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Formulation and Characterization of Glibenclamide Solid Lipid Nanoparticles Formatted by Virgin Coconut Oil and Solid Matrix Surfactant

Mardiyanto[1]*, Najma Annuria Fithri[1], Annisa Amriani[1], Herlina[1], Dwi Purnama Sari[1]

[1]*Department of Pharmacy, Faculty of Mathematics and Natural Science, Sriwijaya University*
Corresponding author: mardiyanto@mipa.unsri.ac.id

Abstract

Glibenclamide has the biopharmaceutics classification system (BCS) class II which has high permeability and low solubility. The solubility of glibenclamide can be enhanced by forming solid lipid nanoparticles (SLN). This research has the aim to prepare and characterize SLN loading glibenclamide. The glibenclamide SLN formula was composed by using the liquid lipid as virgin coconut oil (VCO), PEG 6000 as a solid matrix, tween 80 with various concentrations as a stabilizer, and PEG 400 as co-surfactant. Characterization was conducted by determining the encapsulation efficiency (%EE), size measurement, particle size distribution, and zeta potential of SLN glibenclamide. SLN formation was also tested for its physical stability based on the heating-cooling cycle method. The optimum formula was obtained at the concentration of tween-80 of 1 mg/mL yielding the %EE value of 60.6194%, and pH 6.01. The results of particles diameter analysis were 175.5 ± 10.07 nm with a polydispersity index (PDI) of 0.1270, and zeta potential of +5.9 mV respectively. Stability testing by the heating-cooling cycle method has shown the instability of the SLN glibenclamide form under extreme temperatures and mechanics. It could be concluded that the results of characterization of glibenclamide SLN showed appropriate physical properties for nanoparticulate formulation.

Keywords

glibenclamide, liquid-lipid, solid lipid nanoparticles, PEG-400, PEG 6000

1. INTRODUCTION

Previous studies reported that glibenclamide has been formulated in the form of liposomes, polymeric particles, and patches. The formulation of the lipid particles loaded glibenclamide for antidiabetics has also been investigated using solid lipids but there was not reported yet regarding formulations with liquid lipids (Li et al., 2015; Maretti et al., 2021; Gonçalves et al., 2016). Diabetes Mellitus is one of the most common diseases in the world. Million people with diabetes who are young or old of the world's population indicated that the many people around suffer from diabetics (Boukhors et al., 2003). The number of diabetics in the world is tended to high and the prevalence of diabetes is increasing in line with changes in people's lifestyles which tend to be consumptive and has lacked physical activity. There are several types of diabetics which are known as Diabetes Mellitus (DM) types 1 and 2 also gestational diabetics (Amin et al., 2020; Colmers et al., 2012).

Medicine containing glibenclamide (Figure 1) is used to reduce blood glucose levels in type 2 diabetics by stimulating insulin secretion and are oral hypoglycemic drugs of sulfonylurea groups (Yazar et al., 2002). The available glibenclamide
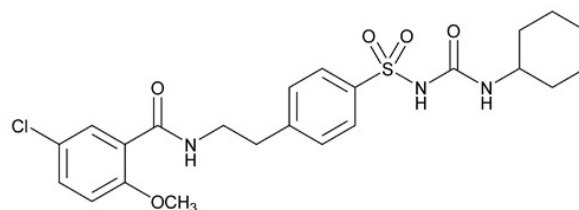
Figure 1. Molecular Structure of Glibenclamide

preparations on the market are as the solid form of tablets but in the form of a solution is not yet available because of the solubility of glibenclamide. Another weakness of glibenclamide is several side effects of the usage i.e. liver and kidney disorders can lead to decreased gluconeogenesis capacity. Besides that, glibenclamide can also interact strongly with such as non-steroidal anti-inflammatory drugs and other drugs that have high protein bonds. A high risk of hypoglycemia also occurs in the geriatric class, nutritional and adrenal disorders, or adrenal insufficiency and combination therapy (Diwan et al.,

2014; Senior et al., 2020).

According to the distribution of glibenclamide in the human body, glibenclamide belongs to Class II Biopharmaceutical Classification System (BCS) which has high permeability but low solubility (Lindenberg et al., 2004). The high permeability of glibenclamide leads to several side effects. The absorption process of drugs included in BCS class II is blocked by the drug dissolution stage so that the bioavailability is reduced which makes it difficult to absorb because of solubility. The preparation of solid lipid nanoparticles is one of the progressive ways to increase the solubility of active pharmaceutical ingredients. The particle size directly affects the drug delivery system as well as can provide pharmacological effects at smaller doses making it more efficient to use (Maritim et al., 2021).

The development of colloid delivery systems such as matrix, micelles, and nanoparticles aims to improve drug delivery. Nanoparticles with special characteristics ie small particle size, large surface area, and the ability to change the surface properties have many advantages over other delivery systems. Smaller particles also have a greater risk of aggregation during the storage and transport of nanoparticle dispersions (Gonçalves et al., 2016). Formulations of the smallest possible size and maximum stability are the challenge of nanoparticle preparations. So far nanoparticle preparations are often prepared with chitosan, poly(lactic-co-glycolic acid) (PLGA), polycarpolactone, and polylactic acid as carriers commonly referred to as polymeric nanoparticles (Ali and Hanafy, 2017). However, nanoparticles may also be prepared using lipids as carriers such as solid lipid nanoparticles (SLN).

