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Dual crosslinked carboxymethyl sago pulp/pectin hydrogel beads as potential carrier for colon-targeted drug delivery

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ABSTRACT: Carboxymethyl sago pulp (CMSP)/pectin hydrogel beads were synthesized by calcium crosslinking and further crosslinked by electron beam irradiation to form drug carrier for colon-targeted drug. Sphere-shaped CMSP/pectin 15%/5% hydrogel beads is able to stay intact for 24 h in swelling medium at pH 7.4. It shows pH-sensitive behavior as the swelling degree increases as pH increases. Fourier transform infrared spectroscopy analysis confirmed the absence of chemical interaction between hydrogel beads and diclofenac sodium. Differential scanning calorimetric and X-ray diffraction studies indicate the amorphous nature of entrapped diclofenac sodium. The drug encapsulation efficiency is up to about 50%. Less than 9% of drug has been released at pH 1.2 and the hydrogel beads sustain the drug release at pH 7.4 over 30 h. This shows the potential of CMSP/pectin hydrogel beads as carrier for colon-targeted drug. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43416.

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INTRODUCTION

A hydrogel is a three-dimensional (3-D) polymeric material which has the ability to absorb more than 20% of its weight of water, swells, and holds the significant amount of water within the structure but remains insoluble in water.[1] Recently, physical crosslinking methods for the synthesis of hydrogel gain higher popularity as they do not require any chemicals agents in the process. One of the common examples is ionic interaction by ionotropic gelation method. Ionotropic gelation method is the crosslinking method which depends on the ability of polyelectrolytes to form crosslinks in the presence of counter ions to form hydrogel beads.[2] Besides, high energy irradiation such as gamma irradiation and electron beam (EB) irradiation has also been used for crosslinking of hydrogel because the process does not involve any toxic additives or catalysts initiate the process.[3,4] EB irradiation has been chosen for crosslinking in this study as it was shown to provide better mechanical properties and less detrimental effects on the product.[4,5] The synthesis of hydrogel using EB irradiation has been demonstrated in some past studies.[6–10]

The unique characteristic of hydrogel that makes it a suitable candidate in drug delivery is their ability to form a "smart" material which can be designed with adjustable responses, to shrink or expand according to the changes in external environmental conditions. This leads to the application of hydrogel in colon-targeted drug delivery. Colon-targeted drug delivery plays vital role in the drug delivery for treatment of local colonic diseases and also systemic diseases such as Crohn's disease, ulcerative colitis, colorectal cancer, and amoebiasis. It offers some advantages because at the region of colon, there is less hostile environment with less diversity as well as intensity of activity compared to stomach and small intestine. In addition, it has longer retention time which is up to 5 days, and it is highly responsive to absorption enhancers, the agents which are responsible to improve the absorption of poorly absorbed drugs.[11] One of the common primary approaches of colon-specific drug delivery (CDDS) is pH-dependent approach that uses the gradient of pH in the gastrointestinal tract. There is a progressive increase in pH from the stomach (pH 1.3–3.5) and small intestine (pH 5.5–6.8) to the colon (pH 6.4–7.0).[12] This range of pH acts as an environmental stimulus for drug release and pH-sensitive polymers are used as the device for drug release because their swelling and deswelling are dependent on the change in pH. For instance, anionic polymers will not swell

in low pH due to the protonation of acidic groups and they will be swollen at high pH due to deprotonation of acidic group.[13]

In Malaysia, about 90% of the sago starch is produced in Sarawak. The development in sago starch research causes the increase in cultivation of sago palm which is up to 60,000 h of plantation. The daily production of sago starch is approximately 24 tons or equivalent to 20 kg of starch/log.[14] The large production of sago starch leads to large production of sago waste, the fibrous residue that left behind after majority of starch has been washed out from sago palm pith.[15] This by-product is considered as one of the least expensive, biodegradable, and most easily obtained renewable natural polymers in Malaysia. The natural polymer of cellulose has been extracted from sago waste through sago pulp extraction and the synthesis of carboxymethyl sago pulp (CMSP) has been demonstrated for controlled drug delivery.[16] The potential of CMSP in controlled drug delivery have been studied. The pH-sensitive CMSP hydrogel prepared from sago waste has been demonstrated for controlled drug delivery.[17,18] The presence of anionic carboxyl groups in the polymer allowed minimum drug release at stomach pH and sustained drug release has been achieved at colonic pH.[17] Besides CMSP, pectin has also obtained high attention for controlled drug delivery due to its nontoxicity, low cost of production as well as its high availability.[19] It is also useful in pH–dependent approach for colon-targeted drug delivery due to its pH-dependent ionization of the carboxyl groups.[20] The studies about synthesis of pectin-based formulations are reported.[21–23]

The model drug, diclofenac sodium has a short half-life, which is about 1–2 h and it needs multiple dosing. Normally, 100–200 mg of drug in separate doses will be administered twice to thrice a day to treat chronic pain caused by arthritis. This actually causes the levels of drug in blood to fluctuate and leads to dose-associated side effects.[24] Based on its short half-life and its side effects like ulceration, bleeding, or perforations of the intestinal wall, controlled release dosage form of diclofenac sodium is needed.[25] Hence, it is expected that this drug can be benefited from this proposed drug delivery system since it is particularly well absorbed in the colon. In addition, diclofenac sodium also helps in colon cancer prevention due to enhanced colon cell apoptosis.[26] Hence, diclofenac sodium is a suitable candidate as the model drug in this drug delivery system.

In this investigation, diclofenac sodium-loaded CMSP/pectin hydrogel beads were formulated by ionotropic gelation using calcium chloride and further crosslinked with EB irradiation to minimize the drug release in acidic stomach pH and allows sustained drug release in the colonic pH. CMSP/pectin hydrogel beads were characterised for its swelling behavior, beads sizes, drug entrapment efficiency (DEE), and *in vitro* release studies.

EXPERIMENTAL

Materials

Sago waste was obtained from Ng Kia Heng Kilang Sagu Industries, Batu Pahat, Johor. Pectin (degree of esterification 63%–66%) was obtained from R&M chemicals (United Kingdom). Methanol, isopropanol and ethanol (95%) were obtained from HmbG Chemicals (Germany), R&M chemicals (United Kingdom), and John Kollin Corporation (United Kingdom), respectively. Sodium chlorite (80% technical grade) and sodium monochloroacetate were from Fluka (Italy) and Fluka (United States), respectively. Anhydrous calcium chloride, sodium hydroxide pellets, glacial acetic acid, potassium dihydrogen phosphate, and potassium chloride were obtained from R&M chemicals (United Kingdom). Hydrochloric acid (37%) was obtained from Merck KGaA (Germany) while disodium hydrogen phosphate was obtained from HmbG Chemicals (Germany). Distilled water was used throughout the study. Diclofenac sodium was obtained from Ningbo Hi-Tech Biochemicals Co. (China).

Isolation of Sago Pulp from Sago Waste

Sago pulp was isolated from sago waste according to method by Pushpamalar and co-researchers.[16] The ground sago waste was dried in oven at 60 °C for 1 h. Twenty grams of sago waste was weighed and added into 500 mL Erlenmeyer flask. The sago waste was suspended in 640 mL of hot distilled water and 4 mL of glacial acetic acid was added. Then, 6 g of technical grade sodium chlorite was added. The conical flask was stoppered and incubated in shaking water bath at 70 °C for 3 h. The mixture was filtered through cheesecloth sieve and washed using cold distilled water until pH of filtrate is 7 and the residue was dried in oven to constant weight.

Preparation of CMSP of DS 0.8

The dried sago pulp was ground and sieved using 0.5 mm^2 test sieve. Five grams of the ground sago pulp was weighed and added to 250 mL Scott bottle containing 100 mL of isopropanol. Ten milliliters of 25% w/v sodium hydroxide was added in dropwise fashion while swirling to mix the content. The mixture was stirred for 1 h and followed by addition of 6 g of sodium monochloroacetate. The mixture was incubated in shaking water bath at 45 °C for 3 h. This mixture was filtered the residue was suspended in 300 mL of methanol overnight. The suspended methanol was neutralized by glacial acetic acid until pH 7 then the residue was washed with 300 mL ethanol, filtered and dried in oven at 60 °C overnight.[16]

Preparation of CMSP/Pectin Unloaded and Drug-Loaded Hydrogel Beads Using Ionotropic Gelation and EB Irradiation

The different ratios of CMSP/pectin mixtures were prepared by adding different concentration of CMSP and pectin as shown in Table I. The mixtures were stirred at 600 rpm for 30 min. The mixture was extruded in droplets into aqueous solution of calcium chloride (10% w/v) under constant stirring by magnetic stirrer through needle of 10 mL hypodermic syringe (21G). The distance between the needle tip and the top of calcium chloride solution was set at 6 cm. The mixtures of polymer were introduced into the crosslinking solution for 2 min and the beads formed instantaneously and it was allowed to cure for 20 min. The beads were separated through filtration, washed with distilled water. For further crosslinking, the beads were irradiated with electron beam irradiation (5 kGy, 10 kGy, 15 kGy, 20 kGy, 25 kGy) by EPS-3000. After EB irradiation, the beads were dried at 37 °C in the oven for about 24 h until constant weight.

