RESEARCH ARTICLE

Controlled release studies of 5-Fluorouracil through poly (vinyl caprolactum-co-vinyl acetate) microspheres

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Novel poly (vinyl caprolactam-co-vinyl acetate) microspheres were crosslinked with *N', N'* methylene bisacrylamide (NNMBA) prepared by free radical emulsion polymerization. This was done by using vinyl caprolactam, vinyl acetate, and NNMBA with varying amounts. 5-Fluorouracil (5-FU) is an anticancer drug which was mixed into these microspheres during *in situ* polymerization. These microspheres were characterized by using differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM) techniques. XRD and DSC results indicated that there was uniform distribution of 5-FU drug particles in microspheres, and SEM pictures suggest that the microspheres are in spherical shape. Both encapsulation efficiency and release patterns are found to be dependent on the amount of crosslinking agent and amount of drug loaded. From the results of drug release kinetic studies, an anomalous and nonFickian behavior was observed in the present studies. Furthermore, *in vitro* release studies indicated the release of 5-FU up to 10 hours.

Key words: *5-Fluorouracil, microspheres, vinyl acetate, vinyl caprolactam*

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).[1-3] Properties of the microspheres have been studied extensively. Recently, there has been a growing interest in temperature-sensitive polymer poly (N-vinyl caprolactam) (PNVCL).[4-7] Both PNIPAAm and PNVCL have the LCST which was near body temperature and, consequently, they may find several biomedical applications.[8] 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.[7-9] PNVCL is also widely used for hair-care and cosmetic applications,[10] used in controlled drug delivery and drug release application and the results have been published by Pong and Wu,[11] Moskvicheva *et al.*,[12] and Vihola *et al.*[13] PNVCL collapses when the temperature exceeds 32°C[14] 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.[15-19] 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

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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 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, FU-5 and FU-1, 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 λ_{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 (λ = 1.5418 A°). 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]

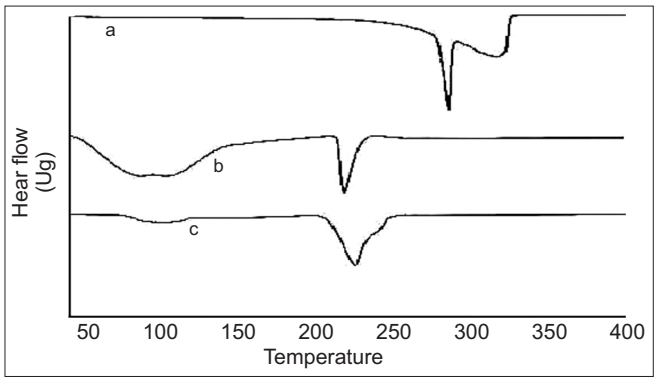

Figure 1: DSC thermograms of (a) 5-FU, (b) plain (VNCL-co-VAC) microspheres, and (c) 5-FU-loaded Poly (VNCL-co-VAC) microspheres

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.[21] 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[21] 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[22] in case of poly (vinyl alcohol) and acrylic acid sequential interpenetrating network for control release of diclofenac sodium.

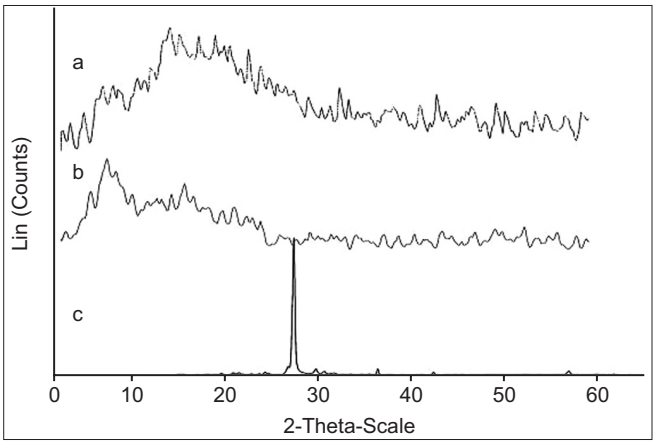

Figure 2: XRD spectra of (a) pure 5-FU, (b) Plain Poly (VNCL-co-VAC) microspheres, and (c) 5-FU-loaded Poly (VNCL-co-VAC) microspheres

