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Indo-Malayan Stingless Bees' Propolis Extract-Loaded Chitosomes: Characterization and Storage Stability Evaluation

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Abstract: ***Therapeutic effects of stingless bees' propolis are continually been explored in nutraceutical studies until recent years. Despite owing a wide range of health-promoting effects, the bioactive compounds composed in the propolis face risk of degradation. This research was intended on synthesizing chitosan-coated liposomes (chitosomes) as a nanocarrier to encapsulate Indo-Malayan stingless bees' propolis extract to protect the bioactive compounds against degradation and enhance its bioavailability upon oral administration. The propolis extract-loaded chitosomes (PEC) were prepared by using the film hydration method and followed by probe sonication for downsizing. The chitosomes were characterized by zetasizer in terms of average size, polydispersity index and zeta potential. The morphology of chitosomes was examined using SEM and determination of functional groups had been performed using FTIR. The performance of chitosomes was evaluated based on encapsulation efficiency, loading capacity and storage stability within one month. Results indicated an increment of encapsulation efficiency of the propolis extract in PEC up to 90.1% and loading capacity of 21.7% compared to uncoated liposomes. The vesicle change rate for PEC at 4^{o}C after one month was 8.4% while its leakage ratio was 9.5%. These rates presented by PEC were significantly lower than uncoated liposomes and made the suspension more stable after one month of storage. Chitosan-coated liposomes have shown promising performance in the protection of encapsulated propolis extract and perhaps will be useful for a wider spectrum of bioactive compounds which have diverse therapeutic effects for human health.***

Index Terms**: *Chitosan, encapsulation efficiency, liposome, propolis, stability***

I. INTRODUCTION

Natural products have historically and persistently been explored for promising new leads in functional food products. In recent years, nutritionists have shown an increased interest in natural antioxidants, which could be used in an unmodified form to replace synthetic substances. In Malaysia, research related to Indo-Malayan stingless bees or locally known as 'lebah kelulut' have been carried out sparsely [1]. Propolis is the natural product from bees' hives that always typically considered as a waste. The potential of the propolis as a functional food product is subtly investigated. Various kinds of beneficial bioactive compounds are composed in the propolis which owns a wide range of health-promoting effects. Flavonoid is one of the most widely occurring polyphenol compounds that can be extracted from the propolis. Flavonoid which possesses potent antioxidant activity has gained significant attention in recent years for their promising health benefits. It is also stimulated by the diverse therapeutic properties as a dietary component which includes anti-proliferative, anti-inflammatory, anticancer, antibacterial, antiviral and antifungal effects [2], [3]. The antioxidant effect is exerted by the functional hydroxyl group composed in flavonoid. It can scavenge free radicals, eliminate highly reactive oxygen species [4] and chelate metal ions [5]. Despite various beneficial health effects comprised of this bioactive compound, the therapeutic use of flavonoids has been remarkably limited due to its low bioavailability [6]. Flavonoid is susceptible to extreme environmental conditions such as pH, oxygen and enzymes existed in the gastrointestinal tract. It faces a risk of degradation because of harsh enzymes in the gastrointestinal tract and consequently, the available circulation time of the active molecule and its bioavailability is limited [7]. To overcome the restrictions offered by the flavonoid itself, efforts at increasing its functionality when administered into the body will be investigated especially on improving its stability. Therefore, the development of modified nanodelivery carriers to improve the bioactivity properties of flavonoid with enhanced bioavailability, long-term stability, and reduced toxic effects is advocated. The recent technology developed to prevent the deterioration of the bioactive compounds when delivered into the body is liposome encapsulation. Liposome is a colloidal, vesicular structure based on phospholipid bilayer. In this structure, an aqueous core is surrounded by lipids arranged in the bilayer configuration. The lipid bilayer protects the drug degradation on the way to the targeted sites during circulation [8]. In spite of that, liposomes have a highly flexible and fragile bilayer membrane. As liposomes tend to attach to each other, aggregation can result in unstable particle size distribution. Another limitation of liposomes is the tendency to leak of entrapped materials over time [9].

