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RESEARCH ARTICLE

Development of nanoliposome formulation of beta-carotene using high speed homogeniser method

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Keywords
Betacarotene
High speed homogeniser
Nanoliposome
Phospholipone 80H

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Abstract

Background: Beta carotene has hydrophobic and unstable properties. Nanoliposomes can package various types of drugs, including hydrophilic and hydrophobic drugs, as well as biological substances such as DNA, RNA, and proteins. This allows for more efficient and targeted drug delivery, making it possible to formulate beta-carotene nanoliposomes to improve efficacy and stability. **Objective:** To formulate and characterise betacarotene liposomes using high speed homogeniser method and to conduct an accelerated stability test on betacarotene liposomes produced using high speed homogeniser method. **Methods:** Nanoliposomes were prepared by high speed homogeniser method with time variation of 3, 6, 9 and 12 minutes and characterised for particle size, zeta potential value, polydispersity index, encapsulation efficiency, and stability. **Results:** The preparation of nanoliposomes produced a yellow, odourless suspension with antioxidant activity, with particle size characteristics measuring 200-300 nm, with a polydispersity index of 0.2-0.5 and potential zeta values ranging from -25 mV to -30 mV, encapsulation efficiency value of 97% and morphology of liposome particles in the form of spherical globules with 500x magnification. **Conclusion:** Formulation with a time variation of 12 minutes has recommended characteristics with spherical shape having good particle size, polydispersity index value, zeta potential value and encapsulation efficiency value.

Introduction

Nanoliposomes are a type of nanovesicles that consist of a phospholipid bilayer, often forming a spherical or oval structure and trapping a liquid core inside (Zarrabi *et al*., 2020). Phospholipids are molecules that have a hydrophilic head (attracts water) and a hydrophobic tail (repels water). The phospholipid head faces towards the outside of the vesicle. At the same time, the tail lies between two phospholipid layers, creating a phospholipid bilayer that separates the central liquid core from the external environment (Liu *et al*., 2022). Nanoliposomes can package various types of drugs, including hydrophilic and hydrophobic drugs and biological substances such as DNA, RNA, and proteins. This allows for more efficient and targeted delivery of drugs (Abbasi *et al*., 2022). Nanoliposomes can protect active substances from degradation before they reach their targets. This can extend the shelf life of drugs and prevent damage by enzymes or environmental conditions. Nanoliposomes can improve the biodistribution of drugs in the body, allowing them to better reach their targets. This can reduce side effects and allow for lower doses. With the surface modification of nanoliposomes, they can be designed to recognise specific targets, such as cancer cells or inflammatory tissues. This enables drug delivery directly to the desired location. Nanoliposomes can be used for drug delivery through various routes of administration, including oral, intravenous, transdermal, and others, according to therapeutic needs. The tiny size of nanoliposomes allows them to pass through biological barriers such as cell membranes

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and mucous membranes more easily. Nanoliposomes can help reduce the toxicity of drugs by directing them to the desired target and avoiding healthy tissues. Nanoliposomes can increase the bioavailability of drugs so that more drugs can be absorbed by the body (Aguilar-Pérez *et al*., 2020; Khan *et al*., 2020; Zarrabi *et al*., 2020; Taléns-Visconti *et al*., 2022).

Beta-carotene is one of the isomers of carotene, which can be found in dark green or dark yellow fruits and vegetables (Sani *et al*., 2019). Some beta-carotene in the human body will be converted into vitamin A as an antioxidant that plays a role in the balance of body functions, such as vision, cell differentiation, immunity, growth, reproduction, and prevention of cancer and heart disease (Li *et al*., 2019). Carotenoids are natural fat-soluble (lipophilic) antioxidants and have low stability as they are sensitive to oxidation, especially when exposed to air, light, and heat. This may cause damage to the beta-carotene molecule. Low bioavailability due to carotenoids affects the interaction with food as well as the conversion of limited carotenoids to retinol in the body, which has several vital functions (Pérez-Gálvez *et al*., 2020; González-Peña & Ortega-Regules, 2023). Beta-carotene has antioxidant activity at low partial oxygen pressures, which is prevalent in peripheral tissues (Anand *et al*., 2022). According to Narsaiah, lipid encapsulation efficiency can be influenced by phospholipid concentration and sonication duration and particle size increases with increasing phospholipid concentration (Narsaiah *et al*., 2014). Meanwhile, temperature in the mixing stage also plays an important role in the preparation process because liposomes from phospholipids have a transition temperature to form liposomes (Putri *et al*., 2017). The mixing method used is the thin-layer hydration method, which is used to produce liposomes by hydrating a thin layer of phospholipid lipids. Furthermore, the liposomes formed are reduced in size using a High Speed Homogeniser, this method can produce small particles with optimal conditions in the formation of semi-solid lipids (Nugroho *et al*., 2020).

