Micellization of Casein-graft-Dextran Copolymer Prepared through Maillard Reaction

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Abstract: Casein is almost insoluble at around pH 4.6, which is its isoelectric point (pI). Grafting copolymer, casein-g-dextran, was prepared through the Amadori rearrangement of the Maillard reaction. The copolymer has a reversible pH sensitive property: micellization at the pI of casein forming a casein core and dextran shell structure and dissociation when pH differs from the pI. The micelles produced at pH 4.6 have a spherical shape and their size is dependent on the Maillard reaction: reaction time, molar ratio of casein to dextran, and molecular weight of dextran used. Typically, the hydrodynamic diameter of the micelles is about 100 nm and the critical micelle concentration is about 10 mg/L. The micelles are very stable in aqueous solution and can be stored as lyophilized powder. The micelles are able to encapsulate hydrophobic compounds such as pyrene.

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INTRODUCTION

Micelles composed of amphiphilic copolymers have been explored as carriers for hydrophobic drugs in aqueous solution. Biopolymers are an interesting alternative to synthetic polymers because of their potential loading for both hydrophilic and hydrophobic drugs. In particular, fabricating polymeric carries without using synthetic chemical reagents and organic solvents is obviously desirable for biomedical applications. Several groups have studied the self-assembly of natural macromolecules to create new matrices dedicated to controlled release applications, such as carrageenan sphere crosslinked by CaCl$_2$ and simple or complex coacervation involving proteins or protein polysaccharide mixtures.

Caseins are the predominant components in milk and they are a family of phosphorylated proteins. The four casein constituents, $\alpha_s$1-, $\alpha_s$2-, $\beta$-, and $\kappa$-casein, exist in proportions of approximately 3:0:8:3:1 by weight in cow milk and their molecular weights are 19,000–25,000 Da. All of the four casein molecules in cow milk are amphiphilic proteins and have no defined structure. The colloidal particles existing...
in cow milk consist of these four casein molecules and calcium phosphate, and the particles are in a size range of 100–300 nm. Casein micelles can be disintegrated by the addition of EDTA,15 and it is widely accepted that calcium is a major factor in maintaining the integrity of the micelles.16 The isoelectric point (pl) of casein micelles is about pH 4.6. Approaching the pl by gradually lowering the pH causes the casein micelles aggregate and form a gel.17–21 Between pH 6.5 and 8, sodium caseinate exists as a polydispersed mixture of four casein molecules.12,13 In the food system, casein has many functions, such as emulsification, water binding, fat binding, and texturization.22,23 These merits endow casein with the ideal matrix to fabricate nanomaterials to carry both hydrophilic and hydrophobic drugs for advanced drug delivery.

The Maillard reaction is a natural, nontoxic reaction that occurs during the processing, cooking, and storage of foods. The Maillard reaction, which conjugates protein and polysaccharide by linking the reducing end of the polysaccharide to the amines in the protein (terminus and amino group of lysine), has been studied extensively.24–30 Many efforts have been made to improve the functions of food proteins by conjugation of polysaccharides through the Maillard reaction. For example, the conjugation of hen egg lysozyme with dextran, galactomannan, or xyloglucan is effective in improving the emulsifying property of the protein and it has been shown that the conjugated lysozyme has new antimicrobial characteristics.31–34 The antioxidant effect and the emulsifying property of ovalbumin are enhanced by the conjugation of galactomannan; the increase in lipid affinity due to the conjugation results in the enhancement of the radical scavenging ability of ovalbumin.31 Casein–maltodextrin conjugates form transparent solutions in low pH and are effective emulsifiers in acidic solutions because the hydrophilicity of the conjugates prevents the aggregation of casein.35

In this work, we prepare the casein-g-dextran copolymer with the Amadori rearrangement of the Maillard reaction to assemble micelles. The pH-sensitive property of the copolymer and the dependence of the micelle size on grafting degree and molecular weight of dextran are characterized with dynamic light scattering. The hydrophobicity of the micelles is studied with pyrene fluorescence.

