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Encapsulation efficiency and controlled release characteristics of crosslinked polyacrylamide particles

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Abstract

Polyacrylamide (pAAm) particles crosslinked with *N*,*N*-methylenebis-acrylamide/ethylene glycol dimethacrylate (NNMBA/EGDMA) have been prepared in water–methanol medium by the dispersion polymerization using poly(vinyl pyrrolidone), PVP as a steric stabilizer. 5-fluorouracil an anticancer drug, has been loaded *in situ* into the crosslinked pAAm particles. Plain as well as drug loaded microparticles have been characterized by differential scanning calorimetry (DSC) and X-ray diffraction studies (XRD) and scanning electron microscopy (SEM). DSC and XRD studies have indicated a molecular level dispersion of the drug in pAAm particles during *in situ* loading and SEM pictures have shown the formation of spherical and oval-shaped particles. *In vitro* release of 5-fluorouracil from the crosslinked pAAm particles has been carried out in 7.4 pH buffer medium. Both encapsulation efficiency and release patterns are found to depend on the nature of the crosslinking agent, amount of crosslinking agent used and the amount of drug loaded. *In vitro* release studies indicated the controlled release of 5-fluorouracil up to 12 h.

Keywords: Crosslinked poly(acrylamide) particles; Dispersion polymerization; 5-Fluorouracil; Controlled release

1. Introduction

Dispersion polymerization is a unique single step polymerization process, capable of forming monodisperse particles in the range of 0.1–15 μm (Cho et al., 2002). During dispersion polymerization, the polymer precipitates out from the initially homogeneous reaction mixture containing monomer, initiator, solvent and stabilizer. The reaction medium for dispersion polymerization is a good solvent for the monomer and stabilizer, but is a poor solvent for the polymer formed. During the process of dispersion polymerization, stabilizer protects the particles by adsorption, steric and electrostatic stabilization (Tseng et al., 1986; Paine, 1990a,b; Paine et al., 1990). Dispersion polymerization has been used to prepare micro or nanoparticles of hydrophobic polymers and core-shell particles using various stabilizers (Sparnacci et al., 2002; Ober and Lok, 1987; Takahasi et al., 1996; Paine, 1990a,b; Corner, 1981). It has also been used to prepare finely dispersed submicron size particles from the water-soluble monomer like, acrylamide in water-alcohol medium (Ray et al., 1995; Ray and Mandal, 1997, 1999; Guha and Mandal, 2004). The most frequently used polymers for the production of particles for controlled release (CR) of drugs are mainly the acrylic acid derivatives, polyalkylcyanoacrylates and polyalkylmethacrylates. However, most of the homopolymer particles have low drug loading capacities, especially for hydrophilic drugs (Harmia et al., 1986). In order to increase the hydrophilicity of particle surface, attempts have been made to prepare copolymers of alkylmethacrylate with various acrylic acid derivatives (Kreuter et al., 1988; Rolland et al., 1986) including acrylamide, acrylic acid, etc.

In this work, we would like to investigate the encapsulation efficiency and CR characteristics of the hydrophilic crosslinked polyacrylamide particles prepared by using PVP as a stabilizer. Polyacrylamide particles crosslinked with two different crosslinking agents, *N*,*N*-methylenebisacrylamide (NNMBA) or ethyleneglycoldimethacrylate (EGDMA) and loaded with 5-fluorouracil were produced by the dispersion polymerization. Release characteristics of 5-fluorouracil from these microparticles in pH 7.4 buffer medium has been investigated. The encapsulation efficiency and CR of 5-fluorouracil from these particles have been studied in terms of the nature of crosslinking agent,

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amount of crosslinking agent used and the amount of drug loaded into particles.

2. Materials and methods

2.1. Materials

Poly(vinyl pyrrolidone) (MW: 360,000) (PVP) was purchased from Aldrich, Milwakee, WI, USA. Acrylamide, *N*,*N*′-methylenebisacrylamide (NNMBA), Ethylene glycol dimethacrylate (EGDMA), potassium persulfate and methanol (AR) were all purchased from s.d. fine chemicals, Mumbai, India. 5-Fluorouracil was purchased from MP Biochemicals, Eschwege, Germany.

