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Article

Chitosan-based Interpenetrating Polymeric Network Microgels for Colon-Specific Drug Delivery of 5-Fluorouracil

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ABSTRACT

pH-responsive interpenetrating polymeric network microgels (IPNMGs) were developed from a combination of a natural carbohydrate polymer, chitosan (CS), with newly synthesized poly(vinyl alcohol)-g-poly(2-hydroxy-4-N-methylamidobenzoic acid) (PVA-g-HMA). The IPNMGs were developed using a water-in-oil emulsion method with glutaraldehyde as a crosslinker. 5-fluorouracil (5-FU), an anticancer drug, was encapsulated successfully into these IPNMGs through an *in situ* method. The grafting and grafting yield for PVA-g-HMA were found to be 103% and 91.63%, respectively. The formation of IPNMGs and the structural interactions of pristine IPNMGs and 5-FU-loaded IPNMGs were characterized by Fourier-transform infrared spectroscopy, differential scanning calorimetry, and X-ray diffraction. Optical microscopy and scanning electron microscopy revealed the IPNMGs to be spherical and 20 μm in size with a smooth surface. The pH-responsive nature of the IPNMGs was examined from equilibrium swelling studies under different pH conditions. The *in vitro* release behavior of the IPNMGs was examined in pH 1.2 and 7.4 buffer media. The *in vitro* release results of the pH-responsive IPNMGs showed that they can be applied to the oral controlled release of 5-FU. The release kinetics of 5-FU was analyzed using the Peppas equation to understand the nature of the drug release mechanism.

Key words: Chitosan, Poly(vinyl alcohol), Graft copolymer, Interpenetrating polymeric network, Anticancer drug, Drug release.

1. INTRODUCTION

In recent years, natural polymers and their blends with synthetic polymers have been used widely in biomedical applications because of their biocompatibility, biodegradability, and stimuli-responsive nature [1]. On the other hand, they have limited applicability due to their poor mechanical properties. The modification of carbohydrate polymers with other synthetic monomers or blending of natural polymers with other modified synthetic polymers has attracted considerable attention in drug delivery applications [2-6] because the mechanical properties can be enhanced without sacrificing their biodegradability, biocompatibility, and responsiveness.

Chitosan (CS), poly[β-(1-4)-linked-2-amino-2-deoxy-D-glucose], is a well-known naturally abundant carbohydrate polymer that possesses pH sensitivity, mucoadhesive properties, biodegradability, biocompatibility, and non-toxicity [7,8]. Over the past decade, different drug delivery carriers have been developed in the form of microgels, capsules, microcapsules, gels, and tablets. On the other hand, the poor mechanical properties of CS have prompted researchers to develop modified CS or blending with other synthetic polymers for drug delivery applications.

Poly(vinyl alcohol) (PVA) is non-toxic, non-carcinogenic, biocompatible [9], can absorb relatively large amounts water, and is a low-cost polymer with good mechanical strength and film-forming properties [10]. PVA has proven to be a better candidate in the class of biomaterials, such as sensors, surgical repair [11], contact lenses, artificial blood vessels, artificial intestines [12], and artificial kidneys [13]. PVA, however, is a water-soluble hydrophilic polymer with long-term temperature and pH stability [14]. Its high solubility in water results in low stability. To avoid this problem, PVA needs to be modified to reduce its solubility by blending [15], copolymerization [16], grafting [17], and cross-linking [18]. PVA is biocompatible, non-toxic and exhibits minimal cell adhesion and protein absorption, which is desired in the biomedical field [19] and pharmaceutical applications [20]. PVA has good swelling properties in water and biological fluids and has a rubbery, elastic nature with mucoadhesive properties, making it highly useful in drug delivery [21]. PVA is capable of simulating natural tissue and can be accepted into the body. Poly(N-methacryloyl-4-aminosalicylic acid) (PHMA) has interesting biomedical applications in drug delivery because the macromolecular nature of the chains allows the ionization of aminosalicylic side groups. PHMA shows calcium complexation ability in bone cement formulations, as well as analgesic and anti-inflammatory activity [22].

