Novel Physical Hydrogels Composed of Opened-Ring Poly(vinyl pyrrolidone) and Chitosan Derivatives: Preparation and Characterization

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ABSTRACT: A novel, physically stabilized hydrogel system composed of chitosan (Chi) or its derivatives [e.g., carboxymethyl chitosan (CMC), sodium carboxymethyl chitosan, or trimethyl carboxymethyl chitosan (TMCMC)] with poly(vinyl pyrrolidone) (PVP) or opened-ring poly (vinyl pyrrolidone) (OR–PVP) were prepared and characterized. TMCMC was synthesized by a novel method with dimethylsulfate as the methylation agent. The synthesized materials were characterized by Fourier transform infrared spectroscopy, ^{1}H-NMR, ^{13}C-NMR, and size exclusion chromatography. The mechanical properties, gel fraction, swelling behavior, and water state of the prepared hydrogels were investigated. Gelation occurred when the OR–PVP and Chi solutions were blended within a few seconds. However, the gelation of the OR–PVP and CMC solutions needed pH adjustment. No gelation occurred when the solutions of TMCMC and PVP or OR–PVP were blended. The quaternization or protonization of $-NH_2$ groups may have prevented the gelation of the solutions. The amino groups of Chi derivatives should have been free to take part in hydrophilic bonds between the two polymers. The physical entanglement of polymeric chains and strong hydrogen bonds between the polymers were considered as mechanisms for the formation of the physical hydrogels. The physical hydrogels showed ionic and pH-sensitive swelling properties. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 2761–2771, 2011

Key words: crosslinking; gelation; hydrogels; polymer synthesis and characterization; swelling

INTRODUCTION

Chitosan (Chi) and its derivatives are well-known biocompatible and biodegradable biomaterials with numerous applications in the biomedical, pharmaceutical, agriculture, packaging, and food biotechnology fields.[1] A major drawback of Chi is its poor solubility in media with pH values above 6. To improve Chi aqueous solubility, many derivatives of Chi have been synthesized; among them, carboxymethyl chitosan (CMC), as an amphoteric polyelectrolyte derivative, has attracted considerable interest in a wide range of biomedical applications, such as wound dressings, artificial bones, and skin.[2] CMC is nontoxic in fibroblast culture assays and can be used for intraperitoneal, oral, or subcutaneous treatments.[3] In addition, CMC is a suitable excipient in ophthalmic formulations for improving the retention and bioavailability of drugs.[4] CMC keeps Chi's advantages, such as blood compatibility, nonantigenicity, biocompatibility, and bioadhesion. In addition, it has a better water solubility, which is appropriate for applications in various biomedical fields.[3] Trimethyl carboxymethyl chitosan (TMCMC), as a new quaternized Chi derivative with a high water solubility and functional carboxylic groups, has also recently received extensive attention.

The synthetic polymer poly(vinyl pyrrolidone) (PVP) has good biocompatibility,[5] and for many years, it has been applied as a safe excipient in drug formulations, for example, as a blood plasma expander[6] and vitreous humor substitute.[7] PVP hydrogels prepared by ionizing radiation show excellent transparency and biocompatibility. They have been used as the main component in temporary wound dressings.[8] PVP hydrogels have weak mechanical properties. Therefore, many researchers have tried to enhance the mechanical behavior of PVP hydrogels by using different blends.[9]

Hydrogels are polymeric materials with a three-dimensional network structure capable of considerable swelling in aqueous media. They are classified as neutral or ionic on the basis of the nature of their side groups. In addition, they can be classified according to the physical structure of their networks as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures, or hydrocolloidal aggregates.[10–12] The study of polyelectrolyte hydrogels responsive to environmental stimuli such as temperature, pH, ionic strength, electric fields,

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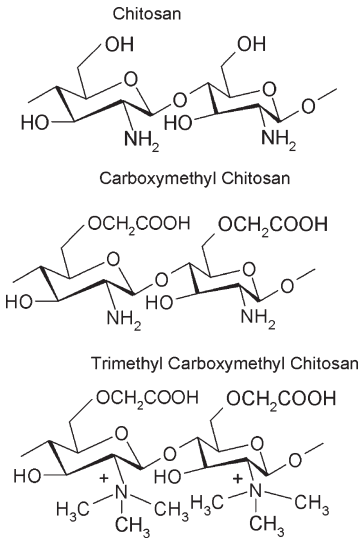

Figure 1 Chemical structures of Chi, CMC, and TMCMC.

light, pressure, sound, or a specific chemical trigger is a very active field of research.[13,14] Polyelectrolyte gels based on polysaccharides and their derivatives have attracted much attention. Because of their properties and comparatively low cost, blended polyelectrolyte gels of polysaccharides and synthetic polymers have been investigated extensively.[15] Many chemically crosslinked hydrogels have been investigated in recent years, such as PVP–Chi hydrogels crosslinked by glutaraldehyde. However, the safety of chemical hydrogels limits their applications because of the toxic residuals of monomer or crosslinkers used for the polymerization.[16] Physical hydrogels have recently received a great deal of interest; this has mainly been due to their safer properties compared to those of crosslinked hydrogels. These agents are toxic compounds that have to be removed/extracted from the hydrogel before application. Physical hydrogels are formed by various reversible links. These can be ionic interactions, such as those in ionically crosslinked hydrogels and polyelectrolyte complexes, or secondary interactions, such as those in Chi/poly(vinyl alcohol) (PVA) complexed hydrogels, grafted Chi hydrogels, and entangled hydrogels. Different methods have been investigated for the preparation of physical hydrogels.

The aim of this study was to develop a physical hydrogel with PVP and Chi derivatives. The concept was to prepare a hydrogel by the interaction of the functional groups of the PVP and Chi molecules. The pyrrolidone ring and Chi amine groups may have had a hydrophilic attraction because of their opposite charges. The first results indicate that these two molecules may need some structure changes or special interaction conditions, such as a suitable pH or heating. The pyrrolidone ring underwent opening under the synthesis conditions; thus, PVP initially had a few opened rings, and its aqueous solution had an acidic pH. In this study, acid–alkaline hydrolysis and heating were used to open more pyrrolidone rings to increase the reactive groups.[17] The substitution of a hydrophilic group on Chi to make CMC and the methylation of the amine group to prepare methylated CMC for reinforcing the interaction of the functional groups on both molecules was another concept that was evaluated in this study (Fig. 1). The chemical structure of the synthesized materials was characterized by Fourier transform infrared (FTIR) spectroscopy, ^{1}H-NMR, and ^{13}C-NMR. Physical hydrogels were prepared and characterized.

