**INTRODUCTION**

Thermoresponsive polymers are macromolecules, which dissolve in cold water but collapse and precipitate upon heating aqueous solution above its lower critical solution temperature (LCST). By crosslinking these types of polymers, hydrogels are obtained which reversibly shrink when heated above the critical temperature. Responsive microparticles may be prepared by conducting the polymerization of the appropriate monomer and a crosslinker in a dispersed medium. The product is latex; in this case, nano-sized gel particles are dispersed in water.

One of the most studied responsive polymers is poly (N-isopropylacrylamide) (PNIPAAm). Latex particles composed of PNIPAAm have been successfully synthesized in aqueous emulsions or dispersions stabilized by a conventional surfactant such as sodium lauryl sulfate (SLS). Properties of the microspheres have been studied extensively. Recently, there has been a growing interest in temperature-sensitive polymer poly (N-vinyl caprolactam) (PNVCL). Both PNIPAAm and PNVCL have the LCST which was near body temperature and, consequently, they may find several biomedical applications. PNVCL is especially interesting due to the fact that it is very stable against hydrolysis. Owing to its stability, PNVCL is expected to be a biocompatible polymer. If the amide bond in the side group is hydrolyzed for harsh strongly acidic conditions, a polymeric carboxylic acid builds up. PNVCL is also widely used for hair-care and cosmetic applications, used in controlled drug delivery and drug release application and the results have been published by Pong and Wu, Moskvicheva et al., and Vihola et al. PNVCL collapses when the temperature exceeds 32°C and therefore, the thermosensitive PNVCL has, presumably, similar characteristic to PNIPAAm.

5-Fluorouracil (5-FU) is the most commonly used chemotherapeutic drug for the treatment of solid tumors of breast, stomach, colon, and pancreas. It has been widely used in drug administration due to its large number of secondary effects that accompany its conventional administration. In this research, novel 5-FU-loaded poly (vinyl caprolactam-co-vinyl acetate) microspheres have been prepared and characterized.
by differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy. The in vitro release studies have been performed in 7.4 pH buffer solutions at 37°C and the results are presented in the present studies.

**MATERIALS AND METHODS**

N-vinyl caprolactam (98%) was purchased from Aldrich Chemicals, Milwauka, USA and Vinyl acetate (VAC), Potassium per sulfate, N,N'-methyl bisacrylamide (NNMBA), Calcium chloride were obtained from S. D. Fine Chemicals, Mumbai, India. 5-FU is purchased from Hi-media chemicals, Mumbai, India.

**Preparation of poly (N-vinyl caprolactam-co-vinyl acetate) microspheres**

Required amount of SLS (1 g) and potassium persulfate (100 mg) were dissolved in 100 ml of distilled water and the mixture was transferred into 250 ml round bottom flask equipped with a stirrer (Remi motor), reflux condenser, and nitrogen inlet. Different compositions of VAC/vinyl caprolactam (VCL) (40/60, 30/70, 20/80 at any cost the total monomer weight is 1 g), keeping NNMBA (200 mg) and 5-FU (20% to that of the monomers content) constant, were added to SLS solution. The mixture was degassed by passing nitrogen gas for 45 min and was heated to 70°C and stirred well at a rotating speed of 800 rpm for 10 hours and then cooled to room temperature and transferred into a beaker containing 5 wt% of calcium chloride solution to break the emulsion. Polymeric microspheres obtained were isolated by centrifuging at 14 000 rpm speed for about 15 min and then filtered under vacuum and allowed to dry at 40°C. Microspheres thus prepared were purified by washing with water thoroughly for 1 hour to remove excess of SLS and NNMBA. The unreacted VCL, VAC present in the microspheres was dissolved in calcium chloride solution and the polymeric microspheres were isolated by centrifuging the mixture. The product were again washed several times with water to remove the unreacted VCL, VAC monomers, NNMBA and then the isolated microspheres were dried under vacuum and allowed to dry at 40°C. Microspheres thus prepared were purified by washing with water thoroughly for 1 hour to remove excess of SLS and NNMBA. The unreacted VCL, VAC present in the microspheres was dissolved in calcium chloride solution and the polymeric microspheres were isolated by centrifuging the mixture. The product were again washed several times with water to remove the unreacted VCL, VAC monomers, NNMBA and then the isolated microspheres were dried under vacuum and the microspheres were designed as FU-2, FU-6, and FU-7. By repeating the above procedure, various formulations were prepared by varying the crosslinker (10, 20, and 30 w%) and the drug content (10, 20, and 30 w%) and were designated as FU-4, FU-2, FU3, respectively.

