ORIGINAL ARTICLE

Biodegradable interpenetrating polymer network hydrogel membranes for controlled release of anticancer drug

B. Mallikarjuna[1,2], K. Madhusudana Rao[3], K. Pallavi[1], K. Chowdoji Rao[4], M. C. S. Subha[1]

[1]Department of Chemistry, Sri Krishnadevaraya University, Anantapur, [2]Department of Chemistry, Government Degree College (A), Rajahmundry, East Godavari, [4]Department of Polymer Science and Technology, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India, [3]Department of Polymer Science and Engineering, Pusan National University, Busan *609-735*, South Korea

Biodegradable interpenetrating polymer network hydrogel membranes of chitosan and gelatin were prepared by solvent casting evaporation method for the controlled release of 5-fluorouracil (5-FU), an anticancer drug. The membranes were characterized by Fourier Transform Infrared Spectroscopy (FTIR), differential scanning calorimeter (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM) and Tensile strength measurements. The FTIR was used to understand the formation of crosslinking and to confirm the absence of chemical interactions between drug and membranes. DSC and XRD studies were performed to understand the crystalline nature of drug after encapsulation into the membranes. SEM was used to study the surface morphology of the membranes. The *in vitro* studies were carried out in phosphate buffer pH 7.4 at 37°C. The results of controlled release tests showed that the amount of 5-FU release increased with the increasing the amount of gelatin in the membrane. Moreover, the release rate of drug increased as the amount of drug loaded in the membranes increased. All the results indicated that the prepared membrane was potentially useful in drug-delivery systems, and the prolonged release rate was observed up to 12 h.

Key words: *5-flurouracil, chitosan, drug-delivery systems, gelatin, hydrogel membrane*

INTRODUCTION

Interpenetrating polymer network (IPN) is a combination of two polymers exhibiting varied characteristics. Whenever an IPN hydrogel is formed from two polymers at a given temperature, the physical phase separation between the component polymers would be almost impossible because of the infinite zero viscosity of the gel. Some of the applications of IPNs include artificial implants, dialysis membranes and drug-delivery systems.[1] IPN hydrogels used as controlled release systems are capable of delivering drugs at constant rate over an extended period. Therefore, the formation of IPN appears to be a better approach.[2] IPN hydrogel has more complicated network structures and possesses improved mechanical properties; in such systems, the crosslinking can be monitored to control the drug-release.[3,4]

Address for correspondence:
Prof. M. C. S. Subha,
Department of Chemistry, Sri Krishnadevaraya University,
Anantapur, Andhra Pradesh, India.
E-mail: mcssubha3@gmail.com

Controlled drug-delivery technology using natural, biodegradable carbohydrate polymers as carriers represents one of the most rapidly advancing areas of science. Chitosan [Poly (1,4-β-D-glucopyranosamine)] is the N-deacetylated polysaccharide from chitin that possesses valuable properties for biomedical applications.[5-7] It is a biopolymer that has the same β-(1–4)-D-glucopyranose units backbone as cellulose, except for the 2-hydroxy is replaced by an acetamide group. Chitosan has been well known as being able to accelerate the healing of the wound in human beings.[8,9] Chitosan has been used in biomedicine because of its favorable characteristics, such as good biocompatibility, and it has been reported to be useful for pharmaceutical preparation.[10] Chitosan films are usually prepared by chemical crosslinking with glutaraldehyde (GA).[11,12]

Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.154716

These films swell under acidic conditions because of the ionization of amino groups but remain in a shrunken state under a neutral condition. Moreover, chitosan has been reported to prolong the retention of the dosage form in the stomach. Therefore, chitosan films and other dosage forms have been exploited for sustained oral drug-delivery in the stomach.[13] The main disadvantages of chitosan in drug-delivery systems are that it is only soluble in dilute acetic acid and has low mechanical properties, and its physical properties are highly dependent on the pH. Therefore, it is difficult for controlled drug-release behavior because of the various pHs of the internal organs of the human body, and it may have negative effects in the human body because of the over-release of drugs.

In order to overcome the above defects of the usage of chitosan and to improve the hydrophilic character of chitosan derivatives, researchers have blended chitosan with a hydrophilic polymer. Gelatin is derived from collagen, a natural protein, which is a fibrous material that occurs in the skin, bones and connective tissues of animals.[14] It is insoluble in water and is solubilized by hydrolysis. The raw materials used for its manufacture are obtained from the bovine bones or porcine skins. The reaction can be carried out at an acid pH level, yielding type A gelatin (which is primarily produced from skins) and at the basic pH level giving type B gelatin (primarily produced from the bovine bones). Gelatin is a heterogeneous product that is a mixture of molecular species, α-, β-, and γ-peptides. Their proportions and molecular weights are dependent upon the nature of the chemical process. Gelatin is biocompatible, biodegradable, edible, and soluble at the body temperature, which undergoes gelation at temperatures just above ambient,[15] which makes it an ideal material for pharmaceutical applications. Various researchers have studied gelatin based IPNs and semi-IPNs. Kosmala *et al*.[16] have studied the gelatin blended with dextran and cross-linked to form enzymatically degradable IPNs as matrices for biodegradable implants. Changez *et al*.[17] have studied the *in vivo* efficacy of antibiotics loaded IPN hydrogels based on poly (acrylic acid) and gelatin for the treatment of experimental osteomyelitis. Liang *et al*.[18] have studied gelatin hydrogels cross-linked with genipin and water-soluble carbodiimide and investigated the crosslinking mechanism, extent of crosslinking and cytotoxicity. Zhanfeng Dong *et al*.[19] have prepared gelatin/alginate blend film for control release. Rokhade *et al*.[4] have also prepared gelatin/sodium carboxymethyl cellulose semi-IPN microspheres for control release of ketorolac tromethamine and various chitosan films have also prepared for controlled drug-delivery. Qun wang *et al*.[20] has prepared the chitosan/polyethylene glycol blend film for control release of ciprofloxacin hydrochloride. Jin *et al*.[21] have prepared the chitosan/poly (ethylene oxide) blend membrane for drug-release.