This study aims to prepare beta-carotene nanoliposomes with different active substances and methods. In the previous study, beta-carotene was encapsulated by ultrasonication. There are still few liposome formulations using phospholipon 80H, a natural emulsifier (phospholipid) derived from soybean oil, so the formulation is something new and can be a reference for the research.

Methods

Materials

The materials used were beta-carotene (Sigma Aldrich), aluminium foil, water for injection (IKApharmindo), 70% ethanol, phospholipon 80H (Sigma Aldrich), chloroform Pa (Merck), methanol Pa (Merck), phosphate buffer solution (1.0 M, pH 7). (1.0 M, pH 7.4), beeswax (Cera Alba), propylene glycol (Merck), tween 80 (Merck), DPPH, ascorbic acid (Merck).

Preparation of nanoliposomes by high speed homogeniser method

Nanoliposomes are produced by a thin-layer hydration method composed of an organic phase and an aqueous phase. The organic phase was prepared by mixing Phospholipon 80H, Beta-carotene, Beeswax (Cera Alba), and Methanol-Chloroform 7:3 using a vortex for 3 minutes. Then after that, it was in rotary for a duration of 1 hour at 45˚C at 100 rpm until caramel-shaped. Then the aqueous phase, which is composed of a mixture of phosphate buffer saline pH 7.4, tween 80 and propylene glycol in a rotary for 30 minutes at 100 rpm. The formed nanoliposome preparation was ultrasonicated for 1 minute with the help of an ice bath to allow cooling of the preparation. After that, mixing was carried out using a Planetary Centrifugal Mixer 2000 rpm for a time variation of 3, 6, 9 and 12 minutes. The resulting preparation was characterised using a particle size analyser to determine the particle size, polydispersity index value and zeta potential value and also determined the percent encapsulation efficiency of the preparation that had been made.

Determination of particle size, polydispersity index and zeta potential

The preparation made was dissolved in water (1:25 and if clear, the second was dissolved at 1:100) and mixed using a vortex for 2-3 minutes, then as much as 2.5 ml of solution was put into the cuvette and then inserted into the PSA holder and then observed. Then the zeta potential analysis was carried out using a zeta sizer, as much as 1 ml of the preparation was put into a special zeta cuvette and then observed using PSA (Chabib *et al*., 2023).

Analysis of percent encapsulation efficiency

The results of the formulation of liquid nanoliposomes of all time variations, then prepared phosphate buffered saline which has been dissolved with distilled water as much as 100 mL then, divided into 4 parts of a 25 mL glass jar, then prepared a float a lyzer which is used for dialysis, insert each sample into the float a lyzer as much as 5 mL then do immersion into PBS

(Phosphate buffered saline) while making circular movements by holding the head of the float a lyzer done for 5-10 minutes until the PBS becomes slightly cloudy, then take the sample in the float a lyzer then injected into a vial of 5 mL in preparation for the preparation of the determination of sample levels (Danish *et al*., 2022). The determination of levels was carried out using UHPLC (Dionex UltiMate 3000), which was then calculated using the following equation:

Encapsulation efficiency % = $\frac{ab-fba}{ab}$ x 100%

Description**:**

ab : amount of beta-carotene

fba : free beta-carotene amount

Nanoliposome morphology assay

Morphological observations of ferrous fumarate nanoliposomes were performed using TEM (Transmission Electron Microscopy) by placing approximately 500 µL of sample solution on a grid in the form of an electric grid and absorbing using filter paper with the help of a vacuum.

Stability test of preparation

The stability test was conducted for 1 month. The resulting nanoliposomes were put into screw-capped glass vials after homogenisation. The stability tests carried out were the accelerated stability test and intermediate stability test. In this test, an accelerated stability test was conducted, and the test results were known by an increase in the rate of chemical degradation and physical changes by storing the sample at temperature conditions of 40°C ± 2°C/75% RH ± 5% RH (ICH Q1A (R2), 2003). Then checked every 3 days. checked every 3 days for 1 month (Ali *et al*., 2013). Checks that particle size, zeta potential and polydispersity index.