Table I. Preparation of CMSP/Pectin Hydrogel Bead with Various Percentage of CMSP and Pectin

Formulation	CMSP DS 0.8 (% w/v)	Pectin DE 63-66% (% w/v)	$CaCl_2$ (% w/v)
1	5	5	10
2	7.5	7.5	10
3	10	10	10
4	5	7.5	10
5	5	10	10
6	7.5	5	10
7	7.5	10	10
8	10	5	10
9	10	7.5	10
10	15	5	10
11	10	2.5	10
12	12.5	2.5	10

For the preparation of diclofenac sodium-loaded hydrogel beads, 10% (w/w) of diclofenac sodium based on total dry weight of polymers (CMSP and pectin) was dissolved in distilled water. The drug solution was used to dissolve CMSP and pectin for the preparation of mixture of CMSP and pectin. This was followed by homogenization of the polymer and drug mixture using homogenizer. The method of calcium crosslinking used in preparation of unloaded hydrogel beads was performed, with the use of calcium chloride (10% w/v) which has been adjusted to pH 3.6. Similarly, the hydrogel beads were rinsed with distilled water and EB irradiated.[17]

Determination of Gel Content in CMSP/Pectin Hydrogel Beads

After EB irradiation, irradiated samples were transferred into individual tea bags and weighed (W_0). Then, they were suspended in beakers containing large amount of distilled water overnight to remove the soluble fraction in hydrogel. The hydrogel beads were then transferred onto petri dishes and dried in an oven at 37 °C until constant weights (W_1). Triplicates of samples were prepared. The percentages of soluble fraction and gel fraction were calculated according to the eqs. (1) and (2), respectively.[6,7]

$$\text{Sol fraction}\,(\%) = [(W_0 - W_1)/W_0] \times 100 \quad (1)$$

$$\text{Gel fraction}\,(\%) = 100 - \text{Sol fraction} \quad (2)$$

Determination Swelling Capacity of CMSP/Pectin Hydrogel Beads in Distilled Water, Hydrochloric Acid Buffer at pH 1.2, Phosphate Buffer at pH 5.5 and pH 7.4

Five hundred milligrams of CMSP/pectin hydrogel beads from each set of sample was used and the weight was recorded as initial weight (at 0th minute). The hydrogel beads from each sample were transferred into tea bags and placed into beaker containing about 80 mL of distilled water. After 30 min, the hydrogel beads were taken out, blotted, and weighed. The weight was recorded. This procedure was repeated every hour until 8th hour, and then last reading was taken at 24th hour to observe equilibrium swelling. This part was repeated using hydrochloric acid buffer at pH 1.2, phosphate buffer at pH 5.5 and pH 7.4. Each sample was prepared in triplicates. The swelling ratio of CMSP/pectin hydrogel beads was calculated based on eq. (3).

$$\%S = \frac{M_t\text{-}M_o}{M_o} \times 100 \quad (3)$$

M_o represents the mass of dry hydrogel at time zero and M_t represents the mass of swollen hydrogel at time t.[6,7]

Determination of Sizes of CMSP/Pectin Hydrogel Beads

Diameter of 50 randomly chosen unloaded and diclofenac sodium-loaded hydrogel beads were determined using a dial caliper (Mitutoyo Series 505). The average bead diameter was calculated.

Determination of DEE of CMSP/Pectin Hydrogel Beads Loaded with 10% Diclofenac Sodium

About 250 mg of diclofenac sodium-loaded CMSP/pectin hydrogel beads were added into Scott bottle containing 50 mL of 1*M* NaOH were shaken for 24 h on orbital shaker (Lab Companion SK-71). The 1*M* NaOH containing drug-loaded hydrogel beads were subjected to ultrasonication with Hielscher UIP 500Hd at 60% amplitude for 1 min. Then, each sample was filtered. The absorbance of each drug-loaded sample was measured using UV-Vis spectrophotometer (Shimadzu UV-1800) at 276 nm following suitable dilutions, with the unloaded sample as blank solution. Triplicates of samples were prepared. A calibration curve for drug in 1*M* NaOH was plotted ($R^2 = 0.9986$). The DEE of each sample was calculated based on the eq. (4).[17]

$$\text{DEE} = \frac{\text{Experimental drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (4)$$

In Vitro Drug Release Studies of Diclofenac Sodium-Loaded CMSP/Pectin Hydrogel Beads

Approximately 2 g of diclofenac sodium-loaded hydrogel beads, which are equivalent to 100 mg of diclofenac sodium were used for dissolution studies. The dissolution studies was carried out in a USP dissolution tester (TDT-08L) by Type I apparatus (rotating basket). The test was performed in 0.1*N* HCl for initial 2 h to simulate stomach environment, followed by dissolution in phosphate buffer at pH 6.8 to simulate intestine and colonic environment, each 900 mL, maintained at 37.0 ± 0.5 °C and 50 rpm. Five milliliters of samples was withdrawn at specific intervals. After the withdrawal of sample, same volume of the release medium was replaced immediately. Each sample was filtered using 50 μm syringe filter and the amount of the drug present in each sample was determined at 276 nm using UV-Vis spectrophotometer (Shimadzu UV-1800) following suitable dilutions. The dissolution media was used as the blank solution. The calibration curves for drug in media of pH 1.2 (R^2= 0.9975) and pH 6.8 (R^2= 0.9990) were plotted. All samples were prepared in triplicates.

Statistical Analysis

Gel fraction, swelling studies, drug particle analysis, DEE, and *in vitro* drug release data were analyzed using ANOVA. Independent *t*-test was also used for beads sizes analyses. SPSS 22

Table II. Gel Fraction of CMSP/Pectin Hydrogel Beads (Selected Formulations)

CMSP/pectin hydrogel beads	Gel fraction (%)[a]				
	5 kGy	10 kGy	15 kGy	20 kGy	25 kGy
10%/10%	5.4991 ± 0.1399	6.0392 ± 0.3213[b]	5.4109 ± 0.1729	5.3471 ± 0.1732	5.2155 ± 0.0048
7.5%/10%	5.097 ± 0.1433	4.6544 ± 0.4417	4.9739 ± 0.1562	5.7528 ±0.2483[b]	4.9485 ± 0.0460
10%/7.5%	5.6095 ± 0.2610	5.4804 ± 0.0832	6.1661 ± 0.0913[b]	5.5058 ± 0.0552	5.2832 ± 0.1240
15%/5%	5.8734 ± 0.2462[b]	4.5944 ± 0.1360	4.9766 ± 0.4267	4.6266 ± 0.3278	4.5991 ± 0.3815

[a] Mean ± SD, $n = 3$.
[b] Significant difference ($P < 0.05$).

software was used for statistical analysis and the confidence level was set at $P < 0.05$.

Characterization of CMSP/Pectin Hydrogel Beads

Fourier Transform Infrared Spectroscopy. The finely ground unloaded and diclofenac sodium-loaded CMSP/pectin hydrogel powder were used for the study on the infrared spectra of each sample between 400 and 4000 cm^{-1} using Varian 640-IR Fourier Transform Infrared (FITR) Spectrophotometer.

Field Emission Scanning Electron Microscope. CMSP, pectin, diclofenac sodium, unloaded hydrogel beads and diclofenac sodium-loaded hydrogel beads were mounted on aluminium stubs and coated with thin layer of platinum using Quorum (Q150RS) sputter coating system for 15 min. The surface morphology of the samples was observed at Hitachi SU8010 Field Emission Scanning Electron Microscope (FESEM) at 3 and 5 kV. The images of all the samples were observed at different magnifications.

Differential Scanning Calorimetry. About 5 mg fine powder of CMSP, pectin, diclofenac sodium, EB irradiated diclofenac sodium, unloaded and diclofenac sodium-loaded CMSP/pectin hydrogel beads were sealed in 50 μL aluminium pan and DSC analysis was performed using Perkin Elmer DSC-4000. The samples were heated from 35 °C to 350 °C with heating rate of 15 °C/min under atmosphere of nitrogen at flow of 20 mL/min.

Thermogravimetric Analysis. Thermogravimetric analysis (TGA) studies were carried out using Mettler-Toledo model TGA/DSC 1. About 3 mg fine powder of CMSP/pectin hydrogel beads on platinum pan was heated at heating rate of 20 °C min^{-1} under flowing nitrogen (20 mL/min) over the temperature range from 60 °C up to 600 °C.

X-ray Diffraction. An X-ray diffraction (XRD) apparatus (Bruker D8 Discover) was used to obtain the XRD patterns of the samples. CMSP, pectin, diclofenac sodium, diclofenac sodium-loaded and unloaded CMSP/pectin hydrogels beads were ground to smaller than 75 μm. About 15 mg of each sample was loaded into the XRD apparatus and obtained the diffractograms over the area $5° < 2\theta < 70°$. The analysis was done with a cobalt target X-ray tube operating at 30 kV and 330 μA.