Preparation SLN is to combine the advantages and avoid the weaknesses of other colloidal nanoparticles such as physical stability, drug protection from degradation, controlled release, and good tolerance. Some of the advantages of SLN are the use of biodegradable physiological lipids to reduce acute and chronic toxicity and to avoid organic solvent in the production method and can increase the bioavailability of drug molecules that have poor water solubility (Hanafy et al., 2007; Jensen et al., 2010; Rupenagunta et al., 2011).

The formula of glibenclamide SLN using VCO (Virgin Coconut Oil) as a carrier with a variation concentration of tween 80 as a stabilizer is to obtain the optimum formula which reveals the stability. The size of the SLN was influenced by the variation of stabilizer concentration. Tween 80 is a non-ionic surfactant that can reduce surface tension while maintaining physical stability of the preparation, thereby increasing the efficiency of encapsulation (%EE) (Sarathchandiran, 2012).

Tween 80 at concentrations of $1 - 3\%$ of the weight of the formula can result in the size of nano-sized particles in the range of $100 - 1000$ nm. Variations of tween 80 were performed to obtain an optimum formula that has a small size and a high %EE. VCO is one of the safest lipids consumed by DM patients. The long-chain fatty acids contained in the VCO are not stored in body tissues but are directly converted into energy. Polyethylene glycol (PEG) 6000 is used as a solid matrix to obtain solid lipid particles, whereas PEG 400 is used

as a co-surfactant to increase drug solubility. The compact PEG 6000 at room temperature is similar to that of solid lipids such as stearic acid which is also solid at room temperature. The melting point of PEG 6000 is between $55 - 63°C$. PEG 6000 can minimize drug loss and also has an advantage for oral drug delivery (Li et al., 2015).

Based on the above description, this research evaluated the formula of glibenclamide SLN of various nanoscale sizes and small size distributions. Characterization of the particle was performed among others: diameter, distribution, and particle size, potential zeta value, and %EE. The physical stability was tested based on the heating-cooling method and mechanical test.

2. EXPERIMENTAL SECTION

2.1 Materials
The materials that were used in this research were glibenclamide (from First MediPharm®), virgin coconut oil (Optima®), tween 80 (Merc®), polyethylene glycol 6000 (Merc®), PEG 400 (Merc®), ethanol 96% (Merc®), and WFI water for injection (Otsuka®).

2.2 Methods
2.2.1 Glibenclamide Preparation
VCO (Virgin Coconut Oil) was melted at 75°C. Furthermore, glibenclamide powder were weighed. Glibenclamide was added into the VCO and stirred until homogeneous using a magnetic stirrer (IKA® C-MAG) at 1000 rpm for 1 hour.

2.2.2 Aquoeus Phase Preparation
The Tween 80, PEG 6000, PEG 400 were weighed according to the formula. After that, Tween 80 and PEG 400 were mixed. PEG 6000 was melted then added the mixture of Tween 80 and PEG 400. All materials were stirred at 1000 rpm for 1 hour at 75°C.

2.2.3 Formula
The formula which was used in this study were three using a variation on the concentration of stabilizer (Fini et al., 2005; Fu et al., 2014).

Table 1. Formula of Glibenclamide SLN

| Ingredients | Function | Concentration (mg/mL) | | |
		F1	F2	F3
GLB	Active substance	0.1	0.1	0.1
VCO	Lipid	1	1	1
Tween 80	Stabilizer	1	1.2	1.4
PEG 6000	Matrix solid	0.5	0.5	0.5
PEG 400	Co-surfactant	0.5	0.5	0.5
WFI (up to)	Solvent	100 mL	100 mL	100 mL

GLB (glibenklamid); VCO (virgin coconut oil), WFI (water for injection)

Based on Table 1, the concentration of Glibenclamide was used at 0.1 g. SLN was a dispersion that typically has a water content of $70 - 99\%$ (w/w) and comprises from 0.1 to 3 (mg/mL) lipids dispersed in aqueous media. Necessity usage of stabilizer as, a surfactant is preferably from 0.5 to 5 (mg/mL).

Based on previous research informed that the optimum formula that has the concentration of Tween 80 as much as 1 mg/mL. Tween 80 concentration variations used were 1, 1.2 and 1.4 mg/mL. The concentration of VCO used is 1 mg/mL of the optimum formula. The concentration of PEG 400 as much as 0.5 mg/mL.

