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Design and optimization of self-nanoemulsifying drug delivery systems (SNEDDS) of ethyl acetate fraction from mangosteen peel (Garcinia mangostana, L.)

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Abstract : Self-nano emulsifying drug delivery system of ethyl acetate fraction from mangosteen peel (EAF-MP) was developed under quality by design approach for improvement of diffusion and precut absorption. Preliminary screening was performed to select proper components combination. Simplex Lattice Design was employed as the statistical tool to optimize the formulation variables. X1 (Tween 80), X2 (PEG 400) and X3 (Virgin Coconut Oil). The system was assessed for transmittance, emulsification time, PH, and drug loading. Following optimization, the value of formulation components (X1, X2, and X3) were 4,61%. 1% and 1,38% respectively (150 mg of EAF-MP). The optimized formulation of EAF-MP had a mean nanoemulsion droplet diameters of 11,6 nm. The stability of the optimized formulation was retained after storage at 25 C for three months.

Introduction

Aging occurs in almost all human body systems and generally all systems is not declining at the same time. Along with the development of science and technology scientifically found that the aging process can be slowed[1]. One of the changes that occur in the dermis layer of skin that has photoaging is the reduced amount of collagen fibers, antioxidants can inhibit this process[2]. Antioxidants are molecules that can work on the skin to reduce the effects of radical oxygen species (ROS). One Indonesian plant that can be used as photoaging is mangosteen (Garciniamangostana L), especially in the skin. The skin of the mangosteen is rich in xanton. The main Xanton is mangosteen. Priya et al[3]. Mangosteen peel extract found traces xanton, isoflavones, tannins, and flavonoids. Xanton had not dissolved in water properties that limit the application of a system that uses water [4], so it requires a drug delivery system that can enhance penetration into the skin with a small size and improve comfort in use.

Nanoemulsions acceptable used for the preparation of cosmetics.Nanoemulsions has a large surface for the absorption and spread good power due to the small particle size so as to allow for uniform distribution in the skin and the ability to penetrated into the skin layer[5]. Nanoemulsion made with drug delivery systems self nanoemulsifying drug delivery system (SNEDDS) allows the large scale manufacture is making is easy, economical manufacturing process so that the main attraction in the industry, as well as thermodynamically stable so as to facilitate storage[6]. Nanoemulsion made using the optimum formula that is using the simplex

lattice design with various concentrations of oil, surfactant and co-surfactant. The simplex lattice design is one method of optimization formula to get the area having no response at and also areas where optimal or the method used to determine the optimization formula on the various differences in the amount of material composition, which in total made the same which is equal to one section [7].This research needs to be developed on an ongoing basis, to obtain optimum formulations nanoemulsi. The success of the research will have an impact on the further development of the potential of natural materials from the development of a pharmaceutical preparation.

2. Experimental

Materials

The main material used in this study was mangosteen peel obtained from Purworejo District, Central Java province. The standard of α-mangostin (Sigma), methanol (Merck), ethyl acetate (Merck),VCO (bagoes), Tween 80 (Brataco), Span 20 (Brataco), PEG 400 (Brataco), Propilenglikol (Brataco), citrus essetial oil (Mustika ratu), kanthil essential oil (Mustika ratu),Cremophor EL (China), distilled water, silica gel F_{254} (Merck).

Procedures

1. Making Raw curve α-mangostin

Stock solution made by dissolving1mg of α-mangostin in 10 ml of methanol P.A. Stock solution was diluted to obtain a concentration of 5ppm. This solution is used to scan a maximum of mangosteen peel wavelength in the wavelength range of 300-400nm. Wavelengths were then used for each measurement uptake throughout the analysis.

2. Solubility studies for oil selection

Seven commonly used oils, virgin coconut oil (VCO), citrus essetial oil, kanthil essential oil, soy bean oil, sun flower oil, corn oil and canola oil, were screened for their properties to dissolve maximum of EAF-MP. An excess amount of EAF-MPwas added into clear screw thread glass vials that contained 10 mL of each oil followed by vortex mixing. Mixtures were shaken for 20 min at 40 °C in a thermostatically controlled shaking water bath, followed by equilibrium for 12 h at 37 °C. Mixtures were then centrifuged at 3000 rpm for 10 min in a microcentrifuge. Aliquots of supernatant were filtered through membrane filter. The filtered sample then diluted with methanol and the amount of EAF-MP was analyzed using .

