs of GG-g-AAm and chitosan semi IPN microspheres. The microspheres formed have been spherical shape with smooth surface.

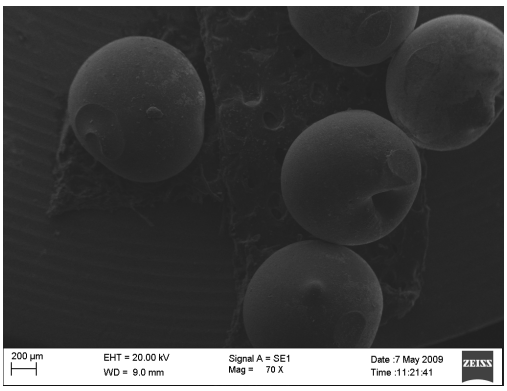


Fig 3: Scanning electron micrograph of CS/GG-g-AAm microspheres.

Encapsulation Efficiency

Effects of GA and graft copolymer content on encapsulation efficiency of drug loaded microspheres are given in table 1. The encapsulation efficiency of 5-FU increases with increasing amount of graft copolymer. This can be attributed to the fact that at higher concentrations, GG-g-poly (AAm) viscosities leading to a less diffuse matrix structure that hinder drug escape from the microspheres during the microsphere formation. GA also effects the encapsulation efficiency of 5-FU. The increasing content of GA for the formation of microspheres decrease trend in encapsulation efficiency was observed. This is due to increase in cross linking density the microspheres will become more rigid thereby reducing the free volume spaces within the polymer matrix.

Swelling studies

Dynamic swelling of the GG-g-AAm microspheres were prepared by using three different concentration of crosslinker as well as three different drug loadings was studied in water by mass uptake measurements with time. Swelling experiments performed in 7.4 pH buffer solutions produced no significant changes and hence, we studied the swelling of microspheres in water (Gallaher et al., 2000). To perform swelling experiments, microspheres were soaked in water; several of them were removed from the swelling bottles at different time intervals and blotted carefully with tissue paper (without pressing hard) to remove the surface-adhered water. The microspheres were then weighed (w_1) on an electronic microbalance (Adam AFP-120L, England accurate to ± 0.0001g). The microspheres were then dried to a constant weight (w_2) in an oven maintained at 40^0C for 5 hours. Swelling experiments were repeated thrice for each sample and average values were used in data analysis. The standard deviations (S.D.) in all cases were < 5 %. The weight % of water uptake was calculated as **Eq:** 1. Drug release rates are influenced by the equilibrium water uptake of the cross linked microspheres (Ritger & Peppas, 1987). The % equilibrium water uptake data of the cross liked microspheres presented in **Table – 1,** indicate that, as the amount of crosslinker (GA) in the polymer matrices increase from 2.5 to 7.5 mL, equilibrium water uptake decreases significantly from 495, 421 & 343 (semi IPN 4, semi IPN 1 & semi IPN 5) respectively. The reduction in water uptake may be due to the formation of a rigid network structure at higher extent of crosslinking. It is also noted that formulations containing higher amount of GG-g-AAm (semi IPN 3) showed higher swelling rates than those formulation containing no amount of GG-g-AAm (semi IPN 0). This is attributed to the extremely hydrophilic nature of GG-g-AAm/CS polymer matrix, leading to higher water uptake.

In-vitro release studies

Drug release kinetics was analyzed by plotting cumulative release data vs time and by fitting these data to the exponential equation of the type (Ritger and Peppas, 1987).

