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Synthesis and characterization of semi-interpenetrating polymer network microspheres of acrylamide grafted dextran and chitosan for controlled release of acyclovir ☆

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Abstract

Semi-interpenetrating polymer network (IPN) microspheres of acrylamide grafted on dextran (AAm-*g*-Dex) and chitosan (CS) were prepared by emulsion-crosslinking method using glutaraldehyde (GA) as a crosslinker. The grafting efficiency was found to be 94%. Acyclovir, an antiviral drug with limited water solubility, was successfully encapsulated into IPN microspheres by varying the ratio of AAm-*g*-Dex and CS, % drug loading and amount of GA. Microspheres were characterized by FT-IR spectroscopy to assess the formation of IPN structure and to confirm the absence of chemical interactions between drug, polymer and crosslinking agent. Particle size was measured using laser light scattering technique. Microspheres with average particle sizes in the range of 265–388 μm were obtained. Differential scanning calorimetry (DSC) and X-ray diffraction (X-RD) studies were performed to understand the crystalline nature of drug after encapsulation into IPN microspheres. Acyclovir encapsulation of up to 79.6% was achieved as measured by UV spectroscopy. Both equilibrium and dynamic swelling studies were performed in 0.1 N HCl. Diffusion coefficients (D) and diffusional exponents (n) for water transport were determined using an empirical equation. In vitro release studies indicated the dependence of drug release rates on both the extent of crosslinking and amount of AAm-*g*-Dex used in preparing microspheres; the slow release was extended up to 12 h. The release rates were fitted to an empirical equation to compute the diffusional exponent (n), which indicated non-Fickian trend for the release of acyclovir.

Keywords: Hydrogels; Crosslinking; Graft copolymer; Interpenetrating polymer networks; Drug delivery systems; Microspheres; Acyclovir

1. Introduction

Hydrogels are the three-dimensional network polymers that are known to swell in aqueous solutions. In the swollen state, they are soft and rubbery, resembling the living tissue exhibiting excellent biocompatibility (Hoffman, 2002). Polymeric hydrogels are of considerable interest as biomaterials in drug delivery research (Coviello et al., 2005; Liu, Lin, Lin, & Liu, 2005; Peppas, Bures, Leobandung, & Ichikawa, 2000; van Tomme, van Steenbergen, De Smedt, van Nostrum, & Hennink, 2005). In pharmaceutics area, carbohydrate polymers are often preferred over synthetic polymers due to their non-toxic, low cost, ease of availability and biodegradability characteristics. The hydrogels of modified carbohydrate polymers have been extensively used in controlled release (CR) applications of pharmaceutical proteins as well as in tissue engineering (Chen et al., 2004; Franssen, Vandervennet, Roders, & Hennink, 1999). Among many methods of modifying the original structure of polymers, graft copolymerization is an easier method, which makes the derived polymer as attractive biomaterials in CR applications (Soppimath & Aminabhavi, 2002).

Among the many natural carbohydrate polymers, dextran (Dex) is a polysaccharide consisting of glucose

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molecules coupled into long branched chains, mainly through 1,6- and some through 1,3-glucosidic linkages. Dextrans are colloidal, hydrophilic and water-soluble substances that have excellent biocompatibility and hence, they do not affect cell viability. Because of these properties, dextrans have been used as blood expanders to maintain or replace blood volume. Dextrans are also used as carriers to study the CR of a variety of therapeutic agents including antidiabetics, antibiotics, anticancer drugs, peptides and enzymes (Hennink, De Jong, Bos, Veldhuis, & van Nostrum, 2004; Kosmala, Henthorn, & Peppas, 2000; Stenekes & Hennink, 1999). Dextrans can be degraded by the dextranase enzyme, which is present in colon (Hovgaard & Brondsted, 1995). Chitin, a poly-β-(1 → 4) linked *N*-acetyl-D-glucosamine, is a polysaccharide widely distributed in nature, whereas chitosan (CS) is obtained by deacetylation of chitin. Chitin and CS are the well-known biocompatible and biodegradable carbohydrate polymers that are widely used in biomedical applications (Berger et al., 2005) including wound dressings and drug delivery systems (Lu, Steenekamp, & Hamman, 2005). Chitosan has many pharmaceutical applications (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004) as a bioadhesive polymer (Agnihotri & Aminabhavi, 2004; Hejazi & Amiji, 2003). However, the biodegradation characteristics of CS are dependent upon the degree of deacetylation.

