Food emulsifier based on the interaction of casein and butyrylated dextrin for improving stability and emulsifying properties

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ABSTRACT

Green hydrophobically modified butyrylated dextrin (BD) was used to modulate casein (CN). The CN/BD complex nanoparticles were formed at different CN-to-BD mass ratios based on a pH-driven technology. The interaction force, stability, and emulsifying properties of complex nanoparticles were investigated. The nanoparticles had a negative charge and a small particle size (160.03, 152.6, 155.9, 206.13, and 231.67 nm) as well as excellent thermal stability and environmental stability (pH 4.5, 5.5, 6.6, 7.5, 8.5, and 9.5; ionic strength, 50, 100, 200, and 500 mM). Transmission electron microscopy demonstrated the successful preparation of complex nanoparticles and their spherical shape. Fourier transform infrared spectroscopy, fluorescence spectroscopy, and dissociation analysis results showed that the main driving forces of formed CN/BD nanoparticles were hydrogen bonding and hydrophobic interaction. Furthermore, the CN/BD nanoparticles (CN/BD mass ratio, 1:1; weight/weight) exhibited the lowest creaming index, and optical microscopy showed that it has the most evenly dispersed droplets after 7 d of storage, which indicates that the CN/BD nanoparticles had excellent emulsifying properties. Butyrylated dextrin forms complex nanoparticles with CN through hydrogen bonding and hydrophobic interaction. The study provided significant information on the improvement of CN properties and the development of emulsions based on CN.

Key words: casein, butyrylated dextrin, interaction, emulsifying

INTRODUCTION

As the most widely sourced food materials and emulsifiers, polysaccharides and proteins are usually used in the food and dairy industries, affecting the texture, stability, and sensory and nutritional properties of food and dairy products (Diaz et al., 2021). Milk proteins have attracted broad interest because of their nutritional, antioxidant, and emulsifying properties (Zhao et al., 2020; Ahn et al., 2022). However, proteins have some drawbacks; they are unstable and tend to aggregate under environmental stressors, and the emulsifying properties of individual proteins have been found to be unsatisfactory (Qin et al., 2022). In recent years, polysaccharides have been used to enhance the stability of proteins under environmental stress and their emulsifying properties by preparing protein–polysaccharide complexes (Meng et al., 2020; Liu et al., 2021b). It is advantageous to study the properties of protein–polysaccharide complexes to determine the effective use of nutrients, proper emulsion preparation, the design of new foods, and improvements in food properties (Ru et al., 2012).

Casein (CN), a major protein in bovine milk, accounts for 80% of milk protein (Pan and Zhong, 2013). Casein can be dissolved in alkaline aqueous solutions, but is insoluble in neutral and acidic aqueous solutions because the CN molecules are dissociated via electrostatic repulsion in alkaline aqueous solutions. In addition, CN can self-assemble into nanoparticles during acidification (Pan et al., 2014). Applications using CN nanoparticles have been limited because CN has poor stability near the isoelectric point of 4.6, tends to aggregate under environmental stressors (pH, ionic strength, and heat), and has poor emulsifying properties. Recently, some researchers found that these problems can be solved by forming nanocomplexes with proteins and polysaccharides (Zhang et al., 2019). Carboxymethyl dextrin has been used to enhance the stability of zein under environmental stress by preparing zein–carboxymethyl dextrin compound nanoparticles. Emulsions stabilized by zein–
carboxymethyl dextrin compound nanoparticles have excellent storage stability (Meng et al., 2020, 2021). Stable carboxymethylcellulose-casein nanocomplexes have been formed and they exhibit excellent ionic strength and stability (Li and Zhong, 2021). It was also discovered that the emulsifying properties of CN and the stability of an emulsion prepared with CN could be enhanced effectively by preparing chitosan–CN complexes (Zhang et al., 2021).

