



# Methoxy poly(ethylene glycol)-grafted-chitosan based microcapsules: Synthesis, characterization and properties as a potential hydrophilic wall material for stabilization and controlled release of algal oil

Hailong Peng<sup>a,b</sup>, Hua Xiong<sup>a,\*</sup>, Jinhua Li<sup>c</sup>, Lingxin Chen<sup>c</sup>, Qiang Zhao<sup>a</sup>

<sup>a</sup> State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, Jiangxi, China

<sup>b</sup> Department of Chemical and Pharmaceutical Engineering, Nanchang University, Nanchang 330031, Jiangxi, China

<sup>c</sup> Key Laboratory of Coastal Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, Shandong, China

## ARTICLE INFO

### Article history:

Received 22 December 2009

Received in revised form 18 June 2010

Accepted 23 June 2010

Available online 30 June 2010

### Keywords:

Methoxy poly(ethylene glycol)

Chitosan

Microcapsulation

Algal oil

Water soluble

## ABSTRACT

Methoxy poly(ethylene glycol)-grafted-chitosan (mPEG-g-CS) was synthesized in an attempt to increase the water solubility of CS, and it was applied as wall material to microcapsulate algal oil (AO). Both Fourier transformed-infrared (FT-IR) spectra and solubility test confirmed that mPEG had been successfully grafted onto CS. The larger particles showed smooth surfaces, whereas smaller ones presented surface folds. The amount of free and total oil were 2.73 and 47.87 g/100 g, respectively, with high encapsulation efficiency of 94.30%. Fourteen fatty acids were detected in free algal oil (FAO) and microcapsulated algal oil (MAO), and the important fatty acids were preserved after microcapsulation. AO contained within microcapsules was protected from UV-light and heat compared with FAO. Release rate of AO was initially rapid and then followed by controlled release with the erosion mechanism playing a dominant role. The mPEG-g-CS proved to be a promising wall material with excellent solubility and film forming ability, and therefore supplied great potential applications in food industry.

Crown Copyright © 2010 Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

It is well known that chitosan (CS) is the second most plentiful polysaccharide, with a structure of poly  $\beta$ -(1  $\rightarrow$  4) *N*-acetyl-D-glucosamine. Due to its good properties of non-toxicity, biocompatibility, biodegradability and film forming ability, CS has been widely applied in various areas, especially in pharmaceutical (Wang et al., 2005), biomedical (Berger et al., 2005) and food fields (Shahidi et al., 1999; Srinivasa et al., 2004, 2007; Chien et al., 2007). However, there are some limitations for CS to be applied because it is soluble only in aqueous acidic solution below pH 6.5, while insoluble in neutral pH and physiological environments. Moreover, some active agents would possibly be damaged in the acidic solution system of dissolved CS. In order to overcome the above problems, a considerable number of different chemical agents for modification of CS have been employed, such as cholesterol (Yuan et al., 2006; Wang et al., 2007), *N*-carboxymethyl (Zhang et al., 2000), linolenic acid (Chen et al., 2003), deoxycholic acid (Lee et al., 2005) and methoxy poly(ethylene glycol) (mPEG) (Sugimoto et al., 1998; Bhattarai et al., 2005).

Because of its hydrophilic flexibility, non-ionic property, biodegradability, biocompatibility, low toxicity and immunogenicity (Mao et al., 2005), mPEG conjugated to CS has been widely applied

in pharmaceutical fields in the forms of microspheres, nanoparticles or polymers to enhance stability, prolong half-life of active agents, and alter pharmacokinetics, tissue distribution and pharmacological properties (Zhu et al., 2007). Furthermore, mPEG-grafted-polyethylenimine-CS (mPEG-g-PEI-CS) can convert  $\omega$ -6 to  $\omega$ -3 fatty acids (Xu et al., 2009). However, to the best of our knowledge, till now there were few reports about the utilization of mPEG-grafted-CS (mPEG-g-CS) as wall material or film for microcapsulation of fatty oils.

Algal oil (AO), produced from unicellular organisms, is a rich source of polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA, C<sub>22:6</sub>,  $\omega$ -3). PUFAs are considered essential for normal growth and development, which play an important role in the prevention and treatment of cardiovascular disease (Wang et al., 2006), hypertension (Narce et al., 1997), inflammation (Treschow et al., 2007), immune disorders (Kelley, 2001) and certain forms of cancer (Wigmore et al., 1996). However, PUFAs can be easily affected by adverse environmental conditions of light, moisture, free radicals and oxygen (Shahidi and Han, 1993). This outcome leads to oxidation and deterioration even at room temperature, and the subsequent formation of unhealthy free radicals, reactive aldehyde, ketone oxidants, and unpleasant fishy off-flavors (Let et al., 2003). All these decrease AO shelf life and limit its commercial utilization. Therefore, improving the stability of AO has received increasing attention from researchers.

\* Corresponding author. Tel./fax: +86 791 6634810.

E-mail address: [huaxiong100@yahoo.com.cn](mailto:huaxiong100@yahoo.com.cn) (H. Xiong).

The aims of this study were to synthesize the mPEG-g-CS in an effort to improve the water solubility of CS, and explore the possibility of using it as wall material to microcapsulate AO. Fourier transformed-infrared (FT-IR) and scanning electron microscopy (SEM) characterizations, solubility, encapsulation efficiency, fatty acid compositions, stabilization and release mechanism were also investigated in detail. The resulted AO microcapsules were demonstrated good physicochemical properties, and the mPEG-g-CS was proved great potentials in food applications.

## 2. Materials and methods

### 2.1. Materials

Algal oil (AO) was provided by Taicheng Bio-tech., Ltd. (Jiangxi, China). Chitosan (CS) (molecular weight  $1 \times 10^6$  Da, deacetylation degree  $\geq 85\%$ ) was supplied by Sankang Health Products Co., Ltd. (Jiangxi, China). MPEG<sub>2000</sub> was purchased from National Starch and Chemical Company (Shanghai, China). *N,N'*-dicyclohexylcarbodiimide (DCC), petroleum ether (PE), diethyl ether (DE), *N*-hydroxysuccinimide (NHS), maleic anhydride (MA), dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The doubly distilled deionized water obtained from a Milli-Q Water System (Millipore, Bedford, MA, USA) was used for all the aqueous solutions.

