Journal of Applied Polymer Science

Hydrogels from Dextran and Soybean Oil by UV Photo-Polymerization

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ABSTRACT: In this work, hydrogels were synthesized by UV photo-polymerization of hydrophilic dextran functionalized with acrylate groups (Dex-A) and hydrophobic acrylate epoxidized soybean oil (AESO). The acrylation of dextran was accomplished by reacting dextran (M_w 70,000 g mol^{-1}) with acryloyl chloride and pyridine. The Dex-A was characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Five rigid hydrogels were prepared using the weight ratios of Dex-A and AESO as 10/90, 20/80, 30/70, 40/60, and 50/50. The hydrogels were characterized by FTIR, thermal gravimetric analyses (TGA) and scanning electronic microscopy (SEM). The experimental results demonstrated that the swelling and release profiles of the Dex-A/AESO hydrogels can be tailored by varying the ratio of Dex-A and AESO thus varying the balance of hydrophilicity and hydrophobicity of the network structures and the crosslinking density. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41446.

KEYWORDS: biopolymers and renewable polymers; gels; photopolymerization; polysaccharides; swelling

Received 21 February 2014; accepted 25 August 2014

DOI: 10.1002/app.41446

INTRODUCTION

Hydrogels are three dimensional hydrophilic polymer networks formed by either chemical or physical cross-linking of (macro) molecules.[1–3] Hydrogels can absorb abundant water, and can have good nutrient permeability and good biocompatibility. Hydrogels can also have tailored mechanical properties ranging from soft to hard depending on their chemical structures and crosslinking density. Hydrogels are widely used in the field of drug delivery and tissue engineering either as a depot system or a nano-sized drug carrier.[2–6] The hydrophilic polymers which can be used for the preparation of hydrogels include natural polymers (e.g., polysaccharides, proteins and peptides, and nucleic acids) and synthetic polymers (e.g., polyethylene glycol based homo- and co-polymers, polyvinyl alcohols, hydroxyethylmethacrylate and acrylic acid based homo- and co-polymers). The chemical composition, functionality and chain topology of the polymers play key roles in the determination of crosslinking density and the balance of hydrophilicity and hydrophobicity of hydrogels.[7] Chemically crosslinked gels normally have stronger mechanical properties than physically crosslinked gels. Chemical crosslinking can be formed via free radial photo-polymerization of (meth)acrylate (macro)monomers with multifunctional crosslinkers.[1,4,6,8–11] Photo-crosslinking has many advantages such as good spatial and temporal control, which makes it an ideal approach for *in situ* formation of hydrogels.[10] During photo-polymerization, photo-initiators (e.g. Iragure 2959[8,12] and 2,2-dimethoxy-2-phenyl acetophenone[13]) absorb ultraviolet or visible light to generate free radicals which initiate polymerizations. Examples of photo-polymerizable macromers include PEG-methacrylate derivatives, PEG-acrylate derivatives, polyvinyl alcohol (PVA) derivatives and polysaccharide derivatives (hyaluronic acid and dextran acrylate derivatives).[4]

Polysaccharides, such as chitosan,[14] dextran,[6,11,15–17] chondroitin sulfate,[12] alginate,[18] heparin,[19,20] agarose,[21,22] and hyaluronic acid,[23] are natural hydrophilic polymers. They have long been used for the preparation of hydrogels in biomedical applications,[24,25] such as for the delivery of protein and peptides in wound healing and for cell cultures in tissue engineering.[5,16,17,25,26] Dextran is a bacterial polysaccharide and consists essentially of α-1, 6 linked D-glucopyranose residues with varying lengths and degree of branching. Dextran has been investigated as biodegradable hydrogels for the delivery of drugs, proteins and imaging agents.[1,6,24,25] Three hydroxyl groups per residue of anhydroglucose in dextran are available for chemical reactions and for functional modification.[13] Various methods have been developed for the preparation of chemically crosslinked dextran hydrogels and dextran hydrogel composites,[24] particularly via free radical photo-polymerization.[6,11,27] For

crosslinking reactions to occur, suitable polymerizable and reactive functional groups such as (meth)acrylate, thiol, or aldehyde are required to be introduced onto the dextran polymer chains. Polymerizable dextran with methacrylate and acrylate groups is by far used the most to obtain chemically crosslinked dextran hydrogels via free radical polymerization. (Meth)acrylated dextrans have been synthesized by a variety of synthetic routes. These include by reacting the dextran with glycidyl(meth)acrylate,[28,29] by using (meth)acryloyl chloride[13] and methacrylic anhydride,[30] and by sequential reactions of dextran with bromoacetyl bromide and sodium acrylate.[11] The degree of substitution of the three hydroxyl groups on dextran anhydroglucose unit can be tailored by adjusting the reaction conditions.[1,13,24]

Plant oils, for example soybean oil, and their derivatives have been used widely in the preparation of many thermoset and thermoplastic polymers for commodity and industrial applications as well as for biomedical applications in recent years.[31–40] Plant oils have the advantages of being renewable, environmentally friendly, biocompatible and relatively inexpensive. Triglycerides are the main ingredient of plant oils with different active sites such as double bonds, ester groups, alkyl carbons and alpha carbons from esters. These active sites can be used to produce polymerizable groups.[32,37] Many polymerizable monomers for example acrylated epoxidized soybean oils (AESO) have been synthesized and used for the preparation of a wide range of polymers.[31,38,39] AESOs are synthesized from epoxidized triglycerides reacting with acrylic acid.[33,39] Epoxidized triglycerides can be found in most plant oils, they can also be prepared from natural unsaturated oils by epoxidation.[36]

In this article, we synthesized novel dextran/soybean oil hydrogels. To the best of our knowledge, this is the first report on the investigation of hydrogels consisting of dextran and soybean oil, which are both from renewable resources. The effects of reaction temperature, reaction time, amount of solvent and molar ratios of the reactants on the acrylation of the dextran were studied. The dextran/soybean oil hydrogels were characterized by Fourier transform infrared spectroscopy (FTIR) and thermal gravimetric analyses (TGA). The pore sizes and the morphology of hydrogels were examined by scanning electronic microscopy (SEM). Swelling tests and drug release studies of the hydrogels were also performed.