Starch, one of the richest renewable biopolymers on the planet, has attracted extensive attention for its vital advantages, such as biocompatibility, biodegradability, and cheap and easy modification (Wang et al., 2020; Gu et al., 2021; Tang et al., 2022). Dextrin is one of the modified starches that has a lower molecular weight than starch, and is prepared from starch using a debranching treatment. Acylated dextrin, a modified dextrin, is an amphiphilic biopolymer with the advantages of easy synthesis, biocompatibility, and lack of toxicity (Liu et al., 2021a). In recent years, acetylated debranched starch has been prepare successfully. For instance, Tang et al. (2017) prepared acetylated debranched rice starch with excellent freeze–thaw stability and swelling ability. Acylated starch has been used to prepare Pickering emulsions, which indicated that butyrylated quinoa starch had an excellent emulsifying ability (Hadi et al., 2020). However, there is scant information regarding the preparation and properties of casein–butyrylated dextrin (BD) complexes.

We prepared CN/BD nanoparticles using pH-driven technology. The purpose of our study was to explore the fabrication, characterization, physicochemical properties, and formation mechanism of CN/BD nanoparticles. In particular, the emulsifying properties of emulsions prepared with CN/BD nanoparticles were studied. In addition, the particle size, zeta potential, and stability of CN/BD nanoparticles were examined, along with the thermogravimetric analysis of CN/BD nanoparticles. The formation mechanism of CN/BD nanoparticles was investigated using fluorescence spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, and dissociation analysis. Also explored were the emulsifying properties of nanoparticles using the creaming index, optical microscopy, and a digital camera. It was hoped that our work would provide some information on the development of novel protein–polysaccharide nanoparticles.

MATERIALS AND METHODS

Because no human or animal subjects were used, this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Materials

Casein was supplied by Sinopharm Chemical Reagent Co. Ltd. Corn starch was purchased from Puluoxing Co. Ltd. N-Butyric anhydride and phytic acid were obtained from Aladdin Biochemical Technology Co. Ltd. Pullulanase (1,000 U/mL) was obtained from Solarbio Science & Technology Co. Ltd. Corn oil was acquired from COFCO Food Marketing Co. Ltd. Other reagents were of analytical reagent grade.

Preparation of Dextrin

The parent dextrin was prepared based on a previous method (Pan et al., 2019). Corn starch (80 g) was added to 1,600 mL of phosphate-buffered solution (pH 5.5), which was gelatinized at 100°C for 60 min. Then, 8,000 U of pullulanase was added to the starch paste to hydrolyze at 55°C for 24 h, after which the starch paste was cooled to 55°C. The sample was then heated promptly at 100°C for 10 min to stop the reaction and centrifuged at 4,500 × g for 20 min. The parent dextrin was acquired by lyophilizing the supernatant.

The dextrin was acquired according to the method of Meng et al. (2020). The parent dextrin (10 g) was dispersed in 200 mL of ethanol solution (water-to-ethanol ratio 1:1), which was stirred at 60°C for 4 h. Subsequently, the sediment was obtained by centrifuging at 4,500 × g for 20 min, which was then added to a 200-mL ethanol solution (water-to-ethanol ratio, 2:1) and stirred at 60°C for 4 h. The sample solution was centrifuged at 4,500 × g for 20 min to acquire the supernatant, which was freeze-dried to obtain dextrin.

Preparation of BD

BD was prepared based on a previous method (Liu et al., 2021a). The dextrin (20 g) was dispersed in 200 mL of deionized water, which was stirred at 40°C for 20 min. Then, the sample was maintained at a pH of 8 to 9 with 1 M NaOH solution. N-butyric anhydride (40% of dextrin, dry basis) was added to the sample to react at 40°C for 4 h. Subsequently, the reaction was stopped by adjusting the pH to 7 and ethanol was used to wash the sample. The sample was centrifuged at 4,500 × g for 15 min and dried at 45°C to acquire BD. The degree of substitution of BD was measured by titration (Xie et al., 2019) and was 0.150 ± 0.008.