Antioxidant assay

The antioxidant activity test of the DPPH (1,1- diphenyl-2-picrylhydrazyl) method begins with measuring the maximum wavelength of DPPH in accordance with the literature of 517 nm using a UV/Vis spectrophotometer. Fifty milligrams of nanoliposome samples were dissolved in a 50 mL volumetric flask to obtain a stock solution of 1000 ppm diluted to 200-1000 ppm, then pipetted 1 mL and added DPPH 0.05 mM as much as 4 mL then vortexed 3 minutes at 80 rpm until homogeneous. After that, the preparation was allowed to stand for 30 minutes under lightproof conditions, and then the absorbance of the sample + DPPH was read on a UV / VIS spectrophotometer. A blank solution using 1 mL of methanol is added to 0.05 mM DPPH as much as 4 mL and then read at a wavelength of three times, the free radical capture activity is obtained from the % yield and concentration of the test compound and then processed using linear regression and obtained the equation value used to calculate the % free radical capture value or IC50 (Wibawa, 2021).

% Rendement = $\frac{AB-AS}{AB}$ x 100%

Description:

AB : Absorbance of blanc

AS : Absorbansi of sample

Results and Discussion

Results of nanoliposome preparation by high speed homogeniser method

The homogenizer process affects the particle size so that a smaller and more uniform size is obtained, namely achieving a small unilamellar vesicle (Lombardo & Kiselev, 2022). The results of the high speed homogeniser minutes 3, 6, 9 and 12 with the speed of each time of 2000 rpm obtained results as in Figure 1. Based on the observation of the length of time homogenise affects the colour of each sample, starting from the left side with a time of 3 minutes has a cloudy yellow colour, time of 6 minutes slightly cloudy yellow, time of 9 minutes slightly cloudy yellow and time 12 minutes has a pale yellow colour. The results of the formulation preparation show different levels of turbidity; it can be influenced by the long homogenise time, causing changes in lipid arrangement, which is initially irregular, then curved to form liposomes

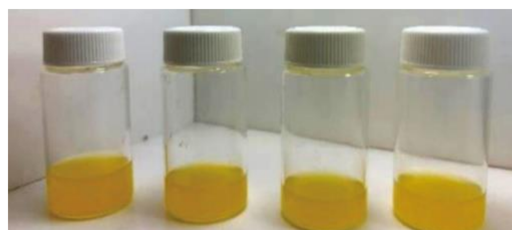

Figure 1: Nanoliposome preparation with homogeniser time variation

Results of particle size, polydispersity index and zeta potential

Particle size is one of the important physical properties to be considered; efforts to reduce particle size are considered to have a good effect on drug delivery to the

target. In the 3-minute time variation, the average particle size is 257.4 nm; in the 6-minute time variation, the average particle size is 250.4 nm; in the 9-minute time variation, the average particle size is 258.5 nm; and in the 12-minute time variation, the average particle size is 221.4 nm. According to these data, the smallest particle size is the nanoparticle formula with a 12-minute time variation. The measurement results show that the nanoliposomes of the four time variations fall into the category of large unilamellar vesicles with a size of 200-500 nm (Liu *et al*., 2022).

The test results of beta-carotene liposomes showed a polydispersity index at the 3rd minute of 0.4 ± 0.01, the 6th minute of 0.4 ± 0.04, the 9th minute of 0.5 ± 0.07, and the 12th minute of 0.3 ± 0.04, so the results of beta-carotene liposome formulation showed good homogeneity because it was not more than 0.7. The DLS technique is more accurate in reading the size distribution of molecules, particles or nanovesicles. The distribution describes how many vesicles are present in each "*segmen*t" of various sizes (Danaei *et al*., 2018). The Polydispersity Index value is also called "*particle size distribution*". This index is dimensionless and scaled, so values smaller than 0.05 are especially noticeable with highly monodisperse standards. PDI values greater than 0.7 indicate that the sample has a very broad particle size distribution and may not be suitable for analysis by dynamic light scattering (DLS) techniques. PI (polydispersity index) values close to zero indicate a monodisperse system, a reading effort is required to achieve the lowest PDI value. The Polydispersity Index (PI) used to observe particle size using Dynamic Light Scattering (DLS) meets the requirements for polydisperse solutions where for particle sizes of 100-300 nm, the PI requirement is <0.3 (Rahman *et al*., 2021). The particle size distribution of beta-carotene nanoliposome preparation can be seen in Figure 2, which shows the results in the form of 4 peaks showing the effect of the length of homogeniser time. Judging from the results that show the same-shaped peaks in each, it shows that the beta-carotene liposome formulation has good particle size distribution and uniformity, the formulation with a time variation of 12 minutes has the smallest PI compared to other formulation time variations.