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To curb these limitations, chitosan, a cationic biopolymer is utilized to modify the surface characteristics of liposomes. It has been used to provide a protective coating layer for liposome encapsulation. Repulsion between differently charged of the bilayer and chitosan coating will arise by electrostatic interaction, which can improve the stability of the liposomes. Chitosan is a typical biological macromolecule synthesized from crustacean shells. It forms a gel-like barrier in the harsh gastric environment leading to a controlled release of bioactive compounds encapsulated in liposomes [10]. Application of chitosan has been extensively utilized in medicine, pharmaceuticals, biotechnology and food industry owing to its properties of biocompatible, biodegradable and low toxicity. In this context, fundamental aspects of liposome preparation were analyzed to obtain the best liposome formulation for a high propolis extract capture. This study reports the characterization of the uncoated propolis extract-loaded liposomes (PEL) and propolis extract-loaded chitosomes (PEC) as well as the evaluation of their performance where special attention has been given to the loading and encapsulation efficiency of the liposomes. Studies related to the storage stability of chitosomes are yet limited specifically when correlated to the propolis extract encapsulation. Hence, an accelerated stability study has been done within one month to evaluate the stability of the chitosomes under different storage time and temperature. Accordingly, this study can impart a better understanding on storage stability of PEC compared to PEL through particle size change rate and leakage ratio. By combining the characteristics of chitosan and liposomes, a specific, prolonged and controlled release drug delivery system may be achieved. A carefully designed nanocarrier for bioactive compound delivery could significantly facilitate the bioactive compound delivery and broaden the range of its possible therapeutic and pharmaceutical applications.

II. MATERIALS AND METHODS

A. Materials

Soy lecithin (L-α-phosphatidylcholine from soybean with ≥ 99% (TLC) in lyophilized powder was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan (with an average molecular weight of 150kDa, degree of deacetylation >80%), cholesterol (from sheep's wool with ≥ 99% purity) and phosphate buffer saline (in tablet form, pH 7.4) were also purchased from Sigma-Aldrich (St. Louis, MO, USA). Aluminium chloride, acetone, chloroform and methanol were obtained from Merck (Darmstadt, Germany). All solvents used were of analytical grade.

B. Extraction of Stingless Bees' Propolis

The sample of propolis was collected from stingless bees' colonies in a rubber small holding plantation in Kuala Nerus, Terengganu. The dried sample was ground and soaked in acetone overnight at room temperature. Then, the sample was percolated gradually for its filtrate and the remaining sample was soaked in 70% methanol. This step was repeated until the filtrate became colorless. The filtrates were pooled altogether, and the methanol was then removed by a rotary evaporator at 45°C to obtain the dry methanolic extract of propolis. The extract was preserved at 4°C prior to usage.

C. Preparation of Propolis Extract-loaded Liposomes (PEL)

Liposomes loaded with propolis extract were prepared by the thin-film hydration method [11] with slight modifications. In brief, the corresponding amount of phosphatidylcholine, cholesterol and propolis extract were dissolved in 10 ml of chloroform: methanol (1:1 v/v). The propolis extract dissolved in methanol (3.0 mg/ml) was mixed with the chloroform solution containing phosphatidylcholine and cholesterol (8:1 w/w). The mixture was then placed on a rotary evaporator to remove the organic solvent by under reduced pressure at 45°C, rotating at 100 rpm. Finally, a dry film was formed in the sidewall of the flask. Afterward, 10 ml of phosphate buffer saline was added to hydrate the film and the mixture was stirred continuously at 40°C for 30 minutes. Subsequently, the liposome suspension was sonicated by a probe sonicator for downsizing [12]. It was carried out with an amplitude of 40% and total treatment of 25 minutes. The flask was plunged into an ice water bath to prevent heating of samples. A pulsed duty cycle was fixed for all treatments with 8s on and 2s off. The liposome suspension was then centrifuged immediately after preparation. The supernatant contained the free unentrapped extract in the suspended stage was separated from the pellet of propolis extract-loaded liposomes by centrifugation for 30 minutes at a speed of 15000 rpm and at 4°C. The collected liposomes were washed with 2 ml of phosphate buffer saline, recentrifuged and resuspended to obtain 2 ml of liposome suspension. The suspension was then vortexed until homogenization [13].

D. Preparation of Propolis Extract-loaded Chitosomes (PEC)

Fresh solutions of chitosan in acetic acid (0.5% v/v) were prepared in different concentrations ranging from 1.0 to 5.0 mg/ml. To ensure complete dissolution, the chitosan solution was sonicated and stirred overnight with mild speed. Afterward, the uncoated liposome suspension was added dropwise into chitosan solution with a ratio of 1:2 (v/v) under softly magnetic stirring overnight at room temperature to produce chitosan-coated liposome suspension [14],[15]. The chitosan-coated liposomes were stored in the refrigerator (4°C) overnight for further usage, in order to allow time for the samples to stabilize at a steady-state.

