CHAPTER III

Chitosan/Guar Gum-g-poly (Acrylamide) Semi IPN Microspheres for Controlled Release Studies of 5-Fluorouracil.
Carbohydrate polymers based interpenetrating networks (IPN’s) are extensively used in pharmaceutical applications [1]. 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 [2-4]. Grafting of vinyl monomers on to natural polymers such as chitosan has been widely accepted. The semi IPN’s of chitosan grafted acrylic 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 (CS) is a polymer of natural origin, which is composed of repeating units of N-acetyl glucosamine and D-glucosamine, being obtained from the deacetylation of chitin, the main component of the exoskeleton of crustaceans [5-7]. 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 [7]. This polymer presents well-documented favourable biological properties such as biocompatibility, biodegradability, and low toxicity, [8-9] and it also displays mucoadhesive properties, [10] 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 [11]. 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 [12-14].

In the present study to improve mechanical properties and chemical stability guar gum grafted poly acrylamide can be added to chitosan. 5-Fluorouracil (5-FU) is an antimetabolic drug, used extensively in cancer chemotherapy [14-20] 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 [21]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 CS/GG-g-PAm. The resulting microspheres are capable of being to control the release of 5-FU more than 12 hours.

Chitosan (CS) and guar gum-g-poly(acrylamide) (CS-GG-g-PAm) 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 µm. 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.

III.2.1. EXPERIMENTAL

The details of the materials and the experimental procedures adopted in the present study have been explained in chapter II under experimental section.
III.2.2. The details of experimental procedures adopted in the present study have been explained in chapter II.3.A.1. Under experimental section.

III.3. Results and Discussions

III.3.1 Differential Scanning Calorimetric study

Differential scanning calorimetric (DSC) curves for 5-FU loaded microspheres and pure 5-FU drug are shown in Figure III.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.

![DSC curve of 5-FU and 5-FU loaded semi IPN 1 microspheres](image)

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

III.3.2 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 III.2. The most intensive peaks of 5-FU are observed at 20 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.

![Figure III.2: XRD curves of 5-FU, semi IPN 1 and 5-FU loaded semi IPN 1](image)

**III.3.3 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 III.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 [23]. 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 III.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 III.3: Scanning electron micrograph of CS/GG-g-PAm microspheres.](image)

III.3.4 Encapsulation Efficiency

Effects of GA and graft copolymer content on encapsulation efficiency of drug loaded microspheres are given in table III.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-PAm 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.

III.3.5. 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 [24]. 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 (w₁) on an electronic microbalance (Adam AFP-120L, England accurate to ± 0.0001g). The microspheres were then dried to a constant weight (w₂) in an oven maintained at 40 °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 % of water uptake was calculated as Eqs: II.5.Drug release rates are influenced by the equilibrium water uptake of the cross linked microspheres (25). The % equilibrium water uptake data of the cross liked microspheres presented in Table III.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&amp;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-PAm (semi IPN 3) showed higher swelling rates than those formulation containing no amount of GG-g-PAm (semi IPN 0). This is attributed to the extremely hydrophilic nature of CS/GG-g-PAm polymer matrix, leading to higher water uptake.
III.3.6 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 [25].

\[
\left( \frac{M_t}{M_\infty} \right) = k t^n \quad \text{Eq. III.1}
\]

Here, \(M_t/M_\infty\) 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°C and these values are given in Table III.2. 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-PAm content of the matrix. Values of \(n\) for microspheres prepared by varying the amount of GG-g-PAm 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 III.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 micro viscosity and closure of micro cavities 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.
Table III.1: Results of % of encapsulation efficiency, mean particle size and water uptake of different formulations.

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

Table III.2: Release kinetics parameters of different formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>k</th>
<th>n</th>
<th>r</th>
</tr>
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<tbody>
<tr>
<td>Semi IPN 0</td>
<td>0.0628</td>
<td>0.369</td>
<td>0.9751</td>
</tr>
<tr>
<td>Semi IPN 1</td>
<td>0.0839</td>
<td>0.478</td>
<td>0.9873</td>
</tr>
<tr>
<td>Semi IPN 2</td>
<td>0.0115</td>
<td>0.680</td>
<td>0.9303</td>
</tr>
<tr>
<td>Semi IPN 3</td>
<td>0.0142</td>
<td>0.540</td>
<td>0.9816</td>
</tr>
<tr>
<td>Semi IPN 4</td>
<td>0.0032</td>
<td>0.265</td>
<td>0.970</td>
</tr>
<tr>
<td>Semi IPN 5</td>
<td>0.0183</td>
<td>0.727</td>
<td>0.9712</td>
</tr>
<tr>
<td>Semi IPN 6</td>
<td>0.0184</td>
<td>0.278</td>
<td>0.9418</td>
</tr>
<tr>
<td>Semi IPN 7</td>
<td>0.0137</td>
<td>0.513</td>
<td>0.9642</td>
</tr>
</tbody>
</table>

III.3.7: 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.

![Graph showing cumulative % release of 5-FU through CS/GG-g-PAm microspheres containing different amount of crosslinking agent](image)

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

**III.3.8: Effect of Drug**

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

III.3.9. Effect of Polymers Ratio

Figure III.6 shows the in vitro release data of 5-FU from the microsphere particles performed with different ratio of GG-g-PAm in the polymeric matrices. The data shows that higher amount of GG-g-PAm containing particles having more encapsulation efficiency and the release studies shown that higher amount of GG-g-PAm containing particles have shown prolonged release characteristics than the microspheres containing lower amount of GG-g-PAm. Generally, the drug release pattern depends on many factors like particle size, crystallinity, surface character, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity and the rate of hydration of the polymeric materials [26]. In the release behavior of polymeric system we can consider the binding affinity of drug and polymer swelling property of GG-g-PAm.
Fig III.6: Cumulative % release of 5-FU CS/GG-g-PAm microspheres containing different amount of GG-g-PAm (semi IPN 0) 0 % GG-g-PAm, (semi IPN 1) 20 wt. % GG-g-PAm, (semi IPN 2) 30 wt. % GG-g-PAm and (semi IPN 3) 40 wt. % GG-g-Pam.

III.4. Conclusions

Carbohydrate polymeric grafted microspheres of GG-g-PAm 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 released in a controlled manner. The swelling studies of microspheres have shown that with an increasing amount of GG-g-PAm 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-PAm. 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.
III.5. References