5-fluorouracil (5-FU) is an antimetabolic drug, used extensively in cancer chemotherapy[22,23] and is an antimetabolite, which is used to prevent the subsequent scarring following trabeculectomy and to improve the prognosis for long-term retinal reattachment. 5-FU is an acidic, water soluble,[24] hydrophilic drug and is an antineoplastic agent of extensive use in clinical chemotherapy for the treatment of solid tumors. It has been widely used in drug administration due to its large number of secondary effects that accompany its conventional administration.

Recently, our group is actively involved in the development of IPN based polymeric systems for the controlled release of various types of drugs. Earlier Yerriswamy *et al*.[25] have been prepared hydroxypropyl methylcellulose/poly (vinyl alcohol) IPN blend microspheres for controlled release of the ciprofloxin hydrochloride. Krishna Rao *et al*.[26] have been developed novel chitosan pH sensitive IPN for the controlled release of cefadroxil. The main objective of this study was to develop a chitosan/gelatin IPN material by crosslinking with GA for controlled release of 5-FU. Chitosan has poor mechanical property, to improve the mechanical property of chitosan, the chitosan blended with a hydrophilic polymer gelatin by crosslinking with the GA, it enhance the hydrophilic nature and modern mechanical strength of the membrane. Further, the developed the membrane has good carrier for the controlled release of 5-FU. The *in vitro* drug-release studies were carried out in buffer medium at pH-7.4. The properties of the membranes were investigated with Fourier Transform Infrared Spectroscopy (FTIR), equilibrium swelling studies, differential scanning calorimeter (DSC), X-ray diffraction (XRD) analysis, *in vitro* drug-release studies, and scanning electron microscopy (SEM). The results are presented here.

EXPERIMENTAL

Material

Chitosan (Mwt 160,000, 85% deacetylated and viscosity 800–2000 cps) was purchased from Aldrich, Milawaukee, WI, USA. 5-FU was purchased from Himedia, Mumbai, India. Gelatin, GA, hydrochloric acid (HCl) and acetone are all of AnalaR grade purchased from S. D. Fine, Mumbai, India. Double-distilled water collected in the laboratory was used throughout this research work. All the chemicals were used as received without further purification.

Preparation of the membranes

The membranes were prepared by a solvent/casting evaporation technique. The 2 wt% of chitosan dissolved in 2% acetic acid and 2 wt% of gelatin (at 40°C) was dissolved in distilled water separately. These solutions were mixed in different proportions [Table 1] to obtain a homogeneous solution and different amounts (10, 15 and 20 wt%) of anticancer drug was loaded into the membranes and the solution was stirred 4 h then the solution was filtered to remove the insoluble particles and the obtained solution was poured on a Teflon plate of 20 cm × 15 cm. The membranes were dried in an oven at 37°C until constant

Table 1: Various formulation parameters used in the preparation of the membranes and data obtained from evaluation of the membranes

Sample code	Chitosan (w/w %)	Gelatin (w/w %)	GA (ml)	Drug (wt %)	Thickness of Membrane (μm)	% Encapsulation efficiency	Tensile strength (MPa)
CG-1	60	40	5	10	170 ± 0.051	53.2 ± 0.8	0.310
CG-2	60	40	5	15	170 ± 0.047	56.7 ± 1.4	0.319
CG-3	60	40	5	20	150 ± 0.016	59.4 ± 0.2	0.334
CG-4	60	40	2.5	15	180 ± 0.023	64.8 ± 0.6	0.267
CG-2	60	40	5	15	170 ± 0.047	56.7 ± 1.4	0.319
CG-5	60	40	7.5	15	190 ± 0.054	55.3 ± 1.8	0.419
CG-6	80	20	5	15	160 ± 0.025	56.9 ± 1.1	0.289
CG-2	60	40	5	15	170 ± 0.047	56.7 ± 1.4	0.319
CG-7	40	60	5	15	150 ± 0.059	59.7 ± 0.9	0.378
CG-8	100	-	5	15	120 ± 0.037	49.5 ± 0.6	0.253

weight. Subsequently the dried membranes were cross-linked by dipping in a glass tray containing acetone/water mixture to which different ratios of GA (2.5, 5 and 7.5 ml) and 1 N HCl (activator) was added for 30 min then the membranes were removed and washed with the distilled water to remove the surface adhered crosslinking agent GA and dried in oven for 24 h at 37°C. The prepared membranes were stored in a closed container for further evaluation. The formulation details are given in Table 1.