EXPERIMENTAL

Materials

Chi [low, medium, and high molecular weight (MW)], with approximately 95% deacetylation, was acquired from Primex (Siglufjorder, Iceland). PVP (K30, K60, and K90) were kindly supplied by BASF (Ludwigshafen, Germany). Pullulan standards (Shodex P-82) were obtained from Shodex Denko (Tokyo, Japan). All other chemicals and reagents were analytical grade.

Synthesis and characterization of CMC

CMC was prepared according to previous reports[18] as follows: Chi (10 g), sodium hydroxide (13.5 g), and solvent (water/isopropyl alcohol) were added to a 600-mL flask to swell and alkalize at 55°C for 1 h. Monochloroacetic acid (15 g) was then dissolved in isopropyl alcohol (20 mL), added dropwise into the reaction for 30 min, and left while it was stirred for 4 h at the same temperature. The reaction was stopped by the addition of 200 mL of ethyl alcohol (70%). The precipitate was filtered and rinsed by 70% ethyl alcohol to desalt and dehydrate before it was vacuum-dried at room temperature.

Sodium carboxymethyl chitosan (NaCMC; 1 g) was suspended in 80% ethyl alcohol aqueous solution (100 mL), and hydrochloric acid (10 mL, 37%) was then added and stirred for 30 min. The solid was filtered and rinsed in 70% ethyl alcohol for neutralization and vacuum-dried.

Synthesis and characterization of TMCMC

A novel method for the preparation of TMCMC was developed with dimethylsulfate as the methylation agent. The basic reaction sequence was comprised of a suspension of 1 g of CMC in dimethylsulfate and water; 1.2 g of NaOH and 0.88 g of NaCl were added, and the solution was mixed at room

temperature for 8 h with a magnetic stirrer. The prepared derivative was purified through a dialysis tube (cutoff = 12,000–14,000 D) against water for 3 days. The final products were obtained by precipitation with acetone. After exhaustive rinsing, TMCMC was centrifuged and vacuum-dried.

Solubility of the Chi derivatives

Known amounts of the Chi derivatives were dissolved in deionized (DI) water. The pH values of the solutions were changed by the addition of a 0.5% aqueous solution of HCl or NaOH. Transmittance (T%) at 420, 500, and 610 nm was measured by a spectrophotometer. When the polymers were dissolved completely, the solution was clear, and T% was 100%. T% was in proportion to the polymer solubility. The solubility was expressed by the value $100 - T$%. A smaller value for $100 - T$% represented better solubility.[20]

Opening of the pyrrolidone ring

To increase the molar equivalents of acid groups, PVP was treated at a temperature of about 130°C in an acidic, neutral, or alkaline solution for 1–3 days.[20] The solution of opened-ring poly(vinyl pyrrolidone) (OR–PVP) was subjected to a dialysis tube (cutoff = 12,000–14,000 D) to remove traces of acid or alkaline residues. PVPs with different molecular weight were treated.

To determine the newly made carboxylic acid groups on OR–PVP, the molar equivalents of the acid groups were compared with the initial value. Then, 0.200 g of OR–PVP was dissolved in 10 mL of DI water and titrated by a standard NaOH solution (0.0984N) with phenolphthalein as an indicator to determine the molar equivalents of acid groups.

Size exclusion chromatography of the synthesized polymers

Size exclusion was performed with a Knauer apparatus (Berlin, Germany). In a PL aquagel–OH mixed-H 8-μm column (25 mm inside diameter) at 25°C with a flow rate of 4 mL/min, degassed DI water or an aqueous solution of an acetate buffer solution of 0.2M acetic acid and 0.1M sodium acetate with a pH value of 4.6 ± 0.05 was used as the eluent. The Pullulan standards and synthesized polymer samples were dissolved in the eluent at a concentration of 0.1% and filtered through a 0.45-μm filter before analysis to remove aggregations.[21] The viscosity of derivative aqueous solutions was measured at 25°C with an Ubbelohde-type capillary viscometer (SCHOTT GERÄT, Mainz, Germany). A narrow calibration system was applied.

Preparation of the physical hydrogels

Physically stabilized hydrogels were prepared by the mixture of aqueous solutions of PVP or OR–PVP and Chi or its derivatives (NaCMC, CMC, and TMCMC) at pH 2–9. Different compositions containing Chi derivatives/PVP with different weight ratios (5–27%) and total polymer concentrations (7–18%) were prepared. Two types of hydrogels, F3-SA1 (NaCMC/OR–PVP) and F3-SB1 (Chi/OR–PVP), with the same total polymer content were selected and characterized.

Determination of the gel fraction

The gel content was estimated by the measurement of the insoluble part of the hydrogel after extraction in distilled water at room temperature for 24 h. The remaining hydrogel was dried, and the gel fraction was measured gravimetrically by application of the following equation:

$$\text{Gel fraction } (\%) = (W_g/W_o) \times 100 \quad (1)$$

where W_g is the weight of the dried gel after extraction and W_o is the weight of the initial dried gel.[22] To clarify the effect of the Chi/PVP ratio on the gel fraction, different amounts of Chi (40, 60, 80, 120, and 160 mg) with a constant amount of PVP were evaluated.

Measurements of the mechanical properties

A plate–plate combination rheometer (Paar Physica MCR300, Anton Paar, Graz Austria) was used to determine the viscoelastic characteristics of the hydrogels. Oscillation frequency sweep measurements were performed at an angular frequency range of 0.1–100 rad/s at a constant temperature of 25 ± 2°C on samples 25 mm in diameter and 2 mm in thickness. Oscillatory stress sweep measurements were examined at a fixed angular frequency of 1 rad/s for a period of 25 min.