EXPERIMENTAL

Materials

Dextran, Leuconostoc spp.($M_w = 70{,}000$ Da) and lithium chloride (BioXtra $\geq 99.0\%$ titration) were purchased from Sigma–Aldrich, dried in a vacuum oven at 50°C over 24 h before use. Acryloyl chloride, pyridine, ethanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), dimethyl sulfoxide d_6 (DMSO)d_6, and 2,2-dimethoxy 2-phenyl acetophenone were purchased from Sigma Aldrich and used without further purification. Acrylate epoxidized soybean oil (AESO) with 8500 ppm mono-methyl ether hydroquinone as inhibitor was purchased from Sigma–Aldrich and used after removing inhibitor by passing through an alumina column. Also Lysozyme (from chicken egg white, $M_w = 14{,}307$ Da) and Carmoisine E122 (red food coloring) were purchased from Sigma–Aldrich. The Bio-Rad protein assay kit was purchased from Bio-Rad.

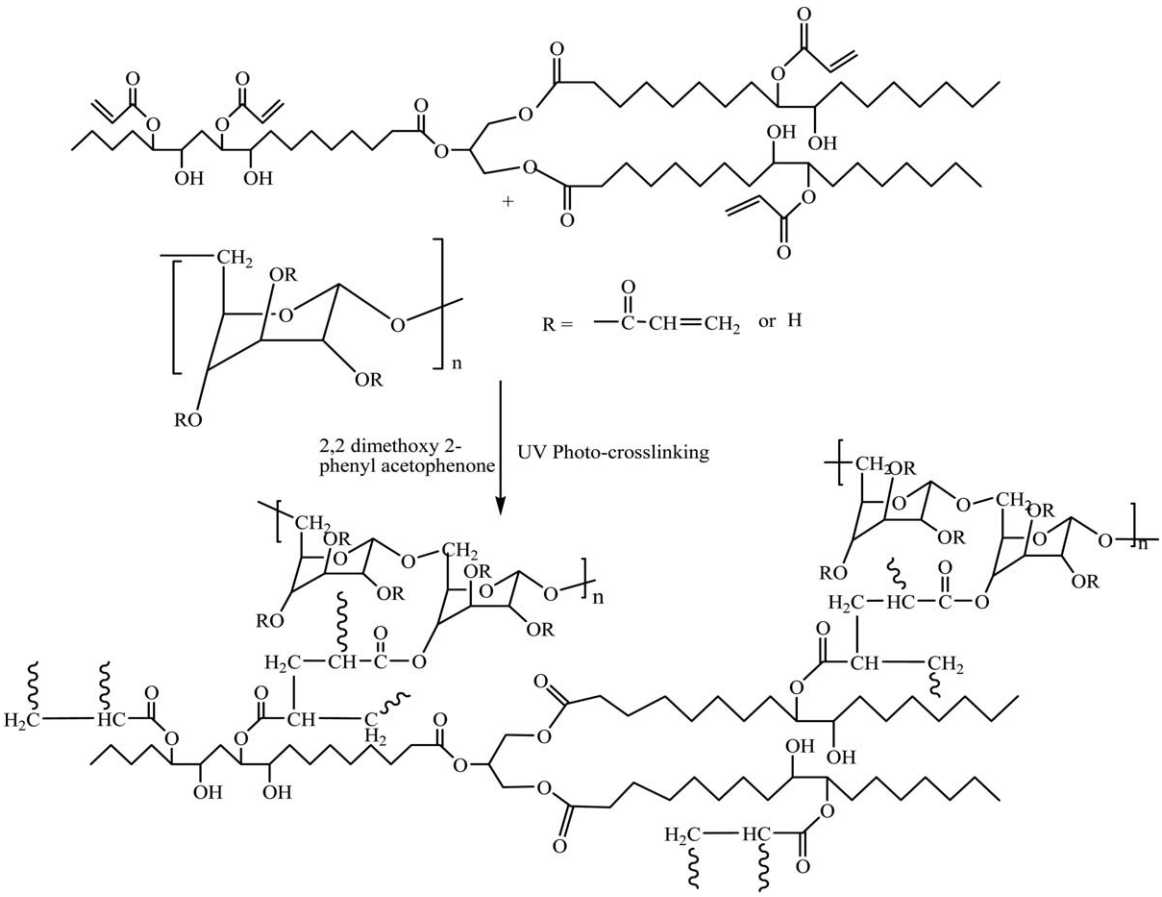
Preparation of Hydrogels

To prepare chemically crosslinked hydrogels via UV photo-polymerization of dextran and soybean oil, unsaturated vinyl functional groups are required in their molecular structures. In this work, the commercially available AESO was used, while dextran was functionalized with acrylate groups using acryloyl chloride following established procedures.[11] AESO has four unsaturated vinyl functional groups in each functionalized triglyceride. The two steps used in this work for the preparation of dextran/soybean oil hydrogels include (1) the synthesis of dextran acrylate (Dex-A) from dextran, and (2) the preparation of hydrogel networks by UV photo-polymerization of Dex-A and AESO (Scheme 1). To optimize the reaction conditions for acrylation of dextran, different molar ratios of pyridine, acryloyl chloride, dextran and DMF solvent were used (Table I). A typical procedure for Reaction 10 in Table I is described below. About 1.5 g LiCl was dissolved in 40 ml DMF and 2 g of dextran was added to the mixture. The mixture in the round-bottom flask was flushed with dry nitrogen for 2 h. The temperature of the oil bath was increased from room temperature to 100°C, a clear solution was formed. The solution was cooled down to room temperature and 2 mL of pyridine was added. After 10 min, 2 mL acryloyl chloride was added to the flask drop wise. The reaction mixture was cooled using an ice bath to maintain the temperature because the reaction was exothermic. A precipitate of dextran acrylate was obtained by adding 20 ml of cold ethanol to the reaction mixture. Dextran acrylate was characterized by NMR and FTIR after being purified by washing four times with cold ethanol and dried under the vacuum at 40°C for 2 days.

In this work, all hydrogels were prepared using AESO (purchased from Sigma Aldrich) and Dex-A (synthesized according to the reaction conditions described in Table I, entry 10). Dex-A and AESO (40% w/v) were reacted in DMSO at various mass ratios (Dex-A/AESO at 10/90, 20/80, 30/70, 40/60, and 50/50) by free radical photo-polymerization. 5% w/w of photo-initiator 2,2-dimethoxy 2-phenyl acetophenone in respect to the total amount of Dex-A and AESO was added and mixed thoroughly. 0.5 mL of the mixtures were added into flat bottom glass vials, irradiated by high intensity UV lamp (DYMAX UV/visible light Flood curing lamps Model 2000 and 400 watt PC power supply, with High intensity flood, 365nm wavelength) at room temperature for 15 min. The mixtures solidified into gels with the thickness about 3–5 mm. Hydrogels were obtained and washed with ethanol and deionized water for 12 h at room temperature to remove unreacted acrylate epoxidized soybean oil, dextran acrylate and DMSO solvent. The hydrogels were dried in the vacuum oven for 3 days at room temperature, then characterized by TGA, SEM and FTIR, then used for swelling and drug release studies.

