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Carboxymethyl sago pulp/carboxymethyl sago starch hydrogel: Effect of polymer mixing ratio and study of controlled drug release

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ABSTRACT: Carboxymethyl sag o pulp (CMSP)/carboxymethyl sago starch (CMSS) hydrogel was synthesized by electron beam irradiation. In the series of hydrogels prepared, 40%/20% CMSP/CMSS hydrogel had the highest gel fraction. The swelling capacity of CMSP/CMSS hydrogel was found to be highest in distilled water, followed by pH 11, pH 7.4, and pH 1.2. Scanning Electron Microscope photographs revealed that the drug-loaded hydrogel had a smoother surface than unloaded hydrogel. Fourier Transform Infrared and Differential Scanning Calorimetry analysis showed the absence of interaction between the hydrogels and the drug. All drug-loaded hydrogels had drug encapsulation efficiency between 63% and 69%. CMSP/CMSS hydrogel swelled and allowed the release of drug at pH 7.4. These properties qualify the hydrogel as a potential candidate for controlled drug release at the ocular and colonic regions. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43652.

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INTRODUCTION

Hydrogels are defined as three-dimensional polymeric networks which have the ability to absorb a large amount of water or biological fluids, but they remain insoluble, because of the presence of physical or chemical cross-links between the individual polymeric chains.[1] The most important characteristic of a hydrogel is the water holding capacity. Hydrogel swells to become an elastic gel upon water penetration. Swelling property depends on the gel fraction, and the quantification of the gel fraction can provide information on the extent of cross-linking of the polymer. The gel content of cross-linked polymers is usually measured by extracting the polymer in distilled water for 16–20 hours and the insoluble polymer which remains is weighed. The percentage ratio of the remaining polymer's weight to the initial polymer's weight is called gel fraction.[2]

Mostly hydrogels formed from natural polymers such as starch, cellulose, pectin are biodegradable, and these hydrogels contain labile bonds which can be broken by hydrolysis, they are advantageous in pharmaceutical applications such as drug delivery.[3,4] In ocular drug delivery system, the hydrogel can act as the polymeric support which has been incorporated with the drug within the polymeric network.[5] The instillation of the ocular insert that causes prolonged drug pre-corneal residence time allows the delivered drug to exhibit its maximum biological action, lower systemic side effects and creates a more distinct effect with lower doses of the drug.[6] This can help to overcome the limitations of eye drops which have low corneal residence time, and low patient compliance.[7] Besides ocular insert, a hydrogel that made up of pH-sensitive polymers is also particularly suitable for colon-targeted drug delivery as it can withstand low pH at the stomach. Therefore, the drug will not be released, and it can be carried down to the small intestine and colon.[8]

Sago palm (*Metroxylon sagu*) is a sustainable agriculture crop in Sarawak, Malaysia.[9] The sago starch production rate is 300 million tonnes/year and meanwhile, a considerable amount of sago waste is produced as a by-product. Sago pulp, which contains cellulose, can be extracted from sago waste and converted to

Carboxymethyl Sago Pulp (CMSP).[10,11] CMSP is prepared through etherification of the hydroxyl groups with sodium monochloroacetate (SMCA) which is used as an etherifying agent, based on Williamson's ether synthesis.[12] Previous studies have demonstrated the formation of hydrogels by cross-linking CMSP using electron beam (EB).[13,14] Besides, it has also been cross-linked with another natural polymer such as pectin to form hydrogel for controlled drug delivery.[15] Based on the previous studies, the usage of CMSP in the preparation of hydrogel for colon-targeted drug delivery appears to be advantageous because of its pH-sensitive property. Based on the results of *in vitro* studies, it was shown to minimize the release of drug at stomach pH, and most of the drug were released at colonic pH.[11,15] Because of the increase in the approach of using natural polymers in pharmaceutical dosage form development, sago starch has also been utilized in the formulation of dosage forms, especially for drug administration.[16] Besides, it has also been utilized for the preparation of wound dressing.[17] Among the functionalization procedures, carboxymethylation of sago starch is the typical process to modify its properties.[18] A derivative of sago starch, CMSS has been reported to exhibit improved properties such as the ability to swell in cold water, improved freeze-thaw stability, and lowered tendency to retrograde.[12] Studies have also been carried out to evaluate its potential as a drug carrier for controlled drug delivery. Similar as CMSP, CMSS was reported to exhibit pH-sensitive behavior as it showed higher swelling at pH 7.4 compared to pH 1.2.[19] Similar results were obtained when CMSS was cross-linked with methacrylic acid using EB irradiation.[20] This report also further demonstrated the potential of CMSS as a drug carrier in the pH-dependent system.

To date, the characteristics of blend hydrogel made from CMSP and CMSS has not yet been reported, and no study has been done on the drug release property. The objectives of this article were to report on the preparation of CMSP/CMSS hydrogel by EB irradiation and study the swelling behavior of formed hydrogel in the various pH medium. The intention was to produce a hydrogel which has the most sustained release of drug in the body parts of interest, especially the ocular and colonic regions. EB irradiation technique has been used in the cross-linking of polymers as it can efficiently replace the chemical cross-linking agents and produce a hydrogel with a high purity that does not need removable of hazardous chemical cross-linker.[21] Ciprofloxacin hydrochloride was used as a model drug for the study of sustained drug release of ocular insert. It is commonly used in the treatment of infectious types of conjunctivitis.[22] Diclofenac sodium was chosen as the model drug for colon-targeted drug delivery system. It is the first nonsteroidal anti-inflammatory agent (NSAID) to be recognized that is a phenylacetic acid derivative.[23]

EXPERIMENTAL

Materials

Sago waste was obtained from Ng Kia Heng Kilang Sagu Industries, Batu Pahat, Johor. CMSS (DS 1.0) was donated by Dr. Kamaruddin Hashim from Malaysian Nuclear Agency, Bangi. Ciprofloxacin hydrochloride and diclofenac sodium were obtained from Dr. Saravanan Muniandy (School of Pharmacy, Monash University Malaysia). Glacial acetic acid, sodium hydroxide pellets, isopropanol, potassium chloride, and potassium dihydrogen phosphate were obtained from R&M Chemicals (United Kingdom). Hydrochloric acid (37%) was obtained from Merck KGaA (Germany) while methanol and di-sodium hydrogen phosphate were obtained from HmbG Chemicals (Germany). Ethanol (95%) was obtained from John Kollin Chemicals (United Kingdom). Sodium chlorite (80% technical grade) and SMCA were obtained from Fluka (Italy) and Fluka (United States) respectively. Distilled water was used throughout the study.

Isolation of Sago Pulp from Sago Waste

Sago waste was oven dried for 3 hours, ground, and sieved through 0.5 mm^2 test sieve. The ground sago waste was pre-dried in the oven at 60 °C for 1 hour. Sago waste (20 g) was suspended in 640 mL of hot distilled water together with 4 mL of glacial acetic acid. Technical grade sodium chlorite (6 g) was added. The 1 L conical flask was stoppered with empty 100 mL of the inverted conical flask and incubated in shaking water bath at 70 °C for 3 hours. The mixture was filtered through cheese cloth sieve and washed with cold distilled water until the pH of the filtrate was 7.0. The residue was dried in oven at 60 °C to constant weight.[10,11]