### 2.2. Synthesis of mPEG-grafted-chitosan (mPEG-g-CS)

The mPEG-g-CS was synthesised via DCC–NHS systems according to the method (Mao et al., 2005; Jeong et al., 2008): mPEG was dissolved in anhydrous dichloromethane and an excess of MA was added under the protection of argon. This mixture was heated at 60 °C for 48 h. After that the reaction mixture was precipitated by DE, filtered and dried in vacuum oven for 3 days. The dried product (mPEG–COOH) was dialyzed against doubly distilled deionized water for 2 days to remove unreacted anhydride. The mPEG–COOH was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> and then DCC–NHS was added. This reaction was carried out for 6 h and the solution was then filtered. The product of mPEG–NHS was precipitated with DE, filtered, and then vacuum dried for 3 days. A predetermined quantity of CS was dissolved in anhydrous DMSO. The mPEG–NHS was also dissolved in anhydrous DMSO and added into CS solution with stirring at room temperature for 24 h. Then the solution was purified by ultra filtration with an Amicon system equipped with a 10,000 MW cutoff membrane. Finally, the dialyzed solution was freeze-dried for following experiments. Spectra of CS, mPEG and mPEG-g-CS were recorded within KBr disks on a FT-IR spectrometer (Nicolet 5700, Thermo Electron Corporation, Waltham, MA, USA).

### 2.3. Solubility

The solubility of the CS and mPEG-g-CS was evaluated at different pH values. CS and mPEG-g-CS (2 g) were dissolved in 0.2% acetic acid solution (2 mg/mL), and the pH of the solution was adjusted by dropwise addition of 1 mol/L NaOH solution. The transmittance was measured by using a UV-spectrophotometer at 600 nm (TU-1901, Purkinje General Instrument Co., Ltd., Beijing, China) to determine the solubility of CS and mPEG-g-CS at different pH solutions.

### 2.4. Preparation of microcapsulation

Sucrose esters (0.3 g) and monoglyceride ester (0.45 g) were dissolved with 10 mL corn oil in a beaker at 70 °C. Then AO

(40 mL) was added into the beaker and continuously stirred at 200 rpm for 30 min under vacuum to form an AO suspension, which was used as the oil phase. The mPEG-g-CS (5 g) was dissolved in distilled water (100 mL) and stirred to form the water phase. Then, the oil phase was added dropwise into the water phase with constant stirring for 10 min and subsequently homogenized at 30–40 MPa using a higher pressure homogeniser (SLS, Shenlu Homogeniser Co., Ltd., Wuxi, China) to form a homogeneous emulsion. Thereafter, the homogeneous emulsion was spray dried using a rotary atomizer at the speed of 20,000 rpm (MDR.P-5, Modern Atomizing Dry Equipment Co., Ltd., Wuxi, China), which was atomized at a feed rate of 2.6 L/h using 70 L/min compressed air at 70 °C. The inlet and outlet air temperatures of the spray dryer were 185 and 90 °C, respectively. The powders of the microcapsule were collected at the bottom of the dryer's cyclone.

### 2.5. Scanning electron microscopy (SEM)

The morphological features of the microcapsules were performed by SEM (Quanta 200 F, FEI, Hillsboro, OR, USA) with an accelerated voltage of 20 kV. Microcapsules were sprinkled onto a double-sided tape and sputter coated with gold. The inner structures were also observed after fracturing the microcapsules using a razor blade.

### 2.6. Total oil content

The microcapsule powders (2 g), doubly distilled deionized water (8 mL) and alealase enzyme (3 mL) were mixed in a water bath (40 °C) for 40 min for breaking the wall of microcapsules. After that, fatty oils were extracted with a solution of PE/DE (20 mL, 1:1, v/v) for 30 min and then filtered to remove the insoluble substances. The solvent was removed by a rotary evaporator and dried at 105 °C for 1 h. Total oil content was expressed by the following equation:

$$\text{Total oil content\%} = \left[ \frac{\text{Weight of total oil}}{\text{weight of microcapsule}} \right] \times 100.$$

### 2.7. Encapsulation efficiency (EE)

Microcapsule powders (2 g) were placed into PE (30 mL). They were mixed with a vortex mixer for 10 min and centrifuged at 12,000 rpm for 15 min. The supernatant was filtered by a filter paper, washed twice with PE, and then evaporated at 60 °C. After that, the solvent-free extract was dried at 105 °C for 1 h and the amount of free oil was weighed gravimetrically. The EE were determined by the following equation:

$$\text{EE\%} = \left[ 1 - \frac{\text{weight of free oil of microcapsules}}{\text{weight of total oil}} \right] \times 100.$$

### 2.8. Fatty acid compositions

AO methyl ester was prepared according to AOAC (1999) method. The fatty acid compositions were analyzed by the capillary gas chromatography (CGC) (6890A Plus, Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector and a column (DB-225, 30 mm  $\times$  0.52 mm ID, 0.25  $\mu$ m, J&W scientific, Palo Alto, CA, USA). Operating conditions were performed as follows: temperature of both the injector and detector were 250 °C; the oven temperature was held at 45 °C for 3 min and subsequently increased to 175 °C at 13 °C/min and kept at 175 °C for 27 min, and then raised from 175 to 215 °C at 4 °C/min and kept at 215 °C for 35 min. Nitrogen was used as the carrier gas.

### 2.9. Peroxide value (POV)

POV is frequently used to measure primary oxidation products and lipid hydroperoxides of fatty oils, which is determined and expressed as meq O<sub>2</sub>/kg oil according to AOCS (1998) method. Microcapsule powders were dissolved in chloroform then mixed with glacial acetic acid and saturated potassium iodide solution. Liberated iodine was titrated with standard sodium thiosulphate (0.01 mol/L) solution using starch indicator (1%).