Preparation of CN/BD Nanoparticles

The CN/BD nanoparticles were fabricated based on the study of Ma et al. (2020) with a little modification. Casein (1 g) was added to 100 mL of deionized
water, which was adjusted to a pH to 12 with a 2 M NaOH solution to obtain the CN solution. The BD solutions of different concentrations were prepared by heating at 100°C for 20 min, and then the pH of the BD solutions was adjusted to 12 with a 2 M NaOH solution. Casein solutions (10 mL) and BD solutions (10 mL) were mixed and stirred at 350 rpm for 1 h. Subsequently, the mixed solutions were adjusted to a pH of 5.5 with a 2% phytic acid solution to obtain CN/BD nanoparticle dispersions with different mass ratios (mass ratios of CN to BD, 3:1, 2:1, 1:1, 1:2, and 1:3, weight/weight). In addition, CN nanoparticles (mass ratio of CN to BD, 1:0) were obtained using this method as well.

**Particle Size and Zeta Potential of CN/BD Nanoparticles**

The particle size and zeta potential of CN/BD nanoparticles were estimated using Nano-ZSE (Malvern Instruments) (Liu et al., 2022). The nanoparticle dispersions were diluted 10 times and the pH of the dispersions was adjusted to 5.5 using a 2% phytic acid solution.

**Transmission Electron Microscopy**

The transmission electron microscopic image of the CN/BD nanoparticle was acquired using transmission electron microscopy (JEM-1400Flash). The phosphotungstic acid was dripped on a copper grid with the diluted sample in order to stain the sample. The samples were studied at 120 kV.

**FT-IR Spectroscopy**

The dextrin (DEX), BD, and CN/BD nanoparticles were investigated using an FT-IR spectrometer (Nicolet 67, Thermo Nicolet) (Li et al., 2021c). The FT-IR spectra were acquired in the scan range of 500 to 4,000/cm.

**Fluorescence Spectroscopy**

Fluorescence spectroscopy (F97, Shanghai Lengguang Technology Co. Ltd.) was used to record the fluorescence of CN nanoparticle and CN/BD nanoparticle dispersions (Li et al., 2018). The nanoparticle dispersions were diluted 10 times and adjusted to a pH of 5.5 using a 2% phytic acid solution. The samples were measured at an excitation wavelength of 280 nm and a range of 300 to 500 nm.

**Dissociation Analysis**

The interaction forces of CN/BD nanoparticles were studied by analyzing the turbidity of CN/BD nanoparticle dispersions. NaCl (500 mM), 0.8% SDS (weight/volume), and 4.8 U of urea were added to the CN/BD nanoparticle dispersions (Li and Zhong, 2020a). Sample turbidity was measured at 600 nm using a UV–visible light spectrophotometer.

**Thermogravimetric Analysis**

Thermogravimetric analysis of the CN/BD nanoparticles was conducted using thermogravimetric analysis–differential thermal analysis (STA449F5, NETZSCH-Gerätebau GmbH; Chang et al., 2018). The powder samples (4 mg) were heated from 25°C to 700°C and the heating rate was 10°C/min.

**pH and Ionic Strength and Stability**

The pH and ionic strength and stability of nanoparticle dispersions were studied based on a former method (Li et al., 2021b). The pH of the nanoparticle dispersions was adjusted to different values (4.5, 5.5, 6.5, 7.5, 8.5, and 9.5), as was the ionic strength (0, 50, 100, 200, 500, and 600 mM).

**Emulsifying Properties of CN/BD Nanoparticles**

**Preparation of Pickering Emulsion.** The mixtures of corn oil and nanoparticle dispersions were used to prepare emulsions (φ = 0.3). The mixtures were homogenized at 12,000 rpm for 2 min using a blender (Ultra-Turrax T18, IKA). Samples were then homogenized at 20 MPa 3 times using a homogenizer (JN3000, JNBIO).

