Journal of Applied Polymer Science

Smart Carboxymethylchitosan Hydrogels that have Thermo- and pH-Responsive Properties

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ABSTRACT: Thermo-responsive poly(*N*-isopropylacrylamide) (poly(NIPAAm)) and pH-responsive poly(*N*,*N*′-diethylaminoethyl methacrylate) (poly(DEAEMA)) polymers were grafted to carboxymethylchitosan (CMC) via radical polymerization to form highly water swellable hydrogels with dual responsive properties. Ratios of CMC, NIPAAm to DEAEMA used in the reactions were finely adjusted such that the thermo and pH responsiveness of the hydrogels was retained. Scanning electron microscopy (SEM) indicated the formation of an internal porous structure for the swollen CMC hydrogels upon incorporation of poly(NIPAAm) and poly(DEAEMA). Effect of temperature and pH changes on water swelling properties of the hydrogels was investigated. It was found that the water swelling of the hydrogels was enhanced when the solution pH was under basic conditions (pH 11) or the temperature was below its lower critical solution temperature (LCST). These responsive properties can be used to regulate releasing rate of an entrapped drug from the hydrogels, a model drug, indomethacin was used to demonstrate the release. These smart and nontoxic CMC-based hydrogels show great potential for use in controlled drug release applications with controllable on-off switch properties. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41505.

KEYWORDS: crosslinking; films; functionalization of polymers; hydrophilic polymers; radical polymerization

Received 6 June 2014; accepted 5 September 2014

DOI: 10.1002/app.41505

INTRODUCTION

Presently, great interest has been paid to stimuli-responsive hydrogels, which can sense and respond to various changes in external stimuli, such as; temperature, light, pH, magnetic, or electric fields and ionic strength.[1–4] Stimuli-responsive hydrogels, also called "intelligent" or "smart" hydrogels, show strong swelling responses at their phase transitions. They are usually in the form of hydrophilic polymeric networks that can swell in water and contain high water content while maintaining their structure. As hydrogels possess water in their structure this enable them to easily uptake hydrophilic drugs into the matrix. One of the key features of these smart hydrogels is they can control the releasing rate of the drug by changing their structure and swelling properties in response to a change in their environmental or an external stimuli.[5]

Carboxymethylchitosan (CMC) is usually prepared via a carboxymethylation reaction of a small portion of the amino and primary hydroxyl groups of the glucosamine unit on the chitosan chain with monochloroacetic acid[6,7] giving rise to a water-soluble chitosan derivative. CMC has been widely studied because of its ease of synthesis, ampholytic character and the potential for use in a wide range of applications. CMC has already been used extensively in various biomedical applications due to its unique properties such as; antibacterial activity, noncytotoxicity, excellent biocompatibility and high water solubility.[8–10] Synthesis of smart CMC-based hydrogels has also been studied, for example, poly(*N*-isopropylacylamide) (poly(NIPAAm)-grafted CMC, poly(ethylene glycol)-grafted CMC,[11] polyacrylamide-grafted CMC,[12] and poly(acrylic acid)-grafted CMC.[13]

Poly(NIPAAm) is a thermo-sensitive polymer and is one of the most studied smart polymers. Poly(NIPAAm) exhibits phase separation at a certain temperature, which is referred to as the lower critical solution temperature (LCST). Poly(NIPAAm) has a LCST of about 32°C in aqueous solution.[9,14–17] When the temperature is below its LCST, poly(NIPAAm) is hydrophilic and soluble in water. The polymer chains are well hydrated and

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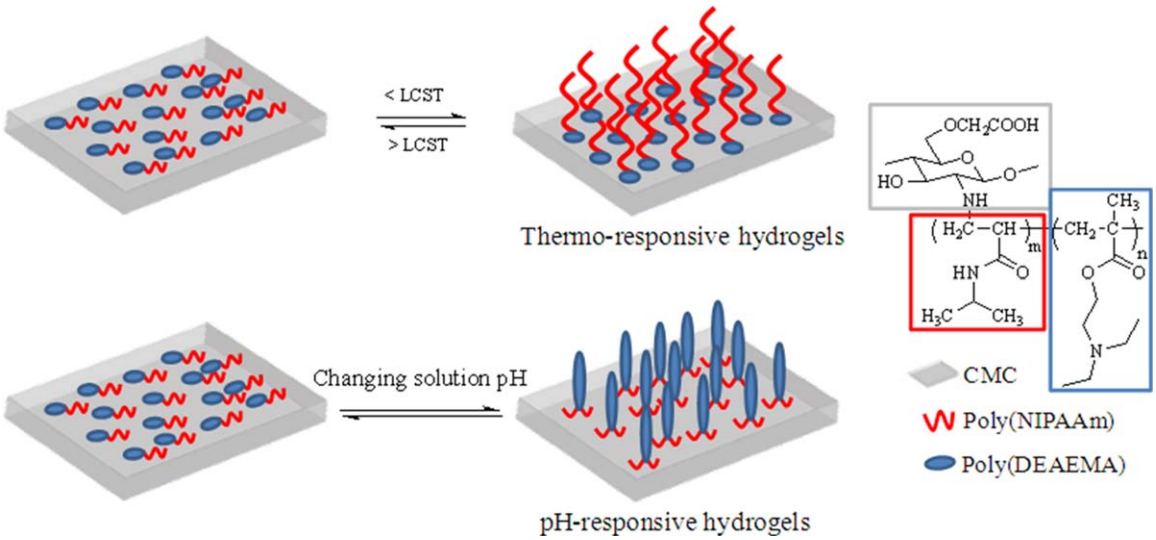