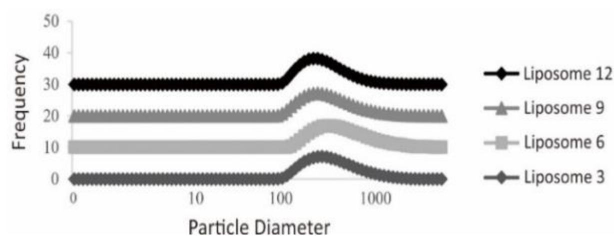

Figure 2: Particle size distribution graph of beta-carotene liposome preparation

The magnitude of the zeta potential of a particle can be used to predict its stability (Ratnasari & Anwar, 2016). (Sekolah Tinggi Teknologi Industri dan Farmasi Bogor *et al*., 2016). The stable zeta potential value ranges from ± 30 mV and the minimum acceptable value is ± 20 mV (Nugroho *et al*., 2020). Beta-carotene nanoliposome preparations with variations in formula time produce negatively charged potential zeta values from 3 minutes, 6 minutes, 9 minutes and 12 minutes, as in Table I.

Table I: Particle size results, polydispersity index values and zeta potential values

Homogenisation time (minutes)	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
3	257.4 ± 0.78	0.4 ± 0.01	-25.0 ± 0.60
6	250.4 ± 4.95	0.4 ± 0.04	-25.9 ± 0.78
9	258.5 ± 1.30	0.5 ± 0.07	-25.2 ± 1.00
12	221.4 ± 8.45	0.2 ± 0.04	-28.9 ± 0.68

The zeta value shows a negative number because it shows the presence of free fatty acids in the preparation so that there is considerable resistance between droplets to combine, which causes the emulsion system to stabilise (Nugroho *et al*., 2020). The zeta potential of nanoliposome has different values; the results of the homogenise time of 3 minutes get zeta results of -25.03 ± 0.60 at minute 6, which is -25.93 ± 0.78, at minute 9, which is -25.17 ± 1.00 and at minute 6 obtained zeta of -28.97 ± 0.68 (Figure 3). The high absolute value of zeta potential indicates a high electrical charge on the surface of liposome nanoparticles, which can cause a strong repulsive force between particles to resist agglomeration. It is also shown that high zeta potential values of about -25 mV or more ensure a high energy barrier for nanosuspension stabilisation. The readings of zeta potential values at minutes 3, 6, 9 and 12 are relatively stable so that the repulsive force to reject particles in beta-carotene liposomes tends to be greater than the

attractive force between particles (Rachmawati *et al*., 2016).

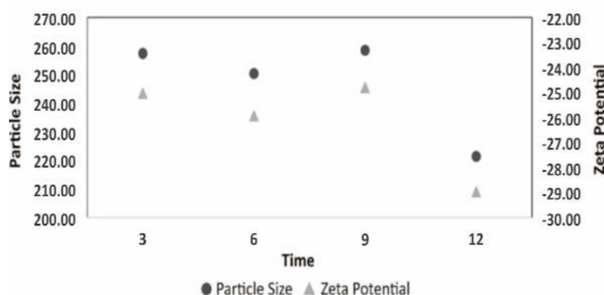

Figure 3: Graph of the relationship between particle size and zeta potential value

Encapsulation efficiency results

Encapsulation efficiency is the percentage of active compound effectively trapped in the inner volume of the liposome of course, its complement up to 100 represents the untrapped compound, which is lost in the aqueous external bulk (Trucillo *et al*., 2020). Beta-carotene nanoliposomes analysed using a float lyzer, there will be penetration of free beta-carotene contained in the formulation, while beta-carotene bound to liposomes will be retained due to the dispersion of a substance into another substance that is mixed or forms a colloid. A good encapsulation efficiency percent value is above 74% (Gomez *et al*., 2019). Nanoliposomes consisting of bilayer phospholipid membranes will have a good effect on the formulation when added with beeswax because when united with liposomes, it is able to increase the permeability and fluidity of the membrane so as to increase the encapsulation efficiency value of liposome preparations, previously measured the standard curve value of beta-carotene with a value of $y = 0.015x-0.0438$ and an R^2 value of = 0.999, from the regression results it is used to calculate the encapsulation efficiency value.

