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Simultaneous nanoparticle formation and encapsulation driven by hydrophobic interaction of casein-*graft*-dextran and β-carotene

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Abstract

Casein-*graft*-dextran copolymer was produced using the Maillard reaction. The copolymer is molecularly soluble in neutral aqueous solution whereas β-carotene is extremely insoluble. By the method of dialysis or evaporation then dispersing in water, the solubility of hydrophobic complex of casein and β-carotene decreases whereas the solubility of dextran increases gradually, then nanoparticles formed with casein and β-carotene core and dextran shell. The particles have spherical shape and are stable in aqueous solution against dilution, pH change, ionic strength change, $FeCl_3$ oxidation and long time storage. The encapsulated β-carotene can be released by pepsin or trypsin hydrolysis. To our knowledge, this is the first report of simultaneous nanoparticle formation and encapsulation induced by hydrophobic interaction. Casein-*graft*-dextran copolymer and the nanoparticle preparation is a green process in which only alkali and ethanol, no other chemicals, were used.

Keywords: β-Carotene; Casein; Copolymer; Dextran; Drug delivery; Nanoparticle

1. Introduction

Inspired by the convenience of organizing molecules via non-covalent associations, self-assembly has undoubtedly been an active and promising field of current chemistry [1,2]. It is well-known that amphiphilic block copolymers can self-assemble in selective solvents to form micelles with a core and a shell containing insoluble and soluble blocks, respectively [3,4]. Micelles can also be prepared in common solvents through non-covalent electrostatic interaction or hydrogen bonding. Kataoka and co-workers [5,6] prepared polyion complex micelles by mixing a charged block polymer with an oppositely charged compound such as ionomer, peptide, and DNA. In recent years, Jiang and coworkers have developed block-copolymer-free strategies to fabricate polymeric nanoparticles. They used homopolymers, random copolymers, and oligomers as building blocks to construct nanoparticles driven by hydrogen bonding complexation [7].

Nanoparticles formed from amphiphilic copolymers have been explored as hydrophobic drug carriers [8,9]. The core made from hydrophobic segments serves as the cargo space for lipophilic drugs, while the shell composed of hydrophilic segments stabilizes the particles in aqueous dispersions [9,10]. The method of hydrophobic drug incorporation employed depends mostly on the method of particle preparation. Commonly used loading methods include dialysis and various solvent evaporation procedures [9,10]. In dialysis method, the drug is added with the copolymer to the common organic solvent that is miscible with water. Then the mixture is dialyzed against distilled water. The drug is encapsulated in the particles when the solvent is changed to water [11–14]. The drug loaded particles can also be prepared by dissolving the drug and copolymer in common organic solvent, then evaporating the solvent and adding aqueous solution [15,16]. For safety purpose, biopolymers are interesting alternatives to synthetic polymers as drug carriers [17]. In particular, fabricating polymeric carries without using synthetic chemical reagents and organic solvents is obviously desirable for biomedical applications [18].

Caseins are predominant proteins in milk. The four casein constituents, α_{S1}-, α_{S2}-, β-, and κ-casein, exist approximately

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in proportions of 4:1:4:1 by weight in cow milk and their molecular weights are 19–25 kDa [19]. All of the four caseins are amphiphilic and have ill-defined structures [20]. Caseins exist as polydisperse particles in neutral pH. In our previous work, dextran was grafted to casein to increase the hydrophilicity through the Amadori rearrangement of the Maillard reaction, which is a nontoxic reaction happening naturally during the processing, cooking, and storage of foods [21]. β-Carotene is a precursor of vitamin A. It was reported that β-carotene can lower the risk of cancer and is able to act as an antioxidant [22]. However, the application of β-carotene is limited by its sensitivity to light, heat, and air/oxygen and by extremely poor solubility in water. β-Carotene can be encapsulated inside carbon nanotubes [23] and Triton X100 micelles [24]; the encapsulation can suppress the light degradation and air oxidation of β-carotene.

The hydrophobic drug carriers composed of amphiphilic copolymers usually form nanoparticles by themself. In this paper, we used casein-*graft*-dextran (casein-*g*-dextran) copolymer which is molecularly soluble at aqueous solution except at pH 4.6 to fabricate nanoparticles. We take the advantages of the high hydrophobicity of β-carotene to induce the interaction with the hydrophobic segments of casein, forming the particle core; the hydrophilic dextran shell makes the particles stable and dispersible in the pH range of 2−12. This is a process of simultaneous nanoparticle formation and encapsulation driven by hydrophobic interaction, which has not been reported before.