Figure 1. Modification of CMC hydrogels with thermo-responsive poly(NIPAAm) and pH-responsive poly(DEAEMA) and ideal swelling/deswelling behavior upon changing its environmental temperature and pH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

completely extended with intermolecular hydrogen bonding between the water molecules and the polymer chains. On the other hand, at a temperature higher than its LCST, the chains abruptly collapse resulting in an increase in hydrophobicity and exclusion of water, resulting in the formation of intermolecular hydrogen bonding among the polymer chains. Preparation of chitosan-based hydrogels or particles containing thermo-responsive poly(NIPAAm) have been widely reported. For example, Chuang et al. reported the preparation of chitosan-poly(NIPAAm) nanoparticle using a self-assembly method and its use in drug delivery and controlled release applications.[18] Wang et al. reported the synthesis of a series of hydrophilic chitosan's grafted with poly(NIPAAm) and the effect of chitosan's molecular weight on self-assembly behavior in order to be potentially used as an anticancer drug carrier.[19] Sun et al. synthesized chitosan-coated alginate/poly(NIPAAm) beads with good swelling and release properties for drug delivery systems in the biomedical field.[20] Synthesis of chitosan-based hydrogels containing other responsive polymers, such as poly(2-acrylamido-2-methyl-1-propanesulfonic acid) (poly(AMPS)), have also been reported.[21] However, the studies with CMC-based hydrogels and smart polymers are quite limited in general.

Another class of stimuli responsive materials is pH-responsive hydrogels, which exhibit phase transition when their environmental pH changes. They usually have weakly acidic or basic functional groups and show pH sensitivity due to the conversion of $-R_2N/-R_2NH^+$ and -COOH/-COO- when the external pH changes.[2] For instance, poly(2-(N,N-diethylamino)ethyl methacrylate) (poly(DEAEMA)) changes in structure and volume when its environmental pH is above or below ~6.5.[22,23]

The aim of this work was to chemically graft poly(NIPAAm) and poly(DEAEMA) to CMC in order to form hydrogels, this was done via radical polymerization and subsequent crosslinking to form semi-interpenetrating polymer networks (semi-IPN). The novel aspect of this work is that this is the first reported example that prepares CMC hydrogels that possess both thermo-responsive poly(NIPAAm) and pH-responsive poly(DEAEMA) in the same gel and most importantly retains satisfactory temperature and pH sensitivities of the smart polymers. Figure 1 shows a schematic of the proposed approach of the smart hydrogels.

In this article a range of properties were studied and the effect of poly(NIPAAm) and poly(DEAEMA) on each of the properties was investigated. The properties tested were: Equilibrium water content (%EWC) of the hydrogels as a function of their environmental temperature and pH. Lower critical solution temperature (LCST) indicated by the volume phase transition of the CMC hydrogels. Surface hydrophilicity of the CMC hydrogels upon addition of poly(NIPAAm) and poly (DEAEMA). Scanning electron microscopy (SEM) was used to observe the cross-sectional morphology of the fully water-swollen hydrogels after lyophilization. In addition, the releasing rate of indomethacin (an entrapped model drug) from the hydrogels was analyzed as a function of temperature and pH. MTT cytotoxicity testing was also investigated as this is an important property as one potential for use these materials is drug delivery applications in biological environments.

EXPERIMENTAL

Materials

Chitosan from crabs ($\overline{M_n}$ 1.4×10^5 g/mol) (Taming Enterprise, 98% deacetylation) was used without purification. *N*-isopropylacrylamide (NIPAAm) (Acros, 99%) was recrystallized in hexane before use to remove inhibitors. 2-(*N, N*-diethylamino)ethyl methacrylate (DEAEMA) (Sigma-Aldrich, 99%) was filtered through silica before used. Monochloroacetic acid ($ClCH_2COOH$), 99% (Acros), 1,6-hexamethylene diisocyanate (HDI)(Acros, 99%), diammoniumperoxodisulphate (APS) (Carlo Erba, 98%), sodium metabisulfate ($Na_2S_2O_4$) (Carlo Erba reagent) and indomethacin

Table I. Composition of the CMC Hydrogels and Their %G and %GE

Sample	CMC (g)	NIPAAm (g)	DEAEMA (g)	molar ratio of CMGA[a]:NIPAAm:DEAEM	%G[b]	%GE[c]
CMC	0.5	-	-	-	-	-
CND0	0.5	0.5	-	1:2:0	44.33 ± 2.11	44.33 ± 2.13
CND1	0.5	0.5	0.205	1:2:0.5	65.24 ± 1.82	46.11 ± 1.67
CND2	0.5	0.5	0.41	1:2:1	86.19 ± 1.66	47.40 ± 0.ss

[a] CMGA is carboxymethyl glucosamine unit in CMC chains. The molecular weight of CMGA is220 g/mol.
[b] %G is grafting percentage.
[c] %GE is grafting efficiency.

were used as received. All other chemicals were analytical-grade and used without purification.

Synthesis of Carboxymethylchitosan (CMC) from Chitosan

Chitosan oligomer obtained from crabs (40 g) was first immersed in isopropanol (500 mL) for 24 h and then in a NaOH solution (40.32 g, 1 mol in 100.8 mL H_2O) for a further 75 min. Monochloroacetic acid (48 g, 0.51 mol in 100 ml H_2O) was added to the swollen chitosan at 60°C with stirring for 5 h. The resultant product was precipitated in an excess of methanol, and then washed with a methanol:H_2O solution (70 : 30 and 80 : 20 v/v, respectively) to remove the salt, and finally filtered and dried at 40°C. The final product appearance was a dried yellow powder, which was characterized by FTIR.

Synthesis of Hexamethylene-1,6-di-(aminocarboxysulfonate) (HDA) as a Water-Soluble Crosslinker

1,6-Hexamethylene diisocyanate (HDI) (13.46 g, 0.08 mol) was added to sodium metabisulfite ($Na_2S_2O_5$) (16.73 g, 0.88 mol) dissolved in water (30 mL) and stirred for 24 h at room temperature. The product was precipitated in acetone and filtered. The precipitant was then re-dissolved in water, followed by filtration to remove insoluble by-product. The product was again precipitated in acetone and dried *in vacuo*.