Measurements of the gel swelling properties

To determine the swelling behavior of the prepared hydrogels, preweighed dried samples were immersed in aqueous media with pH values of 4.5, 7.4, and PBS 7.4 at room temperature for 48 h. At specific time intervals, the swollen hydrogels were removed and blotted with filter paper to absorb excess surface water before they were weighed. The swelling ratios (Q_S) of the test samples were calculated according to the following equation:

$$Q_S = (W_S - W_d)/W_d \quad (2)$$

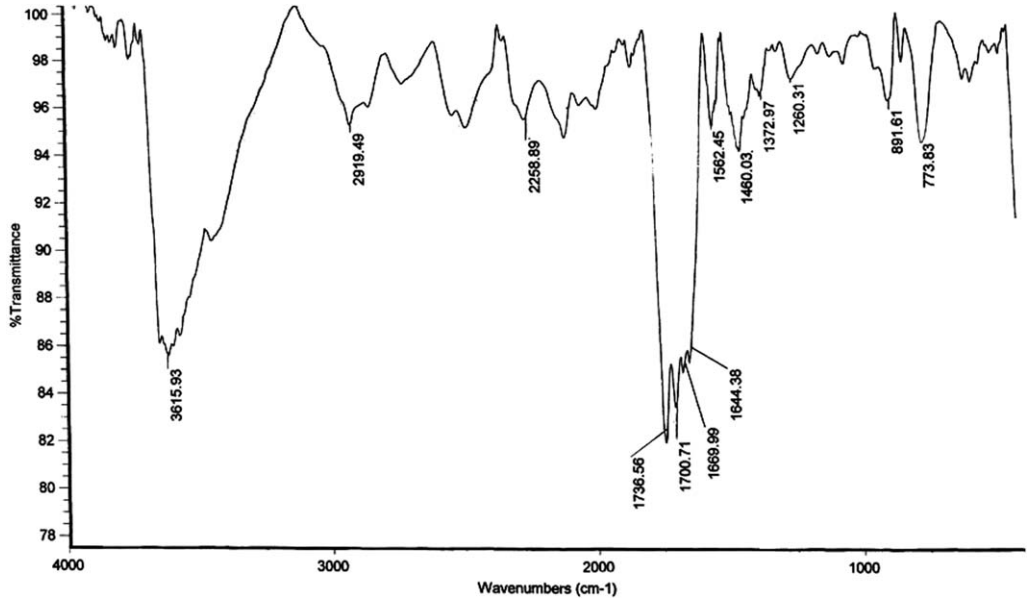

Figure 2 FTIR spectrum of TMCMC.

where W_S is the weight of the swollen hydrogels and W_d is the weight of the initial dried hydrogel. These were measured by a balance after drying at 105°C for 4 h and cooling in a dessicator.[23]

Measurements of the state of water

A differential scanning calorimeter (Metter Toledo DSC823^e, Switzerland) was used for quantitative determination of the amount of freezing and nonfreezing water. The heat of melting in the freezing water (intermediate and free water) was determined from the area under the endothermic curve and was calibrated with pure distilled water as a standard. The fraction of free water in total water was approximated as the ratio of the endothermic peak area for the water-swollen hydrogel to the melting endothermic heat of fusion for pure water. Then, 5–30-mg samples of swollen hydrogel were transferred into a differential scanning calorimetry (DSC) aluminum hermetic pan and sealed tightly to prevent water loss during DSC scanning. The pan was first cooled to −35°C and then heated to 35°C at a rate of 5°C/min. The phase transition of water inside the hydrogel during heating was recorded as the endothermic peak; this was later integrated, with the assumption that the heat of fusion for free water in the hydrogel was the same as that for ice.[24]

RESULTS AND DISCUSSION

Synthesis and characterization of the polymers

The IR spectrum of CMC showed peaks assigned to the saccharide structure at 895 and 1155 cm^{-1} and a characteristic amino peak around 1600 cm^{-1}. The peak at 1741 cm^{-1} clearly showed carboxylic acid groups. The ^{1}H-NMR (in D_2O) spectrum of Chi indicated the following characteristic signals:[25]

1. The signal around 5 ppm was attributed to hydrogen bonds for anomeric carbon (H_1).
2. The signals between 3.4–4.0 ppm were assigned to hydrogen bonds for carbon atoms 3–6 of the glycopiranose unit (H_{3-6}).
3. The signal at 3.18 ppm was assigned to H_2.
4. The methyl moieties of the acetamido groups showed a single signal at 1.99 ppm.

The intensity ratio of the latter two signals mentioned previously allowed the determination of the average degree of acetylation for the purified Chi to be 5%. The ^{13}C-NMR spectrum of CMC showed two characteristic peaks around 165 ppm that confirmed the presence of carboxylic acid groups.

After quaternization of CMC, several peaks appeared at 1644–1740 cm^{-1}; these were assigned to the quaternary ammonium salts (Fig. 2).

In the ^{1}H-NMR spectrum, the presence of peaks at 2.47–3.37 ppm indicated *N*-methylation (Fig. 3). Only the signal at 3.3 ppm was directly related to the occurrence of quaternization; it corresponded to the hydrogen atoms of a methyl group bonded to the quaternary nitrogen.[26]

The degree of quaternization (DQ) for synthesized TMCMC was calculated by comparison of the integral peak at 3.3 ppm with the area below that of the anomeric hydrogen. The DQ percentage was defined as the following equation:

$$DQ\ (\%) = 100 \times A/9B \quad (3)$$

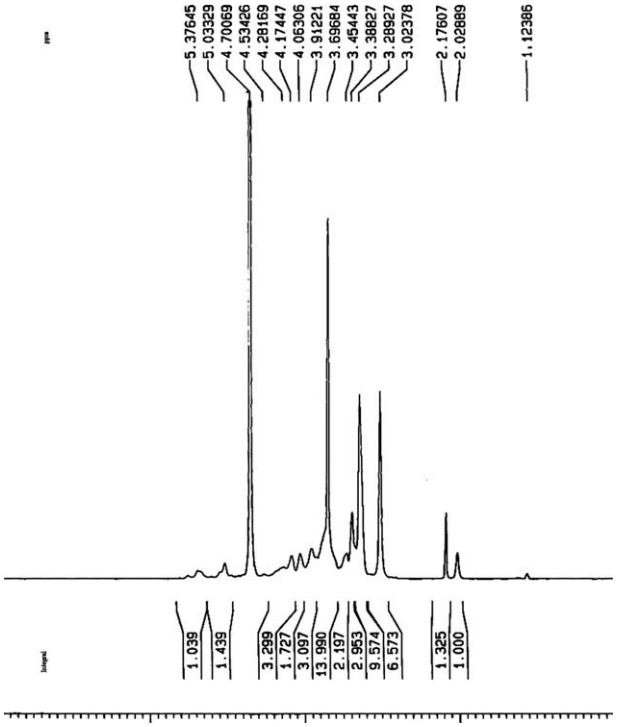

Figure 3 ^{1}H-NMR spectrum of TMCMC in D_2O at 25°C (500 MHz, 300 K).

where *A* is the integral peak at 3.3 ppm [$N^+(CH_3)_3$] and *B* is the integral peak of (H_1) around 5 ppm.

