Indonesian Journal of Pharmaceutics
IdJP
ISSN : 2597-8748

Vol 3, Issue 3, 2021 (149-158)
http://journal.unpad.ac.id/idjp

Preparation of organic-solvent free liposome of *Piper albi* Linn extract in solution and powder form

Eri Amalia[1], Iyan Sopyan[1], Norisca Aliza Putriana [1], Sriwidodo[1], Anas Subarnas[2]

[1]Department of *Pharmaceutics* and Pharmaceutical *Technology*, Faculty of Pharmacy, Padjadjaran University, 45363, Indonesia.

[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, 45363, Indonesia.

e-mail : amalia@unpad.ac.id

Submitted :1/02/2022, Revised :3/02/2022, Accepted 10/02/2022, Published : 11/02/2022

Abstract

White pepper (*Piper albi* Linn) is widely used as a spicy enhancer commodity in many countries. Moreover, it is also known to contain piperine as a potential bioactive substance responsible for numerous beneficial pharmacological activities. Nevertheless, utilization of these bioresources as a product remains less, due to its poorly water-soluble characteristic in nature which affects its low bioavailability. Therefore, our research focused on the preparation of piperine standardized white pepper extract and its modification in the liposome delivery system in both solution and liposome powder form to improve its bioavailability.

The liposome solution was prepared without organic solvent to dissolve the mixture of lipid phase of soy lecithin and cholesterol, thus requiring suitable wet-hydrating the lipid film and overnight self-hydration method for liposome formation. Results showed that the liposome is successfully formed with an average size of 398.7 nm observed by microscopic and particle size analyzer evaluation, and giving encapsulation efficiency of 98.92%±1.17%. Furthermore, the liposome solution was dried by using the spray dry method by employing sucrose as the carrier. This method successfully reveals that sucrose gives maximum protection to the stability of liposome structure, where the rehydration of powder showed a similar spheric shape to the initial liposome solution.

This research gives interesting finding that white pepper is feasible to be processed as a beneficial standardized raw material in liposome delivery system in both solution and powder form, without of employment of toxic organic solvent. Therefore, can be further processed as a commercial solvent-free product in the future.

*Corresponding author,
e-mail : amalia@unpad.ac.id (Eri Amalia)

https://doi.org/10.24198/idjp.v3i3.36140

Keywords: Piperine, *Piper albi* Linn, liposome, extract, organic solvent-free, drug delivery system, spray dry

1. Introduction

White pepper (*Piper albi* Linn) is one of the national commodities mostly as a potential spicy taste enhancer. White pepper contains many substituents such as alkaloids, flavonoids, lignan, aromatic compounds, amides, and essential oil.[1]–[3]. Among them, alkaloid piperine is believed as the most potential bioactive compound with several important pharmacological activities such as antimicrobial, antioxidant, immunomodulatory anti-inflammatory, and anticancer activity [3]. White pepper from kepulauan Bangka Belitung Province- Indonesia is known to have good quality with a higher piperine substituent. The application of white pepper extract and piperine as a product is still limited due to its poor solubility in water (0.004 mg/ml at 18^{o}C) character, thus affect to its low bioavailability in the body and limiting its biological activity [4]. Besides its solubility matter, it is also less in the peppercorn as 3.84–5.35 % [1]. Therefore, modification of piperine is required to improve its bioavailability.

One of the most promising techniques applied is by preparation of a liposome delivery system. A liposome is a unique artificial vesicle composed of a combination of phospholipid which has hydrophilic and lipophilic regions to form a bilayer membrane similar to a natural cell membrane. The liposome structure is special which can be engineered to entrapped lipophilic or hydrophilic active substance on its suitable region of the liposome. [5][6]. Piperin known as a lipophilic substance with a log P value of 2.25 expected to be entrapped in the inner bilayer membrane, and with the hydrophilic head of the structure may improve its release profile when applied as several pharmaceutical dosage forms [4].