2. Materials and methods

2.1. Materials

Casein (technical grade) was from Sigma Chemical Co (St. Louis, MO). β-Carotene ($\geqslant$97%, $\approx$1600 units/mg) was from Fluka Chemie GmbH (Buchs, Switzerland). Dextran (M_w 10 kDa), pepsin (high pure, 3000–3500 NFU/mg) and trypsin (USP, >2500 U/mg) were from AMRESCO Inc (Solon, OH). Casein-*g*-dextran copolymer was prepared by 8 or 20 h Maillard reaction at pH 7.0 with molar ratio of casein to dextran 1:8 as previously reported [21]. Briefly, casein was dissolved in deionized water and the solution was adjusted to pH 7.0 with NaOH, and then dextran solution was added. The mixture solution was lyophilized. The frozen-dry powder was reacted at 60 °C and relative humidity of 78.9%. The resultant products were kept at −20 °C before use. *o*-Phthaldialdehyde assay was used to analyze the grafting degree of casein. Averagely, about 3 and 5.8 dextran molecules were grafted to one casein molecule after 8 and 20 h Maillard reaction, respectively.

2.2. Nanoparticle preparation

β-Carotene was dissolved/dispersed in absolute ethanol with a designed concentration (0.1–5%, w/v) and the solution was sonicated for 10 min. The same volumes of β-carotene ethanol solution and 1 mg/mL casein-*g*-dextran copolymer aqueous solution (pH 7.0) were mixed together, the resultant mixture was stirred for 24 h, and then the solvent of the mixture was totally removed under vacuum at 0 °C. In succession, the sample was re-dispersed in water and the medium was removed again under vacuum at 0 °C. After that, cyclohexane or ethanol was used to wash the sample several times to remove unencapsulated β-carotene completely until the solvent phase was colorless. Finally, the sample was dried under vacuum at room temperature and then was stored at 4 °C before use. In the procedure, the samples were kept in dark to avoid the light degradation of β-carotene.

2.3. Measurements

Dynamic light scattering (DLS) was measured using a laser light scattering spectrometer (Malvern Autosizer 4700, Malvern Instrument, Worcs, UK) equipped with a multi-τ digital time correlator (Malvern PCS7132) and Compass 315 M-100 Diode-Pumped Laser (output power $\geqslant$100 mW, $\lambda_0 =$ 532 nm; Coherent Laser division, Santa Clara, CA) as the light source. All DLS measurements were performed at 25.0 ± 0.1 °C and at 90° scattering angle. The measured time correlation functions were analyzed by the automatic program equipped with the correlator. Apparent *z*-averaged hydrodynamic diameter $\langle D_h \rangle$ (simply written as hydrodynamic diameter) and polydispersity index ($\langle \mu_2/\Gamma^2 \rangle$) [25] were obtained by a CONTIN mode analysis. The copolymer concentration for DLS measurement was shown in casein concentration, which was 1.0 mg/mL in each sample.

ζ-Potentials of the samples were recorded at 25 °C on a ZetaSizer Nano ZS90 (Malvern Instrument, Worcs, UK) based on the technique of Laser Doppler Electrophoresis. The electrophoresis mobility U_E was measured and the zeta potential ζ was calculated by Henry equation [26], $U_E = (2\varepsilon\zeta/3\eta) f(k_a)$, where ε, η, $f(k_a)$ were the dielectric permittivity of the solvent, viscosity of the solution, and Henry's function. The value of $f(k_a)$ here was determined to be 1.5 according to Smoluchowski approximation. The copolymer concentration for ζ-potential measurement was shown in casein concentration, which was 1.0 mg/mL in each sample.

X-ray powder diffraction (XRD) data were acquired from thin layer samples molded with dried powder samples. The measurements were carried out on a X'Pert Pro diffractometer (PANalytical, Almelo, Holland) using Cu$K\alpha_1$ radiation ($\lambda =$ 1.541 Å) and 2θ scattering angle of 8–60°.

Atomic force microscopy (AFM) samples were prepared by drying the solution naturally on freshly cleaved mica surface. Image acquisitions were performed in Tapping Mode on a Digital Instruments Nanoscope IV (Veeco Instruments, Santa Barbara, CA) equipped with a silicon cantilever of 125 μm and an E-type vertical engage piezoelectric scanner. The drive frequency was 248 kHz and the voltage was between 2.0 and 3.0 V. A drive amplitude of 56 mV, a set point of 1.5 V, and a scan rate of 1.0 Hz were used. Height signal and phase signal were acquired simultaneously.

2.4. Release of encapsulated β-carotene through pepsin or trypsin hydrolysis

1 mg of β-carotene/copolymer nanoparticle powder was dissolved/dispersed in 1 mL of 0.1 M HCl. The solution was incubated at 37 °C and then 50 μL pepsin solution (0.2 mg pepsin in 1 mL 0.1 M HCl) was added to initiate the hydrolysis [27]. Aliquot of the hydrolysis solution was taken out at intervals of 15 min. The hydrolysis was stopped by adding 0.5 mL of 1 M NaOH. Then, 1.5 mL of cyclohexane was added and the resultant solution was stirred gently for 1 h to extract released β-carotene. The absorbance of the cyclohexane phase at 455 nm was recorded on a UV–vis spectrometer (Lambda 35, Perkin Elmer, Waltham, MA). The release through trypsin hydrolysis was performed at 0.2 M pH 8.0 Tris-HCl buffer and the hydrolysis was stopped by adding 0.5 mL of 0.2 M HCl; the other conditions were same as pepsin hydrolysis [28].

