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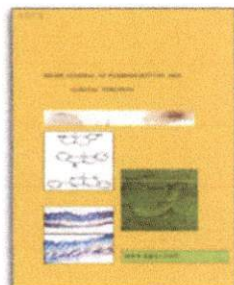
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## PHYSICAL AND CHEMICAL CHARACTERISTICS OF MELOXICAM FROM NANOSTRUCTURED LIPID CARRIERS SYSTEM USING SOME CONCENTRATION RATIOS OF MONOSTEARIN AND ALPHA-TOCOPHEROL ACETATE LIPID MATRIX

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### ABSTRACT

**Objective:** The objective of this research is to develop nanostructured lipid carriers (NLC) of meloxicam (MLX) for topical application using monostearin and alpha-tocopherol as lipid matrix.

**Methods:** MLX-NLC was prepared by high-shear homogenization, and it was characterized for organoleptic, pH, viscosity, particle size and size distribution, zeta potential, crystallinity, morphology, and entrapment efficiency. The six different ratios of solid and liquid lipid matrix were 70:30, 75:25, 80:20, 85:15, 90:10, and 95:5, respectively.

**Results:** The pH value of all formulas met the pH range of topical dosage at 4.5-6.5. Viscosity test showed that a trend toward the decrease in viscosity with the increase in alpha-tocopherol content was observed. The results of particle size test proved that all six NLC system formulas have <1000 nm particle size with a quite narrow particle size distribution. Scanning electron microscope revealed nearly spherical shape NLC with negligible effect ratios of solid and liquid lipid on the particle morphology. The X-ray diffraction result showed the decreasing of peaks intensity of MLX-NLC system. It showed that MLX in NLC system is amorphous. The result showed that the increasing concentration of liquid lipid in NLC system caused the rising entrapment efficiency of MLX.

**Conclusion:** The result of this research indicates that the concentration ratio of monostearin and alpha-tocopherol acetate affects the physical and chemical characteristics of NLC system.

**Keywords:** Nanostructured lipid carriers, Meloxicam, Monostearin, Physical and chemical characteristics, Entrapment efficiency.

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### INTRODUCTION

Meloxicam (MLX) is a non-steroidal anti-inflammatory drug, which inhibits cyclooxygenase-2 selectively, used orally to reduce the symptoms of rheumatoid arthritis. However, the use of oral MLX still showed gastrointestinal side effects, the risk of arterial thrombotic events, kidney malfunction, liver dysfunction at high doses, and long-term treatment. Topical route administration is an alternative to overcome these problems and also provides many advantages, including avoiding gastrointestinal irritation, systemic toxicity is minimal, avoiding hepatic metabolism, the plasma level is stable, and improving patient compliance [1].

MLX is classified as a BCS Class II drug (high permeability and low solubility), has a log p=3.42, and a poor wettability in water, causing difficulties in the pharmaceutical formulations design. Besides, the skin barrier properties also limit the permeability of various pharmaceutical active substances, requiring appropriate drug delivery systems to produce optimal therapeutic effect [2,3].

Nanostructured lipid carriers (NLC) have many advantages such as occlusion and skin hydration, absorption-increasing effects, active penetration enhancement, and controlled release properties. NLC was developed by exchanging the liquid lipid (oil) of oil-in-water (o/w) emulsions by a solid lipid, which can bring many advantages in comparison with a liquid core. The concept of NLC was developed by nanostructuring the lipid matrix, to give more flexibility for modulation

of drug release, increasing the drug loading, and preventing its leakage. The result is a less ordered lipid matrix with many imperfections, which can accommodate a higher amount of drug. The other advantages of using lipids as carrier systems for skin administration are related to their physiological and well-tolerated nature, which reduces the risk of toxicological problems and local irritancy. NLC is a lipid nanoparticle that can increase the penetration of lipophilic drug substance as it has occlusive properties and improves skin hydration. Therefore, MLX is encapsulated in the NLC system to increase its penetration into the skin [4].

### METHODS

#### Materials

MLX was a gift sample from Apex Healthcare Limited; Monostearin (Cutina® GMS) was obtained from BASF; alpha-tocopherol acetate was obtained from Xinchang pharma; Kolliphor® P 188 (poloxamer 188) was obtained from BASF; Tween 80 was obtained from PT KAO; Ethanol p.a., sodium hydroxide, and NaH<sub>2</sub>PO<sub>4</sub> (natrium dehydrogenate phosphate) p.a. were a product of Merck. Aquabidestilata was gift sample from PT. Widatra Bhakti, Pasuruan, Indonesia. All other ingredients used were analytical grade.