2.2. Preparation of drug loaded microspheres

PVP and the initiator, potassium persulfate were dissolved in 50 mL of water–methanol (1:1) mixture taken in a 250 mL round bottom flask equipped with a reflux condenser and a nitrogen inlet. Nitrogen was bubbled through the system for 20 min. The acrylamide monomer (1 g), 5-fluorouracil and crosslinker NNMBA or EGDMA in water were added to the above reaction mixture, stirred well and heated at 70 °C. Turbidity was observed within the first 30 min and the reaction was allowed to continue for 8 h. At the end of reaction time, drug-loaded particles were isolated by centrifuging the reaction mixture at 18,000 rpm. The particles were washed with methanol to remove the unreacted monomer and finally with acetone. The isolated particles were dried at 40 °C. Plain pAAm particles were prepared in the absence of the drug.

2.3. Differential scanning calorimetry (DSC)

DSC curves of 5-fluorouracil; placebo pAAm and 5-fluorouracil-loaded pAAm particles were recorded using Rheometric Scientific differential scanning calorimeter (Model-DSC SP, UK). The analysis was performed by heating the samples at the rate of 10 °C/min under an inert atmosphere.

2.4. X-ray diffraction (XRD)

The X-ray diffraction patterns of 5-fluorouracil, placebo pAAm microparticles and 5-fluorouracil-loaded pAAm microparticles were recorded using a Rigaku Geigerflex diffractometer equipped with Ni-filtered Cu Kα radiation (λ = 1.5418 Å). Dried particles were mounted on a sample holder and the patterns were recorded in the range of 10–50° at the speed of 5°/min to know the crystallinity.

2.5. Scanning electron microscopy (SEM)

SEM images of the plain pAAm particles in dry powder form, dispersed in acetone, dispersed in 7.4 pH buffer medium and dispersed in water were recorded using a JSM 6400 scanning electron microscope (Japan) at the required magnification. A working distance of 39 mm was maintained. The acceleration voltage used was 20 kV with the secondary electron image (SEI) as a detector.

Table 1
Results of % encapsulation efficiency and mean size of pAAm microparticles

Formulation code	AAm (g)	Amount of 5-FU (mg) added at the time of microparticle preparation	NNMBA added (wt.%)	Amount of 5-FU (mg) present in the microparticle ± S.D.[a]
F-1	1	100	2	82 ± 0.061
F-2	1	100	4	78 ± 0.045
F-3	1	100	6	74 ± 0.034
F-4	1	200	4	159 ± 0.026
F-5	1	300	4	240 ± 0.084

5-FU means 5-fluorouracil drug.
[a] S.D. means standard deviation.

2.6. Estimation of encapsulation efficiency

Amount of drug present in the microparticles was determined by dispersing 100 mg of drug loaded microparticles in 10 mL of 7.4 pH phosphate buffer and stirred vigorously to extract the drug from pAAm microparticles. The aqueous solution was filtered and assayed by UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 270 nm. The result of % 5-fluorouracil encapsulation efficiency was calculated using Eq. (1). These results are compiled in Tables 1 and 2, respectively:

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100 \quad (1)$$

2.7. In vitro release study

Dissolution was carried out using a Tablet dissolution tester (LabIndia, Mumbai, India) equipped with eight baskets. Hundred milligrams of microparticles were placed in 900 mL of buffer solution and dissolution rates were measured at 37 °C under 100 rpm speed. Drug release from the microparticles was studied in the intestinal (7.4 pH phosphate buffer) fluids. 5 mL samples were withdrawn at regular intervals of time and replaced by same amount of fresh buffer solution. The sample

Table 2
Results of % encapsulation efficiency and mean size of pAAm microparticles

Formulation code	AAm (g)	Amount of 5-FU (mg) added at the time of microparticle preparation	EGDMA (wt.%) added	Amount of 5-FU (mg) present in the microparticle ± S.D.[a]
F-6	1	100	2	66 ± 0.045
F-7	1	100	4	62 ± 0.025
F-8	1	100	6	56 ± 0.088
F-9	1	200	4	120 ± 0.065
F-10	1	300	4	184 ± 0.058

[a] S.D. means standard deviation.

collected during dissolution experiments were analyzed by using a UV spectrophotometer (Model Anthelie, Secomam, Dumont, France) at the fixed λ_{max} value of 270. Dissolution experiments were carried out in triplicate and the average results of % cumulative release was reported.