2.5. Stability of β-carotene against $FeCl_3$ oxidation

Two kinds of samples, the solution of β-carotene/copolymer nanoparticles and the solution of β-carotene/copolymer nanoparticles hydrolyzed by trypsin, were used to study the stability. Sample solution of 100 μL (1 mg/mL, pH 7.0) was mixed with 100 μL of 20 μg/mL $FeCl_3$ solution. The mixture was incubated at room temperature for a designed time and then $FeCl_3$ was removed by ultrafiltration. 1 mL of water was added to dissolve/disperse the sample and 1 mL cyclohexane was added to extract all β-carotene by vigorous stir until water phase was colorless. The absorbance of the cyclohexane phase was recorded at 455 nm.

2.6. Quantitative analysis of encapsulated β-carotene in the nanoparticles

The method is similar as the release of encapsulated β-carotene using trypsin hydrolysis. The hydrolysis prolonged for 24 h to release β-carotene completely. After the stop of the hydrolysis, cyclohexane was added and the resultant solution was stirred for several hours until the water phase was colorless. The absorbance of the cyclohexane phase at 455 nm was recorded.

3. Results and discussion

We prepared casein-*g*-dextran copolymer by grafting dextran (10 kDa) molecules to casein backbone. The copolymer is soluble in pH 7.0 aqueous solution, but insoluble in ethanol due to the insolubility of dextran side chains. β-Carotene is extremely insoluble in aqueous solution, while its solubility increases to 30 mg/L in ethanol [29]. We mixed same volumes of the copolymer aqueous solution and β-carotene ethanol dispersion together. In 1:1 water/ethanol (v/v) solvent, precipitates composed of the copolymer and β-carotene appeared after 4 days' storage. Control experiments excluded the interaction between dextran and β-carotene; therefore, the precipitates indicate that the interaction between casein and β-carotene exists.

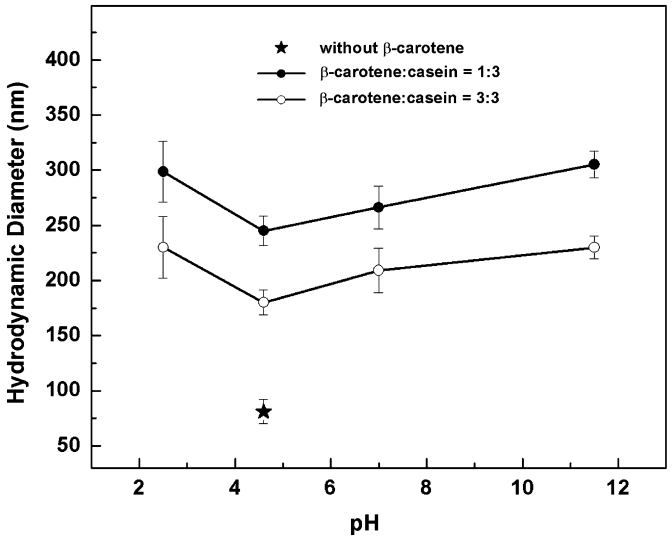

Fig. 1. Hydrodynamic diameter changes of the particles at different pH aqueous solutions. The particles were produced by weight ratios of β-carotene to casein 1:3 and 3:3 in feed and produced without β-carotene.

Freshly prepared copolymer and β-carotene mixture was dialyzed against 70, 80, 90 and 100% water solution (water percentage in water ethanol solvent, v/v) step by step to increase water concentration in the solution gradually. When water percentage increased to 100%, DLS measurement detected particles with hydrodynamic diameter about 200 nm, implying that the nanoparticles formed in the process of increasing water concentration.

The gas pressure of water and ethanol at 0 °C is 4.6 and 12.3 mm Hg [30], respectively, therefore, ethanol evaporates from the sample preferentially. During evaporation process, the percentage of residual ethanol in the sample decreases gradually just as the dialysis process described above. We also used "evaporation method" to fabricate nanoparticles of the copolymer and β-carotene at pH 7.0. After all of the solvent was removed, water was added to the particle sample to produce the particle dispersion in water. Fig. 1 shows the hydrodynamic diameters of the particles with and without β-carotene at different pH aqueous solution. The copolymer alone exhibits pH dependent behavior, that is, no particles form over a broad pH range except at pH 4.6, the isoelectric point (pI) of casein. The copolymer forms particles with hydrodynamic diameter about 80 nm at pH 4.6. When pH of the copolymer solution is not at 4.6, DLS measurement shows no peak in the size distribution curves, indicating that the copolymer dissolves molecularly in the solution. This result consists with our previous study and the explanation is that the electrostatic repulsion of casein and the hydrophilic dextran side chains make the copolymer molecularly soluble in aqueous solution [21]. However, in the presence of β-carotene, the copolymer behaves differently. Nanoparticles form and their hydrodynamic diameter is about 200 nm at pH 7.0, which is similar as the particles produced by the dialysis method. The particles are stable over the pH range of 2.0–12.0; the size of the particles changes, but the scattering light intensity and polydispersity index (<0.2) do not change. Fig. 1 shows that the minima of hydrodynamic diameter occur

