1 **Effects of different polysaccharides on the formation of egg yolk LDL**
2 **complex nanogels for nutrient delivery**

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Abstract

Five polysaccharides, pectin, carboxymethyl cellulose (CMC), gum arabic, carrageenan and alginate, were studied to form complex nanogels with egg yolk low density lipoprotein (LDL). All nanogels were smaller than 85 nm with highly negative zeta potential, while LDL/carrageenan and LDL/alginate nanogels exhibited more heterogeneous size distribution. Fourier transform infrared spectrum suggested that hydrogen bonds, hydrophobic and electrostatic interactions were involved to form nanogels. Overall, significant expansion of nanogels was observed after encapsulation of curcumin, being studied as a model lipophilic nutrient. Fluorescence spectra evidenced that LDL provided non-polar microenvironment for curcumin and polysaccharides played an important role in the encapsulation process. All nanogels showed sustained release of curcumin under simulated gastrointestinal conditions. Furthermore, nanoscale, smooth and spherical ultrafine dry powders of nanogels were obtained by innovative nano spray drying technology. Our study indicated that LDL/polysaccharides may serve as potential oral delivery systems for lipophilic nutrients.

Keywords: Egg yolk; Low density lipoprotein; Nanogels; polysaccharide; Encapsulation; Curcumin

1. Introduction

Development of nanoscale delivery systems for bioactive compounds have received extensive investigation in recent decades in the fields of pharmaceuticals and food. Naturally derived biopolymers have great potentials to form promising nanoparticles as they are biodegradable, biocompatible and GRAS (generally regarded as safe) materials. Among all the biopolymers, protein and polysaccharides are most widely used to form polyelectrolyte nanocomplex (PEC) due to the binding capacity of protein and stabilizing properties of polysaccharides (Jones & McClements, 2011). Many studies have investigated encapsulation and delivery properties of protein-polysaccharides based PEC systems, such as casein with pectin (Y. Luo, Pan, & Zhong, 2015), ovalbumin with cellulose (Wongsasulak, Patapeejumruswong, Weiss, Supaphol, & Yoovidhya, 2010) and zein with chitosan (Y. Luo, Zhang, Whent, Yu, & Wang, 2011).

Egg has been extensively used in the food industry as it possesses various functional properties. As a major component, egg white protein (mainly ovalbumin) has been extensively investigated for its delivery potentials for bioactive compounds (Elzoghby, Samy, & Elgindy, 2012), while only few studies focused on egg yolk proteins (He, et al., 2015; Zhou, Wang, Hu, & Luo, 2016). Low density lipoprotein (LDL) is the main constituent of egg yolk, representing the most dry weight of egg yolk and possessing a naturally occurring micelle nanostructure which promotes its encapsulation and delivery applications (Anton, et al., 2003). Traditionally, egg yolk LDL served as an emulsifier in

food products due to its amphiphilicity (Kiosseoglou, 2003), and there are studies showing that it could be a promising cryoprotective agent (Amirat, et al., 2004; Hu, et al., 2006; Moussa, Martinet, Trimeche, Tainturier, & Anton, 2002). However, egg yolk LDL receives little attention regarding its nutrients delivery property, which may be in part owing to its poor stability under various processing conditions. For instance, both heating and freezing processes have been known to induce the disruption of LDL nanostructure and irreversible gelation and precipitation (Cordobés, Partal, & Guerrero, 2004; Telis & Kieckbusch, 1997).

In our previous work (Zhou, et al., 2016), pectin was applied as a secondary coating by complexing with LDL through hydrophobic and electrostatic interactions to form nanogels and pectin coating was shown to greatly improve the stability of LDL under simulated gastrointestinal (SGI) conditions. Curcumin, as a model hydrophobic compound, was successfully encapsulated into the core of egg yolk LDL and the curcumin-loaded nanogels showed a controlled release profile in SGI conditions. We also demonstrated that Nano Spray Drying technology is a promising approach to transform liquid dispersion of different colloidal nanoparticles to dry powders, which are spherical and homogeneous sub-micro particles when appropriate drying conditions are applied (Hu, et al., 2016; Wang et al., 2016a, 2016b; Zhou, et al., 2016). Besides pectin, there are also many other polysaccharides that have potentials to complex with egg yolk LDL. Therefore, it is of great interest to comprehensively investigate and compare the capability of different polysaccharides to form complex nanogels with egg yolk LDL.