Nevertheless, most of the current liposome preparation is employ organic solvents such as chloroform, methanol, or ethanol during the film hydration process, glass rotary evaporation vessel, and sonication or extruder to reduce the size of liposome, thus not applicable in most industrial facilities. Therefore, this research tries to prepare liposomes of extracted white pepper, in solution and powder form, which is organic solvent-free and in simple technique. This method is promising to be upscaled on a larger industrial scale thus possible to be manufactured as a product that can give benefit for human health.

2. MATERIALS AND METHODS

2.1 Materials

White pepper (*Piper albi* Linn) was obtained from kepulauan Bangka Belitung Province, Indonesia. Piperine with ≥97% purity was purchased from Sigma-Aldrich (Saint Louis, USA), Cholesterol Food grade was purchased from Dyets, Inc (Bethlehem), Soy lecithin was purchased from Cargill Food

(Tianjin, China). Tween 80 (Polysorbate 80) was purchased from PT Brataco (Indonesia), and sucrose was purchased from Merck (Germany).

2.2 Methods

2.2.1 Preparation of Piper albi Linn extract

Piper albi Linn extract was prepared by grinding the peppercorn and passthrough 80 mesh. One hundred grams of powder was extracted by using 250 ml of 95% ethanol and blended for 15 in lower speed and maceration process for 24 hours. Filter the ethanol and evaporate ¾ of the ethanol to remain ¼ portion. Add 1g/10 ml of KOH, to the remaining filtrate, gently shake and let it for 30 minutes. Filter the solution, and add purified water the let precipitation of the piperine. Allow the crystallization process for 24 hours at room temperature protected from light. Separate the supernatant from piperine precipitate. Two types of precipitation will be identified as yellowish-brown mass and glisten yellow crystal, and carefully separated. Weigh each precipitation and performed assay by using HPLC at 342 nm.

2.2.2 Preparation of white pepper extract liposome

Accurately weighed 18 g of soy lecithin and 1.5 g of cholesterol (ratio 12:1) and mixed at 60°C±5°C until homogenous liquid formed. The glisten yellow crystal obtained from the preparation of *Piper albi* Linn extract was accurately weighed in 300 mg and mixed to the lipid phase. The water phase containing 2% of tween 80 was prepared and mixed homogenously at 25°C ±5°C. Half portion of the water phase was poured into a clean glass container. The lipid phase was poured to form a thin layer above the water. Slowly add the remaining water phase on top of the lipid layer to help the hydration. Allow the overnight hydration or until it is completely hydrated. After sufficient hydration, stir the liposome solution by using a magnetic stirrer at a low speed to homogenize and reduce the particle of the liposomes.

2.2.3 Preparation of liposome powder

Accurately weigh sucrose and dissolved in 150 ml of water to make lipid to sucrose ratio 1:6. Transfer the sucrose solution into 300 ml of liposome solution, mixed homogenously by using a magnetic stirrer at low speed. Dry the 300 ml of the mixture by using BÜCHI Mini Spray Dryer B-290 at inlet 123^{o}C, outlet 61^{o}C, aspirator 90% and pump 25%. Keep the powder of liposome at room temperature for further analysis.

2.2.4 Analysis of liposome formation by a microscope

The morphology of white pepper extract liposome solution and rehydration after drying was analyzed by using inverted microscopy phase contrast (Olympus, Japan). The liposome obtained by the above method preparation was observed directly without the addition of immersion oil at 400x magnification.

