Research Article

5-Fluoro Uracil for colon specific drug delivery of a Poly (CarboxyMethyl Cellulose-co-Acryl Amide)/MBA Nano Sized Hydrogel

Mohapatra DK* and Swagatika Mekap

Department of Pharmaceutical Analysis and Quality Assurance
Gayatri College of Pharmacy, Sambalpur, Odisha, Orissa, India.

ABSTRACT

Colon-selective drug delivery systems, not only for local but also for systemic therapy, have been the focus of increasing interest for the last decade. At present, the specific drug delivery to the colon is considered as an important alternative for the treatment of serious diseases such as cancer. The main objective of the present study was to develop novel oral site-specific delivery of 5-FU to the colon with less drug being released in the stomach or small intestine using biodegradable hydrogel, hydrogel nanoparticles and comparing the targeting efficiency of 5-FU to colon from both. Poly (carboxy methyl cellulose-co-acryl amide), P (CMC-co-Am)) normal hydrogel and hydrogel nanoparticles (HN) were synthesized by free radical polymerization using *N, N*-methylene-bis-acrylamide (MBA) as cross-linker, potassium per sulfate as reaction initiator and 5-FU was loaded. HN was found to be degradable in physiological medium and showed comparatively higher swelling in rat caecal medium (RCM). 5-FU entrapment was increased by increasing Am (wt %) monomer feed. *In vitro* release of 5-FU from normal hydrogel and HN in pH progressive medium, it was found that a CMC/Am ratio of 25:75 showed higher release in RCM. The Higuchi model yielded good adjustment of *in vitro* release kinetics. A higher amount of 5-FU reached the colon in HN (58± 2.1%) than normal hydrogel (38± 3.6%) by organ biodistribution studies in albino rats.

Keywords: 5-FU, hydro gel; swelling studies; degradation; nanohydrogel

INTRODUCTION

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome but also for the potential it holds for the systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by per oral delivery, is still deemed to be the ideal site for the delivery of agents to cure the local diseases of the colon[1,2].

Another challenge in developing therapeutically effective products for the treatment of colonic pathologies is the impact of disease on the delivery system[3].Compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying[4]. Multiparticulate approaches tried for colonic delivery include includes formulations in the form of pellets, granules, micro particles and nanoparticles.The use of multiparticulate

drug delivery systems in preference to single unit dosage forms for colon targeting purposes dates back to 1985 when Hardy and co-workers[5] showed that multiparticulate systems enabled the drug to reach the colon quickly and were retained in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems are capable of passing through the GI tract easily, leading to less inter- and intra subject variability. Moreover, multiparticulate systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption[6-8]. Most commonly studied multiparticulate systems for colon specific drug delivery include pellets, granular matrices, beads, micro spheres, and nanoparticles[9-15.] The development of successful colon specific drug. pH- and time- dependent systems. The pH in the terminal ileum and colon (except ascending colon) is higher than in any. Thus a dosage form that disintegrates preferentially at high pH levels has good potential for site-specific delivery into this region[16].

Hydro gels are three-dimensional macromolecular networks that contain a large fraction of water within their structure, do not dissolve and are soft and pliable. These properties are similar to natural tissue and therefore, hydro gels are particularly useful in biomedical and pharmaceutical applications[17,18] In the case of anionic polymeric network containing carboxylic or sulphonic acid groups, ionization takes place, as the pH of the external swelling medium rises above the pKa of that ionisable moiety. The dynamic swelling change of the anionic hydro gels can be used in the design of intelligent controlled release devices for site-specific drug delivery of therapeutic proteins to large intestine, where the biological activity of the proteins is prolonged[19]. Several techniques have been reported for the synthesis of hydro gels. The first approach involves copolymerization/cross linking of co-monomers using multifunctional co-monomer, which acts as cross linking agent. The polymerization reaction is initiated by chemical initiator. The polymerization reaction can be carried out in bulk, in solution, or in suspension. The second method involves cross linking of linear polymers by irradiation, or by chemical compounds[20] several techniques have been reported for the synthesis of hydro gels. The first approach involves copolymerization/cross linking of co-monomers using multifunctional co-monomer, which acts as cross linking agent. The polymerization reaction is initiated by chemical initiator. The polymerization reaction can be carried out in bulk, in solution, or in suspension. The second method involves cross linking of linear polymers by irradiation, or by chemical compounds[21].