**MATERIALS AND METHODS**

**Materials.** Casein was from Sigma Chemical Co. (St. Louis, MO) (technical grade), dextran with molecular weights of 1.5, 6, 10, 35, 62 kDa was from AMRESCO Inc. (Solon, OH) and Amersham Pharmacia Biotech (Uppsala, Sweden). All other reagents were purchased commercially and were used as received. All samples were prepared with deionized water.

**The Solubility of Casein around Its pl.** Five milligrams of casein was added to 5 mL solution with desired pH and NaCl concentration. After mild stirring to reach the equilibrium, the samples were centrifuged at 9000g for 30 min to separate insoluble proteins. The supernatants were assayed for protein concentration by a 280 nm absorbance measurement (Lambda 35, Perkin Elmer, Shelton, CT) at room temperature.

**Maillard Reaction.** Casein was dissolved in deionized water and was adjusted to pH 7.0 with 1.0 M NaOH, and then dextran solution was added dropwise with gentle stirring. The final concentration of casein in the mixture was 8–10 mg/mL, depending on the ratio of casein to dextran and the viscosity of the mixture. The molar ratio of casein to dextran in the mixture was in the range of 1:8 to 8:1. The mixture solution was lyophilized. The frozen-dry powder was reacted at 60°C for a certain time at a relative humidity of 78.9% in a desiccator containing saturated KBr solution. The resultant product was kept at −20°C before use.

**Gel Electrophoresis.** Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was carried out on a gel electrophoresis apparatus (JM250, JM-X Scientific Company, Dalian, China) to analyze the molecular weights of casein and the grafting copolymers. The gel was stained with Coomassie Brilliant Blue.

**OPA Assay.** o-Pthalaldialdehyde (OPA) assay was used to analyze the grafting degree of casein by calculating the decrease of the free amino groups in the protein after the Maillard reaction. The OPA reagent was prepared according to Goodno et al.36 as follows: 80 mg OPA (dissolved in 2 mL 95% ethanol), 50 mL 0.1 M sodium tetraborate buffer solution with pH 9.5, 5 mL 20% SDS, and 0.2 mL 2-mercaptoethanol were mixed together and the mixture was then diluted with water to 100 mL. The OPA reagent was prepared freshly before use.37 The casein-g-dextran copolymer obtained by Maillard reaction was dissolved in water with casein concentration of 3.0 mg/mL. After mixing 0.1 mL of the copolymer solution with 2.7 mL of OPA reagent and incubating for 1 min at room temperature, the absorbency at 340 nm was measured immediately. The working curves were measured at the same condition using L-leucine as a standard and the grafting degree of casein was calculated from the loss in amino groups compared to unreacted controls as reported by Morris et al.38

**Micellization of Casein-g-dextran Copolymer.** The copolymer produced by Maillard reaction was dissolved in water to make the solutions with casein concentrations in the range of 1.0 to 10.0 mg/mL. The pH of the resultant solutions was adjusted to 4.6 with 1.0 M HCl and then the solutions were left at 4°C overnight to induce the copolymer micellization.
**Dynamic Light Scattering (DLS) Measurements.** DLS was used to determine the hydrodynamic diameter ($D_h$) of casein-g-dextran copolymer at differing pH solutions. DLS was measured using a commercial 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 (Coherent Laser division, Santa Clara, CA) (output power $\geq 100$ mW, CW at $\lambda_0 = 532$ nm) as the light source. All the DLS measurements were made at $25.0 \pm 0.1^\circ$C and at a 90° scattering angle. The measured time correlation functions were analyzed by the automatic program equipped with the correlator. The average hydrodynamic diameter ($D_h$) or hydrodynamic radius ($R_h$) and polydispersity index (PDI, $\sigma^2/\mu^2$) were obtained by a CONTIN mode analysis. Samples were measured directly without filtering or other dust-free processing. The copolymer concentration for DLS measurement is shown in casein concentration, which is 1.0 mg/mL in each sample if not otherwise noted.