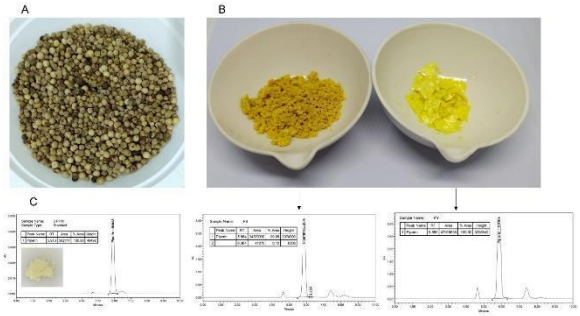

Figure 1. White pepper from Kepulauan Bangka Belitung Province Indonesia used in this experiment (A) has been extracted and yielded in 2 types of material as yellowish-brown mass (B, left picture) and glisten yellow crystal (B, right picture). Piperine content of these 2 types of material was analyzed by HPLC compared to the chromatogram of piperine with purity > 97.0% k (C)

2.2.5 Analysis of liposome powder by a Scanning Electron Microscopy (SEM)

The morphology of white pepper extract liposome powder was examined by a Scanning electron microscope (SEM) using TM3000 Tabletop microscope HITACHI (Japan).

The powder liposome was placed onto carbon tape on top of the stub. Insert the sample stub into the chamber and operate the at a low vacuum 15.0 kV, with an emission current 47800 nA, a filament current 1750 mA magnification. Observed at a magnific of 300x and 2500x by using version 02-03 software.

2.2.6 Characterizations of particle size and zeta potential of the liposome solution

The white pepper extract liposome solution was diluted into 3000 ppm of lipid concentration by using purified water. The size distribution and zeta potential of the liposomal formulations were characterized by dynamic light scattering (DLS) using a Nano Particle analyzer (SZ-100 Nano Partica, Horiba Scientific). The measurement was determined through a helium-neon (He-Ne) laser beam with a scattering angle of 90° and temperature of the holder 25°C. The particle size and zeta potential were analyzed in triplicate and data were analyzed by HORIBA SZ-100 for Windows [Z Type] Ver2.40 software.

2.2.7 Encapsulation efficiency

Ten ml of the solution was let to stand for 48 hours at room temperature in a closed lid tube. Transfer 1 ml of liposome solution taken from the middle part of the solution to 10 ml of volumetric flask. Add 1 ml of water, shake vigorously, and add methanol to volume. The piperine standard was prepared in methanol at a concentration of 5 ppm. Samples and standards are analyzed by using the Waters Alliance HPLC system (5 µm particle size, 250 × 10 mm i.d.) and 20 µl volume injection. The mobile phase consisted of acetonitrile-methanol-water (65:5:30), at a flow rate of 1.0 ml/min refers to a previous study [7]. The results were processed by Empower® 3, software. Chromatographic separations were done on a Jupiter®, C-18 chromatogram was recorded at 342 nm.

Encapsulation efficiency (EE%) was calculated by below formula :

$$EE\% = \frac{Wt}{Wi} \mathbf{x\ 100\%}$$

Where :

Wt = amount of piperine in liposome

Wi = amount of theoretical piperine in white pepper extract liposome

2.2.8 Assay of piperine in white pepper extract liposome powder

Accurately weighed 100 mg of powder liposome piperine and transfer to 10 ml of volumetric flask. Add 3 ml of water, shake vigorously, and add methanol to volume. The standards curve preparation and further analysis were conducted by HPLC refer to as mentioned in the encapsulation efficiency analysis.

3. RESULTS

3.1 *Preparation of Piper albi Linn extract*

Extraction of 100 g white pepper (figure 1A) by using 95% ethanol with 24 hours of maceration process resulting in 1.98 g of piperine or 1.98% (w/w). The purification process gave the result of 2.85 g of yellowish-brown mass containing 43.75% of piperine (figure 1B, left) and 1.21 g of glistening yellow crystal containing 60.97% of piperine (figure 1B, right) as seen in the chromatogram (figure 1C). The yellowish-brown mass from sticky resin-like and has strong pepper characteristic odor, glistening crystals still appear in the mass due to difficulties of complete separation. Meanwhile the glisten yellow crystal has a crystalline, weak pepper characteristic odor, and pungent taste. The further optimization process of extraction is possible to increase the yield of the percentage of piperine.