Considering the implementation of nanohydrogel for anticancer drugs, the polymeric nanohydrogel was prepared and loaded by a model anticancer drug, i.e., 5-fluorouracil (5-FU). 5-FU is a commonly applied anticancer drug in the treatment of colon cancer[22]. Few nanocarriers have been tested for successful therapy by targeting the colon[23–25]. At present, the standard regimen is an intravenous bolus injection of 5-FU modulated by folic acid[26,27]. only few approaches for well administration have been described in the literature. Recently, enzyme-dependent tablet-based systems have been proposed, which might allow an efficient treatment combined with a reduction of adverse effects[28]. However, due to variations in transit time throughout the colon, 5-FU release can be incomplete when the colon specific matrix is not readily disintegrated and the treatment will remain insufficient[29–31]. This can render oral 5-FU treatment insufficient. A size reduction of the carrier system that to nanosized carrier

system might be an option in order to circumvent those problems, since size dependent gastrointestinal retention has been observed earlier[32].

Therefore, the present study was aimed at developing nanosized hydrogel using cross-linked biodegradable and biocompatible polymer P(AA-co-Am) which was loaded with 5-FU for oral delivery to colon and comparing the targeting efficiency of nano hydro gel with normal hydrogel through organ biodistribution in rats. Further, attempts were made to minimize the 5-FU release in the physiological environment of stomach and small intestine and to ensure maximum 5-FU release in the physiological environment of colon.

Experimental and Method

Materials

Acryl amide (Am) and glutaraldehyde were purchased from S.D. Fine Chemicals (Mumbai, India), Carboxy methyl cellulose (CMC) from Himedia Laboratories (Mumbai, India), potassium persulfate (PS) and sodium dodecyl sulfate (SDS) from Loba Chemie (Mumbai, India). *N, N*-Methylene-bis acryl amide (MBA), 3% crystal extra pure AR, was purchased from Sisco Research Laboratories (Mumbai, India). All other reagents used were of analytical grade. 5-FU was a gift sample from Khandelwal Laboratories (Mumbai, India).. 5-FU was a gift sample from Khandelwal Laboratories (Mumbai, India).

METHODS

Synthesis of P (CMC-co-Am) hydro gel

Co-polymerization was carried out in a jacketed reaction vessel having an inlet and an outlet port. The inlet port was connected with the nitrogen gas supply from the nitrogen cylinder. CMC and Am were charged into the reaction vessel at different monomer feeds (wt %) ratios followed by addition of MBA (0.01 M) to the reaction vessel with 20 ml distilled water. The reaction mixture was deaerated by bubbling purified nitrogen through it for an hour. The solutions were stirred at 400–500 rpm. The requisite amount of the initiator PS (0.16 g) in 20 ml distilled water was carefully injected to the reaction mixture. The temperature was maintained at 80◦C for 6 h. Then the reaction mixture was arrested by pouring it into 200 ml methanol (non solvent) under magnetic stirring. The precipitate formed was then filtered and washed with hot distilled water to remove homopolymers. Thereafter, the prepared hydro gel was dried in the oven and weighed.

Synthesis of P (CMC-co-Am) hydrogel nanoparticles

P(CMC-co-Am) hydrogel nanoparticles were prepared by precipitation polymerization[43]. CMC and Am were charged into the reaction vessel at different monomer feed (wt%) ratios followed by addition of SDS (0.02%, w/v) and MBA (0.01 M) in distilled water (50 ml) at room temperature, were purged with nitrogen and stirred for 30 min, and then heated to 70°C.PS (0.16 g) in 20 ml water was added to initiate polymerization. The reaction was maintained at 60°C under nitrogen for 6 h. After cooling to room temperature, the resultant nanoparticles were dialyzed for 1 week to remove surfactant and unreacted molecules. The dialysis water was changed three times every day. The cut off molecular weight of the dialysis membrane was 13 × 103. The dialyzed particle dispersion was condensed by evaporation of water and dried to get hydrogel nanoparticles.

Preparation of rat caecal medium

MaleWistar rats, weighing 200–250 g and maintained on a normal diet, were lightly anesthetized under ether and then killed by decapitation. Central Animal House facility, Gayatri College of Pharmacy, Sambalpur, Odisha, India, approved the study. The caecum was exteriorized, legated at two ends (2.0

cm distances), cut loose and immediately removed from the rat body. The formed caecal bag was then opened, its content weighed, pooled and suspended in two volumes of cold bicarbonate buffered saline (BBS, pH 7.0: NaHCO3, 110 mM; Na2HPO4·12H2O, 20 mM; NaCl, 8.0 mM; KCl, 6.0 mM; CaCl2·2H2O, 0.5 mM; MgCl2·6H2O, 0.4 mM) to give a final caecal dilution of 33% (w/v). The suspension was filtered through 400-mesh grit twice to remove debris. Supernatants were then centrifuged at 15 ?103?*g* for 30 min in order to obtain a clear supernatant containing extra cellular enzymes[33].