**ζ-Potential Measurements.** ζ potentials of the samples were recorded with a ZetaSizer Nano ZS90 (Malvern Instrument, Worcs, UK) equipped with an MPT-2 Autotitrator and a 4 mW He-Ne Laser ($\lambda_0 = 633$ nm) based on the techniques of laser Doppler electrophoresis. The ζ potential was calculated by the Dispersion Technology Software provided by Malvern according to the Smoluchowski approximation.39,40

**Steady-State Fluorescence Measurements.** The fluorescence was measured using a fluorescence spectrophotometer FLS-920 (Edinburgh Instruments, Livingston, UK). Recrystallized pyrene was used as an additional fluorescence probe and its final concentration was $2 \times 10^{-7}$ g/mL. Before the fluorescence measurement, the solutions were stirred for at least 12 h at 4°C after the addition of pyrene. The spectral resolution for both excitation and emission was 1 nm. The emission and excitation spectra were recorded with the excitation and emission wavelengths of 335 and 390 nm, respectively.

**Atomic Force Microscopy (AFM) Measurements.** AFM samples were prepared by drying the solution naturally on freshly cleaved mica at room temperature. Image acquisitions were performed in Tapping Mode on a Digital Instruments Nanoscope IV (Veeco Instruments, Santa Barbara, CA) equipped with a silicon cantilever with 125 µm and an E-type vertical engage piezoelectric scanner. The drive frequency was 240 KHz and the voltage was between 2.0 and 3.0 V. A drive amplitude of 106 mV, a set point of 1.4 V, and a scan rate of 1.0 Hz were used.

**RESULTS AND DISCUSSION**

**Solubility of Casein at pH around Its pI.** It is well known that the solubility of a protein is influenced by medium pH. When pH of the protein solution is near its pI, the net charge of the protein is about zero, thus the protein tends to precipitate. Jahaniaval et al.13 have studied the solubility of casein at different pH values and temperatures, showing that casein placed the minimum solubility around pH 3.5 to 4. We studied the solubility of casein at pH around its pI (about pH 4.6) with respective NaCl concentrations of 0, 0.05, and 0.5 M by the 280 nm absorbance measurement. Our study shows that the absorbency of casein reaches its minimum and casein is almost insoluble when pH is in the range of 4.0–5.0. In addition, the solubility of casein does not increase significantly when NaCl concentration increases to 0.5 M. Furthermore, the addition of dextran to casein solution does not increase the solubility of casein at this pH range. This pH-sensitive solubility behavior of casein and the good solubility of dextran in water over the whole pH range provide the bases for us to fabricate pH-dependent casein–dextran micelles from their graft copolymers produced by the Maillard reaction.

**Preparation of Casein-g-dextran Copolymer with the Maillard Reaction.** The Maillard reaction was used to graft dextran to casein. According to the literature, the Maillard reaction mechanism and products are dependent on the reaction conditions; that is, pH, temperature, time, and relative humidity.25–30 For our solid phase Maillard reaction of casein with dextran at 60°C and a relative humidity of 78.9%, we found that the reaction rate was moderate and the side reactions were not obvious at neutral pH. Therefore, pH 7.0 was chosen in the Maillard reaction studied below. After the Maillard reaction, SDS–PAGE analysis was performed to monitor the molecular weight of the resultant species. Figure 1 shows that two bands from the four casein constituents as indicated in the literature35 appear before the reaction. After the reaction, a smear that exhibits larger molecular weight than casein appears. As the reaction time increases, this smear becomes clearer and wider while the two casein bands become faint. This indicates that, the longer reaction time is, the more dextran molecules conjugate to casein and a larger molecular weight copolymer is produced.

