Journal of Applied Polymer Science

Swelling kinetics, mechanical properties, and release characteristics of chitosan-based semi-IPN hydrogels

Zehra Özbaş, Gülten Gürdağ
Department of Chemical Engineering, Faculty of Engineering, Istanbul University, 34320 Avcilar, Istanbul, Turkey
Correspondence to: Z. Özbaş (E-mail: zehraozbas@hotmail.com)

ABSTRACT: Two series of pH-sensitive semi-interpenetrating network hydrogels (semi-IPN) based on chitosan (CS) natural polymer and acrylamide (AAm) and/or N-hydroxymethyl acrylamide (HMA) monomers by varying the monomer and CS ratios were synthesized by free radical chain polymerization. 5-Fluorouracil (5-FU), a model anticancer drug, has been added to the feed composition before the polymerization. The characterization of gels indicated that the drug is molecularly dispersed in the polymer matrix. The swelling kinetics of drug-loaded gels have decreased with increased HMA content at 37°C in both distilled water and buffer solutions with a pH of 2.1 or 7.4. Elastic modulus of the gels increased with the increase in HMA content and higher CS concentration enhanced the elastic modulus positively. Moreover, cumulative release percentages of the gels for 5-FU were ca. 10% higher in pH 2.1 than those in pH 7.4 media. It was determined that they can be suitable for the use in both gastric and colon environments. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41886.

KEYWORDS: stimuli-sensitive polymers; biomaterials; drug delivery systems

Received 26 September 2014; accepted 9 December 2014
DOI: 10.1002/app.41886

INTRODUCTION

Hydrogels are the crosslinked hydrophilic polymer networks that are not soluble in water or aqueous solutions due to their crosslinked structures, but can retain large amounts of water or biological fluids. These materials resemble many soft living tissues because of their hydrophilic properties.[1,2] Hydrogels can respond to external stimuli such as temperature, pH, ionic strength, light, magnetic field, electric field, etc. as reversible phase transition. These properties make hydrogels an ideal class of materials for medical applications.

The major disadvantage of hydrogels is their poor mechanical properties. Better mechanical properties are displayed by the interpenetrating polymer networks (IPN). IPNs which are classified as semi- or full-IPN, are physical mixtures of at least two polymers without a significant degree of covalent bonds between polymer chains, and they are generally prepared by the polymerization of a monomer in the presence of a crosslinker in the solution of a polymer. In that case, the second polymer will be in the crosslinked state while the other one was in linear form, and the obtained IPN will be called as semi-IPN. If each of two polymers in an IPN gel is in crosslinked form, it will be called as full-IPN. IPN consisting of a synthetic polymer (polyacrylamide, poly(acrylic acid), poly(N-isopropyl acrylamide), etc.) and a natural polymer (chitosan, gelatin, sodium alginate, etc.) could result in materials, which combine the mechanical properties of the synthetic polymer with the biological properties of the natural one.[3–8]

Chitosan (CS) is a linear, nontoxic, and biocompatible polymer consisting of D-glucosamine and *N*-acetyl-D-glucosamine units, and obtained by alkaline *N*-deacetylation of chitin.[9] Properties such as biodegradability, nontoxicity, good biocompatibility and reactive amino and hydroxyl groups make it suitable for use in biomedical and pharmaceutical applications.[10,11] Chitosan as a weak polybase is a pH-responsive polymer due to protonation/deprotonation of the large quantities of amino groups on its chain.[10] Copolymers and terpolymers of AAm have high permeability to hydrophobic and water-soluble solutes and high mechanical strength, according to copolymer composition and crosslink density.[12] HMA is a water-soluble monomer and it possesses functional methylol groups upon polymerization. The methylol groups of PHMA can crosslink and self-condense, and react with functional groups with other polymers.[13]

5-Fluorouracil (5-FU) is one of the oldest antitumor drugs used for the treatment of solid tumors such as breast, pancreas, colon, stomach, liver, and brain cancers.[14–17] The target sites of 5-FU are all organs of the human body, especially gastrointestinal tract.[17,18] Chitosan-based some gels for 5-FU release were used as reported in the literature. The extent of 5-FU release at equilibrium for poly(N-acryloylglycine-chitosan) IPN hydrogels was inversely dependent on the crosslinking degree.[11] CS and

guar gum-g-acrylamide semi-IPN microspheres were able to release 5-FU quite fast and large at the lower amount of glutaraldehyde (GA), while the release was slower at higher GA content.[14] Interpolymeric gel films based on CS and polyvinyl alcohol (PVA), which were crosslinked either chemically with glutaraldehyde or by γ-irradiation were conversely dependent on the crosslinking degree.[19]

In this work, CS-based novel semi-IPN hydrogels (CS:AAm; CS:HMA; CS:AAm:HMA) were prepared with AAm and/or HMA monomers by varying the monomer and CS contents in CS solution. 5-FU, the model drug, has been loaded to the feed composition before the polymerization. For the comparison purpose, AAm and HMA homopolymers and AAm : HMA copolymer gels without CS were also prepared. The semi-IPN gels were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) analysis. The compressive elastic modulus and the equilibrium swelling values of drug loaded gels were compared in order to determine the effect of CS and AAm/HMA content. The release characteristics of the gels for the drug 5-FU were investigated in pH 2.1 and 7.4 for the first time in this work.

EXPERIMENTAL

Materials

CS, AAm, HMA, and 5-FU were purchased from Sigma-Aldrich (St. Louis, MO). HMA was used in the form of aqueous solutions of 48 wt %. The viscosity average molecular weight of CS was determined by the measurements of flow times of solutions with various CS concentrations and found to be 125.600 g mol^{-1}.[20] The degree of deacetylation of CS was found to be 77% by FTIR analysis using characteristic wavenumbers of 1650 cm^{-1} (amide I) and 3450 cm^{-1} (OH stretching vibration).[21] The crosslinker *N,N′*-methylenebisacrylamide (NMBA) was of Merck–Schuchardt (Hohenbrunn, Germany) product. The initiator ammonium peroxydisulfate (APS), the accelerator *N,N,N′,N′*-tetramehylethylene diamine (TEMED), and glacial acetic acid (AAc) were provided by Merck Chemicals Ltd (Hohenbrunn, Germany), Serva Electrophoresis GmbH (Heidelberg, Germany), and Riedel-de Haen (Seelze, Germany), respectively. Potassium chloride, potassium dihydrogen phosphate, sodium hydroxide, and hydrochloric acid solution were used for the preparation of buffer solutions, and all were of Merck Chemicals Ltd products. Sodium chloride (Merck Chemicals Ltd) was used to adjust the ionic strengths of the buffer solutions. Distilled water was used for the preparation and purification of hydrogels, and for the preparation of buffer solutions as well.