Swelling behavior of P(CMC-co-Am)

The equilibrium swellings of the P(CMC-co-Am) HN were determined by swelling the dried HN in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and rat caecal medium (RCM) until equilibrium was attained. The swollen weights of the HN were determined by blotting every hour until equilibrium was attained. The swelling behavior was computed by calculating the percentage swelling (*S*): $S = ((Mt - M0)/M0)$ X100%, where *Mt* and *M*0 are the masses of the swollen and the dry samples, respectively.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR studies of P(AA-co-Am) HN were carried out at room temperature using a FT-IR spectrophotometer (FT-IR Paragon-500) using KBr pellet. All the spectra were recorded in the range of 500–4000 cm^{-1}.

Degradation study

The degradation of P(CMC-co-Am) HN was studied on weight loss basis with about 1.5 g weight, over a period of 60 days[45]. They were conditioned to minimum weight at 37 ?1◦C in an oven containing desiccant prior to being immersed into 100 ml of a simulated physiological isotonic solution (0.154 M NaCl aqueous solution at pH 7.4). The specimens were removed at regular intervals of 14, 30 and 60 days, by being taken out of the solution, blotted on filter paper to remove surface solution and dried in an oven at 50◦C to constant weight in order to determine eventual weight loss, taking an average of two readings.

Transmission electron microscopy (TEM)

The HN were examined by transmission electron microscopy (CM12 Philips, Eindhoven, The Netherlands). Samples were stained with 2% phospho tungistic acid for 10 min, immobilized on copper grids and dried overnight for viewing.

Drug loading

The prepared co-polymer normal hydrogel was loaded with drug (5-FU) by soaking the hydrogel into the drug solution at different drug to P(CMC-co-Am) ratio (Table 1) for 24 h; dried and stored in glass container. Similarly, the prepared HN were loaded with 5-FU by mixing the HN into the drug solution at different drug to P(CMC-co-Am) HN ratio (see Table 2) in the presence of glutaraldehyde (1 ml, 25% aqueous solution) for 6 h. The dispersion was centrifuged to remove air bubbles, dried and stored in glass container.

Drug entrapment efficiency (%)

5-FU-loaded HN of P(CMC-co-Am) and normal P(CMC-co-Am) hydrogel (10 mg) from each batch were dispersed separately in RCM and kept for 24 h, filtered through a Millipore filter and absorbance was measured using UV-Vis spectrophotometer (Genesis-2) at 266 nm. 5-FU contents were determined and expressed in terms of weight of 5-FU per weight of formulation, thus determining the actual entrapment ratio (AER) defined by the following expression:

AER = Measured drug wt. /formulation wt.,

TER = Drug wt./(drug wt. + polymer wt.), Entrapment efficiency (%) = (AER/TER) X100%.

In vitro drug-release study

The *in vitro* release experiment was carried out using a dialysis tube. 5-FU-loaded HN of P(CMC-co-Am) (5 mg) and 2 ml of phosphate-buffered saline (0.1 M, pH 7.4) were put into a dialysis tube (MWCO, 12 kg/mol). Then the dialysis tube was introduced into a vessel with 100 ml dissolution medium and proper simulation of gastro intestinal condition was maintained by altering the pH of dissolution medium at different time intervals following three step-dissolution conditions[46]. To simulate the physiological conditions of GIT: 2 h in 250 ml SGF (3.2 mg/ml pepsinin 0.05 M HCl, pH 1.2), 2 h in 250 ml SIF (10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4) and finally 20 h in 100 ml RCM. The medium was stirred at 100 rpm at 37±0.5◦C. At predetermined time intervals, specified amount of dissolution medium was removed and replaced with the same amount of fresh medium. The amount of 5-FU released from HN was measured with a UV-Vis spectrophotometer at 266 nm. All the dissolution studies were repeated six times. A similar procedure was adopted for the release study of P(CMC-co-Am) normal
hydrogel in the absence of dialysis tube.

In vitro drug-release kinetic mechanism

Different mathematical models may be applied for describing the kinetics of the drug-release process from the HN matrix; the most suited being the one which best fits the experimental results. The kinetics of 5-FU release from HN formulations was determined by finding the best fit of the dissolution data (drug release *vs*. time) to distinct models: first-order and Higuchi [47, 48]:

$Qt = Q\infty\ (1 - e^{-k1t})$, (1)

Where $Q\infty$ is the total amount of drug in the matrix and $k1$ the first-order kinetics constant.