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.[23]

$$(M_t/M_\infty) = kt^n \quad (3)$$

Here, M_t/M_∞ 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

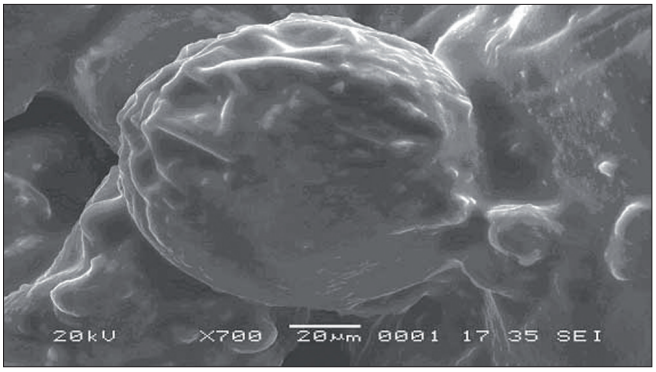

Figure 3: SEM micrographs of Poly (vinyl caprolactam-co-vinyl acetate)

Table 1: Results of % of encapsulation efficiencies for different formulations

Formulation codes	% of VCL in microspheres	% of amount of NNMBA Wt %	Amount of 5-FU Wt %	% of encapsulation efficiency ± SD
FU-1	40	2	10	65±0.9
FU-2	40	2	20	67±0.8
FU-3	40	2	30	72±0.2
FU-4	40	1	20	68±0.9
FU-5	40	3	20	62±0.8
FU-6	30	2	20	61±0.9
FU-7	20	2	20	58±0.8

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 nonFickian 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.[23] 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.[24] 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 regarded 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.

Table 2: Drug release kinetics parameters of different formulations

Semple ID	*k*	*n*	Corrélation coefficient (r)
FU-1	0.1359	0.6953	0.906
FU-2	0.2346	0.8653	0.921
FU-3	0.3113	1.1263	0.913
FU-4	0.1547	0.5874	0.921
FU-5	0.1861	0.6802	0.932
FU-6	0.8312	0.8316	0.938
FU-7	0.2174	0.7474	0.915

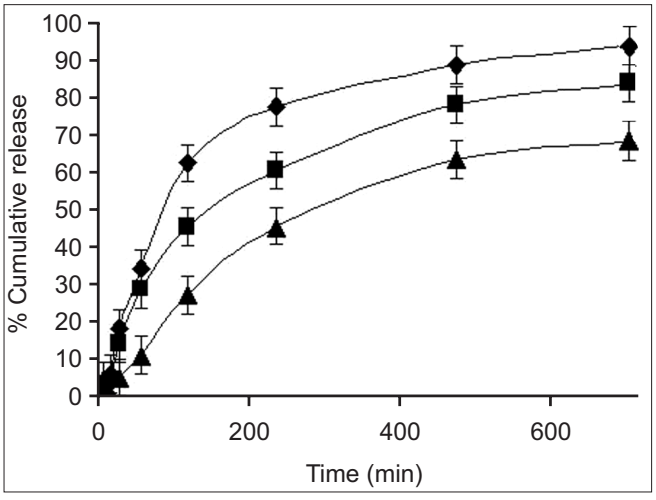

Figure 4: % of cumulative release of drug through Poly (VNCL-co-VAC) microspheres crosslinked with 2 wt% NNMBA and containing 10 (▲), 20 (■), and 30% (♦) of 5-Fluorouracil

Effect of crosslinking

The % of cumulative release data *vs* time plots for varying amounts of crosslinker, NNMBA, 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.[25] A similar observation was made by Krishna Rao *et al*.[26] 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