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \quad \ldots.(4)$$

Here, M_t/M_∞ represents the fractional drug released at time t, k is a constant characteristic of the drug-polymer system, and n is an empirical parameter characterizing the release

mechanism. Using the least squares procedure, we have estimated the values of *n* and *k* for all the seven formulations at 37 ^{0}C and these values are given in **Table 1**. If the value of $n = 0.5$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion. If $n > 0.5$, anomalous or non-Fickian type drug diffusion occurs. If $n = 1$, a completely non-Fickian or more commonly called **case II** release kinetics is operative. The intermediary values ranging between 0.5-1.0 are attributed to the anomalous type transport

The values of *k* and *n* have shown a dependence on the extent of crosslinking, % drug loading and GG-g-AAm content of the matrix. Values of *n* for microspheres prepared by varying the amount of GG-g-AAm in the polymer microspheres of 10, 20 30 and 40 % by keeping 5-FU (20 %) and GA (5 mL GA) constant, ranged from 0.278 to 0.727 leading to a shift of transport from Fickian to anomalous type. The 5-FU loaded particles have the *n* values ranging from 0.278 to 0.513 **Table 2**, indicating the shift from erosion type release to a swelling-controlled, non-Fickian mechanism. This could be possibly due to a reduction in the regions of low microviscosity and closure of microcavities in the swollen state. Similar findings have been observed elsewhere, wherein the effect of different polymer ratios on dissolution kinetics was studied. On the other hand, the values of *k* are quite smaller for the drug-loaded microspheres, suggesting their lesser interactions compared to microspheres containing varying amount of chitosan.

Effect of Cross linker

The % cumulative release data vs time plots for varying amounts of GA i.e. 2.5, 5.0 and 7.5 mL at the fixed amount of the drug (20 %) are displayed in figure 4. The % of cumulative release is quite fast and large at the lower amount of GA (i.e., 2.5 mL), whereas the release is quite slower at higher amount of GA (i.e., 7.5 mL). The cumulative release is somewhat smaller when lower amount of GA was used probably because at higher concentration of GA, polymeric chains become rigid due to the contraction of micro voids, thus decreasing % of cumulative release of 5-FU through the polymeric matrices. As expected, the release becomes slower at higher amount of GA, but becomes faster at lower amount of GA.

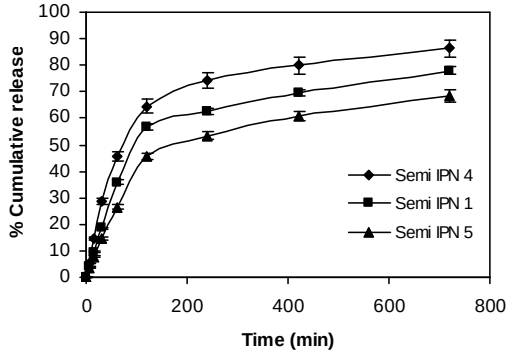


Fig 4: Cumulative % release of 5-FU through CS/GG-g-AAm microspheres containing different amount of crosslinking agent. (Semi IPN 1) 5mL (semi IPN 4) 2.5mL (semi IPN 5) 7.5mL

Effect of Drug

Figure 5 shows the release profiles of 5-FU loaded microspheres at different amount of drug loading. Release data showed that formulations containing the highest amount of drug (30 %) displayed fast and higher release rates than those formulations containing a small amount of 5-FU. A prolonged release was observed for the formulation containing lower amount of drug. In other words, with a decreasing amount of drug in the matrix, it is noticed that the release rate becomes quite slower at the lower amount of drug in the matrix, and this is due to the availability of more free void spaces through which lesser number of drug molecules will transport.

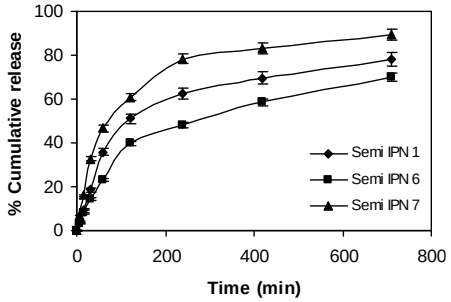


Fig 5: Cumulative % release of 5-FU through CS/GG-g-AAm microspheres containing different amount of drug (semi IPN 1) 20 wt. % drug (semi IPN 6) 100 wt. % drug (semi IPN 7) 30 wt. % drugs.