$Qt = kHt\ 1/2$, (2)

Where kH is the Higuchi rate constant. Furthermore, in order to better characterize the drug-release behavior for the polymeric systems studied, namely to understand the corresponding mechanism,
the Korsmeyer–Peppas semi-empirical model was applied[49]:

$Qt/Q\infty = ktn$, (3)

where $Qt/Q\infty$ is the fraction of drug released at time t , k a constant comprising the structural and geometric characteristics of the tablet and n, the release exponent, is a parameter which depends on the release mechanism and is, thus, used to characterize it[50].

Biodistribution study

For organ distribution studies of the hydrogel system, 24 male albino rats (8 weeks old; 220–250 g) were supplied by Institutional Central Animal House facility and kept under standard laboratory conditions in 12 h light/dark cycle at 25◦C. Animals were provided with standard diet and water ad libitum. They were marked with picric acid solution for easy identification. These animals were divided into 4 groups of 6 rats each. The first group served as control. The second group received 10 mg 5-FU. Animals of the third group were treated with P(AA-co-Am) HN (Batch A1) and animals of fourth group were given normal P(AA-co-Am) hydrogel (Batch A) containing an equivalent amount of 5-FU. The formulations were orally administered in suspension form followed by sufficient volume of drinking water. After 12 h, in all the groups except the third group, the rats were humanely killed. The rats of the third group were killed after 30 h. Stomach, small intestine, and colon were isolated. These organs were homogenized by Micro Homogenizer-II (Mac, India) along with a small amount

of phosphate-buffered saline (pH 7.4); 1 ml of acetonitrile was added to the homogenate and kept for 30 min. Contents were centrifuged and the supernatant liquid was separated. After appropriate dilution of supernatants, the 5-FU content was determined by high-performance liquid chromatography (HPLC) [51] at 266 nm. The 5-FU content in different parts of the GI tract at different time intervals was calculated.

Standard Graph for 5-FU

Estimation of 5-FU was done based on measurement of observance using UV spectrophotometric method 10 mg of 5-FU was taken in 10 ml volumetric flask and mixed with phosphate buffer of pH 7.4. The solution was sonicated for 10 minutes. 1 ml of this solution was diluted upto 50 ml in volumetric flask using Ph 7.4 phosphate buffer. Subsequent dilutions were done and solution was scanned for maximum wavelength for 5-FU i.e 266 nm. Standard graph was plotted (λ max) using the absorbance obtained for linearity, accuracy and precision. The method obeyed Beer's Law in conclusion range 1-40 µg/ml. When a standard drug solution was assayed repeatedly (n=3), the mean error (accuracy) and relative standard deviation (precision) were found to be 1% and 2% respectively.

Drug loading

The prepared co-polymer normal hydrogel was loaded with drug (5-FU) by soaking the hydrogel into the drug solution at different drug to P (CMC-co-Am) ratio (Table 3) for 24 h; dried and stored in glass container. Similarly, the prepared HN were loaded with 5-FU by mixing the HN into the drug solution at different drug to P(CMC-co-Am) HN ratio (see Table 4) in the presence of glutaraldehyde (1 ml, 25% aqueous solution) for 6 h. The dispersion was centrifuged to remove air bubbles, dried and stored in glass container.

Drug entrapment efficiency (%)

5-FU-loaded HN of P (CMC-co-Am) and normal P (CMC-co-Am) hydrogel (10 mg) from each batch were dispersed separately in RCM and kept for 24 h, filtered.

Size distribution of P (CMC-co-Am) hydrogel nanoparticles (HN) by photon correlation spectroscopy (PCS) before and after loading 5-fluorouracil (5-FU)

Batch	Monomer feed (Wt %) CMC/Am	Empty HN size ± SD (nm) (polydispersity)	5-FU-loaded HN size ± SD (nm) (polydispersity)
A1	25:75	16.31 ± 0.55 (0.11 ± 0.02)	25.4 ± 0.93 (0.05 ± 0.03)
B1	50:50	23.5 ± 1.21 (0.12 ± 0.07)	30.4 ± 1.12 (0.08 ± 0.07)
C1	75:25	37.5 ± 1.15 (0.08 ± 0.02)	51.6 ± 1.2 (0.10 ± 0.03)
D1	90:10	53.1 ± 1.8 (0.06 ± 0.03)	80.8 ± 1.12 (0.1 ± 0.02)

Table IV: 5-FU entrapment into P (CMC-co-Am) hydrogel and HN

	P(CMC-co-Am) hydrogel				P(CMC-co-Am) HN			
	Batch A	Batch B	Batch C	Batch D	Batch A1	Batch B1	Batch C1	Batch C1
Monomer feed CMC/Am (wt%)	25:75	50:50	75:25	90:10	25:75	50:50	75:25	90:10
Yield (mean ± SD, %)	66 ±2.31	64 ±3.36	72±2.28	62 ±1.66	35± 3.8	38 ± 3.3	42± 3.6	32± 2.9
Drug entrapped (%)	58(1.7*)	50(1.3*)	47(2.2*)	49(2.1*)	39(1.8*)	30(2.0*)	29 (1.6*)	28(2.0*)

* Coefficient of variance value.

