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Research Article

Highly Porous pH-Responsive Carboxymethyl Chitosan-*Grafted*-Poly (Acrylic Acid) Based Smart Hydrogels for 5-Fluorouracil Controlled Delivery and Colon Targeting

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In the present investigation, new formulations of CMCS/AA hydrogels with varying composition of Carboxymethyl chitosan, acrylic acid, and ethylene glycol dimethacrylate (EGDMA) were prepared by free radical polymerization technique using benzoyl peroxide as catalyst. The bioavailability of 5-FU through the oral route is very limited owing to its rapid metabolism and clearance from the general circulation. Current work was aimed at increasing the bioavailability of 5-FU *via* smart hydrogels and at investigating their potential in delivering 5-FU to target colon cancer. Swelling studies were carried out on dried hydrogel discs in different USP phosphate buffer solutions of various pH values. Porosity and gel fraction of all the samples were measured. 5-FU was used as a model drug and loaded in selected hydrogel samples. The amount of drug loaded and released was determined. Experimental data was fitted to various model equations, and corresponding parameters were calculated to study the release mechanism. Many structural parameters were calculated. The prepared hydrogels were also characterized by FTIR and SEM to study the structure, crystallinity, compatibility, and morphology of the smart hydrogels. The biocompatibility and cytotoxic potential blank and drug-loaded hydrogels were assessed through MTT assay. The prepared hydrogels were found to be an excellent carrier for 5-FU in targeting colon cancer.

1. Introduction

Colon cancer has been reported as a major cause of deaths worldwide [1, 2]. The primary treatment of choice is its surgical removal and early detection and resection be crucial for treating colorectal cancer [3, 4]. However, the recently used chemotherapeutic drugs proposed for the treatment of this disease are related to various toxicities.

The concept of sustained drug release offers advantages in the effective treatment of diseases over the traditional dosage forms. Many sustained release oral solid drug delivery systems such as SR tablets, capsules, and oral pills have been developed. They offer localized drug delivery to the targeted site in the body, improved efficacy, reduced toxicity, improved stability, reduced frequency, optimized drug absorption, and highest patient compliance and convenience. However, the failure of such dosage forms to release of the drug in the predetermined fashion could result in serious consequences [5, 6]. Hydrogels have shown a potential for the delivery of orally administered drugs targeted to colon based on pH control release along the pH change in the human gastrointestinal tract. In response to various stimuli like pH, temperature, and ionic strength, hydrogels show volume changes which are desired characteristics for controlled drug release. By using these drug delivery techniques, numerous local diseases of the colon like infections, ulcerative colitis, Crohn's disease, and carcinomas can be successfully treated. The above-mentioned pathological conditions can be treated by successful delivery of anticancer agents, anti-inflammatory drugs, and antibiotics to the colon [7, 8].

The presence of ionizable functional groups in hydrogels determine the intermolecular interactions between polymer chains and surrounding fluid resulting in pH-dependent swelling of hydrogels [9]. Drug delivery from hydrogels is also influenced by the structural parameters like charge, p*K*a of ionizable groups, degree of ionization, crosslinking degree, and monomeric contents of the network [10, 11].

Hydrogels are cross-linked superabsorbent that has the ability to absorb a large amount of water when placed in buffered or physiological solutions [12]. These materials remained undissolved because of the presence of physical or chemical cross-linkage of polymer chains [13]. Stimuli-responsive hydrogels have attracted considerable attention and are being developed at a prolific rate as drug carrier systems for targeted drug delivery. These hydrogels contract and relax in response to small pH variation of the surrounding medium [14]. These cross-linked networks keep their physical structure stable even when they absorb, swell, and hold a high amount of water or physiological fluids in comparison to thousand times of their dry weights, but eventually, they may disintegrate [15]. Hydrogels show a high biocompatibility in a swollen state due to their soft and rubbery nature resembling living tissues [16]. The swelling behavior of hydrogels can be altered to produce desired characters by controlling various parameters, including parameter composition, crosslinking degree, nature of the crosslinking agent, pH, and ionic strength of the medium [17]. Hydrogels have been the major field of biomedical research and found extensive applications as delivery systems, contact lenses, biomimetic transducing devices, electrophoresis cells, parasternal muscle, artificial lung, and artificial joint biomaterials [18]. Smart polymeric gels have been used as biosensors, actuators, and nanoscale biomedical devices [19, 20]. These are three dimensional cross-linked polymeric structures that have widespread applications in medical, pharmaceutical, environmental, and biological fields due to their nontoxic, biocompatible, and hydrophilic nature [21].

A variety of natural and synthetic polymers comprising suitable functional groups have been found to prepare pH-responsive controlled release drug delivery systems.

Polyacrylic acid hydrogels possess the ability to swell in water many times their original weight. The extensive swelling of polyacrylic acid (PAA) hydrogels is based on the occurrence of carboxyl groups in the polymer network. These groups are highly ionizable, strongly interact with water molecules, and sensitive to the changes in pH and ionic strength of the medium. Since the p*K*a of acrylic acid lies between 4.5 and 5.0, the swelling capacity of PAA hydrogels is minimum at pH below 4 and higher at pH above 5, which is the desired property for oral controlled release drug delivery systems. The crosslinking degree of the polymeric network should be controlled because it determines the macromolecular structure, mechanical strength, phase transition, and other properties of the hydrogels [22].

Carboxymethyl chitosan (CMCS), which has water solubility, contains amino groups (-NH_2) and carboxyl groups (-COOH) simultaneously in the glucosamine units of the chitosan structure. Due to the presence of amino groups in its side chain, CMCS shows a good ionic and pH sensitivity in aqueous solution [6]. Furthermore, it has good physical, chemical, and biological characteristics such as high biocompatibility, bioadhesiveness, nontoxicity, and excellent gel-forming properties. Due to these properties, this derivative of chitosan has widely been applied in biomedical fields as tissue regenerator, as drug and gene carrier, and as antimicrobial material [23].

5-Fluorouracil (5-FU) is one of anticancer drug mostly used in the management of different types of tumors [24], such as colon [25–27], breast [28], pancreatic [29], or gastric cancers [30]. It is an antimetabolite whose mechanism of action mainly include DNA and RNA synthesis inhibition during the S-phase of the cell cycle. Moreover, its metabolism occurs rapidly in the body which in turn rapidly cleared from the body [31–33]. Moreover, due to its limitation of short plasma half-life and poor absorption after oral administration, 5-FU has been selected to be delivered through various sustained release systems [34, 35].

5-FU, a chemotherapeutic agent, is rapidly cleared from the general circulation when administered *via* oral route due to its short half-life. In previous investigations, delivery of 5-FU to most of the cancer types was made possible *via* nanoparticulate carriers. However, most of these nanoparticles encountered a problem due to their stability in general circulation. The aim of the current work was to investigate the potential of delivering 5-FU *via* Carboxymethyl chitosan-*grafted*-Poly (acrylic acid), i.e., Poly (CMCS-*g*-AA) based smart hydrogels to target colon cancer. The objective of the current study also includes the increase in oral bioavailability of 5-FU by incorporating this drug into the smart controlled release matrix of Poly (CMCS-*g*-AA) hydrogels. pH, composition, and degree of crosslinking effects on swelling and drug release were studied in a phosphate buffer solution of various pH values. The best release mechanism was analyzed by fitting drug release data to various mathematical models. Various processing parameters were used to determine the strength, porosity, solvent interaction, and sol-gel fraction of hydrogels. The structure, crystallinity, and surface morphology of prepared hydrogels were analyzed by Fourier transformed infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

2. Experimental Procedures

2.1. Materials. 5-FU (Sigma-Aldrich, Barcelona, Spain, purity 99.80%). Acrylic acid (AA) has purity 99%, and the polymer was Carboxymethyl chitosan (Mw 30000-100000 fluka, Buchs, Switzerland, purity 98.90%). Ethylene glycol dimethacrylate (EGDMA, Merck) and benzoyl peroxide (Merck) were used as crosslinking agent and initiator, respectively. All other analytical grade materials were used.

2.2. Preparation of Hybrid Polymeric Network of Poly (CMCS-Grafted-AA). In the present work, different formulations of hydrogels were prepared with the varying composition of the polymer, monomer, and crosslinker. A previously known method with some modifications was used to prepare the series of hydrogels [6, 23]. A calculated quantity of Carboxymethyl chitosan was added into a predetermined

Table 1: Feed composition of Poly (CMCS-*g*-AA) hydrogels.