**Estimation of drug loading and encapsulation efficiency**

Loading efficiency of 5-FU in the microspheres was determined spectrophotometrically. About 10 mg of the drug-loaded microspheres were placed in 10 ml of buffer solution and stirred vigorously for 48 hours to extract the drug from the loaded microspheres. The aqueous solution was then filtered and assayed by ultraviolet (UV) spectrophotometer (Lab, India, 3000+) at a $\lambda_{max}$ of 272 nm. The results of % of drug loading and % of encapsulation efficiency were calculated, respectively using equations (1) and (2).

\[
% \text{ of Drug loading} = \frac{\text{Amount of drug in beads}}{\text{Amount of beads}} \times 100 \quad (1)
\]

\[
% \text{ of Encapsulation efficiency} = \frac{\text{Actual loading}}{\text{Theoretica 1 loading}} \times 100 \quad (2)
\]

**In vitro release studies**

In vitro release studies have been performed using the tablet dissolution tester (Lab India, Mumbai, India) equipped with eight bowls at 37°C at rotating speed of 100rpm. Drug release from the microspheres was studied in the stimulated 7.4 pH phosphate buffer. Aliquot samples were withdrawn at regular intervals of time and analyzed using UV spectrophotometer.

**X-ray diffraction studies**

XRD measurement of plain drug, plain microspheres, and drug-loaded microspheres were recorded with a Rigaku Geigerflex diffractometer (Tokyo, Japan) equipped with Ni-filtered Cu-Kα radiation ($\lambda = 1.5418$ Å). The dried microspheres of uniform size were mounted on sample holder, and the patterns were recorded in the range from 0 to 50° at the speed of 5°/min.

**Scanning electron microscopy studies**

The microspheres were taken on copper stub and sputtered with gold for 2 min. These gold-coated microspheres were mounted on the Scanning electron microscopy (SEM) instrument (Leica 400, Cambridge, UK) and micrographs were taken at magnification of 700X.

**RESULTS AND DISCUSSIONS**

**Differential scanning calorimetry studies**

DSC tracings of (a) plain 5-FU, (b) plain Poly (VCL-co-VAC) microspheres, and (c) Poly (VCL-co-VAC) microspheres are depicted in Figure 1. The one set-melting peak of 5-FU is observed at 285.16°C.\[20\] However, no characteristic peak of 5-FU is observed in the DSC curves of the drug-loaded microspheres, suggesting that drug is molecularly dispersed in the polymer matrix.\[20\]
X-ray diffraction studies

X-ray diffractograms of (a) placebo microspheres, (b) drug-loaded microspheres, and (c) pure 5-FU drug are shown in Figure 2. These studies are useful to investigate the crystallinity of the drug in crosslinked microspheres. 5-FU has shown the characteristic intense peaks at 2θ of 25° due to its crystalline nature. However, these peaks disappeared in the 5-FU-loaded microspheres, but only peaks observed in placebo polymer matrix were seen. XRD peak depends on the crystal size, but in the present study, for all the drug loadings, the characteristic peaks of 5-FU overlapped with noise of the coated polymer itself. Furthermore, loaded drug is amorphous, which is difficult to measure at the direction limit of the crystal size in the present case. This further confirms that drug is molecularly dispersed at a molecular level in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

Scanning electron microscopy analysis

SEM photographs of single poly (VCL-co-VAC) microspheres were taken at 700X magnification which is shown in Figure 3. From Figure 3, poly (VCL-co-VAC) microspheres show almost spherical and rough surface. Size of the microspheres are found to be around 20 μm.