3.2 *Preparation of white pepper extract liposome*

Preparation of white pepper extract liposome solution by wet-hydrating the lipid film and overnight self-hydration resulting in a yellow milky solution with pH 5.8 (figure 2A).

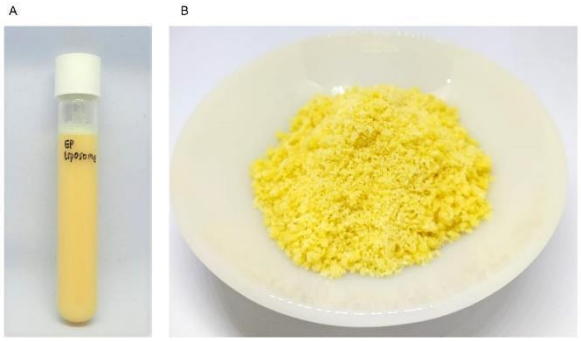

Figure 2. Liposome piperin solution (A) and liposome piperin powder (B)

3.3 *Preparation of Liposomes Powder*

The liposome solution is dried by spray-dried technique resulting in yellow dried white pepper extract liposome powder. The powder has a sweet taste at the beginning and a pungent taste after (figure 2B), indicating the fruitful of the encapsulation process. The dried powder has hygroscopic characteristics and is stored at room temperature for further analysis.

3.4 *Analysis of liposome formation by a microscope*

The white pepper extract liposome was successfully prepared in a spherical shape and micrometer size. No needle-like piperine crystals were observed in the liposome solution, indicating the piperin was successfully entrapped in the hydrophobic region of the liposome (figure 3A). The liposome was observed visually as a large Multilamelar vesicle (MUV) at 400x magnification. The dry powder of the liposome was rehydrated in a concentration of 1 mg/ µl by using purified water and successfully form the spheric liposome (figure 3B). A giant liposome was taken during initial hydration and it was observed that the MUV was formed (figure 3C).