The results of reading the percent encapsulation efficiency in Table II using UHPLC (ultra High-performance liquid chromatography) with the best results of the four beta-carotene nanoliposome formulas found that the results from minutes 3, 6, 9, 12 were 47.38%, 44.62%, 97.47%, 95.51% respectively with the best results in minute 9 with a value of 97.47% ± 0.03, with this value meaning that the results of the test of the percent encapsulation efficiency of liposome preparations are appropriate.

Table II: Encapsulation efficiency values of beta-carotene liposome formulations

Homogenisation time (min)	Percent encapsulation efficiency (%)
3	47.38
6	44.62
9	97.47
12	95.51

Nanoliposome morphology results

This study uses TEM (Scanning Electron Microscope) to see the morphology of nanoliposome preparations, the characteristics and size of liposome vesicles show a round shape like a ball. Testing of beta-carotene nanoliposomes took samples with the best particle size and polydispersity index, the results of the formulation optimisation obtained a time variation of 12 minutes is the best result among other time variations. Liposome morphology affects drug delivery on the ability of nanoliposomes to penetrate the membrane to the target (Ghaferi *et al*., 2020). Based on the results of testing beta-carotene nanoliposomes with the speed homogeniser method, they have diverse shapes; it can be seen in Figure 4 that visible globule liposomes have spherical shapes.

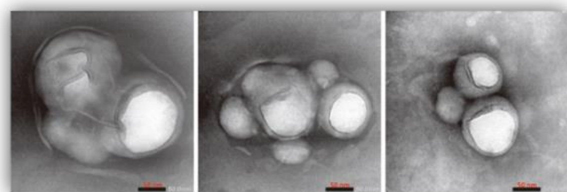

Figure 4: Results of liposome morphology by TEM

Particle shape that is less spherical will facilitate contact between particles so that aggregation occurs, which can increase particle size. The surface of the microparticles, which is generally less smooth and flat, is probably caused by the viscosity of the polymer being too low, which results in the crosslinking force of the microparticle structure formation being less strong so that it shrinks easily and becomes uneven. In addition, shrinkage of microparticles is also possible due to the cooling and drying process that occurs during the freeze-drying process (Robson *et al*., 2018).

Liposome stability results

Stability is a description of the durability of a product in accordance with certain limits during storage and use

and has the same properties and characteristics as at the time of manufacture (Pratiwi *et al*., 2018). Accelerated stability tests are carried out to describe the storage conditions of products that will be circulated over a long period. Samples were placed in a climatic chamber (Binder GmbH, Tuttlingen, Germany) at 40̊ C ± 2̊ C with a relative humidity of 75% ± 5% (Eugresya *et al*., 2018). The stability test has the aim of knowing the effect of storage of liposome preparations at certain temperatures, stability testing includes particle size, polydispersity index, and zeta potential. The stability test is carried out for 1 month by checking every 3 days, but in the stability test that has been carried out, there is an interval of 14 days between days 7 and 21, which is the time interval to see whether the preparation occurs aggregation or not. In the results of the particle size distribution in Figure 5, there is instability in the nanoliposome preparation because, from the beginning, it has varying sizes and uneven polydispersity index values, this instability is due to the sediment that occurs during the process of removing organic solvents by the thin layer hydration method, the possibility of coagulation, phosphate groups and choline groups between vesicles that attract each other. In addition, the large size can occur due to physical instability. Physical instability is caused by the leakage of active compounds from vesicles or the aggregation and fusion of vesicles to form larger particles (Liu *et al*., 2022).