Sample code	CMCS/100 g solution	Acrylic acid/100 g solution	Degree of crosslinking EGDMA Mol (%)	(EGDMA)/ 100 g solution
HS_1	1.25	25.45	0.70	0.178
HS_2	1.25	32.72	0.70	0.229
HS_3	1.25	40.00	0.70	0.280
HS_4	1.20	29.09	0.70	0.203
HS_5	1.40	29.09	0.70	0.203
HS_6	1.60	29.09	0.70	0.203
HS_7	1.60	32.72	0.25	0.065
HS_8	1.60	32.72	0.50	0.196
HS_9	1.60	32.72	1.0	0.327

amount of distilled water under constant stirring at room temperature. A varying amount of ethylene glycol dimethacrylate (EGDMA) and benzoyl peroxide were dissolved in AA. Each of the above solutions were mixed well individually. The two solutions were mixed together thoroughly and the final volume was make 100 g with the addition of distilled water. Finally, the polymerization of the prepared solution was carried out in glass tubes (Pyrex) of 16 mm internal diameter and 150 mm length. The air from tubes was removed through nitrogen bubbling for 15 to 20 minutes and closed with a lid. The capped tubes were kept in a water bath, and the temperature was increased gradually to avoid autoacceleration and bubble formation. The temperature scheme for solution polymerization was 45°C for one hour, 50°C for two hours, 55°C for three hours, 60°C for four hours, and 65°C for 12 hours. After cooling at room temperature, hydrogel samples were taken out from glass tubes and cut into 6 mm discs in length. The hydrogel discs were placed into freshly prepared (50% *v*/*v*) ethanol-water mixture for the removal of uncrosslinked monomer and initiator. The hydrogel discs were washed daily with fresh solution until the pH of the water-ethanol mixture become equal to before washing. The washed discs were removed, placed for drying first at room temperature, and then in a vacuum oven at 40-45°C for one week. Table 1 refers to the feed composition of Poly (CMCS-*g*-AA) hydrogels.

2.3. Physical Appearance. A stable Poly (CMCS-*g*-AA) hydrogels were formed after successful free radical polymerization and components crosslinking. All formulations exhibited strong mechanical strength and unbroken structure.

2.4. Swelling Studies

2.4.1. Dynamic and Equilibrium Swelling Studies. The response of Poly (CMCS-*g*-AA) hydrogels to swelling behavior was analyzed in different USP phosphate buffer solutions (pH 1.2, 5.5, 6.5, and 7.5) to simulate the pH of the colon, respectively, with constant ionic strength. Weighted, washed, and dried discs were placed in solutions of different pH values at 37°C. At regular time intervals, hydrogel discs were removed from the medium, blotted with laboratory paper, and weighed and placed in the same buffer solution. The dynamic swelling ratio of each sample was calculated using the following equation [1].

$$q = \frac{W_h}{W_d}, \tag{1}$$

where W_h refers to the swollen gel weight at time t, and W_d refers to the dry disc weight of the hydrogel disc. To determine the equilibrium ratio, the swelling was continued till the constant weight was obtained. Equilibrium swelling ratio was calculated by using the following relation.

$$S_{(\mathrm{Eq})} = \frac{W_h}{W_d}, \tag{2}$$

where W_h refers to the weight of gel at equilibrium in the hydrated state, and W_d is the dry hydrogel disc weight [22, 23].

2.4.2. Diffusion Coefficient. Diffusion coefficient refers to the quantity of substance diffusing through a concentration gradient across the unit area in unit time. The following equation was used for the calculation of water diffusion coefficients of hydrogels [36].

$$D = \pi\left(\frac{h.\theta}{4.Q_{\mathrm{eq}}}\right)^2, \tag{3}$$

where D refers to the diffusion coefficient of the hydrogels, Q_{eq} refers to the gel swelling at equilibrium, θ refers to the slope of the linear part of the swelling graphs, and h indicates the sample thickness of the dry hydrogel disc before subjected to swelling.

2.5. Characterization of Network Structure of Poly (CMCS-g-AA) Hydrogels

2.5.1. Solvent Interaction Parameters (χ). In order to investigate the compatibility of polymer with the molecules of the surrounding fluid, solvent interaction parameters were measured. The volume fraction of polymers in a hydrated state is the amount of fluid absorbed and retained by the hydrogel. Flory-Huggins theory was used to calculate χ. The following equation was used to calculate χ values.

$$\chi = \frac{1\mathrm{n}(1 - V_{2,s}) + V_{2,s}}{{V_{2,s}}^2}, \tag{4}$$

where $V_{2,s}$ (ml/mol) indicates the volume fraction of the hydrated gel in the equilibrium state, and "χ" refers to the solvent interaction parameters [37].

2.5.2. Molecular Weight between Crosslinks (M_c). Flory-Rehner theory was used to determine the values of molecular weight between crosslinks of Poly (CMCS-*g*-AA) hydrogels. According to this theory, M_c values were increased by increasing the swelling ratio of hydrogels [23, 38]. The following equation was used to measure the molecular weight between crosslinks.

$$M_c = -\frac{d_p v_s \left(v_{2,s}{}^{1/3} - v_{2,s}/2\right)}{1n(1 - v_{2,s}) + v_{2,s} + x v_{2,s}{}^2}, \quad (5)$$

where $V_{2,s}$ which refers to the volume fraction of the polymer was calculated by the following equation.

$$V_{2,s} = \left[1 + \frac{d_p}{d_s}\left(\frac{M_a}{M_b} - 1\right)\right]^{-1}, \quad (6)$$

where d_p and d_s refer to densities (g/ml) of the gel sample and solvent, respectively. M_a and M_b refer to the masses (g) of the hydrated and dry hydrogels, respectively. $V_2, _s$ (ml/mol) indicates the volume fraction of the hydrated gel sample in the equilibrium state, and (χ) refers to the polymer solvent interaction parameters.

2.5.3. Cross-Linked Density (q). Crosslinking density is used for the characterization of cross-linked hydrogels [23, 39]. The following equation was used for calculating (q).

$$q = \frac{M_c}{M_r}, \quad (7)$$

where M_r refers to the molar mass of the repeating unit and is measured by using the following equation.

$$M_r = \frac{m_{CMCS} M_{CMCS} + m_{AA} M_{AA} + m_{EGDMA} M_{EGDMA}}{m_{CMCS} + m_{AA} + m_{EGDMA}}, \quad (8)$$

where m_{CMCS}, m_{AA}, and m_{EGDMA} are the feed masses of the hydrogel components, i.e., CMCS, AA, and EGDMA, respectively, and where M_{CMCS}, M_{AA}, and M_{EGDMA} are the molar masses of CMCS, AA, and EGDMA, respectively.

2.6. Sol-Gel Analysis. For sol-gel analysis, hydrogel samples (3-4 mm in size) dried previously at 45°C in an oven were subjected to soxhlet extraction with deionized water as solvent at an elevated temperature for 4 h. This process was used to remove the uncrosslinked polymer from the gel structure. After the extraction process, the gels were placed again in a vacuum oven for drying at 45°C to constant weight. The gel fraction was calculated by using dry hydrogel disc weight of (W_0) and hydrogel disc weight after extraction (W_{ext}) using the following equation.

$$\text{Sol fraction}(\%) = \left[\frac{W_0 - W_{ext}}{W_0}\right] \times 100,$$
$$\text{Gel fraction}(\%) = 100 - \text{Sol fraction}, \quad (9)$$

where W_0 denotes the hydrogel weight in dry form before the extraction process, and W_{ext} refers to the dried hydrogel disc weight after the extraction process [23].

2.7. Porosity Determination. For the measurement of porosity, solvent replacement method was used. Weighted dried gel discs were placed in absolute ethanol overnight and then weighted again after removing excess ethanol on the surface with blotting paper. The following equation was used for calculating the porosity (%).

$$\text{Porosity} = \frac{(M_h - M_d)}{\rho V} \times 100, \quad (10)$$

where M_h and M_d are weights of hydrated and dried hydrogel discs, respectively. P refers to the density of absolute ethanol, and V indicates the volume of hydrogel disc [40].

2.8. 5-FU Loading of Poly (CMCS-g-AA) Hydrogels. For drug loading and release studies, selected samples were used. Three samples with different AA composition (25.45%, 32.50%, and 40%) and three samples with different crosslinking agent (0.25%, 0.50%, and 1%) were used for drug loading. The drug solution of 5-FU was prepared by dissolving 5-FU in 50% (v/v) ethanol/deionized water solution. Previously weighted dried hydrogel samples were kept in 1% (w/v) drug solution of 5-FU up to equilibrium swelling. After reaching to equilibrium swelling, drug-loaded hydrogel samples were dried first at room temperature and then placed in an oven at 45°C till uniform weight [22, 23].

