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Production and characterization of nanocapsules encapsulated linalool by ionic gelation method using chitosan as wall material

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Abstract

Linalool has been extensively applied in various fields, such as flavoring agent, perfumes, cosmetics and medical science. However, linalool is unstable, volatile and readily oxidizable. A sensitive substance can be encapsulated in a capsule, so encapsulation technology can solve these problems. In this paper, linalool-loaded nanocapsules (Lin-nanocapsules) were prepared via the ionic gelation method and Lin-nanocapsules were characterized. The results of Fourier transformation infrared spectroscopy (FTIR) showed that linalool was successfully encapsulated in the wall materials. Scanning electron microscopy (SEM) results demonstrated that the shapes of Lin-nanocapsules, with smooth surfaces, were nearly spherical. Lin-nanocapsule average particle size was 352 nm and its polydispersity index (PDI) was proved to be 0.214 by the results of dynamic light scattering (DLC). Thermogravimetric results indicated that linalool loading capacity (LC) was 15.17%, and encapsulation could decrease linalool release and increase linalool retaining time under the high temperature. Oscillatory shear and steady-state shear measurements of Lin-nanocapsule emulsions were systematically investigated. The results of steady-state shear showed that Lin-nanocapsule emulsion, which was Newtonian only for high shear rate, was non-Newtonian. It was proved by oscillatory shear that when oscillation frequency changed from low to high, Lin-nanocapsules emulsion changed from viscous into elastic.

Keywords: lin-nanocapsules; flavor; encapsulation; rheology.

Practical Application: Lin-nanocapsules were prepared via the ionic gelation method.

1 Introduction

As a monoterpene compound, Linalool is a major component of essential oils in lots of aromatic species (Elisabetsky et al., 1995), and present in the dried leaves of cinnamomum camphora (Weaver et al., 1991). Due to promising biological activities including cytotoxic (Yang et al., 2014), anti-microbial (Bagamboula et al., 2004), insect-repellant properties (Beier et al., 2014), anti-inflammatory activity (Peana et al., 2002), antihyperglycemic (Weaver et al., 1991; More et al., 2014), antitumorigenic potential (Jana et al., 2014) and sedative effects, linalool as a natural plant-product has been extensively applied in various fields, such as perfumes, cosmetics, flavoring agents and medical science (Re et al., 2000). However, linalool is unstable, volatile and readily oxidizable. These problems may be solved by encapsulation technology because a sensitive substance can be entrapped in a membrane or capsule. It can be separated from the deteriorating circumstance (Xiao et al., 2014; Zhu et al., 2016). Encapsulation can also isolate a substance from the surrounding matter reactions (Matsuno & Adachi, 1993). Chitosan is an ideal polymeric shell component of oily nanocapsules because it has advantageous biological properties, such as biocompatibility (Khalid et al., 2006), biodegradability (Okamoto et al., 2002; Aguirre-Loredo et al., 2017), antimicrobial activity (Li et al., 2008), mucoadhesivity (Anitha et al., 2011), low toxicity (Chaparro-Hernández et al., 2015) and permeability enhancement (Tobío et al., 2000). Sodium tripolyphosphate is an anionic cross-linker; it exhibits non-toxicity and quick gelling ability that make it a favorable cross-linker for ionic gelation of chitosan (Kafshgari et al., 2011). Encapsulation of linalool in chitosan can form nanocapslue, which can be used in food, textile, cosmetics and clinical fields.

The useful information in energy calculation for process, process control and equipment selection, and quality control in the industry, can be provided by rheological data related to flow behavior of semi-solid or liquid materials. Rheology defines as a relationship between the resulting deformation and the stress acting on a given material (Tabilo-Munizaga & Barbosa-Cánovas, 2005). The rheology data have been used in various fields such as cosmetics, perfumery, paint and food industry (Fang, 2010; Curi et al., 2017). The characterization of viscoelastic properties and the science of rheology play an important role in the manufacture of a lot of products. As important quality control tools, flow properties can be used to reduce batch to batch variations and maintain the superiority of the product. However, rheological data about nanocapsule encapsulated linalool has rarely been reported.

In this paper, the linalool-loaded nanocapsules, with chitosan and sodium tripolyphosphate as wall materials, were produced with the ionic gelation method in o/w emulsion. Fourier transformation infrared spectroscopy (FTIR), dynamic light scattering (DLS), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) were used to characterize

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Lin-nanocapsules. The oscillatory shear and steady-state shear measurements of nanocapsule emulsion were systematically investigated.