#### Methods

##### Preparation of MLX-NLC system

The method of this research is high-shear homogenization. MLX-NLC system was made by melting the lipid phase (monostearin and

$\alpha$ -tocopherol) with different lipid ratio (70:30, 75:25, 80:20, 85:15, 90:10, and 95:5), and MLX at 65°C temperature. At the same time, the surfactant solution (Kolliphor® P 188, Tween 80 and pH phosphate buffer 6.0±0.05) was prepared and heated at the same temperature. Then, this heated surfactant solution is dispersed into heated lipid phase using ultra-turrax at 5000 rpm speed for 15 minutes. Furthermore, this surfactant solution was added with phosphate buffer with 6.0±0.05 pH value until the volume reaches 100% b/b. Subsequently, the NLC dispersions were formed simply by cooling the warm pre-emulsion precursor to room temperature in the same container [5,6]. The composition of MLX-NLC system is shown in Table 1.

#### Physical and chemical characterization of MLX-NLC system

##### Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was conducted in the wave number range 4000-400/cm. The sample was combined with KBr and pressed into a pellet. The solid pellet was analyzed using FTIR spectroscopy [7].

##### pH

The electrode was inserted into 10 mL MLX-NLC system and then the number that is shown by pH meter must be written.

##### Viscosity

This viscosity evaluation has done to know the thickness of MLX-NLC system that is resulted from adding other materials, such as surfactants, and the effect of making technique. Viscosity measurement used cone and plate as the tools of the viscometer. 2.0 mL of MLX-NLC system placed in the sample cup, and then the viscometer is turned on and puts it down at a moment until the value is stable.

##### Particle size and size distribution

The examination of particle size and size distribution has done by Delsa™ Nano Submicron Particle Size Analyzer. Then, the tool is turned on and particle size menu is selected. The tool will measure it over 10 minutes. The observed data are the average droplet diameter and polydispersity index (PI). PI illustrates the variation on a sample. The small value of PI (<0.3) indicates that the sample is monodisperse.

##### Zeta potential

Zeta potential was measured using Malvern Instrument® with cell and the appropriate procedure. The instrument can measure on 0.001-40% concentration. The sample is dispersed into the water until it gets the concentration on the optimum intensity of the instrument. The suspension was sonicated for approximately 2 minutes to break agglomerate, and then it is measured.

##### Particle morphology

Particle morphology examination has done by scanning electron microscopy (SEM). This evaluation used to determine the form of particle contained in NLC. The MLX-NLC system was dispersed in CMC-Na gel and spread on glass plates to produce dry NLC system. Then, the dry NLC system was coated with Aurum before counted in vacuum condition.

#### Crystallinity

##### Differential scanning calorimetry (DSC)

Melting temperature measurement has done by DSC. The characterization using this instrument has done by weighing the sample of MLX and monostearin amount at 2-8 mg. Then, this powder inserted into an aluminum pan that is impermeable to air. This pan is heated to the temperature 30-300°C at calorimeter with the temperature rise 10°C every minute.

##### X-ray diffraction (XRD)

The sample is inserted to the holder glass, and the surface is trimmed by plate glass. This sample is placed in X-ray Philips X'Pert diffractometer. The measurement condition was: 40 kV voltage; 30 mA; Divergence slit size 1.0°. Then, it observed over a range of 2 $\theta$  angle from 5° to 50°; 0.15 mm receiving slit. Then, it observed over a range of 2 $\theta$  angle from 5° to 50°.

##### Entrapment efficiency of MLX

Drug E.E. was calculated by determining the amount of drug untrapped (Cf) after removal of NLC system by centrifugation, MLX which is untrapped in NLC system would be dispersed in phosphate buffer with pH 7.4±0.05 as a supernatant. The obtained supernatant was filtered using Millipore Whatman 0.2  $\mu$ m filter paper. This evaluation used UV spectrophotometer. Furthermore, it is calculated using the following formula:

$$EE(\%) = \left( \frac{C_t - C_f}{C_t} \right) \times 100\%$$

Where C<sub>t</sub> is the total amount of drug added in the formulation. The concentration of drug was determined by double-beam ultraviolet (UV)-vis spectrophotometer (Shimadzu UV-1800) with multiple wavelength methods [8].