3. Results and discussion

3.1. Differential scanning calorimetry

DSC tracings of 5-fluorouracil, drug-loaded pAAm microparticles and plain pAAm microparticles are displayed in Fig. 1. The onset melting peak of 5-fluorouracil was observed at 285.16 °C. However, no characteristic peak of 5-fluorouracil was observed in the DSC curves of the drug-loaded microparticles, suggesting that drug is molecularly dispersed in the polymer matrix.

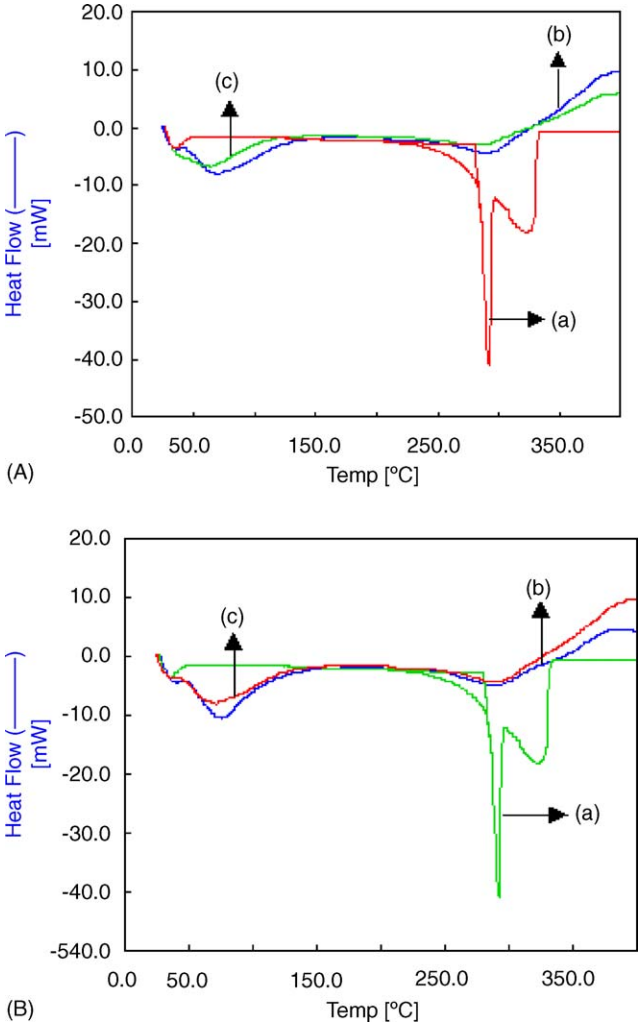

Fig. 1. Differential scanning calorimetric thermograms of (A) plain 5-fluorouracil (a), plain pAAm microparticles (b) and 5-fluorouracil loaded-pAAm microparticles crosslinked with EGDMA; (B) plain 5-fluorouracil (a), plain PAAm microparticles (b) and 5-fluorouracil loaded-pAAm microparticles crosslinked with NNMBA.

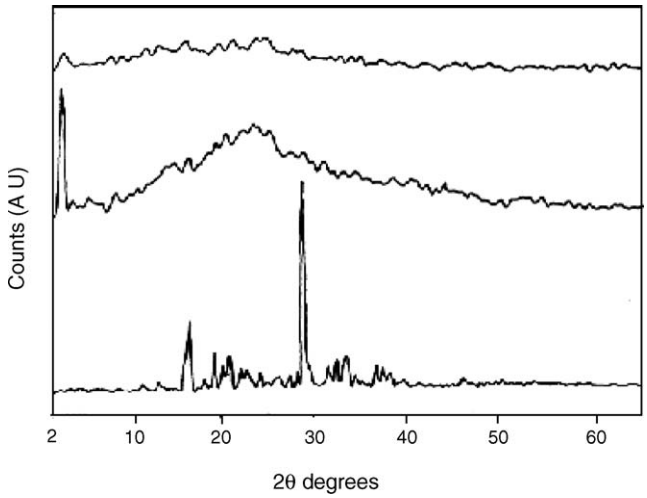

Fig. 2. X-ray diffraction patterns of plain 5-fluorouracil (a), plain pAAm microparticles (b) and 5-fluorouracil loaded-pAAm microparticles.