Through a Millipore filter and absorbance was measured using UV-Vis spectrophotometer (Genesis-2) at 266 nm. 5-FU contents were determined and expressed in terms of weight of 5-FU per weight of formulation, thus determining the actual entrapment ratio (AER) defined by the following expression:

AER = Measured drug wt. /formulation wt.
TER = Drug wt. / (drug wt. + polymer wt.),
Entrapment efficiency (%) = (AER/TER) × 100%.

In vitro drug-release study

The *in vitro* release experiment was carried out using a dialysis tube. 5-FU-loaded HN of P(CMC-co-Am) (5 mg) and 2 ml of phosphate-buffered saline (0.1 M, pH 7.4)were put into a dialysis tube (MWCO, 12 kg/mol). Then the dialysis tube was introduced into a vessel with 100 ml dissolution medium and proper simulation of gastro intestinal condition was maintained by altering the pH of dissolution medium at different time intervals following three step-dissolution conditions [141]. To simulate the physiological conditions of GIT: 2 h in 250 ml SGF (3.2 mg/ml pepsin in 0.05 M HCl, pH 1.2), 2 h in 250 ml SIF (10 mg/ml pancreatin in Sorensen's phosphate buffer, pH 7.4) and finally 20 h in 100 ml RCM. The medium was stirred at 100 rpm at 37±0.5◦C. At predetermined time intervals, specified amount of dissolution medium was removed and replaced with the same amount of fresh medium. The amount of 5-FU released from HN was measured with a UV-Vis spectrophotometer at 266 nm. All the dissolution studies were repeated six times. A similar procedure was adopted for the release study of P (CMC-co-Am) normal hydrogel in the absence of dialysis tube.

In vitro drug-release kinetic mechanism

Different mathematical models may be applied for describing the kinetics of the drug-release process from the HN matrix; the most suited being the one which best *Colon-specific drug delivery of nanosized hydrogel* 1493 fits the experimental results. The kinetics of 5-FU release from HN formulations was determined by finding the best fit of the dissolution data (drug release *vs*. time) to distinct models: first-order and Higuchi [142, 143].

$$Qt=Q\infty(1-e-k1t).....(1)$$

Where $Q\infty$ is the total amount of drug in the matrix and $k1$ the first-order kinetics constant.

$$Qt=kHt1/2,.....(2)$$

Where kH is the Higuchi rate constant. Furthermore, in order to better characterize the drug-release behavior for the polymeric systems studied, namely to understand the corresponding mechanism,the Korsmeyer–Peppas semi-empirical model was applied[144]:

$$Qt/Q\infty=ktn,.....(3)$$

Where $Qt/Q\infty$ is the fraction of drug released at time *t, k* a constant comprising the structural and geometric characteristics of the tablet and *n*, the release exponent, is a parameter which depends on the release mechanism and is, thus, used to characterize it [145].

RESULTS AND DISCUSSION

To achieve long-term oral site-specific drug delivery, it is desirable to develop devices that can be selectively retained at the site of action. Site-specific drug delivery has become a major research endeavor for the pharmaceutical scientists. So the present study aims at developing P (CMC-co-Am) HN loaded with 5-FU as a model drug for oral delivery to colon and comparing the

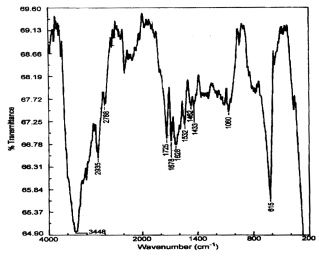
targeting efficiency of HN with normal hydrogel.

FT-IR Spectroscopy

The prepared co-polymer structure is confirmed by IR spectroscopy. The IR of CMC, PAm, and P (CMC-co-Am) are shown in Figs. From the IR spectra of CMC (Fig13), it is evident that it shows a broad absorption band at 3432 cm^{-1} is due to the stretching frequency of the –OH group. The band at 2909 cm^{-1} is due to C-H stretching vibration. The presence of a strong absorption band at 1603 cm^{-1} confirms the presence of COO^- group. The bands around 1423 and 1325 cm^{-1} are assigned to $–CH_2$ scissoring and –OH bending vibration, respectively. The band at 1061 cm^{-1} is due to > CH–O–CH2 stretching.