#### Statistical analysis

Characterization evaluation (pH, viscosity, particle size, zeta potential, and entrapment efficiency of drug) of MLX-NLC system has statistically analyzed using one-way multivariate analysis of variance (MANOVA) method at 95% level. Then, significant difference of NLC system formula was obtained by honestly significant difference test.

## RESULTS AND DISCUSSION

### Physical and chemical characterization of MLX-NLC system

#### FTIR spectroscopy

The analysis of functional group change in MLX-NLC system was done by comparing the IR spectrum of MLX-NLC system with the material IR spectrum, as shown in Fig. 1.

MLX-NLC system with some ratios of the lipid matrix concentration has a similar FTIR spectrum profile. This condition shows that there is no difference in the interaction of some components when forming the NLC system. The characteristic peak of NH<sub>3</sub> and SO<sub>2</sub> group does not appear in the FTIR spectrum of MLX-NLC system. This condition shows that there was a possibility of intermolecular hydrogen bonds between N-H and S=O group with a hydroxyl group of some components. In

Table 1: Composition of MLX-NLC system

Formulation code	MLX (%b/b)	Monostearin (%b/b)	Alpha-copherol acetate (%b/b)	Tween 80 (%b/b)	Kolliphor 188 (%b/b)
F1	1	70	30	3	3
F2	1	75	25	3	3
F3	1	80	20	3	3
F4	1	85	15	3	3
F5	1	90	10	3	3
F6	1	95	5	3	3

NLC: Nanostructured lipid carriers, MLX: Meloxicam



addition, there is no new characteristic peak that appears in the FTIR spectrum of MLX-NLC system. Based on FTIR spectrum result, it could be seen that there was no chemical interaction between MLX with the constituent component of NLC system. There was only physical reaction in NLC system formation. The formation of MLX-NLC system with lipid matrix of monostearin and alpha-tocopherol acetate can be identified from NH<sub>3</sub> group and SO<sub>2</sub> group intensity lost of MLX [7].

**pH**

The pH parameter of MLX-NLC system has a connection with the pH stability of active ingredient and the convenience in topical administration. Topical dosage forms pH applied on the skin should have a value of 4.5-6.5 [9].

The obtained NLC system had a pH value in the range of 5.98-6.07. The histogram of average pH in some formulas of NLC system is shown in Fig. 2.

Statistical analysis using MANOVA showed that there was no significant difference between pH in each formula of NLC system which uses

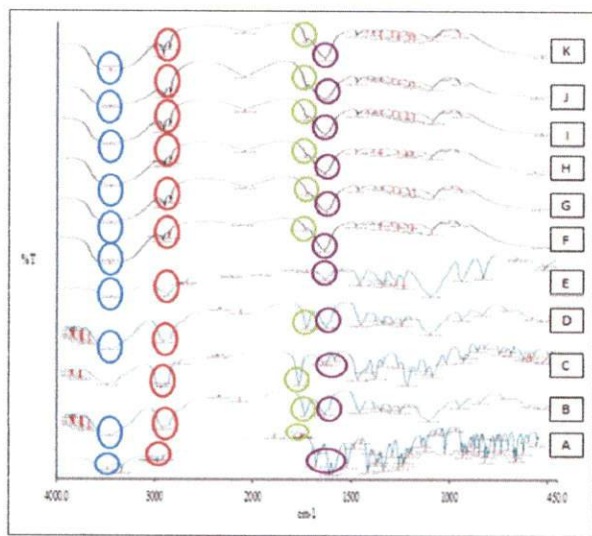


Fig. 1: Fourier transform infrared spectrum of, (a) meloxicam, (b) monostearin, (c) alpha tocopherol acetate, (d) tween 80, (e) poloxamer 188, (f) formula 1 (70:30), (g) formula 2 (75:25), (h) formula 3 (80:20), (i) formula 4 (85:15), (j) formula 5 (90:10), (k) formula 6 (95:5)