2.8.1. Determination of 5-FU Loading. The loading of 5-FU in selected samples was determined by using various methods. For determining the percentage of drug loaded in hydrogels, different methods were applied. The first method used to calculate the amount of drug loaded in hydrogels was by the following equation [41]:

$$\text{Amount of drug} = W_D - W_d,$$
$$\text{Drug loading}\% = \frac{W_D - W_d}{W_d} \times 100, \quad (11)$$

where W_d and W_D are dried hydrogel discs weight before and after placing in a drug solution, respectively. In another method, drug encapsulated in hydrogels was calculated by continually extracting the weighted quantity of loaded gels using 50% (v/v) ethanol/deionized water solution. Every time 25 ml fresh ethanol/deionized water solution 50% (v/v) was used until no drug in the solution was left behind. Drug concentration was calculated spectrophotometrically. The total amount of drug present in extracts was considered as a quantity of drug loaded.

In the third method to calculate the drug loading in the hydrogel, weighted gel disc was placed in a drug solution up to equilibrium swelling. The loaded gel was weighted again after blotting with blotting paper. The weight of drug solution was calculated by measuring the difference in weight before and after swelling of selected gel samples. The volume of drug solution absorbed by gel disc can be calculated by knowing the density and weight of the drug solution. By knowing the volume of drug solution, the amount of drug absorbed by gel disc was calculated.

2.9. In Vitro 5-FU Release Study. Drug release from cross-linked hydrogel samples was calculated using dissolution apparatus (Pharma Test; PT-Dt 7, Germany) linked with UV-vis spectrophotometer (IRMECO, UV-VIS U2020). The

Table 2: Amount of 5-FU loaded and released from selected samples.

Sample codes	Amount of 5-FU loaded (g/g of dry gel)			Amount of 5-FU released (%) (pH of the solution)		
	By swelling	By weight	By extraction	1.2	6.5	7.5
HS_1	0.065	0.069	0.060	27.76	61.79	79.13
HS_2	0.071	0.073	0.069	23.65	69.90	82.56
HS_3	0.072	0.077	0.074	24.99	70.25	83.19
HS_7	0.082	0.083	0.078	26.49	77.69	88.89
HS_8	0.076	0.078	0.072	24.21	70.74	83.93
HS_9	0.059	0.063	0.061	21.37	66.14	77.08

weighted hydrogel disc was immersed in 500 ml dissolution medium at 37°C and at 100 rpm for maintaining a uniform drug concentration. USP phosphate buffer solutions (0.05 M) of variable pH values (1.2, 6.5, and pH 7.5) were used as release medium. 5-FU release study was conducted at λ_{max} 265 nm up to 12 h after regular intervals. Each time 5 ml aliquot of the sample was expelled for UV analysis, and the medium was replaced with fresh USP phosphate buffer solution [41]. Table 2 shows the quantity of 5-FU encapsulated and percent released from selected samples.

2.10. Cell Cultures and Cell Viability Studies. Human cervical (HeLa cells) and African green monkey cell lines (Vero cells) were cultured in a medium comprising RPMI-1640 supplemented with l-glutamine (2 mM), penicillin (100 UmL^{-1}), and streptomycin (100 μg mL^{-1}) containing additionally 10% FBS grown in a 75 cm^2 tissue culture flask and placed under incubation supplied continuously with 5% CO_2 37°C. After achieving 80% confluency, the cells were harvested, seeded, and cultured at 10000 cells/well in a 24-well flat bottom cell culture plate and utilized for cell viability studies [5].

Methyl thiazolyl tetrazolium (MTT) assay was used for cell viability study at various concentrations (1, 2, 4, 6, 8, 10, 15, and 20 μg/ml) for 5-FU in free form and encapsulated in hydrogel form. Cell viability study was conducted in a 24-well plate. Doxorubicin was selected as positive control while cells devoid of formulations were used as negative control, respectively. The cytotoxic potential was determined by placing the drug-loaded discs in a 24-well plate encapsulated with different 5-FU concentration. Cell culture medium was added on the top of the hydrogel discs followed by incubation for 24 h at 37°C. The absorbance at 490 nm was calculated with BioTek Synergy HT (BioTek Instruments Inc.; Winooski, VT) [42]. The cell viability % was calculated using the following formula:

$$\text{Cell viability\%} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100, \tag{12}$$

where A_{sample} and A_{control} indicate the absorbance of the sample and control wells, respectively. The experiments were performed in triplicates. The reported data indicate the mean cell viability ± SD.

2.11. Drug Release Kinetics. Different mathematical models were used to investigate the release kinetics and mechanism applied to drug release data. These models are generally used when more than one phenomenon of drug release is involved. Korsmeyer-Peppas model was used to study the release mechanism from the hydrogels [43]. The equations mentioned below were used for the analysis of drug release.

$$\text{Zero-order kinetics}: F_t = K_0 t, \tag{13}$$

where F_t refers to the fraction of drug release at time t, and K_0 refers to the zero-order release constant.

$$\text{First-order kinetics}: \quad \text{In}(1 - F) = -K_1 t, \tag{14}$$

where F refers to the fraction of drug release at time "t", and K_1 indicates the first-order release constant.

$$\text{Higuchi model}: \quad F = K_2 t^{1/2} \tag{15}$$

where F refers to the fraction of drug release at time "t", and K_2 indicates the Higuchi constant.

$$\text{Korsmeyer-Peppas model}: \quad \frac{M_t}{M \propto} = K_3 t^n. \tag{16}$$

Here, M_t indicates the weight of water imbibed at penetrant time t, $M \propto$ is the mass uptake of water at equilibrium, K_3 refers to the kinetic constant, and n is the exponent describing the swelling mechanism. When $n = 0.45$, order of release is Fickian, but when $0.45 < n > 1.0$, the diffusional mechanism is non-Fickian [23, 44, 45].

2.12. Fourier Transform Infrared (FTIR) Spectroscopy. For FTIR spectroscopy, dried discs of hydrogel samples were powdered using pestle and mortar. Potassium bromide (Merck IR spectroscopy grade) was mixed with powdered material in 1 : 100 proportions and dried at 40°C. The powdered materials were compressed by applying a pressure of 65KN (pressure gauge, Shimadzu) for 2 minutes to a semi-transparent disc of 12 mm diameter. FTIR spectrometer (FT-IR 8400 S, Shimadzu) was used to record the FTIR spectrum over the wavelength range of 4500-500 cm^{-1} [22].

2.13. Scanning Electron Microscopy (SEM). The structural morphology of Poly (CMCS-*g*-AA) hydrogels was analyzed using SEM (Hitachi, S 3000 H, Japan). Hydrogel samples were mounted on an aluminum mount with double adhesive tape and sputtered with gold palladium. The morphology of hydrogel samples was analyzed at an accelerating voltage of 10 KV, with a working distance of 10-25 mm at various magnifications [22].

2.14. Statistical Analysis. The data obtained was analyzed statistically by applying Student's t-test to compare the results and to determine the statistical significant/nonsignificant interpretation at 95% confidence interval, p value less than 0.05 was measured as significant difference in results. Data were displayed as mean ± SD.