E. Physicochemical Characterization

Nanoparticle Morphology

The morphology of uncoated and chitosan-coated liposomes loaded with propolis extract was observed by scanning electron microscopy (S26000-N, Hitachi, Tokyo, Japan) in the solid state. The freeze-dried samples were mounted onto adhesive taped stubs and then coated by a gold film during the metallization process. These samples were sputtered with gold by an automatic auto-coater (JFC 11600) to prevent any charging up of the surface by the electron beam.

Ultraviolet/Visible Spectroscopy

The UV-Vis spectra of propolis extract-loaded chitosomes were obtained by a spectrophotometer (UV-18000 Shimadzu, Kyoto, Japan) in the range of 200-800 nm at 1.0 nm intervals. Samples were measured in a rectangular quartz cuvette with 1 cm path length at 25°C.

Fourier Transform Infrared Spectroscopy

The FT-IR spectrum of lyophilized propolis extract-loaded chitosomes was recorded in the range of 4000-400 cm^{-1} on a FT-IR spectrometer (Spectrum 100 Perkin Elmer, Boston, USA). The sample was deposited in spectroscopic grade potassium bromide (KBr) disks. The spectrum was smoothed, and the baseline was corrected using the spectrometer's built-in software.

Particle Size, Polydispersity Index and Zeta Potential Measurements

Measurements of mean particle size and polydispersity index (PDI) were performed using a dynamic light-scattering instrument (Zetasizer ZS, Malvern Instruments, Worcestershire, UK). The hydrodynamic diameter (z-average) was determined by the intensity distribution of particles while the polydispersity index was measured by a cumulant analysis performed by the same instrument. Multiple scattering effects was prevented by diluting the liposome suspensions with distilled water to a concentration of 3.0% v/v. The samples were then transferred into an electrophoresis cuvette of a laser Doppler microelectrophoresis instrument (Zetasizer ZS, Malvern Instruments, Worcestershire, UK) to measure the zeta potential of the samples. All the measurements were measured in triplicate and results were given as the mean ± standard deviation.

F. Performance Evaluation of Quercetin-loaded Chitosomes

Encapsulation Efficiency and Loading

Encapsulation efficiency is the percentage of bioactive compound that is successfully encapsulated in the liposome. Meanwhile, loading is the amount of bioactive compound loaded per weight of the liposome. The encapsulation efficiency of the propolis extract-loaded chitosomes was determined according to the ultracentrifugation method [13], [16] with slight modifications. The liposome suspension was centrifuged at 15000 rpm at 4°C for 30 minutes to separate the free propolis extract [17]. The encapsulation efficiency was evaluated by taking the supernatant of the centrifuged liposome. The total flavonoids content of the un-encapsulated propolis extract was determined by using the aluminium chloride colorimetric method. 0.5 ml of 2 g/L $AlCl_3$ solution was added to 0.5 ml of the sample followed with one-hour incubation in the dark. The concentration of free un-encapsulated propolis extract was measured through a UV-visible spectrophotometric analysis (UV-18000 Shimadzu, Kyoto, Japan) at 420 nm. Results were expressed as mg of quercetin equivalents (QE) per liter of sample and quantified by using a calibration curve of quercetin, R^2 = 0.9995. The encapsulation efficiency (EE%) and loading (L%) were measured as a percentage of flavonoids entrapped in the liposomes by using the following equations:

$$EE\ (\%) = (W_{total} - W_{free}) \times 100/W_{total} \quad (1)$$

$$L\ (\%) = (W_{total} - W_{free}) \times 100/W_{np} \quad (2)$$

where W_{total} was the total weight of flavonoids in liposome suspension, W_{free} was the weight of free unencapsulated flavonoids in the liposome suspension and W_{np} was the liposome weight. The results presented the mean ± standard deviation of three independent experiment.

Accelerated Stability Study

Physical stability study was conducted to evaluate the ability of the liposomes to retain the encapsulated compound during storage. PEL and PEC suspensions of propolis extract were stored at refrigeration temperature (4±2°C) and room temperature (25±2°C) for a period of one month. Sample from each liposome suspension was withdrawn at definite time intervals. The stability of the formulation was evaluated based on the particle size change rate and leakage ratio.

Particle Size Change Rate

The change rate of nanoparticle size was determined by measuring the mean particle size of each sample as mentioned above [18]. The change rate was calculated by the following equation:

$$\text{Particle size change rate}\ (\%) = (ZA_f - ZA_i)/ZA_i \times 100 \quad (3)$$

where ZA_f was the final mean size of the particle after one month and ZA_i was the initial mean size of the particle.