Preparation of CMSP with Degree of Substitution 0.8

CMSP with a degree of substitution 0.8 was prepared according to the previous publication without any modification.[10] The sago pulp which has dried overnight was ground using a blender and sieved using 0.5 mm^2 test sieve. Ground sago (5 g) was added with 100 mL of isopropanol and 10 mL of 25% w/v sodium hydroxide in a drop-wise fashion. After stirring on an orbital shaker for 1 hour, 6.0 g of SMCA (0.052 mol) was added, and the reaction mixture was placed in a thermostated water bath on a horizontal shaker at 45 °C for 3 hours. Then, the mixture was filtered through Buchner funnel by using laboratory aspirator, and the residue was suspended in 300 mL of methanol overnight. The suspended methanol was neutralized using glacial acetic acid, and the resultant residue was washed with 300 mL ethanol, filtered, and dried in an oven at 60 °C to constant weight.[10,11] Percentages of yield of sago pulp, reaction efficiency of CMSP, and yield of CMSP were calculated as the following eqs. (1–3), respectively:

$$\text{Percentage of yield of sago pulp} = \frac{\text{Sago pulp (g)}}{\text{Sago waste (g)}} \times 100\% \quad (1)$$

$$\text{Reaction efficiency (\%)} = \frac{\text{Weight of CMSP (g)} - \text{Initial weight of sago pulp (g)}}{\text{Weight of sodium monochloroacetate (g)}} \times 100\% \quad (2)$$

$$\text{Percentage of yield of CMSP} = \frac{\text{Actual yield (g)}}{\text{Theoretical yield (g)}} \times 100\% \quad (3)$$

Table I. Preparation of CMSP/CMSS Hydrogels with Different Percentage of CMSP (DS 0.8) and CMSS (DS 1.0)

CMSP/CMSS mixture	% of CMSP DS 0.8	% of CMSS DS 1.0
10%/10%	10	10
20%/20%	20	20
30%/30%	30	30
20%/10%	10	20
20%/30%	30	20
20%/40%	40	20
10%/20%	20	10
30%/20%	20	30
40%/20%	20	40

Preparation of CMSP/CMSS Hydrogels from CMSP And CMSS Solution and Irradiation Procedures

For unloaded hydrogel, CMSP/CMSS mixtures were prepared as shown in Table I and the mixtures were homogenized for 1 hour at 600 rpm using a mechanical stirrer before irradiation.

Determination of Gel Content in CMSP/CMSS Hydrogel with Various Doses of Irradiation

After EB irradiation, irradiated samples were transferred into individual tea bags and weighed (w_0). They were suspended in beakers containing a large amount of distilled water overnight to obtain the formed hydrogels. The hydrogel residues were then transferred to individual plastic bags and dried in the oven at 60 °C until constant weights (w_1) were obtained. The percentage of soluble fraction and gel fraction were calculated according to the following equations:

$$\text{Sol fraction } (\%) = [(w_0 - w_1)/w_0] \times 100 \quad (4)$$

$$\text{Gel fraction } (\%) = 100 - \text{Sol fraction} \quad (5)$$

where w_0 is the weight of initial wet hydrogel and w_1 is the weight of dried hydrogel.[13]

Swelling Behavior of the CMSP Hydrogel in Various pH Media

Approximately 1 g of CMSP/CMSS hydrogel from each sample was weighed, and the weight was recorded as initial weight at 0 minutes. The weighed hydrogel from each sample was then placed in a beaker containing 50 mL hydrochloric acid buffer at pH 1.2. The gels immersed in each beaker were then removed after 30 minutes, blot dried, weighed again, and the observations were recorded. The steps above were repeated after an hour. The reading was continuously taken until the 8th hour for every hour intervals and at the 24th hour. The procedures were repeated by putting the hydrogel into distilled water, phosphate buffer at pH 7.4 and pH 11. The swelling ratio was calculated according to the equation below:

$$\%\ S = \frac{M_t - M_0}{M_0} \times 100 \quad (6)$$

where M_0 is the mass of dry hydrogel at time zero and M_t is the mass of swollen hydrogel at time t.[8]

Preparation of Discs from CMSP/CMSS Hydrogel for Characterization

The hydrogel was dried in the oven at 60 °C overnight. Discs of approximately 6 mm in diameter were obtained by using a hole-puncher. Then weights of 10 discs were recorded by using a dial calliper. The diameter and thickness of the discs were also measured. The average weight (g), diameter (mm), and thickness (mm) of the 10 discs were calculated for each sample.

Loading Drug into the Hydrogel

For drug-loaded hydrogel, 40%/20% mixture were prepared in a similar way with the addition of ciprofloxacin hydrochloride and diclofenac sodium equivalent to 20% of the dry weight of the mixture to give 20% drug content. The mixtures were transferred to plastic bags and air bubbles in the mixture were removed using Henkovac machine. The mixtures were transferred to petri dishes and irradiated with a dosage of 20, 25, and 30 kGy using an ESP-3000 EB accelerator for approximately 15 minutes.

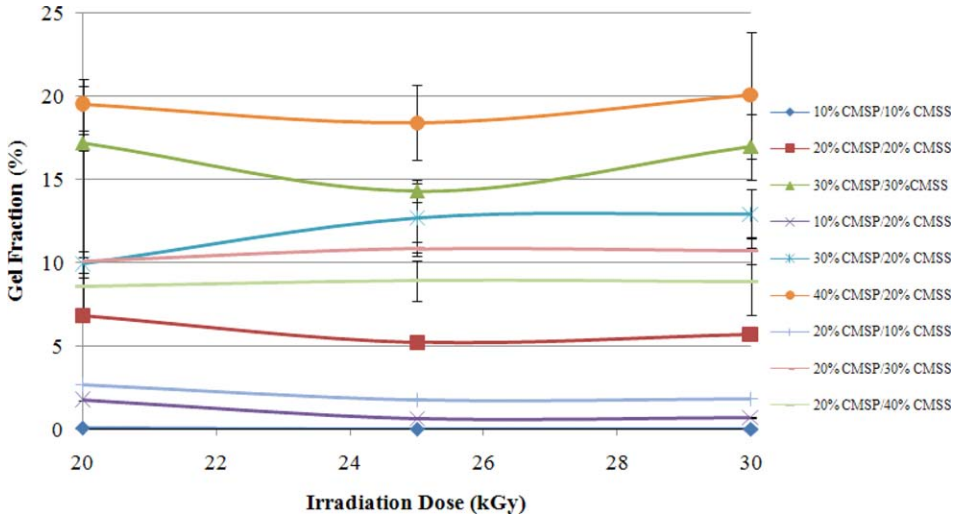

Figure 1. Graph of gel fraction (%) against irradiation dose (kGy) for CMSP/CMSS hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Weight, Diameter, and Thickness of CMSP/CMSS Hydrogel Discs

CMSP/CMSP hydrogel discs	Weight (g)	Diameter (mm)	Thickness (mm)
Unloaded 40%/20% hydrogel, irradiated at 20 kGy	0.03 ± 0.01	5.54 ± 0.12	0.96 ± 0.16
Unloaded40%/20% hydrogel, irradiated at 25 kGy	0.03 ± 0.00	5.55 ± 0.14	0.78 ± 0.08
Unloaded40%/20% hydrogel, irradiated at 30 kGy	0.02 ± 0.00	5.50 ± 0.08	0.72 ± 0.08
Ciprofloxacin hydrochloride loaded 40%/20% hydrogel, irradiated at 20 kGy	0.05 ± 0.01	5.68 ± 0.06	2.38 ± 0.08
Ciprofloxacin hydrochloride loaded 40%/20%hydrogel, irradiated at 25 kGy	0.05 ± 0.00	5.69 ± 0.01	2.23 ± 0.06
Ciprofloxacin hydrochloride loaded 40%/20%hydrogel, irradiated at 30 kGy	0.05 ± 0.00	5.70 ± 0.07	2.45 ± 0.09
Diclofenac sodium loaded 40%/20% hydrogel, irradiated at 20 kGy	0.05 ± 0.00	5.69 ± 0.09	1.52 ± 0.09
Diclofenac sodium loaded 40%/20%hydrogel, irradiated at 25 kGy	0.05 ± 0.00	5.73 ± 0.06	1.53 ± 0.05
Diclofenac sodium loaded 40%/20%hydrogel, irradiated at 30 kGy	0.05 ± 0.00	5.75 ± 0.06	1.59 ± 0.06