3. Selection of surfactants and co-surfactants for solubility ability

Self-nanoemulsifying properties of SNEDDS strongly depend upon the selected lipids, surfactants, and co-surfactants. This mixture gives the possibility to optimize the SNEDDS for a particular drug. The process of dissolving the fractionin a carrier maximized by means sonicator for 15 minutes and left for two days at room temperature. After two days of insoluble fraction which is separated from the soluble fraction by centrifugation at 3000 rpm for 20 minutes. The rest of thefractionsin the form of sediment, separated from the supernatant, fractionated with10 ml of methanol and its concentration measured by spectrophotometry at a wavelength of maximum α-mangostin.

4. Experimental design optimization of EAF-MP loaded SNEDDS

After selecting the best suitable oil, surfactant and co-surfactant in accordance with studies performed. Response surface methodology is a collection of techniques useful when only a few significant factors are involved in optimization[7], such as simplex lattice design. Firstly, a simlpex lattice design was constructed to estimate the best amount of EAF-MP in SNEDDS (150 mg), with combinations from three factors (independent variables) which were the amounts (g) of the component system: surfactant X1 (Tween 80), co-surfactant X2 (PEG 400) and the oily phase X3 (VCO).

5. Characterization of EAF-MP

a. Transmittance

For each assayed medium a batch with samples of the fourteen developed EAF-MPformulations was prepared. Briefly, 100 µL of each EAF-MPwas accurately weighed and palced into 5 mL aquadest. The mixture was homogenized with the aid of a vortex for 30 seconds. Homogeneous mixing results and provide a clear visual display of a sign of the beginning of the creation of SNEDDS. Emulsions have been obtained measured absorbance at a wavelength of 650 nm[8].

b. Emulsification time

500 mL conditioned medium over a magnetic stirrer at 120 rpm. A total of 1 mL SNEDDS optimum contain ethyl acetate fraction of mangosteen peel dropped into the media quickly. Observations were made of the time required by nanoemulsi to form a homogeneous mixture which has been characterized by the mixture of EAF-MPperfectly in the media [8].

c. PH

A total of 100.0 µL diluted with distilled water to 5.0 mL. The solution is taken and placed on a pH meter.

d. Drug Loading

For each assayed medium a batch with samples of the fourteen developed EAF-MPloaded SNEDDS formulations was prepared. Extracted SNEDDS contained EAF-MPwith 20 ml methanol for one day.The mixture was homogenized with the aid of a vortex for 30 seconds. Emulsions have been obtained measured absorbance at a wavelength of α-mangostin.

e. Droplet size and zeta potential measurements

Similarly, the droplet size analysis were performed to the resultant EAF-MP-loaded SNEDDS previously homogenized. Measurements using a Particle Size Analyzer (PSA) to know the size and distribution of nanoparticles.Two drops SNEDDS samples are added 5 mL of distilled water mixed by means of flipping over. After 3 mL was taken and put into a cuvette for analysis. Zeta potential measurements performed with a Zetasizer. 2 drops nanoparticle samples, each added 5 mL of distilled water and mixed by means of flipping over. After it was taken 3 mL and analyzed[9].

f. Stability

Nanoemulsions maintained at a temperature of 37ºC and homogenized by vortex for 30 seconds. Observe every hour for 4 hours to determine its stability and continue until 3 months. Nanoemulsionsbe stable if it does not form lumps or sediment[10].

Result and discussions

1. The standard curve α-mangostin

Standard curvewas madeby usinga specific wavelength. Based on researchobtained bythe wavelength of 243.40nm (fig.1).

2. Solubility study

Result of the solubility of EAF-MPin various essential oils are reported in table.1. This study allows us to identify the suitable oil to load EAF-MP into the formulations. In this case, EAF-MPsolubility was highest in virgin coconut oil (VCO) and was the selected essential oil to the experiments. Screening of appropriate oil is primary requirement of SNEDDS development. Solubility studies were aimed at identifying suitable oil having maximal solubilizing potential for the development of SNEDDDS.According to Shafiq-un-Nabi et al[11]drug solubility in oil on nanoemulsions is the most important component, because it is associated with the ability

nanoemulsi to keep the drug in dissolved form that is highly influenced by the drug solubility in the oil phase. Oil molecules which have a weak adhesion force is easier to stretch bonding process compared with those with a stronger adhesion force. Stretching process required to form the configuration of the oil molecules to capture molecules of the EAF-MP[11].