Acyclovir, previously known as acycloguanosine, has potent inhibitory effects on viruses of the herpes group, particularly herpes simplex virus (HSV, I and II) and herpes zoster varicellaous virus. It also combines inhibitory effects on hepatitis B virus with very low toxicity to mammalian host cells (Haynes, Lambert, & Mitchell, 1996). Various reports have indicated that acyclovir is as effective as or even superior to other antiviral agents with lower host toxicity and milder side effects (Tu, Wang, Yang, Fei, & Li, 2001). Since acyclovir has a short half-life (2–3 h), and its oral dosage forms must be taken five times daily, which is very inconvenient for patients. Hence, researchers have been studying the CR applications of acyclovir (Rossi, Sandri, Ferrari, Bonferoni, & Caramella, 2003a, Rossi, Sandri, Ferrari, Bonferoni, & Caramella, 2003b; Sandri et al., 2004). The principle objective of this study is to develop CR formulations of acyclovir that may be taken twice daily. In this paper, we report the synthesis of pAAm-*g*-Dex to prepare the IPN microspheres with CS crosslinked by GA for the CR of acyclovir. The IPNs are a combination of two or more polymers in a network form that are held together by topological bonds without the formation of covalent bonds between them (Kim & Sperling, 1997). Thus, an IPN structure can be obtained when at least one polymer network is synthesized and/or crosslinked independently in the immediate vicinity of another. As long as the reacting ingredients are blended thoroughly during the synthesis, thermodynamic incompatibility can be made to overcome due to the permanent interlocking of the network segments. Hence, IPN based systems have gained good potential to develop the CR systems.

Earlier, we have reported many IPN-based formulations for the CR of a variety of drugs (Agnihotri & Aminabhavi, 2005; Kurkuri & Aminabhavi, 2004; Rokhade et al., in press; Soppimath, Kulkarni, & Aminabhavi, 2000). In continuation of these studies, we now present synthetic protocols for the preparation of semi-IPN microspheres of acrylamide grafted dextran and CS for the CR of acyclovir. The microspheres formed have been characterized by FT-IR, X-RD and DSC techniques. In vitro release studies have been performed by dissolution experiments. Release data have been discussed in terms of Fickian equation and diffusion parameters.

2. Experimental

2.1. Materials

Acyclovir was obtained as a gift sample from Matrix Laboratories, Hyderabad, India. Dextran, MW ≈ 85,000 was purchased from Hi-media Chemicals Pvt. Ltd., Mumbai, India. CS was procured from Aldrich Chemical Company, Milwaukee, WI, USA. Analytical reagent grade acrylamide, glutaraldehyde solution 25% (v/v), ceric ammonium nitrate, *n*-hexane and light liquid paraffin were all purchased from s.d. fine Chemicals, Mumbai, India. Span®-80 was purchased from Loba Chemicals, Mumbai, India. All the chemicals were used without further purification.

2.2. Synthesis of graft copolymer of dextran–acrylamide

The graft copolymer of dextran and acrylamide was prepared by free radical polymerization. Briefly, 2 g of dextran was dissolved in 70 mL of water and allowed to stir overnight in a 250 mL round-bottom flask. Then, 0.12 mol of AAm was separately dissolved in 20 mL water, which was added to dextran solution and allowed to mix uniformly for 1 h. To this solution, 10 mL of 5 mM ceric ammonium nitrate was added. Polymerization was carried out at 60 °C under a continuous purging of nitrogen gas for 6 h in a water bath with constant stirring. After complete polymerization, a sufficient amount of methanol was added to precipitate the graft copolymer and to remove any homopolymer formed. The polymer was dried under vacuum (60 mmHg pressure) at 40 °C overnight. Mass of the polymer was taken and % grafting efficiency was calculated as

$$\%\ \textit{Grafting efficiency}\ (\%GE) = \left(\frac{W_1 - W_0}{W_2}\right) \times 100, \qquad (1)$$

where W_0, W_1 and W_2 denote weights of dextran, graft copolymer and monomer, respectively. The synthetic scheme for the formation of graft copolymer is shown in Fig. 1.

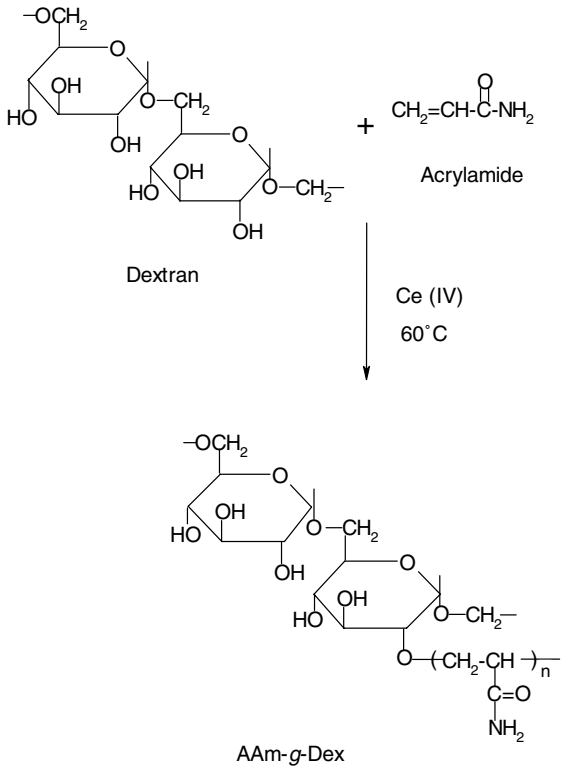

Fig. 1. Schematic representation of the synthesis of AAm-g-Dex.