2 Materials and methods

2.1 Materials

Golden-Shell Biochemical Co., Ltd. (Zhejiang, China) provided chitosan (average molecular weight = 150 000) which used as a wall material; linalool was obtained from Beijing University Zoteq Ltd (Beijing, China). Sodium tripolyphosphate, fatty alcohol-polyoxyethylene ether (AEO9), polyoxyethylene castor oil (EL40) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2 Preparation process of Lin-nanocapsules

The production process of linalool-loaded nanocapsules via ionic gelation method was as follows. STPP was dissolved at 0.03% (w/w) in water. AEO9 and EL40 in a ratio of 1:1 as emulsifier at 0.16% (w/w) and linalool as core material at 0.5% were mixed by magnetic stirring at 500r/min for 10 min. The mixture of emulsifier and core material was added into the STPP solution for 10 min by ultrasonic treatment at 800 W using JY92-2D. This mixture formed the oil phase. Chitosan was dissolved in water with a concentration 0.15% (w/w). Acetic acid was added into the solution of chitosan until its pH was 5.3. This mixture formed the water phase. The oil phase was dropped into water phase at 3d/s by peristaltic pump and ratio of oil phase and water phase was 2:3. The mixture was stirred at 500r/min for further 30 min by magnetic stirring. The preparation principle of Lin-nanocapsule is shown in Figure 1.

2.3 Morphology of Lin-nanocapsules

The morphology of nanocapsules was investigated by Scanning electron microscopy. The SEM was conducted with an S-3400N scanning electronic microscope (Hitachi, Japan). By conductive double-sided tape, electro sprayed micro particles were mounted on metal stubs, and then were coated with gold under an argon atmosphere (Wang et al., 2013).

2.4 Lin-nanocapsule particle size and polydispersity index

Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) was used to determine the polydispersity index (PDI) and particle size of Lin-nanocapsules. A solid state He–Ne laser of

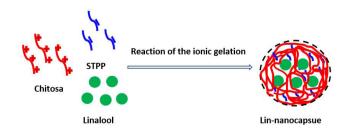


Figure 1. The preparation principle of Lin-nanocapsule.

633.0 nm was used and each sample measurement was carried out with an angle detection of 90° at 25°C.

2.5 FTIR measurement

FTIR was used to prove that linalool was encapsulated. a VERTEX 70 FTIR spectrophotometer (Bruker, Ettlingen, Germany) was adopted to determine the chemical structures of linalool, Lin- nanocapsules and blank nanocapsules. The wavenumber was in the range of 4000 to 500 cm⁻¹. The interaction between sodium tripolyphosphate and chitosan was characterized by the infrared spectra.

2.6 Thermogravimetric analysis

Thermogravimetric analysis was adopted to determine processes such as decomposition and thermal stability, oxidation, and dehydration, and to investigate volatile content (Liu et al., 2013). Thermogravimetry diagram of linalool, Lin-nanocapsules and blank nanocapsules were determined with a TGA-Q5000IR (TA Instruments, USA) thermogravimetric analyzer. The heating rate was 10 °C/min. About 5 mg samples were weighed and were heated from 25 to 600 °C. Nitrogen was used during pyrolysis process at a constant flow of 20ml/min.

2.7 Rheological properties

AR-G2 Rheometer (TA Instrument, US) was used to measure rheological properties of Lin-nanocapsules emulsion. The measuring configuration adopted was a concentric coaxial cylinder. Steady-state shear and oscillatory shear measurements of Lin-nanocapsules emulsion were conducted at 25 °C.

Measurements in steady-state shear condition

For the measurements in the steady-state shear condition, a certain amount of nanocapsules emulsion is loaded in the fixture. In the "steps" options, conditioning step, stepped flow step and post-Experiment step are selected. In conditioning step, initial temperature is set as 25 °C and equilibrium duration is set as 2 min; in stepped flow step, shear rate as variables and the range of Shear rate (S-1) is set as 0.001-1000, data mode is "log", that means data is scanned by logarithmic mode; in post-Experiment step, temperature is set as 25 °C. Changing of apparent viscosity with the shear rate was determined.

Temperature sweep measurement

In the temperature sweep measurement, a certain amount of nanocapsules emulsion was loaded in the fixture. In the "steps" options, conditioning step, temperature sweep step 1, temperature sweep step 2 and post-Experiment step were selected. Conditioning and post-Experiment step was set as same with steady-state shear measurements; in temperature sweep step 1, temperature was set from 20 °C to 40 °C and temperature increment was set as 1 °C/min; in temperature sweep step 2, temperature was set from 40 °C to 20 °C and temperature increment was set as 1 °C/min. Then changing of apparent viscosity with the temperature was determined.