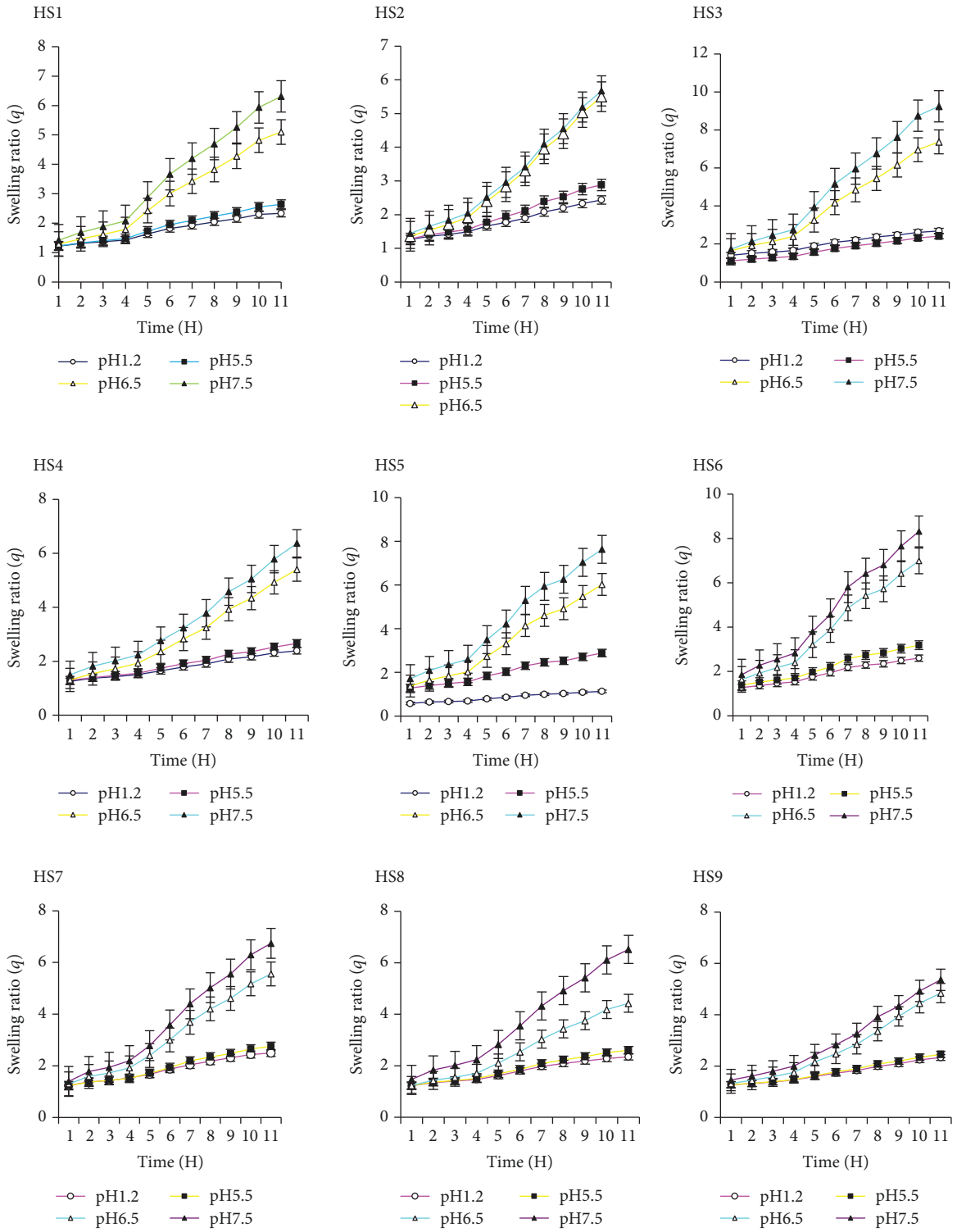

Figure 1: Dynamic swelling coefficient (q) of all hydrogel samples (HS1-HS9) in various 0.05 M USP phosphate-buffered solutions at 37°C.

3. Results and Discussion

3.1. Effect of pH on Swelling and on Drug Release. The pH of the medium and pKa values of the acidic component of the polymer greatly affect the swelling behavior of the hydrogels. This is because the pH of the medium affects the ionization of carboxylic groups that results in huge variation in swelling kinetics of hydrogel. pH effect on swelling and drug release was measured in buffer solutions of pH (1.2, 5.5, 6.5, and 7.5), respectively. The dynamic and equilibrium swelling ratios were found high in the solutions of pH 6.5 and 7.5 as shown in Figure 1. It was found that at low pH values,

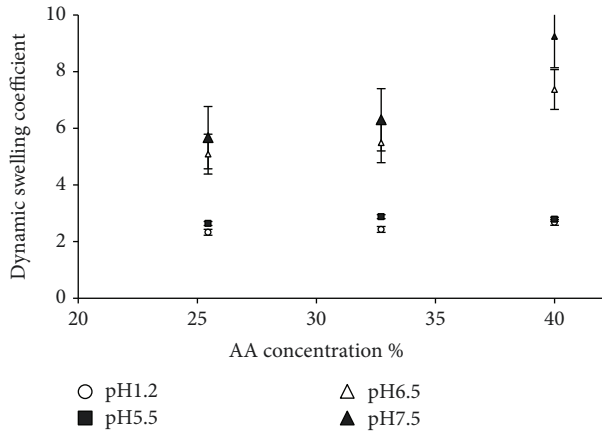

Figure 2: Dynamic swelling coefficient of Poly (CMCS-*g*-AA) hydrogels with different concentration of AA (25.45, 32.72, and 40 g) using EGDMA as crosslinking agent (0.7% of AA) in 0.05 M USP phosphate buffer solutions of different pH at 37°C.

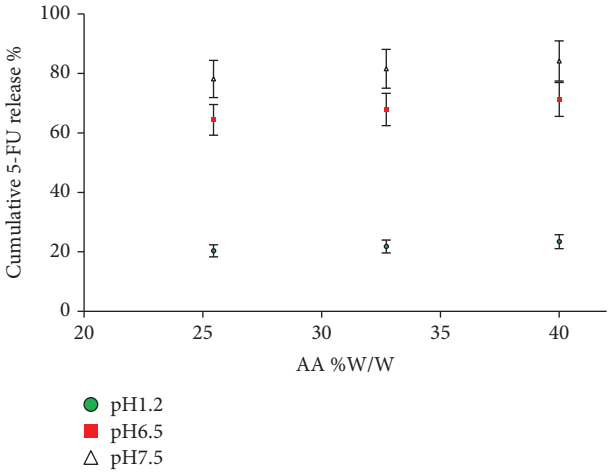

Figure 3: *In vitro* release of 5-FU from Poly (CMCS-*g*-AA) hydrogels using variable contents of AA (25.45, 32.72, and 40 g) and EGDMA as crosslinking agent (0.7% of AA) at various pH values and 37°C in 0.05 M USP phosphate buffer solutions.

CMCS/AA hydrogels remain unionized and did not show significant swelling capability. The pH values in the physiological environment highly change from acidic conditions in the stomach (pH 1-3) to the almost neutral environment in the small intestine (6.37-7.04) and colon (6.63-7.49). Hydrogels containing AA exhibit low swelling degree at gastric pH, but as they pass into the gastrointestinal tract (GI), the swelling degree increases with increase in pH. AA has carboxyl groups, and swelling of the gel is greatly affected due to the presence of ionized charges on the polymer network. The pKa of acrylic acid is 4.26. As the pH of the surrounding medium increases above pKa values, swelling starts due to the ionization of carboxylic groups that result in stretching of coiled molecules. Moreover, at higher pH, the presence of similar ionized charges cause an electrostatic repulsion along the chain which in turn cause the expansion of the originally coiled molecules. In a study conducted by

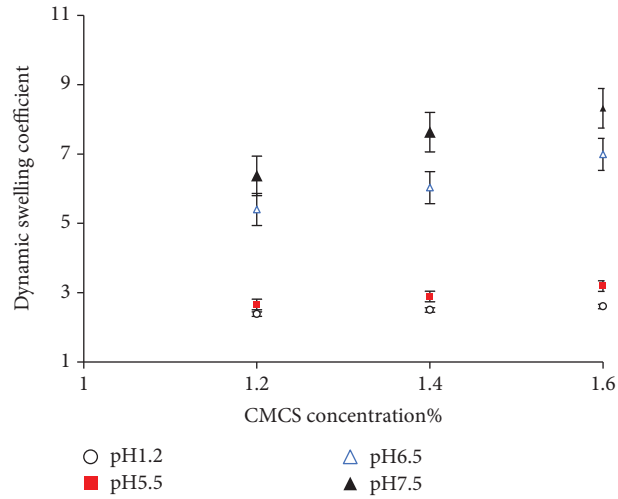

Figure 4: Dynamic swelling coefficient of Poly (CMCS-*g*-AA) hydrogels with different concentration of CMCS (1.20 1.40, and 1.60 g) using EGDMA as the crosslinking agent (0.7%) of AA in 0.05 M USP phosphate buffer solutions of different pH at 37°C.

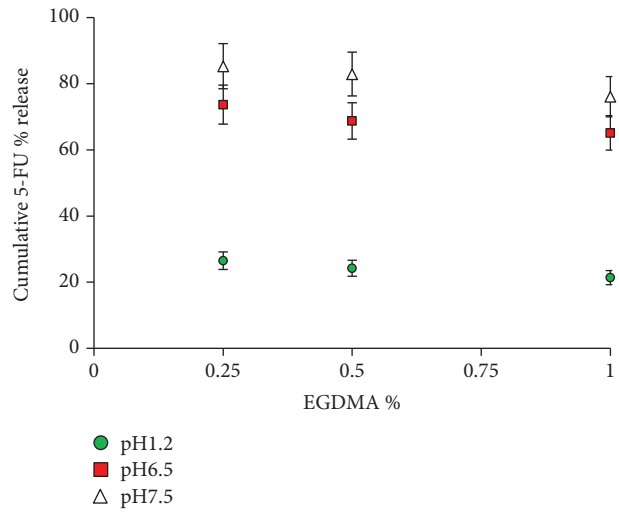

Figure 5: Release of 5-FU from Poly (CMCS-*g*-AA) hydrogels using different contents of EGDMA as crosslinking agent (0.25%, 0.50%, and 1% of AA) at various pH values and 37°C in 0.05 M USP phosphate buffer solution.