2.3. Preparation of semi-IPN microspheres

Semi-IPN microspheres of AAm-*g*-Dex and CS were prepared by emulsion-crosslinking method. Briefly, AAm-*g*-Dex and CS were dissolved in 2% aqueous acetic acid with continuous stirring until a homogeneous solution was obtained. A required amount of drug was directly dissolved in polymer solution and allowed to stir for overnight. This solution was added slowly to the light liquid paraffin (100 g) containing 1% (w/w) Span®-80 under constant stirring at 400 rpm for 10 min. To this w/o emulsion, the required amount of GA was added slowly and stirring was continued for 2 h. Hardened microspheres were separated by filtration and washed with *n*-hexane. The microspheres were vacuum dried at 40 °C for 24 h and stored in a desiccator until further use. Totally, 12 formulations were prepared and the assigned formulation codes are given in Table 1.

2.4. Drug content

Acyclovir content was estimated in distilled water. Microspheres (~10 mg) were ground to powder using an agate mortar and then extracted for 18 h at 25 °C in 50 mL distilled water and sonicated for 1 h (UP 400s, Dr. Hielscher, GmbH, Germany). The solution was centrifuged (Jouan, MR23i, France) to remove the polymeric debris and washed twice to completely extract the drug. The clear supernatent solution was then analyzed by UV spectrophotometer (Secomam, model Anthelie, Paris, France) at the

Table 1
Formulation parameters used for the preparation of microspheres

Formulation codes	Chitosan (% w/w)	AAm-*g*-Dex (% w/w)	Drug loading (%)	GA (mL)
F1	80	20	25	3
F2	80	20	25	6
F3	80	20	25	9
F4	80	20	50	3
F5	80	20	50	6
F6	80	20	50	9
F7	60	40	25	3
F8	60	40	25	6
F9	60	40	25	9
F10	60	40	50	3
F11	60	40	50	6
F12	60	40	50	9

λ_{max} value of 254 nm. The % encapsulation efficiency was calculated as

$$\%\ Encapsulation\ efficiency = \left(\frac{Drug loading}{Theoretical drug loading}\right) \times 100. \quad (2)$$

These data for various formulations are presented in Table 2.

2.5. Particle size measurements

Particle size and size distributions were measured using a laser light scattering technique (Mastersizer-2000, Malvern, UK). Particle size was measured by using a dry sample adopter to calculate volume mean diameter (V_d). These data are also included in Table 2.

2.6. Fourier transform infrared (FT-IR) spectral measurements

FT-IR spectral data were taken on a Nicolet (Model Impact 410, Milwaukee, WI, USA) instrument to confirm the formation of IPN structure and also to find the

Table 2
Results of % entrapment efficiency, volume mean particle size, % water uptake and *n* values

Formulation code	% Entrapment efficiency	Volume mean particle size (μm)	% Water uptake	*n*
F1	48.04	306	495	0.128
F2	57.52	288	406	0.294
F3	63.28	265	226	0.447
F4	70.63	340	287	0.321
F5	74.26	296	229	0.229
F6	79.67	277	195	0.465
F7	37.58	345	664	0.346
F8	46.72	305	501	0.198
F9	59.33	282	495	0.217
F10	65.14	388	415	0.374
F11	72.63	368	392	0.287
F12	76.47	289	281	0.389

chemical stability of the drug in the microspheres. FT-IR spectra of the placebo microspheres, drug-loaded microspheres and the neat drug were obtained. The samples were ground with KBr and pellets were obtained by applying a pressure of 600 kg/cm^2. Spectral scanning was done in the range between 4000 and 500 cm^{-1}.

2.7. Differential scanning calorimetric (DSC) experiments

Differential scanning calorimetry (Rheometric Scientific, Surrey, UK) was performed on the drug-loaded microspheres, placebo microspheres and pristine drug. Samples were heated from 25 to 400 °C at the rate of 10 °C/min in nitrogen atmosphere (flow rate, 20 mL/min).

2.8. X-ray diffraction experiments

Crystallinity of acyclovir after encapsulation was evaluated by X-RD measurements recorded for placebo microspheres, drug-loaded microspheres and pristine drug using X-ray diffractometer (x-Pert, Philips, UK). Scanning was done up to 2θ of 50°.