**Graft Degree Analysis of Casein-g-dextran Copolymer.** As discussed above, SDS–PAGE analysis provides a qualitative result of the grafting reaction. However, a quantitative grafting degree is necessary for us to evaluate the effect of the Maillard reaction. An average lysine residue number of 13 in each casein molecule can be estimated according to the following data: casein is composed of four kinds of molecules, αs1-, αs2-, β-, and κ-casein; the ratio of them is 3:0.8:3:1 by weight; the respective molecular
weights are 23,615, 25,230, 23,983, and 19,007 Da; the number of lysine residues in each casein is 14, 24, 11, and 9, respectively. In addition to the terminus amino group, there are an average of 14 amino groups that can react with the reducing end of dextran in each casein chain. OPA assay was used to analyze the average free amines before and after the Maillard reaction to calculate the average grafting degree of casein. Figure 2 shows the change of the free amino group number with the Maillard reaction time when the molar ratio of casein to dextran is 1:8 and dextran molecular weight is 10 kDa. OPA analysis shows that the measured free amino groups is 12.4 for casein, which is smaller than the 14 calculated above. When casein and dextran mixture undergoes a 2- or 4-h reaction, the number of free amino groups reaches 13.2, a 7% increase compared with casein. We also used a 2,4,6-trinitrobenzenesulfonic acid assay to analyze the grafting degree of casein and obtained a similar result. This phenomenon may be explained as follows: in casein solution, micelle structures exist and some lysine groups are buried in the micelles so they are not able to react with OPA reagent. Our DLS measurement proves that micelles exist in casein solution at pH 9.5, although the light scattering intensity is much smaller than that at pH 6.7. After a 2- or 4-h Maillard reaction, some amino groups of casein conjugate with dextran, resulting in an increase of the hydrophilicity of casein. Therefore, the micelle structure of casein is destroyed and the buried lysine groups are exposed to OPA reagent and more amino groups are measured than the casein–dextran mixture before the Maillard reaction. Recently, Morris et al. reported that the measured value of free amino groups is 0.46 mmol/g for casein using OPA assay and the theoretical value is 0.50 mmol/g, which does not include the terminus amino group. Our OPA result of casein is very similar to theirs if we do not take into account the terminus amino groups. Figure 2 shows that after a 4-h Maillard reaction the available free amino groups decrease with the reaction time, suggesting an increase of grafting degree of casein. After a 20-h Maillard reaction, the number of available free amino groups decreases to 8.2, which means that about 5.8 dextran molecules have grafted to 1 casein molecule on average.

The Influence of pH on the ζ-potential of Casein-g-dextran Copolymer. As we know, a protein carries positive charges when the pH of the solution is lower than its pI and carries negative charges when the pH is higher than its pI. Figure 3 shows ζ-potentials of casein and casein-g-dextran copolymer as a function of pH. The net charge for both casein and casein-g-dextran copolymer is zero at pH 4.6, which is the pI of casein, suggesting that the Maillard reaction does not change the pI of casein. This result may be explained as follows: the Maillard reaction condition that we adopted in the experiment was mild and no yellow color occurred in the reaction product, that is, the Maillard reaction was controlled in its early stage and aminoketose—the Amadori product—was mainly obtained. The structures of the early stage products of the Maillard reaction are shown in Scheme 1. From the data in the literature, we know that the pKₐ values of the secondary amines and the primary amines with similar structure are close, and the ketone group does not change the pKₐ of amine.
from alkali pH to acid pH. So, it is reasonable to speculate that the $pK_a$ of aminoketose is higher than the pI of casein, although it is different from the $pK_a$ of free amine in casein, therefore, aminoketose can not change the pI of casein significantly. Figure 3 shows that the absolute values of the $\zeta$-potential are smaller for the copolymer compared with the same concentration of casein. This ascribes to the dextran that grafts to casein. The highly hydratable dextran, decreases the electrophoresis mobility of the copolymer resulting in a similar $\zeta$-potential.

