Fabrication of Antibacterial Casein-based ZnO Nanocomposite for Flexible Coatings

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Abstract: Flexible hybrid caprolactam-casein/ZnO nanocomposite (CCZ) was prepared by in-situ polymerization **method**. The micro morphology and structure were characterized using DLS, TEM, SEM and FT-IR. The influence of ZnO dosage on the appearance, **hand feeling, mechanical property** as well as antibacterial activity of as-prepared composite films was explored separately. TEM measurements revealed that ZnO NPs **were trapped in the inner layer by casein micelles. SEM indicated** that part of ZnO was deposited on both surface and inside of CCZ film in film-forming process. **Moreover, the films** displayed expected mechanical properties and excellent antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*. This work provided a feasible pathway for fabricating natural polymer-based nanocomposite **antibacterial coating which will has great potential use** in several fields, such as textile, leather, packaging, paper making and indoor wall coating.

Keywords: casein; nano-ZnO; antibacterial activity, flexible, composite film

(Graphical Abstract)

1. Introduction

As an important semiconductor material, zinc oxide (ZnO) has a direct wide band gap energy of 3.37 eV and exciton binding-energy of 60 meV [1]. Up to now, due to its relatively high electron mobility [2], **ZnO has been widely used for sensor, optical communication, piezoelectricity element, flexible transistor, and solar cell** [3], which also **shows** promising applications in electronics, optics and optoelectronics. **In addition, as a green material, ZnO is biocompatible and nontoxic for medical application, food additive [4] and environmental science. ZnO nanoparticles (ZnO NPs) also possess strong antimicrobial activity which is more stable than organic disinfectants or antimicrobial agents** [5]. Thus constructing green and antimicrobial coatings with ZnO NPs provides further potential applications for functional materials in numerous fields [6]. Various natural polymer-based ZnO

nanocomposite coatings have been studied in recent years, such as chitosan/ZnO [7], cellulose/ZnO [8], starch/ZnO [9] and rubber/ZnO [10].

Casein belongs to the phosphoprotein that extracts from raw skim milk by acidification, molecular weights of which range from 19.007 to 25.230 KDa [11, 12]. As a natural polymer, casein is completely biodegradable and has good film-forming properties including excellent adhesive force and strong heat resistance. Various casein-based formulations have been designed for controlled drug delivery, leather finishing and water-based ink [13, 14]. Nevertheless casein film has less extensibility [15, 16] and tends to be easily spoiled by bacteria attack, which might restrict its practical applications. Recently there have been increased interests in producing nanocomposites. In general, inorganic nanoparticles introducing into polymeric matrixes harbor electrical, optical, catalytic, antibacterial property and mechanical property [17, 18]. Up to now, there have few reports studying and constructing a flexible caprolactam-casein/ZnO hybrid coating. In several related reports, casein or casein/ZnO was only used as template or additive. For instance, Maryam Sabbaghan et al. [19] used casein as a template to fabricate nanosheet-like ZnO nanoparticles. Casein micelles could also be acted as capping agent to assist form waxberry-like hierarchical ZnO micro-particles [20]. Ana Rešček et al. [21] added ZnO/casein as an active component into polyethylene/polycaprolactone food packaging films to extend the shelf life of food and to maintain the quality and health safety. In their study, casein/ZnO was prepared through simple mechanical mixing. However, the microstructure and composite film were not studied and analyzed systematically.

In our previous research, SiO₂ [22], and TiO₂ [23] nanoparticles separately were introduced into casein matrix to obtain coating materials with superior performance. Results

showed the casein-based films with higher hydrophobicity, enhanced tensile strength and increase self-cleaning. However, casein's long-term stability was needed to be further solved. In this case, fabricating casein-based nanocomposite to obtain antibacterial and stable hybrid film would be highly valuable. Herein, caprolactam-casein/ZnO nanocomposite (CCZ) was prepared via in-situ polymerization method by introducing commercial ZnO NPs. Caprolactam was intended to enhance the extensibility and flexibility of casein film. Moreover, it also can be attributed to the self-emulsifying function of caprolactam modify casein (CC) and to obtain more uniform hybrid particles [25]. After introducing ZnO NPs, the covering power, mechanical property and antibacterial property were enhanced than pure CC film. The process was advantageous in relative ease method for constructing natural polymer casein/ZnO antibacterial coating material. Meanwhile, the addition of any toxic substances was avoided. The antibacterial coating was also expected to apply in several fields, such as leather, packaging, paper making and special indoor coating.