Characterizations of Polymers and Hydrogels

^{1}H NMR and ^{13}C NMR spectra of dextran and dextran acrylates were recorded using Bruker 400 MHz machine and DMSO-d_6 as the solvent. MnovaLITE software was used to analyze the



Scheme 1. Synthesis of hydrogels from dextran and acrylate epoxidized soybean oil.

spectra and obtain the degree of acrylation using the peaks of vinyl protons (δ 6.28−5.85 ppm) and the anomeric proton (δ 5–4.4 ppm) in dextran acrylates. The peak for DMSO-d_6 was at δ 2.5 ppm which was used as a reference. FTIR spectra of dextran, dextran acrylates and hydrogels were recorded on a PerkinElmer spectrum 100. A scan range of 450 to 4000 cm^{-1}

Table I. The Synthesis of Dextran Acrylates

Entry no.	Dextran (g)	Acryloyl chloride/ anhydroglucose (molar ratio)	Pyridine/ anhydroglucose (molar ratio)	DMF solvent (ml)	Reactem. (°C)	Reaction time (h)	Ratio of peak intensity (FTIR peak, cm^{-1})	
							Peaks at 3027/3400	Peaks at 1733/3400
1	1	2 : 1	2 : 1	40	15	3	0.316	0.218
2	1	3 : 1	3 : 1	40	15	3	0.354	0.266
3	1	6 : 1	6 : 1	40	15	3	0.359	0.298
4	1	6 : 1	6 : 1	20	15	3	0.551	0.523
5	1	6 : 1	6 : 1	40	15	6	0.348	0.275
6	1	6 : 1	6 : 1	40	15	18	0.412	0.318
7	3	6 : 1	6 : 1	40	15	3	0.754	0.627
8	2	3 : 1	3 : 1	40	15	3	0.573	0.523
9	2	3 : 1	3 : 1	40	0	3	0.589	0.541
10	2	1 : 1	1 : 1	40	0	3	0.495	0.411
11	2	4 : 1	4 : 1	40	0	3	0.688	0.676
12	2	6 : 1	6 : 1	40	0	3	0.839	0.823

The effect of reaction conditions on the degree of acrylation, analysed by FTIR.

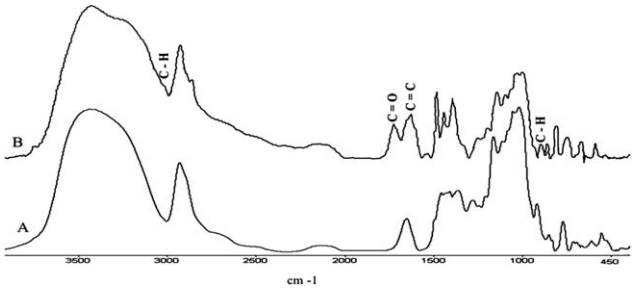


Figure 1. Typical FTIR spectra of dextran (A) and dextran acrylate (B).

was used. The samples were prepared by mixing the polymers with KBr in the ratio of 1/30 w/w and compressed to form a disc. The thermal stability of the hydrogel samples was measured using a SDT Q600 V4.1 Build 59 Module DSC-TGA machine under nitrogen atmosphere. Generally, about 20 mg of a hydrogel sample was heated from 30 to 800°C at a heating rate of 5°C per minute and the mass loss was recorded as a function of temperature. For SEM analysis, the gels were cut in half and coated at both the top surface and bottom surface with a layer of gold using a Polaron E5000 SEM coating unit for 210 s. The gold coated gels were then attached to an agar aluminum stub with an adhesive carbon disc and the SEM images were taken using a Hitachi S-520 scanning electron microscope.

Swelling Test

Dry hydrogels were weighed individually and immersed in 15 mL of deionized water (pH 7) for the time required, the surface water was removed using a paper towel and weighed quickly. The swelling ratios were calculated using the following equation.

$$\text{Swelling ratio} = (W_s - W_\theta)/W_\theta$$

where W_s is the mass of hydrogels after swelling, W_θ is the mass of dried hydrogels. The experiments were performed in triplicate.

Release Studies

Carmoisine E122 (red food coloring) and Lysozyme were used as the model drug molecules for the release studies. Hydrogels with different compositions were prepared according to procedures described in the previous sections. For the release studies of Carmoisine E122 (red food coloring), the dry hydrogels were loaded with 200 μL of 1.5 mg mL^{-1} stock solution and kept in the vacuum oven for 48 h at 20°C. The loaded hydrogels were removed from the oven and washed gently to remove surface dye, then they were placed into 3 mL deionized water. The gels were left for a given time to release Carmoisine E122 red food coloring. At each time point, 0.5 mL solution was removed after gentle shaking and the same volume of fresh deionized water was added. The absorbance was measured at 519 nm, and the concentration of Carmoisine (red food coloring) in the release sample was determined according to the standard calibration curve. All experiments were performed in duplicate. To prepare Carmoisine E122 (red food coloring) standards, a 1.5 mg mL^{-1} stock solution was prepared by adding 0.0015 g of Carmoisine into a glass vial and dissolved in 1 mL of deionized water. The Carmoisine was calibrated by preparing five standards at concentrations ranging from 0.00117 to 0.0187 mg mL^{-1}. The samples were prepared by progressive dilution.