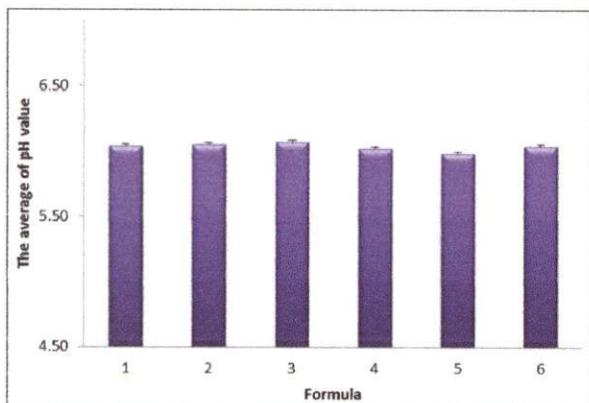


Fig. 2: The histogram of average pH in some formulas of nanostructured lipid carriers system. The data obtained is the average of the pH value ×3 replication formula 3±standard deviation

multiple ratios of lipid matrix which is about 70:30; 75:25; 80:20; 85:15; 90:10; and 95:5 (\*p>0.05). Based on these results, it could be concluded that the ratios of solid and liquid lipid matrix did not affect the pH value of NLC system.

**Viscosity test**

MLX-NLC system which has been produced had viscosity values from 57.87 to 162.67 c.Ps. The histogram of average viscosity in some formulas of NLC system is shown in Fig. 3.

Statistical analysis using MANOVA showed that there were significant differences (\*p<0.05). From *post-hoc* test result, it could be known that F1 is not significantly different with F2, and F2 is not significantly different with F1 and F3, and then F4 is significantly different with all formulas; moreover, F5 is not significantly different with F6. From this result, it can be proven that the increased number of liquid lipid which is added to the formula is capable to decrease the viscosity of NLC system.

Viscosity is a barrier of liquid to flow, if the viscosity of the sample is higher and the barrier to flow is also higher [10]. The viscosity of NLC system is affected by the composition of NLC system formula.

**Particle size and size distribution of NLC**

From the particle examination result of all six NLC system formulas, the particle size was about <1000 nm. The particle size of MLX-NLC system was about 431.1-515.3 nm. In addition, the histogram of average particle size in some NLC formulas is shown in Fig. 4.

Statistical analysis of particle size using MANOVA showed that there was no significant difference between the particle size in each formula of NLC system which uses multiple ratios of lipid matrix 70:30; 75:25;

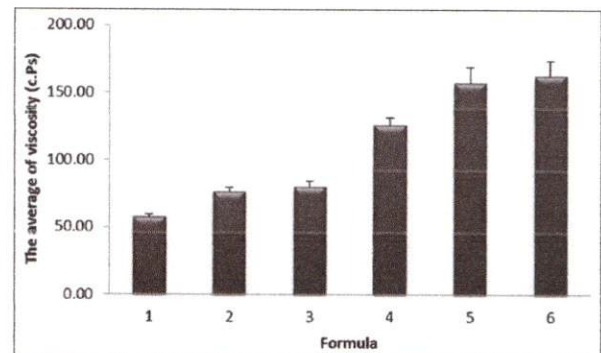


Fig. 3: The histogram of average viscosity in some formulas of nanostructured lipid carriers system. The data obtained is the average of the pH value ×3 replication formula 3±standard deviation

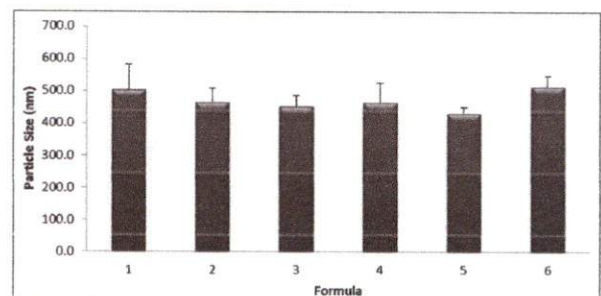


Fig. 4: The histogram of average particle size in some nanostructured lipid carriers formulas in aqueous media using Delsa Nano®,. The data obtained is the average of the pH value ×3 replication formula 3±standard deviation

80:20; 85:15; 90:10; and 95:5 (\*p>0.05). In this study, the results are not in accordance with Khurana *et al.* who found that particle size of NLC prepared using cetyl palmitate (solid lipid) and caprylic acid (liquid lipid) depended on liquid lipid content. A trend toward the decrease in particle size with the increase in content was observed [8]. This is possible because the lipid matrix of different components with different characteristics may give different results. Cetyl palmitate is a wax lipid group, has a highly ordered crystal lattice and monostearin a class of glycerides having less ordered crystal lattice [11,12]. Differences in chemistry, physics, and crystallographic characteristics of the system will affect the characteristics of NLC [11].