RESULTS AND DISCUSSION

Preparation of CMSP/Pectin Hydrogel Beads

Among the 12 sets of formulations of CMSP/pectin hydrogel beads composed of different percentage of CMSP and pectin, some of the formulations resulted in formation of hydrogel beads with irregular shapes after drying process. The formulations of CMSP/pectin of 7.5%/7.5%, 10%/10%, 7.5%/10%, 10%/5%, 10%/7.5% and 15%/5% have resulted in even spherical shapes. This shows that the concentration of both CMSP and pectin are crucial and high concentration of polymers are needed to form regular spherical hydrogel beads. This is because of the presence of higher amount of calcium binding sites that allow formation of more crosslinks.[27] Hence, hydrogel beads with stronger matrix are able to maintain the shapes after drying process. Hydrogel beads with regular spherical shapes are the desirable formulations because they are able to have more controlled and uniform drug release.[28]

Gel Fraction

Under high energy EB irradiation, polymer chains of CMSP and pectin undergo ionization which remove electrons and resulted in the formation of macroradicals. Macroradicals can form on either main chain or side chains of the polymers. On main chain, radicals are formed on C1-C6 atoms of anhydroglucose repeating units of CMSP as well as galacturonic acid repeating units of pectin. The macroradicals formed on main chains will cause random cleavage of glycosidic bonds and leads to chain scissions.[6,29] For the macroradicals formed on the side chains of polymers, intermolecular crosslinking can occur.[6]

Based on gel fraction results as shown in Table II, for different combination of concentration of CMSP and pectin, the irradiation doses that give highest gel fraction are different. Concentration of polymers plays the main role because it determines the distance between macroradicals. At low concentration of polymers, the distance between macroradicals is far that causes lack of opportunity for crosslinking to occur, and therefore, degradation will predominate over crosslinking. Besides, there will be insufficient amount of radicals available for crosslinking.[6] Other than concentration of polymers, irradiation dose is also important in determining the degree of crosslinking because too low irradiation dose will cause less crosslink due to low radiation energy whereas too high irradiation dose will cause more scission and oxidative degradation of polymers. Hence, in order to obtain the highest gel fraction, it is important to determine the

Table III. Average Diameter of CMSP/Pectin 15%/5% Hydrogel Beads

Irradiation dose (kGy)	Average diameter of CMSP/pectin 15%/5% hydrogel beads (mm)[a]	
	Unloaded	Drug-loaded
0	2.13 ± 0.11	2.20 ± 0.13
5	1.78 ± 0.05	1.95 ± 0.09
15	1.81 ± 0.04	1.96 ± 0.06
20	1.80 ± 0.06	1.96 ± 0.08

[a] Mean ± SD, $n = 50$.

appropriate irradiation doses that allow crosslinking to occur predominant over scission.[30]

Swelling Behavior in Various pH Media

In distilled water, the percentage of swelling increase with the immersion time and reached the equilibrium swelling at 24 h [Figure 1(a)]. For CMSP/pectin 10%/10%, 7.5%/10%, 10%/7.5% and 15%/5%, the equilibrium swelling achieved were 791%, 633%, 571%, and 494% respectively. In pH 1.2, for CMSP/pectin 10%/10%, 7.5%/10%, 10%/7.5%, the percentage of swelling increase with the immersion time and reached the equilibrium swelling at 24 h [Figure 1(b)]. The equilibrium swelling percentage achieved were 643%, 466%, 568%, respectively. For CMSP/pectin 15%/5%, the swelling pattern is different as other formulations and it shows fluctuating reading and the swelling percentage achieved 372% at 24 h. In pH 7.4, for CMSP/pectin 10%/10%, 7.5%/10%,10%/7.5%, the percentage of swelling increase with the immersion time, reach maximum swelling percentage of 1195%, 982% and 1051% at 2 or 3 h, followed by drastic reduction in swelling percentage due disintegration of hydrogel after attained maximum swelling (Figure 3). Only CMSP/pectin 15%/5% has increased percentage of swelling as immersion time increase and it reached equilibrium swelling of 986% at 24 h.

The overall results shows that CMSP/pectin hydrogel beads swells maximum in pH 7.4, followed by distilled water and pH

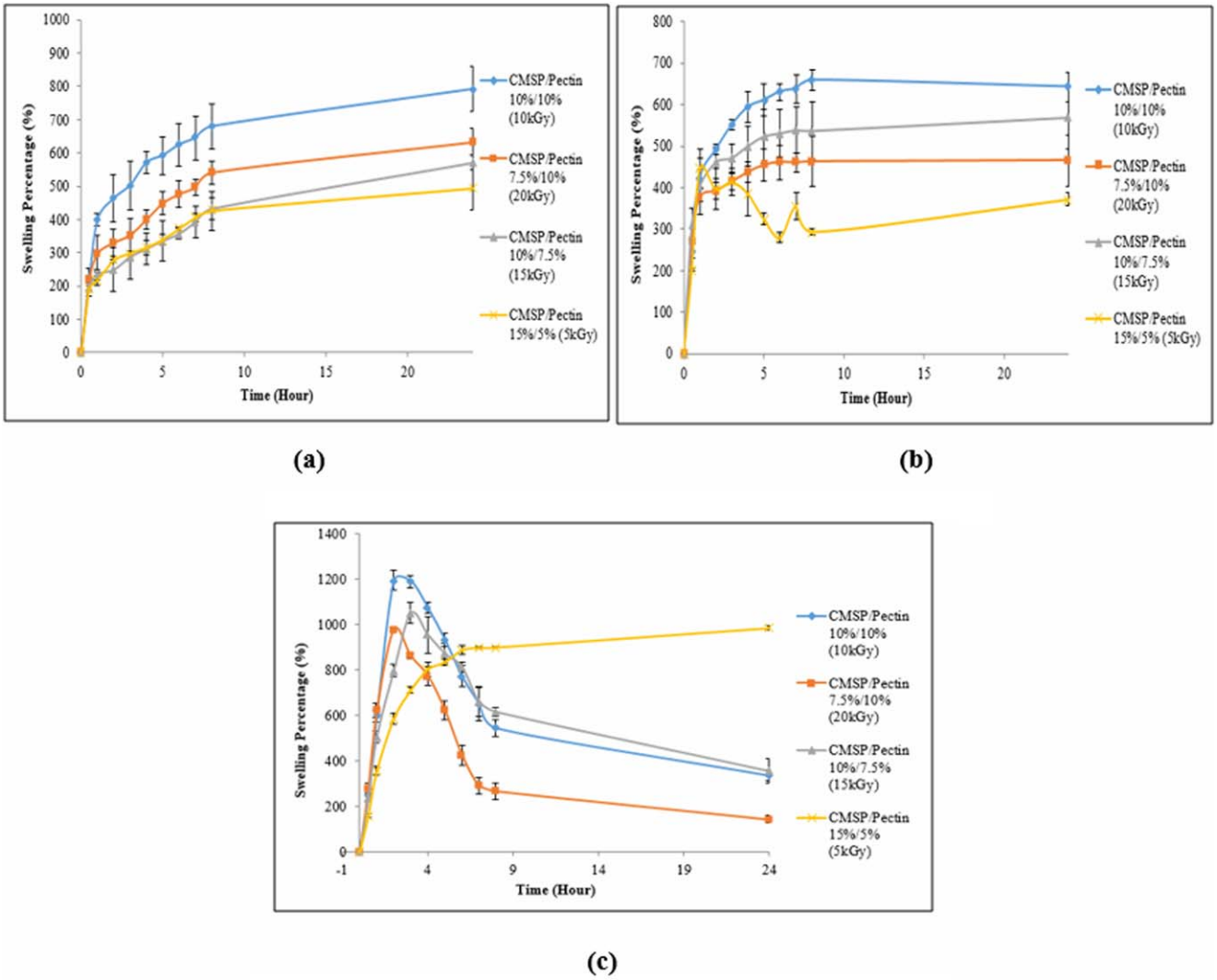

Figure 1. A plot of swelling over time for CMSP/pectin hydrogel beads in (a) distilled water; (b) HCl buffer of pH 1.2, and (c) phosphate buffer of pH 7.4 ($n = 3 \pm$ sd). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