In the present work, specifically, five polysaccharides, i.e., carboxymethyl cellulose (CMC), gum arabic, alginate, carrageenan, as well as pectin as a control, were studied for their capability to prepare LDL/polysaccharide nanogels. Our first objective was to comprehensively characterize the prepared nanogels for physicochemical properties and stabilities under SGI conditions. Then, the encapsulation and nutrient delivery potentials, using curcumin as a model lipophilic compound, were systemically evaluated, including encapsulation efficiency and loading capacity, molecular interactions, as well as controlled release profiles. Finally, nano spray drying was exploited as an innovative technique to obtain ultra-fine nanogels powders and the effects of polysaccharides on the morphology and homogeneity of dry powders were studied by scanning electron microscopy (SEM).

2. Materials and methods

2.1 Materials

Pectin from Citrus peel (galacturonic acid content of >74%), CMC (average M_w ~90,000 Da), gum arabic, carrageenan (kappa type) and alginate were purchased from Sigma–Aldrich Corp. (St Louis, MO, USA). Hen eggs were obtained from Uconn Dairy Bar at the University of Connecticut (Storrs, CT, USA) and stored in a refrigerator and used within two weeks. All other chemicals were of analytical grade and purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA) unless stated otherwise.

2.2 Preparation of LDL-based nanogels

LDL was extracted from fresh hen egg yolk following the same procedures as described in our recent study with 97% purity (Zhou, et al., 2016). The LDL-based nanogels were prepared according to the procedures reported in previous studies (He, et al., 2015; Zhou, et al., 2016). Briefly, LDL and each polysaccharide were dissolved in pure water at 2 mg/mL and were adjusted to pH 4 and 7, respectively. Then, 5 mL polysaccharide solution was titrated into 20 mL LDL solution. The mixture were gently stirred under room temperature for 30 min, followed by pH adjustment to 7.0 and heating at 70 °C for 45 min, and then cooled down to room temperature.

2.3 Nano spray drying of nanogels

Freshly prepared nanogels were spray dried by a Nano Spray Dryer B-90 (Büchi Labortechnik AG, Flawil, Switzerland) under previously optimized drying conditions (Zhou, et al., 2016) as follows: nanogels were diluted with ultrapure water to 0.5 mg/mL; the inlet temperature was set to 100 °C and the air flow rate was 120 L/min; the mesh size was 5.5 µm.

2.4 Characterization of nanogels

Particle size and polydispersity index (PDI) were determined by dynamic light scattering (DLS) measurement using a Nano Zetasizer ZS model (Malvern Instruments, Ltd., Worcestershire, UK) with a 173° scattering angle at 25 °C. Zeta potential was

calculated from the electrophoretic mobility of sample by the same instrument.

Fourier transform infrared (FT-IR) spectra of samples were recorded from a Nicolet™ iSTM5 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA) and analyzed by OMNIC software version 8.0. Dry powder samples were mounted on ATR crystal directly and LDL solution was cast-dried on an aluminum tray previously. All samples were scanned at wavenumber of 500-4000 cm^{-1} with a resolution of 4 cm^{-1} . **Second-derivative FT-IR spectra were also collected within the amide I region (1600 – 1700 cm^{-1}).**

Morphology of spray dried LDL-based nanogels were obtained by a scanning electron microscopy (JSM-6330F, JEOL Ltd., Tokyo, Japan). Dry powders were adhered to conductive carbon tapes directly and then mounted on specimen stub. Prior to observation, samples were coated with a thin gold layer by a sputter coater.

2.5 Stability of nanogels in simulated gastrointestinal conditions

The stability of various nanogels were tested under SGI conditions. Briefly, 1 mL of freshly prepared nanogels was added to 9 mL of either simulated gastric fluid (pH 2 or 4 with 1 mg/mL pepsin) and incubated at 37 °C for 2 h, or simulated intestinal fluid (pH 7.5 with 10 mg/mL pancreatin) and incubated at 37 °C for 4 h. The digested nanogels were characterized for particle size, PDI and zeta potential after each incubation.