In the case of PAm (Fig14), a broad absorption band at 3434 cm^{-1} is for the N –H stretching frequency of the NH_2 group. Two strong bonds around 1679 and 1633 cm^{-1} are due to amide-I (C=O stretching) and amide –II (NH bending). Another band at 1724 cm^{-1} is due to the presence of acid group. The bands around 1398 and 2930 cm^{-1} are for the C–N and C–H stretching vibration. Other bands at 1450 and 1318cm^{-1} are attributed to CH_2 scissoring and CH_2 twisting. NH wagging vibrations occurring at 618, 705, and 816 $cm^{-1,}$ respectively.

(Fig15) Shows the IR spectrum of P (CMC-co-Am). The presence of a broad absorption band at 3448 cm^{-1} is due to the overlapping of –OH starching band of CMC and –NH stretching band of PAm. A band at 1678 cm^{-1} is due to the amide – I band of amide group of PAm. The band at 1603 cm^{-1} of CMC and amide – II band of PAm overlap with each other and lead to a broad band at 1628 $cm^{-1.}$ The presence of a band at 1724 cm^{-1} is due to free acid groups. The above- mentioned bands of CMC-co-PAm are also present in CMC and PAm, but at slightly different frequencies. As PAm was initially removed from the reaction products, the presence of the above bands in the copolymer gives strong evidence of copolymerization.



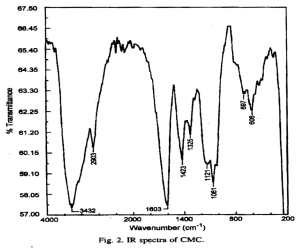


Fig. 2. IR spectra of CMC.

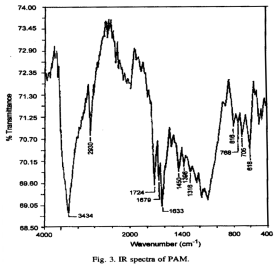
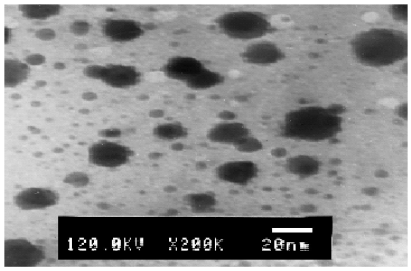


Fig. 3. IR spectra of PAM.

Transmission electron microscopy (TEM) & Size distribution studies

TEM studies of the prepared P (CMC-co-Am) HN were carried out and shown in Fig.24. The particle sizes of the HN were non-uniform and the mean particle size was found to be 40 nm.

Size distribution of HN

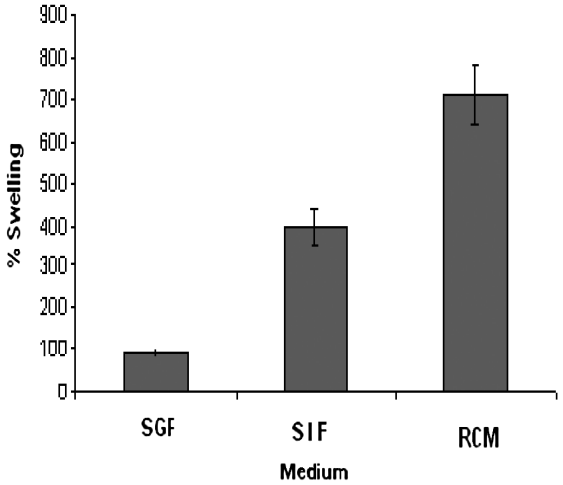


The size distribution of empty and 5-FU-loaded HN was determined using photon correlation spectroscopy (PCS), as shown in Table -3. The sample was illuminated by a laser beam and the particles undergoing Brownian motion were detected. Light scattering by these particles was received by a fibre-optic cable placed at a particular angle and the fluctuations in scattering intensity were analyzed.

The polydispersity (PI) is an important parameter that gives an idea about the reliability of the data obtained with PCS analysis. PI is a dimensionless number extrapolated from values of 0.010 for monodispersed polystyrene standard latex particles up to values of around 0.5–0.7. Values greater than 0.7 are characteristic of samples with a very broad size distribution. The particle size distribution data shows that HN produced were of sub-micrometer size and of low polydispersity (Table 3), which indicated a relatively narrow particle size distribution. A higher increase in particle size was observed in case of 5-FU-loaded HN than that of empty. The monomer feed was also found to be influencing the particle size of HN. With increasing monomer feed of acrylic acid, the size of the HN increased in both empty and 5-FU-loaded HN.