Leakage Ratio

The leakage ratio of propolis extract was determined by measuring the encapsulation efficiency after the storage time compared to the efficiency before storage [19]. The following equation was used to calculate the leakage ratio:

$$\text{Leakage ratio}\ (\%) = (EE_i - EE_f/\ EE_i) \times 100\% \quad (4)$$

where EE_f was the encapsulation efficiency after one month and EE_i was the initial encapsulation efficiency measured before storage.

III. RESULTS AND DISCUSSION

A. Morphology

The morphology of the uncoated liposomes (PEL) and chitosan-coated liposome (PEC) in the presence of encapsulated propolis extract was shown in Fig. 1.

It was found that chitosomes had flat sides with sharp ends. It was different with the morphology observed on the uncoated liposomes which were in spherical shape. This might be attributed to a strong ionic interaction between the positively charged moieties of chitosan and the oppositely charged surface group of phospholipids which resulting in a core compaction. The structural changes of the chitosomes after drying process were also reported before as in [15]. Liposomes were highly flexible, and they had fragile bilayer membranes. This adsorption forced them to come into close proximity and forming a coating layer around the surface [20]. Besides, the vacuum condition applied during the freeze-drying process had overcome the viscoelastic properties of the wall matrix of chitosomes and probably led them to dents and wrinkles.

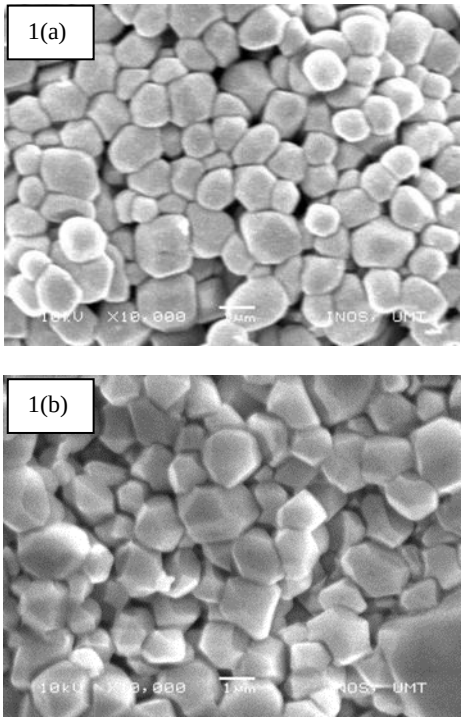

Fig. 1 SEM images with magnification of x10 000 (a) PEL (b) PEC

B. Ultraviolet/Visible Spectroscopy

Fig. 2 presented UV-Vis spectra of free propolis extract, free chitosomes and PEC. The resemblance of spectra between free chitosomes and PEC attributed to the presence of chitosan as the surface coating of liposomes. The characteristic bands of chitosan were absorbed at 224 nm and 275 nm. While the spectrum of free propolis extract showed a significant absorption band at 280 nm. This band was associated with the conjugation in the A-ring of the bioactive compound composed in the extract which was flavonoid [5]. The softly Einstein shifted peak around the same wavelength was also visible in the PEC confirming the formation of nanoparticles and the encapsulation of PE into PEC.

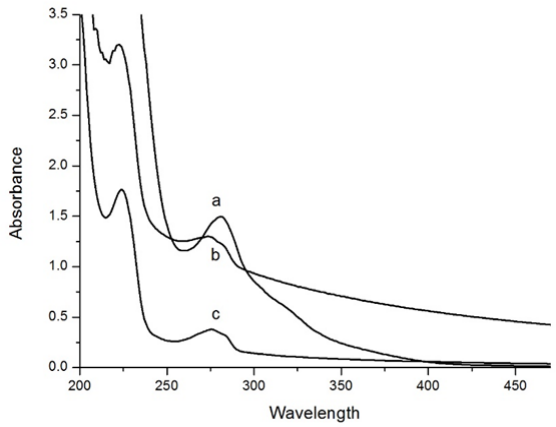

Fig. 2 UV-vis spectra (a) free propolis extract (b) PEC (c) free chitosomes

C. Fourier Transform Infrared Spectroscopy

The FTIR analysis of free chitosomes, PEL and PEC were presented in **Fig. 3**. These FTIR spectra can be used to explain the possible coating mechanism by exploring the interactions between the chitosan and the phospholipid bilayer of the liposomes. The vibrational frequency of the symmetric CH_2 stretching can determine the structural information about the interior region of the phospholipid bilayer while at the interfacial region, the possible vibrational modes are the symmetric stretching of P=O and C=O as well as the asymmetrical N-$(CH_3)_3$ stretching [21].