This paper describes the development of new interpenetrating polymeric network microgels (IPNMGs) through a simple water-in-oil emulsion cross-linking method. First, a graft copolymer was

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synthesized using PVA and HMA monomer. The IPNMGs were then developed from CS and PVA-g-HMA. 5-fluorouracil (5-FU), as an anticancer drug, was encapsulated into these IPNMGs through *in situ* addition into the polymer blends. 5-FU is an anticancer drug that comes under the antimetabolite category and is used widely in cancer chemotherapy [23,24]. 5-FU is used to prevent subsequent scarring after trabeculectomy and to enhance the prognosis for long-term retinal reattachment. In colon cancer therapy, the intravenous administration of 5-FU produces heavier systemic side-effects because 5-FU has cytotoxic effects on normal cells. Numerous pH-sensitive microgels have been developed for the controlled release (CR) of 5-FU [25-28]. To the best of the authors' knowledge, no attempts have been made to formulate microgels of blends of CS with PVA-g-HMA to achieve the CR of 5-FU, overcome the burst release, and enhance drug release in a specific environment. The developed IPNMGs were characterized by Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The *in vitro* release studies of IPNMGs were performed in simulated gastrointestinal (GI) fluids (pH 1.2 and 7.4 buffer media).

2. MATERIALS AND METHODS

2.1. Materials

CS (low molecular weight with 7–85% degree of deacetylation), 5-FU, methacrylic anhydride (MA), and 4-aminosalicylicacid (4-ASA) were obtained from Sigma-Aldrich Chemical Company, USA. Analytical reagent grade potassium persulfate (KPS), PVA (MW=1,25,000), glutaraldehyde (GA), Tween-80, hydrochloric acid, acetic acid, and sodium hydroxide were purchased from S.D. Fine Chemicals, Mumbai, India. Double distilled water was used throughout the experiments.

2.2. Synthesis of 2-hydroxy-4-N-methylamidobenzoic acid (4-HMA) Monomer

4-HMA monomer was synthesized using the same method reported by Elsevier [29]. 4-HMA was synthesized by the selective amidation of 4-ASA with MA at 0°C using acetone as a solvent. To a solution of 5 g of 4-ASA in dry acetone (50 mL), a solution of 7.15 mL MA in 30 mL of acetone was added dropwise under a N_2 atmosphere with constant stirring, and after the reaction (5 h), the solvent was distilled off at reduced pressure, and the solid residue was washed repeatedly with a water: methanol (4:1v/v) solution. The product was recrystallized from methanol: water (2:1) and vacuum dried (yield 60%). Scheme S1 presents a schematic chemical reaction of the HMA monomer.

2.3. Synthesis of PVA-g-HMA Copolymer

The PVA-g-HMA copolymer was synthesized using free radical polymerization method. Briefly, PVA (2.5 g) was dissolved in 50 mL distilled water at 70°C for 2 h to form a clear solution. After cooling the PVA solution, the HMA monomer solution (1 g of HMA dissolved in 10 mL distilled water with 0.2 g of NaOH) was added. Subsequently, 0.15 g of a KPS solution was added and heated to 70°C, and the reaction was continued for 12 h under an inert atmosphere. After the reaction was complete, the reaction mixture was cooled to room temperature and poured into acetone. A white precipitate (graft copolymer) was obtained and dried under vacuum at room temperature (yield 85%). Scheme S2 presents the schematic chemical reaction of the PVA-g-HMA copolymer.

2.4. Preparation of CS/PVA-g-HMA IPNMGs

The CS/PVA-g-HMA blend microgels were prepared using a water in oil (w/o) emulsion method. 2 g of CS was dissolved in 100 mL of a 2% acetic acid solution with constant stirring overnight. To this solution, a clear solution of PVA-g-HMA (0.25 g in 5 mL distilled water) was added and stirred until a homogeneous solution was obtained. The CS and PVA-g-HMA blend solution was added to a 500 mL beaker consisting of liquid paraffin oil (150 mL) with 0.5 (w/v) Tween-80 and agitated with a 3-blade propeller stirrer (IKA EURO STAR digital, Mumbai, India) at 600 rpm. A drop-by-drop solution of a measured quantity (2.5 mL) of aqueous GA and 2.5 mL of HCl were added. Stirring was continued for 5 h, and the microgels formed were separated by filtration and washed with hexane followed by distilled water to remove the adhered liquid paraffin and GA. The washing procedure was repeated to remove all the unreacted crosslinker and surface-adhered paraffin. The product was dried at room temperature for 24 h and used for further experimentation. Different formulations were prepared by varying the compositions, as listed in Table 1. Scheme 1 presents the schematics of the cross-linking reaction of the CS/PVA-g-HMA IPNMGs.