3.2. X-ray diffraction (X-RD)

XRD diffractograms of 5-fluorouracil, placebo pAAm and 5-fluorouracil-loaded pAAm microparticles are displayed in Fig. 2. These traces reveal the crystallinity of the drug after encapsulation in the microparticles. 5-Fluorouracil has shown characteristic intense peaks between 2θ of 16°, 19°, 21° and 29° due to its crystalline nature. However, peaks for plain drug were masked in the drug-loaded microparticles. X-RD diffractogram of 5-fluorouracil-loaded microspheres did not show any characteristic peaks for the drug, indicating that the encapsulated drug is in an amorphous state.

3.3. Scanning electron microscopy

The size and shape of the particles produced depend upon the nature of the polymerization medium, nature of the stabilizer and amount of the stabilizer used in polymerization reaction. Scanning electron micrographs of pAAm microparticles are shown in Fig. 3(a), (b), (c) and (d). SEM micrographs [Fig. 3(a) and (b)] show the formation of polydisperse spherical as well as oval-shaped particles of around 2–3.5 μm size by the dispersion polymerization technique using PVP as a steric stabilizer. The formation of oval-shaped particles is due to the coalescence of similar size particles during the course of polymerization. SEM micrographs [Fig. 3(c) and (d)] indicate the swollen nature of pAAm particles in the buffer and water media. Polyacryalmide particles can also be produced in the nano size (80–300 nm) by other steric stabilizers like PVME, PVME-alt-maliec anhydride, etc. These nano-sized particles will be useful for preparing injectable CR formulations.

3.4. Encapsulation efficiency

Three different concentrations of 5-fluorouracil, i.e., 10, 20 and 30 wt.% were loaded during crosslinking with NNMBA. Results of % encapsulation efficiency are included in Table 1. As the % drug loading has increased, % encapsulation also