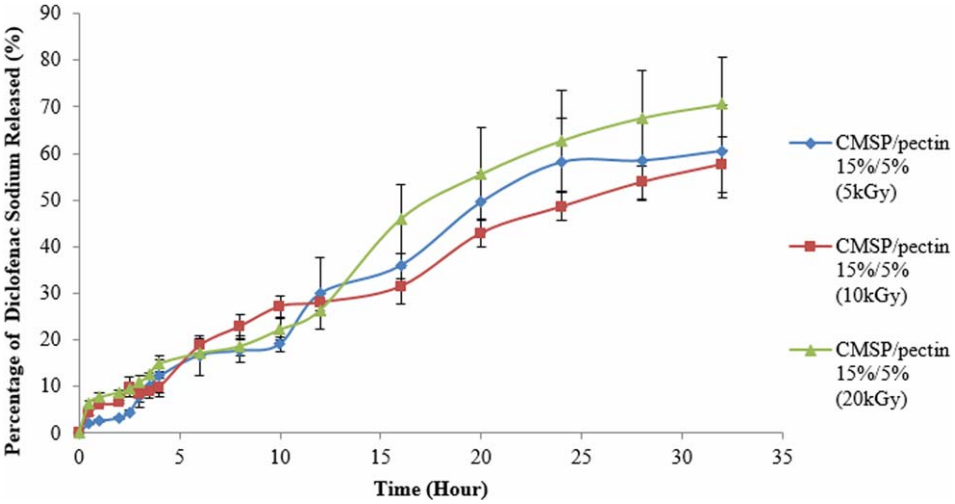

Figure 2. *In vitro* release profile of diclofenac sodium from CMSP/pectin hydrogel beads (n= 3± sd). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

1.2 and thereby CMSP/pectin hydrogel beads could be categorized as pH sensitive hydrogel. The pKa of CMSP is about 4–5, whereas the pKa of pectin about 3–4.[19,31] At pH lower than the polymers (pH 1.2), protonation of carboxylate anions of both polymers does occur thus the swelling degree of hydrogel is low. At the pH higher than pKa of both polymers (distilled water and pH 7.4), the carboxylic groups turn deprotonated and the electrostatic repulsive forces between the negatively charged sites (COO^-) cause polymer chain expansion as well as enhancement in swelling degree.[32,33] Among the four sets of formulations, only CMSP/pectin 15%/5% is the only formulation which stayed intact at the colonic pH of 7.4 for 24 h. The other three formulations started to disintegrate at second or third hour of swelling. This shows that high concentration of CMSP and low concentration of pectin allows the formation of hydrogel beads with ability to have steady increase in swelling degree across the time. It is expected to provide sustained or controlled drug release.

Beads Size Analysis

The significant larger diameter of hydrogel beads without subjected to EB irradiation than hydrogel beads irradiated at different irradiation doses (5 kGy, 15 kGy, and 20 kGy; $P < 0.05$) might be due to EB irradiation which has induced further crosslinking of the hydrogel beads and leads to formation of a more compact matrix.[17] On the other hand, the average diameter as well as diameter deviation of the drug-loaded hydrogel beads are shown to be larger compared to unloaded hydrogel beads and this is applicable to both nonirradiated and EB irradiated hydrogel beads. The diameter of drug-loaded hydrogel beads is significantly larger than unloaded hydrogel beads ($P < 0.05$). When there is presence of drug, the viscosity and density of drug-polymer solution has increased. This causes the lower degree of crosslinking of the polymer network. In addition, the passage of irradiation is also affected by these parameters. As a result, there is increase in the size of hydrogel beads. The increase in diameter deviation can be explained by the distortion of the shape of beads due to presence of drug in the polymer mixture solution. During the formation of hydrogel beads during crosslinking process, the drug molecules of diclofenac sodium disrupt the matrix and they exhibit higher resistance during the extrusion of drug-polymer solution through the needle.[18]

Drug Loading and DEE

The DEE of CMSP/pectin 15%/5% (w/w) prepared with irradiation dose of 5 kGy, 15 kGy and 20 kGy are 50.45% ± 3.41, 47.44% ± 2.19 and 40.89% ± 2.79, respectively. The measurement was done in triplicates and it can be observed that DEE of samples increases with decrease in irradiation dose. The significant lower DEE in hydrogel beads irradiated at higher irradiation dose (20 kGy) compared to 5 kGy might be due to lower degree of crosslinking of the polymers. The lower extent of crosslinking of polymers leads to looser arrangement of the polymeric network that causes some of the drugs molecules entrapped within hydrogel to be leaked out of the network during the formation of further crosslinking under EB irradiation.[33]

The overall results show the low efficiency of encapsulating diclofenac sodium by CMSP/pectin 15%/5% hydrogel beads. High drug loss might have occurred during the process of calcium chloride crosslinking. When the polymer and drug mixture are added into calcium chloride solution, interfacial crosslinking takes place instantaneously followed by gradual gelation process in the interior part of hydrogel beads. Some of the drug molecules could have lost from the surface of hydrogel beads in this process.[34] Besides, high concentration of calcium chloride solution might also contributes to the low entrapment efficiency because calcium chloride which is soluble in water will create high amount of pores on the surface of hydrogel beads and this will cause the drug molecules to freely leach into the crosslinking solution.[35]

In Vitro Drug Release Studies

Based on Figure 2, At the end of second hour, total diclofenac sodium released from CMSP/pectin hydrogel beads irradiated at 5 kGy, 15 kGy, and 20 kGy are 3.29 ± 0.20%, 6.58 ± 0.20% and

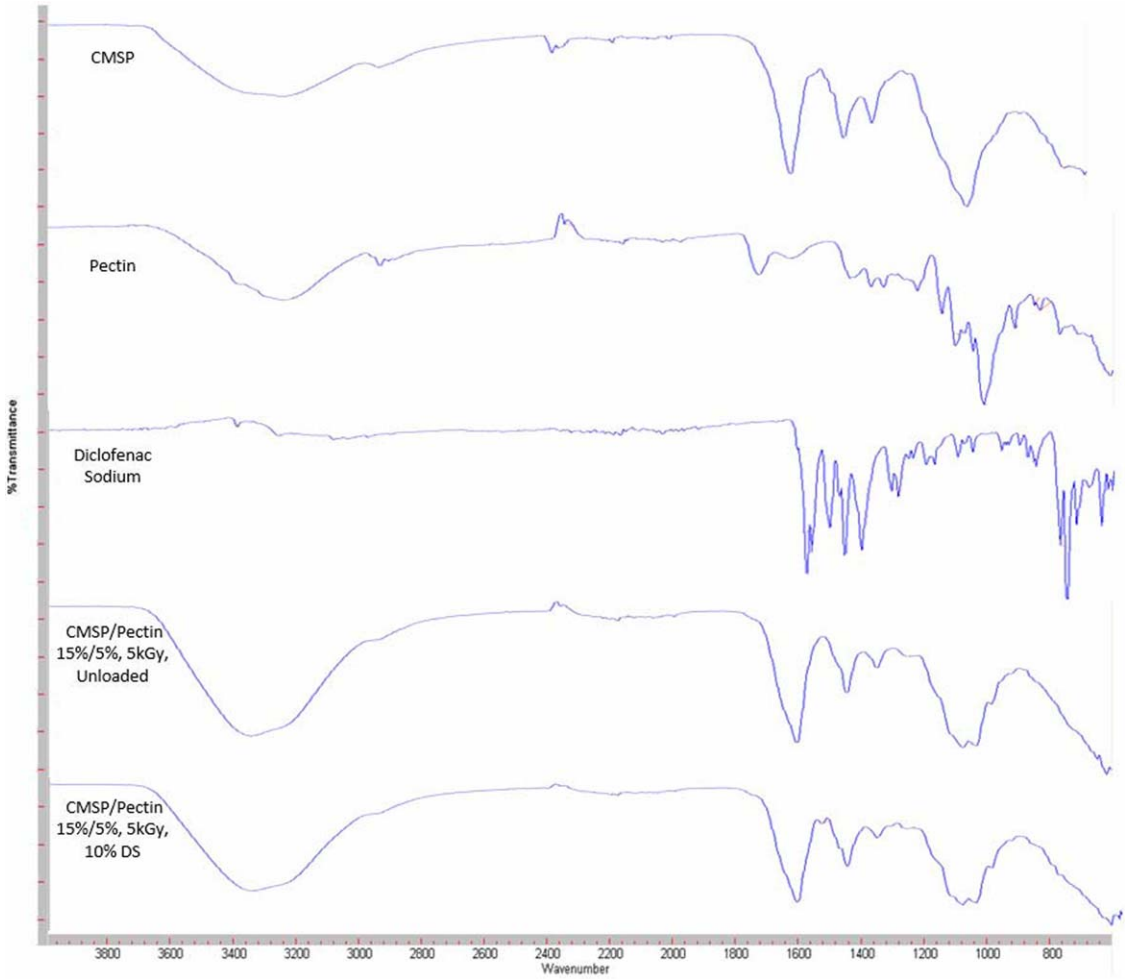

Figure 3. FTIR spectra of CMSP, pectin, diclofenac sodium, unloaded CMSP/pectin 15%/5% hydrogel beads, and diclofenac sodium-loaded CMSP/pectin 15%/5% hydrogel beads. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$8.76 \pm 0.44\%$, respectively. The significant low drug release ($P < 0.05$) from hydrogel beads can be due to low degree of swelling of CMSP/pectin 15%/5% at pH 1.2 The protonated anionic groups of CMSP and pectin cause the hydrogel beads to remain intact in the acidic stage.[17,36] Besides, low drug release might also due to by low solubility of diclofenac sodium at pH 1.2.[37] The small amount of drug detected can be due to the presence of free or loosely bound drugs on the surface of hydrogel beads.[18,36] The low drug release at pH 1.2 is desirable because most of the drug can be protected from being released in stomach and the amount of drug carried in the hydrogel beads to target colon can be maximized.