2.5. Swelling Studies

The pH-dependent equilibrium swelling ratio of the CS/PVA-g-HMA IPNMGs was performed in various pH solutions (1.2, 2, 3, 4, 5, 6, 8, and 9). To perform the swelling experiments, a known weight (10 mg) of the microgels was immersed in 10 mL of different pH solutions to reach equilibrium for 2 days at 37°C. Samples were taken from the pH solutions and blotted with a wiper to remove the surface-adhered medium and weighed on an electronic microbalance (Sartorius, BSA224S-CW, Switzerland) with an accuracy to ±0.1 mg. The percentage equilibrium swelling ratio (% ESR) was calculated using the following equation:

$$\%ESR = \frac{We - Wd}{Wd} \times 100 \quad (1)$$

Where W_e is the weight of the swollen IPNMGs at the equilibrium state and W_d is the weight of the dry IPNMGs.

2.6. Drug Loading and Encapsulation Efficiency

50 mg of 5-FU-loaded IPNMGs was immersed in pH 7.4 phosphate buffer medium for 48 h. The solution was then transferred to an agate mortar along with IPNMGs and agitated. The solution was then filtered with Whatman filter paper. The 5-FU content in the solution was analyzed using a UV spectrophotometer (UV-3092, LABINDIA, India) at λ_{max} = 270 nm. The % 5-FU loading and % encapsulation efficiency of the 5-FU were calculated using the following equations.

$$\%\ 5\text{-FU loading into the IPNMGs} = \left(\frac{\text{Weight of 5-FU in IPNMGs}}{\text{Weight of IPNMGs}}\right) \times 100 \quad (2)$$

$$\text{Encapsulation efficiency of 5-FU} = \left(\frac{\text{Actual 5-FU loading in to IPNMGs}}{\text{Theoretical loading 5-FU in to IPNMGs}}\right) \times 100 \quad (3)$$

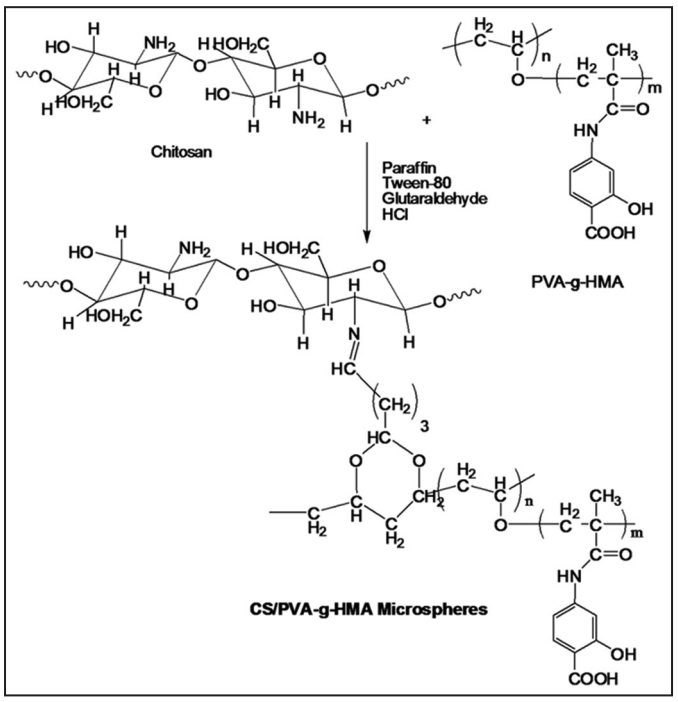
2.7. In vitro Drug Release

In vitro 5-FU release experiments of the CS/PVA-g-HMA IPNMGs were examined using the tablet dissolution system (DS8000, LABINDIA, India) at 37°C and 100 rpm in pH 1.2 and 7.4 phosphate buffer. 100 mg of the microgels were introduced in a basket bowl containing 500 mL of dissolution media (pH 1.2 or 7.4). The medium was rotated at 100 rpm and maintained at 37°C in a thermostat water bath. At pre-determined time intervals, 5 mL of the release medium

Table 1: Feed composition of various formulations.

