Comparison Study Between Encapsulation of *Acalypha indica* Linn Extracts with Chitosan-PCL and Chitosan-OA

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Abstract. Drug delivery is one of the major applications in the biodegradable polymer science. Chitosan is a non-toxic and naturally biodegradable polymer. It is soluble in acidic aqueous media and insoluble in higher pH media. Chitosan has been modified to improve its properties such as stability and the modified derivatives have been widely used in many applications especially for drug delivery. There are several kinds of chitosan modification. This study investigated the modification of chitosan with polyester-types of polymer and the fatty acid-based polymer. The aim of this study is to compare the formation encapsulation of chitosan-oleic acid conjugate (Ch-OA) and chitosan-polycaprolactone (PCL) copolymer for the encapsulation of *Acalypha indica* active compounds for drug delivery by using emulsion-solvent evaporation technique. The crude extracts have been extracted and the phytochemicals inside the crude extracts are less stable in nature. These active compounds need to be encapsulated to stabilize them and delivered well into the body system. As for conclusion, chitosan:PCL is able to form better encapsulation using this method.

1. Introduction

The idea of controlling drug delivery has been proven to improve the delivery of the therapeutic substances by increasing their target to specific cells, tissue or organs [1]. Selecting a good drug carrier is the most promising so that the side effects can be minimized and able to sustain the drug releasing. Drug delivery is one of the major applications of the biodegradable polymers. This is due to the main role of the biodegradable polymers as a controlling and regulating several biochemical and biophysical functions of living cells. The applications of the biodegradable polymers for drug delivery is very promising because of good biocompatibility thus may safe for body system [2]. Chitosan is one of the excellent candidates for polymers drug carrier. It is produced from the natural resources as well as biodegradable and biocompatible plus it comprises the most abundant polymer occurring in nature. Also, chitosan has good mucoadhesion property [3].
However, chitosan has its drawback which is less soluble in neutral or alkaline pH (Bruschi and Luciano, 2015). Thus it needs several modifications of the chitosan backbone (Bhavsar et al., 2017). There are two methods of copolymerization which are blending and grafting method. Blending technique is a quite simple method as this method involves the mechanically mixing of two different polymers whereas grafting involves the chemical bonding of functional groups between the different polymers. The chitosan-modification with another polymer (natural or synthetic polymer) is to boost hydrophilic property, hence increasing its solubility.

*Acalypha indica* is a herbal plant and less get attention and occured as a weed. Nevertheless, there is an interesting benef it to this plant. It was reported that *Acalypha indica* has been used as a traditional medication by native people for hundreds of years ago. It was found that the presence of quebrachitol compound that found inside leaves can act as active inhibition of anti-cancer activity against small cell lung cancer [6]. The encapsulation of the extract by the modification of chitosan copolymer will regulate the secretion of *Acalypha indica* crude extract for the targetted cancer cells. The upcoming outlook on this research can go further to produce a dietary product for controlling the cholesterol level to targetted consumers.

To produce a good and suitable microencapsulated of *Acalypha indica* extracts, freeze drying technique will be selected. This method is one of the quite common methods in the production of microcapsules designed for drug delivery systems and has been applied in pharmaceutical industries [7]. The end product will be in the form of the dry powder thus making the extracts more stable and efficient for transporting to the targetted part in the body system. Less efficient extracts encapsulated may lead to wastage and also the accumulation of phytochemicals in the liver.

## 2. Materials and Methods

### 2.1. Chemical and Materials

Chitosan was obtained from Sigma Aldrich, poly-(ε-caprolactone) (PCL), *Acalypha indica* (AI) extracts were obtained from Universiti Teknologi Malaysia (UTM), ethanol absolute denatured (99. 8% v/v) brand HmbG® Chemicals, polyvinyl alcohol (PVA) (MW 31,000- 50,000 Da), dichloromethane (DCM), and acetic acid glacial were purchased from Sigma Aldrich.