Bukhari et al. 2015, they observed the similar response to pH variation of the buffer solution and noted increased swelling at higher pH values [22]. However, in our study, we also noted swelling at lower and 6.5 pH. This is considered because CMCS is an amphoteric molecule containing both amino and carboxylate functional groups. So the presence of a higher number of carboxylate groups facilitates the increased water uptake at higher pH and higher swelling. On the other hand, while at lower pH carboxylic groups of AA show no swelling behavior due to the presence of deprotonated groups [1]. 5-FU was used as a model drug due to its hydrophilic nature. Effect of pH on 5-FU release was studied by immersing the 5-FU-loaded samples in

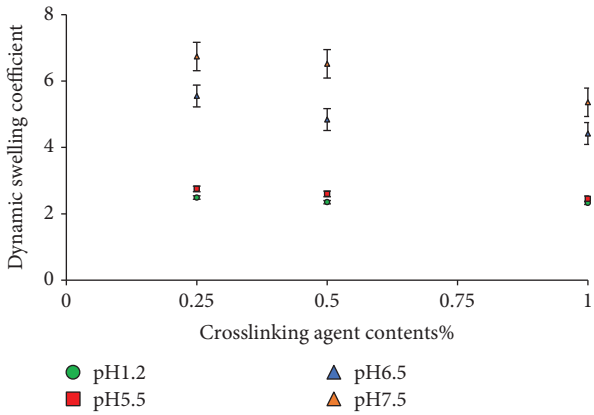

Figure 6: Dynamic swelling coefficient of Poly (CMCS-*g*-AA) hydrogels with different contents of EGDMA as crosslinking agent (0.25%, 0.50%, and 1% of AA) in 0.05 M USP phosphate buffer solutions of different pH at 37°C.

various buffered solutions (pH 1.2, pH 6.5, and pH 7.5). It is observed that by increasing the pH of the medium, drug release and swelling of gel increased [1]. Osmotic pressure inside the gel develops that causes maximum drug to release at higher pH (7.5) as compared to lower pH (1.2) due to more swelling at higher pH.

3.2. Effect of Monomer Contents on Swelling and on Drug Release. To investigate the effect of AA contents on the swelling and on the drug release concentration of AA, AA was varied from 25.45 g, 32.72 g, and 40 g in Poly (CMCS-*g*-AA) hydrogels using EGDMA as a crosslinking agent (0.7% of AA) as shown in Figures 2 and 3. It was noticed that with an increase of AA concentration, drug release and swelling of gel increased due to the availability of more carboxylic groups of AA. This occurred because ionization and electrostatic repulsion along the chain take place that causes an expansion of the originally coiled molecules. Drug release studies were carried out for 12 h. As shown in Figure 3, drug release was observed 79.13%, 82.56%, and 83.19% at pH 7.5, 61.79%, 69.90%, and 70.25% at pH 6.5 and 27.76%, 24.89%, and 24.99% at pH 1.2 with respect to composition of 1.25/25.45, 1.25/32.72, and 1.25/40 [39, 46]. Bukhari et al. 2015 in their study also reported increased swelling and drug release with increasing AA contents owing to the presence of higher numbers of ionized functional groups in the hydrogel network [22].

3.3. Impact of CMCS Contents on Swelling. Three formulations of Poly (CMCS-*g*-AA) hydrogels with varying concentration of CMCS, i.e., 1.20/29.09, 1.40/29.09, and 1.60/29.09 keeping acrylic acid and EGDMA concentration constant (0.7% of AA) were prepared and subjected to swelling studies in different pH solutions. It was noticed that in buffer solutions of low pH values, increase in swelling ratio with increased CMCS concentration is not significant as compared to high pH values. This is because CMCS is an amphoteric molecule that contains a very low amount of carboxylic groups and a high amount of NH_2 groups in its side chain. So at low pH values, carboxyl groups of CMCS are unionized and anion-anion repulsive forces are removed, as a result swelling ratio remains low. At pH values >4, carboxylate groups are ionized, and electrostatic repulsion of carboxyl groups causes an increase in swelling, which further facilitates the swelling and drug release at higher pH values. Figure 4 demonstrates the effect of CMCS contents on swelling ratio in variable buffer solutions [40, 47, 48], while in a study containing AA with gelatin conducted by Bukhari et al. 2015 observed that, with increasing gelatin contents in the feed composition of hydrogels, significant swelling was observed at lower pH values (1.2). This is because gelatin contains an abundant amount of NH_2 groups in its chains which remain in ionized and dominated state at lower pH values. So by increasing its contents in the feed composition, a number of ionized amino groups also increases which leads to higher swelling and drug release at lower pH buffer [22].

3.4. Effect of Crosslinking Ratio on Swelling and on Drug Release. A series of three Poly (CMCS-*g*-AA) hydrogels of variable crosslinking agent contents (0.25%, 0.50%, and 1% of AA) were prepared to monitor the effect of EGDMA on swelling and release kinetics of hydrogels. It was found in Figure 5 that gel swelling decreased with the increase of EGDMA concentration due to the presence of more physical crosslinks between hydrogels. Mechanistically, the influence of increasing crosslinking can be described by the decrease pore size of the network. High cross-linked polymers are less acidic because carboxylate groups are concealed and higher crosslinking ratio reduce the process of ionization. Figures 5 and 6 demonstrate that increase in EGDMA concentration from 0.25%, 0.50%, and 1.0% results in reduction in swelling ratio and drug release % age [6, 49, 50]. Figure 7 indicates the cumulative 5-FU % release with time in phosphate buffer solutions of various pH values at 37°C.

3.5. Polymers Diffusion Coefficient (D). The diffusion coefficient is applied indirectly to monitor solute diffusion into the hydrogel. Fick's law of diffusion was used during membrane permeation method or sorption and desorption phenomenon. It was observed that D decreased with the increasing of AA and CMCS concentrations because swelling of polymer increases as the contents of AA increase. D increased with increasing of crosslinking agent contents. Table 3 shows the D of Poly (CMCS-*g*-AA) hydrogels [49, 50]. In another study reported by Bukhari et al. 2015, observed diffusion coefficient decreases with increasing AA and gelatin contents owing to their more hydrophilic nature. Moreover, with increasing crosslinking agent contents in the feed composition, an increase in diffusion coefficient was observed [22].

3.6. Molecular Weight between Crosslinks (M_c) and Solvent Interaction Parameters (χ). It was found that by increasing AA contents, an increase in the values of molecular weight between crosslinks (M_c) was observed. Higher swelling of the polymer was observed due to impart of AA carboxylic