Determination of Drug Entrapment Efficiency

Approximately 0.05 g of CMSP/CMSS hydrogel that had been loaded with 20% of the drug was measured. The gels were placed into Scott bottles containing 100 mL of 0.1 *M* NaOH solution. All of the Scott bottles were shaken on an orbital shaker at 100 rpm overnight. The next day, the solutions from each sample were filtered and collected in labelled test tubes. The absorbance of each drug-loaded sample was measured at 278 nm (ciprofloxacin hydrochloride) or 276 nm (diclofenac sodium) using a UV-1800 spectrophotometer. The 0.1 *M* NaOH solution with 0.05 g of unloaded hydrogel was used as blank for loaded hydrogel analysis. Triplicates of samples were prepared. A calibration curve for ciprofloxacin hydrochloride and diclofenac sodium in 1 *M* NaOH was plotted ($R^2 = 0.9963$ and 0.9815, respectively). Drug entrapment efficiency (DEE) of each gel was calculated according to the equations below[11]:

$$\text{Drug entrapment efficiency (DEE)}(\%) = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100\% \quad (7)$$

$$\text{Theoretical drug loading}\ (\%) = \frac{\text{Weight of drug added (g)}}{\text{Weight of CMSP and CMSS} + \text{Weight of drug added (g)}} \times 100\% \quad (8)$$

Drug Release Studies

Drug release behavior of drug loaded CMSP/CMSS hydrogel was analyzed by using a UV-visible spectrophotometer. The ciprofloxacin hydrochloride loaded hydrogel was immersed in 100 mL buffer solution at pH 7.4 at 37 °C. At time intervals, 5 mL liquid sample was removed to analyze at 278 nm, while 5 mL fresh phosphate buffer solution was added replacing the removed buffer. These steps were repeated by using a hydrochloric acid buffer at pH 1.2 and phosphate buffer at pH 11. The same procedure was followed for diclofenac sodium loaded CMSP/CMSS hydrogels except for the 5 mL fresh sample removed was analyzed at 276 nm.[3] The calibration curves for ciprofloxacin hydrochloride and diclofenac sodium in media of pH 1.2 (R^2= 0.9977, 0.9968, respectively), pH 7.4 (R^2= 0.9970, 0.9968, respectively), and pH 11 (R^2= 0.9978, 0.9928, respectively) were plotted. All samples were prepared in triplicates.

Characterization of CMSP/CMSS Hydrogel

Scanning Electron Microscope. Discs from unloaded CMSP/CMSS hydrogels, drug-loaded CMSP/CMSS hydrogels, CMSP, CMSS, ciprofloxacin hydrochloride, and diclofenac sodium were selected and coated with gold by using Quorum Q 150A S. Hitachi *S-3400N* SEM (scanning electron microscope) was used to view surface morphology of all these samples.

Fourier Transform Infrared Spectroscopy. The unloaded and drug loaded samples of CMSP/CMSS hydrogel were ground. The fine powder obtained from each sample including drugs was used to study the functional groups present in each sample using Fourier Transform Infrared (FTIR) Spectrometer Varian 670.

Differential Scanning Calorimetry. PerkinElmer Differential Scanning Calorimetry (DSC) 4000 was used for DSC analysis. Each sample (approximately 10 mg) was weighed and kept in a 50 μL aluminium pan in a hermetically sealed condition and heated at a scan speed of 10 °C/min over a temperature range of 35–360 °C in a nitrogen atmosphere having a flow rate of 20 mL/min.

RESULTS AND DISCUSSION

Reaction Efficiency of Extraction of Sago Pulp from Sago Waste and Conversion of Sago Pulp into CMSP

For every 20 g of sago waste used, approximately 14 g of sago pulp was obtained in this experiment. Thus, the percentage of yield of sago pulp was 72.9 ± 6.8%. The extraction residues contain lignin, hemicelluloses, and starch.[24] Besides, the reaction efficiency of CMSP was 65.8 ± 7.5%. Some of the SMCA was reacted by an undesired side reaction with sodium hydroxide and lead to the formation of sodium glycolate.[10] Therefore, only 81.4 ± 4.1% of CMSP was produced from sago pulp.

Gel Fraction

The percentage of gel fraction is the percentage of insoluble part (cross-linked CMSP and CMSS) after immersion in distilled water. During EB irradiation, water that present in the homogenized mixtures increases the mobility of the CMSP and CMSS molecules and allows the diffusion of the macroradicals that minimize the distance between each radical and enable them to recombine efficiently. Moreover, the presence of water increased the concentration of radicals by forming hydrogen atoms and hydroxyl radicals through hydrolysis.[13] Based on the results, when the concentration of CMSP