3. Surfactants for solubility ability

Surfactants work with the mechanism lowering the interfacial tension between the oil phase and the aqueous phase after SNEDDS concentrates are dispersed in dispersing medium so as to form nanoemulsi. The type and amount of surfactant will affect the size of the oil droplets in the water phase. Surfactants were selected that has the ability to interact with molecules capable of dispersing the oil and oil in water to produce nanoemulsi. High HLB value which will facilitate the molecular interaction of oil with the aqueous phase but can complicate the process of mixing the oil with a cosurfactant. Surfactant has the ability to dissolve the highest ethyl acetate fraction was selected as a surfactant in formulations in this study. The ability of surfactants in dissolving the ethyl acetate fraction of mangosteen peel can be seen in table 2. Based on the solubility data is then selected tween 80 as surfactant because it has ability to dissolve EAF-MP the most. The ability of surfactants in dissolving the compound is affected by HLB and interfacial tension. HLB indicate the level of polarity surfactant. The greater the value of increasing polarity HLB surfactant.EAF-MPmost soluble in tween 80 (HLB 15) as the most appropriate polarity tween 80 with the polarities EAF-MP.

4. The ability of co-surfactants in dissolving the EAF-MP

Interest on the formula SNEDDS addition of cosurfactant which is to increase drug loading, self modulatenanoemulsification time and decrease the droplet size nanoemulsi[12]. Co-surfactant connect surfactant molecules and helps the formation of smaller and smaller interfacial tension [13]. Co-surfactant molecules between the surfactant molecules on the surface of the oil globules will form oil globules with a sealed surface by surfactant molecules and co-surfactant. Globules of oil sealed by surfactant molecules and cosurfactant has an increasingly small interfacial tension with water molecules, thereby increasing the oil globule surface interaction with water molecules are characterized by a surface area greater interaction. The surface area of interaction between the oil phase-water the greater will produce droplet sizes are getting smaller.

5. Transmittance, Emulsification time, PH, and Drug Loading measurements of SNEDDS

The concentration of surfactant, co-surfactant and oil of the 14 SNEDDS were assay to be optimized on the basis of Transmittance, Emulsification time, PH, and Drug Loading measurements of SNEDDS. Transmittance indicates that clarity is one of the characteristic properties of the emulsion that need to be measured because the effect on particle size. Based on observations, 3 of the 14 formula shows the formula to see clear SNEDDS with formula 1,5,6 using Tween 80: PEG 400: VCO with a ratio (5: 1: 1), (2,33: 2,33: 2,33), and (5: 1: 1). Meanwhile, the ratio of oil, surfactant and cosurfactant others show a cloudy appearance. Based on the results of the third transmittance measurement formula above has a transmittance value of more than 60%. According to Costa et al [14], nanoemulsion good to have a clear visual sighting with high transmittance, these formulas will be nanoemulsi when emulsification in a water medium.The more clear the emulsion particle size will be smaller and more turbid emulsion the particlesizeincreases.

SNEDDS pH testing aims to determine the safety SNEDDS especially when used on the skin. PH value is too low to cause irritation when the user, while the pH is too high resulting in scaly skin.PH range of topical dosage is 4-6[15]. In the test results, all the formulas have a pH falling within the range of requirements. All the formula have a ph range permitted for use on the skin. Distribution curve ph test data can be seen in the following table 3. The table shows a few scattered points along a straight line.

Testing emulsification time using distilled water with a magnetic stirrer using a speed of 120 rpm. Nanoemulsion spontaneous formation is one important parameter in the formulation SNEDDS. Nanoemulsion expected to be formed quickly when in the water. The measurement results emulsification time of the optimum formula can be seen in the following table 3.Determination emulsification time taken to obtain a picture of ease SNEDDS form the emulsion. Measurements emulsification time at the optimum formula capable of forming nanoemulsi on media distilled water. PEG 400 produces a faster emulsification dispersibility due to better than the propylene glycol, PEG 400 because it has a higher polarity than propylene glycol. Based on the research of Vilas et al [16], dispersion ability of PEG 400 in the category A which are able to produce rapid nanoemulsi which

takes about 1 minute, with a view nanoemulsi clear. These findings are consistent with the results obtained in this study. Emulsification short time mediated by the action of surfactant and co-surfactant that is able to quickly form a layer of oil and water interface. Co-surfactant bigger role in emulsification time is not on droplet size reduction. Co-surfactant will be tucked away and form the spaces between the surfactant so that its structure more bloated but has high fluidity and capable of forming nanoemulsi faster. The ability to improve emulsification at cosurfactant determined on hydrophobic alkyl chain length. The longer the chain, the abilityemulsifikasinya the better[17].

DrugLoadingtestaims todeterminethe amount ofEAFcapabledissolved inSNEDDSformula. The larger thevalue, the volume of drugloadingSNEDDSwhoused the smaller. Determination ofExtractLoadingResultsTest drug loading 175 mg / mL at the optimum formula indicates that the system is not capable of dissolving extract, is characterized by the deposition on three days of observations that used the concentration of EAF-MP is 150 mg/5 ml.

