Encapsulation of biopharmaceuticals improves their stabilities and provides controlled release

Luo, Yangchao, and Wang, Qin.
Nutrition and Food Science, University of Maryland, College Park, U.S. luo142@umd.edu

INTRODUCTION

Indole-3-carbinol (I3C) and diindolylmethane are the two compounds derived from Cruciferous vegetables. These two compounds have been shown to suppress the proliferation of various cancer cell lines. However, the stability of I3C and DIM is one of the major challenges for their applications. Under various conditions, I3C molecules undergo dimerization to form a complex mixture, among which DIM is the major product. Especially, with oral consumption, I3C will dimerize to form DIM in a very short time after it enters acidic condition in stomach. For instance, I3C is not detectable within 1 hour of oral consumption in mice model, and large amount of DIM and other condensation products are formed (Anderton 2004). One recent study pointed out I3C is also not stable in cell culture medium during cellular experiments. Therefore, it is still unclear if I3C itself possesses any beneficial effects or DIM is the effective form of I3C.

Encapsulation technology, as a novel tool, has been drawn increasing attention for its applications in food and pharmaceutical industry. Zein, the prolamine protein from corn, and chitosan, the derivative from natural polysaccharide chitin, are both food biopolymers which have been extensively investigated for their capabilities to encapsulate food bioactives. The complex nanoparticles prepared with the combination of zein and chitosan or its derivative have been developed in our lab as versatile delivery systems for various bioactives (Luo 2011, 2012). Therefore, it is of great interest to study if the encapsulation of I3C and DIM in nanoparticles could provide controlled release and enhance their stabilities. In present study, I3C and DIM will be encapsulated into zein nanoparticles and then further coated with CMCS. Two delivery systems will be compared, in terms of physicochemical properties and their protective effects on stabilities of I3C and DIM. The effect of encapsulation on the dimerization of I3C to DIM will also be monitored during the stability test.

MATERIALS AND METHODS

Materials I3C and DIM were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO). Zein with a minimum protein content of 97% was provided by Showa Sangyo (Tokyo, Japan). CMCS was purchased from Nantongxingcheng Biological Product Inc. (Nantong, Jiangsu Province, China), with a deacetylation degree of 96% and a carboxylation degree of 65%. Other chemical were of HPLC grade, purchased from Sigma-Aldrich.

Preparation of nanoparticles I3C or DIM (5 mg/ml) was dissolved in pure ethanol as stock solution. Zein (5 mg/ml) was dissolved in 70% aqueous-alcohol solution. CMCS (1 mg/ml) was dissolved in pure water. Zein nanoparticles were prepared by a liquid-liquid phase separation method as reported in our previous study (23). Then, the above zein-I3C (-DIM) nanoparticles was coated with CMCS under vigorous stirring until a single phase was formed. Then, 1 mL of calcium chloride solution was added under stirring. The obtained opaque nanoparticles dispersion was freeze-dried. The control nanoparticles were prepared by replacing CMCS and calcium solution with pure water in parallel.

Characterization of nanoparticles Scanning electron microscopy (SEM), X-ray diffraction (XRD), dynamic light scattering (DLS) were used to characterize physicochemical properties of nanoparticles, including morphology, crystalline state, and particle size.

Encapsulation Efficiency (EE) EE is defined as the drug content that is entrapped into nanoparticles, and calculated as follows:

$$\mathrm{EE}(\%) = \frac{\text{Total drug amount} - \text{Free drug amount}}{\text{Total drug amount}} \times 100$$

Briefly, lyophilized nanoparticles were flushed with ethyl acetate for three times and then the elute was dried in presence of DMSO under nitrogen gas. The residue was then suspended in acetonitrile and analyzed by HPLC (Anderton 2004).

Release Profile Release profile of I3C and DIM from nanoparticles were measured in phosphate buffer saline (PBS), according our reported procedure. The released compound content was quantified by HPLC as described above (Luo 2012).

Effects of Encapsulation on Stabilities Photo-stability and thermal-stability of encapsulated compounds were determined under UV light for up to 10 hours and the thermal-stability was performed under 37°C condition, respectively. Free compounds were also tested as control.

RESULTS AND DISCCUSION

Characterization From figure 1, I3C- and DIM-encapsulated zein nanoparticle is successfully fabricated in our study. Both nanoparticles shared similar features of spherical shape and smooth surface (Fig. 1, Z/I and Z/D), with particle size around 250 nm.