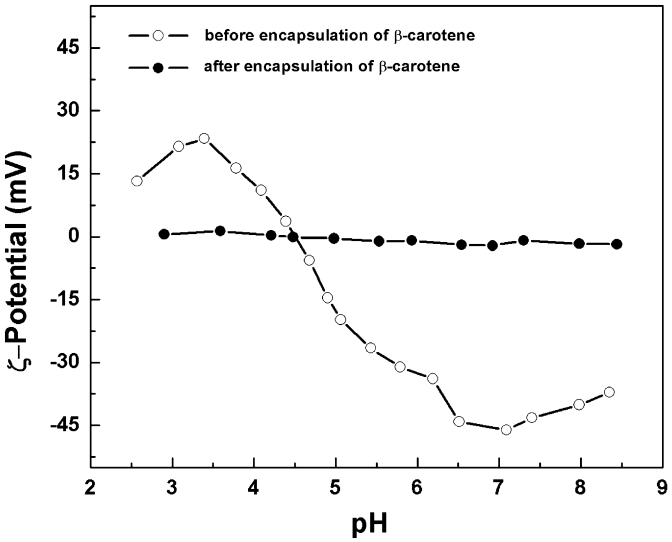

Fig. 2. The pH dependence of ζ-potentials of casein-g-dextran copolymer before and after encapsulation of β-carotene.

at pH 4.6. It is obvious that the electrostatic repulsion of casein causes the particles to expand when pH is away from pI of casein.

Fig. 1 shows that at pH 4.6 the particles composed of the copolymer and β-carotene are larger than the particles composed of the copolymer alone. It is possible that the incorporation of β-carotene increases the volume of the particle core. Two curves of the hydrodynamic diameters of the particles produced by weight ratios of β-carotene to casein 1:3 and 3:3 in feed against pH show parallel variation; the increase of the weight ratio of β-carotene to casein in feed causes the hydrodynamic diameter to decrease about 70 nm in the pH range we studied. The reason is not understood. The particles discussed here were prepared using the copolymer with average grafting number of 5.8. Another copolymer with average grafting number of 3.0 was also used to prepare particles with β-carotene. DLS result does not show significant difference between these two kinds of particles.

Fig. 2 shows ζ-potential curves of the individual copolymer and the nanoparticles composed of the copolymer and β-carotene at different pH. As we know, ζ-potential is directly related to the surface net charges of macromolecules and particles [31]. For the copolymer solution, the charges of casein determine ζ-potentials. The zero ζ-potential appears at pH 4.6 which is the pI of casein. The copolymer shows positive and negative ζ-potential values when the pH of the solution is lower and higher than 4.6, respectively. It is noticeable that the ζ-potential curve of the particles is completely different, that is, the ζ-potential values are about zero in the whole pH range we studied. This indicates that the casein chains are buried in the particle core for reason that their charges cannot influence ζ-potentials. This also indicates that it is the hydrophilic dextran chains, not charges that stabilize the particles in aqueous solution. Considering the result in Fig. 1 that the copolymer alone is molecularly soluble when the pH of the solution is not at 4.6, but the copolymer forms particles with β-carotene in the pH range of 2–12, and our control experiments that individual casein or individual dextran cannot form particles with β-carotene at the same condition, we can conclude that the particles have casein and β-carotene core and dextran shell. This particle structure consists with the result that the solubility of the hydrophobic complex of casein and β-carotene decreases while the solubility of dextran increases during the process of dialysis or evaporation.

Table 1
Quantitative analysis of encapsulated β-carotene in the nanoparticles

Weight ratio[a]	Encapsulated amount[b] (%)	Encapsulation efficiency[c] (%)
1:10	5.4 ± 0.4	54 ± 4
1:1	10.5 ± 0.5	10.5 ± 0.5
5:1	18.8 ± 0.3	4.7 ± 0.1

[a] Weight ratio of β-carotene in feed to casein in feed.
[b] Weight ratio of encapsulated β-carotene to casein in feed.
[c] Weight ratio of encapsulated β-carotene to β-carotene in feed.