2.6 Encapsulation of curcumin into LDL/polysaccharide complex nanogels

Four curcumin/LDL mass ratios, i.e. 0.5%, 1%, 1.5% and 2.5%, were tested to

investigate the encapsulation properties of complex nanogels. Curcumin was first dissolved in ethanol at 2 mg/mL and then added to LDL solution until the curcumin/LDL mass ratio reached the pre-determined percentage. The mixture were stirred at room temperature for 30 min and then nanogels were prepared as described in section 2.2. The curcumin-loaded nanogels were ultrafiltered using Amicon[®] Ultra centrifugal filters (Merck KGaA, Darmstadt, Germany) with a molecular weight cutoff of 10,000 Da. After centrifuging at 8,000 g for 20 min, free curcumin permeated into the receiving reservoir and then the concentration was determined by a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA, USA) with a previously established curcumin standard curve at 430 nm ($R^2 \geq 0.999$). Encapsulation efficiency (EE) and loading capacity (LC) were calculated according to the following equations:

$$EE (\%) = \frac{Total\ Cur - filtrated\ Cur}{Total\ Cur} \times 100\% \quad Eq. 1$$

$$LC (\%) = \frac{Total\ Cur - filtrated\ Cur}{Total\ mass\ of\ nanogels} \times 100\% \quad Eq. 2$$

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where “cur” refers to curcumin, “total mass of nanogels” is the total mass of LDL, polysaccharide and curcumin.

2.7 Fluorescence measurements

The fluorescence spectra of various curcumin-loaded LDL/polysaccharide complex nanogels were recorded using a fluorescence spectrometer LS55 (Perkin Elmer, Waltham,

Massachusetts). The excitation wavelength was set at 420 nm and the emission spectra were collected from 450 to 650 nm. All nanogels were diluted appropriately to meet the instrument sensitivity requirement. Free curcumin in aqueous condition was prepared by dissolving curcumin in pure ethanol at 2 mg/mL and then diluted with water to the identical concentration as in the nanogels.

2.8 Controlled release of curcumin from nanogels

The controlled release of curcumin from LDL-based nanogels was investigated by a dialysis method (Yangchao Luo, Zhang, Cheng, & Wang, 2010). Equal volume of ethanol was premixed with simulated gastric or intestinal fluid to serve as release medium, creating a sink condition for curcumin (Kakkar, Singh, Singla, & Kaur, 2011; Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009). The operation procedures were slightly modified from our previous study (Zhou, et al., 2016). Briefly, 3 mL of 1% curcumin-loaded nanogels solution was sealed in a dialysis bag and immersed into 60 mL of simulated gastric release medium, followed by incubation at 37 °C with gentle shaking for 2 h. At the end of 2 h, the dialysis bag was transferred into 60 mL of simulated intestinal release medium for another 4 h incubation. During the incubation, 1 mL of release medium was withdrawn at pre-determined time intervals to measure curcumin concentration and the same amount of fresh medium was replenished to maintain the constant volume of release medium.

2.9 Statistical analysis

All measurements were conducted in triplicates and the results were expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was conducted to test significance by Duncan test on SPSS version 22. The p-value was set at 0.05.

3. Results and discussion

3.1 Characterization of complex nanogels formed with different polysaccharides

[Table 1]

Generally, all five polysaccharides used in this study were all able to complex with egg yolk LDL to form nanogels with size smaller than 85 nm (Table 1), indicating that egg yolk LDL has great ability to interact with a wide range of polysaccharides. Nanogels fabricated with gum arabic, CMC and pectin were uniform and stable with high absolute value of zeta potential (> 30 mV). Notably, data of LDL/pectin nanogels concurred with our previous results (Zhou, et al., 2016), while LDL/CMC nanogels in the present study showed smaller particle size and higher zeta potential than that in a previously published literature (He, et al., 2015). This may be in part attributed to the greater molecular weight of CMC used in this study, thus providing more carboxyl groups to interact with LDL and forming more compact nanogels. However, nanogels formed with carrageenan and alginate showed significantly larger PDI (> 0.4) and greater absolute zeta potential (> 55 mV) values than other nanogels. The size distribution of various nanogels was further illustrated in Fig. 1. A single peak with sharp shape was observed for nanogels prepared with LDL and gum arabic (Fig. 1A), CMC (Fig. 1B) and pectin (Fig. 1C), while three