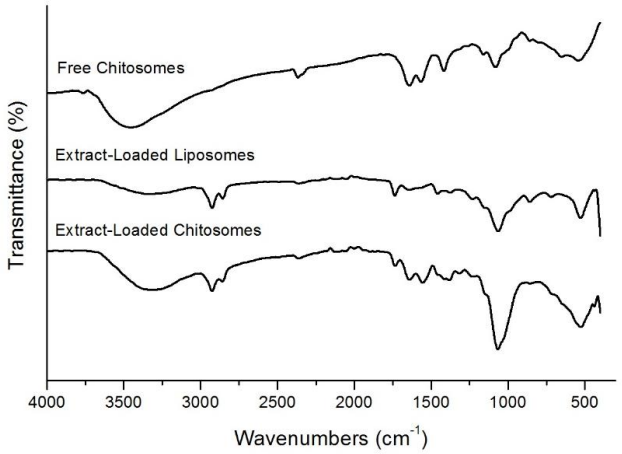

Fig. 3 FT-IR spectra of free chitosomes, PEL and PEC

In view of the spectrum of free chitosomes, it depicted characteristic peaks of chitosan as the liposomal coating. The main absorption bands associated with chitosan were visible at 1564 cm^{-1} showing -NH bending vibration in amide group of the glucosamine. The skeletal vibration at 1080 cm^{-1} involved the C-O stretching was associated with the saccharide structure of the chitosan. The presence of a band at 1639 cm^{-1} indicated C=O while the band at 1417 cm^{-1} owing to CH_2. An overlapped wide band was absorbed at 3452 cm^{-1} due to the stretching vibration of N-H and O-H bonds These characteristic bands of chitosan were also visible in the PEC spectra and suggested that chitosan covered the surface of the propolis extract-loaded liposomes. Based on the spectrum of propolis extract measured in the previous study, the C=O stretching vibration of flavonoids appeared at 1734 cm^{-1} while the C-H bending of aromatic vibrations and the C-O stretching of aromatic ester was visible at 1454 cm^{-1} and1377 cm^{-1}, respectively. These characteristic bands of propolis extract were also existed yet broaden in the spectra of PEL and PEC which illustrated that the extract was entrapped within the liposome bilayer, apparently by hydrogen bonding or hydrophobic interactions between the carbonyl group of propolis extract and phospholipid bilayer [14], [22]. In a comparison of PEL and PEC, the stretching vibration of CH_2 absorbed by both samples at absorption peaks of 2926 cm^{-1} and 2856 cm^{-1} had no significant shift on PEC spectrum and it explained that there was no influence of chitosan coating on the interior of the phospholipid bilayer. On the other hand, the characteristic band of C=O which absorbed at 1735 cm^{-1} was shifted after chitosan coating with increasing intensity.

This band indicated the hydrogen bonding at the interfacial region of PEC between the chitosan and the carbonyl region of the phospholipid bilayer. The increasing hydrogen bonding also led to broadening of the absorption band at 3296 cm^{-1} due to the stretching vibration of N-H and O-H bonds. The mechanism of chitosan onto the liposomal surface was suggested via ionic interactions between the positively charged chitosan (NH_3^+) and negatively charged phosphate of the phospholipid bilayer [23], forming a thin coating layer around the liposomal surface. The band absorbed at 1226 cm^{-1} was attributed to P=O stretching and indicated to the ionic interaction between the different charged groups. This characteristic band was sensitive to the formation of hydrogen bonding where it would be shifted to lower frequencies with increasing hydrogen bonding. Moreover, the chitosan is a hydrophilic polymer, which is compatible with the hydrophilic head of the phospholipid bilayer. A hydrophobic interaction may also possible to occur between the lipid tails and chitosan which allowed the chitosan to come up to the bilayer and fill the empty volume [20].