For the release studies of proteins, a Lysozyme stock solution was prepared by dissolving 0.5 g Lysozyme in 2 mL deionized water. Before loading Lysozyme into hydrogels, each hydrogel was vacuum dried for 48 h. Nearly 200 μL of Lysozyme stock solution was then added to each hydrogel and placed in the vacuum oven and dried at 20°C for 48 h. The Lysozyme loaded hydrogels were then removed from the oven, washed gently with deionized water and then placed in 1 mL deionized water. The gels were left for a given time, at each time point 100 μL of solutions containing Lysozyme was pipetted out of the vial after gentle shaking and added into 5.0 mL of diluted dye reagent. 100 μL of fresh deionized water was added into the vial. The absorbance was measured at 595 nm, and the concentration of Lysozyme in the release sample was determined with reference to a standard calibration curve. All experiments were performed

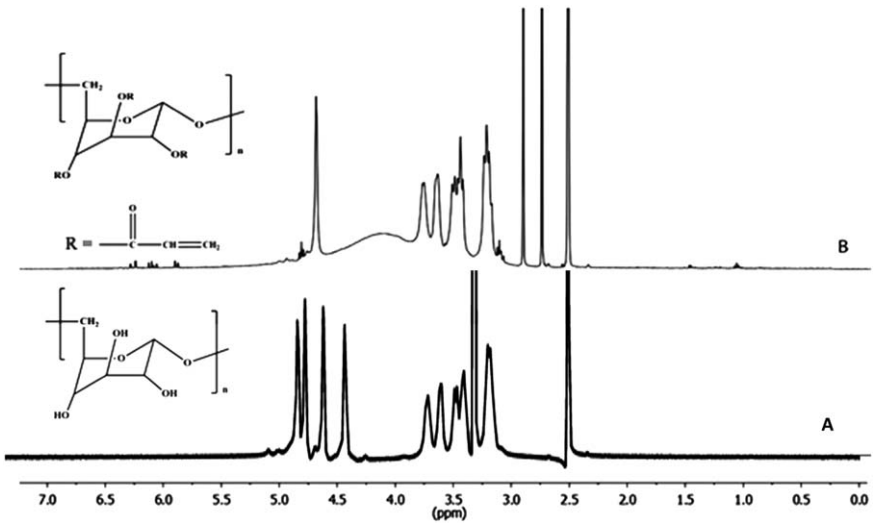


Figure 2. Typical ^{1}H NMR spectra of dextran (A) and dextran acrylate (B).

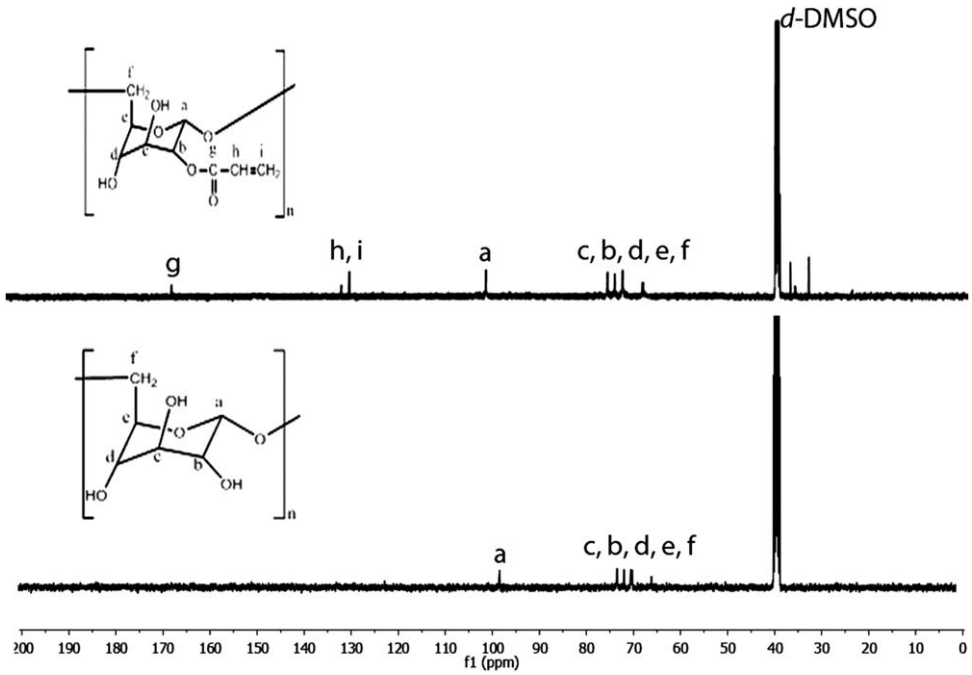


Figure 3. Typical ^{13}C NMR spectra of dextran and dextran acrylate.

in duplicate. For protein analysis, bovine serum albumin from Bio-Rad assay kit was used to establish the standard calibration curve. Briefly, according to the protocols provided by the supplier, 20 mL of deionized water were placed into the standard container and mixed until dissolved. Four diluted protein standards were prepared at concentrations of 0.2, 0.4, 0.6, and 0.8 mg mL^{-1}. For the preparation of the dye reagent, dilute one part reagent to four parts deionized water and filter to remove all particulates. About 100 μL of protein standard was added into a clean dry test tube and 5.0 mL of diluted dye reagent was added to the protein standard and incubated at room temperature for 5 min. The sample should incubate at room temperature for no more than 1 h. The absorbance was measured at 595 nm.

RESULTS AND DISCUSSION

Synthesis and Characterization of Dextran Acrylates

Dextran acrylates were synthesized as described in the experimental section. Zhang et al.[13] synthesized a series of dextran acrylates with different degrees of conversion of hydroxyl groups to vinyl groups by changing the ratio of the reactants, the reaction time, and the temperature. To demonstrate the successful acrylation and to determine the degree of acrylation, FTIR and NMR techniques were used to characterize the resultant polymers. Our experimental results on the synthesis and characterization of dextran acrylates (Table I) are in good agreement with those obtained by Zhang et al.

Comparing the FTIR spectra of the dextran (A) and dextran acrylate (B) (Figure 1), a new strong peak appears at 1733 cm^{-1} in dextran acrylate, confirming the carbonyl group stretch —CO—CH=CH_2, a shoulder peak is also present at 3027 cm^{-1} representing a C—H stretching vibration of the vinyl group in dextran acrylate. Also, the peak for C=C stretching bond in dextran acrylate was found at 1665 cm^{-1}. A small absorption peak at 875 cm^{-1} in dextran acrylate was identified as a vinyl group. The hydroxyl group band (3000–3800 cm^{-1}) from dextran acrylate was broadened due to the incorporation of the acrylate group. Therefore, the ratios of the peak intensities at 1733 cm^{-1} with 3400 cm^{-1} and at 3027 cm^{-1} with 3400 cm^{-1} can be used to represent the degree of acrylation.[13] The higher the intensity ratios of 1733 cm^{-1}/3400 cm^{-1} and 3027 cm^{-1}/3400 cm^{-1}, the higher degree of acrylation of the dextran acrylates in question. The FTIR data (Table I) shows more hydroxyl groups were converted to vinyl groups by increasing the molar ratio of pyridine/acryloyl chloride (entries 1, 2, and 3 in Table I). The FTIR data for entries 3 and 4 in Table I show that less DMF solvent led to an increase in the intensity of the peak ratio at 3027/3400 and at 1733/3400, i.e., a

