Synthesis and Characterization of Novel ZnO/Whey Protein Nanocomposite

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ABSTRACT

Nanocrystalline zinc oxide (ZnO) particles coated with whey protein (WP) were synthesized in the weak basic aqueous solution condition at room temperature. The X-ray diffraction (XRD) and transmission electron microscopy (TEM) measurements confirmed the ZnO/WP nanoscaled composite structure. The average composite granules size was about 300 nm and the embedded ZnO nanoparticles were uniform and monodisperse with an average diameter of 65 nm.

Keywords: ZnO, whey proteins, nanocomposite, synthesis

1 INTRODUCTION

Zinc (Zn) is one of the essential micronutrients and serves important and critical roles in human being growth, development, and well-being. Zn is essential to support child growth, lower the risk of common infections, prevent adverse outcomes of pregnancy, and improve other aspects of human health function. In addition, Zn is also required for the metabolic activity of numerous enzymes in our body and is considered essential for cell division and the synthesis of DNA and protein.

Nonetheless, Zn is deficient in the diet of many segments of the world population. Zn is the number-one nutritional deficiency in U.S. children based on a Tufts University study. More than 50% of poor children and 30% of non-poor children, ages 1 to 5, get less than 70% of the recommended dietary allowance (RDA) of zinc [1-3]. Zn deficiency can be attributed to many reasons including inadequate intake, malabsorption, increased requirements and/or losses, and impaired utilization. Of these, inadequate dietary intake of absorbable Zn is the primary reason.

Phytate, which is present in staple foods such as cereals and pulses, has a strong negative effect on Zn absorption from. Therefore, preventive and therapeutic interventions should be implemented in places where Zn deficiency is likely to be prevalent. If suitable Zn fortificants can be developed to successfully fortify staple foods, it will help alleviate Zn deficiency.

Zn is available in different forms that may be used for supplementation and fortification. The US Food and Drug Administration (FDA) has listed Zn chloride, Zn gluconate, Zn oxide, Zn sterate, and Zn sulfate as generally recognized as safe (GRAS). Among these, ZnO has well been used as foodstuff. It will decompose into Zn ions after going into human body, which were proven in medical papers as the indispensable elements for human body due to Zn deficiency syndrome [4]. Furthermore, ZnO is less expensive than other formation and most commonly used in food industry [5]. Wheat products fortified with ZnO have proven to possess good Zn absorption [6]. Therefore, ZnO appears to be the best choice for dietary Zn fortification.

Recently, nanotechniques have been applied in the food industry [7,8]. Nanoparticles improves the bioavailability of nutratecual compounds, especially poorly soluble substances originated from their subcellular size.

Nanocrystalline ZnO is a very interesting material due to its excellent properties and promising applications in various fields including food industry. Protein-based nanoparticles are particularly interesting because they are relatively easy to prepare and their size distribution can be controlled [9]. Whey proteins (WPs), by-products of cheese manufacturing, are an excellent choice to serve as the delivery matrix for ZnO owning to their functionalities such as gelling, foaming and emulsifying capacity. Besides enhancing the status of these essential micronutrients, Whey protein encapsulated ZnO may also improve the protein content of the fortified diet.

On the other hand, hybrid inorganic-organic nanocomposite materials have currently attracted great interest of researchers because of their multifunctionality induced by combination of different compounds [10]. ZnO incorporated into nanocomposites, when added to foods, will be protected from interactions with food components that may impair their bioavailability and that may result in detrimental reactions. Since WP delivery and controlled release properties have been addressed [11], WP encapsulated nanocomposites may also survive the gastric environment and become available in the intestine and readily absorbed due to their nanoscale size. The incorporation of nanocrystalline ZnO into WPs to form nanocomposite may possess the unique functionalities and potential applications in material science and food sciences.

Herein we report the synthesis of WP-coated nanocrystalline ZnO particles via a simple method under mild basic condition. The formation mechanism of the ZnO/WP nanocomposite is also discussed.
2 EXPERIMENTAL SECTION

2.1 Materials

ZnO nanoparticle was purchased from Sigma-Aldrich (St. Louis, MO). Whey protein isolate (WPI, 98 wt% protein) was obtained from Davisco Foods International, Inc. (Een Prairie, MN). Milli-Q water (resistivity 18.2 MΩ cm) was used for sample preparation. All other reagents are analytical grade and were used without further purification.