### 2.10. Retention ratio of docosahexaenoic acid (DHA)

The contents of DHA in MAO was determined as methyl esters by CGC and evaluated by the retention percentage, namely the ratio of the content of DHA retained to the original one in the microcapsule samples,

$$\text{Retention ratio of DHA}\% = \frac{[(\text{Retained amounts of DHA}) / (\text{initial amounts of DHA})] \times 100.}$$

### 2.11. Stabilization

MAO and FAO were stored under two conditions: (1) at room temperature for 60 days, under the condition of exposure to a UV lamp (25 W at 30 cm distance), and (2) at 55 °C for 27 days in dark, using a heater under the condition of accelerated shelf life test. The stability of AO was evaluated by determining the changes of POV and retention ratio of DHA in samples at predetermined time intervals.

### 2.12. In vitro release

Microcapsule powders (2 g) were placed into Erlenmeyer flasks containing 250 mL release media of SGF (simulated gastric fluid, pH 1.2), PBS (phosphate buffer saline, pH 7.0), and SIF (simulated intestinal fluid, pH 7.4), respectively. The release media were incubated at (37 ± 0.1) °C in a water bath for 48 h with stirring. At predetermined time intervals, samples (5 mL) from each flask were

drawn and replaced by equal volume of media to maintain a constant volume. The AO of drawn samples was extracted with PE (1 mL), filtered and dried. The controlled release of the AO from the microcapsules was expressed as proportion (%) of the amount of AO released from the microcapsules to the initial amount of AO within the microcapsules.

## 3. Results and discussion

### 3.1. Synthesis of mPEG-grafted-chitosan (mPEG-g-CS)

Synthesis reaction and process of mPEG-g-CS is illustrated schematically in Fig. 1. Because one end of mPEG contains a methoxy group and the other end contains a hydroxyl group, activation is required before starting of grafting. The hydroxyl end of mPEG was activated using DCC–NHS system, and then coupled to the amino groups of CS, forming a graft copolymer of mPEG-g-CS. FT-IR spectra of CS, mPEG and mPEG-g-CS are shown in Fig. 2. In the FT-IR spectrum of CS shown in Fig. 2A, the presence of –OH and N–H stretching vibration was confirmed according to the obvious broaden peak at 3397 cm<sup>-1</sup>, in which the –OH stretching vibrations were overlapped by N–H stretching. The absorption of C–H stretching of methyl or methylene group of CS appears at 2906 cm<sup>-1</sup> and the peak at 1649 cm<sup>-1</sup> corresponds to the amide bond. The peak at 1554 cm<sup>-1</sup> corresponds to the symmetrical stretch vibration absorption of amino group and the peak at 1381 cm<sup>-1</sup> corresponds to stretching vibrations of C–N bond, while the stretch vibrations of C–O are found at 1078 cm<sup>-1</sup>. Fig. 2B shows the FT-IR spectrum of mPEG, in which the peaks at 3400, 2879 and 1116 cm<sup>-1</sup> correspond to –OH, C–H and –CH<sub>2</sub>OCH<sub>2</sub>– groups, respectively.

As for the FT-IR spectrum of mPEG-g-CS samples (Fig. 2C), the peaks corresponding to the hydroxyl, amino and amide groups of CS shifted slightly, and their intensities were significantly reduced. The characteristic peaks associated with mPEG were also significantly decreased. The stronger absorbance at 1112 cm<sup>-1</sup> (–CH<sub>2</sub>OCH<sub>2</sub>–) and 2800–3000 cm<sup>-1</sup> (–CH<sub>3</sub>) appeared in mPEG-g-CS. In addition, a characteristic sharp peak at around

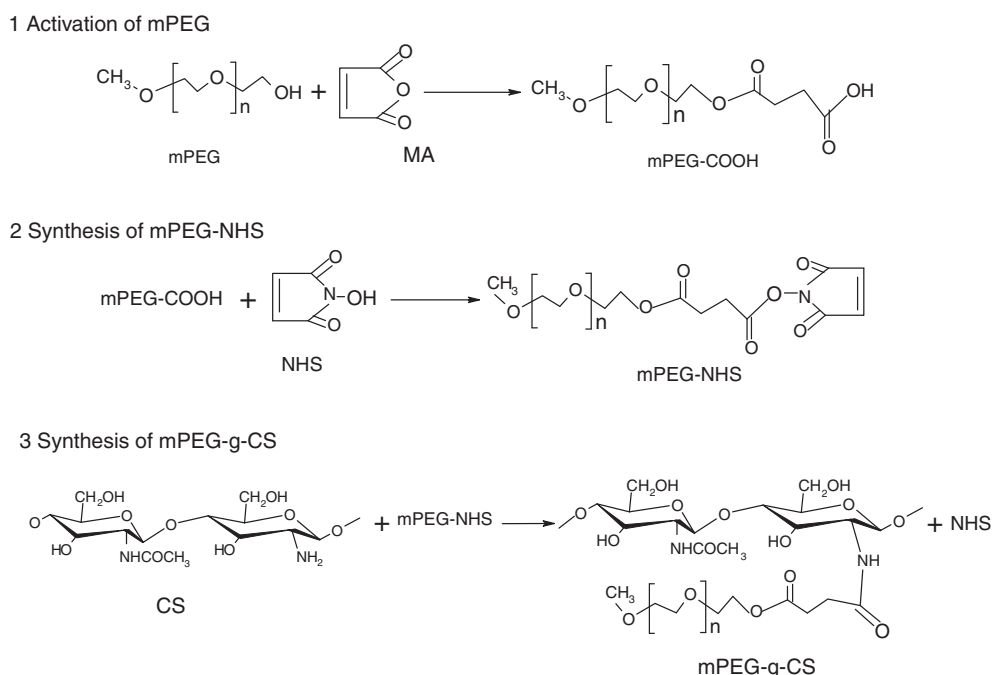


Fig. 1. Chemical schemes for grafting mPEG onto CS.