2. Experiment

2.1 Materials

Casein (CA) was purchased from Zhejiang Huatian CO., Ltd. Caprolactam (CPL) was supplied by Tianjin Kemiou Chemical Reagent Co., Ltd. Triethanolamine (TEA) was obtained from Tianjin Dengfeng Chemical Co., Ltd. Nano ZnO was purchased from Qinhuangdao Taijihuan Nano Products Co. Ltd. All the reagents were analytical grade and used without further purification.

2.2 Preparation of CCZ nanocomposite

Various ZnO NPs concentration was introduced to prepare CCZ nanocomposites, i.e. 0% (CC), 0.1% (CCZ1), 0.5% (CCZ2), 1% (CCZ3), 2% (CCZ4). Different fraction of ZnO and

10.9 g of casein were added to 2.6 g of TEA dissolved in 86 mL of distilled deionized water in a 250 mL three-necked round-bottom flask with constant stirring at 65°C for 2 h. When casein was completely dissolved, the temperature was raised to 75°C, and then 25% concentration of CPL solution was drop-wise added into the system with suitable dropping speed and react for another 2 h. The system was cooled to room temperature and CCZ nanocomposites were obtained. Finally, 20 g of composite emulsion were cast evenly on the polyfluortetraethylene plate and dried at room temperature for 72 h to acquire nanocomposite films. The completely dried films were peeled off from plate and preconditioned in a concentrated sulfuric acid dryer for 24h before test.

2.3 Characterization and Testing

TEM observation of CCZ4 colloidal particles was performed by Tecnai G2-F20 (FEI, America) under an acceleration voltage of 200 KV. Specimen for TEM measurement was prepared by depositing a drop of colloid solution on copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The DLS measurement of CCZ4 nanocomposite was determined by NANO-ZS90 (Malvern, Britain), each specimen was repeated in duplicate. The surface morphology of CC and CCZ nanocomposite films was observed by environmental scanning electron microscopy (ESEM, Q45+EDAX, FEI, America). The FT-IR of CC and CCZ4 films was recorded from 400 to 4000 cm^{-1} using TG-IR-GC instrument (STA449 F3/VERTEX 70/Agilent 5977A, Bruker/NETZSCH/Agilent). The films were tested directly instead of tableting with potassium bromide. Each film was cut into dumbbell-shaped strip and the total length was 80mm. The effective length was 30mm and width in middle was 5mm. The tensile strength (TS) and elongation at break (EB) were measured using Electronic Universal Testing Machine (XK-8012, KunshanXiangke, China).

Each specimen was repeated in triplicate.

Antibacterial activity. The Halo Test method was performed using agar culture-medium. CC and CCZ films were cut into 14mm **circular slices**, sterilized by UV light for 1h, and **then the slices were attached to *S. aureus*-cultured agar and *E. coli*-cultured agar plates. After that the prepared culture were incubated at 37°C for 48 h.** A bacterial inhibition zone was formed around the film. The diameter of the inhibition zone (W_{inh}) was calculated using the following equation:

$$W_{inh} = \frac{d_1 - d_2}{2}$$

where d_1 is the total diameter of **the inhibition zone**, d_2 is the diameter of the film (14 mm) [24].

3. Results and discussion

3.1 TEM Characterization

Fig.1

TEM image shown in Fig.1 indicated that ZnO NPs were trapped by casein latexes in the hybrid emulsion. Irregular spheres with size of about 340-390nm were presented. The average size was analogical with DLS result (Fig.2). Several ZnO NPs showed agglomerations or cluster-like dispersion **inside of the latexes**. Core-shell structure was also verified, of which core represented inorganic materials, shell was organic caprolactam modified casein (see from inset in Fig.1). The thickness of shell and core were approximately 100nm and 160nm respectively. This result demonstrated that core-shell casein-based ZnO nanocomposite was obtained successfully. **DLS result was also attributed to the fact that ZnO NPs individual and agglomerations were dispersed in solution** (the size of ZnO NPs was 30-50 nm).