2.9. Swelling experiments

Equilibrium water uptake by the microspheres was determined by measuring the extent of swelling of the matrix in distilled water. To ensure complete equilibration, samples were allowed to swell for 24 h. Excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed to an accuracy of ±0.01 mg on an electronic microbalance (Mettler, AT120, Greifensee, Switzerland). The hydrogel microspheres were then dried in an oven at 60 °C for 5 h until there was no change in the weight of the dried mass of the samples. The % equilibrium water uptake was calculated as

$$\left(\frac{\textit{Mass of swollen microspheres} - \textit{Mass of dry microspheres}}{\textit{Mass of dry microspheres}}\right) \times 100. \quad (3)$$

Drug release from the crosslinked hydrogel depends upon the extent of water penetration into the matrix. In order to understand the molecular transport of water into crosslinked microspheres, dynamic swelling studies were performed by the microscopic technique (Robert, Bun, & Peppas, 1985; Soppimath & Aminabhavi, 2002). The change in diameter of the microspheres in the presence of distilled water was monitored as a function of time. Experiments were performed in triplicate, but average values were considered for data treatment and calculations.

2.10. In vitro release experiments

Drug release from semi-IPN microspheres with different % drug loading, polymer composition and different extent of crosslinking were investigated in 0.1 N HCl for the initial 2 h, followed by phosphate buffer, pH 7.4, until completion of dissolution. These experiments were performed using a fully automated dissolution tester coupled with a UV system (Logan Instruments Corp., Model D 800, NJ, USA) equipped with six baskets at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500 mL of the dissolution medium maintained at 37 °C. The instrument automatically measures the concentration of drug released at particular time intervals by a UV spectrophotometer coupled with flowthrough cells attached to the instrument, and it then puts the solution back into the dissolution bowl. The acyclovir concentration was determined using the UV spectrophotometer at λ_{max} of 254 nm. These studies were performed in triplicate for each sample, but average values were considered in data analysis.

3. Results and discussion

3.1. Preparation and characterization of microspheres

Graft copolymerization of dextran with acrylamide was attempted by Ce(IV) catalyzed free radical polymerization. The chelate complex formed with the –OH group of dextran decomposes to generate the free radical site, facilitating the grafting to occur at the active site of dextran with the incoming acrylamide monomer. The reaction is shown in Fig. 1. The grafting efficiency was found to be 94%. The mechanism involved is somewhat complicated and the reaction was carried out at 60 °C for 6 h. However, this may create some problem for the facile scaling-up of the preparation. As far as toxicology is concerned, according to Jakupec, Unfried, and Keppler (2005), it was observed that Ce(IV) salts are not biologically stable in the aqueous media. Therefore, cerium species that will freely circulate in the blood as colloidal compounds or protein complexes are likely to contain Ce(III). It is also reported that these ions are non-cytotoxic. Many studies have been reported in the literature to evaluate the safety of glutaraldehyde (GA) and it has been proven that it is non-carcinogenic and safe (Garcia-Sellas et al., 2003). Since many drug formulations are prepared in either w/o or o/w emulsification, hence the method involved in the present study may not pose difficulty in facile scale-up.

The acyclovir-loaded semi-IPN microspheres of AAm-*g*-Dex and CS were prepared by crosslinking with GA. By this method, % encapsulation efficiency ranged between 37.6 and 79.6. However, % encapsulation efficiency showed a dependence on % drug-loading, polymer composition and extent of crosslinking. The % encapsulation efficiency was found to be greater in formulations containing higher drug loadings. This is due to the limited water solubility of the drug thereby, leading to the retention of more of drug particles while preparing the microspheres. As the amount of crosslinking is increased, there is a slight increase in % encapsulation efficiency due to the formation of a rigid net-

work structure, which reduces the possibility of leaching out of the drug during the microsphere preparation. By increasing the content of graft copolymer, a slight decrease in % encapsulation efficiency was observed, which is attributed to greater swelling of the matrix, which will allow leaching out of drug particles during the preparation of microsphere.

Particle size (see Table 2) revealed an increase with increasing amount of graft copolymer. It was found that particle size of F7 (40%, w/w, AAm-*g*-Dex) is higher than that of F1 (20%, w/w, AAm-*g*-Dex) and similar findings were observed for F8 and F9 formulations as compared to F2 and F3. This could be due to the fact that at higher amounts of AAm-*g*-Dex, the viscosity of polymer solution increased, thereby producing bigger droplets during emulsification that were later hardened in the presence of GA. Another interesting observation is that the particle size decreased with increasing extent of crosslinking. Thus, the particle size of F4 (3 mL GA added) is higher than that of F5 (6 mL GA added) and F5 is higher than F6 (9 mL GA added). This is due to the formation of a more rigid polymer network at higher extent of crosslinking. Also, the particle size varied depending upon the drug loading into the microspheres. For instance, formulation F4 exhibits a higher particle size than F1. Similarly, F5 and F6 have higher particle sizes than F2 and F3 formulations. This is due to the accumulation of slightly water-soluble drug crystals in the polymer matrix at higher drug loadings.