Micellization of Casein-g-dextran Copolymer Induced by pH. The hydrodynamic diameter ($D_h$) and scattering light intensity of casein-g-dextran copolymer were measured at differing pH values (Figure 4). The pH of the copolymer samples was adjusted from 6.99 to 2.25 and then was adjusted back to 7.19. The copolymer exhibits a pH-reversible behavior, that is, the intensity goes up to its maximum at pH 4.6 either through the pH increase or the pH decrease process. At pH 4.6, the polydispersity index is about 0.3, which is the smallest in the range of pH 2.25 to 7.19. DLS result shows that the micellization of the casein-g-dextran copolymer occurs at pH 4.6 and the $D_h$ of the micelles obtained is about 80 nm. From the solubility and $\zeta$-potential study shown above, we know that casein is almost insoluble at pH 4.6, its poor solubility is not improved by adding dextran, and the $\zeta$-potential of the copolymer is about zero. Therefore, it is reasonable to think that the copolymer forms micelles at this pH at which insoluble casein forms a core and the highly hydratable dextran, which grafts to casein, locates on the surface of the core to protect them from further aggregation. The copolymer carries charges when pH is not at 4.6 and the electrostatic

FIGURE 3 The pH dependence of $\zeta$ potentials of casein and casein-g-dextran copolymer. The casein concentration for $\zeta$-potential measurement is 2.0 mg/mL in both casein and copolymer samples. The copolymer was prepared by Maillard reaction for 20 h with a molar ratio of casein to dextran of 4:1 and 35 kDa dextran.

SCHEME 1 The structures of early stage products of casein-dextran Maillard reaction.

FIGURE 4 Scattering light intensity of casein-g-dextran copolymer at different pH values. Inset: The size distribution of micelles at pH 4.6 produced in the decreasing pH and increasing pH processes. The copolymer was prepared by Maillard reaction for 20 h with a molar ratio of casein to dextran of 4:1 and 35 kDa dextran.
repulsion makes the copolymer dissociate; therefore, the scattering light intensity decreases sharply. The scattering light intensity of the solution is smaller when adjusting the pH back from 2.25 than that obtained at the same pH before the pH reaches 2.25. This may be caused by NaCl produced in the solution during pH adjustment. To prove this, DLS measurement was performed for the copolymer solution in the presence of 0.1 M NaCl at different pH values; the result shows a similar pH response to that without NaCl (data not shown), but the scattering light intensity of the micelle solution is about 60 at pH 4.6, which is much smaller than the 400 obtained for the micelle solution without NaCl at same pH. This phenomenon is understandable because the micellization of the copolymer is induced by electrostatic interaction, which is very sensitive to ionic strength. The pH dependence of micellization of the casein-g-dextran copolymer makes the micelles potential oral drug carriers, which can dissociate at the stomach (about pH 2) and the intestine (about neutral pH).

**The Influence of Grafting Degree of Casein-g-dextran Copolymer on the Hydrodynamic Diameter, Polydispersity Index, and Scattering Light Intensity of Micelles.** As mentioned above, the grafting degree of the copolymer increases with the Maillard reaction time, therefore, the copolymer becomes more hydrophilic. The self-assembly of the copolymer produced by different Maillard reaction times with the molar ratio of casein to dextran 1:8 and 10 kDa dextran was studied using DLS at pH 4.6. When the reaction time is less than 2 h, the grafting degree is too small to obtain homogeneously dispersed micelles at pH 4.6 and only precipitation occurs. As shown in Table I, the $D_h$ of the micelles decreases with the reaction time between 2 and 4 h; after 4 h, the $D_h$ increases with the reaction time. PDI shows an increase whereas the scattering light intensity decreases continuously when the reaction time increases from 2 to 20 h.