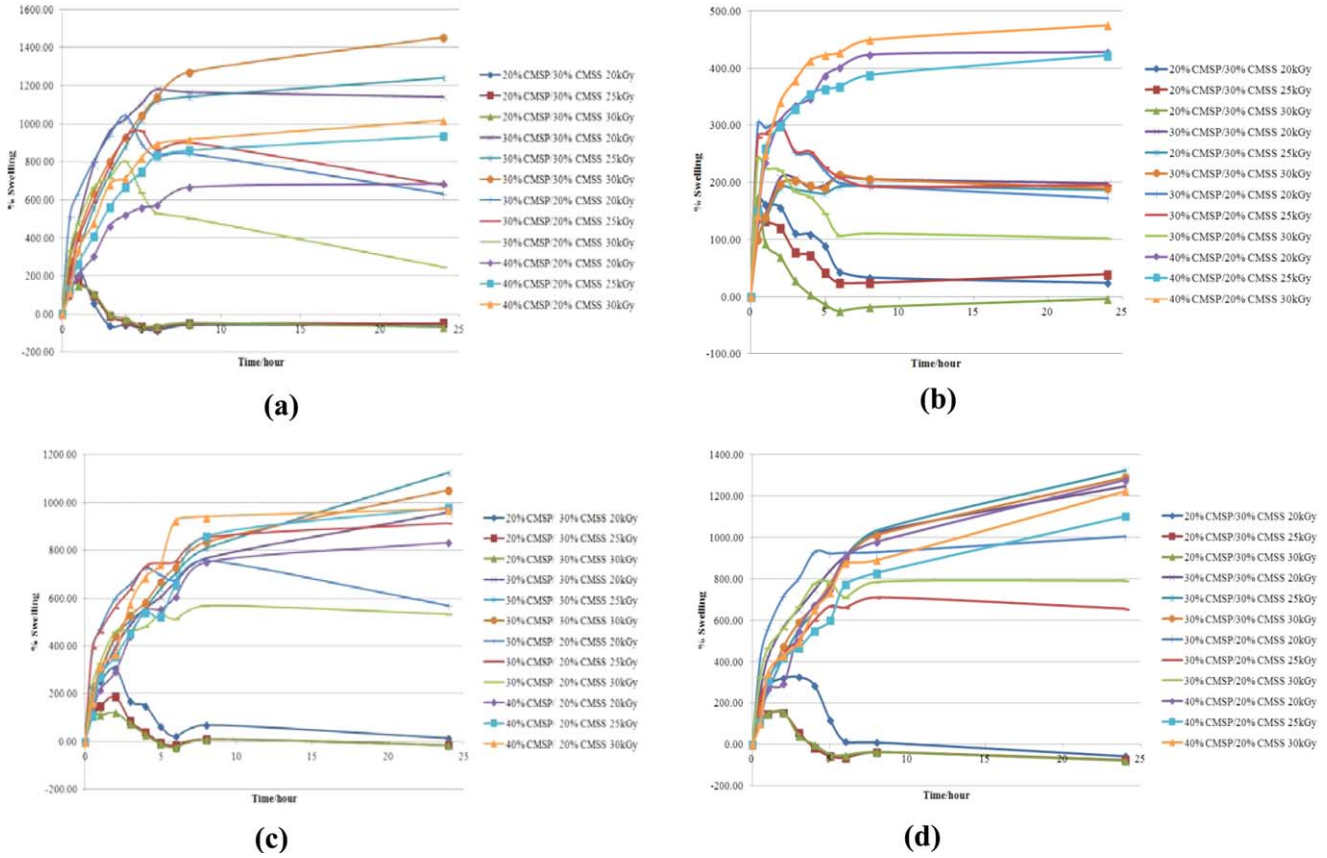

Figure 2. A plot of swelling over time for CMSP/CMSS hydrogels at (a) distilled water; (b) pH 1.2; (c) pH 7.4 and (d) pH 11. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increased, the gel fraction increased. Besides that, when the concentration of CMSS increased, the gel fraction was also increased, but not as significant as CMSP. This can be seen in the difference of the gel fraction of 10%/20%, 20%/10%, 20%/40% and 40%/20% of CMSP/CMSS hydrogels (Figure 1). This is because the distance between the macroradicals that formed during irradiation is far for the formation of the cross-linking bond. Thus, this causes degradation to happen predominantly.[25] Besides, irradiation on a low concentration of polymers will produce insufficient macroradicals and unable to recombine on different chains.[26] The CMSP and CMSS mixtures with higher concentration had higher gel fraction because more free radicals on the side chains of the polymers. The radicals on CMSP and CMSS chains can combine and form the intermolecular cross-linking between the chains, forming an insoluble gel. Hence, an increase in the concentration of polymer can enhance the number of the cross-linking leads to higher gel fraction.

In this experiment, 40%/20% CMSP/CMSS had the highest gel fraction and followed by 30%/30% hydrogel. At a concentration of 30%/20%, 20%/30%, and 20%/40%, the mixture solutions were less viscous, and they gave lower gel content. These 40%/20%, 30%/30%, 30%/20%, and 20%/30% hydrogels were chosen to proceed to the next characterization of swelling capacity test because of their higher gel content which is more suitable for drug-loading.

Physical Characteristic of CMSP/CMSS Hydrogel Discs Results

The diameter of unloaded and drug-loaded CMSP/CMSS hydrogel discs was 5.65 ± 0.12 mm and found to be uniform discus-shaped. The thicknesses of the unloaded, ciprofloxacin hydrochloride are loaded and diclofenac sodium loaded CMSP/CMSS hydrogel discs were 0.82 ± 0.15 mm, 2.36 ± 0.12 mm, and 1.55 ± 0.07 mm, respectively. The small standard deviation of the values showed uniformity in thickness. The unloaded hydrogel discs were thinner and more brittle than the drug-loaded discs because the relatively higher cross-linking in the unloaded hydrogel discs. The weights of the unloaded, ciprofloxacin and diclofenac sodium loaded CMSP/CMSS discs were uniform and the ranges were 0.03 ± 0.01, 0.05 ± 0.00, and 0.05 ± 0.00 g, respectively (Table II).[14]

Swelling Behavior in Various pH Media

The studies of swelling behaviors were done in distilled water, buffer solution at pH 1.2, 7.4, and 11. According to Figure 2, 30%/20% and 20%/30% CMSP/CMSS hydrogels had a lower swelling percentage because they were made up of lower concentration of polymers and had an insufficient amount of cross-linked intermolecular bonds to hold the matrix of hydrogels. These weak intermolecular bonds within the hydrogel allowed a higher amount of water to enter the matrix as the period of time increased. The decrease in the swelling percentage of hydrogel indicated its degradation. However, 40%/20% and

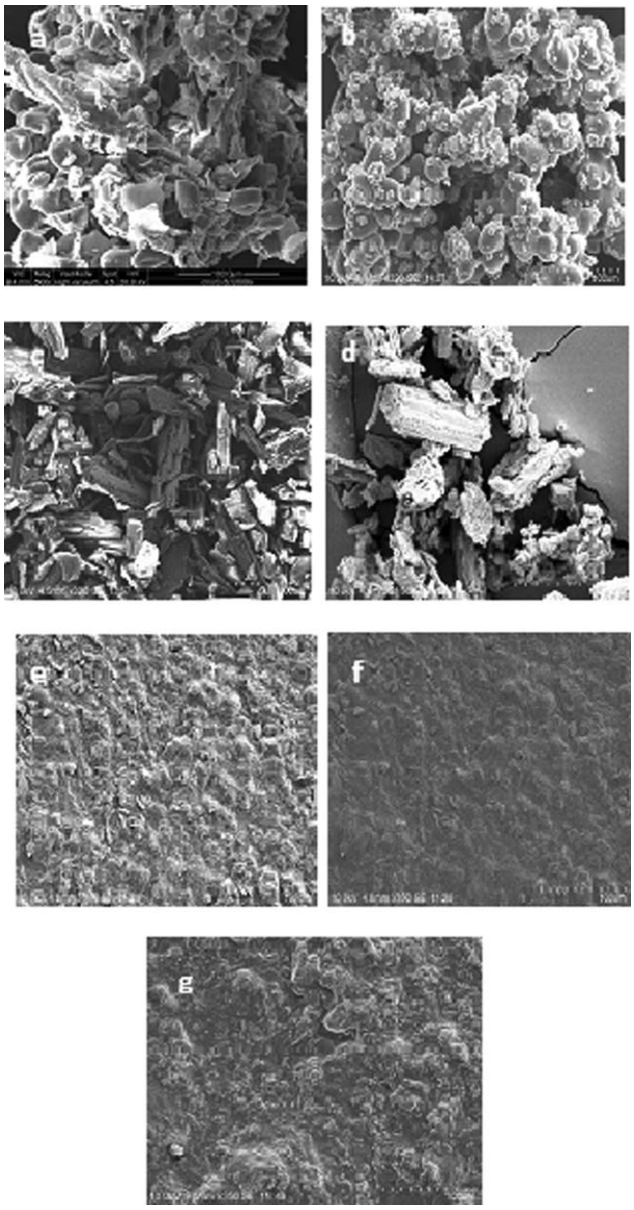

Figure 3. SEM images of (a) CMSP; (b) CMSS; (c) Ciprofloxacin hydrochloride; (d) Diclofenac sodium; (e) 40%/20% CMSP unloaded hydrogel, 25 kGy; (f) Ciprofloxacin hydrochloride loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 25 kGy; (g) Diclofenac sodium loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 25 kGy.

30%/30% CMSP/CMSS hydrogels had higher swelling capacity. This is caused by higher polymers concentration resulted in the greater number of carboxylate groups and cross-linked bonds. Therefore, greater electrostatic repulsion between the adjacent ionized carboxylate groups, resulting in swelling and cross-link leads to a stronger network that holds the water inside space in the matrix. Their swelling percentage can increase up to 24 hours without undergoing any degradation.[13]

Based on the overall results, CMSP/CMSS hydrogel swelled maximum in distilled water, followed by pH 11, pH 7.4, and pH 1.2. CMSP/CMSP hydrogel exhibited excellent swelling capacity in distilled water compared to swelling in buffer solution because it is hydrophilic and sensitive to the presence of salt in the buffer solution.[27] At the same time, these hydrogels are sensitive to pH. Carboxymethyl groups within the gel maintain in equilibrium between the neutral and ionized form. The introduction of an electrolyte disturbs that equilibrium and causes establishment of another one. The dissociation degree is shifted to the left or right side of eq. (9) based on increased pH value of the solution[28]:

$$\begin{gathered}[\mathrm{pH} > 4.3] \\ \mathrm{RCH_2{-}COOH} \rightleftharpoons \mathrm{RCH_2{-}COO^-} + \mathrm{H^+} \\ [\mathrm{pH} < 4.3]\end{gathered} \tag{9}$$

The swelling percentage of the hydrogel in different ratios was the lowest at pH 1.2 because carboxylic groups (-COOH) of hydrogel maintained at protonated state. The protonated state exhibited insignificant electrostatic repulsive force in an acidic environment, hindering it from swelling.[29] In contrast, at