The signals in the region of 3.37–3.56 ppm corresponded to the *O*-methylated sites.

The solubility of CMC is pH-dependent because it contains two functional groups: carboxylic acid and amino groups. At acidic pH values, the carboxylic groups are in acidic form (nonionic), and the protonated amino groups enhance solubility. At neutral pH, the number of amino groups (nonionic) increases, and the solubility is reduced. It is soluble at higher pH values because of the $-COO^-$ groups (NaCMC). TMCMC is soluble for all pH values in the range 1.5–12 because of its permanent positive charge and hydrophilic carboxylic group.

The MW of PVP remained intact during treatment and chemical characterization. Titration showed a 50–100% increase in acid equivalent groups because of the ring opening that was induced in some pyrrolidone rings. The random ring opening of pyrrolidone rings may have facilitated polymer chain configurations to construct hydrogen bonds.

The IR spectrum of OR–PVP showed the characteristic signal of a carboxylic acid group at 1731 cm^{-1}. Also, signals of ^{13}C-NMR at 177 ppm confirmed the ring opening (Fig. 4). The acid equivalent ratio for OR–PVP/PVP was 1.2–1.5 and was dependent on the applied temperature and time of treatment. The size exclusion chromatography study on the PVP and OR–PVP solutions revealed no changes in the MW or the chains of PVP.

The MWs and intrinsic viscosities of Chi, CMC, and TMCMC are presented in Table I. The results suggest that MW of the synthesized derivatives decreased because of the reaction conditions, such as the high temperature and alkaline pH. The derivatives showed a lower intrinsic viscosity in comparison with the initial Chi, although the bulky groups were substituted, but MW decreased under the reaction conditions.

Preparation of the physical hydrogels

We prepared the hydrogels by blending the solutions of pyrrolidone derivatives (PVP or OR–PVP) with the Chi derivatives (Chi, CMC, NaCMC, and TMCMC) and changing the pH values from 2 to 10. The blending solutions composed of different polymer ratios of PVP and Chi did not produce any hydrogels, even when the pH value was changed (from 3.5 to 9), and heating did not help. However, the simple mixing of the OR–PVP and CMC solutions produced hydrogels within a few minutes, just after the addition of a few drops of NaOH (1*N*) to increase the pH value.

The pH value of this solution before preparation of the hydrogel was about 4.5. Increasing the pH value to about 6.5 resulted in the gelation of the system. Blending the solutions of OR–PVP and NaCMC resulted in hydrogel formation within 1 min. The initial pH values of the OR–PVP solution and NaCMC solution were about 6 and 9, respectively, and the mixing of the solutions produced a clear hydrogel with a pH value of 6–6.5. As mentioned earlier, NaCMC should precipitate at pH values

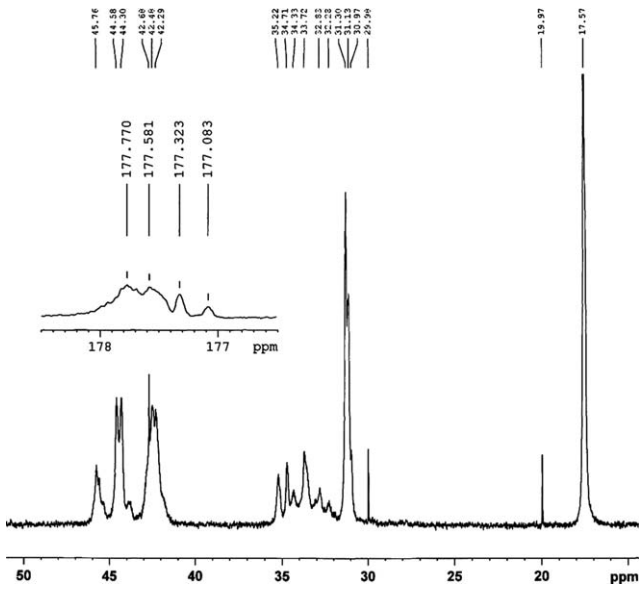

Figure 4 ^{13}C-NMR spectrum of OR–PVP in D_2O at 25°C (500 MHz, 300 K).

TABLE I
Mean MWs and Intrinsic Viscosities of Chi, CMC, and TMCMC

Polymer	M_w (kg/mol)	M_n (kg/mol)	M_w/M_n	MW_P	[η] (dL/g)
Chi	175903	28390	6.196	75247	2.82
CMC	54638	18737	2.916	23613	0.94
TMCMC	29480	14489	2.035	19736	0.25

M_w, weight-average molecular weight; M_n, number-average molecular weight; [η], intrinsic viscosity; MW_P, peak molecular weight.

below 6.88, but in the presence of OR–PVP, a hydrogel formed.

The blending of the OR–PVP and Chi solutions produced a physical hydrogel after just a few seconds in an acidic medium (pH ≈ 6) without the addition of any alkaline solutions. Also, the addition of the TMCMC solution to PVP or OR–PVP solutions at different ratios and pH values (3.5–9.0) did not produce any gels. Hydrogels with different weight ratios of Chi derivatives/OR–PVP (5–27%) and total polymer concentrations (7–18%) were prepared, and the effect of the addition of some humectants were evaluated. The recorded gelling times were from about 4 s to 9 min. Increasing the Chi derivatives ratio in the hydrogel composition resulted in stronger hydrogels and reduced gelation times. The Chi hydrogels were brittle and stronger than the hydrogels containing its derivatives. The use of the PVP with higher MWs (higher *K* values) delayed the gelation process and produced soft and elastic hydrogels (Fig. 5).

Two types of hydrogels, F3-SA1 and F3-SB1, with the same total polymer content, were selected and characterized. Table II shows some formulations of the prepared physical hydrogels.