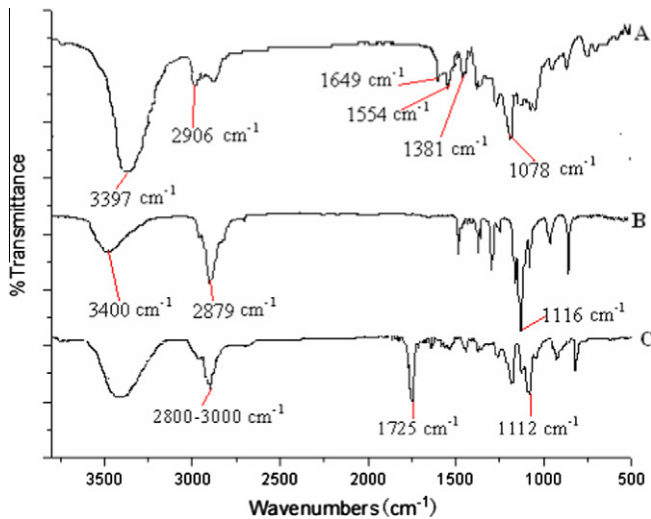


Fig. 2. FT-IR spectra of CS (A), mPEG (B) and mPEG-g-CS (C).

$1725\text{ cm}^{-1}$  belonging to ester carbonyl stretching band ( $\text{C}=\text{O}$ ) was also found. It is assumed that mPEG was successfully grafted onto CS via DCC–NHS systems.

### 3.2. Solubility

The water solubility is shown in Fig. 3. The transparency of CS solution was 50.32% and 17.56% at pH 7.4 and 13, respectively. In contrast, the transparency of mPEG-g-CS solution was still in 90.21% at pH 13, which suggested that the introduction of mPEG onto CS improved water solubility at different pH solutions. The high water solubility is attributed to two factors. One factor may be the decrease of intermolecular interactions, such as van der Waals forces and hydrogen bonding. The other factor may be that the introduction of mPEG onto amino groups can destruct the rigid crystalline domain of CS and thereby disturb the intra and intermolecular hydrogen bonding and lead to the enhancement of hydrophilicity.

### 3.3. Morphology

SEM morphologies of the microcapsules are shown Fig. 4. Those smaller microcapsules presented many folds on the surface (Fig. 4A), whereas the larger ones gave smooth and compact spherical surface with some smaller particles on the surface (Fig. 4B).

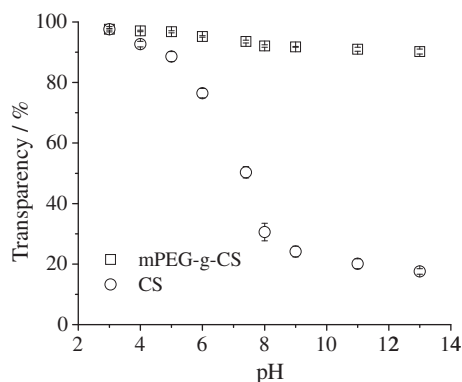


Fig. 3. Solubility changes of mPEG-g-CS (A) and CS (B) in different pH aqueous solutions. Data represent the mean  $\pm$  SD ( $n = 3$ ).

The formation of surface folds or dents is related to the skin forming nature of materials being spray dried, namely, resulting from multiple expansions/contractions due to internal evaporation. This has been studied in detail by Adhikari and Che (Adhikari et al., 2004; Che and Chen, 2010), aiming at the influences of various factors such as the moisture and temperature histories during drying process, or the stickiness and glass transition temperature of the materials. Voids and cores can be seen from the inner structure (Fig. 4C and D), possibly due to the expansion of air bubbles trapped inside the droplet and the movement of the moisture during the non-saturated surface drying period. AO within such voids and cores can be protected from oxidation and improved stability.

### 3.4. Encapsulation efficiency (EE)

In general, acetate buffer at pH 3.0 was used to break the wall of microcapsules. However, such acidic conditions would damage some active agents. In this study, an enzymatic hydrolysis method was employed to break the wall of microcapsules, and the total oils were extracted with PE/DE solution. The amounts of free oils were considered to be equivalent to the PE extracts. The amounts of total and free oil of the microcapsules were 47.87 and 2.73 g/100 g, respectively, and EE was attained up to 94.30%.

### 3.5. Fatty acid compositions

The fatty acid compositions were listed in Table 1 in the order of their number of carbon atoms. A total of 14 compounds of fatty oil were identified from FAO and MAO. The fatty acid compositions of AO after microcapsulation were higher in percent of total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), but lower in PUFAs. Among the SFAs, palmitic acid ( $\text{C}_{16:0}$ ) was the major saturated fatty acid, amounting to 21.44 ( $\pm 0.44$ )% and 21.81 ( $\pm 0.61$ )% in FAO and MAO, respectively. The contents of myristic acid ( $\text{C}_{14:0}$ ) presented at the second highest level, and increased from 10.73 ( $\pm 0.35$ )% to 11.09 ( $\pm 0.31$ )% after microcapsulation. Only oleic acid ( $\text{C}_{18:1}$ ) and palmitoleic acid ( $\text{C}_{16:1}$ ) were identified as the MUFAs, and there were not significant difference in contents between FAO and MAO. In the PUFA fractions, DHA ( $\text{C}_{22:6, \omega-3}$ ) was the major functional fatty acid at levels of 41.59 ( $\pm 1.83$ )% and 39.54 ( $\pm 1.67$ )% in FAO and MAO, respectively. EPA ( $\text{C}_{20:5, \omega-3}$ ) as the second important functional fatty oil was increased from 0.69 ( $\pm 0.04$ )% to 0.99 ( $\pm 0.12$ )% after microcapsulation. The total  $\omega-3$  PUFAs levels were 42.85% and 41.19% for FAO and MAO, respectively, and the ratio of  $\omega-3$  to  $\omega-6$  were found to be 2.38 and 2.77, respectively. As can be seen, AO has higher nutritional value for human consumption and could help prevent the advent of some diseases due to its higher  $\omega-3$  to  $\omega-6$  ratio (Zhao et al., 2010). The results also showed that mPEG-g-CS as water soluble wall material could preserve the important fatty acids of EPA and DHA.