Fig.2

3.2 FT-IR analysis

FT-IR analysis was carried out to demonstrate the chemical structure and interaction between ZnO NPs and casein matrix. As was shown in Fig.3, the characteristic absorption bands of CC were 3276.20cm^{-1} (stretching vibration of N-H bond), 1625.76 cm^{-1} (stretching vibration of -C=O bond which belongs to -CO-NH- on casein backbone) and 1539.68 cm^{-1} (characteristic peak of carboxylate groups, due to the stretching vibration of the C-N bending[27]). CCZ nanocomposite showed the similar characteristic peaks at 3285.20 cm^{-1} , 1629.24 cm^{-1} and 1539.35 cm^{-1} . A new peak around 424.43cm^{-1} was observed due to transverse optical stretching vibration of Zn-O bond [28]. Upon the addition of 2% ZnO NPs, peaks at 3276.20cm^{-1} and 1625.76 cm^{-1} were slightly shifted toward higher wavenumbers, most likely slight changes in casein's structure [29]. According to IR spectrum, the presence of ZnO was verified and there was no obvious effect on chemical structure of casein matrix after introducing ZnO NPs.

Fig.3

Based on the considerations presented above, a feasible mechanism was depicted in Fig. 4. Casein's molecular intertwined with calcium phosphate to form micro clusters structure after casein was dissolved. In the meantime, ZnO was added into the solution, some agglomerated and unagglomerated NPs were cladded inside the casein micelles under the action of appropriate mechanical stirring and heating. Strong mechanical effect urged uncaptured nanoparticles to spread uniformly in the system. Finally, casein could be modified by caprolactam via condensation polymerization. The diminished polarity of casein micelles

became the driving force to get higher emulsifying ability as well as uniform size distribution [25, 26], consequently CCZ nanocomposites were obtained after polymerization.

Fig.4

3.3 SEM analysis

SEM micrographs of the surface and cross-section of CC and CCZ nanocomposite films are provided in Fig. 5. A smooth, nonporous and compact structure was observed on the surface of CC film (Fig. 5a), which was in accordance with other reports [30, 31]. And a rough, porous and discontinuous morphology can be observed in interior part of the film (Fig. 5b). When introduce a small amount of ZnO NPs (only 0.5%), the surface of CCZ film was still smooth and no agglomerates encountered (Fig. 5c). Nevertheless, when increase the nanoparticles dosage, ZnO NPs mainly distributed on the film surface and tended to gather into flowerlike aggregation, as shown in Fig 5e and Fig 5e inset. The size ranged from tens of nanometer to hundreds of nanometer, of which the largest was about 300 nm. Moreover, it was found that the incorporation of ZnO was beneficial for giving a relatively compact and continuous composite film (Fig 5d and Fig 5f). Cross-sections of the films were prepared by fracturing the films using liquid nitrogen which prevented any deformation at the interface, also allowing for easy visualization of the nanoparticles. In Fig 5f, the small holes may be the traces of nanoparticles that remained on the opposite side of the film during fracturing [32].

Fig.5

3.4 Appearance and Mechanical Property

In this research, we found that the presence of ZnO had a great influence on the film appearance as well as the stickiness. Detailed effects were shown in table 1. With an

increasing of ZnO content, covering power of the hybrid film was increased while the stickiness decreased. Consequently, CCZ nanocomposite would be a superior film-forming material and could shield the defects of the substrate, which originated from the reflecting action of ZnO NPs itself. Fig.6 also gave a visual demonstration.

Table 1

ZnO NPs have a relatively high elastic modulus and available surface area thus it frequently acts as a mechanical reinforcement to combine with various polymers. Table 2 presented the tensile strength (TS) and elongation at break (EB) of the composite membranes. Results indicated that the presence of ZnO could significantly enhance mechanical property of CCZ film when ZnO was only 0.5 wt%. ZnO content was increased to 1 wt%, TS of composite membranes reached the highest about 3 times more than that of pure CC film. And EB of membranes containing 2 wt % ZnO was also the highest which increased from 55.23% to 64.77%. It was mainly attributed to an efficient interfacial interaction between ZnO NPs and the matrix, which prompted a good stress transfer [32]. Nevertheless, when ZnO amount was up to 2 wt%, TS was declined. More nanoparticles, more opportunities were possible for ZnO NPs to coalesce with each other, resulting aggregations. The stress concentration would occur and finally caused the reduced mechanical property. It was significant that the mechanical property of CCZ composite had certain improvement by using proper amount of ZnO toughening agent via in-situ synthesis [33,34], which attributed to the fact that ZnO had fine dispersion and compatibility in the casein matrix.