Swelling studies

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

$$\%SR = \left[\frac{W_s - W_d}{W_d}\right] \times 100 \quad (1)$$

Here W_d and W_s were the weights of dried and swollen membranes, respectively.

CHARACTERIZATION TECHNIQUES

Measurement of thickness

Thickness of the membranes was measured at five different places using a digital micrometer (MDC-25S Mitutoyo, Tokyo, Japan) having an accuracy of 0.001 mm.

Drug content

The membrane of specified area (1 cm^2) was cut into pieces and added to 100 ml of phosphate buffer pH 7.4 for complete swelling at 37°C. The swollen membranes were crushed in a glass mortar with pestle. The solution was then heated gently (30°C–40°C) for 2 h to extract the drug completely and centrifuged using a table-top centrifuge (R-8C DX Remi, India) at 3,000 rpm for 10 min to remove polymeric debris. The clear supernatant solution was analyzed for drug content using UV-vis spectrophotometer (LabIndia-UV3000$^+$, Mumbai, India) (λ_{max}) at 270 nm. The average of three determinations was considered.

$$\%\ \text{Encapsulation efficiency} = \left(\frac{\text{Actual loading}}{\text{Theoretical loading}}\right) \times 100 \quad (2)$$

Fourier transform infrared spectroscopy analysis

The FTIR spectra of plain chitosan, plain gelatin, plain 5-FU, plain membrane, and drug loaded membranes was recorded using FTIR spectroscopy Perkin Elmer (model Impact 410, Wisconsin, MI, USA). The samples were crushed with potassium bromide to make pellets under hydraulic pressure of 600 kg/cm^2 and scanned between 4,000 and 400 cm^{-1}.

Morphology observations

The cross-sectional morphologies of the plain and drug loaded membranes were examined using SEM (JEOL, JSM-6360, Kyoto, Japan). Cross-sectional samples were prepared by fracturing membranes in liquid nitrogen. Prior to observation, samples were arranged on metal grids, using double-sided adhesive tape, and coated with gold under vacuum before observation.

Differential scanning calorimetry studies

The sample membranes were heated from 0°C to 400°C at a heating rate of 10°C/min under nitrogen atmosphere using a DSC TA instruments sequential thermal analyzer (Model-SDT Q600, USA) and then thermograms were obtained.

X-ray diffraction studies

The spectra were recorded using a Philips, PW-171, X-ray diffractometer with Cu-NF filtered CuKα radiation. Quartz was used as an internal standard for calibration. The powder X-ray diffractometer was attached to digital graphical assembly and computer with Cu-NF 25 kv/20 mA tube as a CuKα radiation source in the 2θ range 0°–50°.

Tensile strength measurement

The membranes of 15 cm × 15 cm size were firmly fixed to the jaws of tensile tester (Instron, UK) and tensile strength (TS) of the membranes was measured with an extension speed of 20 mm/min.

***In vitro* release study**

In vitro drug-release study was performed in 600 ml of phosphate buffer solution (PBS) pH-7.4 using tablet dissolution tester (LabIndia, Mumbai, India). The membranes of 4.0 cm^2 areas were mounted. The amount of drug-released was determined by withdrawing 10 ml samples at a specific time intervals for 12 h. The volume withdrawn was replaced with an equal volume of fresh PBS; the samples were analyzed in a UV spectrophotometer (LabIndia-UV3000$^+$, Mumbai, India) λ_{max} at 270 nm using PBS as blank. Drug-release kinetics was analyzed by plotting the cumulative release data versus time by fitting these data to the exponential equation of the type.[27]

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

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 squares procedure, we have estimated the values of n and k for all the formulations and these values are given in Table 2. If $n = 0.5$, the drug diffuses and releases out of the polymer matrix following a Fickian diffusion. For $n > 0.5$, anomalous or non-Fickian 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 anomalous type diffusive transport.[28,29]

RESULTS AND DISCUSSIONS

Development of the membrane

The various blend compositions of chitosan with gelatin were cross-linked by GA to form IPNs having three dimensional network structures to facilitate the entrapment for drug of drug-delivery application. When membrane were cross-linked with GA, a bifunctional crosslinking agent, it forms the Schiff's base structures between amine groups of chitosan with another amine group of gelatin polymer stands and aldehyde groups of GA to form and making the network insoluble. The prepared membranes were thin, flexible and smooth.

Table.2: The release kinetic parameters of k, n, r values at pH-7.4.

Formulation codes	*k*	*n*	Correlation coefficient, *r*
CG -1	0.0306	0.616	0.9088
CG -2	0.0449	0.608	0.8473
CG -3	0.0830	0.522	0.9278
CG -4	0.0545	0.578	0.9469
CG -5	0.0585	0.530	0.9527
CG -6	0.0463	0.623	0.9831
CG -7	0.0484	0.601	0.9726
CG -8	0.0437	0.517	0.8533

Swelling studies

The membranes swelling properties were influenced by the amount of gelatin and crosslinker GA. As the amount of gelatin increases, the SR of membranes increase; it may be due to the enhancement of hydrophilic polymer chains by increase with gelatin concentration. In the case of GA crosslinker variation, the SR decreases with the increase of crosslinker content, it may be due to the formation of polymeric chains which become rigid network as a result of contraction of microvoids. The various formulations and their SRs are shown in the Figure 1.