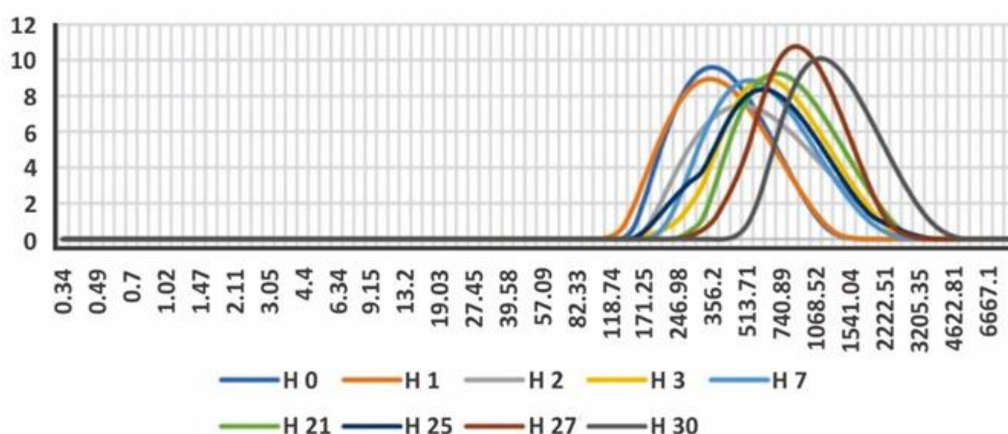

Figure 5: Particle size distribution results of liposome stability test

The results of the zeta potential value readings are in Table III with the potential zeta value the ideal zeta potential value should be required in the range between +30 to -30mV. This range prevents the aggregation of nanoliposome particles (Umbarkar *et al*., 2021).

Table III: Results of zeta potential value readings of liposome stability test

Day to-	Zeta potential (mV)	Day to-	Zeta potential (mV)
0	-33.8±2.83	21	-36.9±0.95
1	-37.1±0.10	25	-43.3±0.00
2	-46.3±0.58	27	-36.8±0.90
3	-37.4±0.60	30	-31.1±0.58
7	-35.6±0.49		

Antioxidant activity test results of beta-carotene liposomes

The parameter used for the DPPH radical capture test is the IC50 (Inhibition Concentration 50) value, which is the concentration of the test fraction needed to capture DPPH radicals by 50%. The IC50 level category states that the value (IC <50 ppm) then the compound is a very strong antioxidant, with strong activity for (IC 50-100 ppm), moderate activity for (IC 100-250 ppm) and weak activity for (IC 250-500 ppm) (Marjoni & Zulfisa, 2017), can be seen in Table IV positive control test results and antioxidant activity of nanoliposome that ascorbic acid as a standard obtained an IC50 value of 8.97 (µg/mL) which means that ascorbic acid has excellent antioxidant activity, while from the IC50 results the beta-carotene nanoliposome formulation obtained a value of 17242.45 (µg/mL) or 17.2 mg/mL which means very large. The effectiveness of antioxidant compounds depends on several factors, the most important of which are structural properties, temperature, characteristics of substrates susceptible

to oxidation, concentration, the presence of synergistic and prooxidant compounds and the physical state of the system. The chemical structure of antioxidants determines their intrinsic reactivity to free radicals and other reactive oxygen species and thus influences antioxidant activity (Munteanu & Apetrei, 2021).

Table IV: Antioxidant activity test results with DPPH

Sample type	Sample concentration	Inhibition (%)	Linear regeression equation	*r*-value	IC50 (µg/mL)
Ascorbic acid	2	6.62	y = 6.0538x – 4.335	0.974344	8.975354
	4	10.48			
	6	37.27			
	8	44.69			
	10	56.55			
Beta-carotene liposome	2000	0.46	y = 0.0003x – 0.1148	0.9952739	17242.45
	4000	1.08			
	6000	1.65			
	8000	2.25			
	10000	3.14			

Conclusion

The formulation with a time variation of 12 minutes has the recommended characteristics with a particle size of 200-260 nm, a polydispersity index value of 0.2-0.5, a zeta potential value of each time variation of -25 mV to -28 mV, and a good encapsulation efficiency value of 47-97%. The encapsulation efficiency value of 47-97%, in the stability test, the data obtained is not good because the results of the particle size distribution are not uniform, the results of the antioxidant test using DPPH are obtained. Antioxidant testing using DPPH obtained IC50 of 17242.45 µg/mL can be said to have poor antioxidant activity.

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References

Abbasi, H., Kouchak, M., Mirveis, Z., Hajipour, F., Khodarahmi, M., Rahbar, N., & Handali, S. (2022). What we need to know about liposomes as drug nanocarriers: An updated review. *Advanced Pharmaceutical Bulletin*, **13**(1), 7–23. https://doi.org/10.34172%2Fapb.2023.009

Aguilar-Pérez, K. M., Avilés-Castrillo, J. I., Medina, D. I., Parra-Saldivar, R., & Iqbal, H. M. N. (2020). Insight into nanoliposomes as smart nanocarriers for greening the twenty-first century biomedical settings. *Frontiers Bioengineering and Biotechnology*, **8**, 579536. https://doi.org/10.3389/fbioe.2020.579536