Encapsulation efficiency

Results of encapsulation efficiency for different formulations as a function of extents of drug lodging, crosslinking and monomer ratio are include in Table 1. % encapsulation efficiency increased systematically with increase in drug loading from 10 to 30% in the matrix, and their encapsulation efficiencies were 65, 68 and 72%, respectively. This increase in encapsulation efficiency is due to the availability of more free void space, through which lesser number of drug molecules will transport. It is noticed that % encapsulation efficiency increased with increase in VCL content in the Poly (VCL-co-VAC) microspheres. For example, for the microspheres containing 20, 30, and 40 wt% VCL and 20 wt% of 5-FU, encapsulation efficiencies were 58, 61, and 68, respectively. For microspheres crosslinked with 10, 20, and 30 wt% NNMBA, encapsulation efficiencies (68, 67, and 62) decreased with increase in crosslinker content in the microspheres respectively. Such a decreasing trend is due to increase in crosslinking density, because the microspheres become rigid, thereby reducing the free volume space within the polymer matrix and hence, a reduction in encapsulation efficiency. A similar observation was also reported by Kurkuri and Aminabhavi in case of poly (vinyl alcohol) and acrylic acid sequential interpenetrating network for control release of diclofenac sodium.

Drug release kinetics

Drug release kinetics is analyzed by plotting the cumulative release data vs time and by fitting these data to the exponential equations of the type:

\[
\frac{M_t}{M_\infty} = kt^n
\]

Here, \(\frac{M_t}{M_\infty}\) represents the fractional drug release 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 square procedure, the estimated

<table>
<thead>
<tr>
<th>Formulation codes</th>
<th>% of VCL in microspheres</th>
<th>% of amount of NNMBA Wt %</th>
<th>Amount of 5-FU Wt %</th>
<th>% of encapsulation efficiency ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU-1</td>
<td>40</td>
<td>2</td>
<td>10</td>
<td>65±0.9</td>
</tr>
<tr>
<td>FU-2</td>
<td>40</td>
<td>2</td>
<td>20</td>
<td>67±0.8</td>
</tr>
<tr>
<td>FU-3</td>
<td>40</td>
<td>2</td>
<td>30</td>
<td>72±0.2</td>
</tr>
<tr>
<td>FU-4</td>
<td>40</td>
<td>1</td>
<td>20</td>
<td>68±0.9</td>
</tr>
<tr>
<td>FU-5</td>
<td>40</td>
<td>3</td>
<td>20</td>
<td>62±0.8</td>
</tr>
<tr>
<td>FU-6</td>
<td>30</td>
<td>2</td>
<td>20</td>
<td>61±0.9</td>
</tr>
<tr>
<td>FU-7</td>
<td>20</td>
<td>2</td>
<td>20</td>
<td>58±0.8</td>
</tr>
</tbody>
</table>
values of ‘n’ and k for all the formulation are given in Table 2. If n = 0.5, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For n > 0.5, an anomalous or non-fickian type drug diffusion occurs. If n = 1, a completely non-Fickian of case II release kinetics is operative. The intermediary values ranging between 0.5 and 1 are attributed to the anomalous type of transport.

The values of k increased with increasing % of loading of 5-FU in the microspheres, but ‘n’ values decreased with decrease in % of loading of 5-FU. This indicates that the interaction between the microspheres and drug are in similar lines studied from the release kinetics Eqn (3) proposed by Ritger and Peppas. The values of exponent ‘n’ ranges between 0.5874 and 1.1263 as calculated from empirical, which indicated that drug release followed by an anomalous nontransport occurs. The correlation coefficient values are in the range of 0.906 to 0.938, suggesting a good fit experimental release data.