D. Effect of Concentration of Chitosan on Characteristics of Chitosomes

The maintenance of particle size following oral route is a critical factor to the fate of the nanoparticle after administration in preventing mononuclear phagocyte recognition and clearance by the reticuloendothelial system [24]. Particles with small size range have relatively high cell uptake with faster drug release and longer half-life in the blood when compared to larger particles [25]. Incorporation of chitosan as a coating on the liposomal surface yielded to an increment of particle size as shown in Fig. 4. The average size of uncoated liposomes (203 nm) was gradually increased up to 346 nm at chitosan concentration of 5.0 mg/ml. The increment of particle size portrayed the formation of the coating layer on the surface of liposomes. As the concentration of chitosan increased, the thickness of the coating layer was also increased. This observation was also recorded as in [26], [27].

Apart from the size, polydispersity index (PDI) is also a key measure in synthesizing liposomes. The index represents the size distribution of the particles where values of <0.25 indicate monodisperse particles are formed and values of >0.5 showing polydisperse particles. The ability of the chitosan coating to hinder aggregation could also be seen in terms of the polydispersity index. According to **Fig. 4**, the relatively high PDI index of uncoated liposomes (0.37) was significantly reduced to 0.18 after coating with chitosan which suggested a uniform distribution of particle size was prepared. This result was supported by the chitosan coating which formed a cage like-barrier that can protect the liposomes from aggregation [27]. Moreover, continuous mechanical force in the preparation of chitosomes by stirring overnight led to a better dispersion of liposomal size. The PDI index, however, was increasing when an excess amount of chitosan added (>4.0mg/ml) owing to the uneven accumulated chitosan deposited on the liposomal surface.

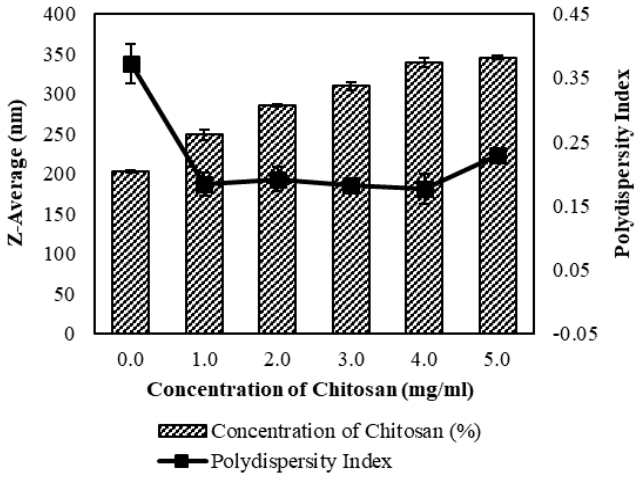

Fig. 4 Z-average and polydispersity index of PEC at different concentration of chitosan

A deeper understanding of the nanoparticle stability requires the analysis of zeta potential values. These values provide predictions about the storage stability of a colloid. The magnitude of zeta potential higher than 30 mV resulted to a better physical stability as the accumulated charge on the surface of liposomes will increase the repulsive force between the particles hence prevent the particles from agglomeration [28]. The prepared samples of PEL and PEC which were different in their composition could affect physicochemical properties such as surface charge and the potential interaction with cells. The zeta potential of PEL (-62 mV) was shifted to the positive charge value (39 mV) due to the shielded surface of the cationic polymer group at the outer surface of the liposomes (results were not shown). These findings were in agreement with results reported previously as in [20], [29]. The cationic properties of sulphate altered the surface charge of the liposomes. The increment shown by zeta potential of PEC was associated with the interaction of the chitosan and the SPC. The cationic properties of sulphate could interact with choline in the polar head group of SPC thereby increased the surface charge of the vesicles. The cationic charge of chitosomes has been shown to assists in the nanoparticle absorption or binding to cell membranes which leads to energy independent translocation [30]. This positive charge PEC would interact with the negatively charged sialic acid group of mucin in the intestine. This process of mucoadhesion could allow a higher chance of particle uptake or absorption since it is detrimental to bioactive compounds delivery. Employment of chitosan as a surface coating for liposomes not only increased its particle size and zeta potential but also increased its loading and encapsulation efficiency as shown in **Fig. 5**. Chitosomes showed the better capability of entrapping the propolis extract. Great differences in encapsulation efficiency related to the composition of vesicle formulation were observed when the efficiency was increased up to 22%.