peaks were observed for those prepared with LDL and carrageenan (Fig. 1D) and alginate (Figure 1E), confirming their heterogeneous size distribution. Namely, two small fractions with one peak smaller than 10 nm and the other one larger than 1000 nm were observed by DLS measurement for those two nanogels. This may suggest the structure of nanogels were destroyed during complexation process, possibly due to the extremely high zeta potential of the two polysaccharides (< -70 mV) at pH 7 that induced excessive electrostatic interactions with egg yolk LDL causing disruption of protein-phospholipid interface. This disruption then led to uncontrolled aggregation and gelation among proteins in LDL during stirring and heating. The strongly negatively charged biopolymers, such as alginate, has been reported before to partially disrupt the phospholipid bilayers by intense electrostatic interactions induced insertion of alginate into the membrane proteins in phospholipid bilayer structure (Cohen, Ban, Chow, & Langer, 1991). Nonetheless, the average sizes of these two kinds of nanogels were still under 100 nm, indicating that the disruption of protein-phospholipid interface may only occur at a small extent.

[Figure 1]

3.2 FT-IR analysis

The FT-IR spectroscopy was carried out to investigate the molecular interactions between LDL molecules and different polysaccharides. As shown in Fig. 2, all the five types of nanogels had a flatter and wider peak at 3290 cm^{-1} compared to a sharp peak at the same position in pure LDL spectrum, despite the fact that characteristic peaks of O-H

215 stretching of each polysaccharide varied from 3234 to 3377 cm^{-1} (**Fig. S1**). This change
216 indicated that hydrogen bond was involved and the water retention in LDL protein-lipid
217 interface was eliminated (Krillov, Balarin, Kosovic, Gamulin, & Brnjac-Kraljevic, 2009;
218 Zhou, et al., 2016). The bands at 2921, 2852 and 1741 cm^{-1} was attributed to asymmetric
219 and symmetric stretching vibrations of $-\text{CH}_2$ group in lipid chains (Krillov, et al., 2009).
220 However, the intensity reduction of these three bands was clearly observed in all nanogels,
221 which may be ascribed to the shielding effect of polysaccharide coating on the surface of
222 LDL molecules forming the nanogels. The characteristic bands of phospholipid at 970
223 and 721 cm^{-1} (Fookson & Wallach, 1978) were only observed in pure LDL molecules, as
224 well as LDL/alginate and LDL/carrageenan nanogels. The exposure of phospholids in
225 these nanogels may indicate the partial disruption of protein-phospholipid interface
226 induced by these two polysaccharides, which well agreed with the DLS results discussed
227 in section 3.1. The shift of characteristic peaks of amide bonds (1400–1700 cm^{-1}) gave a
228 picture of changes in secondary structure of LDL protein. There was little change on the
229 α -helix (1653 and 1541 cm^{-1}) (Byler & Susi, 1986) structure in LDL/gum arabic and
230 LDL/carrageenan nanogels, while in the spectra of the other three types of nanogels the
231 shift of amide I from 1653 to 1616-1598 cm^{-1} was attributed to partial denaturation of
232 LDL protein and hydrophobic interactions induced by heating process (Graff,
233 Pastrana-Rios, Venyaminov, & Prendergast, 1997; Krillov, et al., 2009).

234 **Second-derivative FT-IR spectra of LDL and nanogels were also collected for protein**

secondary structure analysis. As shown in **Fig. S2**, there was no shift at the original β -sheets (1683 cm^{-1}), indicating the polysaccharides coating and heating procedure only partially denatured LDL protein structure. However, LDL/alginate nanogels showed no peak at 1650 cm^{-1} , while original LDL and all the other nanogels had the peak at the same position, suggesting that alginate coating altered the α -helices of LDL. Furthermore, after formation of nanogels, the intramolecular β -sheets (1632 and 1620 cm^{-1}) appeared in all nanogels spectra, which supposed to be originated from the aggregation and unfolding of protein taken place when heating.

