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TEM Analysis of Soy Protein Based Nanoparticles as Nutraceutical Carriers

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In food and pharmaceutical industry, biopolymer nanoparticles (NPs) with various forms and shapes have been applied to nano delivery system (NDS). While food proteins have high nutritional value and the ability to form emulsions and gelations, which allow them as ideal materials for encapsulation of bioactive compounds [1]. Since the size, shape and structure of NDS affect the bioavailability of the active ingredients, characterization of NDS is essential to understand the benefits as well as the NPs development mechanism. Here we discuss suitability of various TEM methods for imaging of soy protein based NPs.

Soy protein isolate (SPI) with about 94.4% protein was used to develop NPs. The SPI proteins were denatured by combination of harsh alkaline and heat treatments [2-3], followed by reducing pH to 7.5, 8.0, 9.0 to induce partial refolding. Then calcium was added as a cross linker by adapting cold gelation method to induce gelation and form NPs [1].

High angle annual dark field (HAADF) scanning TEM (STEM), energy filter imaging (EFTEM) and hole free phase plate (HFPP) imaging [4] techniques were used to analyze the morphology and structure of soy protein NPs. All images were obtained on a JEOL2200FS equipped with a Schottky field emission gun and an in-column omega type energy filter. Fig. 1 shows the typical HAADF STEM images of unstained soy protein NPs developed in PH8 with different calcium concentrations. The data was obtained with incident beam dose of 1.6×10^{-2} electron/nm^2. Some of polypeptide chains (Fig.1a arrow 1) appeared to be linked to form a compact and dense nano-network after introducing 2.5mM calcium, as shown in Fig.1b (marked as arrow 2). With calcium concentration increased to 5mM, NPs with more compact and denser internal structure in a honeycomb shape (arrow 3) were observed as shown in Fig.1c and Fig.2. The core-shell structure of NPs as indicated in Fig.2 (arrow 4) was also observed with HFPP TEM in Fig.3, while honeycomb structure was not clearly revealed with this technique.

For comparison, NPs stained with 2% Uranyl acetate were also imaged using EFTEM and STEM. In bright field TEM and STEM images, the NPs were clearly observed as shown in Fig. 4a and Fig. 4b. Although the contrast of NPs appears to be higher compared to that of unstained NPs, the honeycomb internal structure or porous structure as indicated in Fig.2 was not observed as shown in Fig. 4c. A possible explanation is that the heavy metal bound to the NPs surface produces strong contrast and suppresses the structure details.

In summary, HAADF STEM, which has been exploited both for the contrast enhancement and structural analysis in biology [5], appears to be the favorable method to study the native structure of soy protein based NPs. In a given PH8, the size of NPs ranges from 20 nm to 60 nm depending on the concentration of calcium added. The formation of NPs can be explained by the presence of calcium which increases the ionic strength of dispersion and promotes protein aggregation through

calcium ionic bonding [6]. Therefore, the compactness of inner structure of NPs, which may influence the encapsulation efficiency of the encapsulated core ingredients, could be modulated by adjusting the calcium concentration.

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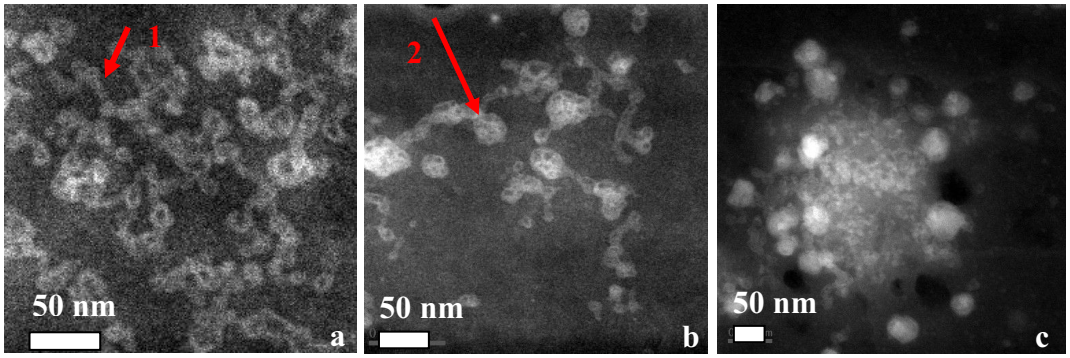

Fig.1. HAADF STEM images of soy protein based NPs developed in PH=8 with 0 mM Ca^{2+} (a); 2.5 mM Ca^{2+} (b), 5.0 mM Ca^{2+}(c).

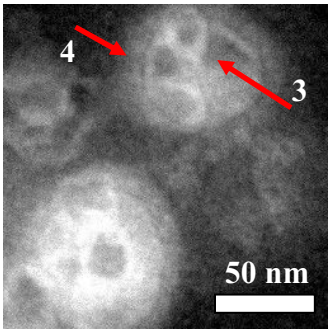

Fig.2. HAADF STEM image of NPs shows core-shell and internal porous structures.

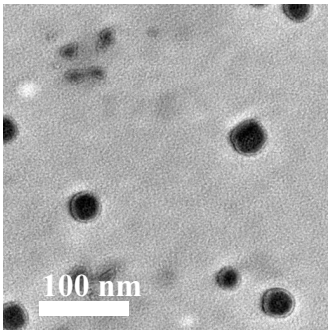

Fig.3. EFTEM image of NPs in HFPP TEM shows core-shell structure.

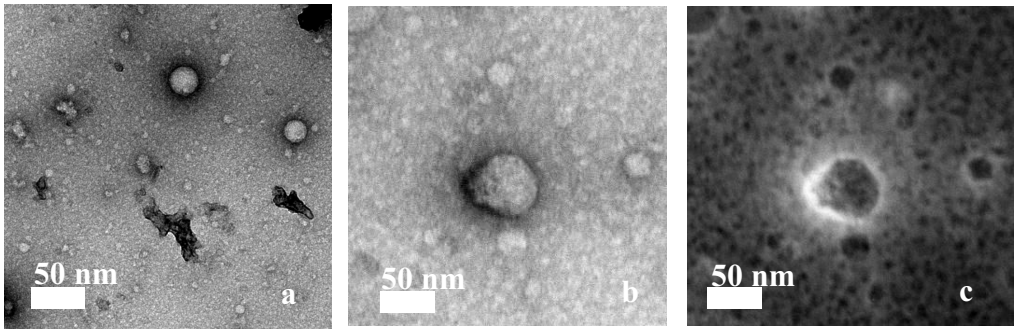

Fig.4. (a) EFTEM, (b) BF STEM, (c) HAADF STEM images of stained NPs.

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