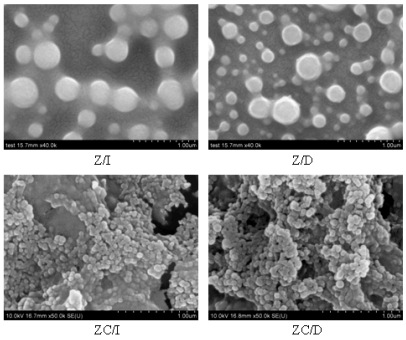

Figure 1 : Morphological observation with SEM

After coated with CMCS, particle size of both nanoparticles decreased to less than 100 nm, and more uniform nanoparticles were formed (Fig. 1, ZC/I and ZC/D). The EE of zein nanoparticles were within 60-70% for both compounds, but significantly increased to almost 80% after nanoparticles coated with CMCS. This observation is similar to our previous study that CMCS coating could improve encapsulation efficiency (Luo 2012). The XRD pattern indicated the crystalline structure of I3C and DIM were converted into amorphous state in nanoparticles, providing the evidence of encapsulation (data not shown).

Release Profile

As shown in Fig. 2, all formulations of nanoparticles showed a first-order release profile, consisting of biphasic trend of burst effect and sustained release. Both I3C and DIM nanoparticles demonstrated similar release profile, the burst effect occurred within 0.5 h of followed by sustained release for more than 6 hrs. The CMCS coating also helped reduce the sustained release for 6.5 hrs, and this effect was more significant in I3C nanoparticles.

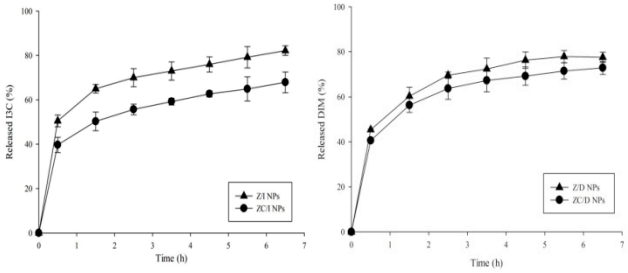

Figure 2: Release profile in PBS.

Effect of encapsulation on stabilities

Fig. 3 shows the thermal stability of I3C after 24 h incubation. The control sample of pure I3C compound demonstrated the fastest degradation rate, followed by I3C encapsulated zein and zein/CMCS nanoparticles. From comparison of initial chromatography of I3C control and after 24 h incubation (Fig. 4A and B), it is suggested that DIM is the major degradation product, along with other two products whose retention time are 22.5 (peak a, LTr) and 31.8 min (peak b, HI-MI). Zein nanoparticles provided little protection of I3C from thermal degradation (Fig. 4C and D). However, after coated with CMCS, the protection effect was greatly improved that only a small amount of DIM and LTr were formed after 24 h. During the thermal stability measurement, the precipitation of zein nanoparticles after 24 h was clearly observed, due to the denaturation of zein protein under heat treatment, resulting in the collapse of nanoparticle structure and thus losing the protection of encapsulated I3C. CMCS coating may decrease the denaturation rate of zein protein and preserve the protective effects. Unlike I3C compound, DIM is much more stable to thermal treatment, as (not shown). DIM control maintained 80% of its original concentration even after 4 days incubation in 37°C condition. The protective effects of zein and zein/CMCS nanoparticles on DIM thermal degradation had similar trends to the protection of I3C (not shown here). Encapsulation also significantly improved UV-stabilities of I3C and DIM, extending their half-life by 2-3 times (not shown here).

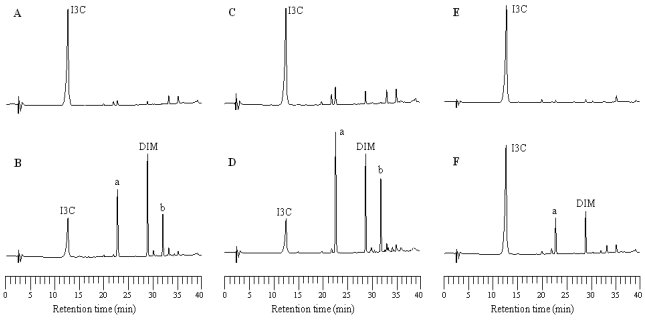

Figure 3: Effects of encapsulation on thermal stability of I3C under 37C. A, C, E represent I3C levels in I3C control, Z/I, ZC/I samples, respectively, at the beginning of incubation (0 day); B, D, F represent I3C levels in I3C control, Z/I, ZC/I samples, respectively, after 24 h under 37C.

CONCLUSION

I3C/DIM zein/CMCS nanoparticles can be prepared under mild conditions, with particle size from 100-250 nm. Encapsulation could provide controlled release and significantly enhance their photo- and thermal stabilities, preventing dimerization of I3C under various harsh conditions.

REFERENCE

- Anderton et al. (2004) *Pharmacokinetics and tissue disposition of indole-3-carbinol and its acids condensation products after oral administration to mice*. Clin. Cancer Res., 10, 5233-41.
- Luo et al. (2011) *Preparation and characterization of zein/chitosan complex for encapsulation of α-tocopherol, and its in vitro controlled release study*. Colloid Surf. B: Biointerfaces 2011, 85, 145−152
- Luo et al. (2012) *Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3*. J. Agr. Food Chem. 60, 836−843