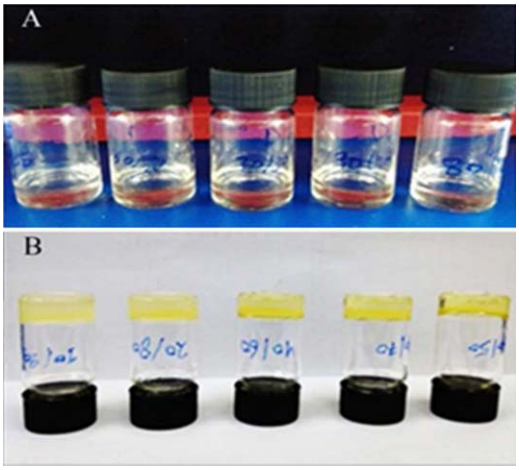


Figure 4. Synthesis of hydrogels from Dex-A and AESO in DMSO at compositions of 10/90, 20/80, 30/70, 40/60, and 50/50 before (A) and after (B) photo-crosslinking. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

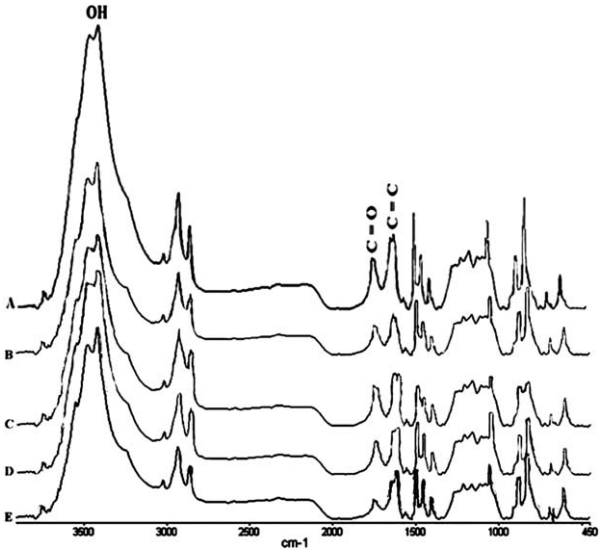


Figure 5. FT-IR spectra of hydrogels prepared from photo-crosslinking of Dex-A and AESO at different compositions: (A) 10/90, (B) 20/80, (C) 30/70, (D) 40/60, (E) 50/50. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

higher degree of acrylation. The FTIR data analysis for entries 5 and 6 in Table I demonstrated that increasing the reaction time resulted in an increase in the intensity peak ratio at 1733/3400 (i.e., an increase in C═O group) and the intensity of the peak ratio from 3027/3400 (i.e., an increase in the C═C group). Comparing entries 8 and 9 in Table I, it showed that a lower reaction temperature led to an increase in the intensity of the peak ratio at 3027/3400 and at 1733/3400, i.e. a higher acrylation degree. Moreover, the data in Table I also show that while the level of acrylate incorporation in dextran acrylates produced at 15°C levelled off as the molar excess of acryloyl chloride and pyridine to anhydroglucose units increased from 2 : 1 to 6 : 1, the level of acrylate incorporation in dextran acrylates produced at 0°C increased linearly as the ratio of acryloyl chloride and pyridine to anhydroglucose units increased from 1 : 1 to 6 : 1. These observations indicated a higher acylation efficiency using acryloyl chloride at 0°C than at 15°C. This could be attributed to an apparent higher reactivity and stability of acrylate chloride at 0°C than at 15°C, thus less side reactions occurred.

Comparing ^{1}H NMR spectra (Figure 2) of the dextran (spectrum A) and dextran acrylate (spectrum B), the proton peaks in

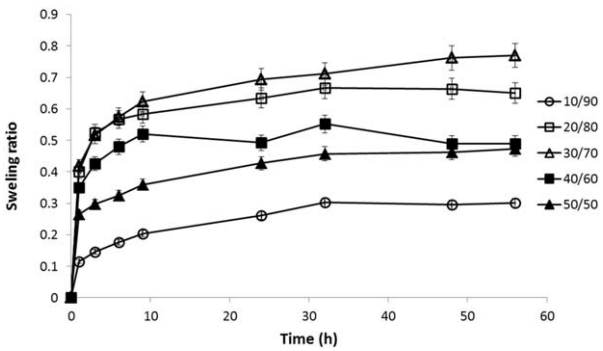


Figure 6. Swelling of hydrogels with different compositions of Dex-A and AESO (10/90, 20/80, 30/70, 40/60, 50/50).

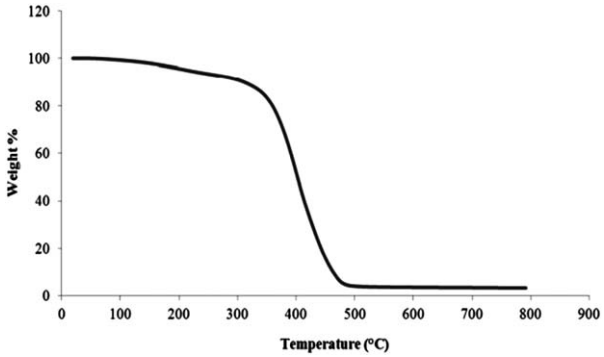


Figure 7. A typical TGA curve of hydrogels prepared from photo-crosslinking of Dex-A and AESO.

acrylic groups (—CH═CH_2) of the dextran acrylate appeared in the δ 6.28−5.85 ppm range, these peaks were not present in the dextran. The peaks in the region of δ 5–4.4 ppm represented the anomeric proton and three hydroxyl protons in the anhydroglucose unit. The hydroxyl protons are not observed in Figure 2(B) due to proton exchange in the relatively less pure product of acrylation. The ^{13}C NMR spectra of dextran acrylate further confirmed the vinyl groups present in dextran acrylate (Figure 3). The carbon of carbonyl (part of the ester linkage) was found at 167 ppm (peak g in Figure 3), and the carbons from C═C in the acrylate dextran were found at 131 and 132 ppm (peak h and peak i in Figure 3), the carbon peaks of dextran are shown at 71, 75, 77, 78, and 103 ppm. The degree of acrylation for the polymers prepared in this work was calculated by ^{1}H NMR to be about one acrylic group in every 50 anhydroglucose units.