Synthesis of Hydrogels

Polymerization reactions were performed in the glass tubes with inner diameters of 1.3 cm and lengths of 15 cm. At first, CS was dissolved in aqueous acetic acid (2 v/v %) to prepare 1% and 2% (w/v) CS solutions by using a magnetic stirrer. Then, AAm and/or HMA were added by keeping the total initial monomer concentration at 0.5 *M*. The crosslinker, *N,N′*-methylenebisacrylamide (NMBA) was used in the preparation of the gels which were prepared only from AAm monomer and the amount of 1 mol % of monomer content in feed. Because of the condensation reaction between the methylol groups of HMA resulting in crosslinking, NMBA was not used in the preparation of the gels with HMA. A constant amount of 5-FU was added to the monomer (and crosslinker) mixture. Then, the mixture was stirred by a magnetic stirrer at room temperature until the drug was completely dissolved. After bubbling N_2 gas for 15 min to remove in order to remove the oxygen from the solution, APS was added first in the amounts of 1 mol % of total monomer content in the feed, and then TEMED in the equal weight of APS. Finally, the glass tubes were immersed in water at 40°C, and held there for 24 h. After the gelation had been completed, the glass tubes were broken carefully without destroying the cylindrical hydrogels. The semi-IPN gels were sliced into small discs with approximately 1 cm lengths, and hydrogel slices were immersed in an excess amount of deionized water for 1 week to remove the residual unreacted monomers. The water was refreshed twice a day. For the comparison, homopolymers of AAm and HMA and AAm : HMA copolymer were prepared without CS under the same conditions as those of CS:AAm, CS:HMA and CS:AAm:HMA semi-IPN hydrogels. The amount of 5-FU entrapped in the hydrogels was determined by UV spectroscopy, and the difference between the amount of the drug initially employed and the drug content in the washing water determined was taken as the amount of 5-FU entrapped in the hydrogel. The resulting drug loaded swollen gels were dried in air for 4 days and then in a vacuum oven until to attain constant weight. The amounts of the components used to prepare the drug-loaded hydrogels were given in Table I. The drug-loaded gels denoted by the symbol F+, and the drug-unloaded ones by F− in the polymer codes. The drug-unloaded gels were prepared by the procedure used for the preparation of drug-loaded ones.

Table I. The Amounts of the Components Used to Form the Drug-Loaded Hydrogels

Polymer code		CS (g)	[AAm] (*M*)	[HMA] (*M*)	5-FU (mg)
CS0:F+	A100:F+	–	0.500	–	75
	A75:H25:F+	–	0.375	0.125	75
	A50:H50:F+	–	0.250	0.250	75
	A25:H75:F+	–	0.125	0.375	75
	H100:F+	–	–	0.500	75
CS1:F+	CS1:A100:F+	0.15	0.500	–	75
	CS1:A75:H25:F+	0.15	0.375	0.125	75
	CS1:A50:H50:F+	0.15	0.250	0.250	75
	CS1:A25:H75:F+	0.15	0.125	0.375	75
	CS1:H100:F+	0.15	–	0.500	75
CS2:F+	CS2:A100:F+	0.30	0.500	–	75
	CS2:A75:H25:F+	0.30	0.375	0.125	75
	CS2:A50:H50:F+	0.30	0.250	0.250	75
	CS2:A25:H75:F+	0.30	0.125	0.375	75
	CS2:H100:F+	0.30	–	0.500	75

Characterization

FTIR Spectroscopy. The FTIR spectra of powdered gel discs with and without drug and plain drug were recorded over the

range 500–4000 cm^{-1}, in a Spectrum One (Perkin–Elmer) spectrometer using potassium bromide (KBr) pellet technique.

X-ray Diffraction. XRD patterns of plain drug, drug-loaded and plain gels were recorded using a DMAX-2200 X-ray diffractometer (Rigaku Company, Japan) with Cu Kα tube at 40 kV and 30 mA to estimate the crystallinity of the drug.

Differential Scanning Calorimetry. A DSC 131 (Setaram, France) was used to determine the thermal behavior of the materials between 50 and 350°C. Heating and cooling rates were 10°C/min. DSC curves of dry gels were obtained from the second heating run after the first run heating to 150°C in order to reset to the thermal history of the gel sample. All samples were analyzed under a continuous flow of dry nitrogen gas (flow rate 40 mL N_2/min).

SEM Observation. The SEM images of drug-loaded and unloaded gels were taken by FEI Quanta 450 FEG model SEM. Before taking the SEM pictures of the gels, they were swollen in distilled water until swelling equilibrium, and then these swollen hydrogels were subjected to the lyophilization and afterward, the dry porous gels were coated with gold using sputter-coating, and their SEM pictures were taken.

Swelling Studies. Swelling behavior of the gels was studied in three different aqueous media: distilled water, buffer solutions with pH 2.1 (KCl-HCl) and pH 7.4 (Na_2HPO_4-KH_2PO_4). The buffer solutions were prepared according to the recipes given by Perrin and Dempsey.[22] The dry gels were held in that medium at 37°C until they attain in swelling equilibrium. Swollen gels were taken out from the swelling medium at regular intervals and blotted carefully with a filter paper to remove the excess surface water. The swelling value (S) was determined by using the equation (1):

$$S\,(\mathrm{g\,H_2O/g\,polymer}) = (W_s - W_d)/W_d \quad (1)$$

where W_s and W_d are the weights of the swollen and dry gels at time t, respectively.

At swelling equilibrium $W_s = W_e$. Thus, the equilibrium swelling value (ESV) is obtained from the equation (2):

$$\text{Equilibrium swelling value (ESV)}\,(\mathrm{g\,H_2O/g\ polymer}) = (W_e - W_d)/W_d \quad (2)$$

where W_e is the weight of the swollen gel attained in swelling equilibrium.

The ionic strength of each buffer solution was adjusted to 0.09 M by adding sodium chloride to the solution.

Elastic Modulus Measurements. The compressive elastic modulus measurements of the swollen gels equilibrated in distilled water were performed by using load and transmission transducers explained in detail by Gürdağ and Öz.[23] The compression elastic moduli of cylindrical gel samples (diameter 4 mm and length 7 mm) were measured after a relaxation time of 30 sec. The compression force applied to the gel (F) and the resulting deformation (λ) were recorded using load and displacement transducers, respectively. Each measurement was performed using at least three samples. Compressive elastic modulus (G) was calculated from the slope of stress–strain curve using the equation (3):

$$\tau = G\left(\lambda - \lambda^{-2}\right) \quad (3)$$

where τ (stress) is the force (F) applied per unit area of undeformed swollen gel sample and where λ is the deformation ratio ($\lambda = L/L_o$, L and L_o are the lengths of the deformed and undeformed sample). The stress was calculated as $\tau = F/A_o$, where A_o is the area of the undeformed swollen gel, and $A_o = \pi r_o^2$, where r_o is the radius of undeformed swollen gel.[23]

Drug Loading and Release Studies. The amount of 5-FU entrapped in the hydrogels was determined by UV spectrophotometer at λ_{max} value of 266 nm. The difference between the amount of the drug initially employed and the drug content in the washing water determined was taken as the amount of 5-FU entrapped in the hydrogels. The concentration of the drug in the washing water was determined from the calibration graph in distilled water.

The drug release experiments were carried out by 10 mL buffer solutions with pH = 2.1 and pH = 7.4 at 37°C. At specific time intervals, 3 mL of buffer solution (aliquot) was taken from the release medium, and the equal volume of fresh buffer solution was added into the release medium to maintain the volume constant. The amount of 5-FU in the aliquot was determined at 266 nm using a UV-VIS Perkin-Elmer Lambda 35 Spectrophotometer. The calibration graphs in pH = 2.1 and pH = 7.4 were used to determine the amount of drug released from the drug-loaded gels. All measurements were carried out in triplicate, and the average values (standard errors < 3%) were considered in calculating the release percentage of 5-FU.