[Figure 2]

3.3 Encapsulation of curcumin into nanogels

[Table 2]

Table 2 showed the characterization of LDL-based nanogels with different curcumin loading ratio. Generally, as curcumin/LDL ratio increased, the particle size and PDI of nanogels increased gradually, while zeta potential of nanogels was relatively stable and was not dramatically affected by curcumin loading (Table 2). Preliminary experiments showed that adding the same amount of ethanol without curcumin did not cause any change in size, PDI or zeta potential of nanogels (data not shown), indicating that the effect of ethanol on the LDL structure was negligible. These results suggested that curcumin was successfully encapsulated into the core of nanogels via hydrophobic interactions without changing the surface structure of nanogels, except causing the

expansion of LDL dimension by about 10-25 nm. Comparing nanogels formed by different polysaccharides, it is notable that carrageenan and alginate had negative effects on the capability to encapsulate curcumin, with dramatically larger size and PDI at the same curcumin loading ratio (Table 2). Furthermore, the size distribution curve in Fig. 1 confirmed that the size of LDL/carragennan and LDL/alginate nanogels was much more heterogeneous and polydispersed than other nanogels. Particularly, when curcumin loading ratio reached 2.5%, nanogels formed by LDL with CMC, carrageenan and alginate precipitated immediately after preparation with an extremely high PDI, demonstrating that the nanogels were overloaded and excessive curcumin adsorbed on the surface of LDL and thus induced overexpansion of LDL micelles. Similar observation was also reported previously (He, et al., 2016) that the size of doxorubicin-loaded LDL/CMC nanogels sharply increased to over 300 nm when the doxorubicin was loaded at a high concentration of 120 µg/mL, due to the surface adsorption of drug on the LDL surface instead of being encapsulated. Therefore, only nanogels formed by LDL with gum arabic, CMC and pectin were selected for further experiments in this study.

[Figure 3]

In terms of encapsulation efficiency and loading capacity, gum arabic, CMC and pectin nanogels showed similar results (Fig. 3.). As curcumin loading ratio increased from 0.5% to 1.5%, encapsulation efficiency of all three types of nanogels remained around 80%, while loading capacity increased gradually from 0.4% to 1.2%, indicating

that curcumin was not saturated in nanogels at these loading ratios. When the loading ratio reached 2.5%, curcumin still did not reach saturation ratio in LDL/gum arabic and LDL/pectin nanogels. However, LDL/CMC was overloaded by curcumin, resulting in rapid precipitation and increased PDI (Table 2). Our previous study showed that by using a pH-driven and organic solvent-free method, the loading ratio was achieved as high as 5% with encapsulation efficiency being almost 100% (Zhou, et al., 2016). This discrepancy in encapsulation efficiency may be explained by the fact that the organic solvent (ethanol) is hard to be completely evaporated during stirring or heating process and thus may interfere with the hydrophobic interactions between curcumin and lipid core of LDL. Such interference may consequently slow down the migration rate of curcumin into LDL micelles and thus lower the encapsulation efficiency. Besides, two different measurement methods of encapsulation efficiency may also account for the discrepancy. Based on the DLS and encapsulation efficiency data, the particle size and PDI of LDL/pectin nanogels exhibited the least changes after curcumin loading without compromising the EE and LC, especially at high loading ratios. To have a better comparison among nanogels in subsequent experiments, the load ratio of 1% was selected as the optimum for LDL/gum arabic, LDL/CMC, and LDL/pectin nanogels, given that they all had similar particle size and PDI values.

3.4 Stability of nanogels in simulated gastrointestinal conditions

[Table 3]

The stability of an oral delivery system in digestive conditions is one of the critical parameters to assess its delivery efficacy. Therefore, three conditions were studied in the present work to better simulate gastric and intestinal conditions. The pH 2 and pH 4 were used to mimic fasting and fed status of gastric conditions, respectively, while the pH 7.5 was tested to simulate intestinal condition since the pH of intestine does not vary too much postprandially (Dressman, et al., 1990). As shown in Table 3, only LDL/pectin nanogels were able to maintain the particle size under 1000 nm after incubation at pH 2, while severe aggregation and precipitation was observed for the other two nanogels in the same condition. At the extreme acidic pH condition, the LDL/CMC and LDL/pectin nanogels became neutral with zeta potential close to zero, due to the protonation of carboxyl groups of polysaccharides. In contrast, the zeta potential of LDL/gum arabic nanogels turned to positive value, which could be explained by the protonation of amino groups on the glycoprotein fraction of gum arabic (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). Our results indicated that electrostatic repulsion was the major contribution to the stability under the condition of pH 2 and meanwhile suggested that pectin coating on the surface of LDL was effective to provide sufficient steric stabilization against aggregation. Under fed gastric status at pH 4, all nanogels slightly swelled by 80-150 nm and zeta potential decreased to around -15 mV, but PDI remained small. These observations together revealed that the polysaccharides were less protonated and the reduction of surface charge was not as severe as at pH 2. When nanogels were incubated in simulated intestinal fluid for 4 h, the screening effects of salts greatly