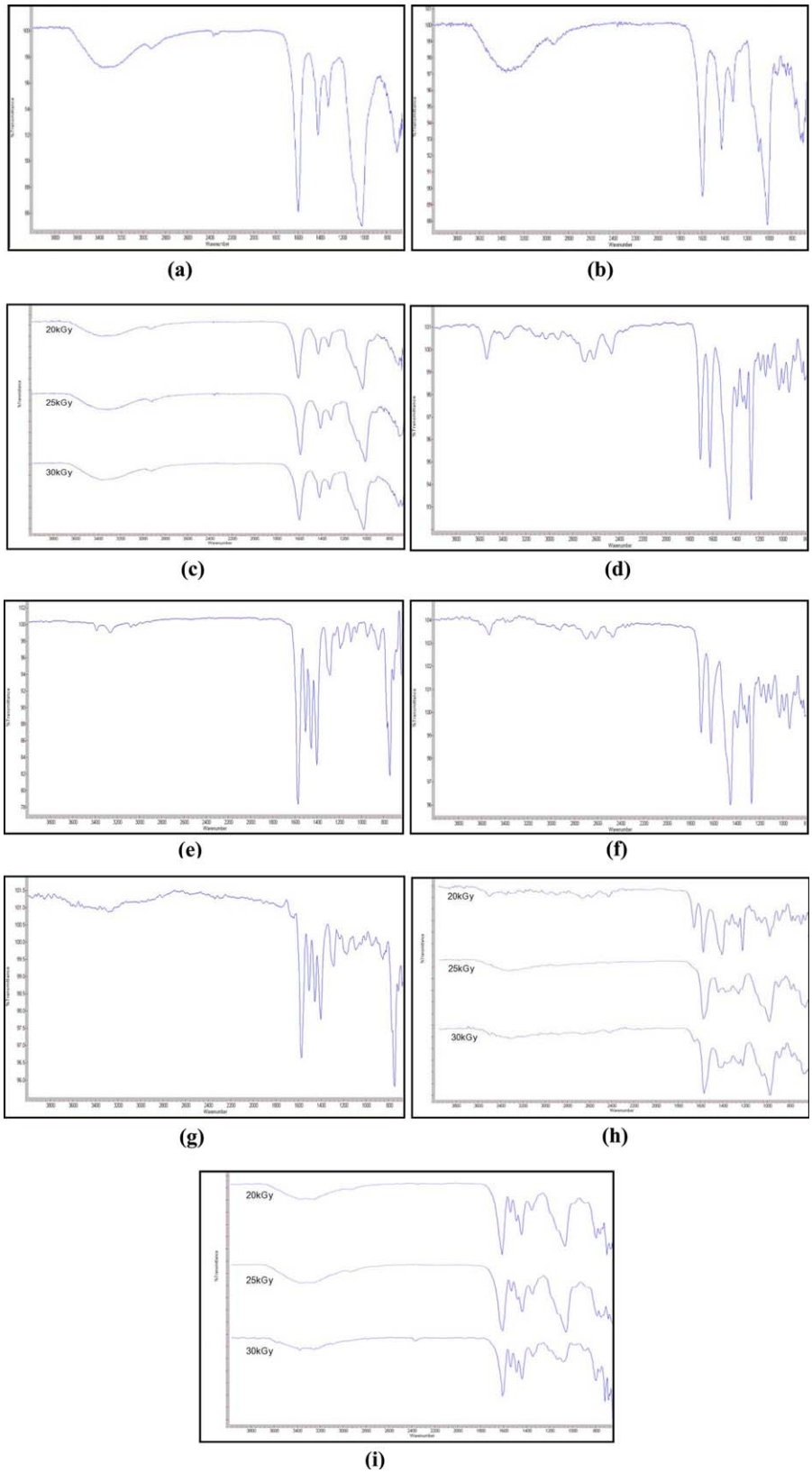

Figure 4. FTIR spectra of (a) CMSP; (b) CMSS; (c) unloaded 40%/20% CMSP/CMSS hydrogel, irradiated at 20, 25, and 30 kGy; (d) Ciprofloxacin hydrochloride; (e) Diclofenac sodium; (f) Ciprofloxacin hydrochloride, irradiated at 25 kGy; (g) Diclofenac sodium, irradiated at 25 kGy; (h) Ciprofloxacin hydrochloride loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 20 kGy, 25 kGy and 30 kGy; (i) Diclofenac sodium loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 20, 25, and 30 kGy. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

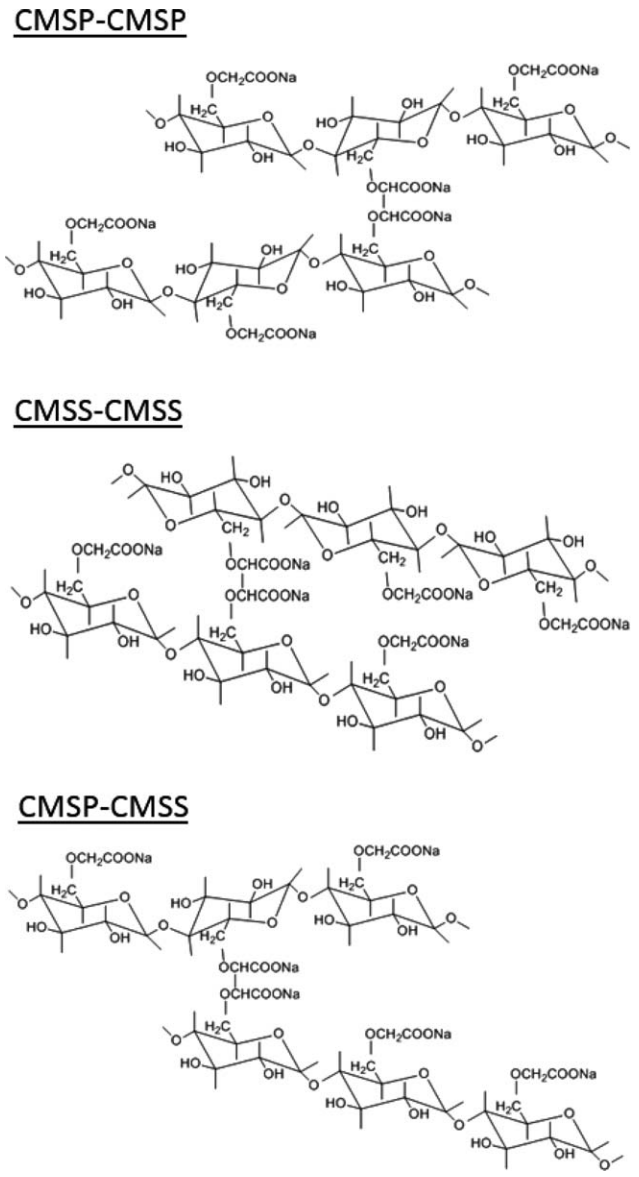

Figure 5. Drawing shows possible cross-linking formed on polymer chains.

neutral pH, the ionized form occurred as pKa of carboxylate groups in CMSP and CMSS are 4.3 and 4.2, respectively.[30,31] At pH above pKa value, an electrostatic repulsion determines the interaction between macromolecules. As there is increase in the degree of ionization, there is increase in the electrostatic repulsion between the adjacent ionized groups, resulting in swelling.[32]

Among all of these four hydrogels with different ratios, 40%/20% hydrogel was chosen for the drug entrapment and release studies. Although 30%/30% CMSP/CMSS hydrogel had the highest swelling percentage in distilled water, pH 7.4 and pH 11, it was not used to proceed to prevent the burst release of the drug because of its high swelling capacity.[33] The 40%/20% hydrogel had an optimum swelling behavior to be used for the controlled release of drug in the colon.

SEM Studies

The morphology of CMSP [Figure 3(a)] is dominated by the presence of smooth torus-like particles within the fibrous network. The shape of CMSS [Figure 3(b)] is round, and the surface is smooth. Ciprofloxacin hydrochloride [Figure 3(c)] and diclofenac sodium [Figure 3(d)] are seen to be in crystal form and they have a plate-like shape. The surface morphology of the unloaded hydrogel is rough and uneven.[3] This could be because of more cross-links within the hydrogel with higher concentration, causing the structures to become wavy and irregular as shown in Figure 3(e). The hydrogel loaded with ciprofloxacin hydrochloride and diclofenac sodium (Figure f and g) have smoother surface compared to the unloaded hydrogel. This could be because of the drug particles that occupy the space between the cross-link within the hydrogel matrix and causes it to have a smoother surface. The successful encapsulation of ciprofloxacin hydrochloride and diclofenac sodium was evidenced based on the appearance of drug particles that are shown to embed within the matrix of cross-linked polymers.