Fourier transform infrared spectroscopy

Figure 2 shows the FTIR spectra of (a) plain chitosan (b) plain gelatin and (c) plain membrane, whereas Figure 3 shows the FTIR spectra of (a) plain drug (b) drug loaded membrane and (c) plain membrane. FTIR studies were performed to confirm the crosslinking between chitosan and gelatin membrane by GA. From Figure 2a it is noticed that chitosan exhibited a peak at 3440 cm^{-1}, which was assigned to stretching vibration of –N-H and –O-H bond. The peak at 2,922 cm^{-1} was due to –C-H stretch vibration and a peak at 1,631 cm^{-1} was due to the –C = O stretch of the amide bond. FTIR spectra of pure gelatin showed – N-H stretching at 3,436 cm^{-1} and –C = O stretching at 1,639 cm^{-1} of amide. The peak 1,545 cm^{-1} was due to –N-H bending vibration of the amide group in gelatin. In the case of plain membrane [Figure 2c], all the peaks of chitosan and gelatin were observed. In addition, a new peak was observed at 1,647 cm^{-1} indicating the –C = N stretching vibration of the imine group of schiff base. The polymer network formed via -C = N- formation due to amino groups reacting with aldehyde groups of GA. This band confirms the crosslinking between chains by GA. From Figure 3a, it is observed that 5-FU shows peaks at 3068 cm^{-1},1660 cm^{-1},1546 cm^{-1} and 1,246 cm^{-1} were due to = C-H, -C = O, -C-N and –C-F stretching vibrations. But in Figure 3b, that is, 5-FU drug loaded membrane showed the peaks at 3443 cm^{-1}, 1645 cm^{-1}, 1337 cm^{-1}, 1247 cm^{-1} due to -O-H,-C = N,-C-O stretching and bending vibrations, it relatively showed the above drug peaks (1660 cm^{-1}, 1343 cm^{-1}, 1246 cm^{-1}) in the drug loaded membrane, which indicates the drug present in the drug loaded membrane. At the same time in Figure 3b no new characteristic absorption bands of drug loaded membranes was observed; this clearly explains there was no chemical reaction between membrane and drug. As a result, the drug did not lose its activity in the drug loaded membranes.

$$CS - NH_2 + GA - CHO + Gel - NH_2 \rightarrow CS - N = C - GA - C = N - Gel$$

Morphology observations

The SEM photomicrographs of the membranes of plain membrane (a) and drug loaded (b) are presented in Figure 4. Analysis of the morphologies of the plain and drug loaded membranes shows that the cross-section of both is smooth and homogeneous, with the absence of any micro phase separation. Again, the results obtained here showed the good compatibility between the membrane and the drug.

Differential scanning calorimetry studies

Differential scanning calorimetry thermograms of (a) plain drug (b) plain membrane (c) drug loaded membrane are presented in Figure 5. In the case of plain membrane and drug loaded membrane a peak was observed at 120°C due to the endothermic transition. Thermogram of plain drug showed a sharp peak at 287.45°C indicating the melting peak. This melting peak was not found in the drug loaded membrane. This indicates the molecular dispersion of 5-FU into the membrane developed.

X-ray diffraction studies

The X-ray diffractograms of (a) plain chitosan membrane, (b) plain membrane, (c) drug loaded membrane and (d) 5-flrurouracil are presented in Figure 6. These studies are useful to investigate the crystallinity of drug in the cross-linked membranes. 5-FU has shown [Figure 6d] characteristic intense peak at 2θ of 29° due to its crystalline nature. However, this peak is disappeared in the drug loaded membrane. In general, XRD peak depends on the crystal size, but in the present study of all the drug loaded formulations, a characteristic peak of 5-FU could overlap with the noise of the coated polymers 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 the drug is dispersed at the molecular level in the membrane and hence, no crystals were found in the drug loaded membrane.

Tensile strength of the membrane

The improved mechanical strength of the membranes was confirmed by TS measurements. The coarse-graining (CG)-8 membrane made of pure chitosan showed TS of 0.253 MPa, while the membranes prepared including chitosan and gelatin have showed higher TS as shown in Table 1. This may be due to the formation of a large number of links among the polymer chains as a result of IPN formation, thereby increasing strength of the matrix. Among the membranes, TS increased with an increase in concentration of GA, indicating an increased strength of the matrix with increasing cross-linking. The mechanical strength values of membranes are shown in Table 1.