### 3.6. Stabilization

Fatty acid is known to be decomposed by light and heat, which limit its application in food fields for human consumption. POV contents and DHA retention ratio of MAO and FAO were determined to analyze the extent of AO oxidation. As is shown in Fig. 5A, the POV contents of MAO and FAO were 4.02 and 12.53 meq/kg under UV-light exposure for 60 days, respectively. The DHA retention ratio of MAO and FAO decreased from 100.00% to 91.52% and from 100.00% to 70.67%, respectively (Fig. 5B). UV irradiation gradually induced AO oxidation, and the oxidation rate of MAO was lower than that of FAO, which resulted from the wall material performed as a permeability barrier to inhibit the formation of singlet oxygen, and hence decreased the oxidative rate of fatty oil.



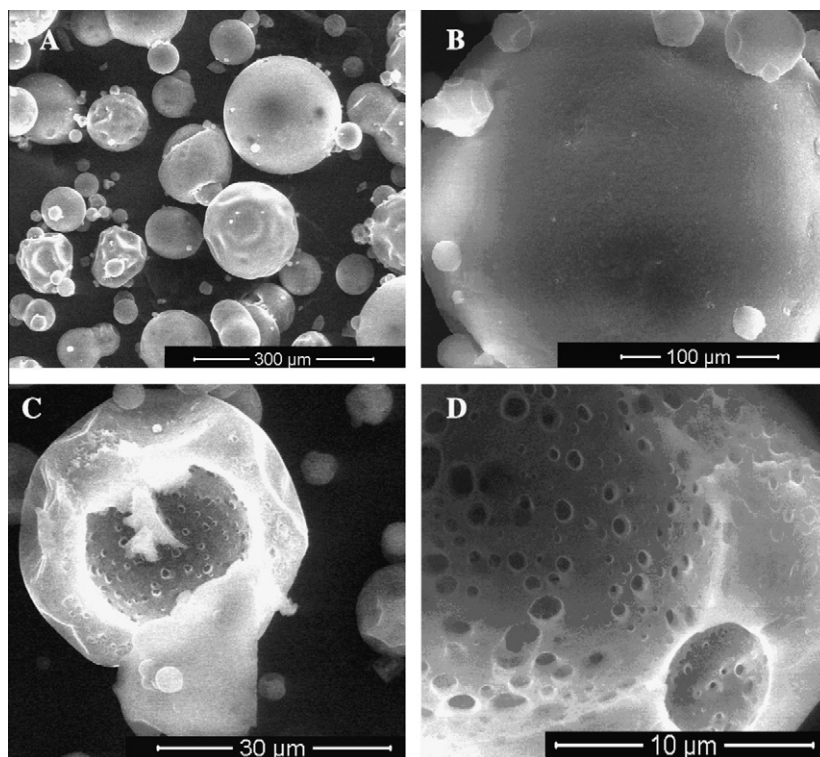


Fig. 4. SEM images of AO microcapsule: surface morphology (A and B) and internal structure (C and D).

Table 1

Fatty acid compositions (g/100 g of samples) of FAO and MAO.

| Fatty acid             | FAO/%                    | MAO/%                    |
|------------------------|--------------------------|--------------------------|
| C <sub>13:0</sub>      | 0.27 ± 0.04 <sup>a</sup> | 1.73 ± 0.04 <sup>a</sup> |
| C <sub>14:0</sub>      | 10.73 ± 0.35             | 11.09 ± 0.31             |
| C <sub>15:0</sub>      | 0.80 ± 0.08              | 0.81 ± 0.09              |
| C <sub>16:0</sub>      | 21.44 ± 0.44             | 21.81 ± 0.61             |
| C <sub>16:1</sub>      | 1.33 ± 0.15              | 1.36 ± 0.11              |
| C <sub>18:0</sub>      | 0.59 ± 0.09              | 1.15 ± 0.14              |
| C <sub>18:1</sub>      | 0.58 ± 0.09              | 0.57 ± 0.08              |
| C <sub>19:0</sub>      | 0.15 ± 0.04              | 0.38 ± 0.13              |
| C <sub>20:4, ω-6</sub> | 0.66 ± 0.08              | 0.28 ± 0.07              |
| C <sub>20:3, ω-3</sub> | 0.33 ± 0.08              | 0.37 ± 0.07              |
| C <sub>20:5, ω-3</sub> | 0.69 ± 0.04              | 0.99 ± 0.12              |
| C <sub>22:5, ω-6</sub> | 17.36 ± 0.78             | 14.60 ± 0.99             |
| C <sub>22:5, ω-3</sub> | 0.24 ± 0.07              | 0.29 ± 0.07              |
| C <sub>22:6, ω-3</sub> | 41.59 ± 1.83             | 39.54 ± 1.67             |
| ∑ω-6 PUFA              | 18.02                    | 14.88                    |
| ∑ω-3 PUFA              | 42.85                    | 41.19                    |
| ω-3/ω-6 ratio          | 2.38                     | 2.77                     |
| ∑SFAs                  | 33.98                    | 37.07                    |
| ∑MUFAs                 | 1.91                     | 1.93                     |
| ∑PUFAs                 | 60.86                    | 56.07                    |
| Total                  | 96.75                    | 95.07                    |

<sup>a</sup> Data represent the mean ± SD (n = 3).