The study above shows that the encapsulation of β-carotene is based on the hydrophobic interaction between β-carotene and hydrophobic segments of casein. Quantitative analysis of encapsulated β-carotene in the particles (Table 1) shows that at the weight ratio of β-carotene to casein 1:10 in feed, the encapsulated amount is about 5.4%, while the encapsulation efficiency is high, reaching about 54%. Increasing the weight ratio of β-carotene to copolymer in feed, the encapsulated amount increases whereas the encapsulation efficiency decreases. This reflects that the binding constant of casein and β-carotene is not a big value. At the weight ratio of β-carotene to casein 5:1 in feed, the encapsulation efficiency is only 4.7%, but the encapsulated amount reaches 18.8%, that is, one copolymer molecule can encapsulate 8.5 β-carotene molecules averagely. Table 1 shows that the encapsulation efficiency cannot reach 100% at any weight ratio in feed. This may be ascribed to that the β-carotene molecules located on the surface of the particles were removed by cyclohexane or ethanol during the wash process.

The stability of β-carotene/copolymer particles against dilution at pH 7.0 was investigated using DLS. We found that decreasing particle concentration from 1000 to 1 mg/L (shown in casein concentration), the size distributions do not change, indicating that the particles do not dissociate. When the particle concentration is less than 1 mg/L, the noise is too strong in DLS measurement, so we cannot obtain the particle dissociation concentration. This is different from the individual copolymer which only forms particles at pH 4.6 with particle dissociation concentration of about 10 mg/L [21]. Fig. 3 displays almost reproducible hydrodynamic diameter distributions of β-carotene/copolymer particles in water monitored over a period as long as 91 days. In the solution with physiological ionic strength, β-carotene/copolymer particles are stable, whereas the individual copolymer particles formed at pH 4.6 tend to dissociation. Perhaps, dextran side chains increase steric hindrance and salt screens inter-copolymer electrostatic attraction, therefore, the individual copolymer prefers to dissolve in molecular state. On the other hand, it is the interaction of casein and β-carotene that causes the high stability of

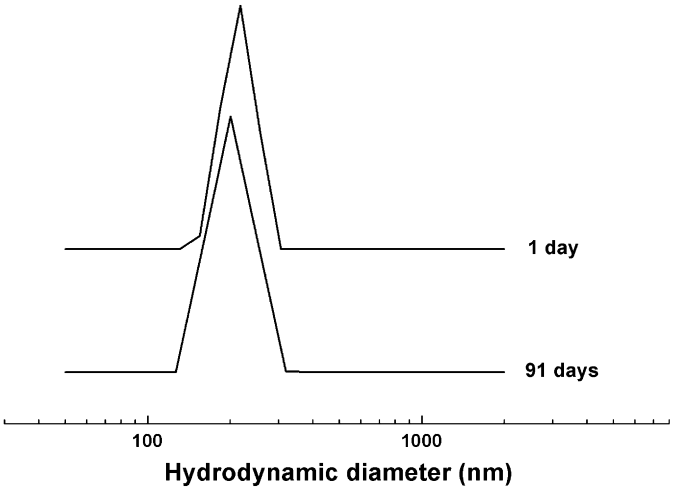

Fig. 3. Hydrodynamic diameter distributions of the particles composed of the copolymer and β-carotene at pH 7.0 after 1 and 91 days' storage.

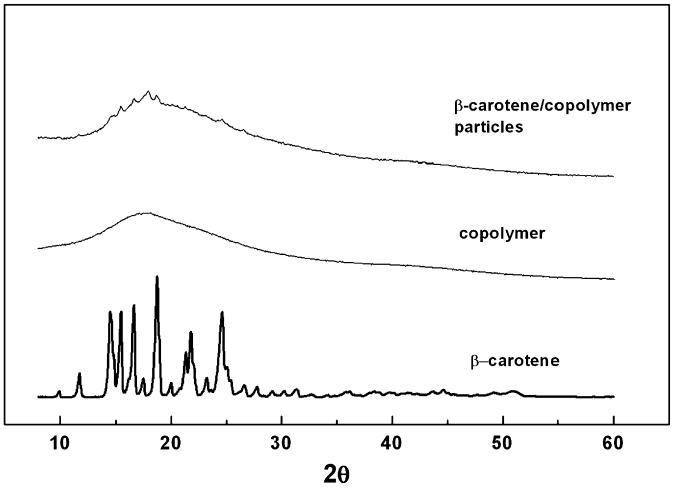

Fig. 4. XRD patterns of β-carotene, casein-g-dextran copolymer before and after encapsulation of β-carotene.

β-carotene/copolymer particles against dilution, storage, ionic strength change and pH change. Another advantage is that the particles can be stored in dried form after preparation as described in experimental section. These are no doubt valuable characters for the practical applications of the particles.