Code	CS (g)	PVA-g-HMA (g)	GA (mL)
CS	2	0	2.5
CS/PVA	2	0.25 (PVA)	2.5
CS/PVA-g-HMA1	2	0.125	2.5
CS/PVA-g-HMA2	2	0.25	2.5

CS-(PVA-g-HMA: Chitosan poly (vinyl alcohol)-g-poly (2-hydroxy-4-N-methylamidobenzoic acid), GA: Glutaraldehyde



Scheme 1: Synthesis and crosslinking chemistry of chitosan poly(vinylalcohol)-g-poly(2-hydroxy-4-N-methylamidobenzoic acid microgels with glutaraldehyde.

was evaluated for the release of the drug from microgels using a UV-Vis spectrophotometer (UV-3092, LABINDIA, India) at l_{max} 270 nm. The experiment was performed in triplicate to calculate the standard deviation.

2.8. Characterization

FTIR spectroscopy of the samples was performed using a PerkinElmer Spectrum-2 spectrometer. The samples were ground thoroughly with potassium bromide to prepare a pellet under a hydraulic pressure of 600 dynes/m^2, and the spectra were scanned from 500 to 4000 cm^{-1}. Nuclear magnetic resonance ($^1H/C^{13}$ NMR) spectra of the 4-HMA monomer were recorded on a 400 MHz instrument (Varian 400 MHz NMR spectrometer) using deuterated dimethyl sulfoxide (DMSO-d_6) as a solvent and tetramethylsilane as an internal reference at room temperature (30 ± 2°C). The spectra were obtained after accumulating 16 scans using a 1% sample in DMSO. XRD (Rigaku mini field goniometer) was performed using Cu-*Kα* radiation (λ =1.5418 Å) at 30 kV and 40 mA over the range, 1.2–50° 2θ. DSC (TA instruments) was performed at a heating rate of 10°C/min in a nitrogen atmosphere (30–600°C). The morphological variations of the IPNMGs were examined by SEM (Table-top mini, SNE-3000M).

3. RESULTS AND DISCUSSION

3.1. Graft Copolymerization of HMA onto PVA

In the present study, the HMA monomer was synthesized using a previously reported procedure [29]. The formation of HMA monomer was confirmed by FTIR, ^1H NMR, and ^{13}C NMR spectroscopy. Graft copolymerization is an important method for polymerizing acrylic monomers onto PVA to obtain the desired responsive properties without sacrificing its original property. Normally, various types of initiators such as ceric ammonium nitrate, ammonium persulfate, potassium persulfate, ferric ion-hydrogen peroxide, and gamma rays are used for the graft copolymerization of acrylic monomers onto the PVA backbone in aqueous medium [30-32].