Strain sweep measurement

The strain sweep experiment, in which a gradually increasing strain was applied at one frequency, was carried out for nanocapsules emulsion to ensure operation in the linear viscoelastic region (LVR). In the strain sweep measurement, a certain amount of nanocapsules emulsion was loaded in the fixture. In the "steps" options, conditioning step, strain sweep step and post-Experiment step were selected. Conditioning and post-Experiment step is set as same with steady-state shear measurements; in strain sweep step, strain was set as 0.1%-10% and frequency was set as 1Hz, then a frequency sweep experiment was performed.

Frequency sweep measurement

The frequency sweep measurement was further carried out within LVR at 1% strain to obtain more precise information on emulsion stability at rest. A certain amount of nanocapsules emulsion was loaded in the fixture. In the "steps" options, conditioning step, strain sweep step and post-Experiment step were selected. Conditioning and post-Experiment step was set as same with steady-state shear measurements; in frequency sweep step, the angular frequency was as variables and the range was 1-1000rad/s. Data mode was "log", which means data was scanned by logarithmic mode. From the phase angle, and these amplitudes of stress and strain, changing of the storage (or elastic) modulus G, the loss (or viscous) modulus G, and complex modulus G* with the oscillation frequency were determined (Luckham & Ukeje, 1999).

3 Results and discussions

3.1 SEM micrograph

SEM was adopted to investigate the nanocapsules morphology. Nanocapsule SEM micrograph is shown in Figure 2a.

It shows that the shapes of nanocapsules are nearly spherical and nanocapsules with smooth surfaces are closely packed. Particle size distribution is comparatively uniform.

3.2 DLC results

DLC results of Lin-nanocapsules are shown in Figure 2b. The particle size data showed a trend of normal distribution. Dynamic light scattering result proved that distribution of particle size was comparatively uniform and the average particle size of nanocapsule encapsulated linalool was 352 nm. The polydispersity index (PDI) was 0.214. Dynamic light scattering results were consistent with the results from SEM.

3.3 FTIR results

FTIR spectra of linalool, Lin-nanocapsules and blank nanocapsules are shown in Figure 3a.

The structure characteristics of nanocapsules were determined through infrared spectroscopy. Characteristic absorption frequencies of sample covered the whole of 500-4000 cm⁻¹ area. Due to C-C, C-O, C-H, and O-H stretching vibrations, peaks at 996, 1450, 2972 and 3569 cm⁻¹ appeared in the FTIR spectrum of linalool respectively. After linalool was encapsulated, FTIR spectrum of Lin-nanocapsules showed that the characteristic absorption peaks at 3569 and 1450 cm⁻¹ disappeared, and a peak appeared at 1098 cm⁻¹ due to the phosphoric acid root and the protonation of amino cross linking effect (Papadimitriou et al., 2008). Peak types of Lin-nanocapsules and blank nanocapsules were similar. The shape and position of the peaks proved that linalool was successfully encapsulated in the wall materials.

3.4 Thermogravimetric analysis

Thermogravimetry diagram of linalool, Lin-nanocapsules and blank nanocapsules are shown as Figure 3b.

The linalool weight loss was 96.50% as show in thermo gravimetric analysis diagram of linalool from 30 to 150°C. Linalool evaporated almost completely at 200°C. Three stages can be observed from the curves of the thermal decomposition process of Lin-nanocapsules and blank nanocapsules. The weight losses

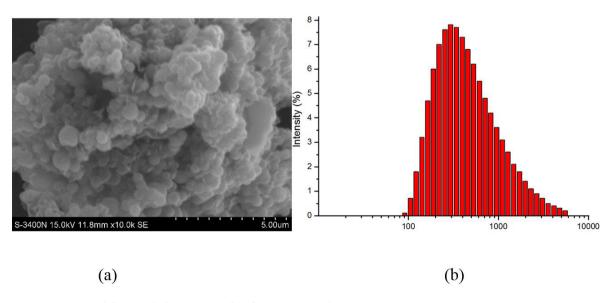


Figure 2. SEM image (a) and dynamic light scattering (b) of Lin-nanocapsules.