Synthesis of CMC Hydrogels Grafted with Poly(NIPAAm-*co*-DEAEMA) Copolymer

An example of the compositions used in the synthesis of CND2 is shown in Table I. Other CMC hydrogels were prepared in a similar fashion with different amounts of reagents. CMC (0.5 g, 0.0022 mol of carboxymethyl glucosamine unit) and NIPAAm (0.5 g, 0.0044 mol) were dissolved in DI water (10 mL) with stirring under N_2 for 30 min at room temperature. After heating to 60°C, APS (0.03 g, 0.0001 mol), a radical initiator, was added to the above solution with continuous stirring for another 30 min. DEAEMA (0.41 g, 0.0022 mol) was then added as a co-monomer to the solution while still maintaining stirring for an additional 45 min. HDA crosslinker (0.05 g, 0.0001 mol) was then introduced into the solution. The mixture solution was cast on to a glass mold and dried at 30°C for 1 day. The hydrogel film was immersed in acetone to dissolve ungrafted homopolymers and unreacted monomers, and then dried at room temperature. The effect of HDA crosslinker content on the properties of CMC hydrogels has been previously reported.[27] Therefore, in this present work, 10 wt % HDA will be used in the crosslinking reaction.

Characterization

Polymers and Hydrogels. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) was conducted on a Bruker NMR spectroscopy operating at 400 MHz. Fourier transformed infrared spectroscopy (FTIR) was performed on a Perkin-Elmer Model 1600 series FTIR spectrophotometer using KBr pellets. Morphological studies of the samples were carried out through LEO 1455 VP scanning electron microscopy (SEM) with an accelerating voltage of 5 kV. To prepare the hydrogels for SEM experiments, samples were swollen in water at 10°C for 24 h and then lyophilized. The dried films were adhered to an aluminum stub and coated with gold. Grafting percentage (%G) and efficiency (%GE) were estimated by the difference in weights before and after the grafting reactions and were calculated according to the following equations:

$$\text{Grafting percentage (\%G)} = \frac{W_g - W_c}{W_c} \times 100 \quad (1)$$

$$\text{Grafting efficiency (\%GE)} = \frac{W_g - W_c}{W_m} x100 \quad (2)$$

Where W_g, W_c, and W_m are the weights of dried polymer-grafted CMC hydrogels, CMC in feed and monomers (NIPAAm and/or DEAEMA), respectively.

Water Contact Angle Measurement

Contact angles (θ) between water and the surface of the hydrogels were measured at room temperature using a sessile drop method on a ramé-hart goniometer model 200-F1. Water was dropped on the surface of the hydrogels and contact angles were quickly determined before swelling commenced. The reported values are the mean average of five different measurements.

Determination of Water Swelling Behavior

Equilibrium water content (%EWC) of the hydrogels was determined by a general gravimetric method. The dried hydrogels were precisely weighed and immersed in distilled water at a given temperature and pH. The swollen hydrogels were periodically removed from the solution and excess water on their surface was bloated off. The %EWC was calculated from the following equation;

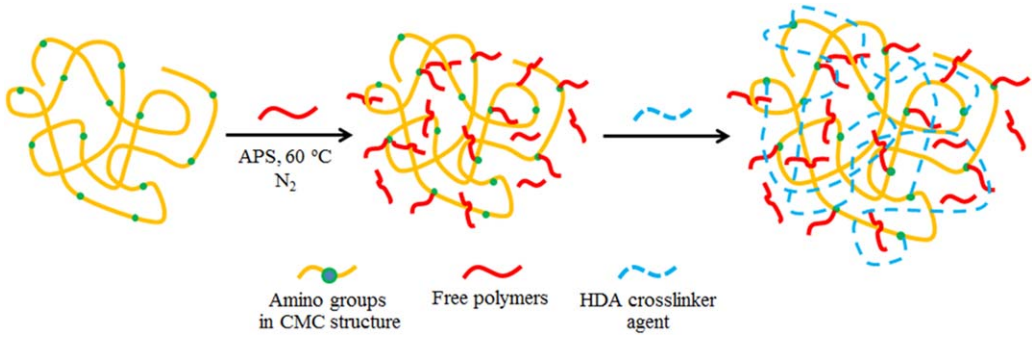

Figure 2. Schematic illustration for the formation of poly(NIPAAm-co-DEAEMA)-grafted CMC hydrogels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

$$\text{Equilibrium water content (\%EWC)} = \frac{W_s - W_d}{W_d} \times 100 \quad (3)$$

Where W_s and W_d are the weights of the swollen and dried samples, respectively.

Determination of Entrapment Efficiencies (%EE) and Drug Loading Efficiencies (%DLE)

The dried hydrogels were submerged in an indomethacin–sodium carbonate (Na_2CO_3) solution (0.500 g of indomethacin in 25 mL of saturated Na_2CO_3 solution) at 10°C for 2 days to fully swell the hydrogels and allow maximum drug uptake. Indomethacin uptake into the hydrogel was determined by measuring the UV absorbance of indomethacin in the solution before and after the swelling experiments using an UV-visible spectrophotometry at a wavelength of 320 nm.