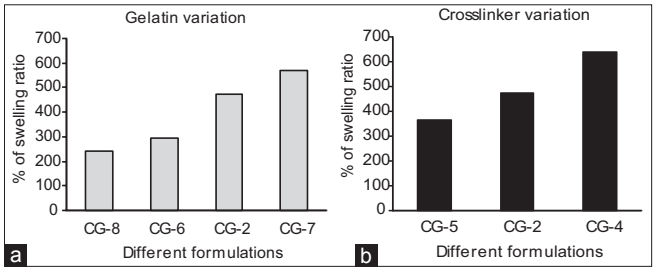

Figure 1: Variation of % swelling ratio with (a) concentration of gelatin and (b) crosslinker

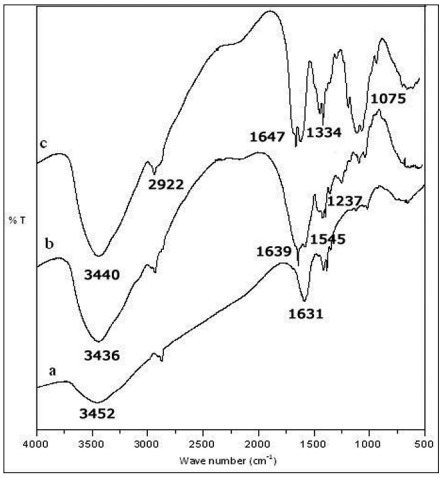

Figure 2: Fourier Transform Infrared Spectroscopy spectra of (a) plain chitosan (b) plain gelatin, and (c) plain membrane

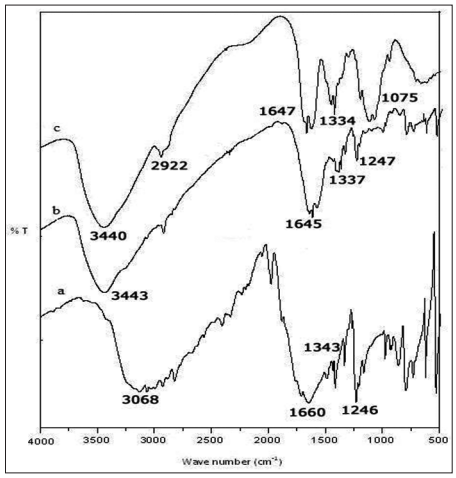

Figure 3: Fourier Transform Infrared Spectroscopy spectra of (a) plain drug (b) drug loaded membrane, and (c) plain membrane

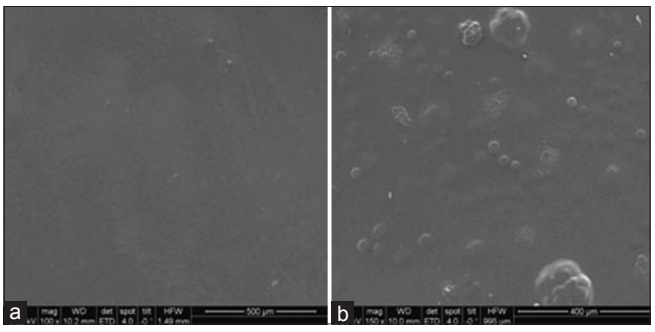

Figure 4: The scanning electron microscopy photographs of (a) plain membrane (b) drug loaded membrane

In vitro release study

Release kinetics parameters of different formulations

The values of *k* and *n* have shown a dependence on the extent of cross-linking, % drug loading, and gelatin content of the matrix. Values of *n* for membranes prepared by varying the amount of gelatin in the membrane of 20%, 40% and 60% by keeping 5-FU (15%) and GA (5 ml) constant, ranged from 0.517 to 0.623 [Table 2], leading to the drug diffuses and release from the polymer matrix following a non-Fickian type diffusion.

Effect of drug loading amount

Figure 7 shows the release profile of 5-FU loaded membranes CG-1, CG-2 and CG-3 at different amounts of drug loading (10 wt%, 15 wt% and 20 wt%, respectively) in PBS pH-7.4 at 37°C. The release data show that the membrane containing higher amount of 5-FU (CG-3) displayed faster and higher release rates than those formulations containing the lower amount of 5-FU. A prolonged release rate was observed in the CG-1 membrane because it contains a lower amount of drug. Notice that the release rate becomes quite slower at the lower amount of drug in the membrane, due to the availability of more free void spaces through which a lesser no of drug molecules will transport.

Effect of crosslinking agent

% cumulative release versus time curves of the membranes CG-2, CG-4 and CG-5 for varying amounts of GA (2.5 ml, 5 ml and 7.5 ml) at a fixed amount of drug (15 wt%), are displayed in Figure 8. The % cumulative release is quite fast and larger at lower amount of GA (2.5 ml) (CG-5), whereas the release is quite slower at higher amount of GA (i.e. 7.5 ml) (CG-4). The % cumulative release is slower when the membrane containing higher amount of GA was used, it may be due to the polymeric chains become rigid due to the contraction of microvoids, thus decreasing the % cumulative release of 5-FU through the membrane.