**Creaming Index.** The fresh emulsions were placed into glass bottles and stored at room temperature for 7 d. The appearance of the emulsions was observed using a digital camera at 0 and 7 d. The creaming index (CRI) was calculated as

\[ \text{CRI} = \frac{H_s}{H_t} \times 100, \]  

where \(H_s\) is the height of the emulsion and \(H_t\) is the height of the serum phase (Liu et al., 2021b).

**Optical Microscopy.** Emulsion morphologies were observed using a microscope (Nikon 80i) with a 40× objective. Emulsion morphologies were observed after dilution.
Statistical Analysis

All values were repeated 3 times. The results are presented as mean ± standard deviation. Statistical differences were determined using SPSS 26 (IBM Corp.). The differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Fabrication of CN/BD Nanoparticles

The particle size and zeta potential of CN/BD nanoparticles at different CN-to-BD mass ratios are shown in Figure 1. As seen in Figure 1A, the particle size of CN/BD nanoparticles was smaller than CN nanoparticles when BD was at a lower level (CN-to-BD mass ratios, 3:1, 2:1, and 1:1). This result might be attributed to the fact that BD and CN formed nanoparticles with a dense structure, resulting from the interaction forces of the CN/BD nanoparticles. However, the particle size of the CN/BD nanoparticles increased significantly and was larger than the CN nanoparticles when BD was at a higher level (CN-to-BD mass ratios, 1:2 and 1:3), which could be attributed to the depletion flocculation caused by the high concentration of BD (Li et al., 2021b).

As shown in Figure 1B, the zeta potential of the CN/BD nanoparticles demonstrated a gradually decreasing trend, which might be attributed to the fact that the hydrophilic parts of BD were coated on the surface of the CN nanoparticles, masking the potential of the CN nanoparticles (Liu et al., 2021c). The transmission electron microscopic image in Figure 1C demonstrates the successful preparation of nanoparticles. In the subsequent stability studies, the CN/BD nanoparticles with a CN-to-BD mass ratio of 1:2 were selected, which reflect the small particle size and high zeta potential.

Characterization of CN/BD Nanoparticles

FT-IR Spectroscopy. The FT-IR spectra of the DEX, BD, and CN/BD nanoparticles are shown in Figure 2A and 2B. The BD appeared at a new characteristic peak of 1,723/cm compared with DEX, which is the characteristic peak of C=O (Tang et al., 2017). The existence of the new characteristic peak indicates that butyryl groups were introduced to dextrin successfully.

The CN nanoparticles emerged with a broad band at 3,281.5/cm, which indicates the stretching vibration of
O–H. Compared with the CN nanoparticles, the characteristic peaks (O–H) of CN/BD nanoparticles were shifted from 3,282.5 to 3,283.5/cm, which proves the existence of hydrogen bonding between CN and BD (Sun et al., 2018). For the CN nanoparticles, the 2 characteristic peaks of the amide I and II bands corresponded to 1,638.5 and 1,535.5/cm. This indicates the stretching of C–N and the bending of N–H, respectively (Yuan et al., 2021). The amide I and II bands of the CN/BD nanoparticles shifted from 1,642.7 to 1,652.72/cm and from 1,535.9 to 1,539.05/cm, respectively, compared with the CN nanoparticles, which proves the

Figure 2. Fourier transform infrared spectra of butyrylated dextrin (BD) and dextrin (DEX), (A) and casein (CN)/BD complex nanoparticles (B). Fluorescence spectra (C) and turbidity (D) of CN and CN/BD complex nanoparticles. The mass ratios of CN to BD are 1:0, 3:1, 2:1, 1:1, 1:2, and 1:3. Different lowercase letters indicate significant differences ($P < 0.05$). Error bars in plot D are standard deviations from triplicate.