3.2. Fourier transform infrared spectral studies

FT-IR spectra of dextran, chitosan and AAm-*g*-Dex are discussed below. In case of dextran, a broad band at 3406 cm^{-1} is due to O–H stretching vibrations. The O–H bending is seen at 1348 cm^{-1}. Aliphatic C–H stretching and bending vibrations are, respectively indicated by bands at 2929 and 1427 cm^{-1}. The C–O–C stretching vibration is present at 1157 cm^{-1}, while bands at 1013 and 1078 cm^{-1} indicate the presence of C–O stretching vibrations. Chitosan shows a broad band at 3426 cm^{-1} due to N–H stretching vibrations. Three bands observed at 1653, 1604 and 1381 cm^{-1} are due to amide-I, amide-II and amide-III, respectively. Bands at 1023 and 1073 cm^{-1} represent the presence of C–O stretching vibration. In case of AAm-*g*-Dex, all the peaks observed in dextran have appeared. A band at 1669 cm^{-1} confirms the presence of C=O stretching vibration, which is not observed in dextran. This confirms the grafting of acrylamide onto dextran.

FT-IR spectra of placebo microspheres, acyclovir and drug-loaded microspheres are also discussed. In case of placebo microspheres, a broad band at 3414 cm^{-1} indicates the presence of both N–H and O–H stretching vibrations of acrylamide, dextran and chitosan. Aliphatic C–H stretching vibrations are observed at 2936 and 2869 cm^{-1}. Aliphatic bending vibration is represented by a band at 1455 cm^{-1}. The N–H bending is observed at 1567 cm^{-1}. A band at 1669 cm^{-1} reveals the overlapping of C=O stretching vibration of acrylamide and C=N stretching vibration of the imine group of Schiff base. This confirms the crosslinking of amine group of chitosan by GA.

In case acyclovir, bands at 3441 and 3312 cm^{-1} reveal the presence of both N–H and O–H stretching vibrations. Bands at 2930 and 2880 cm^{-1} show the presence of aliphatic C–H stretching vibrations. A band at 1696 cm^{-1} shows the presence of C=O stretching vibrations (amide-I). Bands at 1632 and 1388 cm^{-1} represent amide-II and amide-III bands, respectively. In case of drug-loaded microspheres, all the bands observed in acyclovir have appeared, which shows the absence of any chemical interactions between drug and polymer.

3.3. Thermal studies

DSC thermograms of (a) placebo microspheres, (b) drug-loaded microspheres and (c) pristine acyclovir are displayed in Fig. 2. In case of placebo microspheres, a small peak, a broad peak and a sharp peak were observed at 193, 248 and 432 °C, respectively, due to endothermic transition. Thermogram of acyclovir showed a sharp peak at 258 °C and a small peak at 474 °C, indicating its melting. In case of drug-loaded microspheres, all the peaks observed in placebo microspheres are noticed, but there was no peak corresponding to acyclovir, indicating the amorphous dispersion of acyclovir in semi-IPN matrix developed.

3.4. X-ray diffraction studies

X-ray diffractograms of (a) placebo microspheres, (b) drug loaded microspheres and (c) pristine acyclovir were

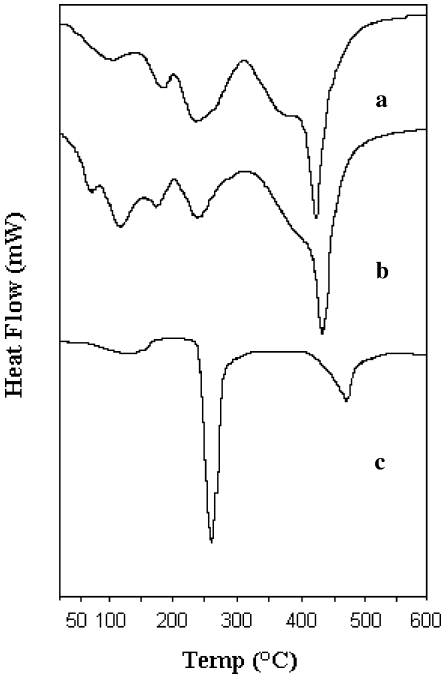

Fig. 2. DSC thermograms of (a) placebo microspheres (b) drug loaded microspheres and (c) pristine acyclovir.

studied. Acyclovir has shown characteristic intense peaks at 2θ of 7° due to its crystalline nature. However, this peak has disappeared in acyclovir-loaded microspheres, but only peaks observed in the placebo polymer matrix were seen. The X-RD peak depends on the crystal size; but in the present study, for all the drug-loaded concentrations, the characteristic peak of acyclovir could overlap with the noise of the coated polymer itself. Further, the loaded drug is amorphous, which is very difficult to measure at a detection limit of the crystal size in the present case. This indicates that drug is dispersed at the molecular level in the polymer matrix and hence, no crystals were found in the drug-loaded matrices.