X-ray powder diffraction (XRD) measurements were performed to study the structure of β-carotene in the particles. Pure β-carotene exhibits strong crystal diffraction peaks (Fig. 4) whose 2θ values consist with the data in powder diffraction database (JCPDS cards, No. 14-0912). Casein-g-dextran copolymer does not show any diffraction peak, but a broad band as of soft polymers. For β-carotene/copolymer particles, some small diffraction peaks appear together with the broad band. All of these small peaks are identical with pure β-carotene diffraction pattern although the intensities are much weaker. The explanation may be that β-carotene in the particles has poor crystal regularity. The interaction of β-carotene with casein may decrease the crystalline order and crystallite size of β-carotene effectively, and this will be more advantageous for nutrient delivery than pure β-carotene. β-Carotene crystallites in the particles may have important effect on particle formation. As discussed above, the hydrophobicity of individual copolymer is too weak to form particles whereas the hydrophobicity of β-carotene is too strong to dissolve in water. It is possible that after interaction with β-carotene as well as the aggregation and crystallization of β-carotene, casein chains are trapped in the aggregates, resulting in the formation of the particles.

Fig. 5 shows atomic force microscopy (AFM) images of both copolymer particles and β-carotene/copolymer particles. Height image (Fig. 5B) and phase image (Fig. 5C) were acquired simultaneously; so did Figs. 5E and 5F. The height mode of AFM reflects the surface property of the object, while the phase mode measures the stiffness or elasticity difference of the object [32]. For β-carotene/copolymer particles, the height image shows globular shape with smooth surface. On average of 40 particles, the diameter is about 110 ± 45 nm and the height is about 6 ± 2 nm, which is much smaller than the hydrodynamic diameter of about 200 nm measured using DLS. As we know, DLS provides the data for the particles swollen in solution, while AFM shows the images of the particles spread and collapsed on mica surface. For the copolymer particles without β-carotene at pH 4.6, the diameter is about 70 nm and the height is about 4 nm, indicating that the copolymer particles are smaller than β-carotene/copolymer particles which consists with the DLS results (Fig. 1).

The phase image of β-carotene/copolymer particles shows both bright and dark areas in each of the particles whereas the phase image of copolymer particles shows dark area only. In phase mode, more rigid material generally results in brighter image [33]. As β-carotene is a stiff molecule and tends to crystallization in the particles proved by XRD analysis, we can deduce that the bright area is the image of β-carotene. The phase image shows that β-carotene disperses inside the particles.

As mentioned above, β-carotene/copolymer particles are stable in the solution with physiological ionic strength. This implies that the hydrophobic interaction between β-carotene and casein is too strong to be destroyed in this condition. In order to release β-carotene encapsulated in the particles, pepsin and trypsin were used to hydrolyze casein. Pepsin and trypsin are proteases; they are able to degrade protein in stomach and pancreas respectively. In our release experiment, cyclohexane as a good solvent for β-carotene was used to extract β-carotene released from the particles. A control experiment verified that β-carotene cannot be released without pepsin or trypsin in the same conditions. Fig. 6 shows the release curves of encapsulated β-carotene under the hydrolysis of pepsin and trypsin. Our control experiment showed that the individual copolymer was almost hydrolyzed by trypsin for 30 min at same condition (data not shown). However, the curves in Fig. 6 show that almost no β-carotene is released during the first 75 min pepsin hydrolysis and first 60 min trypsin hydrolysis. This is understandable as it takes more time for proteases to hydrolyze the casein interacted with β-carotene in the core. The released β-carotene increases suddenly to about 50% when the hydrolysis reactions proceed for 100 min, and then there is a slow release process in both cases. The encapsulated β-carotene can be completely released after 6 h hydrolysis. In our hydrolysis