Chi is a pH-dependent cationic polymer. When the pH value of the Chi solution is below its pK_a (pH < 6.2), Chi is water-soluble and positively charged. The free amino groups (—NH_2) are protonated as —NH_3^+, which causes electrostatic repulsion between the protonated amino groups.

The amino and carboxyl groups form an amphoteric network with oppositely charged structures, which can change the network charges at different pH values. In previous studies, CMC was synthesized for the preparation of blended membranes with poly(ether sulfone), and the composite membranes were found to possess positively charged characteristics at low pH and were negatively charged at high pH values, as measured by the *zeta* potential. The incorporation of amphoteric CMC with a nonionic component has previously been confirmed to potentially improve the charged characteristics of the blend system and, thus, result in pH sensitivity.[27] The pH sensitivity of the special hydrogels prepared in this study is believed to play an important role in biomedical applications.

The blending of the CMC solution (pH 4.5) and OR–PVP solution with only a few drops of 1*N* NaOH was required to initiate the gelation process. The CMC solution had a pH value of 4.5 and contained protonated amino groups, such as —NH_3^+, and —COOH groups. The addition of NaOH increased the pH value to 6–6.5 and increased the number of amino groups (—NH_2); these were necessary to create hydrophilic bonds, such as H bonds, between the chains. The mixture of the NaCMC solution containing amino (—NH_2) and —COO^- groups at a pH value of about 9.0 with an OR–PVP solution having a pH value of about 5.5 resulted in hydrogels with a pH value of 6–6.5. At this pH, NaCMC should have precipitated, but the clear hydrogel may have been due to the existence of hydrophilic attraction between the polymeric chains of the Chi derivatives and OR–PVP in this hybrid network and physical entanglements of the hydrophilic chains.[28,29] The entangled polymeric chains prevented the precipitation of NaCMC at pH values below 6.8 and produced a physical hydrogel. Dextran 6000 has been reported to spontaneously form a hydrogel once concentrated aqueous solutions are incubated at room temperature. Gel formation is most likely caused by crystallization due to the association of chains through hydrogen bonding induced in the concentrated solution.[30] As shown in Table II, a decrease in the total polymer content, such as in

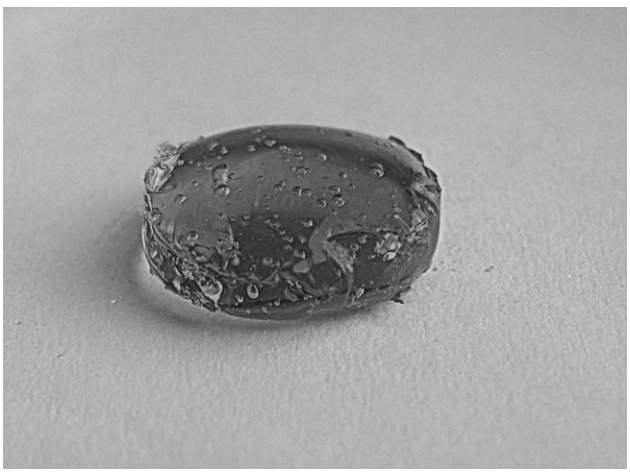

Figure 5 Photo of the prepared physical hydrogel F3-SB1.

TABLE II
Composition and Gelation Times of the Physical Hydrogels

Formulation	Chi (mg)	NaCMC (mg)	OR-PVP 22.67% (mL)	Aqueous medium (mL)	Acetic acid 1% (mL)	Gelation time (s)
F3-SA1	—	120	5.0	2.0	—	47.7 ± 2.5
F3-SA2	—	120	5.0	3.0	—	541.3 ± 10.2
F3-SA3	—	120	5.0	5.5	—	No gelation
F3-SB1	120	—	5.0	1.0	2	8.5 ± 0.5
F3-SB2	60	—	2.5	1.5	1	87.7 ± 0.6
F3-SB3	60	—	2.5	4.0	1	24.0 ± 1.0
F3-SB4	60	—	2.5	5.5	1	46.8 ± 0.7
F3-SB5	60	—	2.5	2.5	1	15.5 ± 0.5
F3-SB6	60	—	2.0	0.5	1	4.4 ± 0.4
F3-SB7	60	—	1.5	0.5	1	5.8 ± 0.3
F3-SB8	60	—	1.0	0.5	1	5.7 ± 0.3
F3-SB9	60	—	3.0	0.5	1	12.8 ± 0.7
F3-SB10	60	—	4.0	0.5	1	13.7 ± 0.3
F3-SB11	60	—	5.0	0.5	1	15.3 ± 0.6

F3-SA3, inhibited gel formation, so in low concentrated solutions, the physical entanglements were not too powerful to stabilize the gel. The IR spectrum of the insoluble part of the physically stabilized hydrogel clearly shows the existence of strong H bonds between the two molecules. As seen in Figure 6, the broadening of the characteristic strong O—H peak at 3300 cm^{-1}, which broadened to below 3000 cm^{-1}, confirmed the establishment of a strong H bond.

TMCMC solutions did not produce hydrogels with PVP or OR–PVP solution at pH values of 1.5–12; this clearly showed that free amino groups were necessary for the creation of the physical hydrogels with OR–PVP. Hence, carboxylic groups may have just increased the solubility of the Chi derivatives and increased the swelling rate of the hydrogels containing CMC and NaCMC.