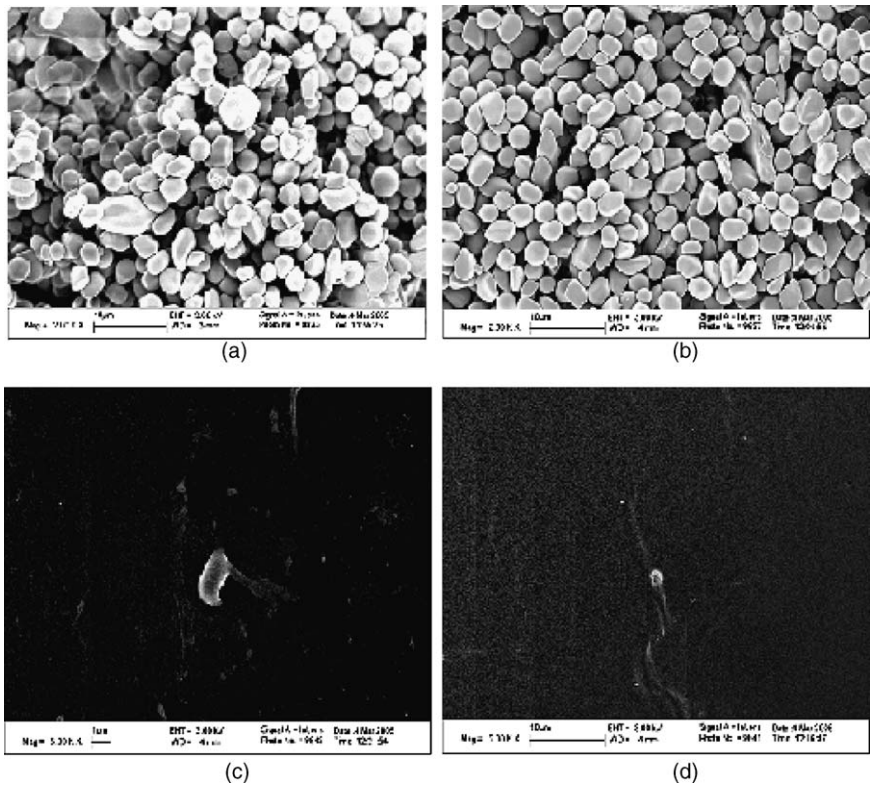

Fig. 3. Scanning electron micrographs of pAAm microparticles, powder form (a), dispersed in acetone (b), dispersed in buffer medium (c) and dispersed in water (d).

increased from 74.89 to 80.24%. With increasing crosslinking, the % encapsulation efficiency decreased. For instance, with an increase of NNMBA concentration from 2 to 6 wt.% entrapment efficiency has decreased from 82.05 to 74.89%. Such a decreasing trend could be attributed to increased croslinking density of the matrix microparticles, which might have become more rigid as a result of reduction in free volume spaces within the polymer matrix; thereby reducing their encapsulation efficiencies. The same trend was also observed for the second crosslinking agent, EGDMA. The % encapsulation efficiency of the formulations prepared with 2, 4 and 6% of EGDMA are, respectively 66.45, 62.25 and 56.88%, which are found to be lower than those observed for microparticles prepared with NNMBA as a crosslinking agent. This may be due to lesser interaction of the hydrophilic 5-fluorouracil with hydrophobic crosslinking agent of the matrix microparticles. These results are presented in Table 2.

3.5. In vitro release study

3.5.1. Effect of drug concentration

Figs. 4 and 5 display the *in vitro* release characteristics of formulations containing different amount of 5-fluorouracil from the PAAm microparticles prepared with 1 wt.% of PVP. Faster release rates were observed from formulations containing higher % of the drug than those microparticles containing the lower amount of drug. Release data showed that formulations containing the highest amount of drug (30%) displayed the faster and higher release rates than those formulations containing the lower amount of 5-fluorouracil. Note that the release rate becomes quite slower at the lower amount of drug in the matrix, due to the availability of more free void spaces through which a lesser number of drug molecules could transport.

3.5.2. Effect of crosslinking agent

The % cumulative release data versus time plots for varying amounts of NNMBA and EGDMA (i.e., 2, 4 and 6 wt.%) at the fixed amount of 5-fluorouracil (10 wt.%) are displayed in Figs. 6 and 7. The % cumulative release is quite fast and large

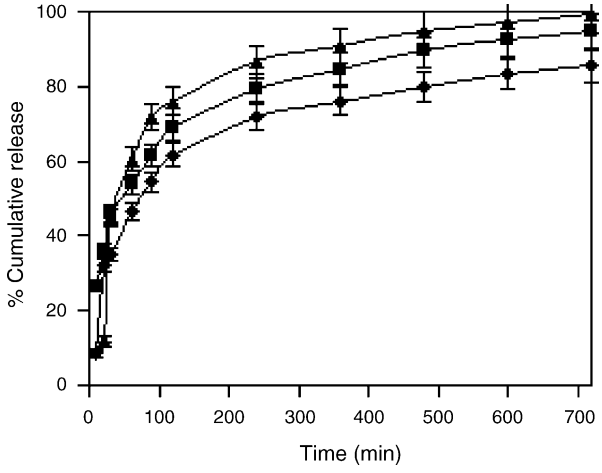

Fig. 4. % Cumulative release of 5-fluorouracil through pAAm microparticles crosslinked with 4 wt.% NNMBA and containing (●) 10%, (■) 20% and (▲) 30% of 5-fluorouracil.