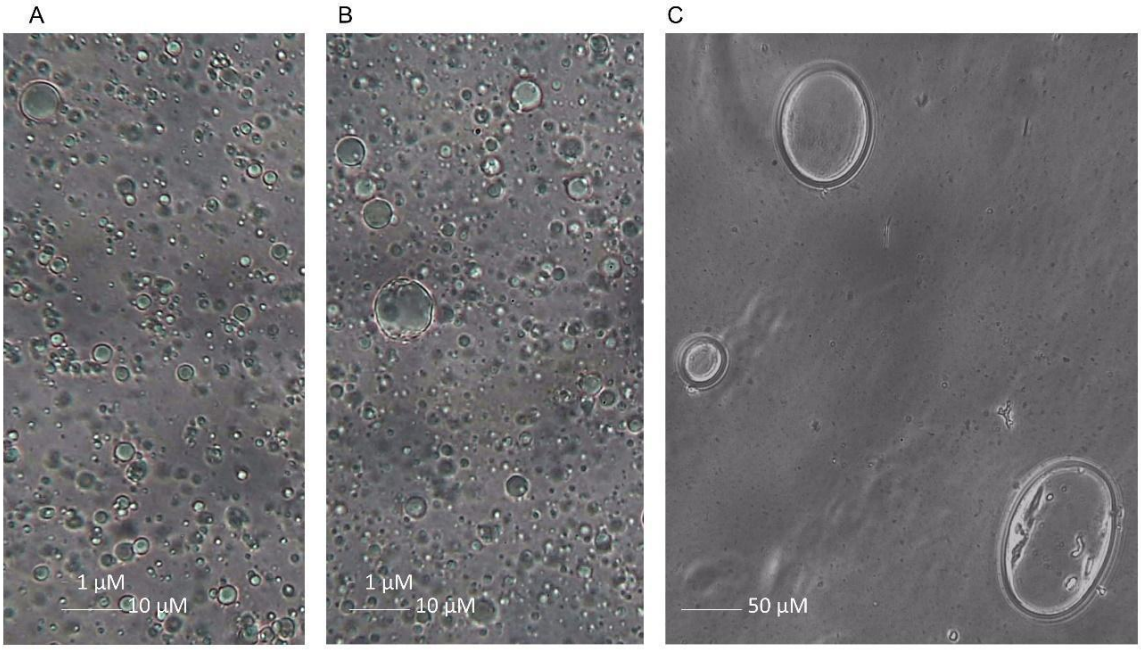

Figure 3. microscopic observation of white pepper extract liposome solution (A) and rehydration of the liposome powder (B) at 400x magnification shows spheric shape. Giant liposome was also taken for observation and shown multilamellar vesicle was successfully formed by wet layer-lipid film hydration technique (C).

Further analysis results in a smaller size of particles are given by using a particle size analyzer instrument.

3.5 *Analysis of liposome powder by a Scanning Electron Microscopy (SEM)*

The morphology of white pepper extract liposome powder examined by a Scanning electron microscope (SEM) shows a surface of liposome protected by sucrose (figure 4 A and 4B). The threshold of small individual liposomes was identified, and they are agglomerated into clump powder during the drying process.

3.6 Characterizations of particle size and zeta potential of the liposome solution

The size distribution of liposomes analyzed by using particle size analysis shows that the mean size is 398.7 nm with a Polydispersion index (PI)= 0,655 (Figure 5A). Histogram shows that 10.0% of particle in 159.3 nm size; 50.0% of particle in 331.0 nm; 70.0% of particle in 464.3 nm; 90.0% of particle in 739.2 nm. Distribution of particles in 3000 ppm lipid concentration shows potential zeta -16.8 mV indicating a threshold of delicate dispersion (figure 5B)

3.7 *Encapsulation efficiency*

The formula of white pepper extract is designed to contain 300 mg of glisten yellow crystal containing only 60.97% of piperine in 300 ml of final liposome solution. This equals 0.6097 mg of piperine/ml of theoretical concentration of white pepper extract in liposome formula. Assay of piperine liposome solution results in 0.6031 mg of piperine/ml, which reveals the encapsulation efficiency of 98.92±1.17%.

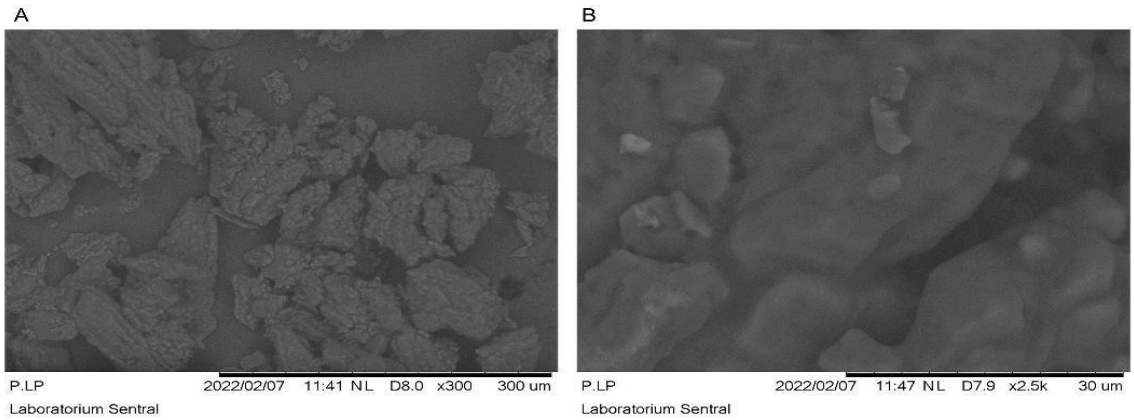

Figure 4. The morphology of white pepper extract liposome powder a Scanning electron microscope (SEM) at 300x magnification (A) and 2500x magnification (B) shows a threshold of small liposome protected by sucrose agglomeration during the drying process.