In pH 6.8 buffer, sustained drug release manner of the three drug-loaded formulations are observed. At pH 6.8, CMSP/pectin hydrogels start to swell higher due to the building up of osmotic swelling force. This is because of the anionic groups of hydrogel which are unprotonated and completely ionized at this pH.[13] The high solubility of diclofenac sodium at pH 6.8 also allows the increase in drug release.[37] At the end of experiment, which is at 32th hour, the total amount of diclofenac sodium released from CMSP/pectin hydrogel beads irradiated at 5 kGy, 15 kGy, and 20 kGy are $60.49 \pm 9.93\%$, $57.67 \pm 5.93\%$ and $70.61 \pm 9.94\%$, respectively. There is no significant difference among the readings of these three sets of samples ($P > 0.05$). The incomplete drug release might be due to the polymeric network which is too compact that retards the release of drug from the formulation into dissolution media.[38] By looking at the sustained drug release profile in this study, main drawback of pectin due to the rapid *in vitro* release has been overcame.[39,40] In addition, no coating on the hydrogel bead is needed to achieve the slow drug release as compared to previous study which requires Eudragit coating on dual crosslinked pectin alginate hydrogel beads to avoid burst release of drug.[41]

FTIR Spectroscopy

FTIR spectrum of CMSP is shown in Figure 3. The strong absorption band at 1584 cm^{-1} shows the presence of COO^-groups. The absorption bands at 1412 and 1321 cm^{-1} represent —CH_2 scissoring and —OH bending vibrations, respectively. The absorption band at around 1011 cm^{-1} is assigned to the C—O stretching vibration of ether group of carboxymethylation of cellulose and also the ether linkage of the 1,4-β-glycoside group (>CH—O—CH<) of the cellulose backbone. There are overlaps in the C—O bands due to these two types of ethers. The absorption at 2927 cm^{-1} indicates the aliphatic C—H stretching

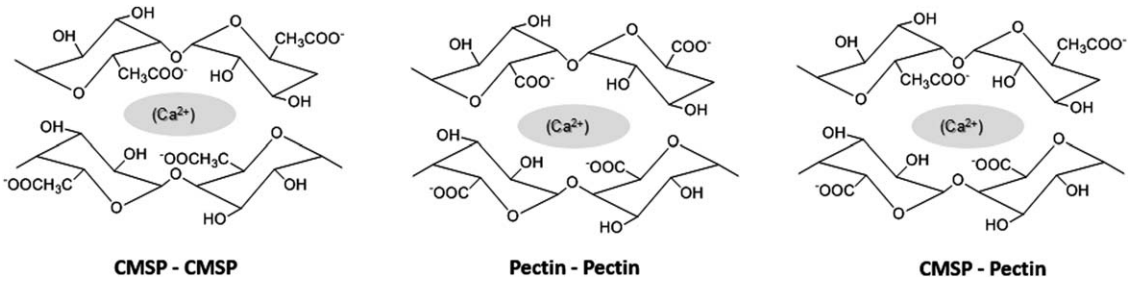

Figure 4. Drawing shows possible crosslinks formed between polymer chains by Ca^{2+} via COO^- groups.

vibration of the carboxymethyl group, whereas the broad peak around 3234 cm^{-1} indicates the stretching vibration of the —OH group. These peaks are well in agreement with those reported in literature.[6,18]

FTIR spectrum of pectin (degree of esterification 63%–66%) is shown in Figure 3. The broad absorption peak at 3249 cm^{-1} represents the stretching of —OH groups. The peak at 2939 cm^{-1} represents C—H stretching vibration. The peaks at 1460 and 1331 cm^{-1} represent $—CH_2$ scissoring and —OH bending vibrations, respectively.[19] Peaks observed at 1639 and 1730 cm^{-1} indicate free carboxyl groups and esterified carboxyl groups, respectively. It is shown that the absorbance is higher at 1730 cm^{-1} than at 1639 cm^{-1}, which is the characteristic of high methoxyl pectin.[42] The absorption band at around 1012 cm^{-1} corresponds to ether (R—O—R) and cyclic C—C bonds in the ring structure of pectin molecules.[43]

FTIR spectrum of unloaded CMSP/pectin 15%/5% hydrogel bead is observed as the combination of the spectra from pure CMSP and pure pectin (Figure 3). Most of the characteristics of pure CMSP and pure pectin discussed previously are present in the spectrum of this calcium crosslinked hydrogel beads. However, there is a slight reduction in the intensity of the peak at 1584 cm^{-1} that represents the carboxylate groups from CMSP. Besides, there is absence of peak at 1639 cm^{-1} that represents the free carboxyl groups from pectin. The reduction of amount of COO^- groups might be due to Ca^{2+} that form crosslinks between the polymer chains via these COO^- groups, as illustrated in Figure 4.[44] Besides, there is absence of peak at about 1730 cm^{-1} which represents esterified carboxyl groups. This suggests that further crosslinking of CMSP/pectin hydrogel beads using EB irradiation has reduced the amount of esterified carboxyl group on pectin chains. When pectin is exposed to EB irradiation, it is hypothesized that pectin macroradicals are formed on the methyl ester group of pectin. For CMSP, macroradicals are also formed on the side chains of polymer. Recombination of macroradicals can occur intra- and intermolecularly through the formation of C—C bonds for both CMSP and pectin chains, as illustrated in Figure 5. The FTIR spectra of all drug-loaded formulations exhibit the peaks which are the summation of the characteristics peaks present in diclofenac sodium and unloaded hydrogel beads and this can be regarded as the superposition of the drug and hydrogel beads.[45] This shows that there is absence of chemical interaction of drug with the polymers as well as the intact nature of diclofenac sodium that present in the crosslinked polymeric network.[46] The reduction in intensity of the peaks of drug in the formulations relative to pure drug is because of the reduction in crystallinity and change into amorphous form of drug in the formulation.[45]

Field Emission Scanning Electron Microscope

Under magnification of 30×, unloaded crosslinked CMSP/pectin 15%/5% hydrogel bead is observed to have spherical shape with smooth surface (Figure 6). No separation into layers of material is observed although the hydrogel bead is made up of two types of polymers. Under magnification of 2000× and 5000×, the irregular structures of CMSP and pectin powder are not observed in the surface morphology of crosslinked hydrogel beads. Instead, the crosslinked matrix is shown to be homogenous and uniform, with regular granular-like structure. This might be due to the structural change of both CMSP and pectin after they crosslinked to form the 3D polymeric matrix.

Under magnification of 30×, diclofenac sodium-loaded 15%/5% hydrogel bead (5 kGy) is also observed to have spherical shape. However, the surface of drug-loaded bead is rougher compared to the surface of unloaded hydrogel beads. The roughness of drug-loaded hydrogel beads is contributed by the encapsulated drug particles of diclofenac sodium that present on the surface of hydrogel beads. Under higher magnification of 2000× and 5000×, the irregular structures of CMSP and pectin are not observed and regular granular-like structures are also observed. The successful encapsulation of diclofenac sodium is evidenced based on the appearance of drug particles that are shown to embed within the matrix of crosslinked polymers.

DSC Analysis

Based on Figure 7(a), the melting point of CMSP is observed at 195.94 °C. The small endothermic peak that appears before the intense peak of 195.94 °C corresponds to the loss of water molecules. For the point at 195.94 °C, it is assumed that the transition is contributed by the structural transitions related to the partial oxidation of the OH groups on the polymer chains and the oxidation is occurs on the OH groups at carbon C-6 which are not substituted by the CH_2COONa groups of CMSP as the OH groups that are directly connected to the sugar rings are difficult to be oxidized.[47] The sharp endothermic peak of pectin at 194.20 °C is assigned to its melting point [Figure 7(b)]. The small endothermic peaks before the melting point can be associated with the removal of bound water as well as glass transition temperature of pectin.[26]

The melting point of unloaded CMSP/pectin hydrogel beads is observed at 181.08 °C [Figure 7(e)]. The melting point is close