reduced the zeta potentials of LDL/CMC and LDL/carrageenan nanogels. But the zeta potential of LDL/gum arabic nanogels was minimally reduced compared to the original curcumin loaded nanogels (Table 2), possibly owing to the buffering effect from glycoprotein in gum arabic. The increase of average size and PDI of nanogels could possibly result from undissolved pancreatin (data not shown), since the size distribution curve of nanogels were hardly shifted but PDI significantly increased. Overall, all the three nanogels exhibited good stability in fed gastric and intestinal conditions.

3.5 Fluorescence spectroscopy analysis

[Figure 4]

The molecular interactions between curcumin and LDL-based nanogels with different polysaccharides were further analyzed by fluorescence spectroscopy. As revealed in Fig. 4A, curcumin encapsulated in nanogels exhibited enhanced fluorescence intensity with a peak at 490 nm, compared to free curcumin dispersed in water showing negligible fluorescence at the same peak position and at 537 nm. Usually, curcumin dissolved in organic solvent showed a strong fluorescence peak at the emission over 500 nm (Barik, Priyadarsini, & Mohan, 2003), while when dispersed in aqueous phase, like water, the fluorescence intensity of curcumin was dramatically reduced due to the more polar microenvironment (Pan, Zhong, & Baek, 2013; Yu & Huang, 2010). Therefore, the blue shift and augmented fluorescence intensity of curcumin in aqueous phase may be explained by its migration from polar microenvironment to the lipid core of LDL via

hydrophobic interactions, thus providing curcumin a strong non-polar microenvironment. The blue shift of curcumin spectrum was also observed when binding with other proteins, such as milk casein (Pan, et al., 2013; Sahu, Kasoju, & Bora, 2008), bovine serum albumin (Barik, et al., 2003) and lysozyme (Z. Li, et al., 2015). Interestingly, significant discrepancies in fluorescence intensity were observed among three nanogels. Curcumin encapsulated in LDL/gum arabic nanogels had the weakest fluorescence intensity, which may be ascribed to the dramatically larger particle size at 1% curcumin loading ratio, compared to the other two nanogels (Table 2). It has been well established that the fluorescence intensity of encapsulated compound is inversely related to the particle size (Stranik, Nooney, McDonagh, & MacCraith, 2007). In addition, the more hydrophilic nature of gum arabic than other polysaccharides may also contribute to the reduction of the fluorescence intensity of curcumin by providing a more polar microenvironment (Garti & Leser, 2001). Conversely, LDL/CMC and LDL/pectin nanogels showed less change in size after curcumin encapsulation and hence a more compact lipid core with stronger non-polar microenvironment was achieved, explaining the greater fluorescence emission at the same curcumin loading.

3.6 Controlled release

Because most of nanogels showed poor stability in fasting gastric condition (pH 2) as discussed in section 3.4, the kinetic release profile was only investigated in fed gastric condition (pH 4), followed by transferring to intestinal condition (pH 7.5). As depicted in

Fig. 4B, free curcumin diffused out much more rapidly compared to curcumin from nanogels, reaching 76% in the first 2 h in gastric phase. The fast diffusion of free curcumin continued after being transferred into simulated intestinal fluid, with complete diffusion at 210 min. Nevertheless, all nanogels showed a sustained release profile of curcumin in both gastric and intestinal conditions. Complexation with pectin and gum arabic provided moderate and similar sustained release of curcumin in gastric condition, with approximately 55% of curcumin was released before entering the intestinal phase. During the incubation in pH 7.5, LDL/pectin nanogels showed a slower release than LDL/gum arabic nanogels. Among three nanogels, LDL/CMC nanogels demonstrated the slowest kinetic release rate and the smallest accumulative release in both digestive conditions. This phenomenon may be attributed to the strongest hydrophobic interactions and the most compact lipid core, which was in a good agreement with the DLS and fluorescence results. Additionally, it is notable that, LDL/CMC nanogels showed relatively faster release in intestinal condition (with about 40% curcumin being released), the major absorption site for nutrients. Our results indicated that LDL/CMC nanogels not only possessed best encapsulation property, but also the optimal release profile for lipophilic nutrients.