3.8 *Assay of piperine in liposome powder*

The dried powder of white pepper extract liposome powder which was analyzed using HPLC (Figure 5C) shows that the assay of piperine was 0.1704 mg/100 mg powder or 0.104%(w/w).

4. DISCUSSION

Piperine is a bioactive substance of white pepper (*Piper albi* Linn) with many beneficial pharmacological effects by in vitro and in vivo laboratory evaluations. Nevertheless, its poor water-soluble characteristic results in low of its bioavailability in the body [8]. Preparation of piperine in a liposome delivery system will encapsulate the compound inside the lipid region of the membrane-like liposome structure. The structure will mask its hydrophobic character inside and give good water dispersion due to the hydrophilic polar head of liposome present in the outer part of the structure for better release and bioavailability profile.

The 24 hours of maceration extraction process yield 1.98% (w/w) of piperine. The result is lower than the previous report which is 3.84–5.35 % [1]. The purest extract result was a glisten yellow crystal containing only 60.97% of piperine (figure 1B, right). The further optimization process of extraction and purification is important to be studied to obtain a higher yield of piperine with better purity.

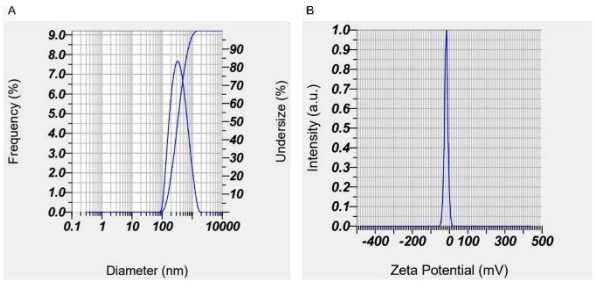

Figure 5. The size distribution of white pepper extract liposome at lipid concentration 3000 ppm analyzed by using particle size analyzer shows that the mean size 398.7 nm with Polydispersion index (PI)= 0.665 (Figure A) and zeta potential value of the liposomal is -16.8 mV (Figure B).

Omitting the organic solvent in the process of liposome formation was intended to prepare organic solvent-free products which may cause problems due to safety issues. Among several types of phospholipid sources, soy lecithin was used due to its abundance availability with less price in the market and especially due to less risk of microbial and viruses contamination [6]. a combination of soy lecithin and cholesterol in the ratio of 12:1 (w/w) is effective to form a stable vesicle liposome with encapsulating efficiency of 98.92±1.17% of the piperine loaded.

The white pepper extract liposome is spherical in the range and mostly observed as LUV and MLV (Figure 2A). Interestingly, smaller particles analyzed by particle size analyzer result in the most particle size in 398.7 nm and zeta potential value -16.8 mV. This indicates the formula is possible to be improved further by the addition of phosphate buffer in suitable pH

Among several methods for tor solvent removal, the spray drying method was employed due to its advantages in a time-efficient and suitable in our formula which is consisted of heat-stable material. Employing sucrose with lipid: sucrose ratio 1:6 successfully protect the structure of liposome during the drying process. Nevertheless, 49.21% of the powder adheres to the inner wall of the drying chamber and thus cannot be collected, thus yielding powder as 50.79%. Improvement of yield can be optimized by using a combination or single carrier such as maltodextrin since its glass transition temperature (Tg) is 162°C higher than sucrose which is 60°C [9]–[11]. This procedure could avoid the sticky characteristic of powder during the drying process which is set at 123°C. Nevertheless, experiment and evaluation need to be performed since it was reported that it often leads to liposomes breaking down and resulting in nonfunctional liposomes in rehydration [12].