6. Optimization of EAF-MP

The aim of the optimization of pharmaceutical formulations is generally to determine the levels of the variables with high quality characteristics may be produced. In order to obtain the best EAF-MP. based on the previous experimental design, data from the caracterization of these were used to determine the levels and interactions of these independent and response variables after generating mathematical relationships between dependent and independent variables by using Design-Expert®version 7.0.0 software. The response representated were transmittance value, emulsification time, PH and drug loading. Observed response are showed in table 3.

Transmittance was used to monitor the process of self emulsification by measuring the transmittance of the solution during dissolution as the emulsification process takes place. A trend toward higher transmittance values was observed with increasing the concentration of surfactant. It could be attributed to the decrease in surface tension and thus smaller droplet size of the SNEDDS.Based on these equations appears that the interaction between the VCO and Tween 80, VCO and PEG 400 provides coefficient with a positive value. It shows that such interaction higher transmittance value SNEDDS. The interaction between the VCO and Tween 80, as well as between oil and PEG 400 positive values indicate that these interactions increase the reflectance values. While the interaction between Tween 80 and PEG 400 increase the transmittance value. The more clear the emulsion particle size will be smaller and more turbid emulsion the particle size increases.

A coefficient value is positive, it means that the component A can increase response ph. Similarly, the coefficients B and C, but the coefficient B is greater than A and A is greater than C. So this indicates that the ability of the components in improving ph Tween 80 greater than the components VCO and PEG 400. The interaction between the VCO with tween 80 provides coefficients are positive means of interaction both increase the value of PH. Similarly nteraction between VCO with PEG 400 also increases the value of PH. While the interaction between Tween 80 with PEG 400 lowers the PH value.

7. Droplet size analysis and Zeta potential

Droplet size analysis and zeta potential examination of the optimized EAF-MP.Observations droplet size nanoemulsion done to ensure nanoemulsi formed droplet size has been smaller than 100 nm. The droplet size distribution is used as a parameter uniformity and reliability of the method of manufacture nanoemulsi. This test is performed on distilled water media. the average size of less than 100 nm nanoemulsi. This proves that the formula SNEDDS made capable of producing nanoemulsi. Theoretically nanoemulsi size greatly influenced by the ratio of surfactant to cosurfactant. The higher the ratio of surfactant to cosurfactant, the smaller the size of nanoemulsi obtained [18]. According to Fernandez et al [19] the particle size at nanoemulsi influenced by the composition of the oil, and a surfactant. SNEDDS oil can increase the ability to carry drugs but causes nanoemulsion size becomes larger so that the ratio of oil to be used is always smaller than the oil[20].PI value (polidispersity index) states nanoemulsi particle homogeneity. PI value varies from 0.0 to 1.0 and a value of 0, the closer the more homogeneous particle[21]. In this research PI value 0,21.PI values obtained from testing the size and particle size distribution of distilled water media. It concluded that nanoemulsi a uniform particle size distribution and a method of making nanoemulsi have good reliability. Average size was 11.3 nm.

Zeta potential in this research is-5,07. Value in the range of ± 30 mV is the limit value capable of maintaining the stability of the emulsion because the value is close to neutral reduce the likelihood of the particles to form aggregates.

8. Observations Stability Nanoemulsion

Physical stability is an important parameter that must be met for optimum formula SNEDDS illustrate the feasibility of the preparation on storage. SNEDDS preparation stability was tested by observing the physical stability of the preparation with the active substance after storage at room temperature for 3months.

Table 1.Testing Results Surfactants and co-surfactant solubility Ethyl Acetate Fraction AgainstSkin Mangosteen

Oils	Oilcontentsof mangosteen peelethyl acetate fraction(mg /10mL)
Citrus Oil	0,98
Corn Oil	0,19
Virgin Coconut Oil	1,56
Canola Oil	0,21
Soy Bean Oil	0,32
Sun Flower Oil	0,23
Kanthil Oil	0,34

Table 2.Testing Results Surfactants and co-surfactant solubility Ethyl Acetate Fraction AgainstSkin Mangosteen

Surfactant and Co-surfactant	Surfactants and co-surfactantcontentsof mangosteen peelethyl acetate fraction (mg /10mL)
Tween 20	8,2
Tween 80	8,3*
Span 20	8,2
Cremophor El 40	7,6
PEG 400	8,13*
Propilenglikol	8,07