Gel fraction

Recently, hydrogels of polysaccharides and synthetic polymer blends prepared by irradiation or chemical crosslinking have been widely studied. The irradiation and chemical crosslinking of hydrogels may result in the simultaneous crosslinking of synthesized polymers, the degradation of polysaccharides, and the grafting of polysaccharides to crosslinked polymer chains; this can eventually lead to the formation of hydrogels. Physically crosslinked hydrogels usually contain fewer degradation products.[31] Gel fraction studies on physically crosslinked hydrogels have helped in determining the best ratio of the two components. In this study, different Chi/OR–PVP percentages (3.2, 4.8, 6.4, 9.6, and 12.8%) were used to prepare hydrogels, and the gel fractions of the hydrogels were investigated

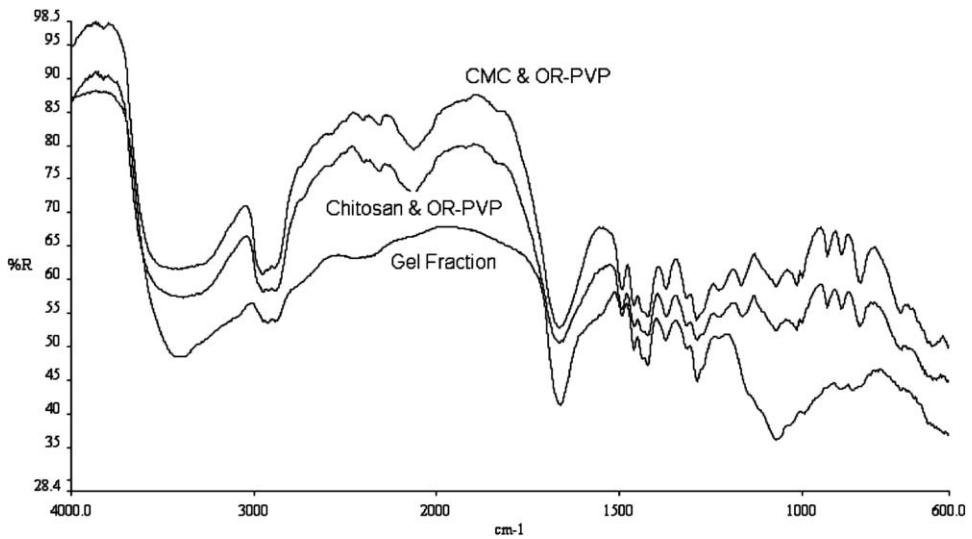

Figure 6 FTIR spectra for the physical blends of CMC/OR–PVP, Chi/OR–PVP, and the insoluble part of the prepared physical hydrogel. %R= % Relative intensity.

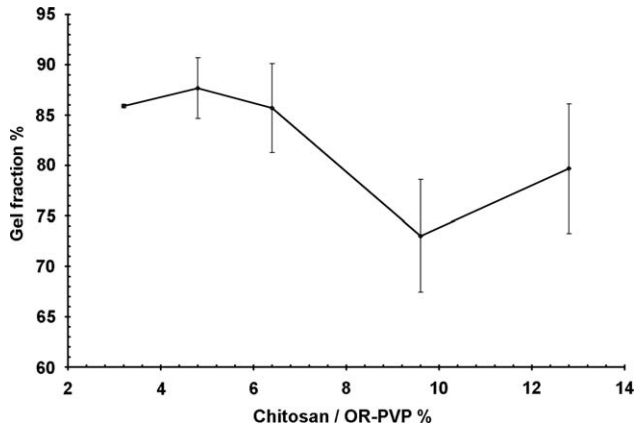

Figure 7 Influence of the Chi/OR–PVP ratio on the gel fraction of the F3-SB1 hydrogel.

in an aqueous solution with a pH value of 7.4. As depicted in Figure 7, the largest gel fraction was obtained for a Chi/OR–PVP ratio of 4.8%. The increase in the gel fraction by the Chi percentage from 9.6 to 12.8% may have been due to the collision of unbounded Chi chains in the hydrogel network, which could not be extracted in the medium.

The IR spectra of the insoluble part that remained at the end of this test clearly showed that strong hydrogen bonding needed to be considered the formation mechanism for this physically crosslinked hydrogel and the physical entanglements of the hydrophilic chains. The amino groups of the Chi derivatives easily settled between the carbonyl groups of the pyrrolidone rings to result in a physical stabilized hydrogel. Figure 8 depicts the scheme of the hydrogel formation.

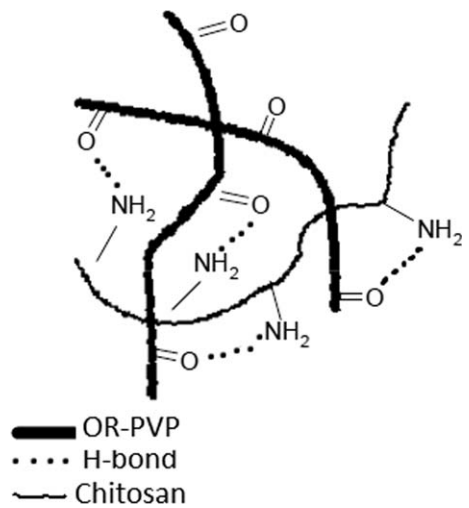

Figure 8 Scheme of the physical hydrogel formation by hydrogen bonds.

Mechanical properties

Dynamic viscoelastic functions, such as the dynamic shear elastic modulus or storage modulus (G'), viscous modulus or loss modulus (G''), and dynamic viscosity (η') were calculated by the following equations as a function of time and angular frequency:

$$G' = (\sigma/\gamma)\cos\delta \quad (4)$$

$$G'' = (\sigma/\gamma)\sin\delta \quad (5)$$

$$\eta' = G''/\omega \quad (6)$$

where σ is the shear stress, γ is the shear deformation, and ω is the angular frequency, and the phase shift or phase angle δ is defined by $\delta = \tan^{-1} G'/G''$ and indicates whether a material is a solidlike or liquidlike component. A gel is defined in rheological terms, where G' and G'' are frequency-independent and tan δ is less than 1; this is in contrast to a liquidlike material, where tan δ is greater than 1. When G' is equal to G'' at the crossover point, this means that the polymer is as elastic as the viscous components.[32–34]

Typical mechanical spectra of the F3-SA1 and F3-SB1 hydrogels are presented in Figure 9; G' and G'' are plotted as a function of oscillating frequency.