5. CONCLUSION

The solution and powder liposome were successfully prepared without applying organic solvent for dispersion of lipid phase to form lipid bilayer liposome structure. Selection of type and concentration of each concentration of material used is important for maximum encapsulation of the piperine. Self-hydration of the wet layer-lipid film is suitable for the formation of liposomes without organic solvent. Sucrose is known to be suitable as a carrier to protect liposomes during the drying process. Our experiment result is important as the initial finding for liposome preparation without organic solvent in simple hydration technique thus can be applied larger industrial scale.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work was supported by the Directorate for Research, Community Service, and Innovation (DRPMI) Universitas Padjadjaran. The present study was supported by the Head lecture acceleration grant (Hibah Percepatan Lektor Kepala) Universitas Padjadjaran, no 48/UN6.3.1/PT.00/2021. We would also thank Rizqa Nurul A. and Ferris Dzaky RN. for technical support during the experiment.

REFERENCES

1. Athfiyah SN, Budiastra IW, Sutrisno, Purwanto YA. Prediction of Piperine content in white pepper by NIR spectroscopy using Partial Least Square (PLS) method Prediction of Piperine content in white pepper by NIR spectroscopy using Partial Least Square (PLS) method. IOP Conf. Series: Earth and Environmental Science. 2020. 1–5.
2. Singh S, Kapoor IPS, Singh G, Schuff C, De Lampasona MP, Catalan CAN. Chemistry, Antioxidant and Antimicrobial Potentials of White Pepper (Piper nigrum L.) Essential Oil and Oleoresins. *Proc. Natl. Acad. Sci., India, Sect, B.Biol.Sci.,* 2013;8(3):357-366.
3. Stojanović -Radić Z, Pejčić M, Dimitrijević M, Aleksić A, Kumar NVA, Salehi B, Cho WC, and Sharifi-Rad J. Review : Piperine-A Major Principle of Black Pepper : A Review of Its Bioactivity and Studies. *Appl. Sci*, 2019; 9 (4270):1-29.
4. National Center for Biotechnology Information. PubChem Compound Summary for CID 638024, Piperine. from https://pubchem.ncbi.nlm.nih.gov/compound/Piperine, Retrieved February 10, 2022.
5. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome : classification, preparation, and applications. *Nanoscale Res. Lett.*, 2013; 8(102):1–9.
6. Kan P, Tsao C, Wang A, Su W, and Liang H. A Liposomal Formulation Able to Incorporate a High Content of Paclitaxel and Exert Promising Anticancer Effect. *J. of Drug Deliv.*, 2011; 2011: 1–9.
7. Setyaningsih D, Santoso YA, Hartini YS, Murti YB, Hinrichs WLJ, Patramurti C. Isocratic high-performance liquid chromatography (HPLC) for simultaneous quanti fi cation of curcumin and piperine in a microparticle formulation containing Curcuma longa and Piper nigrum. *Heliyon*, 2021; 7:1-8.
8. Salsabila H, Fitriani L, Zaini E, Recent strategies for improving solubility and oral bioavailability of piperine. *Int. J. Appl. Pharm, 2021; 13(4):* 31–39.
9. Fongin S, Kawai K, Harnkarnsujarit N, Hagura Y. Effects of water and maltodextrin on the glass transition temperature of freeze-dried mango pulp and an empirical model to predict the plasticizing effect of water on dried fruit site. *J. Food Eng.*, 2017; 210: 91–97.
10. Roe KD and Labuza TP. Glass transition and crystallization of amorphous trehalose-sucrose mixtures. *Int. J. Food Prop.*, 2005; 8:559–574
11. Hussein NR. Spray-dried liposomes : A study of the effect of carbohydrate carrier and concentrations on liposome size and drug entrapment. *J. Med. Sci.*, 2019; 23(3): 345–353.
12. Karadag A, Ozcelik B, Sramek M, Gibis M, Kohlus R, Weiss J. Presence of Electrostatically Adsorbed Polysaccharides Improves Spray Drying of Liposomes. *J. Food Sci.;2013:* 7