Figure 3. Thermogravimetric–differential thermal analysis of butyrylated dextrin (BD), casein (CN), and CN/BD complex nanoparticles. The mass ratios of CN to BD are 3:1, 2:1, 1:1, 1:2, and 1:3. DTG = differential thermogravimetric.
existence of a hydrophobic interaction between CN and BD (Wei et al., 2021). Taken together, the hydrophobic interaction and hydrogen bonding promoted the formation of the CN/BD nanoparticles.

**Fluorescence Spectroscopy.** The fluorescence spectra of CN and CN/BD nanoparticles are shown in Figure 2C. The CN nanoparticles exhibit an emission peak at 337 nm, which is attributed to the tryptophan residues in the CN molecule. The fluorescence intensities of the CN/BD nanoparticles decreased gradually with an increase of BD, which may result from the fact that the binding of BD to the hydrophobic site of CN led to a change in the environmental polarity of tryptophan; the formation of the CN/BD nanoparticles caused the tryptophan to be masked (Sun et al., 2018). These results prove that BD and CN formed complex nanoparticles through hydrophobic interaction, which is consistent with the results of FT-IR spectroscopy.

**Dissociation Analysis.** Casein and CN/BD nanoparticle turbidity in the different dissociating solvents (SDS, NaCl, and urea) is shown in Figure 2D. The turbidities of all CN/BD nanoparticles in SDS and urea were decreased significantly, where the degree of decrease for urea was greater than that of SDS. The turbidity of the CN/BD nanoparticles in the NaCl solvent showed an ascending trend, and the degree of increase was less than the degree of decrease of all CN/BD nanoparticles in SDS and urea. These phenomena indicate that hydrogen bonding and hydrophobic interaction were the driving forces behind CN/BD nanoparticles formation; the electrostatic interaction was a hindering force (Li et al., 2021b; Wei et al., 2021). The driving forces for the formation of the CN/BD nanoparticles were greater than the hindering force. In short, the formation of the CN/BD nanoparticles was driven by hydrogen bonding and hydrophobic interaction, which was consistent with the results of FT-IR spectroscopy.

**Stability of CN/BD Nanoparticles**

**Thermal Stability.** The thermogravimetric and derivative thermogravimetric curves are shown in the Figure 3. As seen in Figure 3A, BD shows 3 main
weightlessness steps. The first step occurred at 30 to 160°C, and was attributed to the evaporation of free water. The second step occurred at 160 to 250°C, and was attributed to the decomposition of BD. The third step occurred at 250 to 380°C, and was attributed to the further decomposition of BD (Chang et al., 2018).

Casein showed 2 main weightlessness steps. The first step occurred at 30 to 160°C, and was attributed to the evaporation of free water. The second step occurred at 160 to 460°C, and was attributed to the CN main chain as well as the breaking of S–S, O–O, and O–N in the CN molecule (Jiang et al., 2021).

As shown in Figure 3B, CN and BD showed maximum degradation peaks at 316°C and 223°C, respectively. All the CN/BD nanoparticles (mass ratios of CN to BD are 1:0, 3:1, 2:1, 1:1, 1:2, and 1:3) showed maximum degradation peaks at 326, 323, 319, 284, and 283°C, respectively. The maximum degradation peaks of the CN/BD nanoparticles with mass ratios of 3:1, 2:1, and 1:1 shifted to the right compared with the maximum degradation peaks of CN and BD, which indicates that thermal stability was improved by the complexation of CN and BD (Xu et al., 2021).

As shown in Figure 3B, CN and BD showed maximum degradation peaks at 316°C and 223°C, respectively. All the CN/BD nanoparticles (mass ratios of CN to BD are 1:0, 3:1, 2:1, 1:1, 1:2, and 1:3) showed maximum degradation peaks at 326, 323, 319, 284, and 283°C, respectively. The maximum degradation peaks of the CN/BD nanoparticles with mass ratios of 3:1, 2:1, and 1:1 shifted to the right compared with the maximum degradation peaks of CN and BD, which indicates that thermal stability was improved by the complexation of CN and BD (Xu et al., 2021).

