Carboxymethylcellulose–Chitosan-Coated Microneedles with Modulated Hydration Properties

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ABSTRACT: Microneedles containing sodium carboxymethylcellulose (CMC) formulations were fabricated to include an external chitosan (CS) layer to modulate their hydration profile, an important parameter affecting their application as intradermal delivery devices and their storage. The microfabrication process was carried out under conditions that enabled the formation of polyelectrolyte complexes between these oppositely charged macromolecules. CMC–CS microneedles were characterized by water uptake in a humid environment, contact angle measurements, dissolution in aqueous solutions, and protein-release profiles. The results demonstrate that the microneedles containing CMC–CS formulations displayed suppressed moisture sensitivity in water vapors compared to their unmodified CMC counterparts while the maintaining quick protein-release characteristics required for their uses. This approach also showed the potential for sustained protein-release applications, as the CMC–CS formulations could be combined in layers to fabricate multicompartment microneedle coatings with delayed release characteristics.
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Key words: biological applications of polymers; drug delivery systems; polyelectrolytes

INTRODUCTION

Microneedle technology offers an attractive alternative to traditional parenteral injections and hypodermic syringes, affording the potentially easier, safer, and more effective delivery of vaccines and therapeutic proteins.[1–6] A simple patch-based system containing microneedles works when it is pressed on the skin like a band aid so that microneedles, which are typically several hundred micrometers long, can penetrate beyond the skin's outer layer, the stratum corneum. Microneedles are commonly fabricated to contain a water-soluble physiologically active formulation in the form of a coating or even their entire body so that the drug or vaccine can be released by dissolution in a highly hydrated environment of the skin. Thus, the water solubility of microneedle formulations is a key element of the technology, as it allows the drug or vaccine to be released upon administration to the skin. However, the resulting moisture sensitivity of such solid formulations can present considerable challenges during their administration or storage under high humidity conditions; this leads to blunt or bent tips and potential hurdles with their insertion into the skin.[7,8] To alleviate these issues, storage at a reduced water vapor pressure[9] and the use of various excipients, such as sucrose or trehalose, have been considered essential for stabilizing and controlling the water sensitivity of drug or vaccine formulations.[10,11]

In this study, we explored the potential of polyelectrolyte complexes, which can be formed on the surface of hydrophilic formulations in a microfabrication process, to modulate the hydration properties of microneedles. To this end, sodium carboxymethylcellulose (CMC; Fig. 1), one of the polymers most commonly used in the microfabrication process for the encapsulation of drugs and vaccines,[12,13] appeared to be an attractive material because of its polyanionic properties and its broad spectrum of uses in biomedical applications.[14,15] Chitosan (CS; Fig. 1), a naturally occurring polysaccharide, which has been widely investigated for life sciences applications because of its biodegradability and other important characteristics,[16–19] was used in this study because it could be easily protonized to achieve polycationic behavior. Moreover, CMC–CS polyelectrolyte complexes have already attracted attention as various drug-delivery carriers and biomaterials[20,21] but have not yet been explored as microneedle fabrication agents.

In this article, we report on the fabrication of microneedles containing CMC–CS compositions and the study of their moisture sensitivity and functional properties, such as their ability to dissolve and release their protein-containing formulations.

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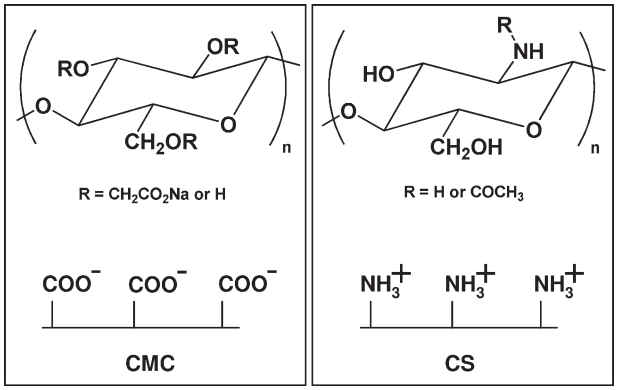

Figure 1 Chemical formulas and schematic presentations of ionized forms of CMC and CS.