As reported previously, for surfactant-free particles the average surface area stabilized by one hydrophilic moiety should be a constant. More grafts on a copolymer can stabilize a larger surface area, leading to smaller micelles; therefore, the decrease of $D_h$ when the reaction time changes from 2 to 4 h can be explained. On the other hand, the copolymer becomes soluble when the grafting degree is high enough because the dextran grafts can prohibit the micellization of casein at pH 4.6, as reported for casein–maltodextrin conjugates that were prepared using the Maillard reaction and formed transparent solutions in low pH. Our SDS–PAGE analysis (Figure 1) shows a wide smear and two casein bands after the Maillard reaction, suggesting that the grafting reaction is not homogeneous; that is, some casein molecules have a very high grafting degree, whereas some casein molecules do not graft any dextran. In this situation, the micellization of the copolymer still occurs when the reaction time is between 4 and 20 h; however, the PDI increases and the intensity decreases with the reaction time because the core of the micelles becomes less compact and some of the casein molecules with a high grafting degree may not take part in the micellization. Finally, after a 20-h Maillard reaction, the resultant solution is transparent at pH 4.6.

**The Influence of Molecular Weight of Dextran and Molar Ratio of Dextran to Casein on the $D_h$ of the Micelles at pH 4.6.** A series of dextran with respective molecular weights of 1.5, 6, 10, 35, and 62 kDa was used to graft to casein with an 8-h Maillard reaction. DLS measurements were performed to investigate the influence of the molecular weight of dextran and the molar ratio of dextran to casein on the $D_h$ of the copolymer micelles at pH 4.6 (Figure 5). For the 1.5 and 6 kDa dextran, precipitation occurs no matter what the molar ratio is. This means that these dextran molecules are so small that the hydrophilicity of the copolymer is too weak to support homogeneously dispersed micelles at pH 4.6. With 10 kDa dextran, the precipitation at pH 4.6 can be prohibited provided the molar ratio of dextran to casein is 1:2 or more. As shown in Figure 5, when the molar ratio of dextran to casein increases from 1:2 to 1:1, the $D_h$ of the micelles decreases. Using 35 or 62 kDa dextran, homogeneously dispersed micelles at pH 4.6 can be obtained when the molar ratio of dextran to casein is 1:6 or more, suggesting that the minimum grafting

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<th>Reaction Time (h)</th>
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<th>$D_h$ (nm)</th>
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*The copolymer was prepared by Maillard reaction with a molar ratio of casein to dextran 1:8 and 10 kDa dextran.*
degree needed to prohibit the precipitation of casein at pH 4.6 is lower for longer dextran. For the 35 kDa dextran, the $D_h$ shows a decrease followed by an increase when increasing the molar ratio of dextran to casein. This change of $D_h$ may result from two opposing factors: the higher grafting degree of the copolymer is able to support more surface area favoring smaller micelles; meanwhile, the higher grafting degree makes the core less compact and also makes dextran crowd in the shell and take a more extended conformation, leading to a larger $D_h$. With the 62-kDa dextran, a monotone increase of $D_h$ with the molar ratio of dextran to casein is observed, suggesting that the latter factor becomes dominant. Compared to the $D_h$ of the micelles composed of the copolymers with 10, 35, and 62 kDa dextran at the same molar ratio, it is clear that the $D_h$ increases generally with the molecular weight of dextran.

The Dissociation Concentration of Casein-g-dextran Copolymer Micelles at pH 4.6. The dissociation of the copolymer micelles was investigated by monitoring the $D_h$ at pH 4.6 after a successive dilution. Figure 6 shows that the dissociation concentration or critical micelle concentration (CMC) of the micelles is about 10 mg/L. When the concentration is lower than 10 mg/L, the peak with a $D_h$ of about 80 nm disappears and the scattering light intensity is very weak. The lower CMC of the micelles means a higher thermodynamic stability. Generally, the CMC value of macromolecules is smaller than that of small molecules. Compared with some block copolymer CMC values, polylactic acid-b-poly(ethylene oxide) 35 mg/L, poly($\beta$-benzyl-$\gamma$-aspartate)-b-poly(ethylene oxide) 5–18 mg/L, and polystyrene-b-poly(ethylene oxide) 1–5 mg/L, the stability of casein-g-dextran copolymer micelles is similar to that of block polymer micelles.