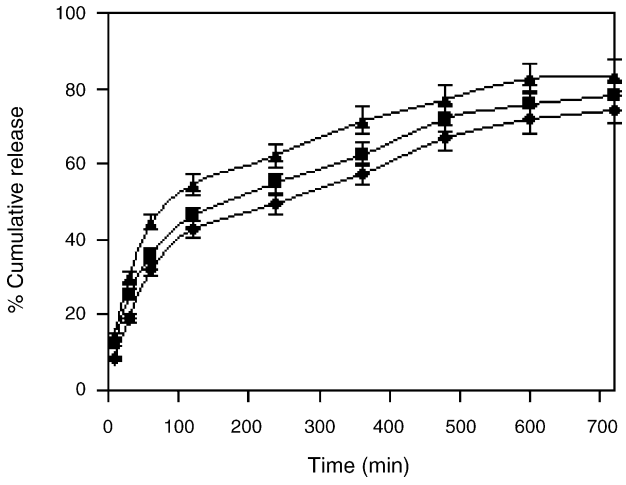

Fig. 5. % Cumulative release of 5-fluorouracil through pAAm microparticles crosslinked with 4 wt.% EGDMA and containing (●) 10%, (■) 20% and (▲) 30 wt.% of 5-fluorouracil.

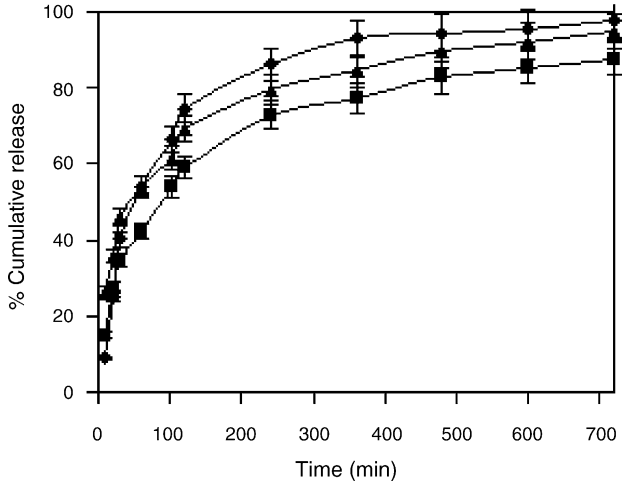

Fig. 6. % Cumulative release of 5-fluorouracil through pAAm microparticles containing 10 wt.% of 5-fluorouracil and crosslinked with (●) 2%, (▲) 4% and (■) 6 wt.% of NNMBA.

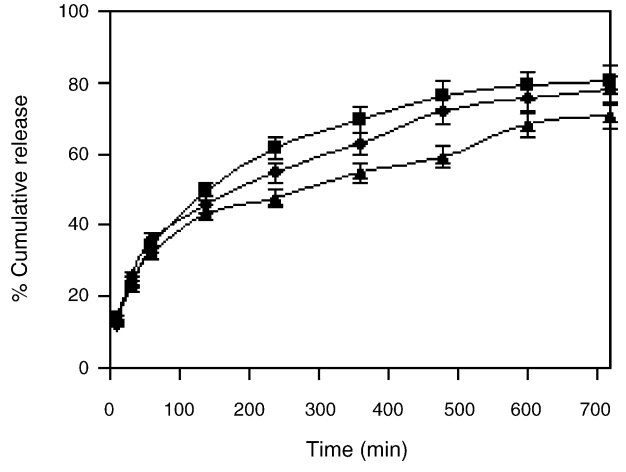

Fig. 7. % Cumulative release of 5-fluorouracil through pAAm microparticles containing 10 wt.% of 5-fluorouracil and crosslinked with (■) 2%, (●) 4%, and (▲) 6 wt.% of EGDMA.

when a lower amount of NNMBA or EGDMA is used, whereas the release becomes quite slower at higher amount of NNMBA or EGDMA (i.e., 6 wt.%) in microparticles. Thus, cumulative release is slower at higher amount of crosslinking agent because at higher concentration of crosslinking agent, the polymeric chains would become more rigid due to the contraction of microvoids. This would decrease the swelling, which will further decrease the % cumulative release of 5-fluorouracil through the microspheres. As expected, the drug release becomes slower at the higher amount of the crosslinking agent, but it becomes faster at lower amount of *N*,*N*′-methylene bisacrylamide (NNMBA) and ethyleneglycol dimethacrylate (EGDMA).