EXPERIMENTAL

Materials

CMC United States Pharmacopeia–National Formulary (USP/NF grade, low viscosity, Hercules, Wilmington, DE), CS (low molecular weight, degree of deacetylation = 91.7%, viscosity = 46 cps for a 1% w/v solution in 1% w/v acetic acid, Aldrich, Milwaukee, WI), bovine serum albumin (BSA), sodium phosphate dibasic heptahydrate, sodium phosphate monobasic, potassium phosphate monobasic (Sigma, St. Louis, MO), sorbitan monolaurate, Tween-20 (Tween; TCI America, Portland, OR), and Dulbecco's phosphate buffered saline (PBS) without calcium and magnesium (HyClone Laboratories, Logan, UT) were used as received.

The 1% w/v CS stock solution was prepared by dispersion of the polymer in deionized water and the addition of 0.1*M* hydrochloric acid under stirring until its complete dissolution (pH 5). We prepared the 2% w/v CMC stock solution by stirring the polymer powder in deionized water until complete dissolution. Additional dilutions were made by the dilution of the stock solution with deionized water. Solutions of CMC and CS were filtered through γ-irradiated, 0.45-μm Millex syringe filters (Millipore, Billerica, MA) before use.

Turbidimetric titration

The turbidimetric titration of 0.02% w/v CMC with 0.2, 0.5, and 1.0% w/v CS was performed in deionized water at ambient temperature by measurement of the transmittance (*T*) of the mixture at 420 nm [ultraviolet–visible (UV–vis) spectrophotometer, Hitachi U-2810, San Jose, CA] in 2-mL cuvettes with a 1-cm path length with deionized water or phosphate buffer solution as a reference. The initial solutions were filtered with 0.2-μm Millex filters before titration. The solution was vortexed for 15 s after the addition of the titrant and monitored in the spectrophotometer until a stable turbidity reading (±0.1% *T*) was obtained.

Microneedle fabrication

Microneedle arrays were produced in a two-stage process. First, arrays of 50 metal shafts were manufactured by the chemical etching of titanium foil with hydrofluoric acid and were bent out of plane at a 90° angle. The design was similar to that of previously reported stainless steel microneedles.[12,22] Each shaft was 600 μm long, and the arrays had dimensions of 1 × 1 cm^2. Next, a micro-dip-coating process was performed at ambient temperature to coat the tips of these shafts with CMC and CS formulations to fabricate the microneedles. The coating formulation was fed to a 50-microwell reservoir with a Genie Plus syringe pump (Kent Scientific, Torrington, CT). A microneedle array was secured on an array holder and then attached to an X–Y–Z micropositioning system with alignment pins and holders. With the micropositioning system, the coating procedure was performed for all compositions by submersion of the shafts into the wells in the coating reservoir and then their immediate removal; this allowed contact between the microneedle and formulation for no longer than 1 s. Each submersion was followed by a drying step, in which the arrays were purged with anhydrous nitrogen gas for 7 s. A stereozoom microscope (STZ-45-BS-FR) with a digital camera (Caltex Scientific, Irvine, CA) was used to monitor the process.

Typically, the arrays were coated with a 1.5% w/v CMC and 0.2% w/v Tween formulation in deionized water to achieve a loading of 160–170 μg of CMC per array. Some of the CMC-coated arrays were then additionally coated with a 1% w/v CS solution in deionized water (pH 5) with two coating cycles. The arrays were then dried in a desiccator containing silica gel until a constant weight was reached, typically in 24 h.

BSA-containing microneedles were prepared with coating formulations containing 0.5% w/v BSA, 1.5% w/v CMC, and 0.2% w/v Tween in 5 m*M* phosphate buffer (pH 7.4) with 10 coating cycles to achieve a loading of 25 μg/array of BSA and 75 μg/array of CMC. They were then dried and additionally treated with 0.5% w/v CS solutions in deionized water (pH 5) with two coating cycles.