RESULT AND DISCUSSION

FTIR Studies

FTIR spectra of AAm and HMA homopolymers without CS and their counterparts with CS, AAm-HMA copolymers without CS, chitosonium acetate and the drug 5-FU were given in Figure 1(a,b). The stretching vibrations of N-H and O-H are indicated by the broad and intense bands at 3426 and 3419 cm^{-1} in the spectrum of drug-unloaded AAm homopolymer (AAm:F−) and drug-unloaded HMA homopolymer (HMA:F−), respectively.[24] These bands became broader with the increase in HMA content, namely the amount of OH groups in the copolymer gel. In the spectra of AAm homopolymer, the characteristic absorption bands are seen at 1662 cm^{-1} (ν C═O, amide I), at 1615 cm^{-1} (N–H bending, amide II), and at 1321 cm^{-1} (ν C–N, amide III).[25,26] In the spectra of HMA homopolymer, the band attributed to C–O stretching is seen at 1021 cm^{-1} and the band at 1281 cm^{-1} is ascribed to the stretching of C–N (amide III) bond in HMA homopolymer which is seen at 1321 cm^{-1} in the spectra of AAm homopolymer.[27] In the case of copolymer gels, the sharp band at about 1541 cm^{-1} is attributed to the stretching of N–H bond in HMA, respectively, and they confirm the presence of HMA in the copolymer gel structure.[24] Also, the band at 1271 cm^{-1} is attributed to the stretching of C–N (amide III) bond. In addition, the characteristic peak around 1021 cm^{-1} arises from the ether group (–CH_2O–) of HMA homopolymer and copolymer

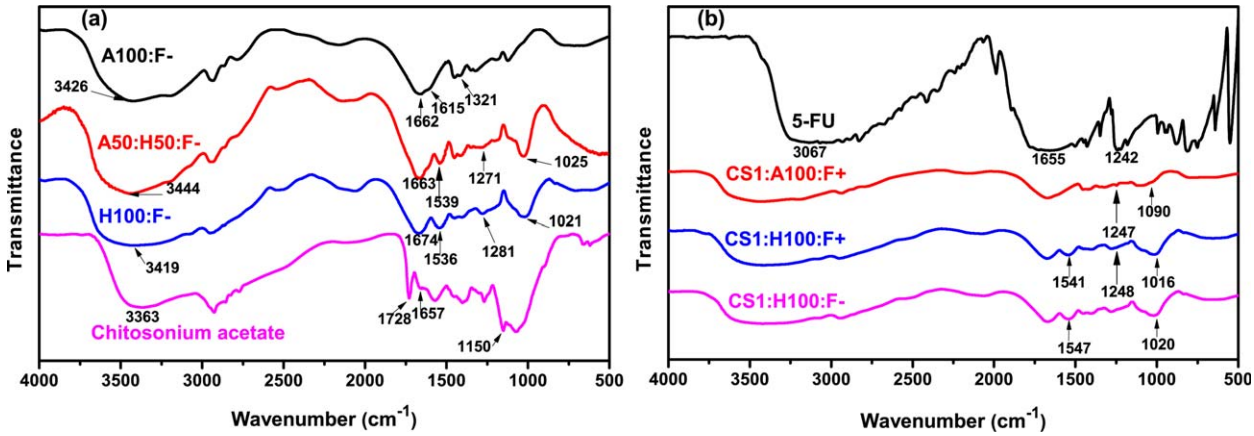


Figure 1. FTIR spectra of (a) chitosonium acetate and the gels without CS and (b) 5-FU, 5-FU-loaded, and -unloaded semi-IPN gels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

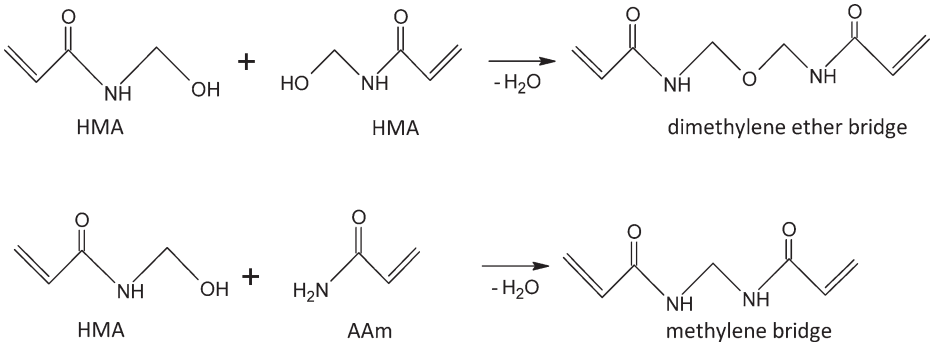
indicated the crosslinking reaction between two N-methylol groups.[28,29] In case of our polymers, the condensation reaction between either methylol groups of HMA or N-methylol of HMA and $-NH_2$ groups of AAm are presented in Scheme 1.

CS dissolved in aqueous acetic acid solution exists in the chitosonium acetate structure ($CS\text{-}NH_3{}^+COO^-$).[30–32] For that reason, its FTIR spectrum is given in Figure 1(a) instead of that of CS. The absorption bands at 1728 and 1657 cm^{-1} are assigned to the stretching vibration of carbonyl groups ($\nu_{C=O}$ for ester carbonyl and amide I) of chitosonium acetate.[30] The band at 3363 cm^{-1} is attributed to the stretching vibrations of O-H and N–H bonds in chitosonium acetate, which are overlapped.[30,33,34] The broadness of the band at 3363 cm^{-1} indicates the presence of intermolecular hydrogen bonds[30,34] between CS namely chitosonium acetate chains. The band at 1150 cm^{-1} in the spectrum of chitosonium acetate is attributed to the saccharide structure, while the strong peak at 1070 cm^{-1} is due to the C–O stretching vibration in CS.[30,35]

In the case of CS-containing semi-IPN gels [Figure 1(b)], the band at 1728 cm^{-1} in the spectrum of chitosonium acetate disappeared due to the dilution of ester carbonyl with a large amount of stretching vibration of amide carbonyl ($\nu_{C=O}$) of the homopolymers of AAm/ HMA and the copolymer of AAm-HMA. The characteristic peak for saccharide at 1150 cm^{-1} in the spectrum of chitosonium acetate disappeared in the spectra of semi-IPN gels. The shift in the amide I band in the spectrum of chitosonium acetate from 1657 cm^{-1} to the higher band (1670 cm^{-1}) in the spectra of semi-IPN gels confirms the presence of intermolecular hydrogen bonds between CS and AAm/ HMA homopolymer and copolymer network. In addition, the peak at around 1090 cm^{-1} in the spectra of CS : AAm semi-IPN gels due to stretching vibration of C–O bond confirms the presence of CS. The characteristic bands at 3067, 1655, and 1242 in the spectra of 5-FU indicate the N–H stretching vibration, C=O stretching vibration and C–F stretching bands, respectively, and they are given in Figure 1(b).[16] There are no apparent differences between the spectra of drug-loaded and unloaded gels. The band at around 1247 cm^{-1} in the spectrum of drug-loaded gels confirms the presence of the drug[16] and shows the absence of any chemical interactions between the drug and the polymer.

XRD Studies

XRD study helps to determine the crystallinity of a material. XRD analysis of pure 5-FU and, drug-loaded and unloaded gels are given in Figure 2. The intensive peaks of pure 5-FU are observed at 2θ of 16, 29, and 31°, confirming its crystalline nature. These peaks are not seen in XRD patterns of drug-loaded gels, indicating that 5-FU is dispersed at a molecular level in the polymer matrix and drug-loaded gels are in an amorphous structure.[14,15,36,37]



Scheme 1. The reaction of N-methylol groups to form dimethylene ether bridge and methylene bridge.