In vitro release studies
Effect of 5-flourouracil
5-FU is a water-soluble drug and therefore, it is difficult to encapsulate it into hydrophobic polymers by solvent exchange process. In the present case, release profiles ranging from 62 to 81% could be achieved for different copolymer compositions; these releasing properties are quite higher than those reported in the earlier literature. The % of encapsulation efficiency data are presented in Table 1. Figure 4 displays the drug release characteristics of the formulations containing different amounts of drug. The above results indicate that 5-FU release is up to 10 hours. From these groups, it is noticed that the faster release rates have been observed for the formulations containing higher amount of 5-FU than those microspheres containing lower amounts of 5-FU in the matrix. Release data with regard to drug content is in the order of 30 > 20 > 10% of 5-FU. A prolonged drug release was observed for formulation containing lower amount of 5-FU. It is understood that the release rate becomes quite slower when a lower amount of drug is present in the matrix, probably due to the availability of more free-void spaces, through which lesser number of drug molecules could possibly transport. Generally, drug release through microspheres depends upon the particle size, polymer crystallinity, surface character, molecular weight, polymer composition, etc. Hence in the present study, the above said parameters may be responsible for the release trend with respect to drug content in the microspheres.

Effect of crosslinking
The % of cumulative release data vs time plots for varying amounts of crosslinker, NNMB, i.e., 10, 20, and 30 w% at a fixed amount of drug (20%) are depicted in Figure 5. The % of cumulative release is quite fast and large at lower amount of NNMBA, whereas the release is quite slower at higher amount i.e., at 30% NNMBA. This is because at higher concentration of NNMBA, the polymeric chains will become rigid due to higher contraction of microvoids, thereby giving a decrease in % of cumulative release of the drug. A similar observation was made by Krishna Rao et al. in case of the drug release studies on 5-FU drug through poly (vinyl alcohol)/poly (acryl amide-co-acrylamidoglycolic acid) polymer matrices.

Effect of vinyl caprolactam
Effect of VCL content on encapsulation efficiency and

---

Table 2: Drug release kinetics parameters of different formulations

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>k</th>
<th>n</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FU-1</td>
<td>0.1359</td>
<td>0.6953</td>
<td>0.906</td>
</tr>
<tr>
<td>FU-2</td>
<td>0.2346</td>
<td>0.8653</td>
<td>0.921</td>
</tr>
<tr>
<td>FU-3</td>
<td>0.3113</td>
<td>1.1263</td>
<td>0.913</td>
</tr>
<tr>
<td>FU-4</td>
<td>0.1547</td>
<td>0.5874</td>
<td>0.921</td>
</tr>
<tr>
<td>FU-5</td>
<td>0.1861</td>
<td>0.6802</td>
<td>0.932</td>
</tr>
<tr>
<td>FU-6</td>
<td>0.8312</td>
<td>0.8316</td>
<td>0.938</td>
</tr>
<tr>
<td>FU-7</td>
<td>0.2174</td>
<td>0.7474</td>
<td>0.915</td>
</tr>
</tbody>
</table>

---

Figure 4: % of cumulative release of drug through Poly (VNCL-co-VAC) microspheres crosslinked with 2 wt% NNMB and containing 10 (▲), 20 (■), and 30% (♦) of 5-Flourouracil

Figure 5: % of cumulative release of the drug through Poly (VNCL-co-VAC) microspheres crosslinked with 20 wt% of 5-Flourouracil and containing 1 (▲), 2 (■), and 3% (♦) of NNMBA
in vitro release of 5-FU was investigated and the data are given in Table 1. In vitro release profiles of 5-FU for formulations prepared by taking different amounts of VCL with 20% of 5-FU and 2% NN MBA are shown in Figure 6. Higher release rates were observed for the formulations prepared with higher amounts of VCL (i.e., 40%) than those formulations prepared using lower amounts of VCL. Slower drug release is observed from formulations prepared with lower amount of VCL, which is due to the hydrophilic nature of both the drug and VCL in the copolymer. Similar observation was reported by Prabaharan et al. in case of Chitosan-g-Poly(N-Vinyl caprolactam).

CONCLUSIONS

Novel types of 5-FU-loaded Poly (VCL-co-VAC) microspheres were prepared by emulsion polymerization using SLS as a surfactant. 5-Flourouracil, anti-cancer drug, was chosen as a model drug to investigate the release studies. The microspheres prepared were characterized by DSC, X-ray diffractometry, and Scanning electron microscopy. DSC studies indicate that 5-FU is molecularly distributed in the microspheres. The in vitro drug release studies were carried out in pH 7.4. Higher the drug loadings, faster the release rates have been observed from in situ drug-loaded microspheres of this study.

REFERENCES