Effect of Polymers Ratio

Figure 6 shows the *in vitro* release data of 5-FU from the microsphere particles performed with different ratio of GG-g-AAm in the polymeric matrices. The data shows that higher amount of GG-g-AAm containing particles having more encapsulation efficiency and the release studies shown that higher amount of GG-g-AAm containing particles have shown prolonged release characteristics than the microspheres containing lower amount of GG-g-AAm. Generally, the drug release pattern depends on many factors like particle size, crystallanity, surface character, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity and the rate of hydration of the polymeric materials, etc (Ratner et al., 1997). In the release behavior of polymeric system we can consider the binding affinity of drug and polymer swelling property of GG-g-AAm.

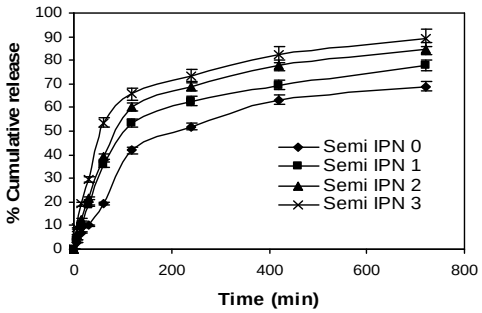


Fig 6: Cumulative % release of 5-FU CS/GG-g-AAm microspheres containing different amount of GG-g-AAm (semi IPN 0) 0 % GG-g-AAm, (semi IPN 1) 20 wt. % GG-g-AAm, (semi IPN 2) 30 wt. % GG-g-AAm and (semi IPN 3) 40 wt. % GG-g-AAm.

Table 1. Results of % of encapsulation efficiency, mean particle size and water uptake of different formulations.

Formulation code	CS/GG-g-AAm ratio	GA (mL)	5-FU (mg)	% Encapsulation efficiency ± S.D.	Mean particle size (μm) ± S.D.	% Water uptake
Semi IPN 0	100:0	5	20	48.5±1.2	146±6	395
Semi IPN 1	80:20	5	20	49.3 ± 1.1	158 ± 5	421
Semi IPN 2	70 :30	5	20	51.6 ± 0.8	160 ± 7	420
Semi IPN 3	60 :40	5	20	53.8 ± 1.2	185 ± 9	464
Semi IPN 4	80 :20	2.5	20	48.2 ± 0.8	168 ± 5	495
Semi IPN 5	80:20	7.5	20	46.5 ± 0.9	112 ± 8	343
Semi IPN 6	80:20	5	10	52.4 ± 1.1	156 ± 6	458
Semi IPN 7	80:20	5	30	56.9 ± 1.5	155 ± 4	486

Table 2. Release kinetics parameters of different formulations

Formulation code	*k*	*n*	Correlation coefficient, *r*
Semi IPN 0	0.0628	0.369	0.9751
Semi IPN 1	0.0839	0.478	0.9873
Semi IPN 2	0.0115	0.680	0.9303
Semi IPN 3	0.0142	0.540	0.9816
Semi IPN 4	0.0032	0. 265	0.970
Semi IPN 5	0.0183	0.727	0.9712
Semi IPN 6	0.0184	0.278	0.9418
Semi IPN 7	0.0137	0.513	0.9642

CONCLUSIONS

Carbohydrate polymeric grafted microspheres of GG-g-AAm and blended with chitosan were prepared and characterized by differential scanning calorimetry, scanning electron microscopy and particle size distribution. DSC thermograms have confirmed the uniform molecular distribution of the drug molecules in the microspheres. SEM micrographs exhibited a spherical morphology of the prepared microspheres. The drug was release in a controlled manner. The swelling studies of microspheres have shown that with an increasing amount of GG-g-AAm in the microspheres, water uptake has increased. This effect is correlated with the release rates of the drug though the microspheres containing different amount of GG-g-AAm. The microspheres have lower densities and hence, these could be retained in the gastric environment for more than 10 hrs which would help to improve the bioavailability of 5-FU.

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