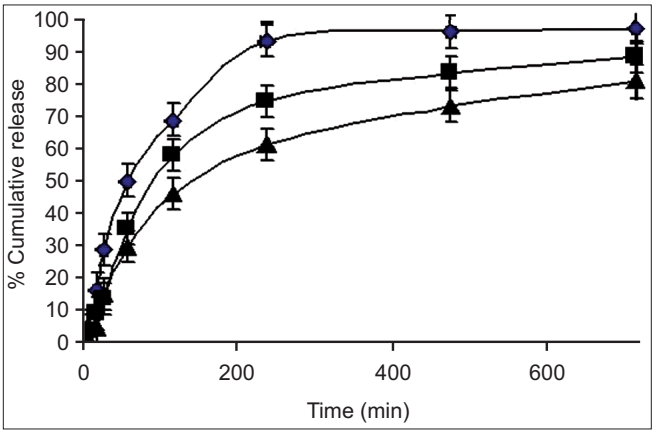

Figure 5: % of cumulative release of the drug through Poly (VNCL-co-VAC) microspheres crosslinked with 20 wt% of 5-Flourouracil and containing 1 (♦), 2 (■), and 3% (▲) of NNMBA

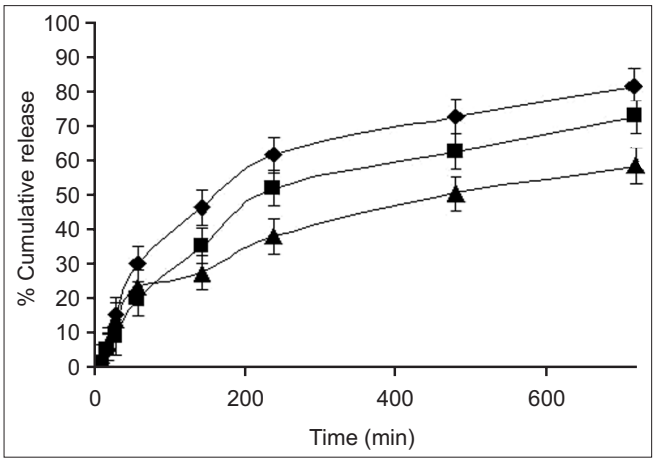

Figure 6: % of cumulative release of 5-FU drug through Poly (VNCL-co-VAC) microspheres crosslinked with 2 wt% NNMBA and 20% 5-FU for different ratios of vinyl caprolactam, i.e., 20 (▲), 30 (■), and 40% (♦)

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% NNMBA 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.*[27] 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