### 2.2. Encapsulation of *Acalypha indica* Linn extracts with chitosan-PCL

PCL was emulsified in an organic phase of DCM (0. 5 % w/v), PVA was dissolved in deionized water (2 % w/v), and chitosan was dissolved in aqueous 0.5 % v/v acetic acid (0.2 % w/v). Stirring were done for 24 hours under vigorous stirring without applying heat except PVA heated under 60°C. The preparation process of polymer solutions was done in a fume hood to ensure the environmental conditions under controlled.

Next, the preparation of microparticles with and without *Acalypha indica* extracts were performed by emulsion-solvent evaporation method according to El. Hadi et al. (2019) with slight modification. 5 mL emulsions of PCL were transferred into an empty 50 mL-sized tube followed by 5 mL of *Acalypha indica* extracts, 5 mL of chitosan and 5 mL of PVA. The solutions were homogenized under ultrasonic homogenizer (Fisher Scientific FB705,700 watts) with amplitude 90 % for 5 minutes to form a water-in-oil (W/O) emulsion. After that, the solutions were stirred moderately on the magnetic stirrer for 24 hours under room temperature in a controlled environment. During stirring, the particles start to ageing and forming microencapsulation. After 24 hours, the solutions was centrifuged under 13,000 rpm for 1 hour using a mini centrifuge (Eppendorf, speed x 1,000). After centrifuge, two layers of supernatant and precipitate were formed. The particles were separated from the solution. The next step was the washing step. The solvents were removed and replaced by deionized water. Then the precipitates and the deionized water were mixed by vortex. Again, the solution was centrifuged under 13,000 rpm for 1 hour. After that, the centrifugation steps were repeated several times until the solutions turn the pH to neutral using a blue litmus paper as an indicator. Then the samples are ready for lyophilization. The blank microparticles encapsulation were prepared using similar procedure without the addition of *Acalypha indica* extracts.
2.3. Encapsulation of chitosan-OA conjugate

2.3.1. Synthesis of N-oleoyl-chitosan (Ch-OA). N-oleoyl chitosan was prepared by a method described by Yan et al. with slight modification [8]. Briefly, 100 mg of low molecular weight chitosan (44869, Sigma-Aldrich, USA) was dissolved in 10 ml of 0.01 M hydrochloric acid solution through magnetically stirring at 28 ± 1°C. 50 mg of oleic acid (0.18 mmol), 69 mg of N-(3-dimethylaminopropyl)-N′-ethylenediamide hydrochloride, EDC (0.36 mmol, 2 equiv, Merck, USA) and 41 mg of N-hydroxysuccinimide, NHS (0.36 mmol, 2 equiv, Merck, USA) were dissolved in 5 ml of dimethyl sulfoxide (Fisher Scientific, UK). Both chitosan and oleic acid solutions were allowed to stir for 2 h at 28°C. Then, these solutions were mixed and stirred for 48 h at 28°C. The formed CS-OA conjugate was dialyzed (Standard regenerated cellulose membrane, Spectra/Por® 1, molecular weight cut off 6-8 kD, Spectrum Lab, USA) with dimethyl sulfoxide for 24 h and subsequently with deionized water for another 48 h at 28°C to remove the excess EDC, NHS, and oleic acid. It was freeze-dried (-40 °C, 0.1 mbar, Alpha 1-2 Ldplus, Martin Christ, Germany) and the chitosan-OA conjugate in dry form was kept in desiccator at 28 ± 1°C until use.

2.3.2. Encapsulation of Acalypha indica Linn extracts with chitosan-OA. The chitosan-OA conjugate was dissolved in the 0.5% acetic acid. The stirring of chitosan-OA took 24 hours to fully dissolve. Then, 0.1 % w/v of Acalypha indica extracts were mixed with 0.4 % w/v chitosan-OA conjugate and vortex. 2 % w/v of PVA was added into the solution in dropwise. Then, the next steps were continued exactly as for chitosan-PCL.