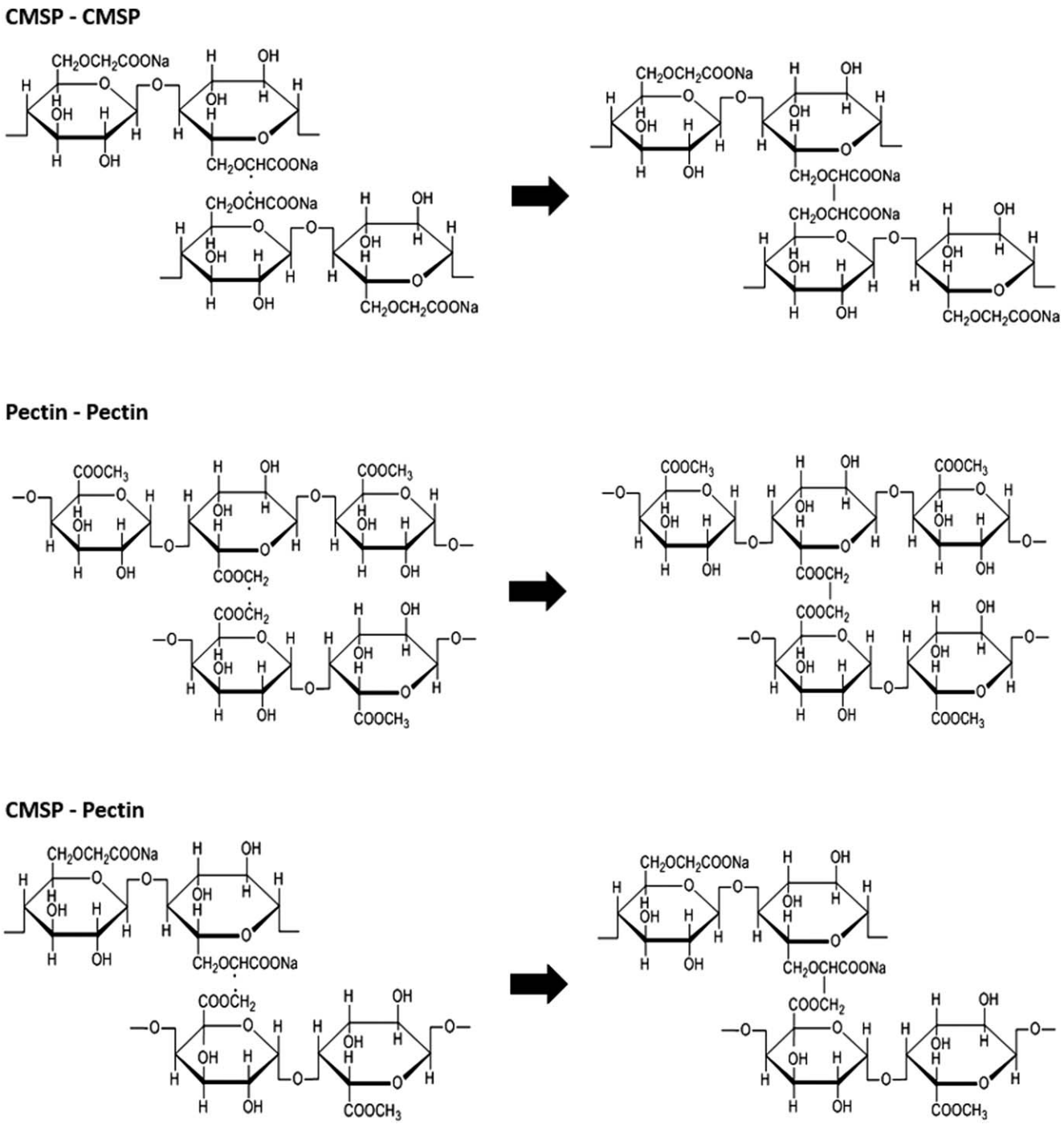

Figure 5. Drawing shows possible radicals formed on polymer chains and recombination of polymers.

to the melting point of both CMSP and pectin, showing that this blend hydrogel retains the thermal characteristics of the individual components. The melting point is closer to the melting point of CMSP than pectin and this might be due to the higher composition of CMSP within the hydrogel beads compared to pectin.[48]

From Figure 7(c), the sharp endothermic peak of diclofenac sodium is observed at 301.01 °C, indicating the melting point of stable crystalline drug of diclofenac sodium. The small endothermic peaks before the sharp peak at 301.01 °C are due to loss of water molecules.[49] The DSC thermogram of diclofenac sodium irradiated at 5 kGy [Figure 7(d)] is shown to be similar to the DSC thermogram of nonirradiated drug, shows that EB irradiation does not cause any change in the stability of the drug crystals.

DSC thermogram of diclofenac sodium-loaded CMSP/pectin hydrogel beads is shown in Figure 7(f). Only the endothermic peak that represents the melting point of crosslinked hydrogel

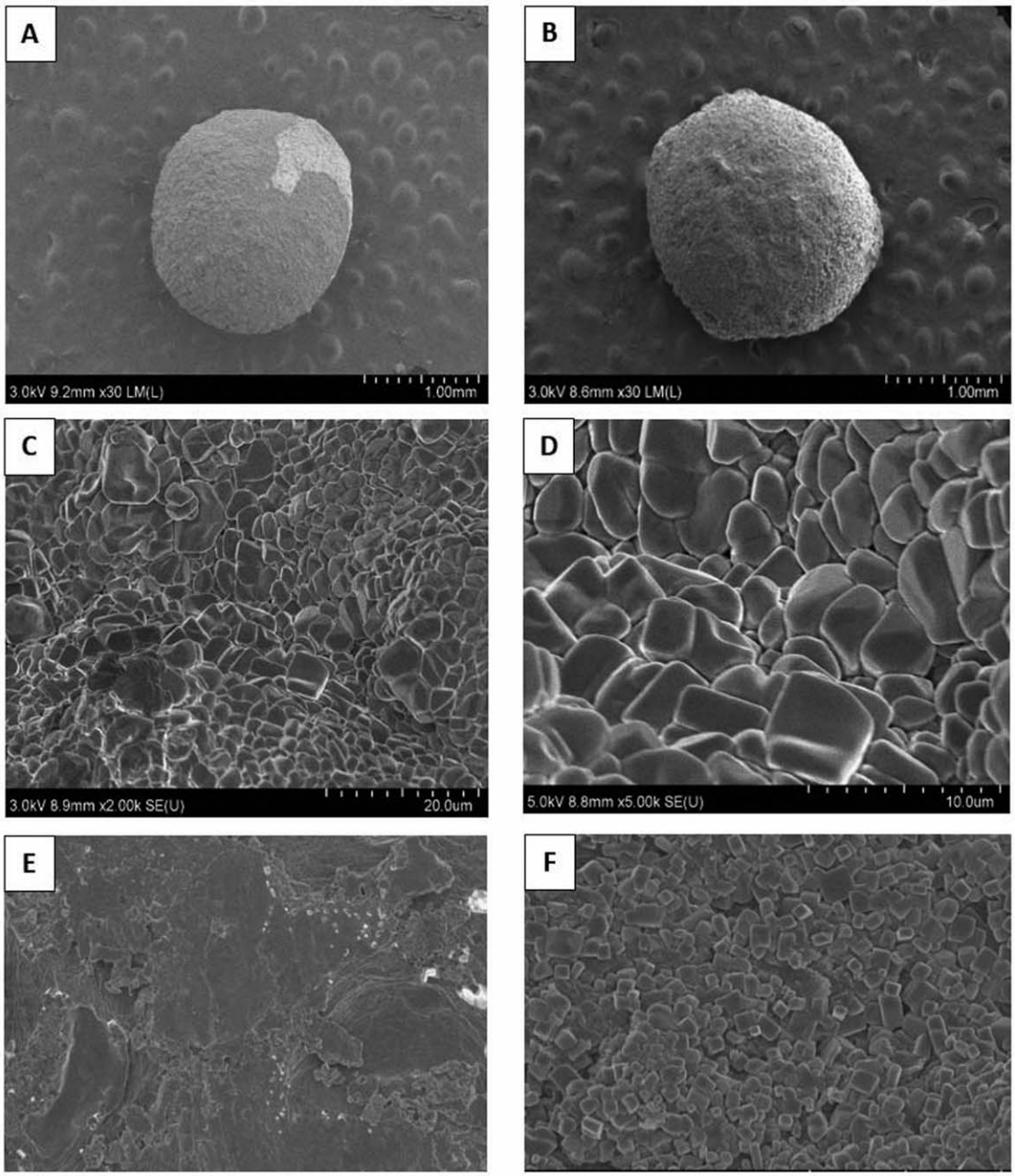

Figure 6. Surface morphology of (a) unloaded 15%/5% CMSP/pectin hydrogel beads under ×30 magnification, (b) diclofenac sodium-loaded 15%/5% CMSP/pectin hydrogel beads under ×30 magnification (c) unloaded 15%/5% CMSP/pectin hydrogel beads under ×2000 magnification, (d) unloaded 15%/5% CMSP/pectin hydrogel beads under ×5000 magnification, (e) diclofenac sodium-loaded 15%/5% CMSP/pectin hydrogel beads under ×2000 magnification, (f) diclofenac sodium-loaded 15%/5% CMSP/pectin hydrogel beads under ×5000 magnification.

beads is shown at 186.00 °C. The absence of any sharp endothermic peak close to the reported melting point of diclofenac sodium clearly shows that most of the drug molecules present in the system are in amorphous state. The conversion of crystalline state to amorphous form might be due to the intimate contact between drug and polymer during their physical mixing. Consequently, the crystalline structure of drug has been disrupted and they are homogenously dispersed within the hydrogel beads.[46,50,51]