1. Wu C, Zhou S, Au-yeung SC, Jiang S. Volume phase transition of spherical microgel: Particals. Angew Makramol Chem 1996:240:123-36.
2. Lowe TL, Tenhu H. Interactions of thermally responsive polyelectrolyte lattices with low molar mass organic molecules studied by light scattering. Macromolecules 1998;31:1590-4.
3. Lowe TL, Benhaddaou M, Tenhu H. Partially fluorinated thermally responsive lattices of linear and crosslinked copolymer. Polym Sci B Polym Phys 1998;36:2141-52.
4. Eisele M, Byrchard W. Hydrophobic water-soluble polymers dilute solutions properties of poly (1-vinyl-2-piperidone) and poly (N-vinyl caprolactam). Macromol Chem Physic 1990;191:169.
5. Makhaeva E, Thanh LT, Starodoubtsev SG, Khokhlov AR. Thermo shrinking behavior of poly(N-vinyl caprolactam) gels in aqueous solution. Macromol Chem Physic 1996;197:1973-82.
6. Gao Y, Au-yenung SC, Wu C. Interaction between Surfactant and Poly (N-vinyl caprolactam) Microgels. Macromolécules 1999;32:3674-7.
7. Lau AC, Wu C. Thermally Sensitive and Biocompatible Poly (N-vinyl caprolactam): Synthesis and characterization of high molar mass linear chains. Macromolecules 1999;32:581-4.
8. Galaev IY, Mattiasson B. Smart polymers and what they could do in biotechnology and medicine. Trends Biotechnol 1999;17:335-40.
9. Laukkaen A, Hietala S, Maunu SL, Tenhu H. Poly(N-vinyl caprolactam) Microgel particles grafted with amphiphilic chains. Macromolecule 2000;33:8703-8.
10. Goddsrd ED, Guber JV. Principles of polymer science and technology in cosmetics and personal care. New York: Marcel Dekker; 1999. p. 217-74.
11. Pong S, Wu C. Poly (N-vinyl caprolactam) microgels and its related composites. Macromol Symp 2000:159:179-86.
12. Makvicheva EA, Tkacuk NE, kuptsova SV, Dugina TN, Strukova SM, Krish YE, *et al*. Immobilized enzymes and cells in poly(N -vinyl caprolactam)-based hydrogels. Appl Bioche Biotechnol 1996;88:1-3.
13. Vihola H, Laukkanen A, Hirvonen J, Tenhu H. Binding and release of drugs into and from thermosenesitive poly (N-vinyl caprolactam) nanoparticless Eur J Pharm Sci 2002;16:69-74.
14. Kirsh YE. Water-soluble poly (n-vinylamides). Chichester: Wiley; 1998.
15. Matsuyama H, Teramoto M, Urano H. Analysis of solute diffusion in poly(vinyl alcohol) hydrogel membrane J Memb Sci 1997;126:151-60.
16. Heidelberger C. Cancer medicine. 2nd ed. Philadelphia: Lea and Febiger; 1961;801.
17. Waxman S, Scanlon KJ, Greenspan EM. Clinical interpretation and practice in chemotheraphy. New York: Raven Press; 1982. p. 38.
18. Sommadossi P, Gewirtz DA, Diasio RB, Aubert C, Cano JP, Goldman ID. Rapid catabolism of 5-fluorouracil in freshly isolated rat hepatocytes as analyzed by high performance liquid chromatography. J Biol Chem 1982;257:8171-7.
19. Einmhl S, Zigani M, Varesio E, Heller J, Veuthey JL, Gurny R. Concomitant and controlled release of dexamethasone and 5-fluorouracil from poly(orthoester). Int J Pharm 1999;189:185-98.
20. Babu VR, Sairam M, Hosamani KM, Aminabhavi TM. Development of 5-fluorouracil loaded poly (a crylamide-co-methylmethacrylate) novel core-shell microspheres: In vitro release studies.Int J Pharm 2006;325: 55-62.
21. Denizli A, Kiremitçi M, Piskin E. Subcutaneous polymeric matrix system poly (HEMA-BGA) for controlled release of an anticancer drug (5-fluorouracil): I. Synthesis and structure, Biomaterials 1988;9: 257-62.
22. Kurkuri MD, Aminabhavi TM. Poly (vinyl alcohol) and poly (acrylic acid) sequential interpenetrating network pH-sensitive microspheres for the delivery of diclofenac sodium to the intestine. J Control Release 2004;96:9-20.
23. Ritger PL, Peppas NA. A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J Control Release 1987;5:23-6.
24. Reddy MK, Babu VR, Krishna Rao K, Subha MC, Rao CK, Sairam M, *et al*. Temperature sensitive semi-IPN microspheres from sodium alginate and N-isopropylacrylamide for controlled release of 5-fluorouracil. J Appl Polym Sci Symp 2007;107:2820-9.
25. Vihola H, Laukkanen A, Tenhu H, Hirvonen J. Drug release characteristics of physically cross-linked thermo sensitive poly(N-vinylcaprolactam) hydrogel particles. J Pharm Sci 2008;97:4783-93.
26. Krishna Rao K, Kiran Kumar A, Rao MK, Subha MC. Yong-Ill Lee Semi-IPN hydrogels based on Poly (vinyl alcohol) for controlled release studies of chemotherapeutic agent and their Swelling characteristics. Polym Bull 2008;61:81-90.
27. Prabaharn M, Grailer JJ, Steeber DA, Gong S. Stimuli responsive chitosan-graft-poly (N-vinyl caprolactam) as promising material for controlled hydrophobic drug delivery. Macromol Biosci 2008;8:843-51.

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