Preparation of Hydrogels by UV Photo-Polymerization

The photo-crosslinking reactions of the dextran acrylate (sample 10 in Table I) with acrylate epoxidized soybean oil were conducted in DMSO and initiated by 2,2-dimethoxy 2-phenyl acetophenone upon irradiation with UV–visible light at room temperature (19°C) (scheme 1). It was observed that the temperature of the atmosphere was increased to 60°C during gelation reactions. Hydrogels were prepared at the composition of dextran acrylate/ acrylate epoxidized soybean oil as 10/90, 20/80, 30/70, 40/60, and 50/50 (Figure 4). The successful incorporation of dextran into acrylate epoxidized soybean oil was demonstrated by FT-IR analysis of the hydrogels before and after photo-crosslinking. As shown in Figure 5, the relative absorption intensity at 1742 cm^{-1} (representing the C═O) and at 1635 cm^{-1} (representing the C═C) varies for the gels with different compositions. It shows that increasing dextran acrylate in the hydrogels led to a decreased absorption at 1742 cm^{-1} and increased absorption at 1635 cm^{-1}. This could indicate a decreased crosslinking density. The maximum absorption peak for C═O and minimum absorption peak for C═C are found for the hydrogel with 30/70 (Dex-A/AESO) composition, indicating a high crosslinking density in this hydrogel.

Swelling Test of Hydrogels

The swelling test data for Dex-A/AESO hydrogels, at the five compositions of 10/90, 20/80, 30/70, 40/60, and 50/50, in deionized water (pH 7) are shown in Figure 6. The swelling behavior

Table II. Thermal Stability of Hydrogels Prepared by UV Photo-Crosslinking

Hydrogels composition (Dex-A/AESO)	Weight loss (wt %)			5% weight loss temperature (°C)
	≤105°C	105–300°C	305–470°C	
10/90	Stable	5.89	86.10	231.29
20/80	Stable	6.45	86.43	250.36
30/70	Stable	5.87	84.06	251.91
40/60	Stable	8.74	82.47	214.93
50/50	Stable	8.10	83.27	195.93

of hydrogels is related to the functionality, the balance of hydrophobic and hydrophilic properties, and the crosslinking density of the polymer networks. In Dex-A/AESO hydrogels, the AESO is the hydrophobic part, while the Dex-A is the hydrophilic part of the copolymer network. The hydrogel with 10/90 (Dex-A/AESO) composition shows the lowest swelling but with an excellent structural integrity during the test because it has a high hydrophobic content of AESO (90%) thus a high crosslinking density. By increasing the hydrophilic Dex-A in the hydrogel to 20% and 30%, the swelling ratios of the hydrogels increased. However, increasing the hydrophilic Dex-A further to 40 and 50%, the swelling ratios decreased slightly. Overall, the hydrogel 30/70 (Dex-A/AESO) showed the highest swelling ratio at about 0.8. This could be the result of the combined effect of the crosslinking density and the balance of the hydrophobicity and hydrophilicity of the hydrogels. The hydrogels prepared in this work contain hydrophobic soybean oil more than 50%. Therefore, the swelling ratio of the hydrogels is not high comparing to other more hydrophilic hydrogels reported in the literature. The application of the hydrogels with a low swelling ratio can be used for the fabrication of biomedical devices as they normally have better mechanical properties than the hydrogels with a high swell ratio. In addition, some hard tissues require good mechanical properties and a low swelling. Therefore, this type of hydrogels with a low swelling ratio could also find the applications in tissue engineering and drug delivery.

Thermal Gravimetric Analysis (TGA)

TGA was used to evaluate the thermal stability of the hydrogels under a nitrogen atmosphere.[23] A typical TGA curve obtained is shown in Figure 7 and the data are also summarized in Table II. The results show that all hydrogels were thermally stable at temperature below 105°C. The mass loss of the hydrogels was observed at two temperature regions (105–300 and 305–470°C). The hydrogels with 30/70 and 10/90 compositions showed higher thermal stability at the temperatures between 105 and 300°C which could be due to their more compact structure compared to other compositions. However, hydrogels with 40/60 and 50/50 compositions showed a higher thermal stability at the temperatures between 305 and 470°C. The hydrogels with compositions 20/80 and 30/70 have the highest 5% mass lost temperature at about 250°C.

Release Studies from UV Photo-Crosslinked Hydrogels

Carmoisine E122 (red food coloring) and Lysozyme were used as the model drug molecules to perform the release studies on the photo-crosslinked Dex-A/AESO hydrogels. Carmoisine is an azo-type organic dye compound with a molar mass of 502 Da, Lysozyme is a protein biopolymer with 129 amino acids linked together by peptide bonds and its molar mass is 14,307 Da. Therefore, the size and hydrodynamic volumes of Carmoisine and Lysozyme can mimic small bioactive molecules and large therapeutic biopolymers respectively. The amounts of Carmoisine E122 (red food coloring) released from hydrogels with compositions of 10/90, 20/80, 30/70, 40/60, and 50/50 at 20°C was determined using the standard calibration curve established. For example, Carmoisine release during 1 h for the hydrogel 10/90 sample gave an average reading of 0.697 absorption and this can be used with the gradient equation derived from the Carmoisine E122 (red food coloring) calibration curve. The equation for the Carmoisine E122 calibration gradient was calculated at $y = 30.836x - 0.0236$. The equation can be rearranged

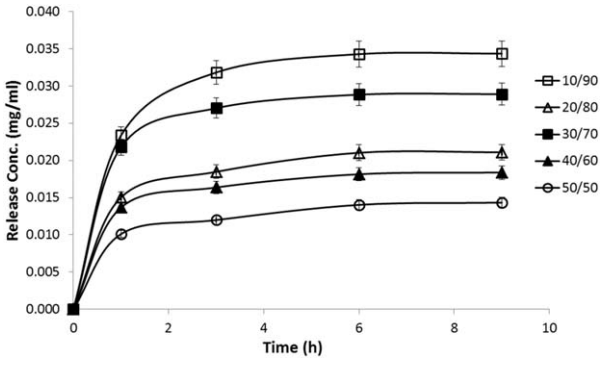


Figure 8. Carmoisine E122 (red food coloring) release from hydrogels with different compositions of Dex-A and AESO (10/90, 20/80, 30/70, 40/60, 50/50).