3.5.3. Nature of crosslinking agent

Figs. 6 and 7 show the *in vitro* drug release patterns of pAAm microparticles containing different amounts of NNMBA or EGDMA. Comparatively, a better encapsulation efficiency was observed for the hydrophilic NNMBA crosslinking agent than the hydrophobic EGDMA. This is attributed to lesser interaction of the hydrophilic drug with the hydrophobic crosslinking agent when present in the microparticles. Generally, the drug release through microparticles depends upon particle size, polymer crystallanity, surface character, molecular weight, polymer composition, swelling ratio, degradation rate, drug binding affinity, rate of hydration, etc (Ratner et al., 1996). Prolonged drug release was observed from the microparticles containing hydrophobic crosslinking agent than that of the hydrophilic crosslinking agent. EGDMA, being hydrophobic in nature, the swelling of microparticles crosslinked with EGDMA, will thus be lesser in the buffer medium than those prepared with NNMBA.

3.5.4. Drug release kinetics

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

$$\left(\frac{M_t}{M_\infty}\right) = kt^n \tag{2}$$

Here M_t/M_∞ represents the fractional drug release at time t, k a constant characteristic of the drug–polymer system and the exponent, 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 nine formulations and these values are given in Tables 3 and 4. If $n = 0.5$, the drug diffuses and releases from the polymer matrix following a Fickian diffusion. For $n > 0.5$, an anomalous or non-Fickian

Table 3
Release kinetics parameters of different formulations

Formulation code	k	n	Correlation coefficient, r
F-1	0.1283	0.346	0.806
F-2	0.0682	0.508	0.974
F-3	0.0494	0.660	0.813
F-4	0.0382	0.594	0.862
F-5	0.0277	0.438	0.947

Table 4
Release kinetics parameters of different formulations

Formulation code	k	n	Correlation coefficient, r
F-6	0.0301	0.524	0.951
F-7	0.0525	0.438	0.964
F-8	0.0637	0.430	0.945
F-9	0.0479	0.467	0.989
F-10	0.0637	0.378	0.970

type drug diffusion occurs. If $n = 1$, a completely non-Fickian or Case II release kinetics is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type transport (Ritzer and Peppas, 1987).

The values of k and n have shown a dependence on the extent of crosslinking as well as the % drug loading in the matrix. Values of n for the microparticles prepared by varying the amount of NNMBA (i.e., 2, 4 and 6 wt.%) by keeping 5-fluorouracil (10 wt.%) i.e., 0.346–0.660 in the microparticles has shifted drug transport from Fickian to anomalous type. The microparticles containing different amounts 5-fluorouracil have the n values ranging from 0.346 to 0.594 (see Table 3) and the microparticles croslinked with EGDMA are in the range of 0.378–0.524 (see Table 4), indicating a shift from erosion type release to a swelling-controlled, non-Fickian type of mechanism. This is possibly due to a reduction in the regions of low microviscosity and closure of microcavities in the swollen state. On the other hand, the values of k are quite smaller for drug-loaded microspheres, suggesting their lesser interactions compared to those micropsheres prepared by varying amounts of NNMBA and EGDMA. The correlation coefficient r values of microparticles crosslinked with NNMBA and EGDMA are in the range of 0.806–0.974 and 0.951–0.989, respectively (Tables 3 and 4).

4. Conclusions

Crosslinked poly(acrylamide) particles prepared by dispersion polymerization were used for the controlled release of 5-Fluorouracil. The size of the particles was found to be in the range of 2–3.5 μm. DSC and XRD studies have indicated a molecular level dispersion of the drug in the prepared poly(acrylamide) particles during *in situ* loading. SEM pictures have shown the formation of spherical and oval-shaped particles. Higher encapsulation efficiency up to 82.05 was achieved for particles crosslinked with NNMBA, whereas prolonged drug release was observed when EGDMA was used as a crosslinking agent. Nature as well as the amount of crosslinking agent and the amount of drug loaded into the microparticles have shown an effect on encapsulation efficiency as well as on *in vitro* release of 5-fluorouracil from the pAAm microparticles. Drug release studies indicated the controlled release of 5-fluorouracil up to 14 h.

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