Entrapment Efficiency (%EE) and Drug Loading Efficiency (%DLE) were calculated according to the following equations;

$$\text{\% Entrapment Efficiency (\%EE)} = \frac{\text{Weight of the entrapped drug in the hydrogel}}{\text{Weight of the loaded drug}} \times 100 \quad (4)$$

$$\text{\% Drug Loading Efficiency (\%DLE)} = \frac{\text{Weight of the entrapped drug in the hydrogel}}{\text{Weight of the dried film}} \times 100 \quad (5)$$

Studies in the *In Vitro* Drug Release Behavior

Indomethacin release behavior from the hydrogels was studied as a function of solution temperature and pH. To study the effect of the solutions pH on drug release behavior, the drug-loaded hydrogels were submerged in different buffer solutions at 25°C and at pH's of 3, 7, or 11. Similar experiments were also performed in a phosphate buffer saline (PBS) solution with pH 7.4 at 10, 30, or 50°C to study the effect of solution temperature on drug releasing profile. The drug concentration in the releasing media was periodically measured *via* UV-vis spectrophotometry (320 nm).

$$\text{\%Drug release} = \frac{\text{weight of released drug at a given time}}{\text{the total absorbed drug in hydrogel}} \times 100 \quad (6)$$

Cytotoxicity Testing

Cell culture experiments were carried out using mouse fibroblast cells. A cell suspension of 1×10^5 cells/mL L929 in minimum essential medium (MEM) was seeded in a 96-well plate and incubated at 37 ± 1°C with 5.0 ± 0.1% CO_2 and 95 ± 5% relatively humidity for 24 ± 2 h to obtain a confluent mono-layers of cells. The dried hydrogels were sterilized in an autoclave at 121°C for 15 min. A "Thermanox" (Nunc) coverslip and a polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC) were used as negative and positive control materials, respectively. After incubation, the viable cells were stained with MTT (3-(4, 5-dimethylthiosol-2-yl) 2, 5-diphenyltetrazolium bromide) and incubated for another 2 h. Then, MTT was removed and dimethylsulfoxide (DMSO) was added in each well. Finally, the absorbance was measured using a microplate reader at a wavelength of 570 nm.

RESULTS AND DISCUSSION

The primary aim of this work was to synthesize dual responsive CMC hydrogels containing thermo-responsive poly(NIPAAm) and pH-responsive poly(DEAEMA) polymers. Previous researchers have reported the preparation of chitosan hydrogels containing thermo- and/or pH-responsive properties.[24–26] However, the limited solubility of chitosan in water and common organic solvents have inhibited extensive studies and utilization, especially for biomedical applications. In the work described here we have reported a novel smart CMC-based hydrogel crosslinked with a water-soluble crosslinking agent to form a stable semi-IPN. Amino functional groups in CMC actively reacted with aminocarboxysulfonate groups of HDA to form urea linkages.[27–29] The originality of this work is that we here demonstrate a simple and efficient process for preparing highly water-swollen and stable CMC hydrogels that sense changes in their environment. Incorporation of poly (NIPAAm) and poly(DEAEMA) into the CMC semi-IPN provided dual responsive properties to the hydrogels (Figure 1).

It should be noted that ungrafted poly(NIPAAm) and poly (DEAEMA) homopolymers and the copolymers might also be formed due to the existence of ammonium sulfate radicals in the solution, which served as free radical initiators in the solution. To form the hydrogels, these polymers were left in the solution without extraction in order to simplify the hydrogel preparation process. These polymers were thus embedded in poly(NIPAAm-*co*-DEAEMA)-grafted CMC using a water-soluble HDA crosslinker to form semi-IPN. Therefore, it was envisioned that ungrafted free polymers that might exist in the solution were physically locked in the CMC hydrogels without

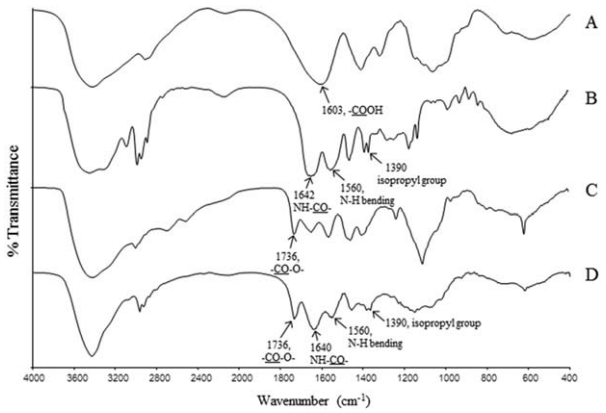

Figure 3. FTIR spectra of (A) CMC, (B) poly(NIPAAm), (C) poly (DEAEMA) (D) poly(NIPAAm-*co*-DEAEMA)-grafted CMC hydrogel (CND2 hydrogel)

covalent bonding. Figure 2 shows schematic illustration for the formation of poly(NIPAAm-*co*-DEAEMA)-grafted CMC hydrogels in the presence of the free polymers interlocked in the structure.

To confirm that the polymerization of poly(NIPAAm-*co*-DEAEMA) was also initiated from CMC chains, polymerization of poly(NIPAAm-*co*-DEAEMA) in the presence of CMC was prepared, followed by repetitive extractions of the ungrafted polymers and unreacted monomers from CMC without cross-linking reaction step. ^{1}H-NMR spectrum of the product shown in the supporting information exhibits characteristic signals of poly(NIPAAm-co-DEAEMA); 1.18 ppm of methyl protons of NIPAAm ($CHC\underline{H}_3$) and DEAEMA ($CH_2C\underline{H}_3$) and 1.5−2.2 ppm of methylene and methine protons in poly(NIPAAm) and poly (DEAEMA) backbones (Supporting Information Figure S1). The presence of these newly formed signals indicates that polymerization of poly(NIPAAm-*co*-DEAEMA) was initiated from CMC and covalently grafted on CMC chains.