MAO and FAO were stored under the condition of the accelerated shelf life test (Verardo et al., 2008). The shelf life of the food stored at 55 °C for 13.5 and 27 days was equivalent to that stored at room temperature (20–25 °C) for about 9 and 18 months, respectively. Fig. 6 shows that the changes of POV contents and DHA retention ratio for MAO and FAO during storage at 55 °C for 27 days. Oxidation of the FAO seemed to occur in faster rate and the POV contents reached 25.81 meq/kg, as well as, the DHA retention ratios also changed significantly and decreased from 100% to 59.06% on the 27th day. In contrast, the POV content was 10.45 meq/kg and DHA retention ratio only decreased from 100%

to 86.87% for the MAO. It was possible that a complex between the carboxy groups of fatty acids and the hydroxy groups of mPEG-g-CS might be formed, which increased the melting point of fatty acids and stabilized against heat effects. Therefore, the physical incorporation of fatty acids into the mPEG-g-CS microcapsules remarkably improved its stability and extended its shelf life.

### 3.7. In vitro release

The release behaviours of AO from microcapsules in SGF, PBS and SIF media are shown in Fig. 7. Release rate of AO was dependent on pH value of media conditions, with slower release kinetics at higher pH, which might be related to amino groups of protonation of CS that resulted in a soluble, and positively charged polysaccharide and therefore led to faster swelling in acidic medium (Peng et al., 2010). A fast release was found at the beginning of 3 h incubation, and then followed by a slower release. The fast release might be due to the initial “burst effect”, during which AO released from the microcapsules surface and surface voids/cores. Only about 82.54%, 81.13% and 80.03% of AO was released from microcapsules in SGF, PBS and SIF media after 48 h incubation, respectively. The relatively sustained release property of AO would maintain the effective concentration for a long time and improve the bioavailability of AO in body.

In order to gain insight into the release mechanism of AO from microcapsules, the release time curve was calculated using Peppas and Sahlin model as follows (Peppas and Sahlin, 1989):

$$M_t/M_\infty = K_1 t^m + K_2 t^{2m} \quad (1)$$

where,  $M_t/M_\infty$  is the fraction of active agents release at time  $t$ ,  $K_1$  is the Fickian kinetic constant,  $K_2$  is the erosion rate constant and the value of  $m$  is 0.5. If the ratio of the diffusion to erosion ( $K_1/K_2$ ) is greater than 1, the active agents release will occur mainly by diffusion. If the ratio is less than 1, the active agents release will occur predominantly by relaxation/erosion. If the ratio is equal to 1, the

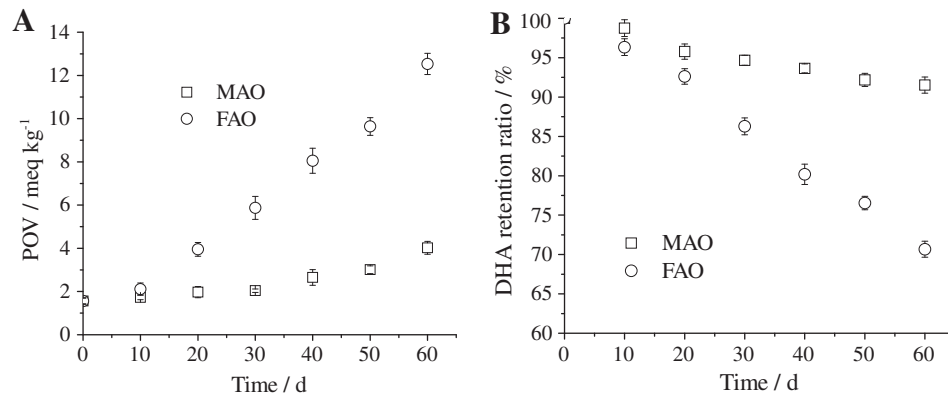


Fig. 5. The changes of POV (A) and DHA retention ratio (B) of FAO and MAO stored under UV-light exposure. Data represent the mean  $\pm$  SD ( $n = 3$ ).

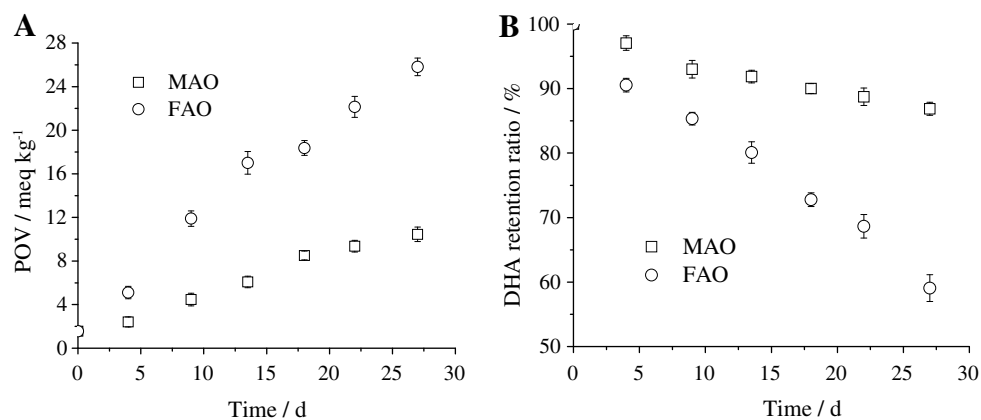


Fig. 6. The changes of POV (A) and DHA retention ratio (B) of FAO and MAO stored in a laboratory heater at 55 °C. Data represent the mean  $\pm$  SD ( $n = 3$ ).

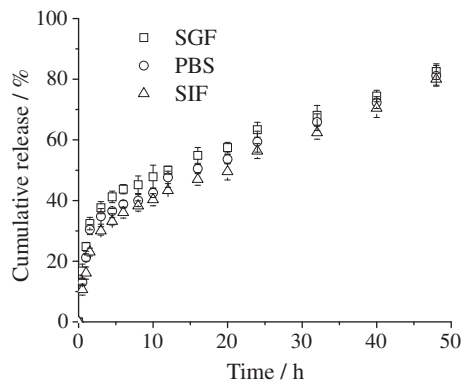


Fig. 7. *In vitro* percentage cumulative release of AO from microcapsules in SGF, PBS and SIF media at  $(37 \pm 0.1)$  °C. Data represent the mean  $\pm$  SD ( $n = 3$ ).

diffusion and erosion might coexist for the release mechanism (Toti and Aminabhavi, 2004; Crcarevska et al., 2008). The values of  $K_1$  were 1.54, 1.03 and 0.58 for AO microcapsules in SGF, PBS and SIF media, respectively; while accordingly, the values of  $K_2$  were 22.16, 19.10 and 16.24, respectively. All the ratios of  $K_1/K_2$  were less than 1, implying that AO release from microcapsules occurred dominantly based on the relaxation/erosion mechanism.