2.4. Surface morphology study
The surface morphology studies was carried out using a scanning electron microscope (SEM) model JEOL JSM-IT 100 InTouchScope™. The samples were deposited on a Nissin EM conductive carbon tape and coated. Then the coated samples were placed on a brass holder under the vacuum holder. The voltage were accelerated under 5 kV.

2.5. Encapsulation efficiency (EE%) identification.

2.5.1. Preparation of standard curve. The standard sample of known concentrations were prepared and inspected in the range 200-1100 nm wavelength to identify the wavelength with a maximum peak of absorbance indicates the most dominant active compounds. Ethanol absolute as the blank solution. After the wavelength of the maximum absorbance obtained, five standard samples of known concentrations (0.02, 0.05, 0.1, 0.15 and 0.2 % w/v) were tested according to the previous wavelength. The linear relationship and the UV absorbance (y) were created according to the standard curve of spectrophotometry and the equation obtained and further used to find the unknown concentration of the extracts being encapsulated in the microcapsules.

2.5.2. Releasing the Acalypha indica extract from the microcapsules using ethanol. The microcapsules with Acalypha indica extracts were dispersed in the 99.98% ethanol and centrifuged using a mini centrifuge (Eppendorf, speed x 1,000) under 13,000 rpm and 1 hour to release the Acalypha indica extracts from the microcapsule suspension into the ethanol. After centrifuge, the supernatant was taken and transfer into a cuvette for testing. The UV absorbance (y) of the free Acalypha indica extracts were tested by UV-vis spectrophotometer. To obtain the concentration (x) of encapsulated extract, the Y value were substituted into the linear equation from the standard curve.

2.5.3. Determination of encapsulation efficiency (EE%). Finally, the value of EE% were calculated using Equation 1 and the data were tabulated.

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EE\% = \frac{\text{Amount of extract entrapped in the microcapsules (g)}}{\text{Total extract added (%w/v)}} \times 100\%
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3. Results and Discussions
Encapsulation is the most desired method to stabilize the active compounds and prolong the shelf life. After undergoes emulsion-solvent evaporation process, the samples were freeze dried to prevent polymer degradation [9]. Ultrasonic homogenization device was selected to mix the solutions forming O/W emulsion as the intermediate form before hardening forming microparticles [10]. Table 1 shows the percentage of encapsulation efficiency of chitosan-PCL higher than chitosan-OA. Morphological studies by scanning electron microscope (SEM) supports the encapsulation efficiency data to show that chitosan-PCL manage to form better encapsulation in spherical shape (Figure 2 and 3).

| Chitosan modification | Encapsulation efficiency (EE%) |
|-----------------------|-------------------------------|
| Chitosan-PCL          | 59.83                         |
| Chitosan-OA           | 20.00                         |

**Table 1.** The encapsulation efficiency (EE%) of chitosan-PCL and chitosan-OA.

**Figure 1.** Chitosan/PCL microcapsules without *Acalypha indica* extract.

**Figure 2.** Chitosan/PCL with *Acalypha indica* extract encapsulations.
Figure 3. SEM image for the chitosan-OA loaded Acalypha indica microcapsules.

Figures 1 and 2 show that the encapsulations occurred in the chitosan-PCL microparticles without and with Acalypha indica extracts by 5 minutes ultrasonic homogenization. The particles formed in smooth and spherical shape and uniformly size. This is due to the vibration of ultrasonic probe mixed the PCL, chitosan, and PVA reduce the sizes, homogenize and easy to self-assemble forming tiny droplets of microparticulates [11]. When Acalypha indica extracts were added, the microcapsules formation slightly changed and increased in size as shown in Figure 2. This morphological surface characteristics supported by Lu et.al [12]. Conversely, the shape of chitosan-OA as shown in Figure 3 does not form particle shape and due to that, the performance in encapsulations less efficient than chitosan-PCL encapsulations.

4. Conclusions
In conclusion, chitosan-PCL managed to form microencapsulations in spherical shape by solvent-evaporation method and the chitosan-PCL encapsulation gave higher encapsulation than chitosan-OA. SEM image showed the spherical shape of encapsulation which is one of the important characteristics of encapsulations.

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