3.5. Water uptake studies

Drug release rates are influenced by the equilibrium water uptake of the crosslinked microspheres (Ritger & Peppas, 1987). The % equilibrium water uptake data of the crosslinked microspheres presented in Table 2 indicate that, as the amount of GA in the matrices increases from 3 to 9 mL, equilibrium water uptake decreases significantly from 664% to 195%. 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 amounts of AAm-*g*-Dex showed higher swelling rates than those formulations containing lesser amounts of AAm-*g*-Dex. The formulation F7 (40%, w/w, AAm-*g*-Dex) exhibits higher swelling than F1 (20%, w/w, AAm-*g*-Dex). Similar observations were made for formulations F8 and F9 as compared to F2 and F3. This is attributed to the extremely hydrophilic nature of AAm-*g*-Dex matrix, leading to higher water uptake.

Dynamic swelling studies were performed by monitoring the changes in microsphere diameter, D_t, as a function of time using an optical microscope. Fig. 3 displays the plot of normalized diameter, D_t/D_0, (D_0 is initial diameter of the microsphere) as a function of time for different amounts of GA added. It is evident that the normalized diameter decreases with increasing amount of GA, which could be due to the rigid network formed at a higher amount of GA. Fig. 4 shows the plot of D_t/D_0 vs t for different amounts of AAm-*g*-Dex in the matrix. It is observed that the normalized diameter increases with increasing amount of AAm-*g*-Dex, probably due to the extremely hydrophilic nature of AAm-*g*-Dex, which enhances the water transport rate as well as the extent of water uptake from the microspheres. The results of equilibrium swelling diameter, D_∞ normalized to the original diameter, D_0 are presented in Table 3.

The dimensional changes of the microspheres due to swelling (i.e., volume change ΔV_t with time with respect to initial volume, V_0) have been analyzed to calculate diffusion coefficient, D_v of water molecules (Harogoppad & Aminabhavi, 1992).

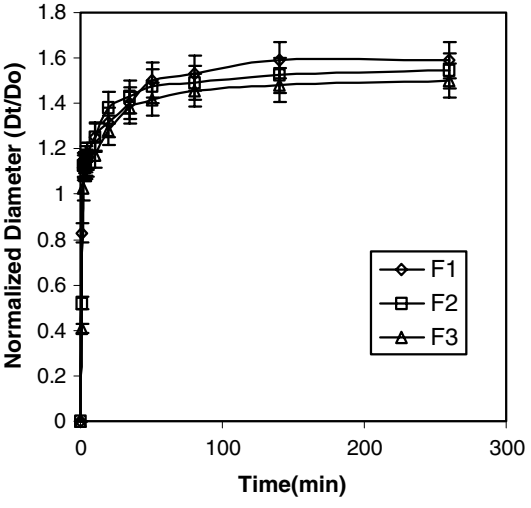

Fig. 3. Plot of D_t/D_0 vs time, t is the effect of extent of crosslinking of formulations F1 (3 mL GA), F2 (6 mL GA) and F3 (9 mL GA).

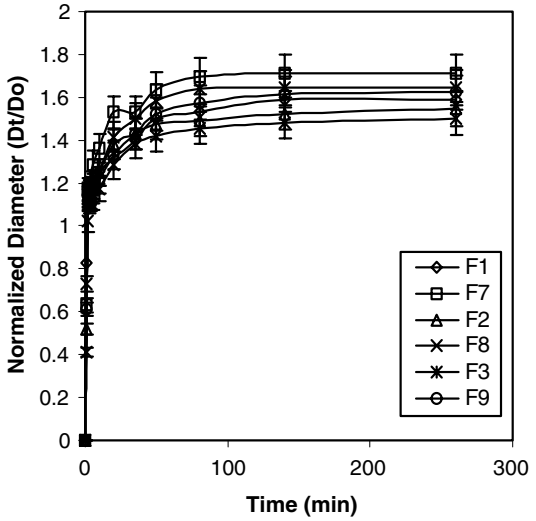

Fig. 4. Plot of D_t/D_0 vs time, t is the effect of amount of AAm-*g*-Dex on formulations F1 (20%), F7 (40%), F2 (20%), F8 (40%), F3 (20%) and F9 (40%) containing AAm-*g*-Dex.