Ali, M. S., Alam, M. S., Alam, N., Anwer, T., & Safhi, M. M. (2013). Accelerated stability testing of a clobetasol propionate-loaded nanoemulsion as per ICH guidelines. *Scientia Pharmaceutica*, **81**(4), 1089–1100. https://doi.org/10.3797/scipharm.1210-02

Anand, R., Mohan, L., & Bharadvaja, N. (2022). Disease prevention and treatment using β-Carotene: The ultimate provitamin A. *Revista Brasileira de Farmacognosia*, **32**, 491–501. https://doi.org/10.1007/s43450-022-00262-w

Chabib, L., Suryani, A., Dewi, L. S., Noviani, H., Maharani, W. H. P., & Indraswari, A. A. (2023). Pineapple fruit extract (*Ananas comosus* L. Merr) as an antioxidant and anti-acne agent made with the nano-emulsion gel delivery system. *Pharmacy Education*, **23**(2), 126–132. https://doi.org/10.46542/pe.2023.232.126132

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*, **10**(2), 57. https://doi.org/10.3390/pharmaceutics10020057

Danish, M. K., Gleeson, J. P., Brayden, D. J., Byrne, H. J., Frías, J. M., & Ryan, S. M. (2022). Formulation, characterisation and evaluation of the antihypertensive peptides, isoleucine-proline-proline and leucine-lysine-proline in chitosan nanoparticles coated with zein for oral drug delivery. *International journal of molecular sciences*, **23**(19), 11160. https://doi.org/10.3390/ijms231911160

Eugresya, G., Avanti, C., & Uly, S. A. (2018). Formula development and physical-pH stability test of facial wash gel preparations containing ethanol extract of kesambi bark. *Media Pharmaceutica Indonesia*, **1**(4), 181–188. https://doi.org/10.24123/mpi.v1i4.769

Ghaferi, M., Asadollahzadeh, M. J., Akbarzadeh, A., Shahmabadi, H. E., & Alavi, S. E. (2020). Enhanced efficacy of PEGylated liposomal cisplatin: In vitro and in vivo evaluation. *International Journal of Molecular Sciences*, **21**(2), 559. https://doi.org/10.3390/ijms21020559

Gomez, A. G., Syed, S., Marshall, K., & Hosseinidoust, Z. (2019). Liposomal nanovesicles for efficient encapsulation of staphylococcal antibiotics. *ACS Omega*, **4**, 10866–10876. https://doi.org/10.1021/acsomega.9b00825

González-Peña, M. A., & Ortega-Regules, A. E. (2023). Chemistry, occurrence, properties, applications, and encapsulation of carotenoids—A Review. *Plants (Basel, Switzerland)*, **12**(2), 313. https://doi.org/10.3390/plants12020313

Khan, A. A., Allemailem, K. S., Almatroodi, S. A., Almatroudi, A., & Rahmani, A. H. (2020). Recent strategies towards the surface modification of liposomes: An innovative approach for different clinical applications. *3 Biotech*, **10**, 163. https://doi.org/10.1007/s13205-020-2144-3

Li, X., Wu, M., Xiao, M., Lu, S., Wang, Z., Yao, J., & Yang, L. (2019). Microencapsulated β-carotene preparation using different drying treatments. *Journal of Zhejiang University-Science B*, **20**, 901–909. https://doi.org/10.1631/jzus.B1900157

Liu, P., Chen, G., & Zhang, J. (2022). A review of liposomes as a drug delivery system: Current status of approved products, regulatory environments, and future perspectives. *Molecules*, **27**(4), 1372. https://doi.org/10.3390/molecules27041372

Lombardo, D., & Kiselev, M. A. (2022). Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics*, **14**(3), 543. https://doi.org/10.3390/pharmaceutics14030543

Marjoni, M. R., & Zulfisa, A. (2017). Antioxidant activity of methanol extract/fractions of senggani leaves (*Melastoma candidum* D. Don). *Pharmaceutica Analytica Acta*, **8**(8), 1000557. https://doi.org/10.4172/2153-2435.1000557

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, **22**(7), 3380. https://doi.org/10.3390/ijms22073380

Narsaiah, K., Jha, S. N., Mandge, H. M., Jha, J., & Manikanatan, M. R. (2014). Optimization of microcapsule production by air atomization technique using two-fluid nozzle. *Agricultural Research*, **3**, 353–359. https://doi.org/10.1007/s40003-014-0123-9