Swelling behavior

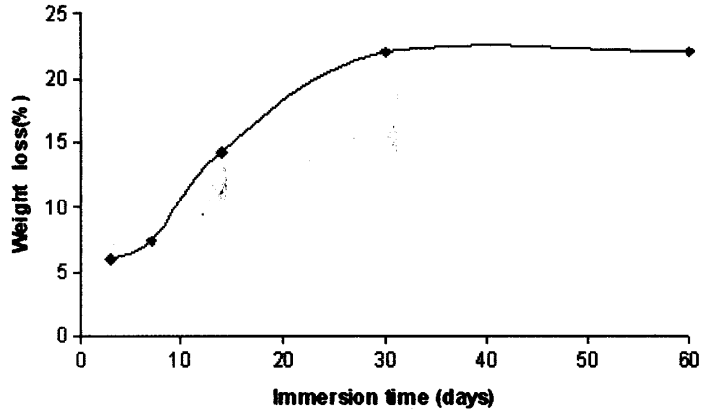
Hydrogel is the combination of the chains and as the prepared polymer is made up of P (CMC-co Am), the acid group is bound to their polymer chains, from where the H+ comes off and combines with OH− to form H_2O. The charge is compensated by cations that enter the gel together with another OH−; thus, charge neutrality is maintained. The increased cation concentration gives rise to an osmotic pressure that causes the gel to swell/deswell. An equilibrium ionic gel occurs when the elastic restoring force of the network balances the osmotic forces. In the hydrogel formed by the homogenous co-polymer of CMC and Am with MBA as cross-linker, the acidic groups bound to the polymer chains are carboxyl groups, which made the gels pH sensitive. The swelling behavior of the co-polymer in physiological solutions were studied and it was found that the prepared co-polymer showed comparatively higher swelling in RCM which has a higher pH, as shown in Fig.30.



Degradation study

To date investigators have focused on controlling degradation behavior of hydrogels, as well as on enhancing their biological interactions with body components, in order to design and tailor appropriate vehicles for drug delivery. Controlling degradation

behavior has been one of critical issues in general biomaterials research, and has been widely investigated to date. In general, especially for drug-delivery applications, biomaterials need to be cleared from the body once they complete their roles in the body, and degradable materials could be ideal for this purpose.



The degradation studies of the prepared HN were studied on the basis of % wt.loss in simulated physiological isotonic solution (0.154 M NaCl aqueous solution at pH 7.4) at regular intervals of 3, 7, 14, 30 and 60 days. From the degradation study of P (CMC-co-Am) HN over a period of 60 days (Fig. 31), it was found that with increase in the immersion time, the weight loss increased to become stabilized after about 30 days and extended up to 60 days. The degradation period of the prepared HN reflected its strength, which might be due to effective cross-linking of molecules. The slow and continual degradation behavior of cross-linked HN might be attributed to the attachment points that only allow complete disintegration of the cross-linking molecules once all attachment points are lost.

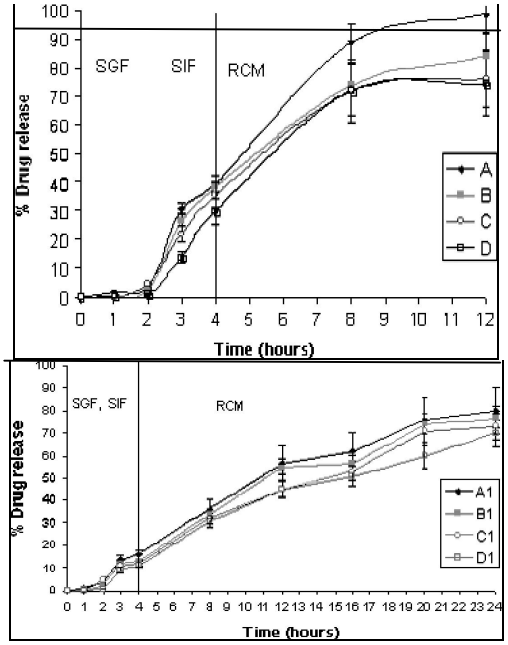
Drug entrapment studies

Entrapment studies of drug (5-FU) into P (CMC-co-Am) normal hydrogel and HN were carried out in RCM. Among the formulations, monomer feed (wt %) ratio 25:75 (CMC/Am) showed comparatively higher drug entrapment, both in hydrogel and HN. Comparatively higher percentages drug entrapments were found in P (CMC-co- Am) hydrogel. Drug loading increased by increasing monomer feed (wt %) of acrylamide both in hydrogel and HN. Low coefficient of variance (<2.2%) in %5-FU entrapment indicates uniformity of drug entrapment in different batches as per Table 4. The yield from all the batches of hydrogel was found to be in the range of 61–73% and that of HN was found to be 28–38%.