3.7 Nano spray drying of nanogels

[Figure 5]

It has been reported before that dehydration processes, such as traditional spray

drying, may significantly change the morphology and structure of egg yolk LDL/polysaccharides complex coacervates (Souza, Rojas, Melo, Gaspar, & Lins, 2013), therefore all five LDL/polysaccharide nanogels dispersions were included in this experiment to explore the potential protective effects of polysaccharides on the dehydration of LDL nanogels. In order to obtain nano-sized ultrafine dry powders of nanogels, nano spray drying technology was utilized to minimize uncontrolled protein denaturation and gelation during the drying process, although the surface structure and particle size distribution may still be changed as several nanogels droplets may be pressed to form one powder particle during spray drying process. Fig. 5 depicts the representative SEM images of nano spray dried powders of different nanogels. Overall, except LDL/gum arabic nanogels, all other four nanogels showed spherical-shaped structure with particle size mostly smaller than 200 nm. Notably, LDL/gum arabic nanogels presented the most exceptional morphology with highly dented external surface and several large microparticles (Fig. 5A). The deep concavities were also observed both by nano and traditional spray drying technologies in many other studies on gum arabic-based formulations (Bertolini, Siani, & Grosso, 2001; Kim & Morr, 1996; X. Li, Anton, Arpagaus, Belleteix, & Vandamme, 2010), which could be explained by the rapid shrinkage and evaporation of water molecules retained among gum arabic molecules (Rosenberg, Kopelman, & TALMON, 1985). The spray-dried powders of the other four nanogels showed relatively smooth surface structure. Comparatively, the LDL/CMC and LDL/pectin nanogels were more uniform than LDL/carrageenan and LDL/alginate

nanogels (Fig. 5B to E), which was in accordance with size distribution and PDI of freshly prepared nanogels (Table 1 and Fig. 1B to E). Our results suggested that nano spray drying technology is an innovative and feasible technique to produce nano-scale ultrafine dry powders of LDL/polysaccharides complex nanogels.

4 Conclusion

In summary, nanogels were successfully prepared between five different polysaccharides and egg yolk LDL, and their encapsulation and delivery potentials were evaluated, using curcumin as a model lipophilic bioactive compound. Gum arabic, CMC and pectin were more suitable to form nanogels with LDL, while the ultrafine nano spray dried powder cannot be obtained using LDL/gum arabic nanogels. It was found that curcumin loading dramatically increased the size and PDI of nanogels and each polysaccharide had its own critical curcumin loading ratio to keep the nanogels small and homogeneous. Nanogels prepared with LDL and CMC provided the most non-polar microenvironment for curcumin, which improved the encapsulation capability and decreased the kinetic release rate in gastric condition, showing the highest potential for oral delivery of curcumin.

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Fig. 1. Size distribution by intensity of original LDL nanogels (blue) and curcumin-loaded nanogels (red). GA, gum arabic; CMC, carboxymethyl cellulose; PEC, pectin; CAR, carrageenan; ALG, alginate; LDL, egg yolk low density lipoprotein.

Fig. 2. FT-IR spectra of spray dried LDL nanogels and air dried LDL.

Fig. 3. Encapsulation efficiency (A) and loading capacity (B) of LDL nanogels. Percentage in legends refers to curcumin/LDL mass ratio. Data sharing different letters within the same nanogels formulation are significantly different ($p < 0.05$).

Fig. 4. Characterization of 1% curcumin-loaded nanogels and free curcumin control. Fluorescence spectra (A) and the kinetic release under simulated gastrointestinal conditions (B).

Fig. 5. SEM images of spray dried nanogels. (A) LDL/gum arabic; (B). LDL/CMC; (C) LDL/pectin; (D) LDL/carrageenan; (E) LDL/alginate. Scale bars are 500 nm.

Table 1. Characterization of LDL nanogels formed with different polysaccharides. Data sharing different superscript letter are significantly different (p<0.05).