Multicompartment coating on the microneedles was created by sequential coatings with BSA–CMC formulations, as described previously, with 10 coating cycles and then with 0.5% w/v CS with 10 coating cycles, and then, the procedure was repeated to achieve a loading of 45 μg of BSA and 130 μg of CMC. The microneedles were dried for 2 h after each step of the coating procedure.

Analysis of the microneedles

Quantitative analysis of the coating was performed with UV–vis spectrophotometry (Hitachi U-2810

spectrophotomer) and size-exclusion high-performance liquid chromatography (Hitachi LaChrom Elite system) equipped with an Ultrahydrogel 250 size-exclusion column (Waters Corp., Milford, MA) with 0.1 × PBS with 10% v/v acetonitrile as a mobile phase. Each coated array was placed in an individual plastic weigh boat, and then, 0.5 mL of 0.1 × PBS was added to dissolve the coating.

Water-uptake experiments

The kinetics of water vapor uptake was measured gravimetrically at 50 or 100% relative humidity (RH) and ambient temperature with analytical balances (AL 204, Mettler Toledo, Columbus, OH). Coated arrays were dried in a desiccator in the presence of silica gel for 2 days until a constant weight was reached. Eighteen arrays were put in an aluminum boat and kept in a desiccator in saturated water vapors over deionized water (100% RH) or a saturated solution of magnesium nitrate in water (50% RH). RH was measured with a calibrated traceable humidity pen (Controlled Co., Friendswood, TX). To reduce evaporation during the gravimetric measurement, the boat containing the arrays was covered with aluminum foil. Three measurements were taken for each time point.

Changes in the shape and size of the coating during water vapor uptake were monitored with a Macrozoom 125 Photomicrography Imaging System coupled with a Zeiss Focus Block Stage, a ring-light illuminator, and a 3 MP Moticam 2300 CMOS digital camera (Bunton Instrument Co., Inc., Mount Airy, MD). The microneedle array was mounted onto a custom-built stage with a white background and secured with double-sided tape. Advanced 3.2 image analysis software (Motik, Xiamen, China) was used to characterize the coating size and shape.

Contact angle measurements

Static contact angle measurements were conducted by the coating of titanium foil with the same formulations that were used for microneedle fabrication. The titanium foil was cleaned with water, isopropyl alcohol, water, and ethanol and then dried under a nitrogen flow. The CMC and CMC–CS films were deposited onto the titanium surface with the dip-coating technique used for the preparation of the microneedles. A 3-μL droplet of deionized water was placed on the horizontally aligned coated foil. The droplet shape was recorded with a digital camera (Caltex Scientific, Irvine, CA) connected to a stereozoom microscope (STZ-45-BS-FR). The images were then processed, and the contact angles were calculated with Motic Images Advanced 3.2 software. At least 10 experiments were performed for each sample ($n = 10$).