G' was higher than G'' for both hydrogels, and the slow slope of G' and G'' against the oscillating frequency suggested elastic behavior in the weak hydrogels.[35,36]

Swelling behavior of the F3-SA1 and F3-SB1 hydrogels

It is well known that swelling or deswelling in response to pH changes is a typical phenomenon of polyelectrolyte hydrogels, but nonionic polymer networks, such as PVP hydrogels, are not considered pH-sensitive. The F3-SA1 and F3-SB1 hydrogels

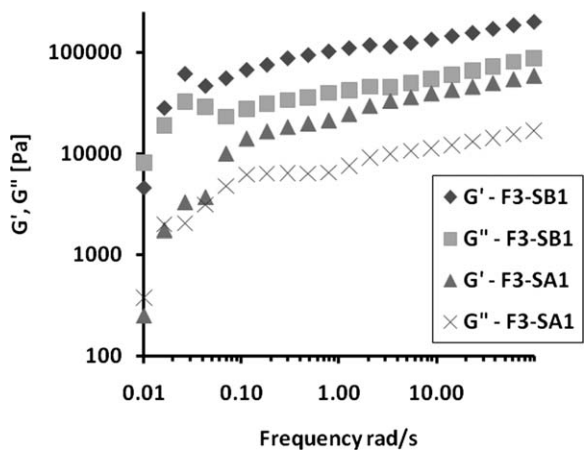

Figure 9 Frequency dependence of G' and G'' of the F3-SA1 and F3-SB1 hydrogels.

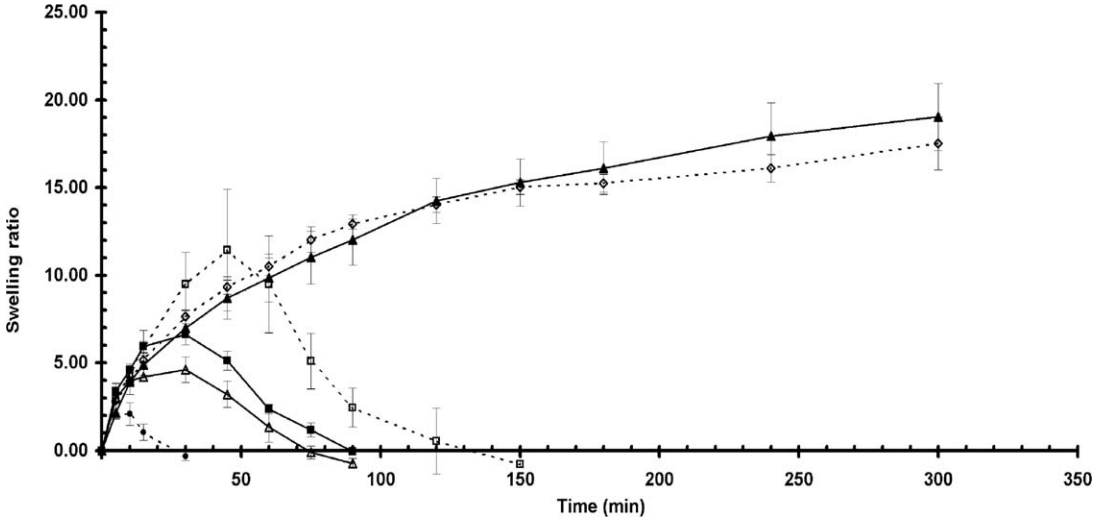

Figure 10 Swelling characterization of (◇) F3-SB1 at PBS pH 7.4, (□) F3-SB1 at pH 4.5, (▲) F3-SB1 at pH 7.4, (■) F3-SA1 at PBS pH7.4, (●) F3-SA1 at pH 4.5, and (△) F3-SA1 at pH 7.4.

were allowed to swell in 50 mL of an aqueous solution at pH 4.5, 7.4, and PBS 7.4 solutions at room temperature. The results indicate that both hydrogels swelled rapidly at a pH value of 4.5 before dissolving and losing their hydrogel networks. The maximum swelling ratios of the F3-SA1 and F3-SB1 hydrogels at acidic pH were about 2 and 12, respectively, but the F3-SA1 hydrogel dissolved more rapidly.

As depicted in Figure 10, the F3-SA1 hydrogel swelled up to 600% of its starting weight and showed pH- and ionic-dependent swelling properties. However, it lost its structural network and dissolved in the media at pH values of 4.5 and 7.4, respectively. F3-SB1 swelled more, up to 1700% of its starting weight, and showed a pH-dependent swelling; it kept its structure at pH 7.4 and reached an equilibrium swelling ratio within 5 h.

Theoretically, when polyelectrolyte chains form a hydrogel containing ionizable groups due to intermolecular coulombic repulsion, hydrogen bonding, polar forces, and mobile counter ions develop a large swelling pressure. As a result, the polymer interactions are enhanced and cause a very high sorption rate.[37] The oppositely charged groups of CMC enabled the system to keep a strong electrostatic attraction in the hydrogel network and led to a higher electrostatic force in the early stages of sorption.[38] This may have affected the equilibrium time and existence of a hydrophilic ionizable group, such as —COOH on the CMC chain, which was responsible for the F3-SA1 hydrogel dissolving at pH 7.4. As a result, the penetration of the medium with pH 4.5 into the hydrogel network amino ($—NH_2$) groups converted them to $—NH_3^+$; this decreased the H-bond power and, consequently, dissolved both hydrogels.

State of water

In general, three types of water are present in hydrogels:

1. Nonfreezing/bound water: These terms refer to water molecules that are immobilized by the functional groups of polymer molecules through hydrogen bonds. This kind of water showed no endothermic peak in the DSC analysis for the temperature range −70 to 0°C.
2. Intermediate/secondary bound water: water molecules that interact weakly with polymer molecules. This kind of freezing water had a melting point below 0°C.
3. Free water: water molecules that do not take part in hydrogen bonding with the polymer molecules. They have a greater degree of mobility in comparison with the other two types of water molecules. Free water is freezing water that shows a melting point of 0°C. It has a transition temperature, enthalpy, and DSC curves similar to those of pure water.[39,40] Free water is responsible for the permeation, diffusion, and physical and chemical reactions that occur in a hydrogel between the active ingredients and different materials used in the formulation; thus, information on changing the fraction of free water through swelling of the hydrogel in different media is important when considering the hydrolysis interactions and stability of active ingredients. This information also helps in predicting the hydrogel swelling behavior in different media.