**pH and Ionic Strength and Stability.** The pH stability of the CN/BD nanoparticles is shown in Figure 4A and 4B. At pH 4.5, the nanoparticles had the largest particle size and lowest potential, which we attribute to the low potential of CN around the isoelectric point. At pH 5.5, the nanoparticles had the smallest particle size and a high potential. As the pH changed from 6.5 to 9.5, the size and potential of the nanoparticles increased gradually, and was attributed to the fact that the structure of CN became loose because of the dissociation of CN under alkaline conditions (Li and Zhong, 2020b).

The ionic strength and stability of CN/BD nanoparticles is shown in Figure 4C and 4D. The particle size of nanoparticles with an ionic strength of 50, 100, and 200 mM was reduced significantly compared with nanoparticles with an ionic strength of 0 mM, which indicates that the hindering strength (electrostatic interaction) of the nanoparticles was screened with the increase in ionic strength (50–200 mM) to form nanoparticles with dense structures. The particle size of the nanoparticles increased significantly with an ionic strength ranging from 500 to 600 mM, a result of a reduction in electrostatic repulsion between nanoparticles (Li and Zhong, 2021). The potential of nanoparticles reduced gradually with an increase in ionic strength, which was a result of the electrostatic shielding effect of the salt ions.

### Emulsifying Properties

**Creaming Index.** The creaming index and appearance of CN/BD nanoparticles are depicted in Figure 5. The results indicated that the creaming index of all the nanoparticles increased gradually with an increase in storage time, and there were significant differences among nanoparticles with different mass ratios during the 7 d. This was mainly a result of the aggregation and flocculation of droplets as time increased, thus making the emulsion unstable and stratified. The nanoparticles with a mass ratio of 1:1 had the lowest creaming index, which indicates that it had excellent emulsification. The phenomenon might be attributed to the fact that weak spatial repulsion resulted in an unstable emulsion when the additional amount of BD was low, and the bridging flocculation resulted in an instability of the emulsion when the additional amount of BD was large (Lv et al., 2021). We noted that the emulsion prepared with CN nanoparticles demonstrated oiling off and it was unstable. The change in emulsion appearance was consistent with the creaming index.

**Optical Microscopy.** Figure 6 shows the optical microscopic images of all emulsions. The droplets of all fresh emulsions were dispersed evenly except for those prepared from CN nanoparticles and from CN/BD nanoparticles with a mass ratio of 1:1 (Figure 6D). After 7 d of storage, the droplet size of all emulsions had an increasing trend, and the emulsion prepared from the CN/BD nanoparticles with a mass ratio of 1:1 had evenly dispersed droplets that were smaller, which was consistent with the creaming index result. In short, the
Figure 6. Optical microscopic views of emulsions stabilized by casein and casein/butyrylated dextrin complex nanoparticles with mass ratios of 1:0 (A), 3:1 (B), 2:1 (C), 1:1 (D), 1:2 (E), and 1:3 (F).
emulsion prepared from the CN/BD nanoparticles with a mass ratio of 1:1 had the best emulsifying properties.

CONCLUSIONS

In our study, CN/BD nanoparticles with a small particle size (160.03–231.67 nm) were acquired successfully using pH-driven technology. The complexation of CN and BD improved the thermal and environmental stability (pH and ionic strength). FT-IR spectroscopy, fluorescence spectroscopy, and dissociation analysis results show that the main driving forces of the formed CN/BD nanoparticles were hydrogen bonding and hydrophobic interaction. Furthermore, the emulsion stabilized by CN/BD nanoparticles with a mass ratio of 1:1 had the lowest creaming index and the most evenly dispersed droplets after 7 d of storage, which indicates that these CN/BD nanoparticles (with a mass ratio of 1:1) had excellent emulsifying properties. The complexation of CN and BD improved the emulsifying property of CN. Overall, our study provides significant information for the development of stable protein–polysaccharide particles based on pH-driven technology.

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