In the FTIR spectra of the HMA monomer (Figure S1), a characteristic peak was observed at 3373 cm^{-1} for the -N-H stretching vibrations along with a broad peak at 3206–2816 cm^{-1} for -COOH, Ar-OH, Ar-CH, aliphatic =CH, and C-H stretching vibrations, and the peak at 1685 cm^{-1} was assigned to the carbonyl group of the carboxylic free residue of HMA. In addition, bands at 1649 and 1625 cm^{-1} for -CONH- bending vibrations and -CH_2=C stretching vibrations were observed with peaks at 1598, 1528, and 1499 cm^{-1} for the aromatic -CH bending vibrations. Figures S2 and S3 present the ^1H-NMR and ^{13}C NMR spectra of HMA, respectively. The vinyl group of protons corresponding to b was 5.5 and 5.8 ppm. The non-equivalent -CH groups in the aromatic ring, f, k, and j, are shown in the spectra at 7.2, 7.5, and 7.7 ppm. A sharp singlet can be seen at 1.9 ppm, and a broad singlet can be observed at 3.4 ppm for -CH_3 (a) group aromatic -OH protons. The peaks at 10 and 11.4 ppm correspond to the -OH group of carboxylic acid and -NH proton of amide group, respectively. For ^{13}C NMR, the peaks were assigned, as presented in Figure S3. The characterization results confirmed the formation of HMA. The synthesized HMA monomer was grafted further onto the PVA chains using a simple conventional radical initiation system. First, persulfate anion radicals were formed when heated to the reaction mixture at 70°C. A radical was then abstracted hydrogen from PVA to form an alkoxy radical on the substrate. The resulting active substrate radically initiated polymerization of the HMA monomer to yield the graft copolymer. The grafting and grafting yield of the synthesized graft copolymers were 103% and 91.63%, respectively. Graft copolymerization was confirmed by FTIR, DSC, and XRD. In Figure S4, curve (a), the absorption at 1651 cm^{-1} corresponds to a carbonyl functional group in PVA (i.e., less % of vinyl acetate). For the HMA monomer grafted onto the PVA backbone in curve (b), the peak at 1630 cm^{-1} was assigned to the C=O stretching (amide I) of O=C-NHR, while the band at 1585 cm^{-1} was assigned to the NH bending (amide II) (NH_2). In pure PVA, the band at 1137 cm^{-1} was due to the C-O stretching vibrations. After grafting, a broad peak at 1096 cm^{-1} was assigned to the C-O stretching vibrations. The above spectra confirmed that the HMA monomer was grafted onto PVA backbone. Grafting was also confirmed by DSC and XRD. Figure S5 shows the DSC thermograms of PVA and PVA-g-HMA. The peak at approximately 290°C on the DSC curve was assigned to the melting of PVA. After grafting the HMA monomer onto PVA, the peak was shifted to a higher temperature (320°C). The increase in peak temperature indicated that the structure of the PVA chains was changed by the introduction of HMA chains. This was confirmed for the formation of graft copolymer. Figure S6 shows the XRD patterns of (a) pure PVA and (b) PVA-g-HMA. Pure PVA exhibits crystalline peaks indexed to the 001 plane at 16°, 100 at 19, 101 at 20°, and 200 at 23° 2θ [33]. After grafting the HMA monomer onto the PVA backbone, an additional sharp peak appeared at 32° 2θ. Grafting affected the crystal structure of PVA. Therefore, XRD also confirmed the grafting of the HMA monomer onto PVA.

3.2. Preparation of Microgels from CS and PVA-g-HMA Blends

Many studies have developed microgels by combining natural polymers and synthetic polymers and their modified polymers using a water-in-oil emulsion cross-linking method [25-28]. In the present work, IPNMGs were developed using a simple emulsion cross-linking method. First, a mixture of the polymer blend solutions was mixed with paraffin oil to form a W/O emulsion. The emulsion was stirred using a three-blade mechanical stirrer at a constant rpm to obtain uniform aqueous polymer droplets in the oil phase. The emulsion droplets formed were highly spherical and uniform in size, due to interactions between the protonated amine groups of CS and the acid functionality of the graft copolymer chains through complexation. Tween-80 also supported the stability of the aqueous polymer droplets. Finally, an IPN structure was developed in the microgels by the addition of a GA solution in the presence of an acid catalyst. Figure 1 shows photographs of IPNMGs, clearly indicating that the microgels were spherical and uniform in nature. Scheme 1 presents the cross-linking reaction of GA with CS and PVA-g-HMA. 5-FU as a model anticancer drug was loaded into these IPNMGs through an *in situ* method. The encapsulation efficiencies of all formulations were calculated as shown in Table 2.