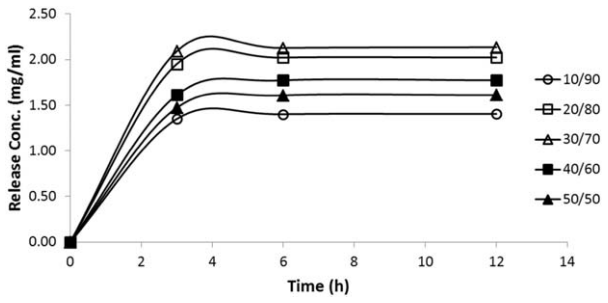


Figure 9. Protein lysozyme release from hydrogels with different compositions of Dex-A and AESO (10/90, 20/80, 30/70, 40/60, 50/50).

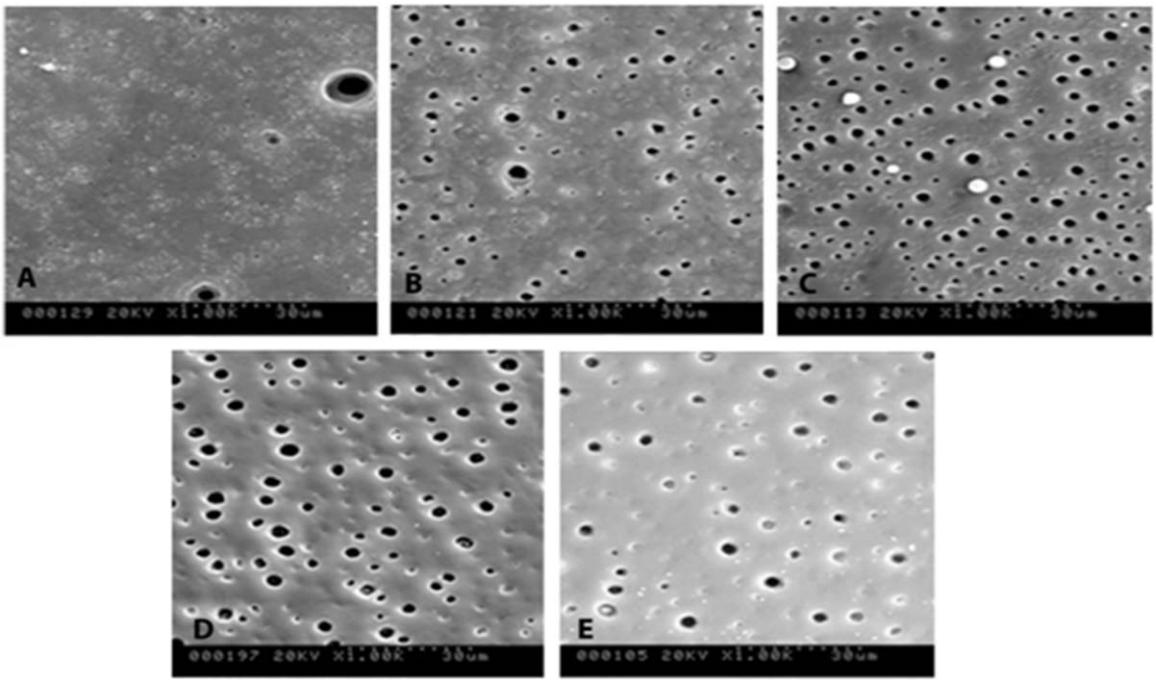


Figure 10. SEM images of photo-crosslinked hydrogels with different compositions of Dex-A and AESO. (A) 50/50; (B) 40/60; (C) 30/70; (D) 20/80; (E) 10/90. (×1000 magnification).

to the form $x = (y - c)/m$ to make x the subject and calculate the concentration of the unknown.

$$\text{Released Carmoisine} = \frac{0.697 + 0.0236}{30.836} = 0.0233 \text{ mg/ml}$$

Figure 8 shows that the release of Carmoisine from hydrogels with different compositions all reached to a plateau at about 6 h, but their release rates and extents are different. In general, increasing the dextran acrylate in the hydrogels resulted in a decrease in the release rate, for example the hydrogel with composition 10/90 shows the highest release and 50/50 shows the lowest release. The amount of protein Lysozyme released was calculated using the standard calibration curve of the protein. Comparing to Carmoisine E122 release studies, the release of Lysozyme from all hydrogels also reached to a plateau at about 6 h. Figure 9 shows the Lysozyme release from hydrogels at 20°C. In general, increasing the dextran acrylate in the hydrogels resulted in an increase of protein release. The hydrogel with 30/70 composition showed the highest release of proteins and 10/90 shows the lowest release. This is different from the observation for Carmoisine release. This result could be due to the combined effect of cross-linking density, the balance of hydrophilicity and hydrophobicity of the hydrogel and the interaction of the guest molecules with the host polymers. Moreover, the release behaviors are directly related to the size (hydrodynamic volume) of the guest drug molecular. As the hydrogel swells, the mesh size increases and the drug can penetrate into and diffuse out of the hydrogel more easily. Properties of the hydrogels, for example hydration, surface area to volume ratio and ionic interaction between the guest molecules and the hydrogels also affect the rate of diffusion.[24]

Table III. Pore Size and Porosity of Hydrogels Prepared by UV Photo-Crosslinking

Hydrogel composition (Dex-A/AESO)	Average pore sizes (~μm)	Porosity percentage (%)
10/90	3.26 ± 0.81	3.27 ± 0.58
20/80	4.19 ± 0.33	4.44 ± 0.40
30/70	3.80 ± 0.52	10.65 ± 0.62
40/60	3.05 ± 0.56	3.76 ± 0.45
50/50	2.07 ± 0.83	5.28 ± 0.81

The Morphology of Photo-Crosslinked Hydrogels

The morphology of hydrogels (pore size and porosity) is an important factor in determining the gel performance in tissue engineering and drug delivery applications.[25] In this work, pore size and porosity of photo-crosslinked Dex-A/AESO gels were determined using SEM analysis by JMicrovision software and normalized to the number of pores present in a viewing field (porosity). By this method, the porosity is determined by the area of the material versus the area of the pores visible in SEM images, and the pore size is the average diameter of the pores in the viewing area. Top surface structures of hydrogels consisting of Dex-A and AESO with different ratios, are shown in Figure 10. The surface of the hydrogels shows large pores with different size and porosity for hydrogels with different compositions. Generally, with increasing dextran acrylate content, the pore size on the surface increased. For example in the hydrogel with composition of 10/90 [Figure 10(E)] the average pore size is ~3.26 μm, but the percentage porosity is equal to ~3.27%. For the 20/80 hydrogel [Figure 10(D)], the average pore size increased to ~4.19 μm and the