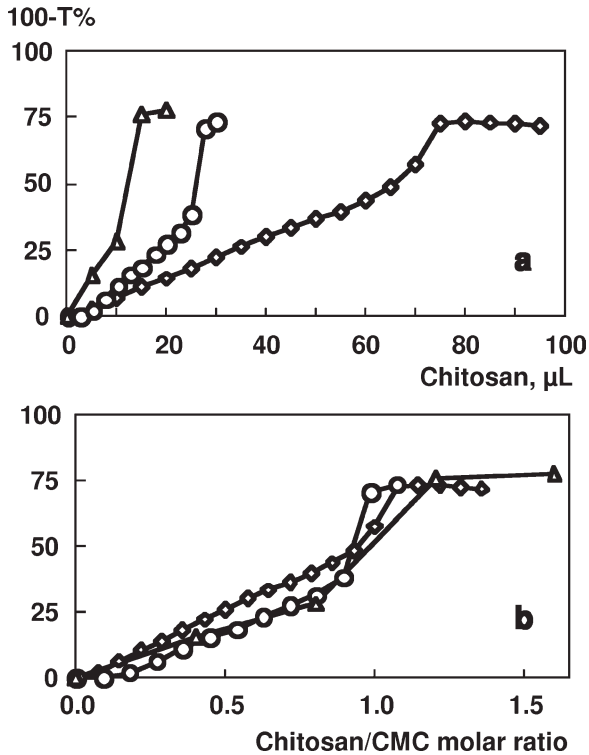

Figure 2 Turbidimetric titration of CMC with (◇) 0.2, (○) 0.5, and (△) 1.0% w/v CS in deionized water. The amount of titrant added is shown as the (a) volume of solution or (b) as a molar ratio between the repeating units of CS and CMC (0.02% w/v of CMC, 1 mL initial volume, 22°C).

Protein-release experiments

We performed a release study under ambient conditions by placing the microneedle arrays in 0.5 mL of PBS (pH 7.4). The solution was refreshed after each time point was taken. We analyzed the amount of BSA released from the microneedles via UV–vis spectrophotometry by obtaining the optical densities at 280 nm.

RESULTS AND DISCUSSION

CMC–CS complex formation in solution

Although the ability of CS to form polyelectrolyte complexes with CMC has been previously established,[20,23] it was important to investigate the complex formation under the conditions that were used for coating the microneedles. Figure 2 shows the results of the turbidimetric titration of CMC in aqueous solutions with various concentrations of CS. As shown in Figure 2, the formation of water-insoluble complexes was observed; this started from the beginning of the titration, with curves characterized by a steeper slope for higher concentrations of the titrant [Fig. 2(a)]. The first abrupt change in the slope was observed when the molar ratio of the repeating units reached approximate

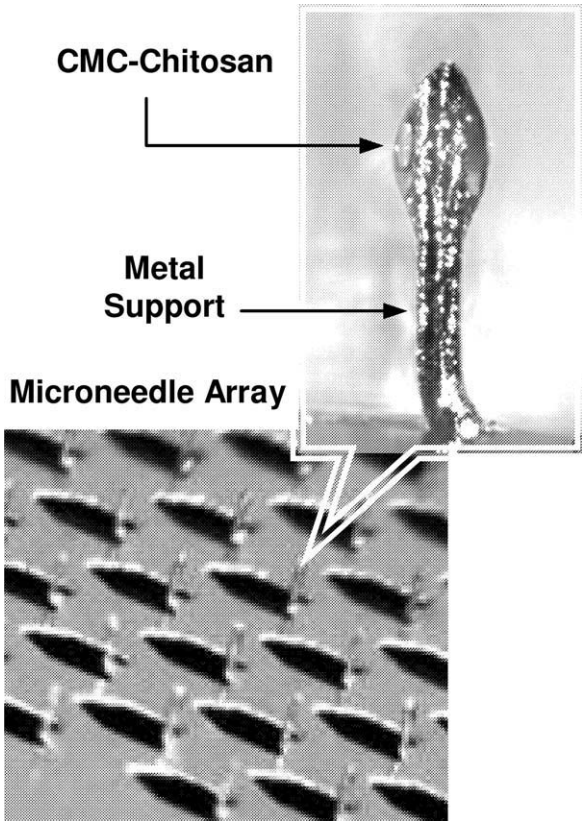

Figure 3 Optical microscopy images of the microneedles with CMC–CS coatings and microneedle arrays (length of the microneedles = 600 μm).

unity [Fig. 2(b)] and was considered an end point of the titration.[24] The results confirmed the formation of water-insoluble stoichiometric polyelectrolyte complexes in water when CS was added in the range of concentrations between 0.2 and 1% w/v. As surfactants are typically used for the fabrication of microneedles,[12,25] the titration was also performed in the presence of 0.2% w/v Tween; this revealed no significant differences compared to the surfactant-free systems.