Table 3
Transport data of water in microspheres

Formulation code	Equilibrium normalized diameter (D_∞/D_0)	n	$D_v \times 10^5$ (cm^2/s)	$u \times 10^3$ (cm/s)
F1	1.591	0.141	3.93	3.64
F2	1.545	0.284	3.24	2.95
F3	1.501	0.364	1.91	1.58
F4	1.792	0.293	5.54	4.97
F5	1.686	0.433	4.33	3.49
F6	1.641	0.397	3.17	2.33
F7	1.714	0.340	4.48	4.53
F8	1.643	0.227	3.68	3.87
F9	1.623	0.189	2.46	1.86
F10	1.923	0.438	6.93	7.21
F11	1.674	0.272	4.87	5.47
F12	1.444	0.368	3.98	3.82

$$\left(\frac{\Delta V_t}{V_0}\right) = \left(\frac{4\left(\frac{\Delta V_\infty}{V_0}\right)}{D_0}\right)\left(\frac{D_v}{\pi}\right)^{1/2} t^{1/2} \quad (4)$$

$$D_v = \left[(1.773 \times \text{Slope})\frac{V_0 D_0}{4\Delta V_\infty}\right]^2 \quad (5)$$

Here, ΔV_∞ represents the change in volume at equilibrium condition. Eq. (5) is used to calculate the values of D_v from the slope of the initial linear plots of $\Delta V_t/V_0$ vs $t^{1/2}$. These data are also included in Table 3.

The solvent front velocity, u, of the advancing boundary for the spherical microspheres was calculated using the relation

$$u = \left(\frac{dv}{dt}\right)\frac{1}{A}. \quad (6)$$

Here, dv/dt is the change in volume of the microsphere per unit time and A is the area of the microsphere. The results of u are also included in Table 3. The values of diffusion coefficients and solvent front velocity decrease with increasing crosslink density, i.e., by increasing the amount of GA from 3 to 9 mL, a considerable decrease in diffusion coefficients from 6.93×10^{-5} to 1.91×10^{-5} cm^2/s, and similarly, decrease in solvent front velocities from 5.47 to 1.58 cm/s are observed. However, by increasing the amount of AAm-*g*-Dex, diffusion coefficients as well as solvent front velocities increased due to the extremely hydrophilic nature of AAm-*g*-Dex matrix. Molecular transport through microspheres is thus dependent upon the extent of crosslinking and the present results support that more rigid crosslinked matrix does not expand much as compared to loosely crosslinked matrix. At lower amounts of GA, the network is loose and has a high hydrodynamic free volume to accommodate more of solvent molecules, thereby causing matrix swelling. Water uptake in hydrogels depends upon the extent of hydrodynamic free volume available as well as hydrophilic functional groups for water to establish hydrogen bonds. Higher water uptake values observed at lower levels of crosslinking and vice versa observed in the present systems confirm the formation of semi-IPN due to matrix crosslinking.

Dynamic swelling data of all the formulations have been fitted to an empirical equation (Robert et al., 1985; Soppimath et al., 2000) of the type

$$\frac{D_t}{D_\infty} = kt^n. \quad (7)$$

Here, k is rate constant and n is an exponent parameter that represents the type of transport. Least-squares estimations of the values of n from the dynamic swelling data after fitting to Eq. (7) are presented in Table 3. The values of n range between 0.141 and 0.438 with increasing GA from 3, 6 and 9 mL, respectively. These values are in agreement with our earlier reported data (Rokhade et al., in press; Soppimath et al., 2000; Soppimath & Aminabhavi, 2002; Korsmeyer & Peppas, 1981), indicating the non-Fickian diffusion trends.

3.6. In vitro release studies

To understand the release of acyclovir from the semi-IPN microspheres of AAm-*g*-Dex and CS, in vitro release experiments were carried out in gastric and intestinal pH conditions. The % cumulative release vs time plots for the drug-loaded microspheres for formulations F1, F2 and F3 are compared in Fig. 5 to investigate the extent of crosslinking on in vitro release profiles. The F1 shows higher release rate than F2 and similarly, F2 exhibits higher release rate than F3. This is due to the formation of a more tightly crosslinked rigid network structure as the amount of crosslinking agent has increased from 3 to 9 mL.

Effects of polymer ratio in formulations F1, F7, F2, F8, F3 and F9 on release rates are presented in Fig. 6. The

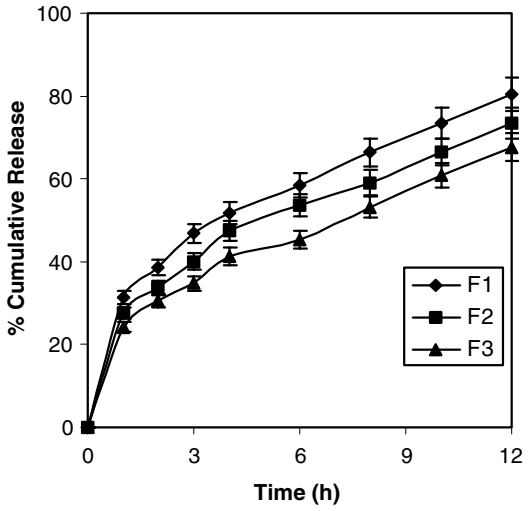

Fig. 5. Effect of crosslinking on in vitro release profile of formulations F1 (3 mL GA), F2 (6 mL GA) and F3 (9 mL GA).