Statistical analysis of PI using MANOVA showed that there were significant differences between PI in each formula of NLC system which uses multiple ratios of lipid matrix 70:30; 75:25; 80:20; 85:15; 90:10; and 95:5 (\*p<0.05). From *post-hoc* test result, it could be known that F5 is significantly different with F1, F3, and F6. However, F6 is significantly different with F2. In addition, F1 and F6 have PI value which is larger than the other formulas. Moreover, F2, F3, F4, and F5 have more homogeneous particle size and they have particle size distribution which is narrower than the F1 and F6. F1 and F6 have broader particle size distribution than the others formula. This is possible because the F1 has a lower viscosity than other formula NLC systems, Stokes's law states that the value of the viscosity is inversely proportional to the speed of sedimentation [10]. Sedimentation velocity describes as the speed of formation of agglomerates. So that on systems with lower viscosity will be easier to occurrence of agglomeration. This resulted in the F1 is obtained PI value greater than other formulas. The high value of PI F6 is possible because most of the components of the lipid matrix is monostearin. Monostearin powder is generally in  $\beta$ -polymorph form and it will transform to  $\alpha$ -polymorph after going through the process of melting lipid.  $\alpha$ -polymorphic form monostearin is metastable, which will transform into  $\beta$ -polymorph at room temperature. The phenomenon of polymorph rapid change affects the instability of NLC system, such as the transformation of structure particle, agglomeration, and sedimentation in aqueous phase [13]. This condition can affect the formation of NLC system with polydisperse particle size distribution and higher tendency to agglomeration.

**Zeta potential**

Zeta potential measurement is an indirect measurement of NLC physical stability; it also affects the release kinetics and biological process of a nanoparticle. Zeta potential is the electrical potential on sliding area, which is defined as the electrical charge of outer particle surface where the opposite charge still able to strongly bound with other particles when they move in an electrical field.

Zeta potential measurement obtained 10.4-16.5 as a negative value. The histogram of average zeta potential in all NLC system is shown in Fig. 5.

Based on MANOVA, statistical analysis result showed that there are significant differences between zeta potential in each formula of NLC system that uses multiple ratios of lipid matrix 70:30; 75:25; 80:20; 85:15; 90:10; and 95:5 (\*p<0.05). On *post-hoc* analysis, it could be known that there was a difference between F1 and F6 with F2, F3, F4, and F5. The result was almost equal to the particular size and PI results, where F1 and F6 had smaller zeta potential than the other formulas. On the other hand, the value of particle size and PI was larger than the other four formulas.

**The examination of crystallinity**

*The X-ray diffraction data*

X-ray diffraction analysis is used to know the crystallinity change of active substance and lipid component in NLC system. X-ray diffraction's pattern of MLX, monostearin, and some NLC formulas are shown in Fig. 6.

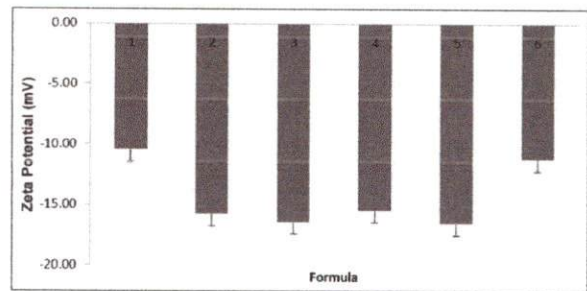
Based on the X-ray diffraction result, the diffractogram of MLX has some sharp peak at 25°, 15°, 18°, 19°, and it has some wide specific peak at

13° and 23°. Moreover, monostearin has some sharp specific peak at 5°, 19° and wide specific peak at 23°. At the results of all formulas with different ratio of solid lipid and liquid lipid, it was known that NLC system's peak intensity has decreased. The result showed that NLC system was amorphous. The condition indicated that MLX was entrapped in NLC system.