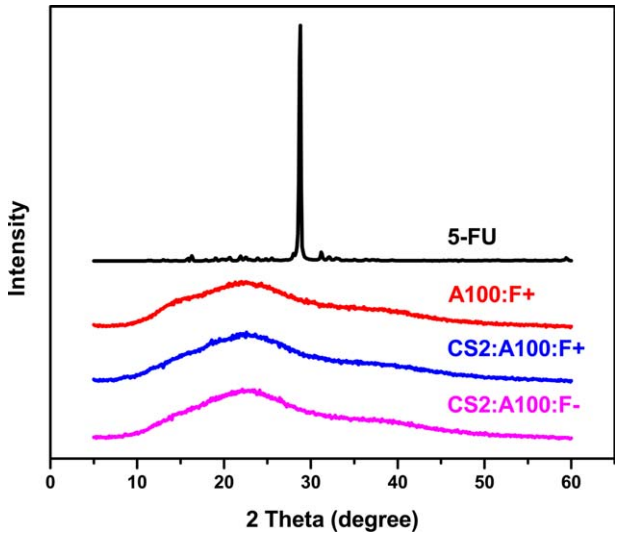


Figure 2. XRD patterns of pure 5-FU, drug-loaded, and -unloaded gels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DSC Studies

DSC thermograms of pure 5-FU, drug-loaded and unloaded gels are given in Figure 3. The sharp peak in the DSC curve of pure 5-FU at 285°C indicates the melting of the drug. In case of drug-loaded gels, no characteristic peak for 5-FU was observed, indicating that the drug is molecularly dispersed in the polymer matrix.[14,15,36,37] In the DSC curve of A100:F+, there is only one endothermic peak at 222°C. It is known that polyacrylamide, used in the gel synthesis, decomposes in three steps.[38,39] In the first step between 150 and 220°C, the loss of adsorbed and bound water takes place. In our case, DSC curves were obtained in the second heating run to delete the thermal history of the samples. For that reason, this peak due to moisture loss is not seen in the DSC curve. In the second decomposition step, the decomposition of polyacrylamide occurs at 220–298°C by the release of ammonia by the reaction between $–NH_2$ groups of acrylamide and –NH group of crosslinker (NMBA).[38,39] The third decomposition step is due to entire main chain breakdown of polyacrylamide (298–440°C). The presence of the endothermic peak in DSC curve of A100:F+ can be attributed to the second step decomposition of polyacrylamide by the release of small molecule (ammonia).[40,41] The presence of CS (CS2:A100:F+ and CS2:A100:F−) was shifted the endothermic decomposition peak from 222°C to higher temperature (232°C).

SEM Images

SEM images of CS2:A100:F−, CS2:A100:F+, CS2:A50:H50:F+ and CS2:H100:F+ are given in Figure 4. There are no big differences between the SEM images of drug-loaded and -unloaded CS2:A100 gels and both the polymers exhibit porous structure. However, when the HMA was incorporated in the polymer structure, the porosity of the gels disappeared and the gels had smoother surfaces. This finding is consistent with the lower swelling values of gels with HMA.

Swelling Studies

The swelling values of the AAm and/or HMA homopolymers in distilled water and the buffer solutions with pH 2.1 and 7.4 at 37°C are given in Table II. The swelling medium and the contents of AAm and HMA in the monomer feed were changed in order to determine the influence of pH and the type and content of the monomer on the swelling of the polymer network. The equilibrium swelling values of the gels in distilled water decreased significantly with an increase in HMA content of gels. This can be attributed to an increase in the formation of crosslinking due to the condensation reaction between the methylol groups of HMA. The same decrease in the equilibrium swelling values of gels with HMA content is shown at pH 2.1 and 7.4, the swelling value of gels had no distinct. This is expected behavior, and attributed to nonsensitive pH changes in the monomers. For a nonionic gel, the ionic strength in the neutral swelling medium is smaller because of the ionic strength of distilled water is zero than that in acidic and basic medium, therefore having the higher swelling capacities in distilled water.[30]

CS is a cationic polyelectrolyte and this polyelectrolyte is a weak polybase with a pKa around 6.5, and weak polyelectrolyte gels's swelling capacity is dependent on pH due to the functional ionizable groups change with the pH of the swelling medium.[30,42] Gels with CS polymer have high swelling values in acidic medium due to the repulsion forces between the chains with the protonated amine groups ($–NH_3^+$) and their swelling capacities diminish at high pH due to disappearance of repulsion forces between polymer chains and protonation of amine groups ($–NH_2$) of CS.

Table II also shows the swelling values of the semi-IPN gels, which were prepared by the polymerization of monomer/monomer mixture in 1(w/v) % and 2 (w/v) % CS aqueous acetic acid solution, in distilled water and in the buffer solutions with pH = 2.1 and pH = 7.4. Both osmotic pressure and repulsion forces between the same charged (+) CS chains cause the high swelling values in acidic medium for the semi-IPN gels containing CS. As can be seen, the swelling values of semi-IPN gels are

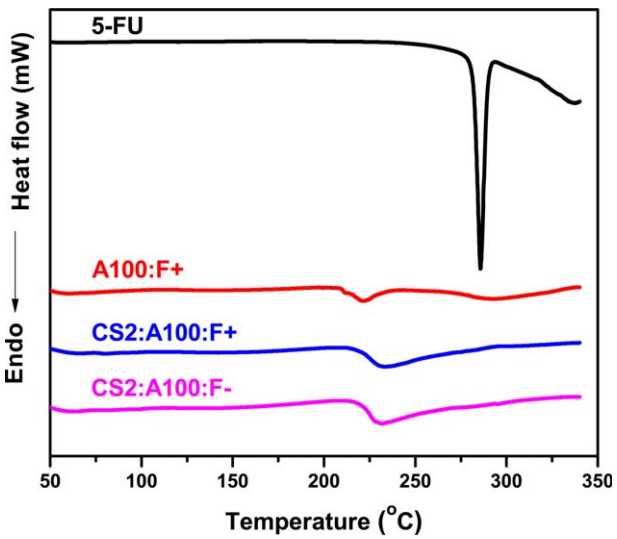


Figure 3. DSC thermograms of pure 5-FU, drug-loaded, and -unloaded gels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