The Peppas and Sahlin model can be rewritten as:

$$M_t/M_\infty = K_1 t^m [1 + (K_2/K_1) \times t^m] \quad (2)$$

The AO percentage due to Fickian diffusion mechanism (F), is clearly calculated as:

$$F = 1/[1 + (K_2/K_1) \times t^m] \quad (3)$$

which leads to the ratio of relaxation/erosion (R) over Fickian contribution as:

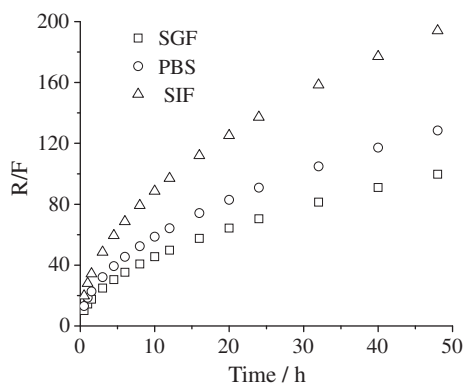
$$R/F = (K_2/K_1) \times t^m \quad (4)$$

The percentage contributions of Fickian diffusion and relaxation (R/F) of AO release from microcapsules are shown in Fig. 8. The results also indicated that the contribution of relaxation/erosion occurs throughout the entire dissolution time period in different media, which is corresponding to the results of the  $K_2/K_1$  values.

Peppas and Sahlin's equation ( $M_t/M_\infty = K_1 t^m + K_2 t^{2m}$ ) is only an empirical equation, and the release mechanism of drugs from sustained-release preparation can be explained according to  $K_1$  and  $K_2$ . The release of drugs from sustained-release preparation accompanied with diffusion, erosion release and other mechanisms. Generally speaking, in the initial time, the concentration of drugs is high at the local of the controlled release preparation and the release mechanism is Fickian diffusion. Following the initial time, the concentration of drug at the local of preparation is reduced greatly, and the erosion effect of excipients (wall of microencapsulation) plays the predominant role. At that time, drug release mechanism is also involved in the erosion of excipients. So, both the first ( $K_1$ ) and second ( $K_2$ ) terms are related to the diffusion and erosion mechanisms.

#### 4. Conclusions

The water soluble mPEG-g-CS copolymer was successfully synthesized. The MAO using mPEG-g-CS as the hydrophilic wall mate-



**Fig. 8.** The ratio of the contribution of R and the contribution of F is plotted as a function of AO release time ( $t$ ) in SGF, PBS and SIF media.

rial exhibited higher EE, better UV-light stability and thermostability, and longer shelf life compared with those of FAO. The *in vitro* release of AO from the microcapsules showed an initial burst release followed by a slowly sustained release with erosion mechanism. Based on these superior properties, the mPEG-g-CS would be a promising water soluble wall material or film for food industry.

### Acknowledgements

This research project was supported by the National 863 High Tech. Program of China (2008AA10Z332), the Key Projects in National Science & Technology Pillar Program during the 11th Five-Year Plan Period (2006BAD27B04), the Programs of State Key Laboratory of Food Science and Technology of Nanchang University (SKLF-KF-201006, SKLF-TS-200818 and SKLF-MB-200809), the Department of Science and Technology of Shandong Province of China (2008GG20005005), One Hundred Person Project of the Chinese Academy of Sciences (20090462), and the National Natural Science Foundation of China (20975089).

### References

- Adhikari, B., Howes, T., Bhandari, B.R., Troung, V., 2004. Effect of addition of maltodextrin on drying kinetics and stickiness of sugar and acid-rich foods during convective drying: experiments and modelling. *Journal of Food Engineering* 62, 53–68.
- AOAC, 1999. Official Methods of Analysis, sixteenth ed. Association of Official Analytical Chemists, Washington DC.
- AOCS, 1998. Official Methods and Recommended Practices of the American Oil Chemists' Society, fifth ed. Champaign, Illinois, Washington, DC.
- Berger, J., Reist, M., Chenite, A., Felt-Baeyens, O., Mayer, J.M., Gurny, R., 2005. Pseudo-thermosetting chitosan hydrogels for biomedical application. *International Journal of Pharmaceutics* 288, 17–25.
- Bhattacharj, N., Ramay, H.R., Gunn, J., Matsen, F.A., Zhang, M.Q., 2005. PEG-grafted-chitosan as an injectable thermosensitive hydrogel for sustained protein release. *Journal of Controlled Release* 103, 609–624.
- Chen, X.G., Lee, C.M., Park, H.J., 2003. O/w emulsification for the self-aggregation and nanoparticle formation of linolenic acid modified chitosan in the aqueous system. *Journal of Agricultural and Food Chemistry* 51, 3135–3139.
- Chien, P.J., Sheu, F., Yang, F.H., 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering* 78, 225–229.
- Che, L.M., Chen, X.D., 2010. A simple non-gravimetric technique for measurement of convective drying kinetics of single droplets. *Drying Technology* 28, 73–77.
- Crcarevska, M.S., Dodov, M.G., Goracinova, K., 2008. Chitosan coated Ca-alginate microparticles loaded with budesonide for delivery to the inflamed colonic