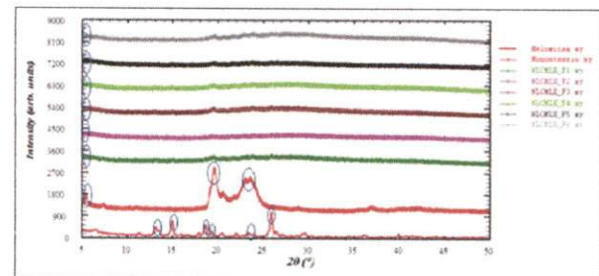
**DSC**

DSC analysis provides data such as thermogram to determine the melting point of a sample. The result of these data is shown in Fig. 7.

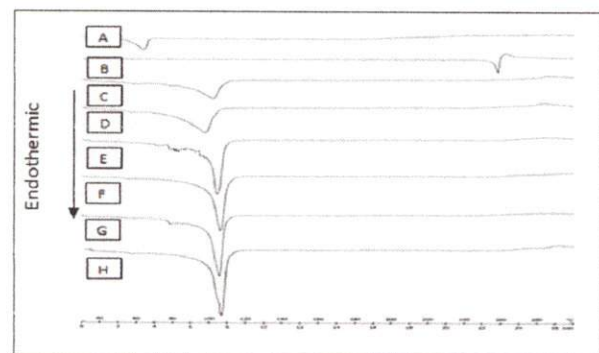
The melting point of NLC has decreased when it is compared with the melting point of the bulk lipid. This decrease occurs because there is a foreign molecule (molecule of the active substance or surfactant) which is dissolved in the lipid matrix. The melting point and crystallinity will



**Fig. 5: The histogram of some nanostructured lipid carriers formulas average zeta potential in aqueous media using Malvern Instrument®. The data obtained is the average of the pH value x3 replication formula 3±standard deviation**



**Fig. 6: The Examination result of meloxicam, monostearin, formula 1 (7:3), formula 2 (7.5:2.5), formula 3 (8:2), formula 4 (8.5:1.5), formula 5 (9:1), and formula 6 (9.5:0.5) X-ray diffraction**



**Fig. 7: The examination result of differential scanning calorimetry, (a) monostearin, (b) meloxicam, (c) formula 3 (8:2), (d) formula 5 (9:1), (e) formula 4 (8.5:1.5), (f) formula 6 (9.5:0.5), (g) formula 2 (7.5:2.5), (h) formula 1 (7:3)**

change after they are formed in NLC system. The DSC of NLC system result shows that enthalpy result ( $\Delta H$ ) is lower than enthalpy ( $\Delta H$ ) bulk lipid [4]. Based on DSC result, the melting point of NLC system was bigger than the bulk lipid. This result is opposite with NLC system's theory. This condition is possible because MLX active substance (257.34°C) has higher melting point than the solid lipid (63.58°C); it affects the melting point of NLC system. This research used MLX as a drug model; it is because MLX has some specific sharp peak which show that MLX is a crystalline drug (based on the X-ray analysis results). In literature, it is mentioned that if there is a crystalline drug in particle or a dispersion, DSC method cannot be used to know the changes of melting characteristics of NLC system compared with bulk lipid. A small amount of crystalline active substance can produce a wide area [14]. Therefore, it is possible that the crystallinity of active substance affects melting characteristics of NLC system and influences the DSC result inappropriate with the NLC's theory. The DSC result showed that the melting point has decreased when it was compared with the melting point of active substance. In F1 and F6, the melting point was higher than the other formulas. This condition could happen because of particle size effect. The decrease of melting point in NLC system is determined by the particle size which is getting smaller. Therefore, this phenomenon is called the "effect of particle size." The decrease of melting point can be described by Gibbs-Thomson equation [5].

#### The examination of particle morphology in NLC system

The examination result of particle morphology in NLC using SEM is shown in Fig. 8.

Based on the observation of particle morphology using SEM with a magnification of 10,000 times, each formula showed that the particle is almost spherical shaped. This particle size could produce the controlled release and avoid the agglomeration. A particle with a sphere shape has a little contact with media which causes longer diffusion process than the non-spherical particle. The spherical surface particle has little contact between particles that causes smaller agglomeration risk.