Microfabrication of the CMC- and CMC–CS-coated microneedles

Microneedles containing CMC and CMC–CS formulations were fabricated with the previously described micro-dip-coating process of titanium supports, which were obtained by chemical etching.[3,22] Coating solutions containing 1.5% w/v CMC and 0.2% w/v Tween in deionized water were used to achieve CMC loadings of 150 and 170 μg per array. To prepare the CMC–CS formulations, the microneedle arrays were additionally coated with 0.05–1% w/v solutions of CS. Optical microscopy images of the CMC–CS-coated microneedles are displayed in Figure 3.

Water uptake of the CMC- and CMC–CS-coated microneedles

The ability of the CMC- and CMC–CS-coated microneedles to take up water was investigated in water vapors at ambient temperature. The optical microscopy inspection of the CMC-coated microneedles before [Fig. 4(a)] and after exposure to a 100% RH environment for 2 h [Fig. 4(b)] revealed some changes in both the coating shape and volume. The overlay of planar projections [Fig. 4(c)] of dry (white area) and swollen (black area) microneedles showed noticeable smoothing of the contours and an increase in the area indicating some swelling of the coating.

Comparative studies on the hydration of the CMC and CMC–CS coatings were further conducted gravimetrically. The results of water uptake at the 100% RH level are shown in Figure 5. For both the CMC and CMC–CS formulations, the water sorption profile was characterized with a rapid hydration of the polymer film in the first 30–50 min; this was followed by a much slower rate of hydration. The results clearly demonstrate that the surface treatment of the CMC-coated microneedles with CS suppressed the uptake of water by approximately 30%. Although a significant reduction in the swelling was observed, this additional coating was not capable of leveling the sorption off; this may have indicated the rupture of the polyelectrolyte complex coating due to osmotic pressure; this was to that previously observed for microspheres coated with a polyelectrolyte coating.[26]

The effect of the CS coating on the sorption kinetics was even more pronounced in experiments conducted at 50% RH, in which the water uptake for the CS-treated microneedles was reduced by more than 75% (Fig. 6). Moreover, contrary to the results

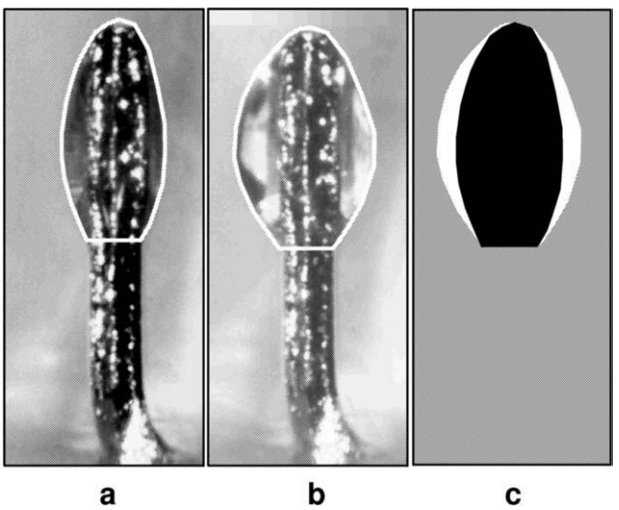

Figure 4 Optical microscopy images of the CMC-coated microneedles (a) before and (b) after exposure to water vapor for 2 h and (c) their overlap (100% RH, 150 μg of CMC per array).