2.2 Preparation of ZnO/WP nanocomposite

0.05 M buffer tris(hydroxymethyl) aminomethane at pH 8 was prepared by dissolving 50 mL 0.1 M Trizma base and appropriate 0.1 M HCl aqueous solution, then diluted to 100 mL with distilled water. Appropriate amount of (24 mg) WP was added into 20 mL previously made buffer with stirring. After complete dissolution, WP Solutions was firstly heated at 80 °C for 30 min. After cooling to room temperature, 0.8 mL 0.5 M Zn(NO$_3$)$_2$ aqueous solution was added drop-wise into WP solutions under constant stirring. The reaction was proceed at 40 °C for 5 h after addition of Zn(NO$_3$)$_2$. Then, the obtained precipitate was centrifuged at 10,000 rpm for 10 min and collected, washed with deionized water several times to remove the byproducts. After drying in vacuum at 30 °C for 4 h, the final white powder product was obtained.

2.3 ZnO/WP nanocomposite characterization

The overall crystallinity of the product was examined by a powder X-ray diffraction unit (Scintag Pad V with a Ge solid-state detector; Cu Kα radiation) with the solid specimens mounted on a low background quartz holder. Detailed microstructure analysis was carried out using a transmission electron microscopy (TEM, PhilipsCM120). A particle size analyzer (90Plus, Brookhaven Instruments Corporation, New York, USA) was used to determine the granular average diameter distribution of ZnO/WP composite. The differential scanning calorimetry (DSC) was carried out on a TA Instruments Modulated DSC system. Measurements were conducted from 20 to 500 °C, at a heating rate of 10 °C min$^{-1}$ under N$_2$ atmosphere.

3 RESULTS & DISCUSSION

The evidence for phase structure of the ZnO/WP composite was obtained by X-ray powder diffraction (XRD) pattern, as shown in Fig. 1. All the diffraction peaks marked with star can be indexed to those of hexagonal ZnO. After refinement, the lattice constants, \(a = 3.251 \text{ Å}, c = 5.210 \text{ Å}\), are obtained, which was very close to the reported value for ZnO (\(a = 3.253 \text{ Å}, c = 5.209\), JCPDS card, No.80-0075). The broadening of the ZnO XRD peaks suggests that the grain sizes were on nanometer scale. The average particles size was estimated to be 70 nm using the Scherrer equation [12]:

\[
D = \frac{(K\lambda)}{\beta \cos \theta}
\]

(1)

Where, \(K\) is the shape factor of the average crystallite, \(\lambda\) is the wavelength for the Kα$_1$ (1.540 56 Å), \(\beta\) is the full width at half-maximum of the diffraction line and \(\theta\) is the Bragg’s angle. The rest XRD diffraction peaks should arise from WP.

Fig. 1 XRD pattern of the synthesized ZnO/WP composite

The morphology of the ZnO/WP composite was investigated by TEM. Fig. 2 shows a typical TEM image of the ZnO/WP composite. The dark part is ZnO and the pale part is WP. It clearly shows that we obtained a true composite of ZnO and WP, i.e., a WP granule of about 300 nm embedded with several ZnO nanoparticles. These nanoparticles are uniform and monodisperse with average diameters of 65 nm, which correlates very well with XRD results (70 nm). The ZnO/WP nanoparticles were stable under the electron beam in vacuum used for TEM, suggesting a strong bond between ZnO and WP. The corresponding selected area electron diffraction (SAED) pattern of ZnO nanoparticle is shown in the inset of Fig. 2. It reveals bright spots, suggesting well-crystallized diffraction pattern of ZnO.

Fig. 2 TEM image of the ZnO nanoparticles embedded in the WP matrix; Inset: selected area electron diffraction pattern of ZnO nanoparticle.
particles. The SAED pattern can be indexed as a hexagonal crystal structure (wurtzite), which is in agreement with the XRD result.