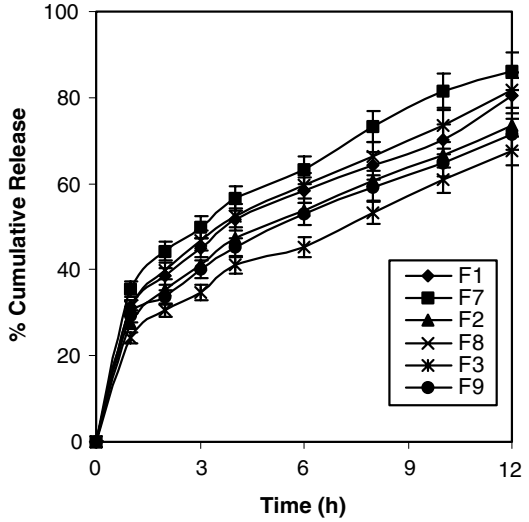

Fig. 6. Effect of polymer ratio on in vitro release profile of formulations F1 (20%), F7 (40%), F2 (20%), F8 (40%), F3 (20%) and F9 (40%) containing AAm-*g*-Dex.

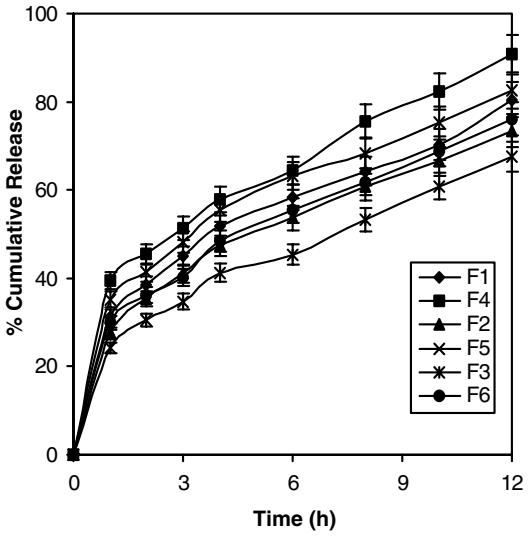

Fig. 7. Effect of% drug loading on in vitro release profile of formulations F1 (25%), F4 (50), F2 (25%), F5 (50%), F3 (25%) and F6 (50%).

% cumulative release is higher in case of F7 than F1 because as the AAm-*g*-Dex content in the polymer matrix increases, swelling of the matrix also increases due to hydrophilic nature of AAm-*g*-Dex. Similarly, F8 and F9 show higher release rates than F2 and F3. The effect of % drug loading on in vitro release profiles for formulations F1, F4, F2, F5, F3 and F6 are displayed in Fig. 7. The formulation F4 exhibits a higher release rate than F1. Similarly, F5 and F6 show higher release rates than F2 and F3. Thus, the release rates vary depending upon the amount of drug present in the matrices, i.e., release is higher for those formulations having higher amount of drug and vice versa. During the first 2 h of drug release, dissolution was performed in 0.1 N HCl, wherein we could observe a burst release effect. Such burst effect is observed in all formulations, but the release of drug was extended up to 12 h. However, none of the formulations showed 100% drug release.

In order to establish a link between drug release and molecular transport parameters, we have fitted the release data to an empirical equation (Ritger & Peppas, 1987)

$$\frac{M_t}{M_\infty} = kt^n. \tag{8}$$

Here, k and n have the same meanings as indicated in Eq. (7). The n values calculated by using Eq. (8) are also included in Table 2. The n values for microspheres range from 0.185 to 0.481, indicating that drug release in the microspheres follows non-Fickian trends, as was also suggested in earlier reports (Korsmeyer & Peppas, 1981; Rokhade et al., in press; Soppimath et al., 2000). The n values for microspheres crosslinked with 3 mL of GA are smaller than those observed with 6 and 9 mL of GA. This is because of the loose crosslinking of the network matrix, thereby leading to greater swelling. The values of n are higher for microspheres crosslinked with 9 mL of GA due to a tighter crosslinking of the IPN matrix.

4. Conclusions

This work demonstrates the usefulness of semi-IPN microspheres in size ranges of 265–388 μm prepared from acrylamide grafted dextran and CS and used in the CR of acyclovir. It is found that the drug encapsulated microspheres prepared by water-in-oil emulsion method could successfully extend the release of acyclovir. However, the initial burst effect with a cumulative release ranging from 20% to 40% is attributed to the sudden release of drug from the matrices, which later extended up to about 12 h with a cumulative release of up to 80%. However, % cumulative release rates depend upon the nature of the formulated product. The % encapsulation efficiency up to 80% was observed depending upon the nature of the matrix. Diffusion coefficient and diffusional exponents calculated from the empirical equations have indicated the non-Fickian nature of transport of drug through the matrices developed.

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