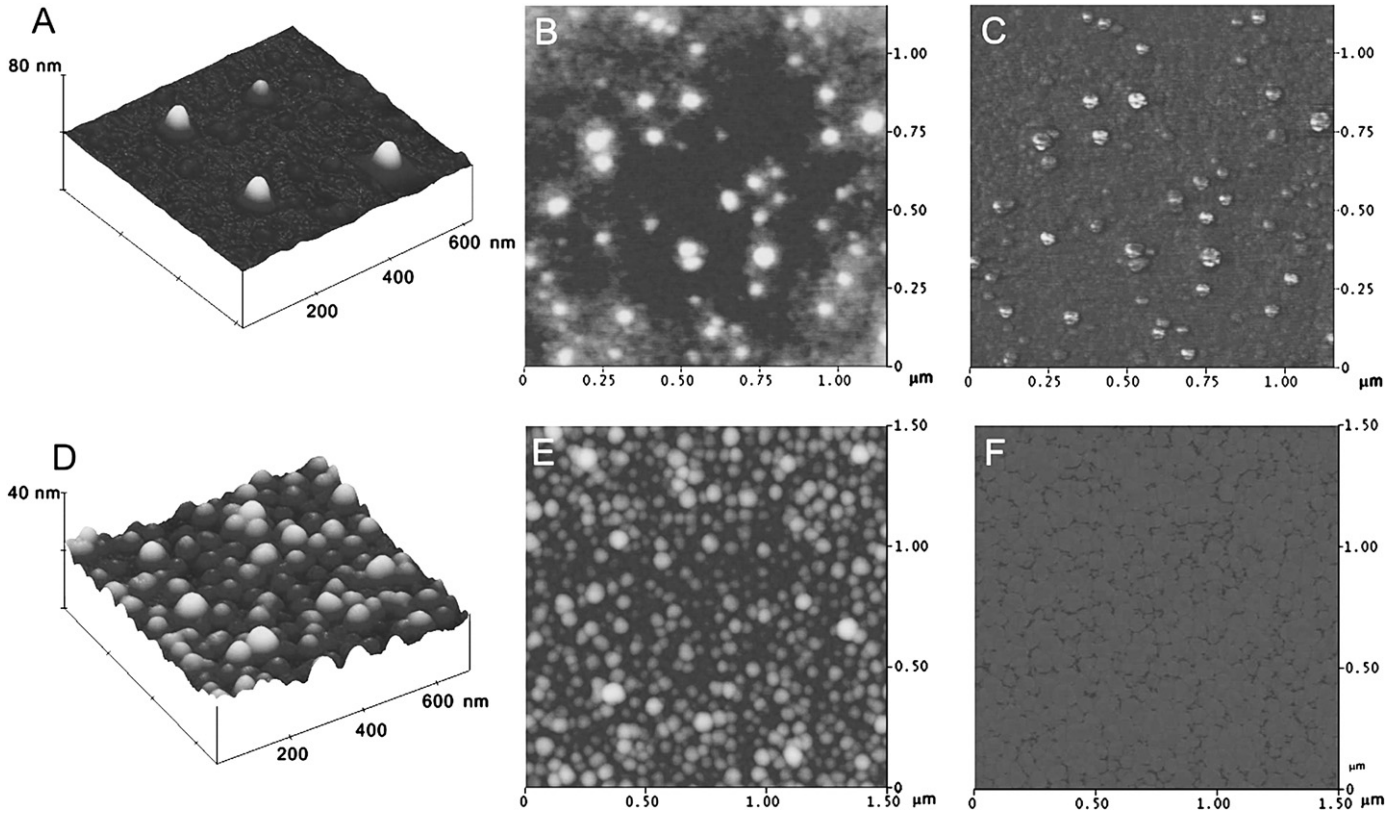

Fig. 5. AFM height images (A, B) and phase image (C) of the particles composed of β-carotene and copolymer at pH 7.0; AFM height images (D, E) and phase image (F) of the particles composed of copolymer alone at pH 4.6.

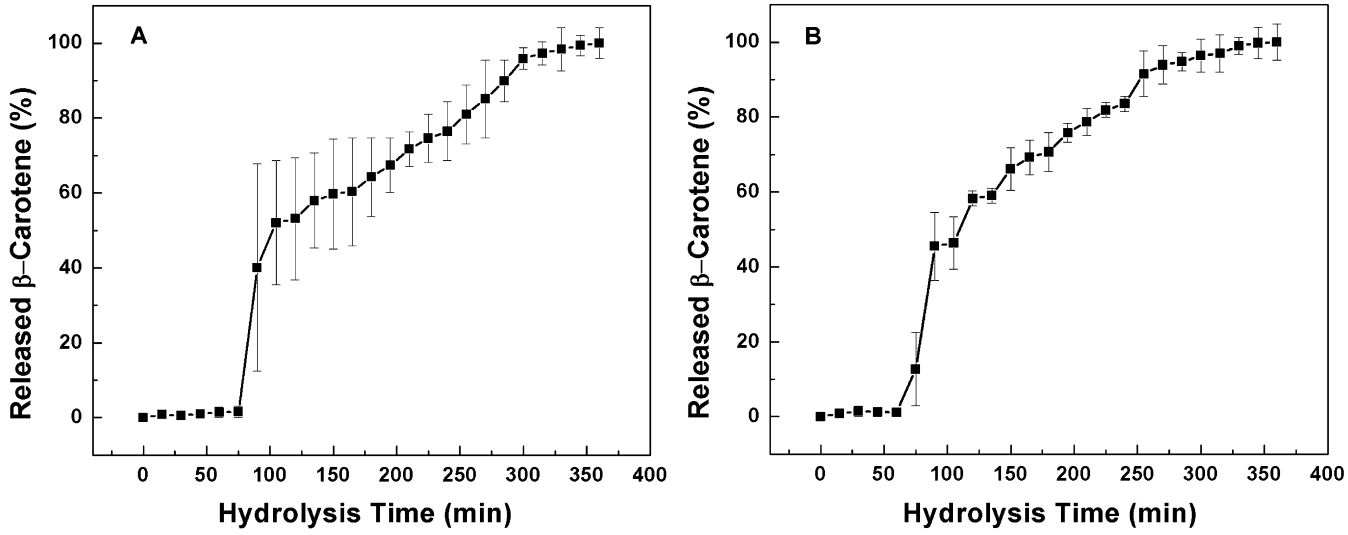

Fig. 6. Release of β-carotene from the particles using (A) pepsin and (B) trypsin hydrolysis.