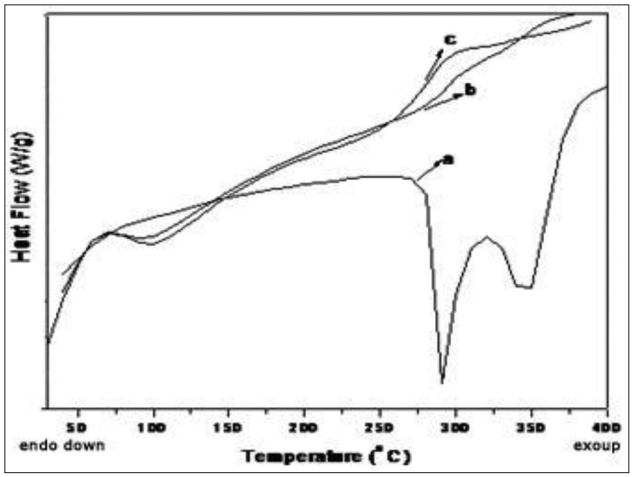

Figure 5: Differential scanning calorimeter thermograms of (a) plain drug (b) plain membrane, and (c) drug loaded membrane

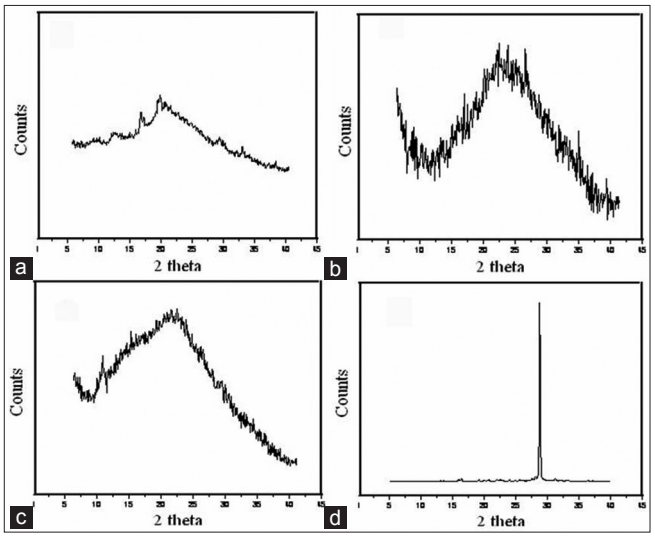

Figure 6: The X-ray diffraction (a) plain chitosan membrane (b) plain membrane (c) drug loaded membrane, and (d) plain 5-flurouracil

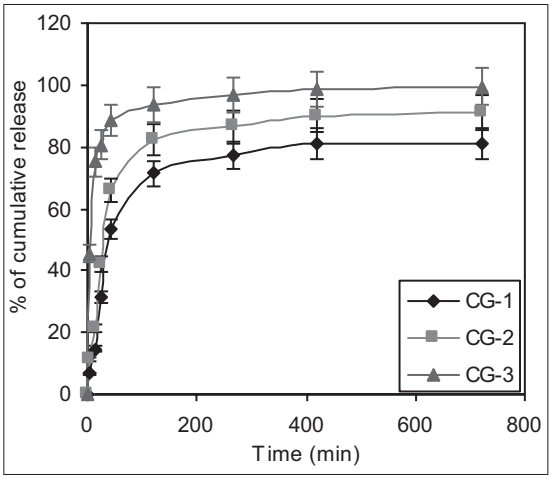

Figure 7: % cumulative release of 5-fluorouracil through the membrane containing different amounts of drug 10 wt% (coarse-graining (CG)-1), 15 wt% (CG-2) and 20 wt% (CG-3) at pH - 7.4

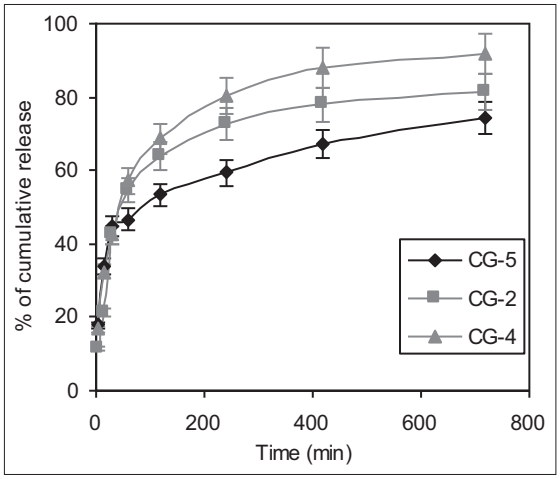

Figure 8: % cumulative release of 5-fluorouracil through the membrane containing different amounts of crosslinker 2.5 ml (coarse-graining (CG)-4), 5 ml (CG-2) and 7.5 ml (CG-5) at pH - 7.4

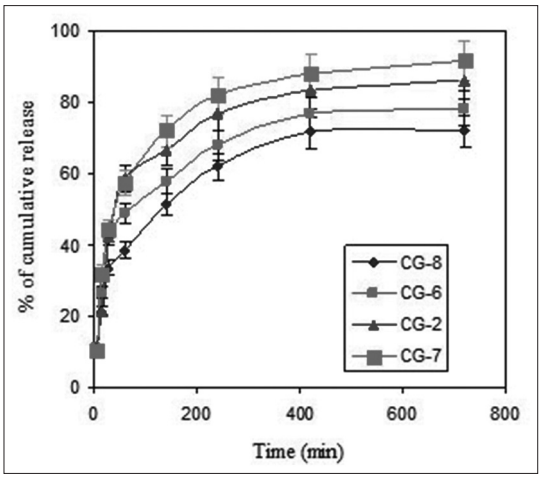

Figure 9: % cumulative release of 5-fluorouracil through the membrane containing different amounts of gelatin 0 wt% (coarse-graining (CG)-8), 20 wt% (CG-6), 40 wt% (CG-2) and 60 wt% (CG-7) at pH - 7.4