In this study, partially swollen hydrogels were prepared by the immersion of a definite amount of dried hydrogel in solutions with pH values of 4.5

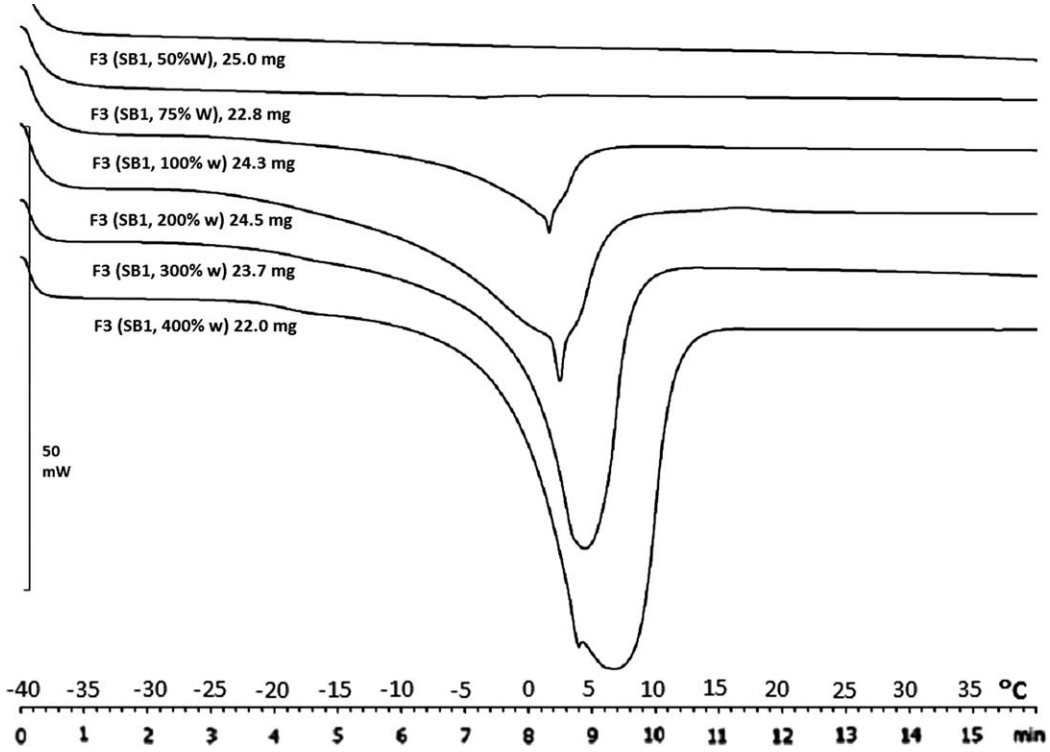

Figure 11 Series of DSC endothermic profiles for the F3-SB1 hydrogel partially swollen in water.

and 7.4 at room temperature. At specific time intervals, the swollen hydrogels were removed from the swelling medium and blotted with a piece of filter paper to absorb excess water on the surface; they were then weighed and immediately analyzed by DSC. Therefore, the total wet mass of the hydrogels (M_{TOTAL}) could be calculated as follows:

$$M_{TOTAL} = W_d + (W_S - W_d) \quad (7)$$

where W_d is the weight of the initial dried gel and W_S is the weight of the swollen hydrogel.

The total water content of the hydrogel (X_{TW}) was calculated as follows:

$$X_{TW} = 1 - (W_d/M_{TOTAL}) \quad (8)$$

$$X_{BW} = X_{TW} - (Q_{endo}/Q_f) \quad (9)$$

where X_{BW} is the bound water fraction in the hydrogel, Q_{endo} is the heat of fusion for freezable water in the hydrogel as obtained from the DSC scans (J/g), and Q_f is the heat of fusion of pure water (−333 J/g).[41]

A series of DSC scans for the hydrogels partially swollen in DI water are shown in Figure 11. The gradual expansion of the endothermic peaks indicated the increasing amount of free water fractions for the hydrogels. This figure clearly shows no DSC endothermic peak at up to 50% swelling, so no free water could be considered at this point.

Figure 12 presents the swelling ratios and X_{BW} values of the F3-SB1 hydrogel after immersion in media with pH values of 4.5 and 7.4. X_{BW} decreased slightly with increasing swelling ratio. This could be explained by the simultaneous increase in the free water fraction (X_{FW}) when X_{TW} was increased. In other words, the increase in total water at this stage was caused solely by the increase in free water in the hydrogel. The water imbibed later tended to stay relatively far from the charged groups, which were already surrounded by the water molecules absorbed earlier. For this reason, this part of the water was more likely to be the free water when determined through DSC.

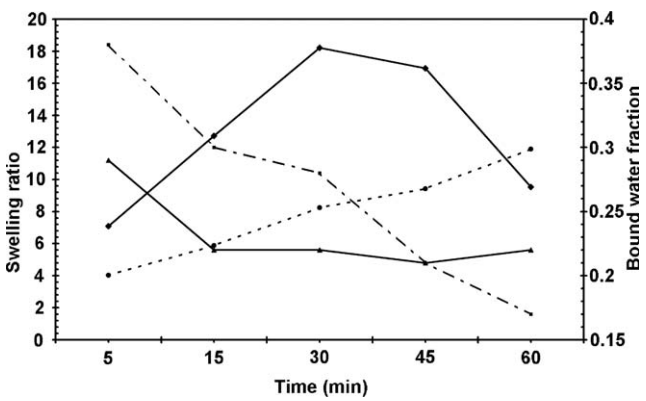

Figure 12 Swelling ratio of the F3-SB1 hydrogel at (◆) pH 4.5 and (●) pH 7.4 and X_{BW} of F3-SB1 hydrogel at (▲) pH 4.5 and (■) pH 7.4.

Although X_{BW} at the first point (5 min) for pH 7.4 was higher than for pH 4.5, this was probably due to the lower swelling ratio. The hydrogel at pH 4.5 had more protonated sites to take in water rapidly and keep the X_{BW} constant.

CONCLUSIONS

A physical hydrogel prepared through the physical entanglement of hydrophilic chains stabilized by a strong H bond between Chi derivatives and treated PVP was introduced in this article. The amino groups of Chi derivatives should have been free to take part in hydrophilic bonds between the two polymers. Quaternization or protonated ($-NH_2$) groups prevented hydrogel creation. A novel method was developed to synthesize TMCMC. It was shown that any hydrophilic substitution, such as carboxyl methylation, increased the hydrophilic characterization of the hydrogels and increased the swelling rate of the hydrogels. The physical hydrogel prepared by common and safe ingredients through a fast and easy process without the addition of any chemical agents and with a pH value of 6–7 seemed to be a good candidate for the preparation of delivery systems, such as implants, drug depots, or *in situ* gelling systems.

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