Characterization of Poly(NIPAAm-*co*-DEAEMA)-Grafted CMC Hydrogels

To synthesize the smart hydrogels, poly(NIPAAm) was first initiated from CMC chains via radical polymerization using APS as an initiator. Amine radicals (•NH-) can be formed on CMC chains in a mild condition at 60°C, giving rise to radical initiating sites for poly(NIPAAm) grafted on CMC. In the case of CND0, the reaction was terminated after 30 min, without subsequent addition of DEAEMA monomer. In the cases of CND1 and CND2 (Table I), suitable amounts of DEAEMA monomer was sequentially introduced to the mixtures and the polymerization continued for another 45 min, leading to the formation of poly (NIPAAm-*co*-DEAEMA)-grafted CMC hydrogels. Grafting

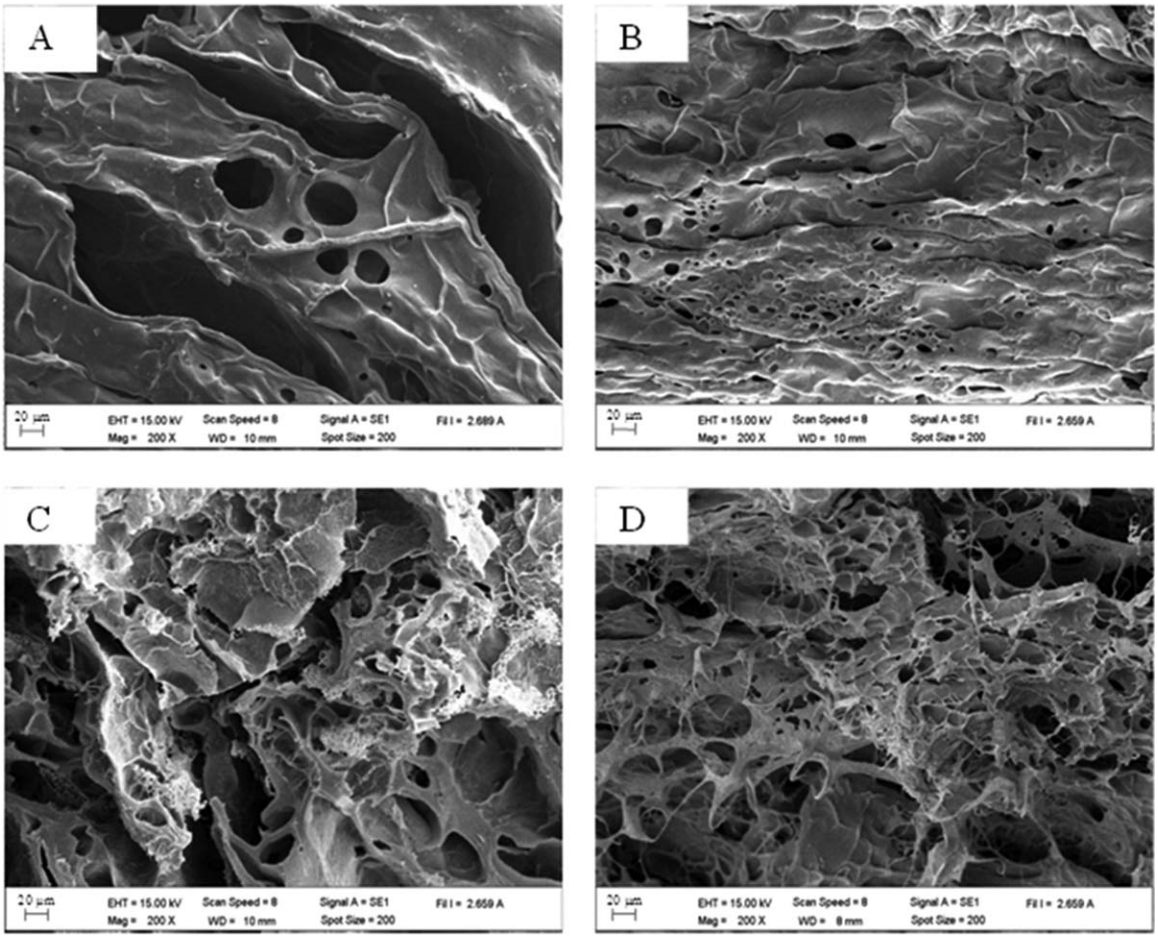

Figure 4. Cross-sectional morphologies of (A) CMC, (B) CND0, (C) CND1 and (D) CND2 hydrogels

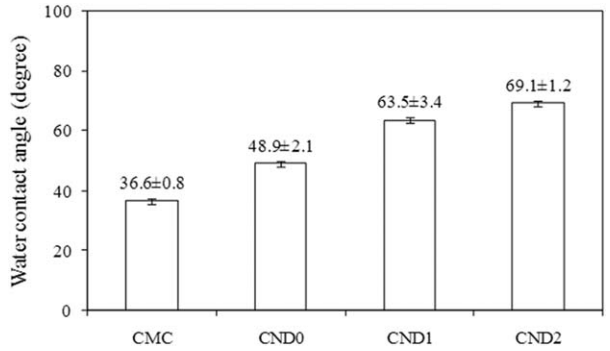

Figure 5. Water contact angles of CMC, CND0, CND1 and CND2 hydrogels

efficiencies (%GE) of poly(NIPAAm-*co*-DEAEMA) copolymers ranged between 44% and 48%, while their grafting percentages (%*G*) nn the CMC chains were in the range of 44–86% (Table I). It was found that both %GE and %*G* increased when poly (DEAEMA) was present in the hydrogels (CND1 and CND2 as compared with CND0). This was attributed to the lengthening of the grafted polymers on the CMC chains as the reaction time was extended. The increase in DEAEMA loading in the reaction (CND2) also enhanced both the %*G* and %GE of the hydrogels.

The functional groups of CND2 were characterized by FTIR (Figure 3). Poly(NIPAAm) and poly(DEAEMA) homopolymers were separately synthesized for analysis of the functional groups of each homopolymer [Figure 3(B,C)]. FTIR spectrum of CND2 exhibited the characteristic absorption signals of ester linkages of poly(DEAEMA) at 1736 cm^{-1} (O—CO— stretching) and those of amide functional groups of poly(NIPAAm) at 1642 cm^{-1} (NH—CO— stretching) and 1560 cm^{-1} (N—H bending). In addition, the characteristic signal of the isopropyl group was also observed at 1390 cm^{-1} in CND2, indicating the presence of NIPAAm moieties in the hydrogel. FTIR spectrum of CND1 shows a similar pattern to that of CND2 in Figure 3(D).