Thermogravimetric Analysis

All the samples tested show a characteristic three-step thermal degradation and the first step, occurs at about 80 °C represents the water loss and it is evaluated as about 10%–14% of the initial mass.[52]

For CMSP [Figure 8(a)], in second step, at the temperature of about 180 °C to 340 °C, degradation that covers about 20% of weight loss is corresponded to the loss of carbon dioxide from the polysaccharide. In third step, weight loss of about 16%

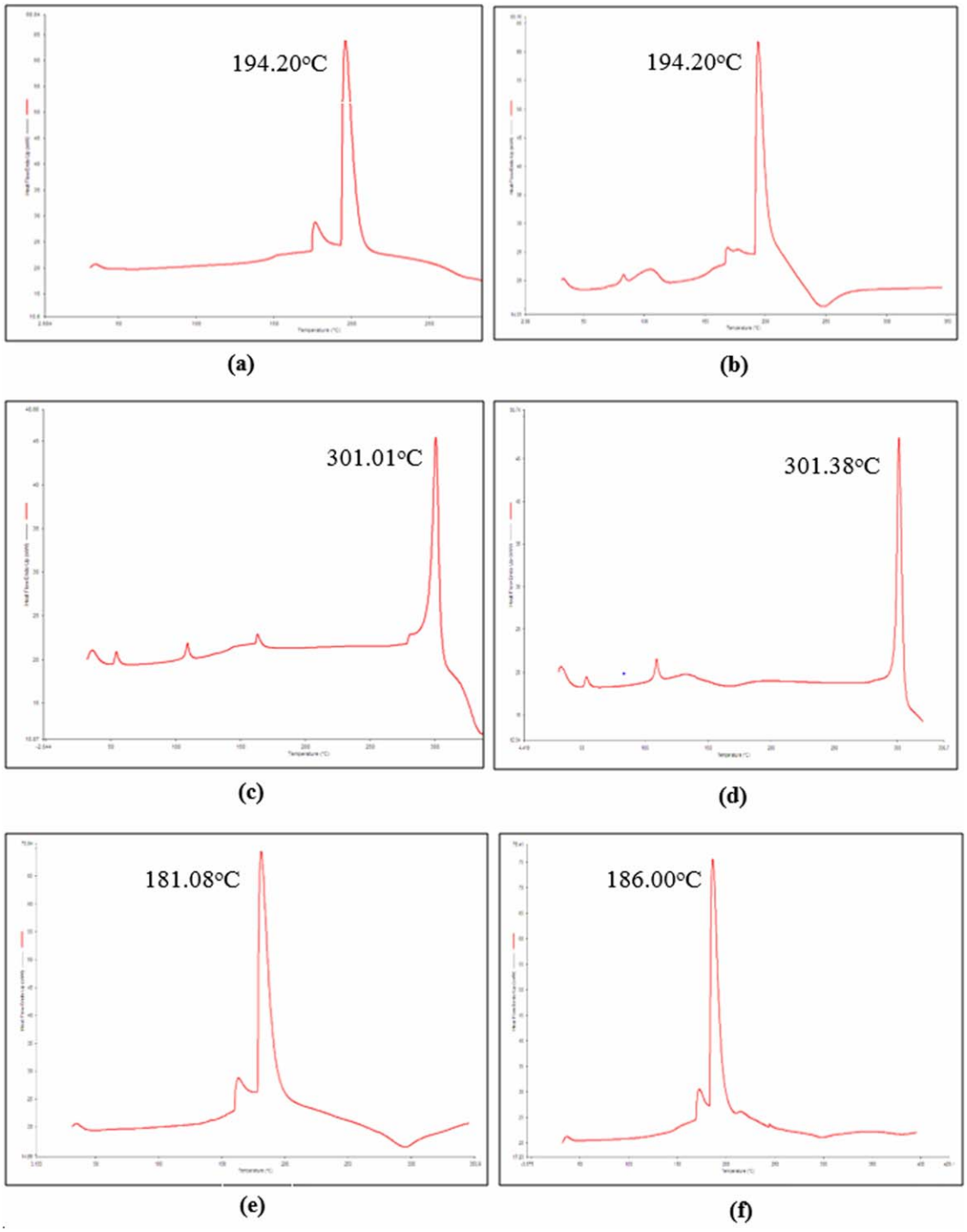

Figure 7. DSC thermograms of (a) CMSP, (b) pectin, (c) diclofenac sodium, (d) diclofenac sodium (5 kGy), (e) unloaded CMSP/pectin 15%/5% (5 kGy), and (f) diclofenac sodium-loaded CMSP/pectin 15%/5% (5 kGy). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

occurs within temperature range of 340 °C to 600 °C. These two steps of decomposition are due to depolymerization with the production of water, CO, CO_2 as well as CH_4. Decarboxylation of the COO^- group in CMSP also occurs in this range of temperature.[53] The maximum weight loss of CMSP was observed to occur at around 340 °C.

In pectin [Figure 8(b)], the second step, at the temperature of 190 °C to 280 °C, degradation that covers about 37% of weight loss is corresponded to pyrolitic decomposition. This comprises of primary and secondary decarboxylation that involves the acid side group as well as a carbon in the ring. Lastly, the weight loss that covers about 24% of initial mass in third step which

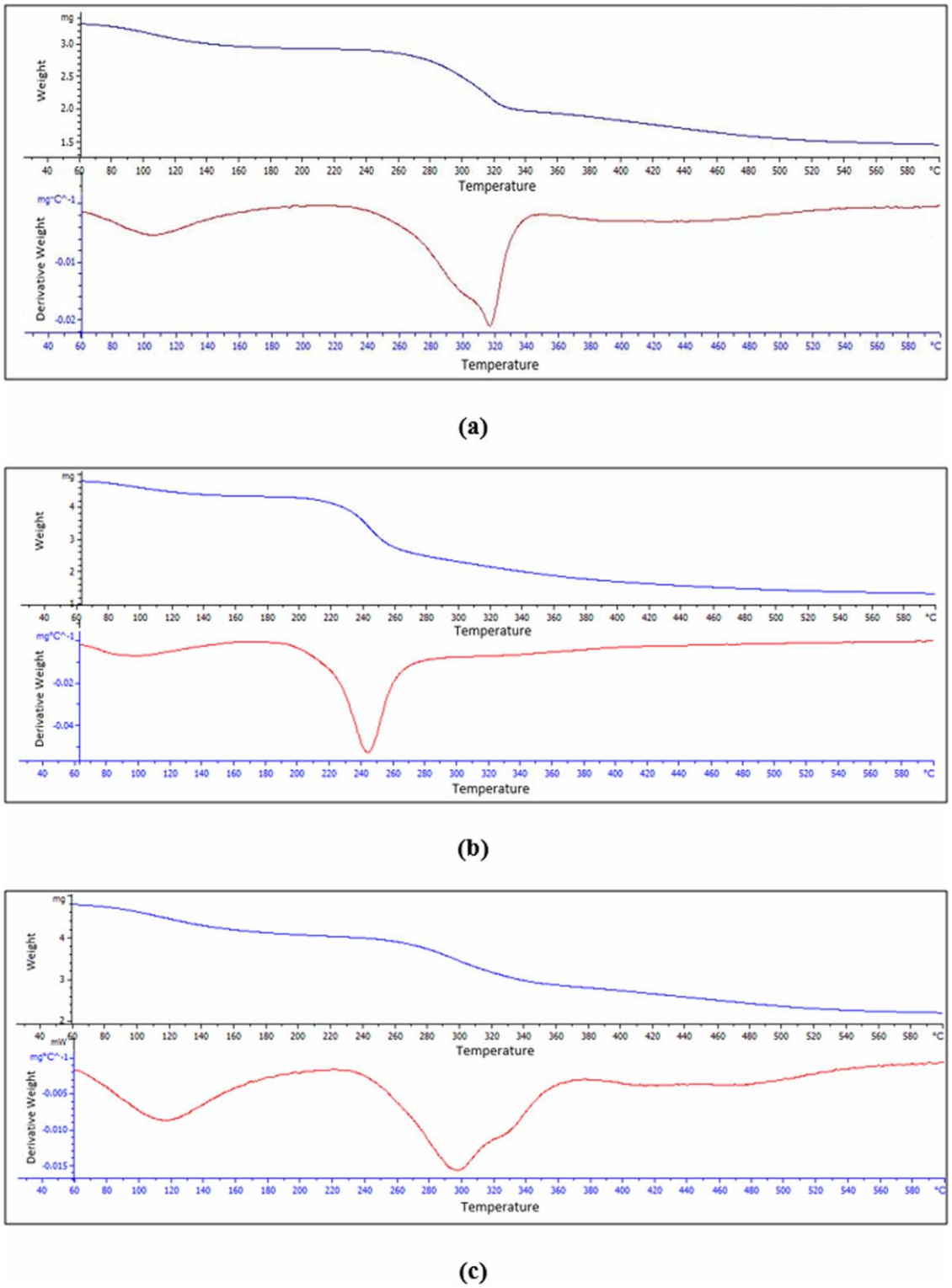

Figure 8. Thermogram of (a) CMSP, (b) pectin, and (c) unloaded CMSP/pectin 15%/5% hydrogel beads irradiated at 15 kGy. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

occurs at 280 °C represents the oxidation region.[45] The maximum weight loss of CMSP was observed to occur at around 245 °C.