Nugroho, B. H., Ningrum, A. D. K., Pertiwi, D. A., Salsabila, T., & Syukri, Y. (2020). Utilization of fig leaf extract (*Ficus carica* L.) based on liposome nanotechnology as an antihyperglycemia treatment. *EKSAKTA: Journal of Sciences and Data Analysis*, **19**(2), 216–229. https://doi.org/10.20885/eksakta.vol19.iss2.art12

Pérez-Gálvez, A., Viera, I., & Roca, M. (2020). Carotenoids and chlorophylls as antioxidants. *Antioxidants*, **9**(6), 505. https://doi.org/10.3390/antiox9060505

Pratiwi, L., Fudholi, A., Martien, R., & Pramono, S. (2018). Physical and chemical stability test of SNEDDS (Self-nanoemulsifying drug delivery system) and nanoemulsion ethyl acetate fraction of *Garcinia mangostana* L. *Majalah Obat Tradisional*, **23**(2), 84. https://doi.org/10.22146/mot.28533

Putri, D. C. A., Dwiastuti, R., Marchaban, M., & Nugroho, A. K. (2017). Optimization of mixing temperature and sonication duration in liposome preparation. *Journal Pharmaceutical Science Community*, **14**(2), 79–85. https://doi.org/10.24071/jpsc.142728

Rachmawati, H., Rahma, A., Shaal, L. A., Müller, R. H., & Keck, C. M. (2016). Destabilization mechanism of ionic surfactant on curcumin nanocrystal against electrolytes. *Scientia Pharmaceutica*, **84**(4), 685-693. https://doi.org/10.3390/scipharm84040685

Rahman, H., Wisnuwardhani, H. A., & Rusnadi, R. (2021). Optimization of silver nanoparticle synthesis using kawista leaf extract (*Limonia acidissima* Groff). *Jurnal Farmasi Klinik Dan Sains*, **1**(1), 31. https://doi.org/10.26753/jfks.v1i1.671

Ratnasari, D., & Anwar, E. (2016). Characterization of transfersome nanovesicles as "rutin" carriers in the development of transdermal preparations. *Jurnal Farmamedika*, **1**(1), 12–18. https://doi.org/10.47219/ath.v1i1.40

Robson, A.-L., Dastoor, P. C., Flynn, J., Palmer, W., Martin, A., Smith, D. W., Woldu, A., & Hua, S. (2018). Advantages and limitations of current imaging techniques for characterizing liposome morphology. *Frontiers in Pharmacology*, **9**, 328115. https://doi.org/10.3389/fphar.2018.00080

Sani, M. F. H., Setyowati, S., & Kadaryati, S. (2019). Effect of processing techniques on beta-carotene content in broccoli (Brassica oleracea L.). *Ilmu Gizi Indonesia*, **2**(2), 133–140. https://ilgi.respati.ac.id/index.php/ilgi2017/article/download/108/40

Taléns-Visconti, R., Díez-Sales, O., de Julián-Ortiz, J. V., & Nácher, A. (2022). Nanoliposomes in cancer therapy: Marketed products and current clinical trials. *International Journal of Molecular Sciences*, **23**(8), 4249. https://doi.org/10.3390/ijms23084249

Trucillo, P., Campardelli, R., & Reverchon, E. (2020). Liposomes: From Bangham to supercritical fluids. *Processes*, **8**(9), 1022. https://doi.org/10.3390/pr8091022

Umbarkar, M., Thakare, S., Surushe, T., Giri, A., & Chopade, V. (2021). Formulation and evaluation of liposome by thin film hydration method. *Journal of Drug Delivery & Therapeutics*, **11**(1), 72–76. https://doi.org/10.22270/jddt.v11i1.4677

Wibawa, A. A. C. (2021). Total antioxidant capacity of methanol extract of cocoa beans (*Theobroma cacao*. L) using Uv-Vis spectrophotometric method. *Hydrogen Jurnal Kependidikan Kimia*, **9**(1), 30. https://doi.org/10.33394/hjkk.v9i1.3794

Zarrabi, A., Alipoor, M. A. A., M., Khorasani, S., Mohammadabadi, M.-R., Jamshidi, A., Torkaman, S., Taghavi, E., Mozafari, M. R., & Rasti, B. (2020). Nanoliposomes and tocosomes as multifunctional nanocarriers for the encapsulation of nutraceutical and dietary molecules. *Molecules*, **25**(3), 638. https://doi.org/10.3390/molecules25030638