Polysaccharides	Size (nm)	PDI	Z-potential (mV)
Gum arabic	76.97±5.16 ^a	0.213±0.005 ^a	-30.9±0.97 ^a
CMC	63.54±0.17 ^b	0.222±0.003 ^a	-50.4±0.25 ^b
Pectin	57.80±1.70 ^b	0.252±0.010 ^b	-41.3±1.80 ^c
Carrageenan	84.86±2.35 ^a	0.463±0.007 ^c	-55.6±0.40 ^d
Alginate	76.52±8.93 ^a	0.436±0.022 ^c	-58.6±2.45 ^d

Table 2. Characterization of LDL nanogels with different curcumin loading ratio. Data sharing different superscript within the same nanogels formulation are significantly different (p<0.05).

Polysaccharides	Curcumin/LDL mass ratio (%)	Size (nm)	PDI	Z-potential (mV)
Gum arabic	0.5%	100.2±9.6 ^a	0.221±0.006 ^a	-27.8±0.53 ^{ab}
	1%	131.5±6.1 ^b	0.275±0.002 ^{ab}	-27.5±1.60 ^a
	1.5%	178.9±20 ^c	0.352±0.078 ^c	-30.6±1.15 ^c
	2.5%	207.3±7.3 ^d	0.379±0.085 ^c	-29.8±0.62 ^{bc}
CMC	0.5%	64.39±4.2 ^a	0.232±0.011 ^a	-49.4±0.30 ^{bc}
	1%	68.35±0.3 ^a	0.257±0.002 ^a	-50.8±0.45 ^c
	1.5%	94.61±1.6 ^a	0.337±0.022 ^b	-48.3±2.42 ^{ab}
	2.5%	475.3±60 ^b	0.528±0.051 ^c	-46.2±0.60 ^a
Pectin	0.5%	59.3±0.62 ^a	0.258±0.002 ^a	-37.3±1.26 ^c
	1%	63.1±0.05 ^a	0.262±0.008 ^a	-35.1±1.75 ^c
	1.5%	90.7±2.83 ^b	0.312±0.033 ^a	-28.9±1.33 ^a
	2.5%	123±7.97 ^c	0.248±0.007 ^a	-32.6±2.06 ^b
Carrageenan	0.5%	101.8±8.7 ^a	0.473±0.003 ^a	-50.4±0.21 ^a
	1%	116.4±2.8 ^a	0.502±0.006 ^a	-53.7±0.75 ^b
	1.5%	206.1±1.9 ^b	0.409±0.030 ^a	-53.8±0.43 ^b
	2.5%	545.4±65.3 ^c	0.731±0.084 ^b	-57.3±1.09 ^c
Alginate	0.5%	90.5±1.1 ^a	0.447±0.003 ^{ab}	-53.3±10.8 ^a
	1%	138.1±63.7 ^{ab}	0.484±0.012 ^b	-60.8±0.45 ^a
	1.5%	367.9±32.5 ^b	0.403±0.033 ^a	-57.4±0.93 ^a
	2.5%	804.2±179 ^c	0.978±0.036 ^c	-61.0±0.81 ^a

Table 3. Stability of LDL nanogels incubated in simulated gastrointestinal conditions. Data sharing different superscript letter within the same nanogels formulation are significantly different (p<0.05).

Polysaccharides	Conditions	Size (nm)	PDI	Zeta potential (mV)
Gum arabic	pH 2	1165 ± 249 ^a	0.579 ± 0.044 ^a	20.8 ± 0.65 ^a
	pH 4	228.4 ± 19.3 ^b	0.259 ± 0.006 ^b	-14.2 ± 0.81 ^b
	pH 7.5	118.7 ± 1.40 ^c	0.333 ± 0.008 ^c	-28.8 ± 0.70 ^c
CMC	pH 2	6544 ± 402 ^a	0.131 ± 0.036 ^a	3.41 ± 0.43 ^a
	pH 4	136.1 ± 2.36 ^b	0.164 ± 0.002 ^a	-13.7 ± 0.65 ^b
	pH 7.5	145.5 ± 9.13 ^b	0.430 ± 0.018 ^b	-26.5 ± 0.69 ^c
Pectin	pH 2	423 ± 21.4 ^a	0.271 ± 0.004 ^a	-1.17 ± 0.09 ^a
	pH 4	141.0 ± 1.11 ^b	0.184 ± 0.002 ^b	-16.4 ± 0.46 ^b
	pH 7.5	164.5 ± 2.72 ^b	0.430 ± 0.009 ^c	-23.5 ± 0.70 ^c