#### MLX entrapment efficiency

In a number of drugs, the solubility in liquid lipid is greater than in solid lipid, and the addition of liquid lipid to the solid lipid can damage the crystal lattice in the matrix of lipid nanoparticle, and then it can increase the entrapment efficiency of active ingredient [15]. Entrapment efficiency increases by increasing the concentration of liquid lipid. In NLC system, high solubility of active ingredient in liquid lipid plays an important role in increasing the entrapment efficiency of active ingredient [8].

The measurement of entrapment efficiency in the sixth formula of NLC system showed the high result (>70%). The measurement result of entrapment efficiency of MLX in NLC system was 74.50-86.22. The histogram of average percentage entrapment efficiency of some NLC formulas is shown in Fig. 9.

Statistical analysis of particle size using MANOVA showed that there was a significant difference between the formulas of NLC system. From *post-hoc* test, it could be known that there were significant differences between the percentages of entrapment efficiency in F1 and F2 with F3, F4, F5, and F6. F1 had the highest percentage of entrapment efficiency, and F6 had the lowest percentage of entrapment efficiency. This result is consistent with the concentration theory of liquid lipid which can produce the big entrapment efficiency, because of the active substance solubility in liquid lipid increases [8].

#### CONCLUSION

The MLX-NLC system with monostearin and alpha-tocopherol acetate as lipid matrix is characterized by small particle size, low crystallinity, spherical morphology of particle, and high MLX entrapment efficiency. Increasing the liquid lipid concentration can reduce the viscosity of the NLC system and improve the entrapment efficiency while there is no effect on the particle size and morphology characteristics. Concentration

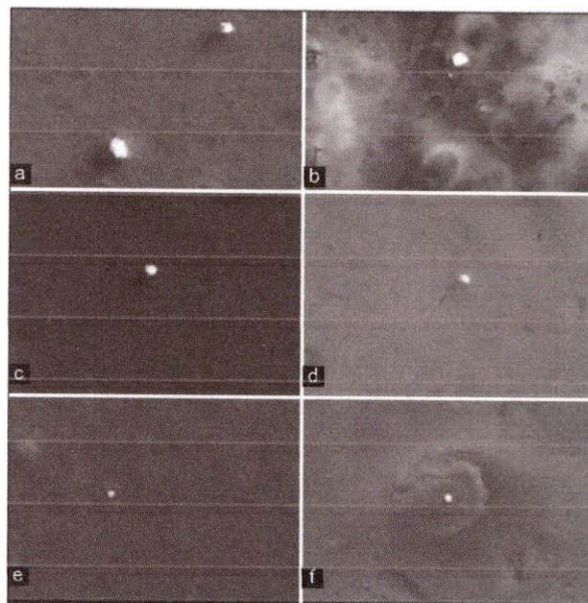


Fig. 8: The test result of particle morphology in nanostructured lipid carriers system with  $\times 10,000$  magnification using scanning electron microscopy. (a) Formula 1 (7:3), (b) formula 2 (7.5:2.5), (c) formula 3 (8:2), (d) formula 4 (8.5:1.5), (e) formula 5 (9:1), (f) formula 6 (9.5:0.5)

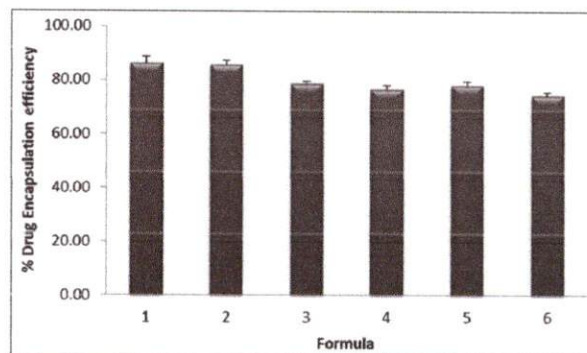


Fig. 9: The histogram of average % entrapment efficiency of all nanostructured lipid carriers' system formulas. The data obtained is the average of the pH value  $\times 3$  replication formula  $3 \pm$  standard deviation

ratio also affects the pH, PI, and the zeta potential, but the value is not equal with the addition of a lipid component. The results were possible because of the nature of the solid lipid.

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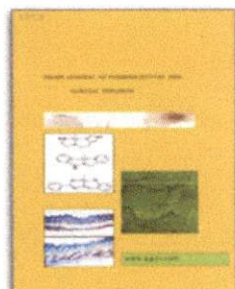
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