Surface Morphology Studies of the Hydrogels

Figure 4 shows cross-sectional morphologies of the fully water-swollen hydrogels after lyophilization. SEM images of the hydrogels without poly(DEAEMA) [CMC and CND0 in Figure 4(A,B), respectively] showed relatively dense morphologies with low degrees of porosity, while those of the poly(DEAEMA)-containing hydrogels exhibited open and porous structures [CND1 and CND2 in Figure 4(C,D), respectively]. It was hypothesized that poly(DEAEMA) enhanced water swellability of the hydrogels, resulting in the formation of abundant internal micropores in the swollen matrix. The size of these micropores ranged between 10 and 50 μm in diameter.

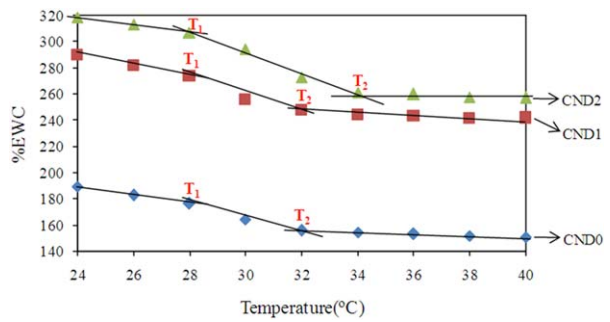

Figure 6. Temperature dependence of EWC of CND0 (◆), CND1 (■), and CND2 (▲) hydrogels in pH 7-aqueous solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Studies in Water Contact Angles of the Hydrogels

Surface wettability of the hydrogels was investigated by measuring the water contact angles of poly(NIPAAm) and poly (NIPAAm-*co*-DEAEMA) grafted on to CMC, compared with that of the unmodified CMC hydrogel. In contact angle measurements an increasing water contact angle implies the decrease in surface hydrophilicity of the material. According to the results in Figure 5, the water contact angle increased from 36.6° to 48.9°. This was attributed to the presence of hydrophobic isopropyl groups in the structure of poly(NIPAAm), resulting in changes in the surface hydrophobicity of the hydrogels. Addition of poly(DEAEMA) in the hydrogels even further increased their surface hydrophobicity as indicated by the steady increase seen in the water contact angles. This was attributed to the migration of hydrophobic diethyl groups in the poly(DEAEMA) units to the polymer-air interface during the crosslinking process. It should be noted that this hydrophobic characteristics was observed only on the hydrogel surface. In contrary, the hydrogels showed an enhancement in water swellability when grafted with poly(DEAEMA) (this is discussed in further detail in a latter section). This was attributed to the presence of tertiary amino groups in the DEAEMA unit, which thus enhanced degree of hydrophilicity in the bulk of the hydrogels.

Determination of the Phase-Transition Temperature (LCST) of the Hydrogels

Effect of poly(NIPAAm) and poly(NIPAAm-*co*-DEAEMA) on EWC and phase transition temperature (LCST) of the CMC hydrogels was investigated (Figure 6). It was observed that as the DEAEMA concentrations increased in the hydrogels, %EWC also increased. The improvement in water swellability of the hydrogels upon addition of poly(DEAEMA) was attributed to the increase in hydrophilic amino groups in the hydrogel structure, which essentially increased the water absorbing capability of the samples.

The phase-transition temperature, indicated by the presence of LCST, is the temperature at which there is a drastic decrease in the EWC of a hydrogel. The EWC change (ΔEWC) was measured by increasing the solution temperature from 24°C to 40°C ($\Delta EWC = EWC^{\circ}_{24} - EWC^{\circ}_{40}$) for CND0, ΔEWC was 38, and those of CND1 and CND2 were 47 and 61, respectively. This indicated a sharper volume phase transition when poly(DEAEMA) was grafted to the hydrogels. LCST of these hydrogels was ~32°C, which was attributed to the existence of a continuous alkylamide sequence of thermo-responsive poly(NIPAAm) in the structure. It should be noted that CMC hydrogels without poly(NIPAAm) did not show a phase transition temperature.

Water Swelling Properties of the Hydrogels

Temperature Dependence of Equilibrium Water Content (EWC) of the Hydrogels. The effect of solution temperature on the water swelling behavior of the hydrogels is shown in Figure