3.2.1. FTIR spectra

The formations of the IPNMGs were confirmed by FTIR spectroscopy. Figure 2 shows the FTIR spectra of 5-FU, CS, CS/PVA, CS/PVA-g-HMA, and drug-loaded CS/PVA-g-HMA microgels. The CS/PVA, CS/PVA-g-HMA, and drug-loaded CS/PVA-g-HMA MGs showed a broadband at 3616–3068 cm^{-1} (due to -OH stretching and overlap with the peak of a –NH stretching) (see for all curves -NH stretching except curve (a)). In curve (c), pure CS MGs present two characteristic absorption peaks at 1634 cm^{-1} and 1518 cm^{-1}, which were assigned to an amide functional group, i.e., acetylated amine, and a free amine I functional group, i.e., deacetylated amine, respectively. CS blended with PVA cross-linked with GA (curve (d)) showed peaks at 1451 and 1384 cm^{-1} for the N-H bending and C-N stretching vibrations. On the other hand, a strong band at 1138 cm^{-1} was observed for the C-O-C stretching vibrations. In curve (c) and (d), bands at 2954, 2923, and 2049cm^{-1} (due to the aliphatic -CH, $-CH_2$, and C=C stretching vibrations), at 1255 and 1159 cm^{-1} (due to O-H in-plane bending and -OH stretching vibrations in cyclic alcohols), and at 1708 cm^{-1} (due to the presence of carbonyl groups in carboxylic acid) were observed. A relatively intense peak at 2853 and 890 cm^{-1} was assigned to the aliphatic C-CH_3 stretching vibrations and -CH bending vibrations in substituted ethylene systems in the grafted copolymer. Peaks at 1634 and 1549 cm^{-1} were observed, which confirmed the grafting reaction of HMA on PVA. In addition, peaks at 1075 and 1632 cm^{-1} represent the stretching vibrations of the C-O and C=N bond, confirming the formation of CS GA crosslinks. This revealed the formation of a Schiff's base and acetal linkage between CS and PVA-g-HMA, respectively, by GA. In the case of pure CS, cross-linking leads to the formation an imine bond or ionic bonds, whereas bands at 1521, 1462, and 1377 cm^{-1} (C=C, $-CH_2$, and $-CH_3$ bending vibrations) were observed.

3.2.2. DSC and XRD studies of IPNMGs

The physicochemical states of the biological active molecules present in the MGs were examined by DSC and XRD. Many bioactive agents are crystalline and should give a high-intensive endothermic peak in DSC and crystalline peaks in the XRD pattern. After the encapsulation of bioactive molecules into the particular polymeric matrix, the crystalline nature collapsed because, during encapsulation, the crystalline phase of the drug molecules will be converted to a molecular state. From DSC (Figure 3a), 5-FU (a) showed an endothermic peak at 270°C due to melting. On the other hand, such a melting peak was not observed for the 5-FU-loaded and pristine IPNMGs, indicating the molecular level

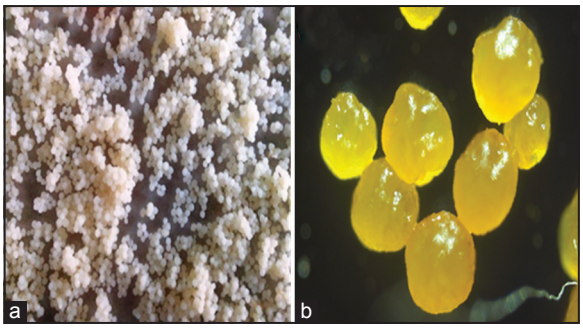


Figure 1: Photographs of (a) swollen chitosan/poly(vinyl alcohol) (CS/PVA-g-HMA) interpenetrating polymeric network microgels (IPNMGs) and (b) CS/PVA-g-HMA IPNMGs

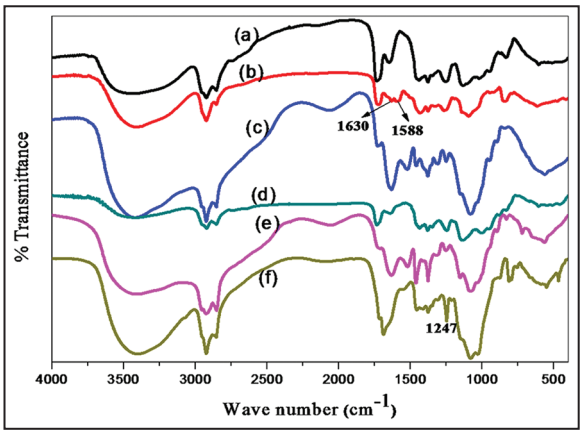


Figure 2: Fourier-transform infrared spectra of (a) poly(vinyl alcohol (PVA), (b) PVA-g-poly(2-hydroxy-4-N-methylamidobenzoic acid) PVA-g-HMA, (c) chitosan (CS), (d) CS/PVA microgels (MGs), (e) CS/PVA-g-HMA interpenetrating polymeric network MGs (IPNMGs), and (f) 5-Fluorouracil-loaded CS/PVA-g-HMA IPNMGs.

distribution of 5-FU in the microgel networks. XRD also confirmed the molecular level dispersion of 5-FU in the IPNMGs (Figure 3b). 5-FU in Figure 3b (c) showed a highly crystalline XRD pattern peak at 38° due to polymorphism and melting. On the other hand, a crystalline peak was not observed in the case of 5-FU-encapsulated IPNMGs and pristine IPNMGs, which indicates that 5-FU was highly dispersed in the IPNMGs at the molecular level.