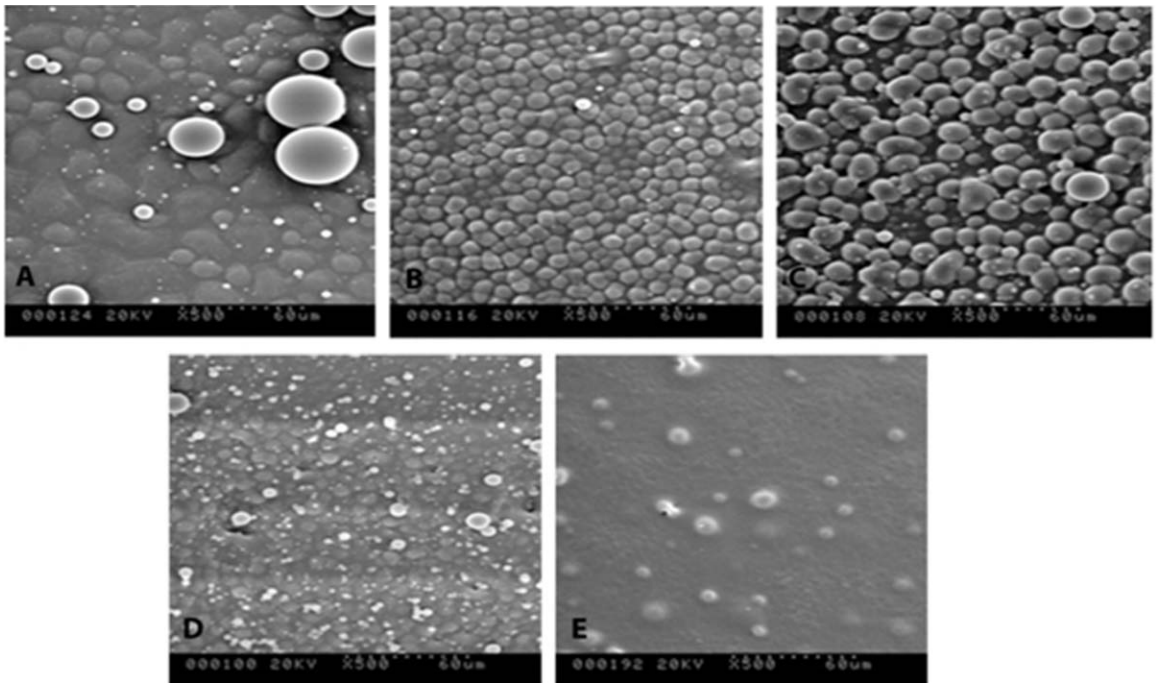


Figure 11. SEM images of the bottom surfaces of hydrogels at different compositions of Dex-A and AESO. (A) 50/50; (B) 40/60; (C) 30/70; (D) 20/80; (E) 10/90. (×500 magnification).

percentage of porosity also increased to ~4.44%. For the hydrogel with 30/70 composition [Figure 10(C)], the average pore size decreased to ~3.8 μm, but the percentage of porosity increased to ~10.65% i.e. the number of the pores presents as a percentage of the total viewing field. In the 40/60 composition hydrogel [Figure 10(B)], the average pore size was ~3.05 μm and the percentage of porosity was equal to ~3.76%. In the 50/50 composition hydrogel [Figure 10(A)], the average pore size was equal to ~2.07 μm and the porosity percentage was equal to ~5.28%. Table III shows the summarized pore size and porosity percentage of hydrogels prepared by UV photo-crosslinking at different compositions. SEM results showed that the hydrogel with 30/70 composition has the highest porosity, while this hydrogel showed the highest swelling compared to all the other hydrogels.

The bottom surfaces (Figure 11) of hydrogels with different compositions were also examined. While the top surface of the hydrogel showed pores at a magnification of 1000× (Figure 10), the bottom of the hydrogels (Figure 11) does not show any visible pores. One possible reason for the absence of pores on the bottom surface is because the solvent escaped upwards to form the pores on the top surface but left the bottom surfaces without any pores during the drying procedures. Another possible reason is due to the lack of penetration of UV through the depth of the hydrogel. In other words, the efficiency of photo-crosslinking depends on the thickness of the hydrogel.

CONCLUSION

A new class of hydrogels from dextran and acrylate epoxidized soybean oil were prepared by UV photo-polymerization at compositions of 10/90, 20/80, 30/70, 40/60, and 50/50 (Dex-A/AESO). These hydrogel networks were analyzed by FT-IR and the results showed that increasing the dextran acrylate percentages from 10 to 30, resulted in an increase in the degree of cross-linking density, however, for the 40 to 50 percentage the degree of cross-linking decreased. The maximum cross-linking density occurred at the 30/70 composition of Dex-A and AESO. The swelling property of the hydrogels was examined as a function of the Dex-A/AESO ratio in deionized water (pH 7). The composition had a major influence on the swelling of the hydrogels. The swelling ratio increased with an increase in the percentage of the hydrophilic dextran acrylate and it reached equilibrium faster. The swelling study showed that the hydrogel with 30/70 (Dex-A/AESO) composition had the highest swelling. All hydrogels prepared demonstrated good thermal stability at temperatures below 105°C. Carmoisine E122 (red food coloring) and the protein Lysozyme were used as the model drug molecules to examine the release behaviors from hydrogels at room temperature. For the Carmoisine release study the hydrogel with 10/90 (Dex-A/AESO) composition demonstrated a higher release rate. However, when using protein Lysozyme the hydrogel with the 30/70 (Dex-A/AESO) composition demonstrated a higher release rate. This could be attributed to the size (hydrodynamic volume) of the guest molecules and the interactions of the guest molecules with the host polymers. The morphology of the hydrogels was investigated by SEM. The images showed clear differences in morphology between the top and bottom surfaces of hydrogels. Pore size and porosity were significantly influenced by the composition of the hydrogels. The largest pore sizes were observed for the hydrogel with 20/80 composition, but the highest percentage of porosity was observed for the hydrogel with 30/70 composition.

ACKNOWLEDGMENTS

The HCDP programme of the Ministry of Higher Education and Scientific Research of the Kurdistan Region Government—Iraq is

thanked for providing financial support of the Master studentship to Rebaz A. Omer. Dr Andreas Endruweit from the University of Nottingham is acknowledged for kindly lending the UV curing equipment.

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