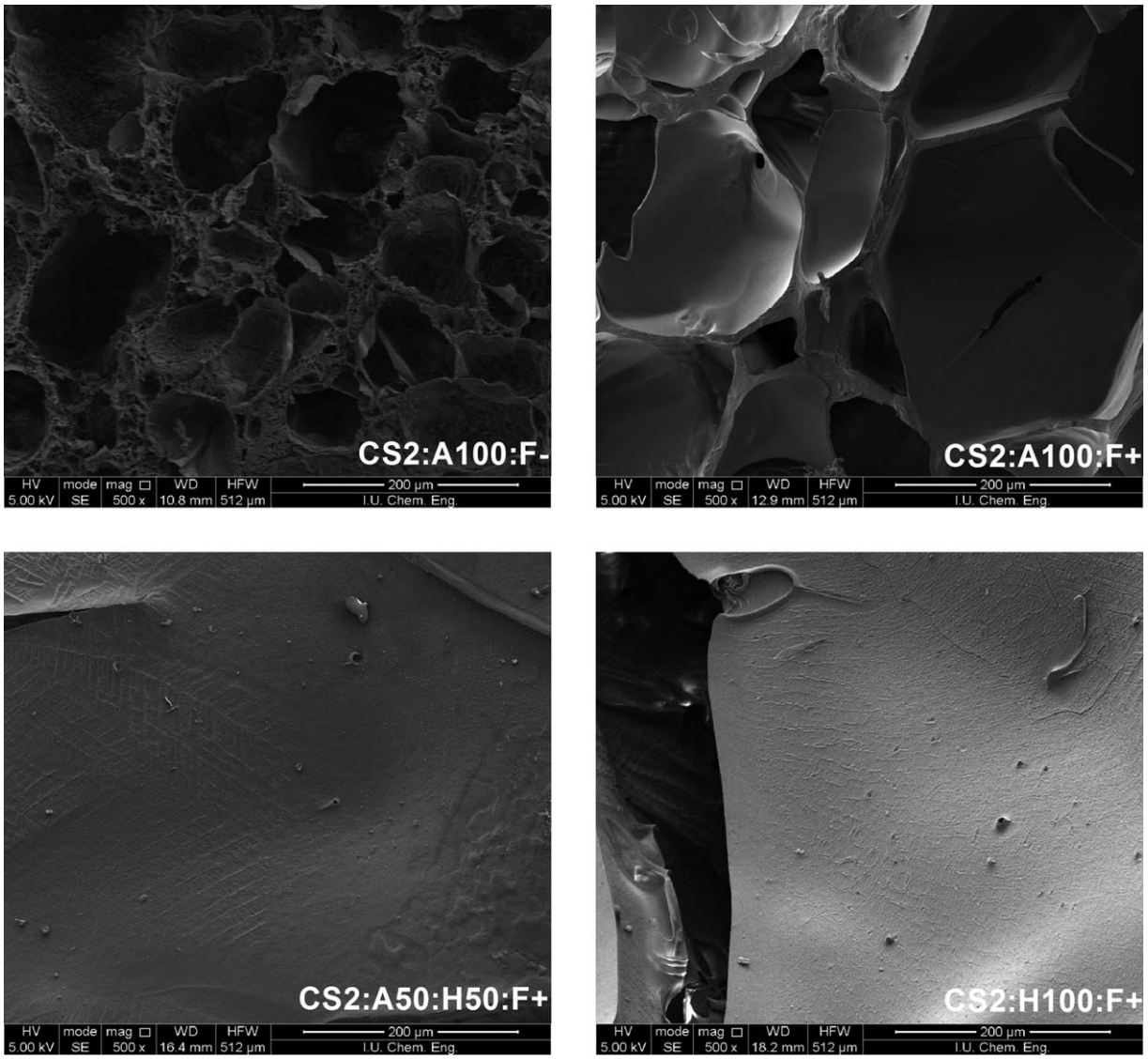


Figure 4. SEM images of CS2:A100:F−, CS2:A100:F+, CS2:A50:H50:F+, and CS2:H100:F+.

higher in acidic medium compared to those in distilled water and in buffer solution with pH = 7.4, since the protonation degree of CS at pH = 2.1 is in the highest amount. For nonionic gels, the equilibrium swelling values in distilled water are higher than that in acidic and basic medium. The driving force of the swelling process is the maximum difference between the osmotic pressures the inside and outside of the gel. A reduction of osmotic pressure causes a decrease in swelling for nonionic gels. The swelling studies show that ESVs of semi-IPN gels in distilled water are lower than those at pH 2.1, but higher than those at pH 7.4. Chitosan has a positive charge in acidic solutions due to the presence of protonated amino groups along its backbone and the swelling values are high at low pH because of the repulsive forces of the charged groups in the semi-IPN gels. However, at high pH, the degree of protonation of CS is very low. For that reason, the swelling of semi-IPN gels in distilled water (pH 6.5) is lower than that in acidic medium.[30]

The effect of HMA concentration on the swelling value of semi-IPN's is similar to that on the gels without CS. Increasing the HMA content of the gels decreased the swelling value of semi-IPN's for all swelling media. Such a reduction in swelling values is due to the formation of a rigid network structure with increasing HMA because of the increase in crosslinking by a condensation reaction between two $–HNCH_2OH$ (N-methylol) groups or –NH and $–HNCH_2OH$ groups that increased crosslinking density.[43] Poly(N-t-butylacrylamide-co-N-hydroxymethyl acrylamide)[43] and poly(acrylic acid-co-acrylamide)[28] gels show the same decrease trend in swelling values because of the condensation of highly reactive N-methylol groups which cause crosslinking. Hence, the crosslink density of gels has a great influence on the swelling values as well as the release rate of the drug. In addition, semi-IPN gels exhibit higher swelling values with increasing CS content. This finding can be attributed to the increase in positive charges ($-NH_3^+$) in polymer chains and repulsion forces between them.

Table II. The Equilibrium Swelling Values of the Drug-Loaded Gels at 37°C in Distilled Water, and Buffer Solution with pH 2.1 and pH 7.4

		Equlibrium swelling value (ESV) (g H_2O/g polymer)		
Polymer code		Distilled water	pH 2.1	pH 7.4
CS0:F+	A100:F+	36.4 ± 0.9	31.1 ± 1.0	30.9 ± 1.0
	A75:H25:F+	13.4 ± 0.6	11.0 ± 0.5	11.1 ± 0.5
	A50:H50:F+	7.6 ± 0.3	6.5 ± 0.3	6.4 ± 0.2
	A25:H75:F+	6.0 ± 0.2	5.3 ± 0.2	5.1 ± 0.2
	H100:F+	5.3 ± 0.2	3.9 ± 0.1	3.9 ± 0.1
CS1:F+	CS1:A100:F+	22.3 ± 0.8	36.0 ± 1.1	18.5 ± 0.8
	CS1:A75:H25:F+	4.1 ± 0.2	21.6 ± 0.9	3.3 ± 0.1
	CS1:A50:H50:F+	2.8 ± 0.1	17.5 ± 0.7	2.4 ± 0.1
	CS1:A25:H75:F+	2.5 ± 0.1	10.6 ± 0.4	2.3 ± 0.1
	CS1:H100:F+	2.2 ± 0.1	3.1 ± 0.1	2.0 ± 0.1
CS2:F+	CS2:A100:F+	27.8 ± 0.9	40.3 ± 1.0	22.7 ± 1.0
	CS2:A75:H25:F+	5.2 ± 0.2	34.4 ± 0.9	4.3 ± 0.2
	CS2:A50:H50:F+	4.0 ± 0.2	24.1 ± 0.8	3.0 ± 0.1
	CS2:A25:H75:F+	3.6 ± 0.1	20.7 ± 0.7	2.7 ± 0.1
	CS2:H100:F+	2.8 ± 0.1	13.5 ± 0.5	2.5 ± 0.1

Table III. Diffusional Exponent (n), Swelling Constant (K), and Correlation Efficient (R) of CS0 Gels in Distilled Water and Buffer Solutions

	Distilled water			pH 2.1			pH 7.4		
Polymer code	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*
A100:F+	0.6584	8.18	0.9813	0.7192	6.00	0.9969	0.7376	5.56	0.9944
A75:H25:F+	0.5224	55.53	0.9952	0.5122	67.00	0.9961	0.5525	71.48	0.9885
A50:H50:F+	0.5331	47.24	0.9979	0.5856	32.30	0.9972	0.6121	32.87	0.9989
A25:H75:F+	0.5740	46.44	0.9991	0.6437	32.90	0.9993	0.6545	33.22	0.9991
H100:F+	0.5595	52.78	0.9925	0.6276	38.12	0.999	0.6041	46.25	0.9988

Table IV. Diffusional Exponent (*n*), Swelling Constant (*K*), and Correlation Efficient (*R*) of CS1 Gels in Distilled Water and Buffer Solutions

	Distilled water			pH 2.1			pH 7.4		
Polymer code	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*
CS1:A100:F+	0.5197	21.88	0.9970	0.7083	4.00	0.9981	0.5281	28.31	0.9767
CS1:A75:H25:F+	0.5027	74.83	0.9895	0.5643	5.31	0.9952	0.5270	75.04	0.9300
CS1:A50:H50:F+	0.5138	71.93	0.9941	0.6202	6.98	0.9982	0.5741	45.70	0.9958
CS1:A25:H75:F+	0.5387	64.13	0.9995	0.574	12.19	0.9982	0.5773	49.30	0.9998
CS1:H100:F+	0.5198	57.36	0.9884	0.5411	23.72	0.9795	0.5478	54.33	0.9891

Table V. Diffusional Exponent (*n*), Swelling Constant (*K*), and Correlation Efficient (*R*) of CS2 Gels in Distilled Water and Buffer Solutions