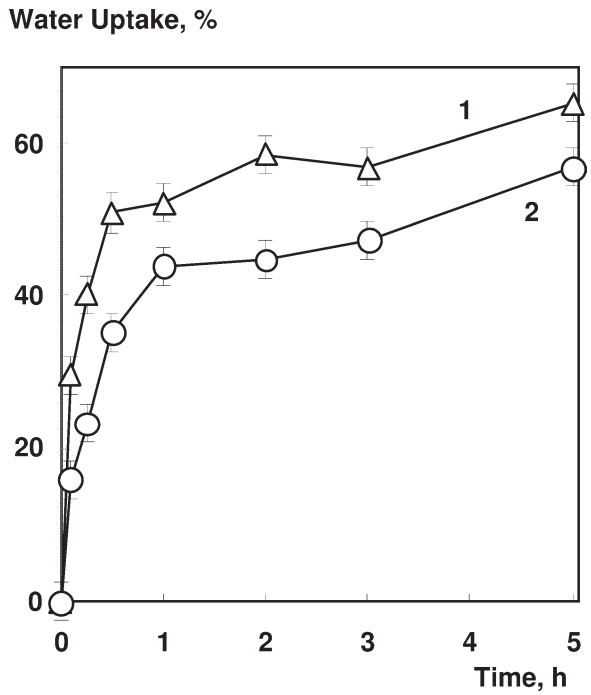

Figure 5 Kinetics of water vapor uptake by microneedle coatings containing (1) CMC and (2) CMC–CS formulations (170 μg of CMC per array, 22°C, 100% RH). The data represent the mean values, and the error bars indicate the standard deviation in each series ($n = 4$).

for the CS-treated microneedles at 100% RH and CMC microneedles at 50 and 100% RH, no rapid initial swelling was observed for the microneedles containing the polyelectrolyte complex; this suggested its integrity under the conditions used.

Most of the tested formulations displayed a very quick initial uptake of water (Figs. 5 and 6). Some of the factors that could have potentially contributed to this phenomenon include irregularities in the coating shape (larger surface area), which disappeared as the swelling progressed [Fig. 4(a,b)] or potential differences in the structure of the external and internal layers resulting from their various residence times in the coating solution during the microfabrication process; these require further investigation.

Characterization of the CMC–CS surfaces with contact angle measurements

We anticipated that the treatment of microneedle coatings with CS would alter their surface characteristics because of the formation of the polyelectrolyte complex, which displays water insolubility in aqueous solutions (Fig. 2). Changes in the hydrophilicity of the CMC coatings upon their modification with the cationic polyelectrolyte were followed by contact angle measurements of water with CMC–CS films deposited on metal foil with the dip-coating technique used for the preparation of the microneedles. Figure 7 demonstrates that the deposition of CS led to some increase in the hydrophobicity of the coating; however, the effect was not significant, and the surface remained highly hydrophilic. The presence of surfactant in the CMC formulations, which is required for the microfabrication process,[12,25] may have contributed to the maintenance of the hydrophilic characteristics. These results suggest that the effect of CS on the water-sorption properties of the microneedles was not due to the surface hydrophobization but rather to the formation of polyelectrolyte complex membranes and the resulting changes in the water permeability, which were similar to those previously described for microspheres.[26,27]

Protein release with the CMC- and CMC–CS-coated microneedles

As coated microneedles are designed to deliver their drug payload by dissolving solid formulations and releasing the drug in the environment of the skin, it was important to evaluate whether the CS coating had an effect on the release profiles in aqueous solutions. The CMC and CMC–CS microneedles were prepared to contain 25 μg of BSA per array, and the kinetics of protein release in PBS (pH 7.4) were evaluated.

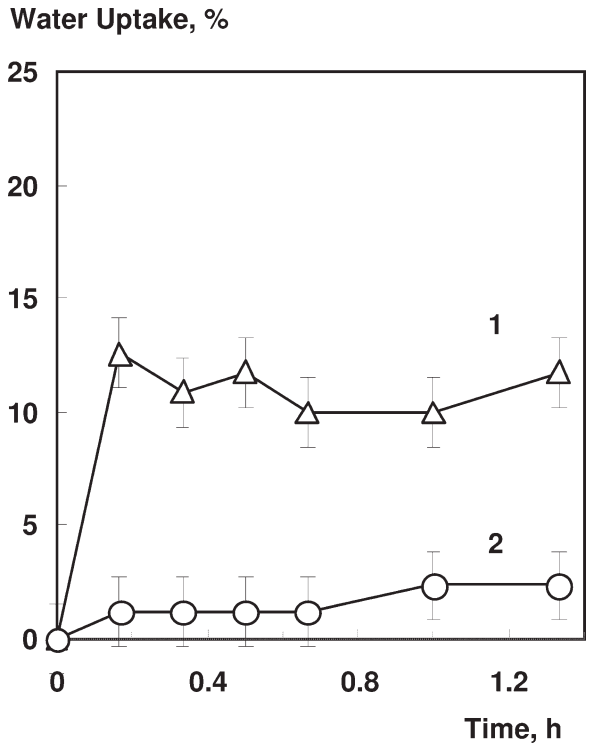

Figure 6 Kinetics of water vapor uptake by microneedle coatings containing (1) CMC and (2) CMC–CS formulations (170 μg of CMC per array, 22°C, 50% RH). The data represent the mean values, and the error bars indicate the standard deviation in each series ($n = 4$).