FTIR Spectroscopy Analysis

Figure 4(a) represents the FTIR spectrum of CMSP. The strong absorption band at 1600 cm^{-1} confirms the presence of COO^- group, proofing that the sago pulp has undergone carboxymethylation. The IR spectrum of CMSS is shown in Figure 4(b) is quite similar, except the absorption band at 1020–1100 cm^{-1} for CMSP that represents β-1,4-glucosidic bonds and absorption band at 1010 cm^{-1} for CMSS that represents α-1,4-glycosidic bonds.[13,18] The FTIR spectrum of 40%/20% unloaded hydrogels are shown in Figure 4(c). The absorption peak at 1020–1100 cm^{-1} from CMSP and 1010 cm^{-1} from CMSS are overlapping so the hydrogel has a broader peak at 1010–1100 cm^{-1}, representing the combination of two peaks. The unloaded hydrogels contain the functional groups that present in CMSP and CMSS, indicating that it retains similar properties as CMSP and CMSS.

Figure 4(d,e) shows the IR spectrum of ciprofloxacin hydrochloride and diclofenac sodium respectively. The peaks of ciprofloxacin hydrochloride and diclofenac sodium are well in agreement with those reported in literature.[16,34–37] Based on Figure 4(f,g), there is a slight drop in intensity of absorption bands of irradiated drugs, showing the small change of drug from the crystalline form into more amorphous form.[38] However, the functional groups present in both irradiated ciprofloxacin hydrochloride and diclofenac sodium remained the same before and after irradiation. This indicates that the structure and functional groups of the drug were not affected because of irradiation. Based on Figure 4(h,i), the drug-loaded hydrogels have all the functional groups present in unloaded hydrogels and drug showing that the drug is entrapped inside the hydrogel, and there is no interaction between the drug and the hydrogel.

Figure 5 shows the cross-linking that could be formed in the polymer chains of CMSP and CMSS. In order to form the radicals at the side chain (R-O-CH-COO^-), glycosidic bonds of the cellulose and its derivatives have to be ruptured. In the FTIR spectrum [Figure 4(a–c)], can be observed that the intensity of the absorption bands at 1422 and 1326 cm^{-1} decreases upon

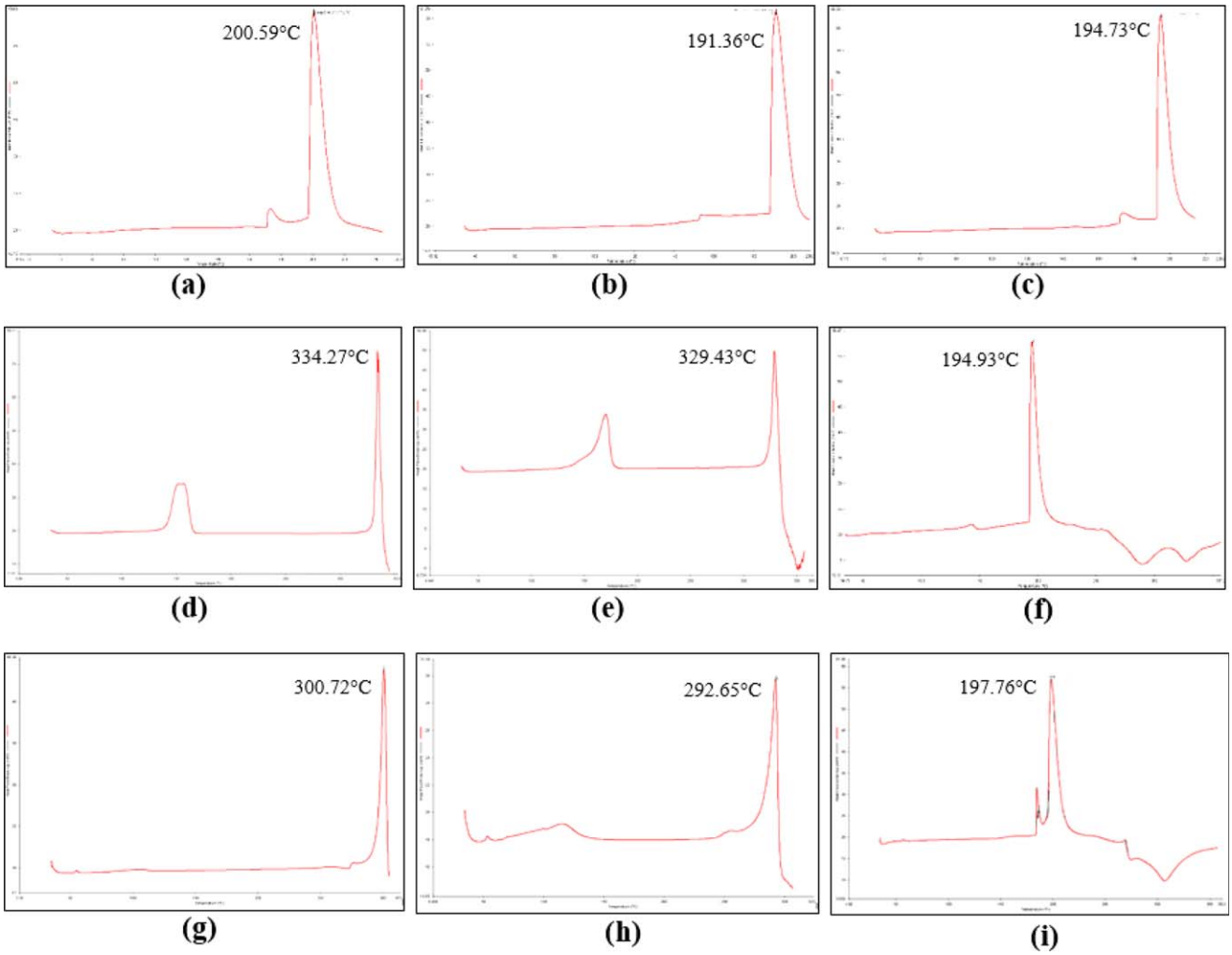

Figure 6. DSC thermograms of (a) CMSP; (b) CMSS; (c) 40%/20% CMSP unloaded hydrogel, irradiated at 25 kGy; (d) ciprofloxacin hydrochloride; (e) irradiated ciprofloxacin hydrochloride; (f) ciprofloxacin hydrochloride loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 25 kGy; (g) diclofenac sodium; (h) irradiated diclofenac sodium; (i) diclofenac sodium loaded 40%/20% CMSP/CMSS hydrogel, irradiated at 25 kGy. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increase in cross-linking. This observation indicates that more –OH and $–CH_2$ groups are involved in cross-linking reaction because of the formation of radical –OH and $–CH_2$ groups at the side chain of CMSP and CMSS molecules. Besides cross-linking, chain scission reaction will also occur. If the radicals formed in >CH-O-CH< of main chain (glucose ring), chain scission of CMSP and CMSS molecular chains will happen. This will lead to the decrease in intensity bands at 1010–1100 cm^{-1}. The reduction in the intensity at approximately 1600 cm^{-1} also shows the increase of cross-linking because the COO^- is involved in the side chain and lead to the formation of radicals. Figure 4(c,h,i) show that the intensity of these peaks is decreasing when the irradiation doses are increasing in all unloaded hydrogels, ciprofloxacin hydrochloride loaded hydrogel and diclofenac sodium loaded hydrogel, respectively. This might be because of the different amount of cross-linking in the hydrogel. As irradiation increases, the cross-link density will increase because higher energy EB results in formation of more free radicals which can lead to more cross-linking to occur.[39,40]