	Distilled water			pH 2.1			pH 7.4		
Polymer code	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*	*n*	*K* (10^{-3})	*R*
CS2:A100:F+	0.6763	12.41	0.9866	0.7035	4.14	0.9975	0.6462	7.84	0.9872
CS2:A75:H25:F+	0.5583	50.55	0.9715	0.7232	3.30	0.9931	0.5123	60.22	0.9604
CS2:A50:H50:F+	0.5210	61.04	0.9996	0.6760	5.26	0.9956	0.5826	58.52	0.9437
CS2:A25:H75:F+	0.5046	67.84	0.987	0.6794	5.01	0.9963	0.5770	43.59	0.9988
CS2:H100:F+	0.5104	75.21	0.9697	0.6104	11.43	0.9988	0.5752	44.43	0.9996

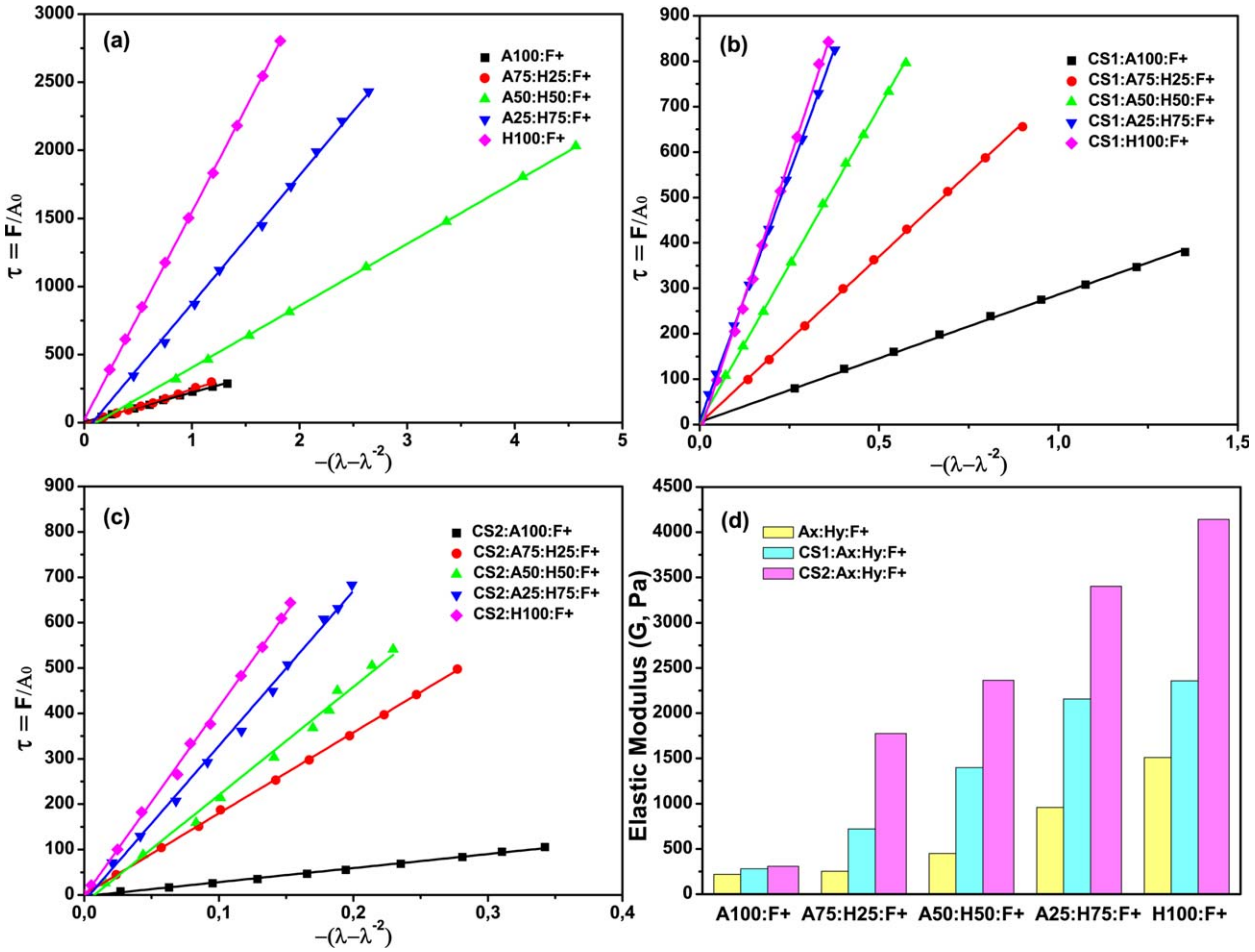


Figure 5. Stress versus strain graphs for drug loaded gels (a) CS0, (b) CS1, and (c) CS2 and (d) the effect of CS and monomer(s) concentration in the feed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Diffusion Kinetics Through the Gels

In order to study the effect of the composition of hydrogels on the kinetics of water uptake of hydrogels, the water uptake data were fitted using the following models. The part of the water absorption curve with a fractional water uptake (M_t/M_e) less than 0.60 was analyzed with the equations (4) and (5):

$$F = M_t/M_e = K \cdot t^n \quad (4)$$

$$\ln(M_t/M_e) = \ln K + n \cdot \ln t \quad (5)$$

M_t is the mass of water absorbed at any time t, M_e is the mass of water absorbed at equilibrium, K is the characteristic swelling constant of the hydrogel, and n is the swelling exponent characterizing the mechanism of diffusion of solvent into the network. The slope of the line obtained by plotting $\ln(M_t/M_e)$ versus $\ln(t)$ shows the values of n and K. There are three types of diffusion depending on the relative rates of diffusion and polymer relaxation. The first one is Fickian type diffusion ($n = 0.5$), in which the diffusion rate, R_{diff}, is clearly slower than the relaxation rate of polymer chains, R_{relax}, ($R_{diff} << R_{relax}$). Thus, the swelling is controlled by the diffusion of water into the polymer. The second one is Case II diffusion

Table VI. Drug Loading Capacity of Gels

Polymer code		Loaded drug (mg 5-FU/g polymer)
CS0:F+	A100:F+	16.8
	A75:H25:F+	14.5
	A50:H50:F+	12.1
	A25:H75:F+	10.7
	H100:F+	9.7
CS1:F+	CS1:A100:F+	31.3
	CS1:A75:H25:F+	27.3
	CS1:A50:H50:F+	19.5
	CS1:A25:H75:F+	15.2
	CS1:H100:F+	13.7
CS2:F+	CS2:A100:F+	42.7
	CS2:A75:H25:F+	34.7
	CS2:A50:H50:F+	25.0
	CS2:A25:H75:F+	16.5
	CS2:H100:F+	14.7