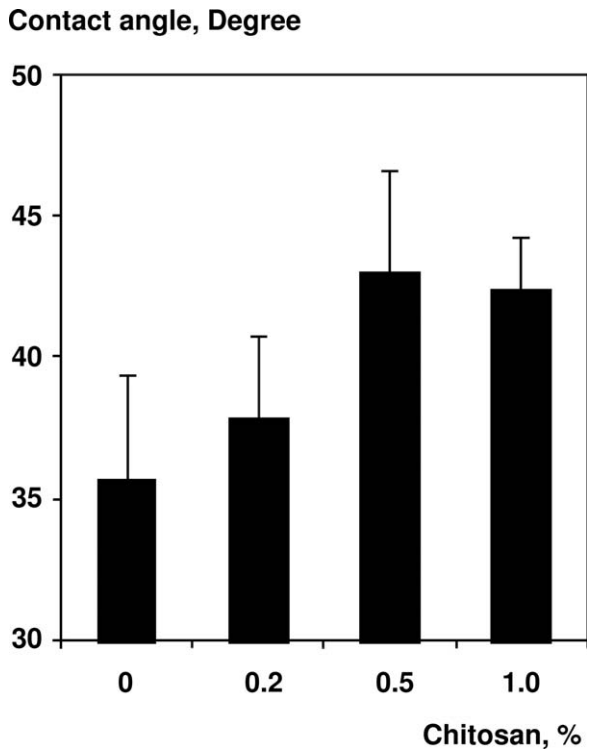

Figure 7 Contact angles of water on the CMC film and CMC films modified with various concentrations of CS. The films were deposited on titanium foil with a dip-coating process at 22°C. The data represent the mean values, and the error bars indicate the standard deviation in each series ($n = 10$).

Figure 8 shows that although the CS coating had some effect on the release profile at concentrations of the polyelectrolyte above 0.05% w/v, all of the microneedles released the protein sufficiently rapidly, with more than 80% of the drug dissolved within the first 10 min. These results demonstrate that the CS treatment modulated the moisture sensitivity of the CMC coatings and still provided acceptable release profiles for their pharmaceutical applications.

Finally, the feasibility of multicompartment microneedle coatings, in which layers of CS are superimposed on BSA containing CMC formulations to sustain protein release, was also evaluated. The results for the microneedles containing two CMC–BSA/CS compartments demonstrate that such a coating design extended the total BSA release time by approximately 18-fold compared to the CMC microneedles (Fig. 9). Thus, it appears that this approach can be used if the prolonged release of a protein drug is desirable.

Although CMC has been routinely used in the fabrication of microneedles,[12] the use of CS in such devices, to our knowledge, constitutes a new approach. CS has been widely investigated for biomedical applications in the past because of its degradability in the presence of lysozyme, an enzyme prevalent in the human body;[28] however, its safety profile can vary depending on the application,[28] and determination of the biocompatibility of CS–CMC microneedles will require further investigation.