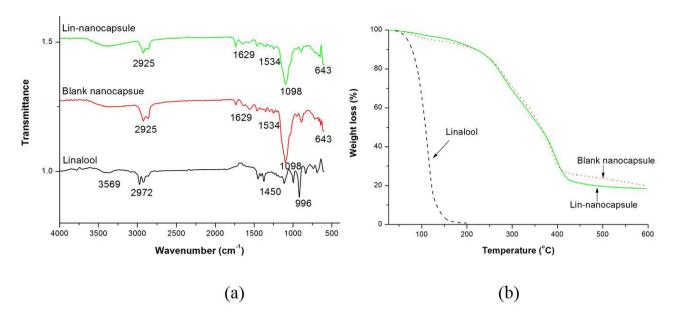


Figure 3. FTIR spectra (a) and Thermogravimetric analysis diagram (b) of linalool, Lin-nanocapsules and blank nanocapsules.

in the first stage of Lin-nanocapsules and blank nanocapsules were 2.08% and 3.7% respectively, mainly due to evaporation of moisture from 30 to 100 °C. For Lin-nanocapsules and blank nanocapsules, the weight loss mainly occurred from 100 to 400 °C. The values of weight loss were about 64.56% and 65.34% respectively, which is because of the loss of hydrogen bonds between free amino and the N-acetyl groups (Grant et al., 1990) and the decomposition and depolymerization of CS glucosamine units. In the third stage, core materials evaporated due to the destruction of wall material structure, which is a reason for the Lin-nanocapsules weight loss. In addition, the depolymerization of hydrogen bonds between the free amino groups and N-acetyl, and CS glucosamine unit decomposition are also reason of Lin-nanocapsules and blank nanocapsules, and the weight loss was about 13.2% and 7.5%, respectively. The thermo gravimetric curve also shows that encapsulation can reduce linalool release and increase linalool retaining time at high temperature. The thermal decomposition weight loss of various samples in different temperature ranges is showed in Table 1.

The total weight loss of Lin-nanocapsules and blank nanocapsules was nearly 77.76% and 72.84%.

Loading capacity (LC) was defined as core material encapsulated in nanocapsules as shown Equation 1.

$$LC(\%) = \frac{\text{Encapsulated Linalool weight}}{\text{Nanocapsules weight}} \times 100$$
 (1)

According to the literature (Xiao et al., 2014), Equation 2 can be obtained.

$$\frac{77.76\% - LC}{1 - 2.08\% - LC} = \frac{72.84\%}{1 - 3.70\%} \tag{2}$$

The LC of linalool can be calculated according to Equation 2 and the value was 15.17%.

3.5 The rheological behavior

Static rheological measurements

As a function of shear rate, the viscosity variation of the nanocapsule emulsion was shown as Figure 4a.

Lin-nanocapsules emulsion was non-Newtonian. However, at high shear rates, it can change to Newtonian as shown in Figure 4a. Because initially nanocapsules were dispersed disorderly in an emulsion, nanoparticles were elongated with the flow direction under the shear field action. Due to space rearrangement of nanocapsules was happened in emulsion system, nanocapsules had a directional arrangement. At the same time, interaction of the nanoparticles gradually reduced, energy consumption and the internal friction decreased accordingly, so apparent viscosity and flow resistance decreased with shear rate increase, until stable (Gao et al., 2004).

Temperature sweep measurement

The temperature-viscosity diagram of Lin-nanocapsules emulsion is showed in Figure 4b.

Figure 4b indicated that the decreasing trend of apparent viscosity with the rise in temperature. Because the arrangement of nanoparticles was affected by temperature and activity ability enhancement of particle was enhanced with rise in temperature, interaction between particles reduced and liquidity increased. Therefore, the apparent viscosity decreased. Because viscous flow activation energy of chitosan was particularly large and viscosity of sample was sensitive to temperature, product stability was affected by these factors (Sakurai et al., 2000). In the process of production, high viscosity can lead to difficult conveying processing of sample, while low viscosity of sample can lead to difficult shaping and overlapping temperature circular sweep curves. The safety temperature range of the sample was 20 \sim 40 °C, so 25 °C was selected as test temperature of the sample.

Strain sweep measurement

A representative rheogram is shown in Figure 5a. LVR was obtained from a strain sweep experiment.

The results of strain sweep measurement manifested that the linear viscoelastic region of the Lin-nanocapsules emulsions was performed at a fixed frequency of 0.1%-10%. Therefore, 1% was selected for the dynamic frequency sweep measurements

Table 1. The thermal decomposition weight loss of linalool, Lin-nanocapsules and blank nanocapsules.