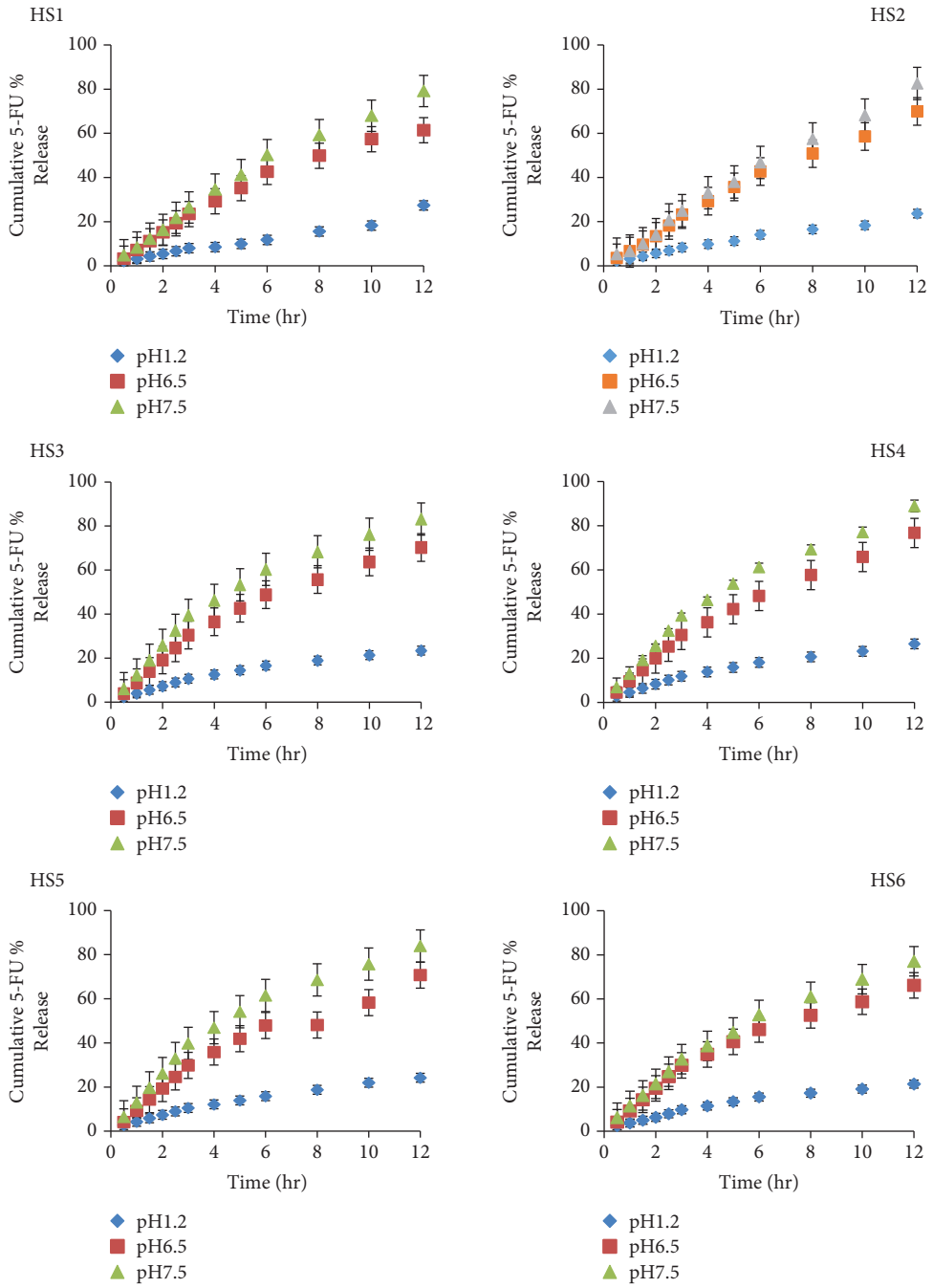

Figure 7: Cumulative 5-FU % release from Poly (CMCS-*g*-AA) hydrogels with time at various pH values and 37°C in 0.05 M USP phosphate buffer solution.

group into the polymer network. Similarly, with increasing CMCS contents in the feed composition, M_c values also increased accordingly owing to its amphoteric nature and higher swelling. Cross-linked density (q) is also related to the values of AA and M_c as shown in Table 3. Solvent interaction parameters (χ) were used to monitor the effect of solvent interaction between polymer and solvent. It was observed that the higher the (χ) values, the weaker the interaction between solvent and polymer. Similarly, another study conducted by Bukhari et al. 2015 found that molecular weight increases with increasing AA contents. While the authors did not observe the effect of gelation on M_c in their study [22, 23].

3.7. Sol-Gel Fraction Analysis. The sol-gel fraction was used to measure the amount of uncrosslinked fraction of the polymer in the hydrogel. It was found that gel-fraction of hydrogels increased along with the increased contents of AA, CMCS, and crosslinker. Sol-fraction of hydrogels was noticed to decrease along the increased contents of CMCS,

Table 3: Molecular weight between crosslinks (M_c), cross-linked density (q), and solvent interaction parameter values (χ).

Samples code	$V_{2,s}$	χ	M_c	M_r	q	$D\ 10^{-7}$ (cm^2/sec)
HS_1	0.134	-0.551	541.22	84.12	6.39	0.134
HS_2	0.116	-0.545	659.73	81.81	8.06	0.115
HS_3	0.111	-0.542	736.59	80.29	9.18	0.110
HS_4	0.145	-0.553	475.82	79.69	5.96	0.149
HS_5	0.127	-0.547	561.07	82.83	6.76	0.127
HS_6	0.123	-0.545	545.37	85.79	6.36	0.122
HS_7	0.133	-0.549	529.40	81.28	6.52	0.132
HS_8	0.149	-0.556	408.51	81.69	5.01	0.149
HS_9	0.167	-0.564	337.53	82.13	4.12	0.167

AA, and EGDMA. Bukhari et al. 2015 observed in their study that the gel fraction of the formulations increases with the increasing AA, gelatin, and crosslinker contents in their work [22]. Figures 8(a)–8(c) depicts the effect of polymer, EGDMA, and monomer contents on gel-fraction of the hydrogel. Table 3 also shows the sol-gel fraction values of Poly (CMCS-*g*-AA) hydrogels.

3.8. Porosity Measurement. The effect of CMCS and AA on porosity is reported in Table 4. It was noticed from the results in Table 4 that with increasing CMCS and AA contents, porosity increases due to an increase in viscosity of the hydrogel solution. This increase in viscosity efficiently prevents the escaping of bubbles from the solution that in turn results in an increase of porosity due to the formation of interconnected channels. While on increasing the concentration of EGDMA, porosity decreases due to the increasing physical crosslinks between CMCS and AA. Increase in crosslinking agent contents results in increased crosslinks between monomer and polymer which result in decreased porosity.

3.9. Drug Release Mechanism. The drug release constant (k) and regression coefficient (r) were obtained for zero order, first order, Higuchi model, and Korsmeyer-Peppas to investigate the drug release kinetics [43]. The model that best turns the release data were monitored. The selection criterion for the most appropriate model was based on the best goodness of fit designated by the values of (r) nearer to 1. Regression coefficient (r) values obtained from Poly (CMCS-*g*-AA) hydrogels at different concentrations of AA and degree of crosslinking are shown in Tables 5 and 6. Drug release kinetics indicated that the release of drug was best explained by Higuchi equation. The "r" values of Higuchi model at variable monomeric composition and at variable crosslinking degree displayed the highest linearity followed by first-order release designated that the drug release occurs through diffusion-controlled mechanism [51]. Effects of monomer contents and crosslinking degree on "n" values are shown in Tables 7 and 8, respectively. All samples displayed non-Fickian fashion at all pH values. This indicates that drug release occurs through swelling and relaxation of the polymer. A similar result was found with microparticles of tramadol hydrochloride [52].

3.10. Cell Viability Study. MTT assay was used for the determination of *in vitro* compatible nature and cytotoxicity of drug-loaded hydrogels. Figure 9(a) shows the *in vitro* cytocompatibility against Vero cells. The biocompatibility of Poly (CMCS-*g*-AA) hydrogels was evaluated by using hydrogel samples with two different concentrations (HS_1 and HS_6). It was noticed from the results in Figure 9(a) that the hydrogel samples at lower and higher polymeric contents have good cytocompatibility with no detectable cytotoxicity.

HeLa cells cultured previously were subjected to MTT assay for the determination of cell cytotoxicity. Figure 9(b) indicates the cytotoxic potential of 5-FU in free and encapsulated form at various concentrations. It is clear from the results that 5-FU has dose-dependent cytotoxic potential and the % cell viability decreased with increasing dose per well. It is also clear that 5-FU exhibit high toxicity in pure form in comparison to encapsulated form.

The cell viability study showed the biocompatibility of hydrogels. It also shows that 5-FU has retained its cytotoxic potential after loading into the hydrogel matrix.

3.11. Solid State Properties of Prepared Hydrogels

3.11.1. Fourier Transformed Infrared (FTIR) Spectroscopy. Figure 10 ((A), (B), and (D)) shows that the peak at 1752 cm^{-1} is assigned to stretching of the carboxylic acid. A peak at 2990 cm^{-1} is related to C-H stretching, while the peaks at 3575 cm^{-1} refer to the stretching of OH group of carboxylic acid. In Figure 10(C), the medium peaks at 1030-1070 cm^{-1} are assigned to stretching vibration of C-O-C and C-O-H bands of 5-FU. A weak band at 1453 cm^{-1} is due to the C-N stretching of bands of 5-FU. Two peaks at 1560-1630 cm^{-1} are assigned to carboxylate $C=O$ stretching. The peaks at 1460-1375 cm^{-1} are attributed to the stretching of CH_2 and OH groups, respectively. FTIR analysis has confirmed that the hydrogel network was not a physical blending, and chemical linkages have been formed during the polymerization reaction.

3.11.2. Scanning Electron Microscopy (SEM). The surface and cross-sectional morphology of the interpenetrating Poly (CMCS-*g*-AA) hydrogels was analyzed by scanning electron microscopy. The surface and cross-sectional morphology of the Poly (CMCS-*g*-AA) hydrogels is shown in Figure 11.

(Figures 11(c) and 11(d)) shows the unloaded and loaded SEM micrographs at high resolution. The SEM micrographs of the Poly (CMCS-*g*-AA) hydrogels indicate the pores or channels that facilitate the penetration of the fluid carrying drug (5-FU) into the interpenetrating network of the hydrogel.

4. Conclusion

Highly porous with excellent pH-sensitive behavior hydrogels based on Poly (CMCS-*g*-AA) were successfully developed by free radical polymerization technique for targeting colon cancer owing to the basic environment of the colon.