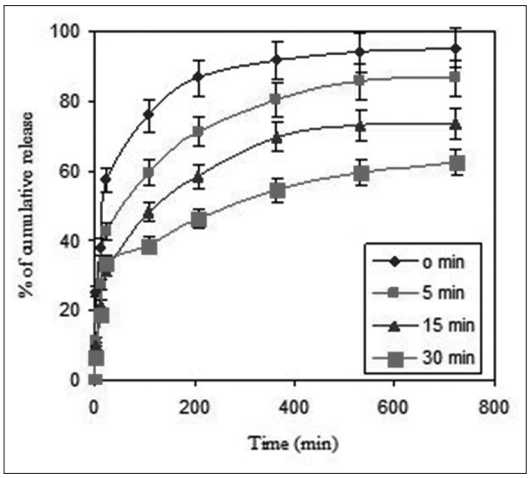

Figure 10: % cumulative release of 5-fluorouracil through coarse-graining-2 membrane containing with different crosslinking times 0 min (♦), 5 min (■), 15 min (▲) and 30 min (■) at pH - 7.4

Effect of gelatin content in membranes

To understand the release profiles of 5-FU from the cross-linked membranes CG-8, CG-2, CG-6 and CG-7 with different gelatin concentrations (0 wt%, 20 wt%, 40 wt% and 60 wt%) were studied in pH-7.4 at 37°C. From Figure 9; it was observed that the highest cumulative release was obtained in CG-7 formulation, which has 60 wt% of gelatin. On the other hand, the least cumulative release was observed, the formulation containing the lower amount (20 wt%) of gelatin, this is due to the hydrophilic nature of gelatin. When the amount of gelatin increased in the membrane, the drug-release was increased, and a lower cumulative release was observed for the formulation containing pure chitosan (CG-8). It may be due to the increase of the hydrophilic nature and loses the polymer network by the increase of gelatin concentration in the membrane. Hence, with an increase of gelatin concentration the hydrophilic nature of the membrane was increased and the increased % cumulative release was observed.

Effect of crosslinking with time

Drug loaded CG-2 membrane cross-linked at different time intervals were produced by solvent/casting evaporation method. The % cumulative release data versus time plots of keeping the amount of crosslinker constant by varying the time with different intervals was shown in Figure 10. The amount of drug-release is very fast at lower time crosslinking membrane whereas slow release rates were observed at higher time crosslinking membrane because as the time of crosslinking increases the membrane rigidity increased, so the % of cumulative release of the drug was slow.

CONCLUSIONS

5-flurouracil drug loaded membranes based on chitosan/ gelatin, were prepared by a solvent/casting evaporation method, with 5-FU as a model drug. We studied the membrane structures and characteristics, especially its potential capacity in drug-delivery system. The chemical and morphological characterizations showed a good compatibility between the membrane and drug. The results of controlled release tests showed that the amount of 5-FU release increased with an increase of gelatin, amount of drug and decreased with an increase of crosslinker. Thus, we can control the drug-release rate through changing some influence factors of the drug loaded membrane. The mechanical property of the membrane is also good. By observing all the results, the hydrogel membrane was a quite promising for controlled release of anticancer drug. The prolong release rates of 5-FU were observed up to 12 h. The membrane can lead to a successful application for localized drug-delivery *in vivo* or *in vitro* environment.

ACKNOWLEDGMENTS

One of the authors (B.M) thanks the Council of Scientific and Industrial Research for providing financial support under the CSIR-JRF (File.No. 09/383 (0043)/2009-EMR-1). Government of India. New Delhi.

REFERENCES

1. Kulkarni RV, Sa B. Novel pH-sensitive interpenetrating network hydrogel beads of carboxymethylcellulose-(polyacrylamide-grafted-alginate) for controlled release of ketoprofen: Preparation and characterization. Curr Drug Deliv 2008;5:256-64.
2. Changez M, Burugapalli K, Koul V, Choudhary V. The effect of composition of poly (acrylic acid)-gelatin hydrogel on gentamicin sulphate release: *In vitro*. Biomaterials 2003;24:527-36.
3. Kulkarni AR, Soppimath KS, Aminabhavi TM, Rudzinski WE. *In-vitro* release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. Eur J Pharm Biopharm 2001;51:127-33.
4. Rokhade AP, Agnihotri SA, Patil SA, Mallikarjuna NN, Kulakarni PV, Aminabhavi TM. Semi-CG microspheres of gelatin and sodium carboxy methyl cellulose for controlled release of ketrolac tromethamine. Carbohydr Polym 2006;65:243-52.
5. Molinaro G, Leroux JC, Damas J, Adam A. Biocompatibility of