The granular size distribution of ZnO/WP composite examined with a particle size analyzer is shown in Fig. 3, which indicates that the composite granules are uniform and the granular size distributes mainly at about 300 nm. This is consistent with the TEM observance result.

![Histogram of granular size distribution of ZnO/WP nanocomposite](image)

**Fig. 3** Histogram of granular size distribution of ZnO/WP nanocomposite

For the UV-Vis absorbance measurement, the as-prepared powder sample was ultrasonically dispersed in distilled water before examination. The room temperature optical absorbance spectrum is shown in Fig. 4, which indicates that the sample is transparent in the visible region.

![UV-visible absorbance spectra of the ZnO/WP nanocomposite](image)

**Fig. 4** UV-visible absorbance spectra of the ZnO/WP nanocomposite

A sharp absorbance peak located at about 362 nm correspond to the band gap of 3.42 eV. This is almost in accordance with the value of bulk ZnO [13], suggesting excellent crystal quality of the ZnO nanoparticles. So, no blue shift was observed in the UV-Vis absorbance spectrum, revealing the nanoscaled ZnO particles were not small enough to show quantum confinement related effects. An asymmetric tail can also be found on the peak shoulder side with higher wavelength, induced by light scattering. Since the nanocrystalline ZnO sample is not soluble in water, some incident light may be scattered by the dispersed particles due to inhomogeneous distribution.

To investigate the thermal properties of the samples, differential scanning calorimetry (DSC) of pure WP and as prepared ZnO/WP nanoscaled composite were carried out at nitrogen atmosphere, as shown in Fig. 5. The DSC curves indicate that both samples have a broad endothermic peak around 100 °C, which should be attributed to the loss of absorbed water. A small endothermic peak centered at 250 °C can also be observed for the two samples. This peak may arise from the loss of chemical binding water. WP and ZnO/WP nanoscaled composite both show endothermic peak around 310 °C, which is associated with the decomposition of whey protein. However, the peak for ZnO/WP nanoscaled composite is much stronger and sharper than that of pure WP, revealing the decomposition of whey protein is remitted in some degree. This suggests there may exist some interactions, such as electrostatic attraction, hydrogen bonding or O-Zn-O bonding, between protein molecules of WP and ZnO. The stabilization of ZnO/WP nanoscaled composite under the electron beam for TEM measurements confirms also the existence of the interaction between WP and ZnO. Similar behavior has been reported for polymer-inorganic composites [14].

![DSC curves of WPI and Zn/WPI nanocomposite](image)

**Fig. 5** DSC curves of WPI and Zn/WPI nanocomposite.

The formation mechanism of the ZnO/WP composite may be proposed as follows. During our synthesis, WP solution was first heated at 80 °C for 30 min. Under this condition, WPs denature into soluble WP aggregates. This solution is adjusted to weak basic, PH = 8, with buffer. At this PH value, WP solution (the isoelectric point is 5.2 for β-lactoglobulin (BLG), the major component of whey proteins) is negatively charged. Therefore, electrostatic attraction
between the positively charged $\text{Zn}^{2+}$-water complex and the carboxylic groups of the negatively charged protein forms a composite. The WP may also coordinate $\text{Zn}^{2+}$ ions via either primary amino groups or sulfhydryl group. The configuration of the whey proteins in the aggregate solution is that of random coil. These random coils behave as organic matrices holding many hydrated Zn cations on the negatively charged amino acids. Deprotonation of these Zn aquo-complexes results because binding of water to Zinc increases the acidity of the water. The buffer consume the released protons, keeping the zinc complex hydrolyzing continuously. This deprotonation of water molecules associated with the Zn cations produces Zn hydroxides that presumably then lose additional protons to form ZnO. With the reaction continuing, the formed ZnO nanoparticles bind with whey proteins.

4 CONCLUSIONS

In summary, we report a facile process to synthesize ZnO/WP nanoscaled composite of about 300 nm. The ZnO/WP composite has been characterized by XRD, UV-Vis, DSC and TEM. TEM images show that typical sample is composed of uniform WP coated ZnO nanoparticles. The average size of ZnO nanoparticles is 65 nm. The TEM and DSC results indicate a strong binding between ZnO and WP.

REFERENCES