3.2.3. SEM studies of IPNMGs

The morphology of IPNMGs was characterized by SEM. Figure 4 shows the CS/PVA-g-HMA IPNMGs cross-linked with GA. The IPNMGs were smooth, spherical, and 20 μm in diameter.

3.3. pH-Responsive Behavior of IPNMGs

The pH-responsive swelling characteristics of the MGs were analyzed by their equilibrium swelling values in various pH solutions at 37°C. As shown in Figure 5, the % ESR of the CS-based MGs was very high at pH 1.2. This is because the amino groups present in CS became ionic at lower pH; thus, ion-ion repulsion forces developed in the network structure of the MGs. Hence, the network structure could expand at lower pH. On the other hand, the maximum swelling was observed for both CS and CS-PVA MGs at pH 6 due to pKa of CS [5]. For IPNMGs (CS-PVA-g-HMA1 and CS-PVA-g-HMA2), however, the swelling increased significantly with increasing pH. This was due to the

Table 2: Percentage encapsulation efficiency and *in vitro* release parameters of various formulations.

Formulation code	% EE±SD	pH 1.2			pH 7.4		
		n	*k*	r^2	*n*	*k*	r^2
CS	48.3±1.6	0.32	1.03	0.9298	0.39	1.13	0.9846
CS-PVA	53.2±2.1	0.36	1.02	0.9991	0.43	1.10	0.9912
CS-(PVA-g-HMA1)	61.5±1.4	0.42	0.83	0.9639	0.46	0.96	0.9894
CS-(PVA-g-HMA2)	64.0±1.1	0.45	0.54	0.9195	0.48	0.64	0.9247

SD=standard deviation (n=3). CS-(PVA-g-HMA: Chitosan poly (vinyl alcohol)-g-poly (2-hydroxy-4-N-methylamidobenzoic acid)

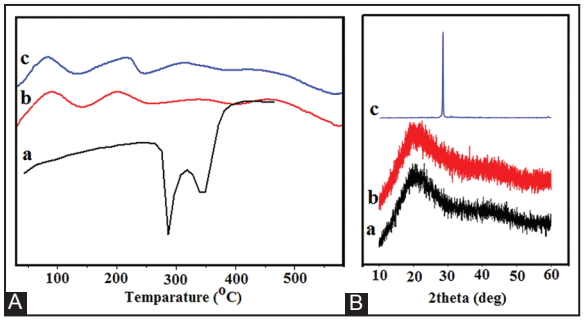


Figure 3: (A) Differential scanning calorimetry thermograms of (a) 5-Fluorouracil (5-FU), (b) 5-FU-loaded interpenetrating polymeric network microgels (IPNMGs), and (c) pristine IPNMGs and (B) X-ray diffraction patterns of (a) pristine IPNMGs, (b) 5-FU-loaded IPNMGs, and (c) pure 5-FU.

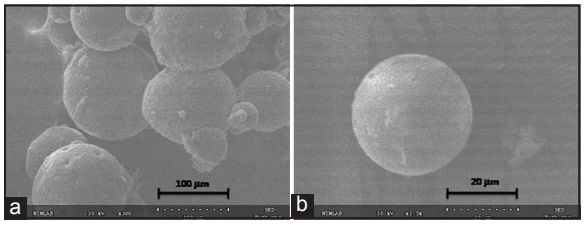


Figure 4: Scanning electron micrographs of interpenetrating polymeric network microgels (IPNMGs) (a) group of IPNMGs and (b) single IPNMGs.