thermosensitive chitosan-based hydrogels: An *in vivo* experimental approach to injectable biomaterials. Biomaterials 2002;23:2717-22.
6. Park IK, Kim TH, Park YH, Shin BA, Choi ES, Chowdhury EH, *et al*. Galactosylated chitosan-graft-poly (ethylene glycol) as hepatocyte-targeting DNA carrier. J Control Release 2001;76:349-62.
7. Suh JK, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. Biomaterials 2000;21:2589-98.
8. Cho YW, Cho YN, Chung SH, Yoo G, Ko SW. Water-soluble chitin as a wound healing accelerator. Biomaterials 1999;20:2139-45.
9. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, *et al*. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. Biomaterials 1999;20:1407-14.
10. Peppas NA. Hydrogels and drug delivery. Curr Opin Colloid Interface Sci 1997;2:531-7.
11. Li Q, Dunn ET, Grandmaison EW, Mattheus FA, editors. In: Applications and Properties of Chitosan. Lancaster PA: Technomic; 1997.
12. Hsien TY and Rorrer GL. Effects of Acylation and Crosslinking on the Material Properties and Cadmium Ion Adsorption Capacity of Porous Chitosan Beads. Separation Science and Technology 1995;30:2455.
13. Chandy T, Sharma CP. Chitosan beads and granules for oral sustained delivery of nifedipine: *In vitro* studies. Biomaterials 1992;13:949-52.
14. Ferdinando JC. Formulation solutions softgels. Pharm Manuf Packing 2000;10:69-73.
15. Ikada Y, Tabata Y. Protein release from gelatin matrices. Adv Drug Deliv Rev 1998;31:287-301.
16. Kosmala JD, Henthorn DB, Brannon-Peppas L. Preparation of interpenetrating networks of gelatin and dextran as degradable biomaterials. Biomaterials 2000;21:2019-23.
17. Changez M, Koul V, Dinda AK. Efficacy of antibiotics-loaded interpenetrating network (IPNs) hydrogel based on poly (acrylic acid) and gelatin for treatment of experimental osteomyelitis: *In vivo* study. Biomaterials 2005;26:2095-104.
18. Liang HC, Chang WH, Liang HF, Lee MH, Sung HW. Crosslinking structures of gelatin hydrogels crosslinked with genipin or a water-soluble carbodiimide. J Appl Polym Sci 2004;91:4017-26.
19. Dong Z, Wang Q, Du Y. Alginate/gelatin blend films and their properties for drug controlled release. J Memb Sci 2006;280:37-44.
20. Wang Q, Dong Z, Du Y, Kennedy JF. Controlled release of ciprolfloxin hydrochloride from chitosan/polyethylene glycol blend films. Carbohydr Polym 2007;69:336-43.
21. Jin J, Song M. Chitosan and chitosan-PEO blend membranes crosslinked by genipin for drug release. J Appl Polym Sci 2006;102:436-44.
22. Einmahl S, Zignani M, Varesio E, Heller J, Veuthey JL, Tabatabay C, *et al*. Concomitant and controlled release of dexamethasone and 5-fluorouracil from poly (ortho ester). Int J Pharm 1999;185:189-98.
23. Fournier E, Passirani C, Colin N, Breton P, Sagodira S, Benoit JP. Development of novel 5-FU-loaded poly (methylidene malonate 2.1.2)-based microspheres for the treatment of brain cancers. Eur J Pharm Biopharm 2004;57:189-97.
24. Ermis D, Yüksel A. Preparation of spray-dried microspheres of indomethacin and examination of the effects of coating on dissolution rates. J Microencapsul 1999;16:315-24.
25. Yerriswamy B, Prasad CV, Reddy CL, Mallikarjuna B, Rao KC, Subha MC. Interpenetrating polymer network microspheres of hydroxyl propyl methyl cellulose/poly (vinyl alcohol) for control release ciprofloxin hydrochloride. Cellulose 2011;18:349-57.
26. Krishna Rao KS, Naidu BV, Subha MC, Sairam M, Aminabhavi TM. Novel chitosan base pH sensitive interpenetrating network microgel for the controlled release of cefadroxil. Carbohydr Polym 2006;66:333-44.
27. Ritger PL, Peppas NA. A simple equation for description of solute release. II. Fickian and anomalous release from Swellable devices. J Control Release 1987;5:37-42.
28. Ramesh Babu V, Krishna Rao KS, Sai Ram M, Naidu BV, Mosamani KM, Aminabhavi TM. pH sensitive interpenetrating network microgels of sodium alginate-acrylic acid for the controlled release of ibuprofen). J Appl Polym Sci 2006;99:2671-8.
29. Sanli O, Ay N, Isiklan N. Release characteristics of diclofenac sodium from poly (vinyl alcohol)/sodium alginate and poly (vinyl alcohol)-grafted-poly (acrylamide)/sodium alginate blend beads. Eur J Pharm Biopharm 2007;65:204-14.

How to cite this article: Mallikarjuna B, Rao KM, Pallavi K, Rao KC, Subha M. Biodegradable interpenetrating polymer network hydrogel membranes for controlled release of anticancer drug. Asian J Pharm 2015;9:129-36.

Source of Support: Nil. **Conflict of Interest:** None declared.