This was possible because the extract was not only incorporated into the phospholipid bilayer but also in between of the liposomal surface and the chitosan coating. In fact, PEC (2.0 mg/ml) showed the highest incorporation capability with a loading of 21.7% and encapsulation efficiency of 90.1% than PEC (1.0 mg/ml) as a consequence of the higher chitosan concentration. Chitosan coating at this concentration also yielded non-flocculating chitosomes. However, the loading and encapsulation efficiency were gradually reduced with the increasing concentration of chitosan higher than 2.0 mg/ml. The reducing efficiency of chitosomes with the increasing chitosan concentration was also remarked in [15]. There was a white precipitate observed in the suspension with the higher concentration of chitosan. Probably, a high amount of chitosan perturbed the lipid bilayer, leading to bilayer destabilization and reduce the capacity of the chitosomes to encapsulate the propolis extract. Since the optimum encapsulation efficiency was yielded at 2.0 mg/ml, this formulation of chitosomes was chosen and to be further evaluated in storage stability study.

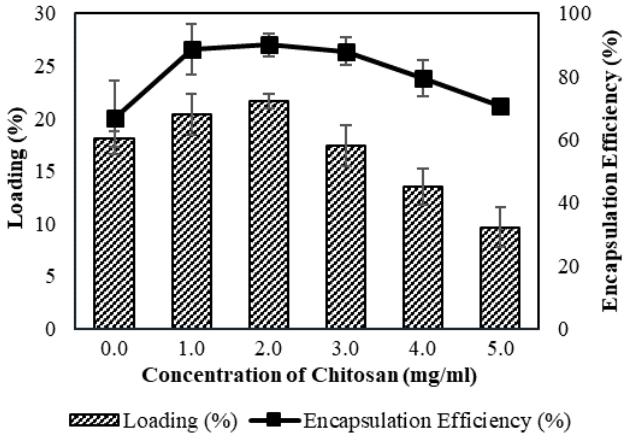

Fig. 5 Loading and encapsulation efficiency of PEC at different concentration of chitosan

E. Effect of Storage Time and Temperature on Stability of Chitosomes

Small sized of nanoparticles are mostly metastable which can result in a breakdown of the colloidal dispersion. It means that the size of particles can become larger over time because small liposomes tend to merge to reduce the high curvature energy attributed to the large bending of the lipid bilayer [31]. Effective liposomes must be stable during the storage period and remain at the appropriate size range before reaching the site of action. In order to compare the physical stability of the coated and uncoated liposomes, PEL and PEC with the same PE loading were stored at different temperature (4°C and 25°C) in one-month duration. According to the graph constructed in **Fig. 6**, the overall mean particle size was increasing within the storage period. However, the increment of particle size could be controlled by storing the liposome suspension in a cooler surrounding. The particle size of PEL and PEC stored at 25°C increased abruptly after the third week. On the contrary, the liposomes stored at 4°C had a gradual increment within the storage period. This result demonstrated that the liposomes stored at lower temperature were more stable than liposomes stored at a higher temperature. This was because the hydrolysis rate of phospholipid increased when the storage temperature increased. Liposomes stored at higher temperature tend to increase its hydrodynamic size and become unstable. Based on **Fig. 7**, the lowest particle size change rate was depicted by PEC with 8.4% at 4°C which ascribed the highest storage stability was achieved. Moreover, at both temperatures, the particle size change rate of PEC was lower than PEL and it suggested that the chitosan coating improved the stability of the liposomes even in high storage temperature. To further examine the stability of PEL and PEC at 4°C, the leakage ratio based on encapsulation efficiency was measured and analysed.