CONCLUSIONS

Microneedles containing CMC formulations treated with CS were fabricated under conditions that enabled the formation of polyelectrolyte complexes between these macromolecules. CS-treated CMC microneedles displayed reduced hydration, as demonstrated by the water uptake; however, contact angle measurements did not reveal noticeable alterations in their surface hydrophobicity. Protein-release experiments showed that the treatment of the microneedles with one layer of CS practically did not affect their dissolution rate; this is an important parameter required for their effective biomedical application. Thus, the fabrication of the CMC–CS-coated microneedles allowed suppression of their water sensitivity while maintaining the main functional characteristics and resulted in a potential improvement of their storage and application characteristics. Moreover, the formation of multiple CMC–CS compartments within

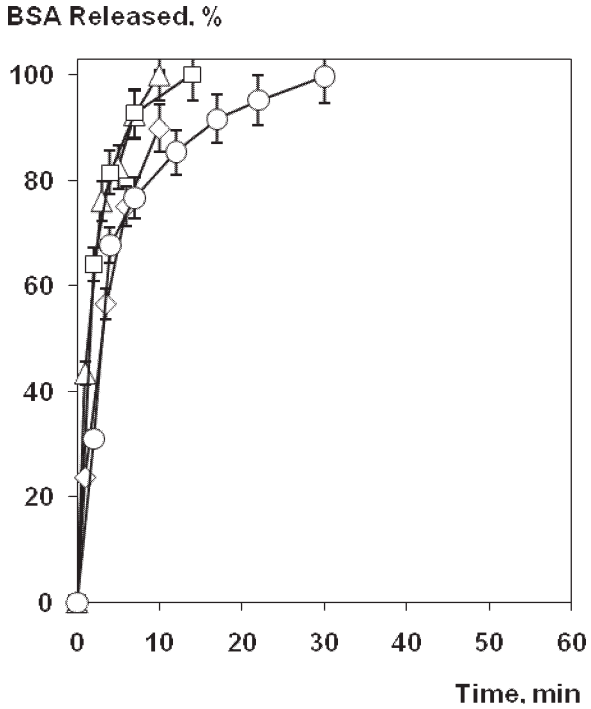

Figure 8 BSA release profiles for the (△) CMC-coated microneedles and CMC-coated microneedles treated with (□) 0.05, (◇) 0.5, and (○) 1% w/v aqueous CS solutions. The microneedles contained 25 μg/array of BSA and 75 μg/array of CMC (release media: PBS, pH 7.4, 22°C). The data represent the mean values of the duplicates, and the error bars indicate the standard deviation in each series.

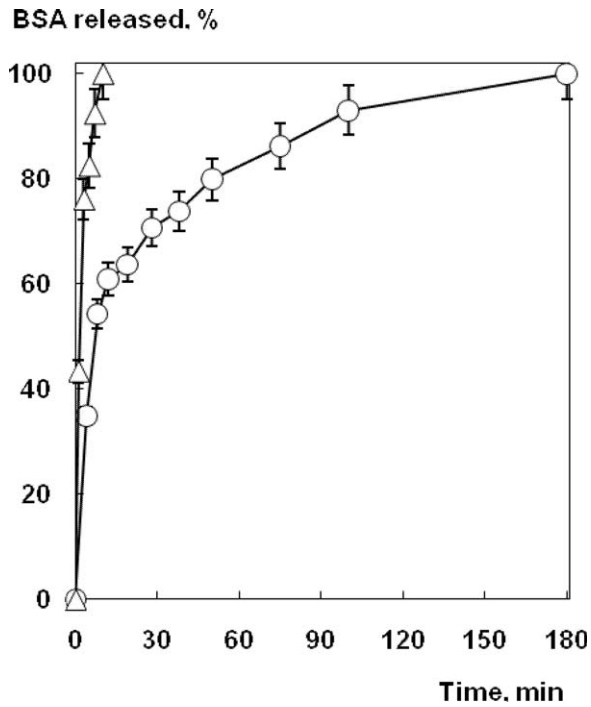

Figure 9 BSA release profiles for the (△) CMC-coated microneedles and (○) multicompartment CMC–CS microneedles. The microneedles contained 45 μg of BSA and 130 μg of CMC. The details of multicompartment microneedle fabrication are in the text (release media: PBS, pH 7.4, 22°C). The data represent the mean values of the duplicates, and the error bars indicate the standard deviation in each series.

the microneedle coating also demonstrated the potential of the approach for the achievement of formulations with sustained release profiles.

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