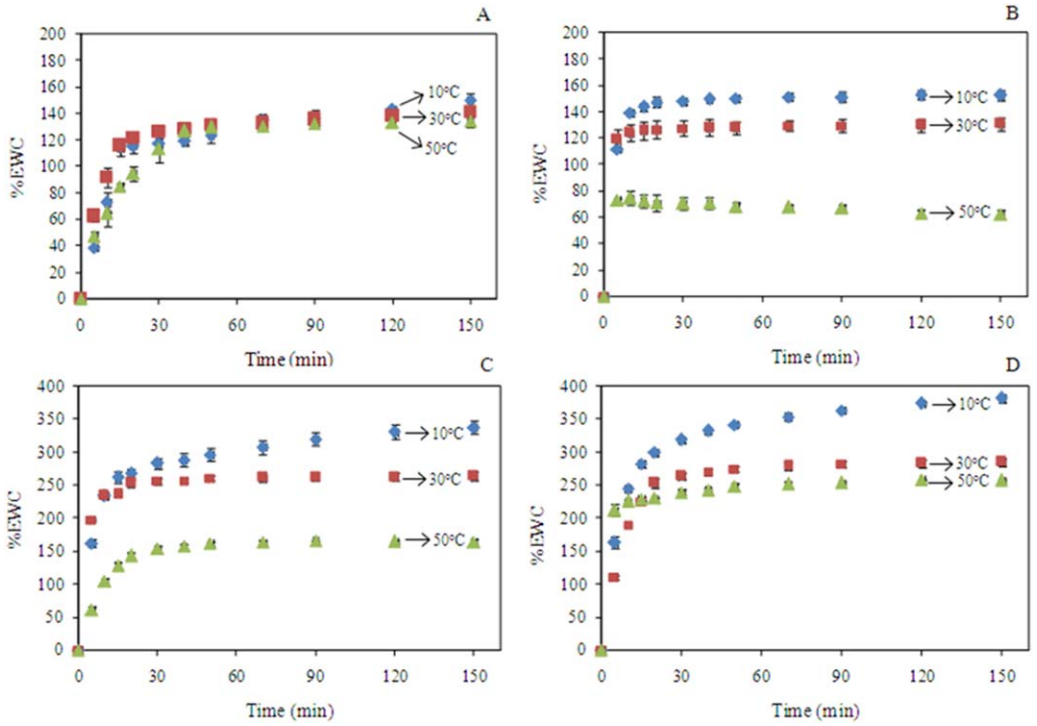

Figure 7. Equilibrium water content (%EWC) of (A) CMC (B) CND0, (C) CND1, and (D) CND2 hydrogels as a function of solution temperature (◆10, ■ 30, and ▲50°C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

7. The experiments were performed in three solutions with different temperatures (10, 32, and 50°C). The temperatures chosen were based on the hypothesis that thermo-responsive poly (NIPAAm) can swell in solutions at temperatures below its LCST and deswell at those above its LCST. In Figure 7(A), CMC hydrogel (the control sample) had no temperature dependence due to the absence of poly(NIPAAm) in its structure. Incorporation of poly(NIPAAm) into the hydrogels [Figure 7(B)] produced two distinct swelling responses. The gels at 10°C swelled normally but collapsed and did not swell at 50°C, indicating responsive behavior to changes in their environmental temperature. Amide groups (-NHCO-) in poly(NIPAAm) structure formed strong hydrogen bonding among the hydrophobic segments at the temperature above its LCST (50°C) and prefer to form intermolecular hydrogen bonding with surrounding water below its LCST (10°C).

Incorporation of hydrophilic poly(DEAEMA) to the CMC hydrogels enhanced water swellability when compared with those without poly(DEAEMA). %EWC significantly increased from 60–150% in the hydrogels without poly(DEAEMA) [Figure 7(B)] to 168–334% in those with poly(DEAEMA) [Figure 7(C)]. Increasing poly(DEAEMA) concentration in the hydrogels even further enhanced their %EWC (257–382%) [Figure 7(D)]. It was rationalized that the presence of amino groups in DEAEMA units promoted the formation of hydrogen bonds between the hydrogel and the surrounding water, thus giving the enhancement of their water swellability. The increase in water swellability of the CMC hydrogels grafted with poly (DEAEMA) was in good agreement with those observed in SEM results, indicated by high degree of internal porosity of the poly(DEAEMA)-containing hydrogels.

pH Dependence of Equilibrium Water Content (EWC) of the Hydrogels

The hydrogels water swelling behavior as a function of solution pH is shown in Figure 8. The experiments were carried out in three solutions with different pHs (3, 7, and 11) to investigate EWC of the hydrogels in acidic, neutral and basic pH, respectively. CMC hydrogel (control sample) exhibited a slight response to a change in solution pH, probably due to the presence of amino and carboxylic acid groups in the CMC chains [Figure 8(A)]. After addition of poly(NIPAAm) in the hydrogels, their EWC increased due to the presence of poly(NIPAAm) in the swollen state at the temperature below its LCST (the experimental temperature was about 25°C) [Figure 8(B)]. Grafting poly(DEAEMA) to the CMC hydrogels enhanced their water swellability. For instance, in a pH 11 solution, %EWC significantly increased from 290% in CND0 (without poly (DEAEMA)) [Figure 8(B)] to 520% in CDN1 [Figure 8(C)] and up to 535% in CND2, when the percentage of poly(DEAEMA) in the hydrogels was increased [Figure 8(D)]. This was attributed to the formation of carboxylate (COO^-) in CMC structure and the presence of tertiary amines units when poly(DEAEMA) is in basic conditions, resulting in an enhancement in water swelling properties of the hydrogels.

Effect of Temperature and pH Changes on Releasing Rate of Indomethacin

CND2 hydrogels showed high %EWC and a good response to both temperature and pH changes. Therefore, this was the chosen hydrogel to be used for the controlled release study of indomethacin (the model drug). The controlled release was varied by altering the solution temperature and pH. Entrapment efficiency (%EE), drug loading efficiency (%DLE), and releasing

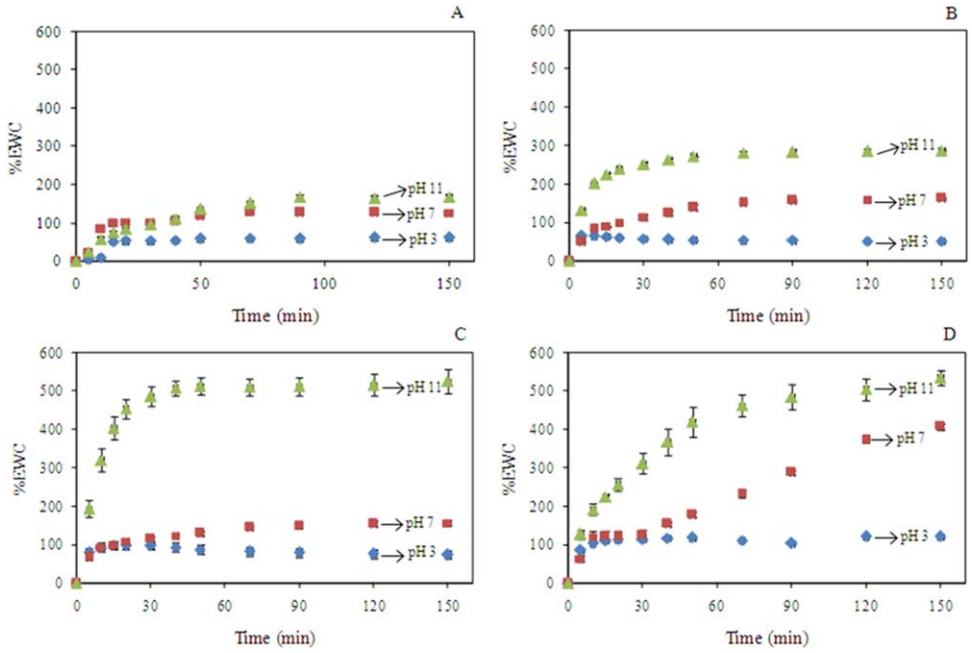

Figure 8. Equilibrium water content (%EWC) of (A) CMC, (B) CND0, (C) CND1, and (D) CND2 hydrogels as a function of solution pH (◆3, ■ 7, and ▲ 11). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