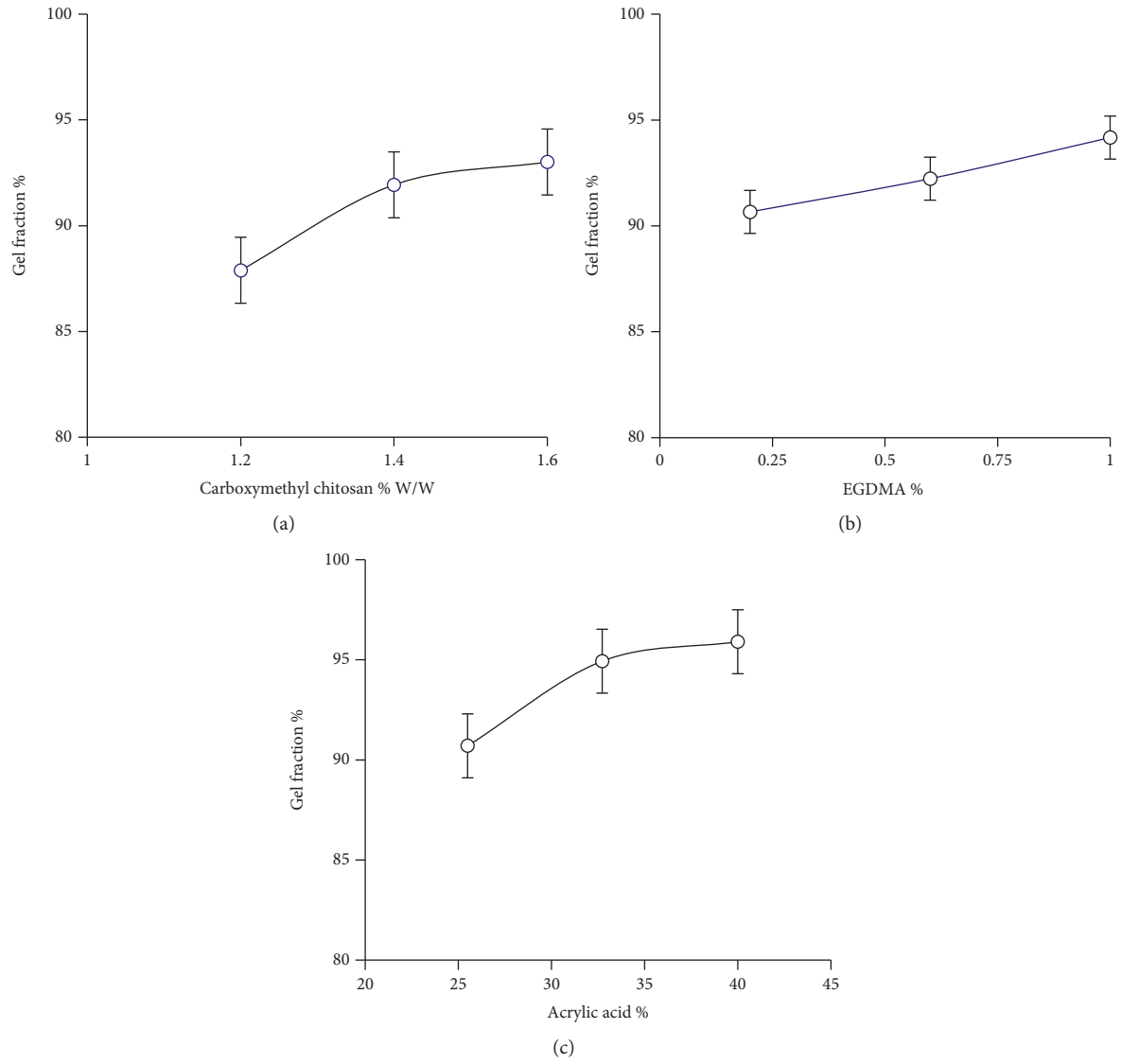

Figure 8: Effect of different contents on gel fraction (a) CMCS concentration, (b) EGDMA concentration, and (c) AA concentration.

Table 4: Porosity and Sol-gel fraction analysis of different formulations.

Sample code	Degree of crosslinking EGDMA Mol (%)	Porosity (%)	Gel fraction (%)	Sol fraction %	p value
HS_1	0.70	13.11	90.70	9.30	0.047
HS_2	0.70	19.02	94.93	5.07	0.041
HS_3	0.70	22.98	95.90	4.10	0.005
HS_4	0.70	9.27	87.89	12.11	0.049
HS_5	0.70	11.50	91.93	9.07	0.005
HS_6	0.70	17.34	93.01	6.99	0.05
HS_7	0.25	24.66	90.66	9.34	0.043
HS_8	0.50	20.10	92.23	7.77	0.047
HS_9	1.0	12.54	94.17	5.83	0.046

Table 5: Effect of different concentration of AA on the drug release kinetics of Poly (CMCS-*g*-AA) hydrogels in solutions of different pH using EGDMA as crosslinking agent (0.7% of AA).

Samples code	AA contents	pH	Zero-order kinetics		First-order kinetics		Higuchi model	
			K_0 (h^{-1})	r	K_1 (h^{-1})	r	K_2 (h^{-1})	r
HS_1	25.45	1.2	1.59	0.99	0.018	0.99	0.068	0.982
		6.5	5.37	0.97	0.087	0.98	0.232	0.996
		7.5	6.52	0.98	0.125	0.99	0.279	0.991
HS_2	32.72	1.2	1.70	0.98	0.019	0.98	0.072	0.990
		6.5	5.69	0.98	0.094	0.99	0.244	0.991
		7.5	6.76	0.99	0.133	0.97	0.287	0.981
HS_3	40	1.2	1.82	0.95	0.021	0.96	0.079	0.992
		6.5	5.78	0.95	0.103	0.99	0.252	0.996
		7.5	6.66	0.93	0.149	0.99	0.292	0.992

Table 6: Effect of degree of crosslinking on the drug release kinetics of Poly (CMCS-*g*-AA) hydrogels in the solutions of different pH values.

Samples code	EGDMA contents	pH	Zero-order kinetics		First-order kinetics		Higuchi model	
			K_0 (h^{-1})	r	K_1 (h^{-1})	r	K_2 (h^{-1})	r
HS_7	0.25%	1.2	1.99	0.95	0.023	0.972	0.087	0.997
		5.5	5.94	0.96	0.109	0.998	0.255	0.998
		7.5	6.73	0.94	0.154	0.995	0.295	0.993
HS_8	0.50%	1.2	1..84	0.97	0.021	0.984	0.079	0.998
		5.5	5.26	0.93	0.089	0.973	0.230	0.985
		7.5	6.53	0.92	0.144	0.995	0.288	0.989
HS_9	1%	1.2	1.65	0.94	0.018	0.960	0.072	0.992
		5.5	5.18	0.93	0.086	0.987	0.227	0.992
		7.5	6.07	0.95	0.117	0.998	0.264	0.997

Table 7: Effect of different contents of AA on the drug release mechanism of Poly (CMCS-*g*-AA) hydrogels in solutions of different pH values using EGDMA as crosslinking agent (0.7% of AA).

Samples code	AA contents	pH	Release exponent (n)	r	Order of release
HS_1	25.45	1.2	0.728	0.995	Non-Fickian
		5.5	0.937	0.997	Non-Fickian
		7.5	0.912	0.992	Non-Fickian
HS_2	32.72	1.2	0.746	0.994	Non-Fickian
		5.5	0.953	0.991	Non-Fickian
		7.5	0.936	0.983	Non-Fickian
SH_3	40	1.2	0.733	0.989	Non-Fickian
		5.5	0.897	0.973	Non-Fickian
		7.5	0.817	0.972	Non-Fickian

Table 8: Effect of degree of crosslinking on the drug release mechanism of Poly (CMCS-*g*-AA) hydrogels in solutions of different pH.