For unloaded CMSP/pectin 15%/5% (5 kGy) [Figure 8(c)], the second step at 190 °C to 360 °C could be due to degradation that covers about 25% of weight loss. In third step, the weight

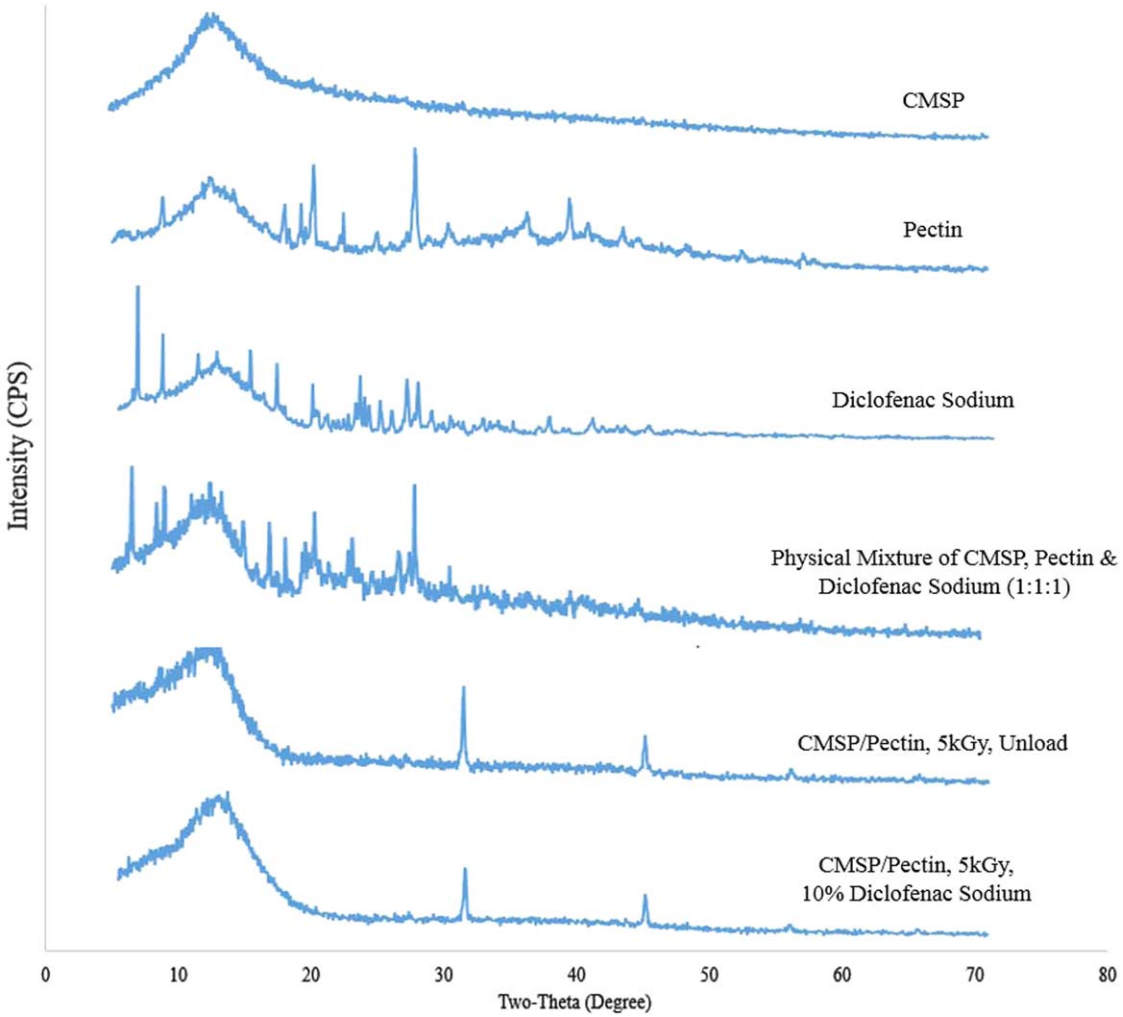

Figure 9. X-ray diffractograms of CMSP, pure pectin, diclofenac sodium, and physical mixture of CMSP, pure pectin, and diclofenac sodium (1:1:1 ratio), unloaded CMSP/pectin 15%/5% hydrogel beads and diclofenac sodium-loaded CMSP/pectin 15%/5% hydrogel beads. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

loss that covers about 24% of initial mass occurs at 360°C. Similar as individual polymers of CMSP and pectin, decomposition that occurs in these two steps involves depolymerization and decarboxylation of the polymers.

The maximum weight loss of CMSP/pectin hydrogel beads was observed to occur at about 300 °C, which is the temperature in between at which the maximum weight loss of CMSP (320 °C) and pectin (245 °C) occurred. Enhanced thermal stability of crosslinked hydrogel beads has been observed. At this stage, the amount of weight loss is 24% and this is lower than the weight loss of CMSP and pectin individual polymers in this stage, which involves 30% and 37% weight loss respectively. The enhanced thermal stability might be caused by the strengthening of amorphous region on crosslinking which lead to the higher stability of the hydrogel.[55–57]

XRD Analysis

From Figure 9, CMSP has been shown to have amorphous nature as no prominent peaks are observed. When carboxymethyl groups are introduced to the sago pulp, the regularity of CMSP chains are disrupted and reduced the crystallinity resulted in more amorphous region is produced. The occurrence of broad absorption peak at 13° diffraction angle indicates the remaining crystal region of CMSP which has not been fully destroyed.[58] For pectin, the presence of sharp peaks at 2θ equal to 8.8°, 12.30°, 20.2°, 27.9° and 39.6° show the crystalline behaviour.[59,60] Diclofenac sodium powder shows a XRD pattern of a crystalline material, with intense sharp peaks at 2θ equal to 6.5°, 8.4°, 15.0°, 17.0°, 19.7°, 23.3°, 26.8° and 27.7°.[61] The physical mixture of CMSP, pectin and diclofenac sodium shows the crystalline peaks from pectin as well as diclofenac sodium combined with amorphous peak of CMSP, indicating the absence of drug-polymer interaction.[62]

Based on X-ray diffractogram of unloaded CMSP/pectin 15%/5% hydrogel beads, it can be observed that the broad peak of CMSP at about 13° (2θ) still presents but the sharp peaks at 8.8°, 12.30°, 20.2°, 27.9°, and 39.6° (2θ) which representing the crystalline pectin are absent. This observation could be due to large modification in the crystalline structure of pectin after the crosslinking process. It has reduced crystallinity and it turns to

have more amorphous character as the regularity of the packing of original pectin chains has been destroyed. The crosslinked hydrogel beads show new intense peaks at 31.6°, 45.3° and 56.4° (2θ). The presence of new peaks and disappearance of characteristic peaks of original polymer of pectin suggests the occurrence of physical and chemical modifications, showing the formation of the tridimensional hydrogel polymeric network due to crosslinking.[63] These are attributed to the process of crosslinking during ionotropic gelation and EB irradiation.[60] Diclofenac sodium-loaded CMSP/pectin 15%/5% hydrogel beads do not show prominent crystalline peaks, suggesting the presence of diclofenac sodium as molecular dispersion in the hydrogel beads. This is in agreement with the results of DSC study which indicates the amorphous nature of entrapped drug in the hydrogel beads.[46]

CONCLUSION

CMSP and pectin are able to form hydrogel beads through ionotropic gelation process using calcium chloride. CMSP/pectin 15%/5% hydrogel beads is the only formulation which can form rigid spheres and it is able to remain intact and reach equilibrium swelling within 24 h in colonic pH of 7.4. The hydrogel beads are pH sensitive that had low swelling degree in acidic medium and higher swelling degree in neutral and alkaline medium shows its potential to minimize drug release in stomach and allows sustained drug release in colon. The loading of diclofenac sodium into the hydrogel beads causes the change of drug from crystalline form into amorphous form. This is a desirable property for the enhancement of drug solubility. This crosslinked hydrogel of CMSP and pectin are shown to have no interaction with diclofenac sodium in terms of the change in functional groups and thermal stability. The result of *in vitro* drug release study shows the potential application of CMSP/pectin hydrogel beads as drug carrier of colon targeted drug as it allows low amount of drug release in stomach and it provides higher and sustained drug release in colonic environment. This can be helpful to overcome the limitation of conventional dosage dorm which requires high frequency of oral administration other than causing side effects.

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