DSC Analysis

The melting point of CMSP and CMSS are close to each other, which are 200.59 °C and 191.36 °C respectively (Figure 6). There is only presence of one sharp peak in all unloaded hydrogel, where the melting point of unloaded CMSP/CMSS hydrogels is around 195 °C, the temperature in between the melting point of CMSP and CMSS. The presence of one peak indicates the presence of cross-linked CMSP/CMSS polymer, and it shows that all the CMSP and CMSS cross-linked and did not present as an individual polymer but as a cross-linked polymer. Pure and irradiated ciprofloxacin hydrochloride have a melting point at about 330 °C. Besides, pure and irradiated diclofenac sodium have a melting point at about 300 °C. The similar melting points between the pure drugs and irradiated drugs show that the EB irradiation did not cause any drastic change in the structure of the drug. The thermograms of drug-loaded samples show one sharp peak at around 195 °C, indicating the melting point of the hydrogel, and a small peak was observed in the thermograms of drug-

Table III. Drug Entrapment Efficiency (DEE) of Ciprofloxacin Hydrochloride and Diclofenac Sodium Loaded CMSP/CMSS Hydrogel

Drug-loaded CMSP/CMSS hydrogel	Mean of ciprofloxacin hydrochlorideentrapment efficiency (%)	Mean of diclofenac sodium entrapment efficiency (%)
40%/20%, 20 kGy	66.80 ± 2.94	63.51 ± 0.82
40%/20%, 25 kGy	64.73 ± 7.50	68.17 ± 0.47
40%/20%, 30 kGy	64.06 ± 4.97	68.93 ± 4.82

loaded hydrogels, showing the melting point of the respective model drug. This reveals the combination of hydrogel and the drugs without any shifting, additional and disappearing of the peak, proving that the thermostability of the hydrogel was not affected by irradiation and loading with the drug. It also shows that there is no drug–polymer interaction when ciprofloxacin hydrochloride and diclofenac sodium are incorporated into the polymer matrix.[41]

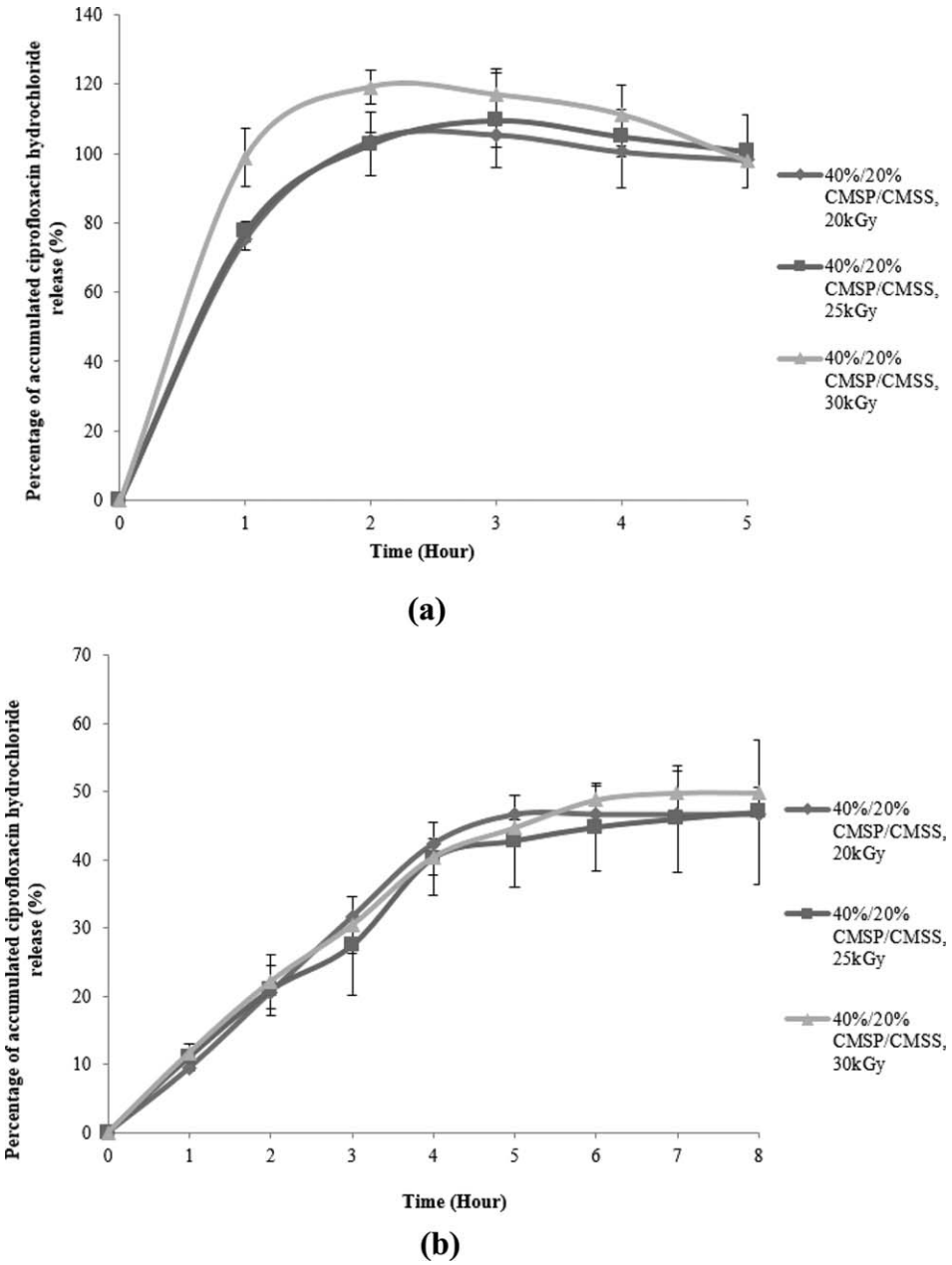

Figure 7. Graph of percentage of accumulated ciprofloxacin hydrochloride release (%) against time (hour) for 40%/20% CMSP/CMSS hydrogel loaded with diclofenac sodium at (a) pH 1.2 and (b) pH 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