behavior of indomethacin from the CND2 hydrogels were investigated using UV-visible spectrophotometry. It was found that %EE and %DLE were 24.74% and 1.56%, respectively. The

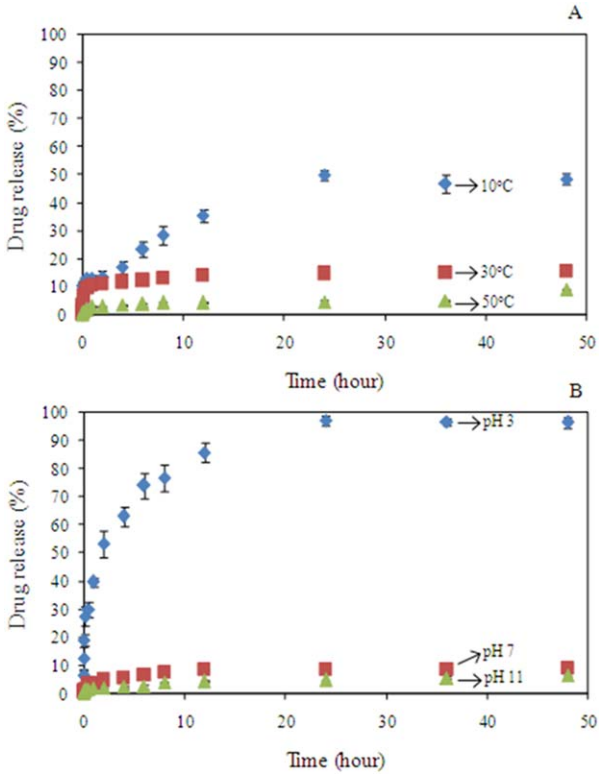

Figure 9. Releasing behavior of indomethacin of CND2 hydrogels in buffer solutions in different temperatures and pHs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

indomethacin releasing profiles of the hydrogel were performed in water with varying solution temperature (10, 30, and 50°C) and pH (pH 3, 7 and 11) [Figure 9(A,B), respectively]. After 48-h observation, the release of indomethacin was measured. The most indomethacin was released at the temperature below its LCST (10°C) with 49% being released, compared to 9% released when the temperature was above its LSCT (50°C). The higher percentage drug release at the temperature below its LCST was attributed to the swelling state of poly(NIPAAm) in the hydrogels that was below its LCST (10°C) (Figure 7), resulting in an increased volume and larger pore sizes. This enabled the buffer solutions to diffuse and exchange in the associated but unbound water of the hydrogels, thus allowing the drug to be dissolved and released due to osmotic pressure. Above the LCST (50°C), in contrast, the hydrogel structure has collapsed chains, resulting in the drug being entrapped in the hydrogel and a low drug release percentage was obtained. It was rationalized that only the drug on the surface of collapsed hydrogels was released.

The drug releasing behavior of the hydrogels was also dependent on the solution pH due to the presence of pH-responsive poly(DEAEMA). After 48 h, a high percentage of drug release was observed in the acidic pH solution (96%), the release seen in neutral and basic pH solutions (10% and 6.7%, respectively) was much lower. This observation is unexpected in terms of the structure of the hydrogel but not so when the properties of indomethacin are taken into account. The rate at which a drug goes into the solution when it is dissolved in an acidic or basic medium is proportional to the solubility of the drug, and also the pKa of the drug, which results in pH-dependant solubility differences.[30] Indomethacin has a solubility of −4.62 (logS) and a pKa of 4.5, it is also stable in neutral or acidic conditions but

decomposes in strong alkali media.[31,32] This information fits with the results seen in Figure 8(B), the solution of pH 3 showing a high release percentage as the solution is below the pKa of the indomethacin. The other two pHs are well above the pKa meaning the drug is in a form that is unable to escape the hydrogel matrix, which results in the low release percentages (10 and 6.7%).

Cytotoxicity

Cytotoxicity is an important characteristic of materials intended for use in biomedical applications. The cell viability of all tested materials, CMC, CND0, and CND2 hydrogels, was more than 70% when compared with the blank sample, indicating that all CMC hydrogels were not toxic to the mouse fibroblast cells. This result shows that these smart hydrogels have good potential for use in biomedical-related applications.

CONCLUSIONS

CMC hydrogels grafted with thermo-responsive poly(NIPAAm) and pH-responsive poly(DEAEMA) were prepared via a radical polymerization and crosslinked to form a semi-IPN with high water swellability. These hydrogels had high internal porosity, leading to the enhancement in their surface area and thus promoting their water swellability. They exhibited responses to the changes in their solution temperature and pH. These responsive properties of the smart hydrogels as well as the non-toxic nature of the materials means these smart hydrogels have excellent potential to be used as triggering mechanisms with controllable on–off switch properties for the controlled release of therapeutic drugs and other delivery applications. The hydrogels can be produced with a wide range of EWC allowing the desired properties to be tailored to each use.

ACKNOWLEDGMENTS

The authors acknowledge The National Research Council of Thailand (NRCT) (R2556B049) and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education for financial support. The authors also thank Associate Professor Dr.Voravee Hoven from Chulalongkorn University for assistance in water contact angle measurements.

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