Fig.6

3.5 Antimicrobial activity

Fig.7 and Table 1 showed the inhibition activity of CC and CCZ films against *E. coli* and *S. aureus* and the diameter of inhibition zone could directly explain the effect of antibacterial property. There was no resistance to bacteria for CC film. However, the participation of ZnO NPs endowed the CC film with antibacterial activity. Further increase of ZnO content caused an obvious inhibition zone against *S. aureus* and *E. coli* after incubating 2 days. Moreover, the Gram-negative bacteria *E. coli* was less affected by ZnO than the Gram positive bacteria *S. aureus*. The nature of the cell wall structure was one of possible reasons for the sensitivity. *S. aureus* is composed of multilayers of peptidoglycan with an abundant amount of pores that renders them more susceptible to reactive species, leading to the cell disruption. The cell wall of *E. coli* is relatively thin and is mainly comprised of peptidoglycan, while the outer layer consists of lipopolysaccharide, lipoprotein, and phospholipids, which would be less vulnerable to the attack of reactive species [24]. Therefore, CCZ films displayed better antibacterial activity against *S. aureus* than *E. coli*.

Generally, ZnO displayed a broad spectrum of antibacterial activity through some mechanisms: ZnO may release Zn^{2+} , which could penetrate through the cell wall of bacteria and reacted with the cytoplasmic content and then kill bacteria. ZnO NPs were known that they could generate a strong oxidizing agent, hydrogen peroxide (H_2O_2), which damaged the cell membrane of bacteria. Even though there were no clear evidences to explain the mechanism, it was convinced that the presence of ZnO nanoparticles cause damages to the membrane wall [35]. However, there was a drawback for CCZ composite films, a lower water resistance. This would be a focus of our future research.

Fig.7

4. Conclusion

Caprolactam-casein/ZnO hybrid material was prepared by in-situ polymerization method.

ZnO NPs were dispersed in casein matrix and trapped in the inner layer. The size of hybrid latex was approximately 360nm and irregularly spherical shape was obtained. With increase of ZnO concentration, the strong interaction occurred between ZnO and casein matrix. The tensile strength, elongation at break and covering power were enhanced with the incorporation of ZnO NPs. Moreover, CCZ films displayed excellent antibacterial activities against *E. coli* and *S. aureus*. CCZ films had significant potential use in textile, leather and papermaking industry with an economic point of view.

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Table 1 Appearance, softness, viscosity, mechanical property and antimicrobial activity of CC and CCZ nanocomposites

Sample	Appearance	Softness	Stickiness	Tensile strength (MPa)	Elongation at break (%)	W _{inh} (mm)	
						E. coli	S. aureus
CC	Yellowish, Transparent	Flexible	Viscous (+++)	0.49	55.23	0	0
CCZ1	Yellowish, Transparent	Flexible	Viscous (+++)	0.97	52.51	4.5	9.2
CCZ2	White, Covering (+)	Flexible	Viscous (++)	1.61	56.14	4.5	16.2
CCZ3	White, Covering (+)	Flexible	Viscous (++)	1.65	57.66	7.0	15.2
CCZ4	White, Covering (++)	Flexible	Viscous (+)	1.53	64.77	7.0	13.0

“+” represent degree

Fig.1 TEM image of CCZ4 composite, the inset shows core-shell structure of latex

Fig.2 Particle size of CCZ4 colloid emulsion

Fig.3 FT-IR spectra of CC and CCZ4 nanocomposites

Fig.4 Mechanism for the formation of the CCZ latexes

Fig.5 SEM surface spectra of a) CC; c) CCZ2; e) CCZ4 and cross section spectra,
b)CC; d) CCZ2; f) CCZ4

Fig.6 Appearance of CC and CCZ nanocomposite films

Fig.7 Anti-bacterial property of CC and CCZ nanocomposites (a-e) *E.coli* and (a'-e') *S. aureus*: (a, a') CC (b, b') CCZ1, (c, c') CCZ2, (d, d') CCZ3, (e, e') CCZ4

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Fig.7

(Graphical Abstract)