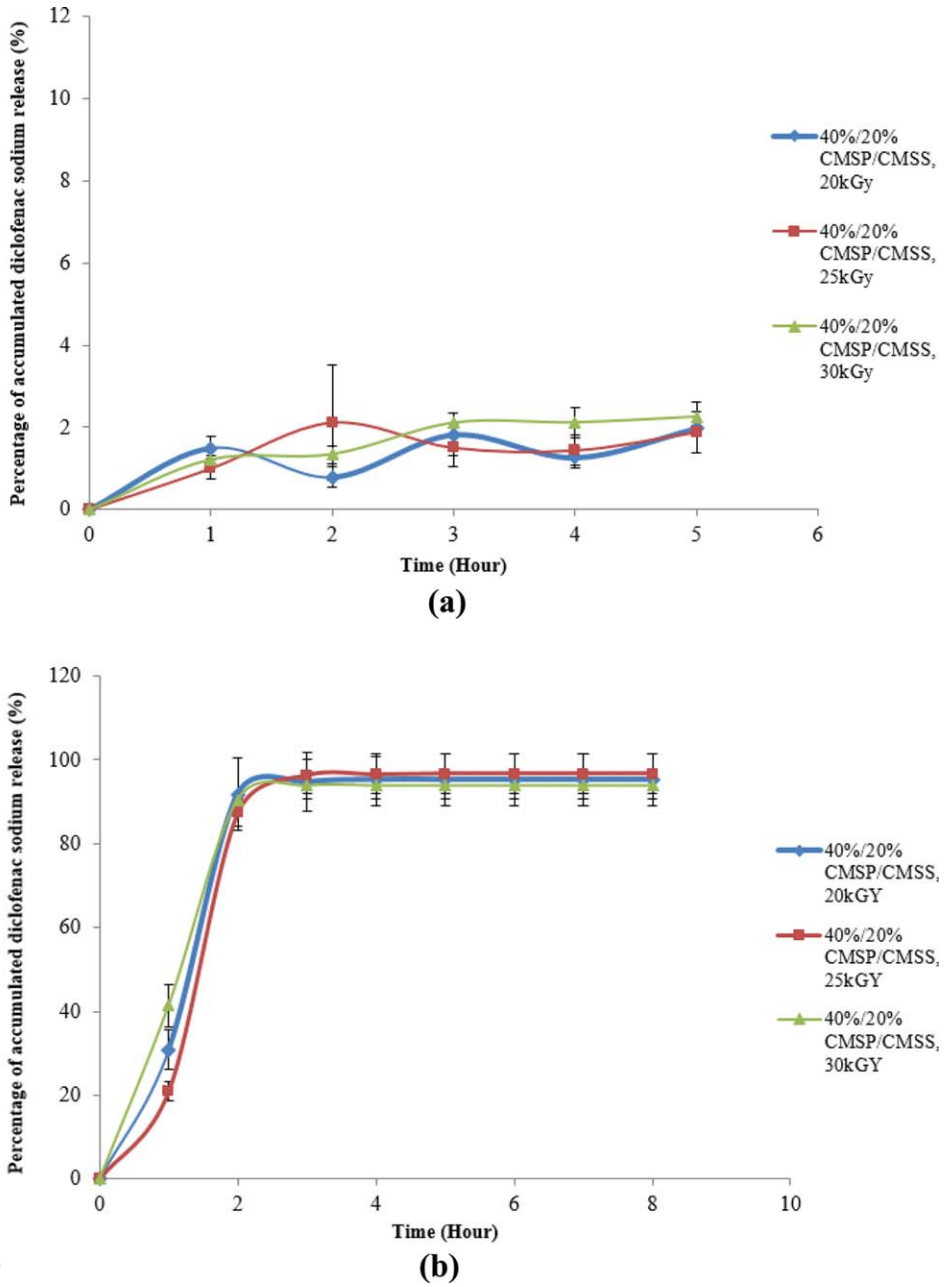

Figure 8. Graph of percentage of accumulated diclofenac sodium release (%) against time (hour) for 40%/20% CMSP/CMSS hydrogel loaded with diclofenac sodium at (a) pH 1.2 and (b) pH 7.4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Drug Entrapment Efficiency

DEE is determined to study how efficiently the drugs ciprofloxacin hydrochloride and diclofenac sodium are entrapped in the CMSP/CMSS hydrogel discs.

In this study, *in situ* loading was used because the polymer precursor solution, CMSP/CMSS mixture was mixed with the drug for the formation of hydrogel network and drug encapsulation to occur simultaneously. The efficiency of drug encapsulation was determined by allowing degradation of labile covalent bonds through the breakdown of the hydrogel in NaOH solution.[42] *In situ* loading method is chosen because it is expected to give a better encapsulation of drug and allows higher drug content within network of the hydrogel compared to postloading method.[43]

In situ drug loading allows drug encapsulation efficiency up to 82.65%.[44] However, in this study, DEE of ciprofloxacin hydrochloride and diclofenac sodium was only about 63–69% (Table III). During EB irradiation, some of the drugs might be degraded and caused the DEE to be less than 100%.[24] Another possible reason of low DEE is the insufficient level of cross-link density of hydrogel. High cross-link density is important to form more pores and space available for drug entrapment.[42] Besides, high cross-link density allows the formation of the matrix which is more rigid and less loose, allows a better

entrapment of drug.[45] In this study, the drug might be loosely trapped in the hydrogel and caused it to be lost quickly, resulting in the decrease in DEE.

Drug Release Studies

Figure 7(a) shows the release profile of ciprofloxacin hydrochloride from 40%/20% CMSP/CMSS hydrogel at pH 1.2. Although the swelling degree of the hydrogel is minimum at this pH, there was a sharp increase in the drug release since the beginning of time and the drugs are completely released within 2 hours. This is because of the high solubility of ciprofloxacin hydrochloride in acidic pH and all drugs diffused out of the hydrogel as the released drug.[46]

Figure 7(b) shows the release profile of ciprofloxacin hydrochloride from 40%/20% CMSP/CMSS hydrogel at pH 7.4. There was a gradual increase in the drug release since the beginning of time until 6th hour. The drug release attained maximum level of drug release after 6th hours. However, the drug-loaded discs were only able to release about 50% of the drug within the hydrogel. It might be because of the low aqueous solubility of ciprofloxacin hydrochloride at physiological pH, which is pH 7.4. This is because of its overall neutral charge as a zwitterion at pH 7.4, and the presence of its dual aromatic rings. Thus, the entrapped ciprofloxacin hydrochloride is hardly dissolved, resulting in white and crystalline precipitates.[46] The only part of the drug was able to diffuse out of the hydrogel as the released drug.

Diclofenac sodium is a salt of the weak acid. Thus, its solubility depends on the ionization constant, pKa and the pH of the dissolution medium.[47] It also undergoes an intramolecular cyclization in acidic conditions which is found in gastric juices and lead to its inactivation. Because of the intramolecular cyclization, Na^+ is lost and causes the solubility of the drug to decrease.[48] When the surrounding media with pH below pKa (4.0), the active ingredient is mostly in its free acid form, which is less soluble than the salt. Hence, it is insoluble in the hydrochloric acid buffer at pH 1.2. The low solubility of drug prevents it to dissolve and diffuse out from the hydrogel and enter the release medium. Based on Figure 8(a), very little amount of drug was released in pH 1.2 buffers. This is because of the minimum swelling capacity of the hydrogel at pH 1.2. Therefore, when the hydrogel passes through the stomach, it will minimize the release of drug and prevent the adverse effect in the stomach from occurring.

The percentage of release of diclofenac sodium at pH 7.4 is shown in Figure 8(b). At pH 7.4, the amount of drug released was little which was 20–40% during the first hour and increased drastically to approximately 95% for the second hours. This result is corresponding to the swelling studies of CMSP/CMSS hydrogel which indicated that the hydrogel had higher swelling capacity at pH 7.4 and allowed the drug to be released. After the 2nd hour, the percentage of drug release remained constant throughout the next few hours. This suggested that the total drug released in 2 hours.

CONCLUSIONS

Subjecting CMSP and CMSS solution under EB irradiation resulted in cross-linking that forms a clean hydrogel. High concentration of CMSP causes higher gel fraction of the hydrogel. Higher CMSS concentration also increases the cross-link density, but it is not as significant as CMSP. The 40%/20% CMSP/CMSS hydrogel has the highest gel fraction of 20%, and this hydrogel could absorb 930% of water/g of the hydrogel. CMSP/CMSS hydrogel is sensitive to pH and swells better at pH 7.4 and 11. The smooth surface morphology of drug-loaded hydrogel prepared from *in situ* loading of drug and cross-linking using EB irradiation allows the drug to be embedded within the networks, showing that this is a useful method to entrap the drug within the hydrogel. FTIR reveals that all of the functional groups present in hydrogel and drugs are maintained in the drug-loaded hydrogel, indicating that their structures are not affected by irradiation, and there is no interaction between the polymers and drugs. DSC results show that the thermo-stability of hydrogel and drugs are maintained as well. The 40%/20% CMSP/CMSS hydrogel with three different irradiation dosages have DEE between 63% and 69%. For ocular drug delivery, high swelling of CMSP/CMSS hydrogel at pH 7.4 is advantageous as it allows the slow and steady release of ciprofloxacin hydrochloride which can last for 6 hours. For colon drug delivery, the percentage of release of diclofenac sodium in the hydrogel is less than 3% at pH 1.2 and more than 90% at pH 7.4 and pH 11. CMSP/CMSS hydrogel allows the release of drug which can sustain up to 2 hours at pH 7.4. The 40%/20% CMSP/CMSS hydrogel has a potential to be used as a drug delivery vehicle at the ocular and colonic regions.

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