Samples code	EGDMA contents	pH	Release exponent (n)	r	Order of release
HS_7	0.25%	1.2	0.712	0.990	Non-Fickian
		5.5	0.867	0.981	Non-Fickian
		7.5	0.795	0.979	Non-Fickian
HS_8	0.50%	1.2	0.697	0.996	Non-Fickian
		5.5	0.846	0.998	Non-Fickian
		7.5	0.796	0.972	Non-Fickian
HS_9	1%	1.2	0.710	0.981	Non-Fickian
		5.5	0.850	0.968	Non-Fickian
		7.5	0.798	0.988	Non-Fickian

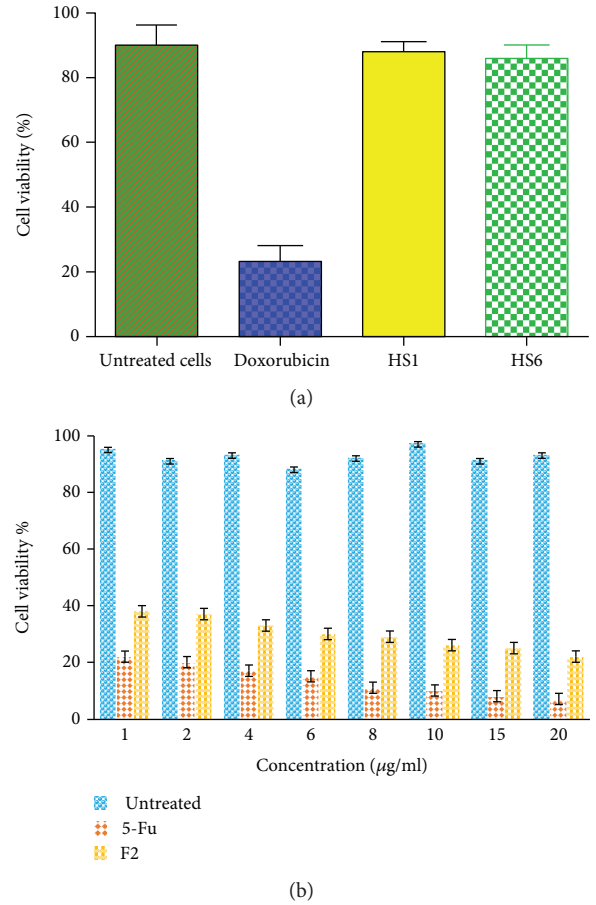

Figure 9: *In vitro* cytocompatibility sketch of bare Poly (CMCS-*g*-AA) hydrogels against Vero cell lines (a). *In vitro* cell cytotoxicity of 5-FU-loaded Poly (CMCS-*g*-AA) hydrogels against HeLa cell lines (b). Data reported indicates the mean ± SD of ($n = 3$) independent experiments. The data of control and experimental groups was analyzed statistically with one-way ANOVA. The data was found statistically significant with $^{***}p$ value of <0.001.

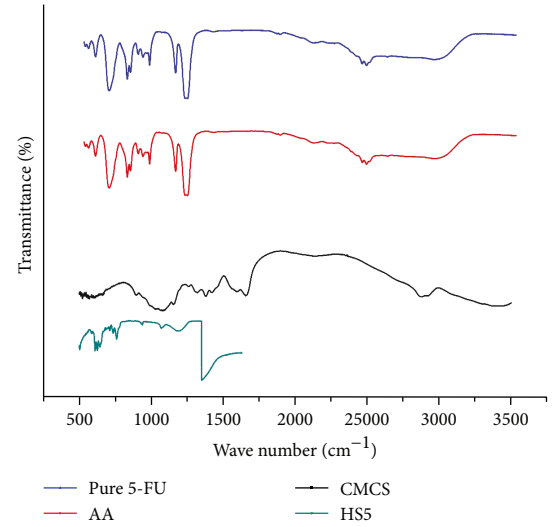

Figure 10: FTIR spectra of (A) 5-FU, (B) AA, (C) CMCS, and (D) Poly (CMCS-*g*-AA) hydrogel (unloaded).

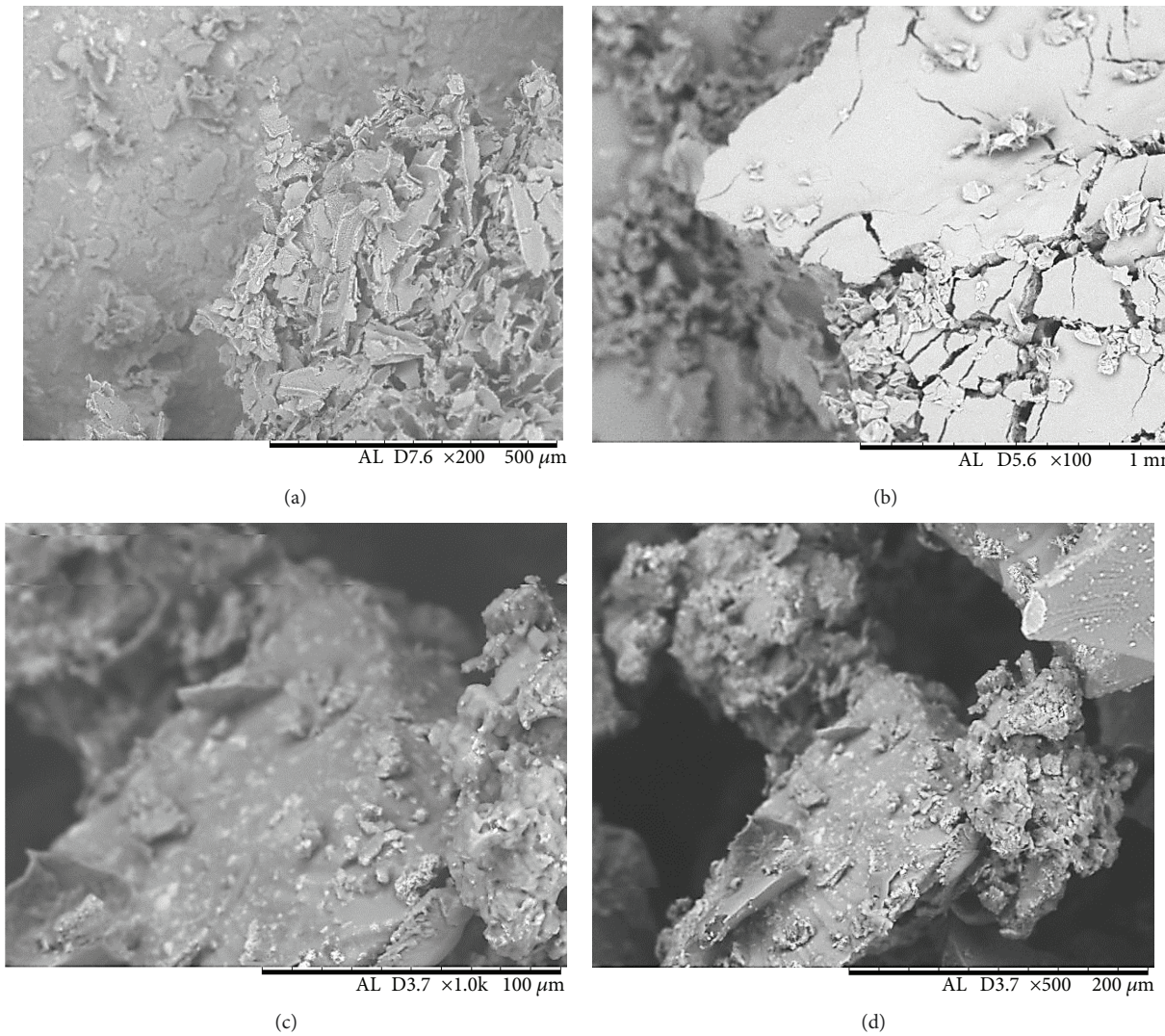

Figure 11: SEM micrographs of Poly (CMCS-*g*-AA) hydrogels. Surface morphology of blank hydrogel sample (a). Surface morphology of drug-loaded hydrogel sample (b). Cross-sectional morphology of blank hydrogel sample (c). Cross-sectional morphology of drug-loaded hydrogel sample (d).

It was concluded that with increasing concentration of AA, the swelling of hydrogel and release of 5-FU increased and decreased with increasing concentration of EGDMA. This increased at higher pH was suggested due to the availability of more ionized carboxylic groups of AA. Since CMCS contain carboxylic groups in its side chain, so it also facilitate swelling and drug release at higher pH values. It was observed that gel fraction and porosity increased by increasing the concentration of AA and CMCS. It was concluded that the release of 5-FU decreased with increasing ratio of EGDMA, and an increase in release was observed with increase in the concentration of AA. Swelling characterization of Poly (CMCS-*g*-AA) hydrogel was studied by investigating diffusion coefficient, solvent interaction parameters, and molecular weight between crosslinks and cross-linked density. MTT assay confirmed the biocompatible nature of Poly (CMCS-*g*-AA) hydrogels against Vero cells while the cytotoxic potential of 5-FU-loaded gels was confirmed against HeLa cancer cell lines. The structural confirmation and morphology of Poly (CMCS-*g*-AA) hydrogels were investigated by FTIR and SEM, respectively. FTIR spectroscopy confirmed the formation of chemical linkages between the functional groups while SEM results indicated the porous nature of the hydrogel. The results confirmed that Poly (CMCS-*g*-AA) hydrogel can be effectively used as pH-responsive controlled released vehicle for drug delivery in targeting colon cancer.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no potential conflict of interest.

Acknowledgments

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