n vitro release studies

In vitro 5-FU release from different batches of P (CMC-co-Am) normal hydrogel and HN was performed in pH progression medium at 37 ± 0.5◦C. Both P (CMC-co-Am) hydrogel and HN were showing comparatively higher 5-FU release with increasing pH of medium. Among all the batches of hydrogel, batch A with CMC/Am (wt %) ratio 25:75 showed comparatively higher 5-FU release in the pH progression medium (Fig. 34). Similarly, in the case of HN, batch A1 with CMC/Am (wt %) ratio 25:75 showed faster 5-FU release (Fig. 35). Hence, the monomer feed of CMC was found to play a major role in controlling the release rate of drug. In both the cases, comparatively much higher 5-FU release was observed in RCM than in other media. This may be due to the higher swelling capacity of the system in RCM (Fig. 30). The release of 5-FU was controlled up to 12 h in case of hydrogel, and up to 24 h in case of P(CMC-co-Am) HN. The nano size of the HN might play a

major role in controlling the release rate of 5-FU from the co- polymeric matrix.



In vitro drug-release kinetics mechanism

The drug-release mechanism from swellable HN matrices was found to be complex. Although some processes might be classified as either purely diffusional or purely erosion controlled, many others could only be interpreted as being governed by both. The analysis of experimental data in the light of the Korsmeyer–Peppas equation (equation (3)), as well as the interpretation of the corresponding release exponent values (*n*), leads to a better understanding of the balance between these mechanisms. For formulation A1, *n* was determined to be equal to 0.701. Notwithstanding this value is pointing to an anomalous (non-Fickian) diffusion mechanism; both Higuchi's model (Fickian) and first-order kinetics yielded similarly good quality adjustments. For formulations B1–D1 the diffusional exponent value (*n)* ranged from 0.549 to 0.608 (Table 3), indicating that the release mechanism of 5-FU from these HN matrices was an anomalous (non-Fickian) transport, which suggests that both diffusion of the drug in the hydrated matrix and its own erosion modulate drug release. For these systems, the Higuchi kinetics model yielded remarkably good adjustment.

Table-Fitting results of experimental release data of P (CMC-co-Am) HN (formulations A1–D1) to different kinetic equations Formulation First order

Formulation	*K*1 (h−1)	First order *R*2
A1	0.116 (0.049)	0.9063 (0.009)
B1	0.061 (0.071)	0.8469 (0.0015)
C1	0.066 (0.101)	0.8654 (0.002)
D1	0.067 (0.01)	0.8608 (0.004)

Table: Higuchi

Formulation	Higuchi	
	*k*H (% h−1)	*R*2
A1	31.241 (1.212)	0.9689 (0.001)
B1	13.131 (1.021)	0.9474 (0.0006)
C1	11.126 (1.094)	0.9448 (0.0016)
D1	11.042 (0.904)	0.9431 (0.0011)

Table: Korsmeyer–Peppas

Formulation			
	k_{KP} (% h−*n*)	*n*	*R*2
A1	14.421 (1.129)	0.701 (0.106)	0.9231 (0.014)
B1	10.083 (0.996)	0.574 (0.115)	0.9036 (0.068)
C1	11.896 (0.094)	0.608 (0.091)	0.9113 (0.036)
D1	8.993 (1.028)	0.549 (0.121)	0.8996 (0.017)

CONCLUSION

The aim of this study was to prove the occurrence co-polymerization by various techniques. Variations of the synthetic parameters resulted in co-polymer with variation in the length and number of PAm chains. With increase in PAm chain of the co-polymer, the viscosities of the co- polymer gradually increases. The IR spectra of the co-polymer after extraction of homo polymer also provide strong proof of co-polymerization. Morphological studies of the co-polymer, CMC and PAm, also support co-polymerization. TGA result show different thermal decomposition pattern for the base polysaccharides and the corresponding co-polymers. X-ray diffraction patterns show the presence of crystallinity in case of CMC and PAm, which reduces on copolymerization due to disruption of the original ordered structure.

The designed colon-specific delivery of 5-FU from P (CMC-co-Am) HN may reduce the side-effects of the drug caused by its absorption from the upper part of the GI tract. The experimental results demonstrated that P (CMC-co-Am) hydrogel nanoparticles have a greater potential than P (CMC-co-Am) normal hydrogel to be used as a drug carrier for an effective colon-targeted delivery system.

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