and analysis processes, some of β-carotene might be exposed on the surface of the particles and then was extracted by cyclohexane. In the absence of cyclohexane, it may take more time for β-carotene to leave the particles. However, from Fig. 6 we can expect that β-carotene will be released from the particles in gastroenteric system *in vivo*. DLS result of the degradation of β-carotene/copolymer nanoparticles under trypsin hydrolysis without using cyclohexane was shown in supporting material.

β-Carotene is unstable to heat, light, and air/oxygen [34]. The behavior of β-carotene in organic solvents has been studied using electrochemical, optical absorption spectroscopy, etc. [24,35]. β-Carotene can easily lose two electrons by electrochemical or chemical oxidation with $FeCl_3$, I_2, etc. The reaction can be monitored by measuring the absorbance of β-carotene around 460 nm in CH_2Cl_2. In this study, $FeCl_3$ oxidation was investigated to examine the stability of β-carotene encapsulated in the particles and released from the particles by trypsin

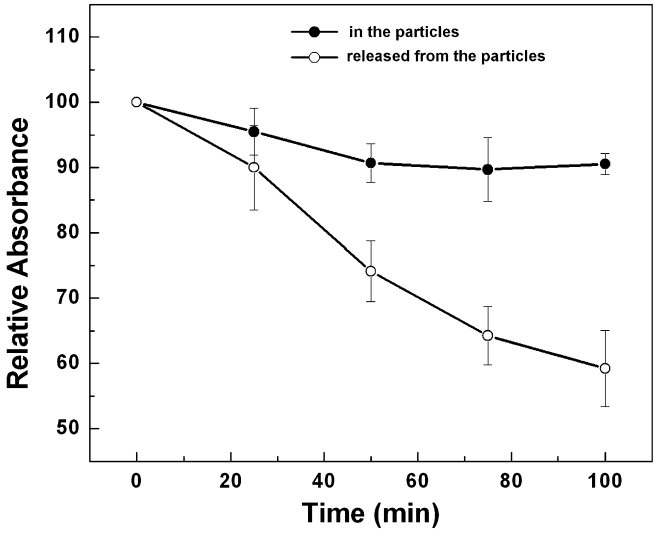

Fig. 7. The stability of β-carotene against $FeCl_3$ oxidation studied by measuring 455 nm absorbance of β-carotene extracted in cyclohexane.

hydrolysis. After β-carotene reacted with $FeCl_3$ and then was extracted by cyclohexane, the absorbance at 455 nm, the maximum absorbance of β-carotene in cyclohexane, was measured. Fig. 7 shows the data of both encapsulated and released β-carotene. The decrease of the absorbance levels off after 50 min reaction with $FeCl_3$ for the encapsulated β-carotene, while the absorbance decreases continually and more quickly with the reaction time for the β-carotene released through 4 h hydrolysis. For the encapsulated β-carotene, the initial decrease of the absorbance at first 50 min may result from the β-carotene located on or close to the surface of the particle core where $FeCl_3$ is accessible; no further decrease of the absorbance indicates that the β-carotene located inside the particle core is properly protected from $FeCl_3$ oxidation. After release through trypsin hydrolysis, the reactivity of β-carotene with $FeCl_3$ is similar as the naked β-carotene (data not shown). This verifies that β-carotene becomes much more stable after being encapsulated.

4. Summary

Casein-g-dextran copolymer is soluble in pH 7.0 aqueous solution in molecular state whereas β-carotene is extremely insoluble. By the method of dialysis or evaporation then dispersing in water, 50% ethanol solvent of the copolymer and β-carotene mixture was changed to 100% aqueous solvent. During this process, the solubility of hydrophobic complex of casein and β-carotene decreases whereas the solubility of dextran increases gradually, forming the particles with casein and β-carotene core and dextran shell. To our knowledge, this is the first report of simultaneous nanoparticle formation and encapsulation induced by hydrophobic interaction. Casein-g-dextran copolymer and copolymer/β-carotene particle preparation is a green process in which only alkali and ethanol, no other chemicals, were used.

The particles have spherical shape and their hydrodynamic diameter is about 200 nm at pH 7.0 solution. The particles can be stored in dried form. The aqueous dispersion of the particles is stable against dilution, pH change, ionic strength change, $FeCl_3$ oxidation, and long time storage. The encapsulated β-carotene can be released by pepsin or trypsin hydrolysis. These characters of the particles provide a possibility for practical applications of the particles to deliver unstable and hydrophobic nutrients and drugs.

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Supporting material

The online version of this article contains additional supporting material.

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