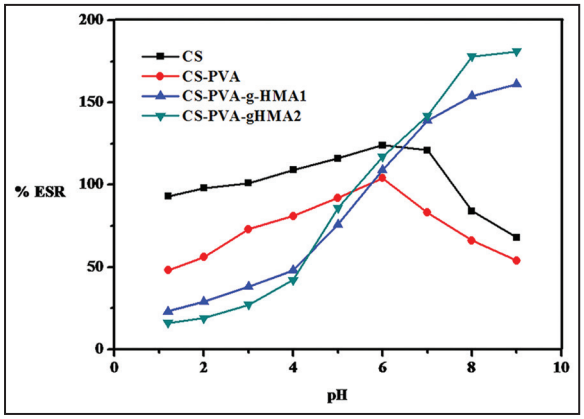


Figure 5: Swelling studies of interpenetrating polymeric network microgels at various pH solutions.

ionization of carboxylic groups of HMA onto PVA. Therefore, HMA can play an important role in the swelling behavior under different pH conditions. The graft polymer ratio also affected the pH swelling of IPNMGs. With increasing concentration of graft copolymer in the IPNMGs, the carboxylic functionality also increased, which was caused by the improved swelling behavior with increasing pH. This is due to the more ionic repulsion indicating the highly expanded network structure of IPNMGs. Finally, the swelling results confirmed the pH sensitivity of IPNMGs.

3.4. In vitro Drug Release

In vitro release studies of all formulations were performed in both pH 1.2 and 7.4 media (GI tract [GIT] conditions). Figure 6a and 6b shows the release profiles for all formulations. The initial burst release was not observed for the CS/PVA-g-HMA IPNMGs, indicating that, during the formation of the IPNMGs, the PVA-g-HMA could cover all drug molecules in the network structure of MGs. With the exception of CS, all other formulations showed high release profiles in GIT fluids. The release of 5-FU depends mainly on the contraction and expansion of the networks of IPNMGs. The contraction and expansion of the network of IPNMGs can be explained based on the pH swelling of IPNMGs, as discussed above.

The release profiles were fitted according to the well-known Peppas equation [34] to understand the release mechanism of 5-FU from IPNMGs. The following Peppas equation was used to calculate the parameters of the release profiles.

$$\frac{Mt}{M\infty}=kt^n \quad (4)$$

Where $\frac{Mt}{M^\circ}$ is the fractional 5-FU release at time *t*; *k* is a constant, and *n* is an exponent. Table 2 lists the calculated values of *n* and *k* for all formulations. In general, *n*=0.5 represents Fickian diffusion (drug diffuses and is released from the polymer matrix), *n*>0.5 indicates anomalous or non-Fickian type, and *n*=1 shows completely non-Fickian diffusion. Intermediary values between 0.5 and 1.0 were attributed to the anomalous type of diffusive transport. In the present study, *n* values were 0.325–0.48, which were attributed to diffusive transport.

4. CONCLUSION

pH-sensitive IPNMGs were developed from CS and PVA-g-HMA blends using a W/O emulsion method. The grafting and grafting yield of PVA-g-HMA were found to be 103% and 93%, respectively. This graft copolymer could play an important role to achieve the formation of IPNMGs with the pH-responsive nature for the successful encapsulation of 5-FU loading. SEM and optical microscopy showed that the IPNMGs were spherical, ~20 µm in size, with a smooth surface. The molecular level distribution of 5-FU present in the IPNMGs was confirmed by DSC and XRD. From the *in vitro* release results, IPNMGs are the most effective for the oral delivery of 5-FU because they could protect 5-FU from the acidic conditions of the stomach (pH 1.2) and most of the drug release in the intestinal conditions (pH 7.4). The newly developed IPNMGs of the present study could provide a way to utilize pH-sensitive CR of bioactive molecules through the oral route.

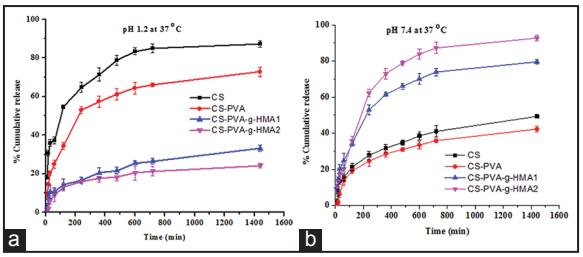


Figure 6: *In vitro* release studies of 5-Fluorouracil through interpenetrating polymeric network microgels formulations in (a) pH 1.2 and (b) 7.4 h 37°C.

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