- mucosa. *European Journal of Pharmaceutics and Biopharmaceutics* 68, 565–578.
- Kelley, D.S., 2001. Modulation of human immune and inflammatory responses by dietary fatty acids. *Nutrition* 17, 669–673.
- Mao, S.R., Shuai, X.T., Unger, F., Wittmar, M., Xie, X.L., Kissel, T., 2005. Synthesis, characterization and cytotoxicity of poly(ethylene glycol)-grafted-trimethyl chitosan block copolymers. *Biomaterials* 26, 6343–6356.
- Narce, M., Freaux, J.M., Dardel, V., Foucher, C., Germain, S., Delachambre, M.C., Poisson, J.P., 1997. Fatty acid metabolism, pharmacological nutrients and hypertension. *Biochimie* 79, 135–138.
- Jeong, Y., Kim, D.G., Jang, M.K., Nah, J.W., 2008. Preparation and spectroscopic characterization of methoxy poly (ethylene glycol)-grafted water-soluble chitosan. *Carbohydrate Research* 343, 282–289.
- Lee, K.Y., Kwon, I.C., Jo, W.H., Jeong, S.Y., 2005. Complex formation between plasmid DNA and self-aggregates of deoxycholic acid modified chitosan. *Polymer* 46, 810–8112.
- Let, M.B., Jacobsen, C., Frankel, E.N., Meyer, A.S., 2003. Oxidative flavour of fish oil enriched milk. *European Journal of Lipid Science and Technology* 105, 518–528.
- Peng, H.L., Xiong, H., Li, J.H., Xie, M. Y., Liu, Y.Z., Bai, C.Q., Chen, L.X., 2010. Vanillin cross-linked chitosan microspheres for controlled release of resveratrol. *Food Chemistry* 121, 23–28.
- Peppas, N.A., Sahlin, J.J., 1989. A simple equation for the description of solute release III Coupling of diffusion and relaxation. *International Journal of Pharmaceutical* 57, 169–172.
- Shahidi, F., Arachchi, J.K.V., Jeon, Y.J., 1999. Food applications of chitin and chitosans. *Trends in Food Science and Technology* 10, 37–51.
- Shahidi, F., Han, X.Q., 1993. Encapsulation of food ingredients. *Critical Review in Food Science and Nutrition* 33, 501–547.
- Srinivasa, P.C., Ravi, R., Tharanathan, R.N., 2007. Effect of storage conditions on the tensile properties of eco-friendly chitosan films by response surface methodology. *Journal of Food Engineering* 80, 184–189.
- Srinivasa, P.C., Ramesh, M.N., Kumar, K.R., Tharanathan, R.N., 2004. Properties of chitosan films prepared under different drying conditions. *Journal of Food Engineering* 63, 79–85.
- Sugimoto, M., Morimoto, M., Sashiwab, H., Saimotob, H., Shigemasa, Y., 1998. Preparation and characterization of water-soluble chitin and chitosan derivatives. *Carbohydrate Polymers* 36, 49–59.
- Toti, U.S., Aminabhavi, T.M., 2004. Modified guar gum matrix tablet for controlled release of diltiazem hydrochloride. *Journal of Controlled Release* 95, 567–577.
- Treschow, A.P., Hodges, L.D., Wright, P.F.A., Wynne, P.M., Kalafatis, N., Macrides, T.A., 2007. Comparative Biochemistry and Physiology Part B 147, 645–656.
- Verardo, V., Ferioli, F., Riciputi, Y., Iafelice, G., Marconi, E., Caboni, M.F., 2008. Evaluation of lipid oxidation in spaghetti pasta enriched with long chain-3 polyunsaturated fatty acids under different storage conditions. *Food Chemistry* 10, 1006–1012.
- Wang, C.C., Harris, W.S., Chung, M., Lichtenstein, A.H., Balk, E.M., Kupelnick, B., 2006. n-3 Fatty acids from fish or fish oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary-and secondary-prevention studies: a systematic review. *American Journal of Clinical Nutrition* 84, 5–17.
- Wang, Q., Du, Y.M., Fan, L.H., 2005. Properties of chitosan/poly(vinyl alcohol) films for drug controlled release. *Journal of Applied Polymer Science* 96, 808–813.
- Wang, Y.S., Liu, L.R., Weng, J., Zhang, Q.Q., 2007. Preparation and characterization of self-aggregated nanoparticles of cholesterol-modified-carboxymethyl chitosan conjugates. *Carbohydrate Polymers* 69, 597–606.
- Wigmore, S.J., Ross, J.A., Falconer, J.S., Plester, C.E., CarterTisdale, M.J., Tisdale, C.D., 1996. The effect of polyunsaturated fatty acids on the progress of cachexia in patients with pancreatic cancer. *Nutrition* 12, S27–S30.
- Xu, Z.G., Wan, X.P., Zhang, W., Wang, Z., Peng, R., Tao, F., Cai, L., Li, Y., Jiang, Q., Gao, R., 2009. Synthesis of biodegradable polycationic methoxy poly (ethylene glycol)-polyethylenimine-chitosan and its potential as gene carrier. *Carbohydrate Polymers* 78, 46–53.
- Yuan, X.B., Li, H., Yuan, Y.B., 2006. Preparation of cholesterol-modified chitosan self-aggregated nanoparticles for delivery of drugs to ocular surface. *Carbohydrate Polymers* 65, 337–345.
- Zhang, L., Guo, J., Zhou, J., Guang, Y., Du, Y., 2000. Blend membranes from carboxymethylated chitosan/alginate in aqueous solution. *Journal of Applied Polymer Science* 77, 610–616.
- Zhao, F., Zhuang, P., Song, C., Shi, Z.H., Zhang, L.Z., 2010. Amino acid and fatty acid compositions and nutritional quality of muslin the pomfret, *Pampus punctatissimus*. *Food Chemistry* 118, 224–227.
- Zhu, S.Y., Qian, F., Zhang, Y., Tang, C., Yin, C.H., 2007. Synthesis and characterization of PEG modified N-trimethylaminoethylmethacrylate chitosan nanoparticles. *European Polymer Journal* 43, 2244–2253.