Table 3. Formula and evaluation with simplex lattice design model

No.	Tween 80	PEG 400	VCO	Transmitan (%)	Emulsification time (seconds)	PH	Drug Loading
1	5	1	1	67,89	4,7	6,28	99,15
2	1	1	5	3,21	8,2	7,06	99,23
3	3	3	1	43,74	6,4	4,92	98,87
4	1	1	5	1,8	8,9	7,26	99,25
5	2,33	2,33	2,33	72,11	5,5	6,23	99,36
6	5	1	1	71,95	4,3	6,05	99,26
7	1,67	1,67	3,67	6,48	3	6,32	99,13
8	1	3	3	11,29	7,1	6,78	98,74
9	3,67	1,67	1,67	47	6,4	4,82	98,68
10	1,67	3,67	1,67	3,23	3,9	6,41	99,19
11	3	1	3	32,94	3,3	4,44	98,53
12	3	3	1	52,18	6,3	4,82	99,09
13	1	5	1	1,95	3,7	4,79	99,34
14	1	5	1	1,9	3,2	4,95	99,35

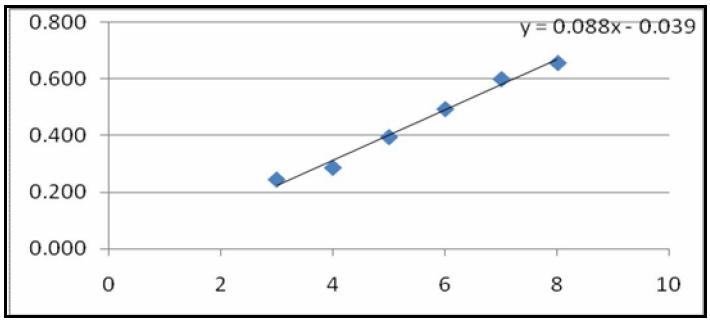
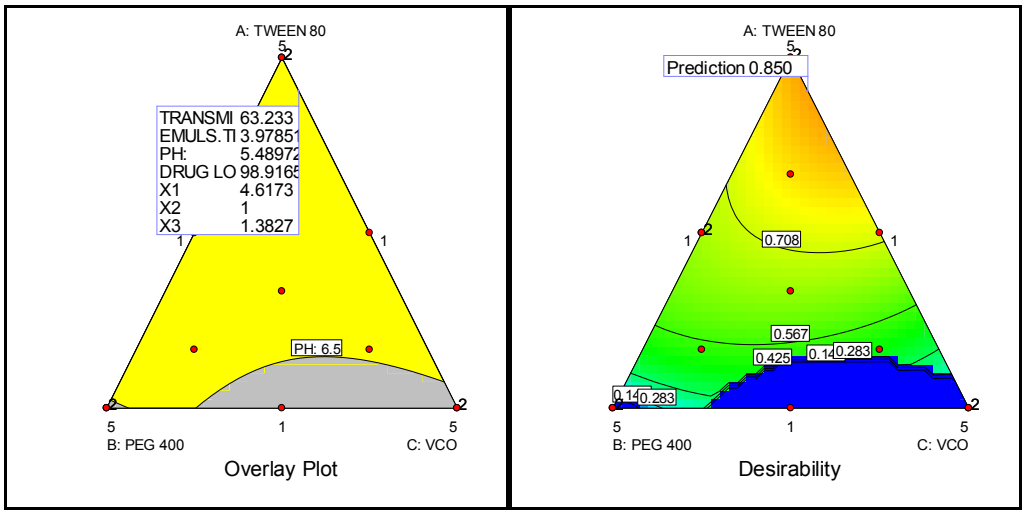


Figure 1. Equation of calibration curve of α-mangostin standard using Spectrophotometer method



a b

Figure 2. Ternary Phase Diagram of Ethyl Acetat Fraction Mangosteen Peel Optimum

9. Conclusion

This study report an approach on the use of a Simplex lattice design and response surface methodology in the optimization of self nanoemulsifying drug delivery system of EAF-MPfor evaluation. This study demonstrates the suitability of SNEDDS as a topical delivery system for EAF-MP. Based on the solubility test, the active ingredient chosen is EAF-MP, oil select is the VCO, tween 80 as surfactant selected and PEG 400 as cosurfactant. Following optimization, EAF-MP loaded SNEDDS composed of EAF (150 mg), Tween 80 (4,61%), PEG 400 (1%) and VCO (1,38%) was selected. Transmittance, emulsification time, pH, drug loading, mean droplet size, potential zeta assay. Under the experimental conditions used in this study, formulations containing fraction up to 150 mg remained monodispersed exhibiting a droplet size at the 11,3nm range. SNEDDS ethyl acetate fraction characterized and showed good emulsification time, also the particle size and zeta potential. Also showing good physicochemical stability after long-term storage conditions.

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