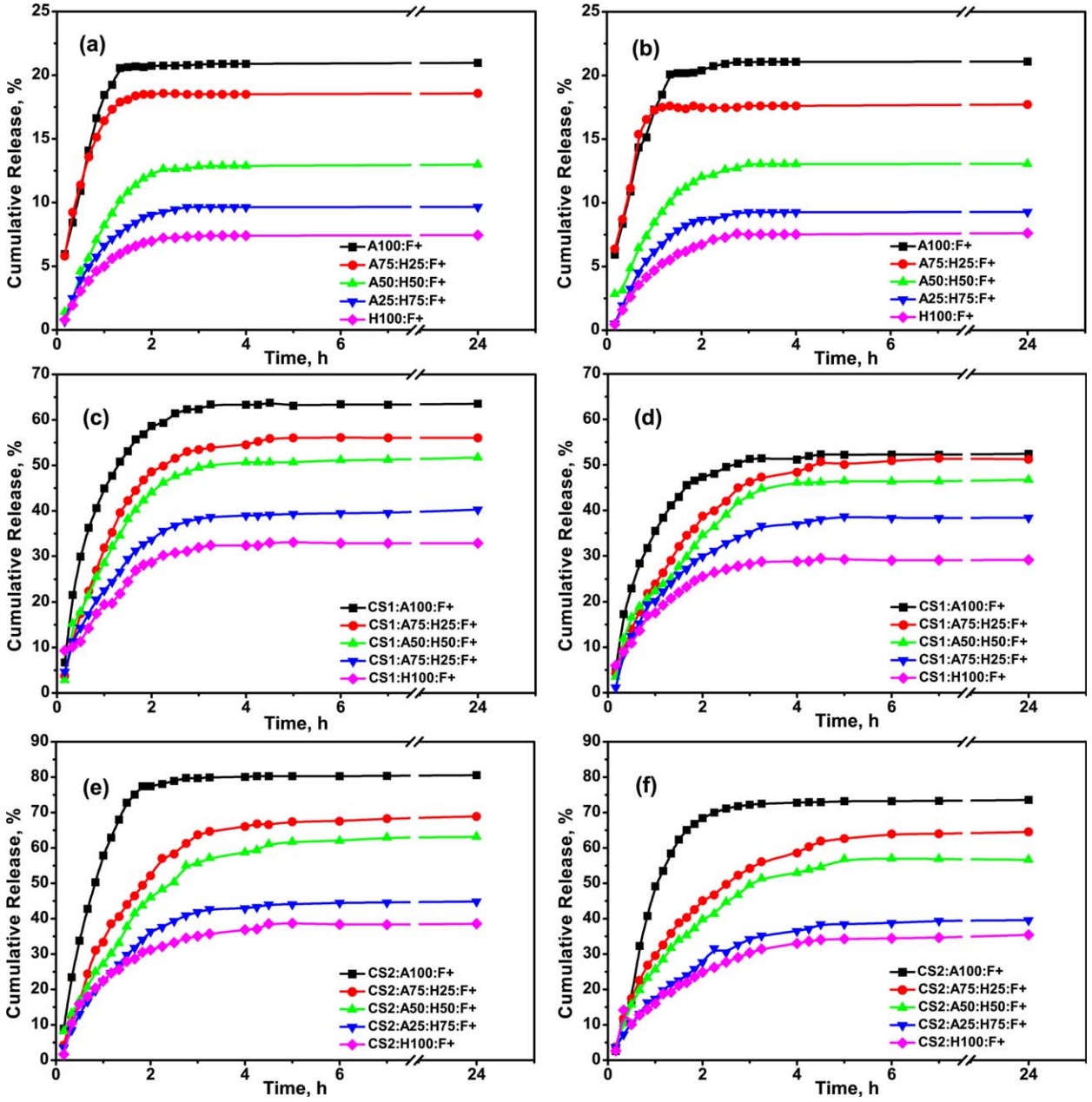


Figure 6. Cumulative drug release values of the gels as a function of time at 37°C in buffer solutions with pH 2.1 (a, c, and e) and 7.4 (b, d, and f). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

($n = 1.0$), where the diffusion of water is very rapid compared to relaxation of polymer network ($R_{diff} >> R_{relax}$), with relaxation occurring at an observable rate. The third class is non-Fickian type or anomalous diffusion, while the value of n in the range $0.5 < n < 1.0$, where both the diffusion of water and the relaxation of polymer network control the overall rate of water uptake.[44,45]

The parameters K, n and correlation coefficient (R) were calculated and listed in Tables III–V. As seen in Tables III–V, the diffusion exponent (n) values for gels are between 0.51 and 0.74. This indicates that the transport of water into all gels occurs by non-Fickian diffusion that is both diffusion- and relaxation-controlled system. In general, the values of n decreased with the decrease in the HMA content of drug loaded CS0, CS1, and CS2 gels in distilled water and buffer solution with pH 2.1 and 7.4. In addition, the n values for drug loaded CS1 gels in distilled water and the buffer solution with pH 7.4 indicate a diffusion that is close to Fickian type.

Elastic Modulus

The stress–strain graphs of drug loaded gels in the swollen state at 25°C are shown in Figure 5. There is a linear relationship between the stress and strain data for the polymers with or without CS, and the correlation coefficients change between 0.986 and 0.999. Compressive elastic modulus (G) was calculated from the slopes of the

straight lines obtained in the graphs of stress (τ) versus strain ($\lambda - \lambda^{-2}$). The compressive elastic moduli (G) of the samples are shown in Figure 5 as a function of the CS and monomer content.

At high AAm concentrations (0.5 *M*), the existence of CS didn't change the compressive elastic modulus values of the samples effectively. *G* values of the gels enhanced with the increase in HMA content of the polymer matrix and the effect of HMA was highly remarkable in the gels with high CS content (CS2 gels). The increase in *G* values with HMA content can be attributed to the molecules to condense, through the reaction of reactive methylol groups which cause an increase in crosslinking density.[23] The presence of interchain interactions between CS chains and AAm / HMA homopolymer and copolymer chains creates intramolecular hydrogen bonds.[46] Hence, CS gels have a lower ESV than the gels without CS. The *G* values increased with the introduction of CS and its content. From the stress–strain curves of the hydrogels, the compressive elastic moduli of CS2 gels containing 2% (w/v) CS are higher than those of the other gels. This can be attributed to the increase in hydrogen bonding at higher CS content.

Drug Loading and Release Studies

The effect of increasing concentrations of CS and AAm on the drug loading capacity is shown in Table VI. The amount of drug loaded to the gels increased with the presence of CS and AAm in the gel structure and it decreased with the increase in HMA concentration in the gels because of the additional crosslinking.

The effect of pH on drug release was studied at two pHs, pH 2.1 and 7.4, in the buffer solution. The release percentages of drugs from the gels are given in Table V and the cumulative drug release values of the gels in the buffer solutions with pH 2.1 and 7.4 at 37°C are given as a function of time in Figure 6.

There is no difference between the release percentages of the gels without CS in both buffer solutions (pH 2.1 and 7.4) and they are lower than those of CS-containing gels as expected because of the nonsensitivity to pH of gels without CS. The release percentages of 5-FU from CS1 and CS2 semi-IPN gels at pH 2.1 are higher than those at pH 7.4 since the synthesized gels contain amino groups. At pH 2.1, the degree of swelling increased and led to higher drug release. Under near-neutral condition at pH 7.4, the swelling of the gels is low and for that reason, the drug release is low. The formulations with higher HMA content decreased the release rate than those with higher AAm content. This is due to the formation of a more rigid network structure caused by the additional crosslinking.[23] The cumulative release values of AAm-containing gels are quite high, whereas they are lower for the gels with high HMA content. In addition, a higher amount of drug release was observed when the CS content of the gel increased. Drug release increased with the increase in drug loading. The comparison of the amounts of drug release percentages at the same pH showed that the equilibrium release is dependent on the contents of AAm and/or CS of the polymer network.

CONCLUSION

CS-based semi-IPN hydrogels have been prepared by free radical polymerization and characterized by FTIR, XRD and DSC methods. FTIR spectra of drug loaded gels show the absence of any chemical interactions between the drug and the polymer. DSC thermograms and XRD patterns have confirmed the uniform distribution of the drug molecules in the polymer matrix. It was found that the equilibrium swelling values of CS-containing semi-IPN gels in acidic medium (pH = 2.1) are higher than those at pH 7.4 due to the protonation of amino groups of CS. In addition, the increase in HMA content of the polymer has negatively affected the equilibrium swelling values of semi-IPN hydrogels. The swelling of drug-loaded gels followed a non-Fickian type diffusion. It was observed that the compression elastic modulus of the drug-loaded gels increased with the increase in HMA content because of the additional crosslinking. In addition, the higher CS content in the semi-IPN gels enhanced the swelling capacity as well as mechanical strength. The amount of drug loading increased with the increase in AAm and CS content of gels. The crosslink density of gels increased with the HMA content of the gel due to the condensation reaction between its methylol groups and it led to decrease in the functional groups which may interact with the drug. The release rate of the drug depended upon the extent of crosslinking and percentage of drug loading. The obtained results encourage the use of AAm and/or HMA-containing CS-based semi-IPN hydrogels in the release of 5-FU.