Stability of Casein-g-dextran Copolymer Micelles in Aqueous Solution. The micelles produced with the copolymer at pH 4.6 are very stable. Figure 7 shows that a reproducible hydrodynamic diameter distribution of the micelles was obtained when it was monitored over a period as long as 66 days. Another advantage is that the micelles can be stored as lyophilized powder because lyophilization and rehydration of the micelles do not change the size distribution significantly (Figure 8). This is no doubt a valuable characteristic for the practical application of the micelles.

AFM Image of the Micelles. The AFM image (Figure 9) proves the expectation that the micelles have a spherical shape. The diameter of the micelles is about 75 nm and the height is about 10 nm on average, which is much smaller than the hydrodynamic diameter of about 80 nm measured using DLS. This difference is ascribed to a large amount of water contained in the micelles and the shrinkage of the micelles after water evaporation. Obviously, DLS provides the data for the micelles swollen in solution, while AFM shows the images of the micelles spread and col-
lapsed on a mica surface. The big difference between the diameter and the height of the micelles in the AFM image indicates that the copolymer micelles are very soft.

**Hydrophobicity of the Core of Casein-g-dextran Copolymer Micelles.** Pyrene has much lower solubility in water (about $10^{-7} \text{ M}$) than in hydrocarbon (0.075 M); it significantly transfers into hydrophobic regions once the hydrophobic association occurs in aqueous solution. Pyrene has been widely used to monitor the association and micellization of polymers in solution because its photophysical character changes when it transfers from a polar environment to a nonpolar one. The hydrophobicity of the copolymer micelles formed at pH 4.6 was investigated by examining the intensity ratio of 338 to 333 nm ($I_{338}/I_{333}$) in a pyrene excitation spectrum (Figure 10). The $I_{338}/I_{333}$ value of pyrene is 0.71 when the concentration of casein in the copolymer solution is 5 mg/L.
Although the CMC of the micelles measured using DLS is about 10 mg/L, the value of $I_{338}/I_{333}$ does not show an increase until the copolymer concentration is higher than 50 mg/L of casein. This apparent discrepancy was reported previously and the reason is that, at such low concentrations, the volume fraction of the formed hydrophobic cores is very small and the relative amount of pyrene transferred into the hydrophobic cores is not enough to make a significant change in $I_{338}/I_{333}$. When the copolymer concentration is increased further, more hydrophobic cores form and the $I_{338}/I_{333}$ value increases. The $I_{338}/I_{333}$ value reaches 1.79 at 5000 mg/L of casein of copolymer solution; that is to say, the micelle core formed at pH 4.6 is very hydrophobic. Therefore, the micelles can be used to encapsulate hydrophobic compounds.

CONCLUSION

Natural biomacromolecules, casein and dextran, are used to prepare casein-g-dextran copolymer through the Maillard reaction, a chemical- and solvent-free reaction. The copolymer has a reversible pH-sensitive property: micellization at the pI of casein with a casein core and dextran shell structure and dissociation when pH differs from the pI of casein. The hydrophobic attraction and electrostatic attraction caused by charge fluctuations at the pI of casein drive the micellization of the copolymer. The micelles produced at pH 4.6 by the copolymer have a spherical shape and their size is dependent on the grafting degree of the copolymer and the molecular weight of dextran used. The micelles are able to encapsulate hydrophobic compounds such as pyrene, and the micelles are potential oral drug carriers that are nontoxic and can dissociate in the gastrointestinal tract.

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