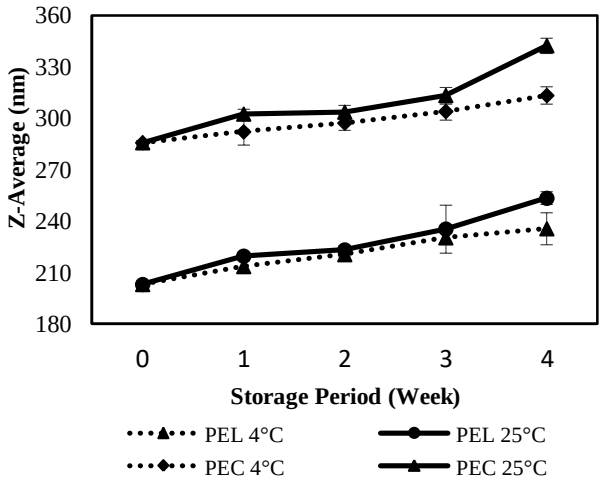

Fig. 6 Z-average of PEC within one month of storage at the different temperature

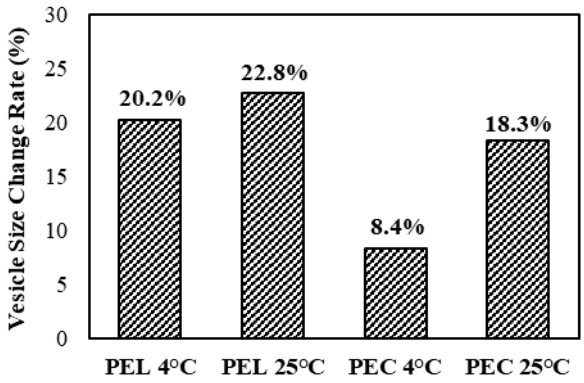

Fig. 7 Vesicle size change rate of PEC at different temperature

The effect of chitosan coating on the change of the encapsulation efficiency and the leakage ratio of PEC in comparison with PEL were presented in **Fig. 8** and **Fig. 9** respectively. In general, both samples showed a gradual reduction in the encapsulation efficiency within the storage period. However, PEC after one-month storage yet showing a high encapsulation efficiency with 81.6% and was significantly higher than PEL (53.0%).

On top of that, the leakage ratio of PEC was at a very low percentage (9.5%) compared to PEL with 20.7%. This notable difference in ratio implied the applicability of the chitosan coating in retaining the encapsulated material which was higher compared to the uncoated liposomes. The incorporation of chitosan coating showed a minimal effect on storage stability over the investigated incubation time and more than 90% of originally PE was still intact in the PEC fraction. In comparison to PEL, uncoated liposomes were found to be less capable to hold the propolis extract. These results highlighted the efficacy of chitosan coating on keeping the liposomes stable during storage so that the maximum amount of encapsulated bioactive compounds could be administered to the site of action.

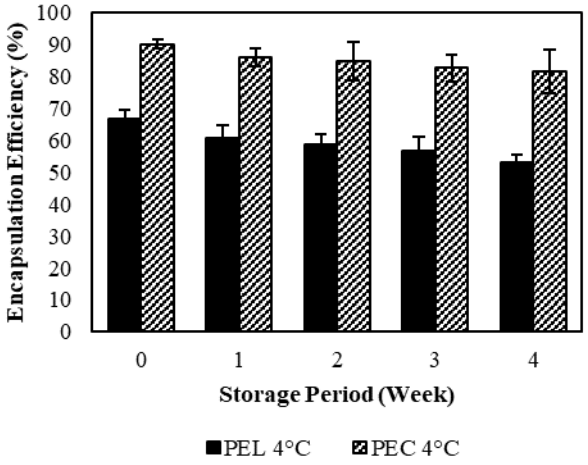

Fig. 8 Encapsulation efficiency of PEC within one month of storage

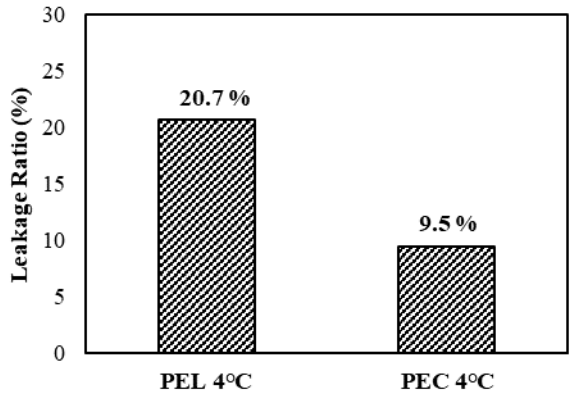

Fig. 9 Leakage ratio of PEC within one month of storage

IV. CONCLUSION

This study demonstrated an approach to prepare homogenous propolis extract-loaded liposomes with chitosan coating in a colloidal system and to evaluate the liposome performance as a function of encapsulation efficiency, loading and storage stability. Propolis-loaded chitosomes (PEC) have been successfully prepared with high encapsulation efficiency which was 90.1% and loading capacity of 21.7%. These percentages were enhanced after being coated with chitosan. The particle size change rate and leakage ratio of PEC within one month showed significantly lower percentages compared to PEL when stored in 4°C with 8.4% and 9.5% respectively. These results proved that good physical stability of liposome suspension was achieved and particle aggregation during storage was not likely to occur, due to the electrostatic repulsion among the particles. The performance presented by the chitosomes synthesized in this study make them appeal to be a promising nanocarrier system to increase the bioavailability and stability of the encapsulated bioactive compound. This technology of liposome encapsulation and chitosan coating studies provide useful knowledge and will be highly attractive in biotechnology and biopharmaceutical industries especially in dealing with nutraceuticals.

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