ACKNOWLEDGMENTS

This work was supported by the Research Fund of Istanbul University, Project No: 11970.

REFERENCES

1. Peppas, N. A.; Khare, A. R. *Adv. Drug Del. Rev.* **1993**, *11*, 1.
2. Soppimath, K. S.; Aminabhavi, T. M.; Dave, A. M.; Kumbar, S. G.; Rudzinski, W. E. *Drug Dev. Ind. Pharm.* **2002**, *28*, 957.
3. Changez, M.; Burugapallı, K.; Koul, V.; Choudhary, V. *Biomaterials* **2003**, *24*, 527.
4. Alvarez-Lorenzo, C.; Concheiro, A.; Dubovik, A. S.; Grinberg, N. V.; Burova, T. V.; Grinberg, V. Y. *J. Control. Release* **2005**, *102*, 629.
5. Risbud, M. V.; Hardikar, A. A.; Bhat, S. V.; Bhonde, R. R. *J. Control. Release* **2000**, *68*, 23.
6. Chen, S.; Wu, Y.; Mi, F.; Lin, Y.; Yu, L.; Sung, H. *J. Control. Release* **2004**, *96*, 285.
7. Ekici, S.; Saraydin, D. *Drug Delivery* **2004**, *11*, 381.
8. Lopes, C. M. A.; Felisberti, M. I. *Biomaterials* **2003**, *24*, 1279.

9. Bhattarai, N.; Gunn, J.; Zhang, M. *Adv. Drug Del. Rev.* **2010**, *62*, 83.
10. Kim, S. Y.; Cho, S. M.; Lee, Y. M.; Kim, S. J. *J. Appl. Polym. Sci.* **2000**, *78*, 1381.
11. El-Sherbiny, I. M.; Lins, R. J.; Abdel-Bary, E. M.; Harding, D. R. K. *Eur. Polym. J.* **2005**, *41*, 2584.
12. Pulat, M.; Tan, N.; Onurdağ, F. K. *J. Appl. Polym. Sci.* **2011**, *120*, 441.
13. Macias, E. R.; Rodriguez-Guadarrama, L. A.; Cisneros, B. A.; Castaneda, A.; Mendizabal, E.; Puig, J. E. *Colloids Surf. A Physicochem Eng. Asp.* **1995**, *103*, 119.
14. Sekhar, E. C.; Rao, K. S. V.; Raju, R. R. *J. Appl. Pharm. Sci.* **2011**, *01*, 199.
15. Rao, K. S. V.; Rao, K. M.; Kumar, P. V. N.; Chung, I. *Iran Polym. J.* **2010**, *19*, 265.
16. Olukman, M.; Sanlı, O.; Solak, E. K. *J. Biomater. Nanobiotechnol.* **2012**, *3*, 469.
17. Chouhan, R.; Bajpai, A. K. *J. Mater. Sci. Mater. Med.* **2009**, *20*, 1103.
18. Grem, J. L.; Nguyen, D.; Monahan, B. P.; Kao, V.; Geoffroy, F. J. *Biochem. Pharmacol.* **1999**, *58*, 477.
19. Abdelaal, M. Y.; Abdel-Razik, E. A.; Abdel-Bary, E. M.; El-Sherbiny, I. M. *J. Appl. Polym. Sci.* **2007**, *103*, 2864.
20. Kasaai, M. R. *Carbohyd. Res.* **2008**, *343*, 2266.
21. Baskar, D.; Kumar, S. T. S. *Carbohydr. Polym.* **2009**, *78*, 767.
22. Perrin, D. D.; Dempsey, B. Buffers for pH and Metal Ion Control; Chapman and Hall: London, **1974**.
23. Gürdağ, G.; Öz, G. M. *Polym. Adv. Technol.* **2009**, *20*, 216.
24. Chen, J.; Liu, M.; Liu, H.; Ma, L. *Mater. Sci. Eng. #C* **2009**, *29*, 2116.
25. Kasgoz, H.; Ozgumus, S.; Orbay, M. *Polymer* **2003**, *44*, 1785.
26. Rao, K. S. V.; Kumar, A. B. V. K.; Rao, K. M.; Subha, M. C. S.; Lee, Y. *Polym. Bull.* **2008**, *61*, 81.
27. Saeed, A.; Georget, D. M. R.; Mayes, A. G. *React. Funct. Polym.* **2010**, *70*, 230.
28. Liu, Z. S.; Rempel, G. L. *J. Appl. Polym. Sci.* **1997**, *64*, 1345.
29. Xie, L.; Liu, M.; Ni, B.; Wang, Y. *Ind. Eng. Chem. Res.* **2012**, *51*, 3855.
30. Sarmad, S.; Yenici, G.; Gürkan, K.; Keçeli, G.; Gürdağ, G. *Smart Mater. Struct.* **2013**, *22*, 1.
31. Quijada-Garrido, I.; Iglesias-Gonzalez, V.; Mazon-Arechederra, J. M.; Barrales-Rienda, J. M. *Carbohydr. Polym.* **2007**, *68*, 173.
32. Wang, N.; Chen, Y.; Kim, J. *Macromol. Mater. Eng.* **2007**, *292*, 748.
33. Peng, H.; Xiong, H.; Li, J.; Xie, M.; Liu, Y.; Bai, C.; Chen, L. *Food Chem.* **2010**, *121*, 23.
34. Hemant, K. S. Y.; Shivakumar, H. G. *Trop. J. Pharm. Res.* **2010**, *9*, 197.
35. Milosavljevic, N. B.; Kljajevic, L. M.; Popovic, I. G.; Filipovic, J. M.; Kalagasidis Krusic, M. T. *Polym. Int.* **2010**, *59*, 686.
36. Sairam, M.; Babu, V. R.; Naidu, B. V. K.; Aminabhavi, T. M. *Int. J. Pharm.* **2006**, *320*, 131.
37. Babu, V. R.; Sairam, M.; Hosamani, K. M.; Aminabhavi, T. M. *Int. J. Pharm.* **2006**, *325*, 55.
38. Merlin, D. L.; Sivasankar, B. *Eur. Polym. J.* **2009**, *45*, 165.
39. Ferfera-Harrar, H.; Aiouaz, N.; Dairi, N.; Hadj-Hamou, A. S. *J. Appl. Polym. Sci.* **2014**, *131*, 39747.
40. Biswal, D. R.; Singh, R. P. *Carbohydr. Polym.* **2004**, *57*, 379.
41. Zhou, C.; Wu, Q. *Colloids Surf. B: Biointerfaces* **2011**, *84*, 155.
42. Martínez-Ruvalcaba, A.; Sánchez-Díaz, J. C.; Becerra, F.; Cruz-Barba, L. E.; González-Álvarez, A. *Express Polym. Lett.* **2009**, *3*, 25.
43. Kaçmaz, A.; Gürdağ, G. *Macromol. Symp.* **2006**, *239*, 138.
44. Cavusoglu, F.; Kasgoz, H. *Polym. Composite* **2011**, *32*, 2062.
45. Deen, G. R.; Chua, V.; Ilyas, U. *J. Polym. Sci. Part A: Polym. Chem.* **2012**, *50*, 3363.
46. Kim, S. J.; Shin, S. R.; Kim, N. G.; Kim, S. I. J. *Macromol. Sci. Part A* **2005**, *42*, 1073.