Samples	The first stage		The second stage		The third stage	
	Temperature range (°C)	Weight loss (%)	Temperature range (°C)	Weight loss (%)	Temperature range (°C)	Weight loss (%)
Linalool	30-150	96.50	150-200	3.50	-	-
Blank nanocapsules	30-100	3.70	100-400	65.34	400-600	7.50
Lin- nanocapsules	30-100	2.08	100-400	64.56	400-600	13.20

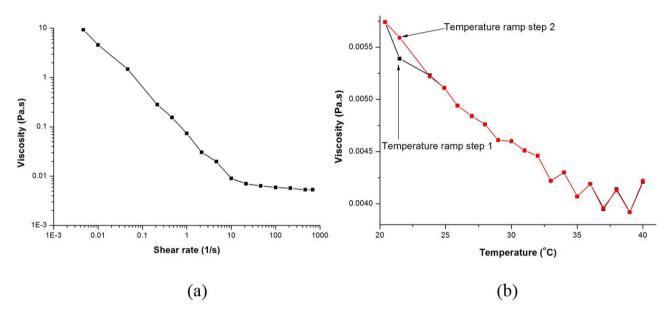


Figure 4. The apparent viscosity plots of Lin-nanocapsule emulsion with shear rate (a), and the temperature-viscosity diagram of Lin-nanocapsules emulsion (b).

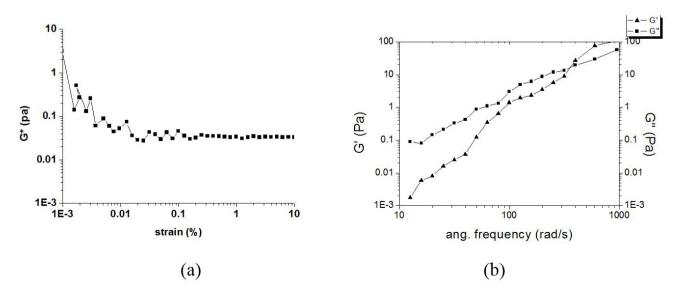


Figure 5. The complex modulus plots (a) with strain, and the storage and loss moduli plots (b) with frequency (10 to 1000 rad/s).

from 10 to 1000 rad/s. The curves of G' and G" with frequency were obtained.

Frequency sweep measurement

The frequency sweep measurements were conducted to evaluate the change of the loss modulus and storage modulus at different oscillation frequency. The log–log plots of storage and loss moduli with frequency for Lin-nanocapsules emulsion are presented as Figure 5b.

G, as elastic modulus, is a number that measures a substance's resistance to being deformed elastically when a force is applied to it. A stiffer material will have a higher elastic modulus. G," as loss modulus representing the viscous portion, measures the energy dissipated as heat. As shown in Figure 5b, Lin-nanocapsules emulsion took on viscous property (G" > G") when oscillation frequency was low. However, when oscillation frequency was high, it took on elastic property (G" > G"). G" and G" were frequency dependent and both of them increased with the rise of frequency.

When G' equals G', the point is 356 rad/s. It means the transition of the viscosity and elasticity. It corroborated that, at high frequencies, the underlying nanocapsule emulsion structure was broken. Because space rearrangement of Lin-nanocapsules happened in emulsion system with the increase of oscillation frequency, the intermolecular distance was reduced and the interaction force between the molecules was enhanced. This led to the increase of the viscosity.

4 Conclusions

In the article, chitosan and STPP was adopted as wall materials. Lin-nanocapsules were produced by ionic gelation method in o/w emulsion, and were characterized with SEM, DLS, FTIR and TGA. The result of SEM demonstrates that the shapes of Lin-nanocapsules, with smooth surfaces, were nearly spherical. The result of DLS proved that PDI was 0.214 and the average Lin-nanocapsule size was 352 nm. FTIR result showed that linalool has been encapsulated in the wall materials. TGA result indicated that the linalool LC was 15.17%. Encapsulation can decrease linalool release and increase the linalool retaining time at high temperature.

Lin-nanocapsule emulsion rheology was studied in detail. Lin-nanocapsule emulsion was investigated systematically using oscillatory shear and steady-state shear measurements. The results of steady-state shear showed that Lin-nanocapsule emulsion, which was Newtonian only for high shear rate, was non-Newtonian. Temperature sweep measurement indicated that temperature circular sweep curves were overlapping basically. The safety temperature range of the sample was 20-40 °C. The results of strain sweep measurement manifested that the linear viscoelastic region of the Lin-nanocapsules emulsions was performed at a fixed frequency of 0.1%-10%. It was proved by oscillatory shear that when oscillation frequency changed from low to high, Lin-nanocapsules emulsion changed from viscous into elastic.

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