2.2.4 Preparation of Solid Lipid Nanoparticles

The preparation of solid lipid nanoparticles was initiated with preparing the lipid phase (Gonçalves et al., 2016). The lipid phase preparation was performed by dispersing glibenclamide powder into a heated VCO above the water bath (Memmert®) at 75°C. Furthermore, making the water phase by dissolving Tween 80, PEG 6000, and PEG 400 was stirred (1000 rpm) for 1 hour with a temperature of 75°C. The lipid phase was then dispersed into the water phase drop by drop. The mixture was stirred using the magnetic stirrer for 6 hours. The emulsion formed was dispersed into an ad WFI of 100 mL, then homogenized using a bath sonicator (Elmasonic® S180H) for 10 min to form a homogeneous SLN dispersion. The SLN dispersion results were stored in a sealed the light-shielded container at room temperature.

2.2.5 Characterization of Solid Lipid Nanoparticles

2.2.5.1 Purification and Determination of Encapsulation Efficiency (EE)

SLN samples of 10 mL were centrifuged (Hettich EBA BS®) at 12,000 rpm for 30 min so that 2 phase phases were absorbed and the unabsorbed phases were obtained. Performing phase separation that was not absorbed on the membrane, then 10 mL WFI was poured into the phase that stacked on the membrane. The repeating centrifugation was done three times. This treatment was carried out until particles were obtained as supernatant (Khan et al., 2021).

The determination of %EE was performed by calibrating curves with concentrations of 50, 100, 150, 200, and 250 ppm, respectively, of a 1000 ppm glibenclamide parent solution. Measurements were made at a wavelength of 301 nm. The absorbance result is made in the line equation (y = a + bx) with y as absorbance and x as the concentration. Measurement of absorbance by using Spectrophotometer UV-Vis (UV-1700 Shimadzu®) in the supernatant phase of glibenclamide SLN and subsequently carried out to obtain the free drug, then the determination of EE percent by using the formula in Equation 1.

$$\%EE = \frac{\sum drug\ in\ formula - \sum drug\ in\ supernatant}{\sum drug\ in\ formula} x 100\% \quad (1)$$

2.2.5.2 Determination of pH

Determination of pH was done by dipping pH meters (LuthronpH-Electrode®) into the glibenclamide SLN solution of each formula. Furthermore, pH reading was done by monitoring the results listed on the pH meter screen. Perform this treatment three times (triple) to determine the stability of the preparation.

2.2.5.3 Measurement of Diameter, PDI, and Zeta Potential

The apparatus used to determine the diameter, particle size distribution and the potential zeta is particle size analyzer (Horiba Scientific® SZ-100). Measurement of diameter average, particle size distribution and, zeta potential was performed by using the DLS method (Price et al., 2020). The SLN samples were diluted and taken as much as 50 μL, then fed into a PSA quad. Measurement of particle diameter and zeta potential were done with 90° and 173° scattering angles. The morphology of particles was determined on ambient conditions by scanning electron microscopy (SEM) using a Carl Zeiss® Microscope. The concentration of particles was known that affected the NP properties, therefore the suspension of NP can be diluted 1:100 in WFI.

2.2.6 Physical Stability Test

A physical stability test was performed by mechanics and temperature methods (Almanassra et al., 2021). Samples were centrifuged (Hettich EBA BS®) at 3000 rpm for 30 minutes for 3 cycles. The treatment was similar to the treatment of gravity for separation by mass. The Observation was made by monitoring the occurrence of separation of each cycle. The test of Heating-cooling Method was performed by using a refrigerator (Toshiba®) storing the glibenclamide SLN at 4°C ± 2°C for 24 hours. After that the sample was transferred into an oven (IMU55L®) which was 40°C ± 2°C for 24 hours (1 cycle), then the test was performed 6 cycles and the presence or absence of phase separation to identify the physical stability of the preparation by organoleptc and pH measurement.

2.2.7 Data Analysis

Diameter, size distribution, and zeta potential particle were processed directly by PSA. PDI calculations and storage stability tests were processed using Microsoft Excel®. The obtained %EE data were first tested for Shapiro-Wilk normality, then normally distributed data was statistically analyzed using ANOVA (one way) method to see the difference of each result of formula. Subsequent follow-up tests were conducted with Post Hoc Tukey and LSD (Least Significant Difference) tests on the SPSS®24 program. The result of pH stability measurement was also done statistical test using correlation analysis by Minitab®17 program.

3. RESULTS AND DISCUSSION

3.1 Materials Preparation

Material preparation is an important step in the preparation of solid lipid nanoparticle preparation (SLN). The lipid phase is virgin coconut oil (VCO) which is preferable (Shakeel et al.,

2014). Preparation of the material carried out includes the preparation of the water phase and lipid phase (Chantaburanan et al., 2017). Glibenclamide is dispersed into the VCO as much as 3.7 g. Glibenclamide solubility in VCO 6.30 ± 0.007 mg / mL (1 g of solids or 1 mL of liquid in several mL solvents). The statement shows that the solubility of glibenclamide in VCO can be said to be soluble. The choice of VCO as a lipid phase because VCO contains 48% lauric acid, which is medium-chain fatty acids (MCFA) is easily absorbed by the body, so it can directly enter the metabolism produce energy, and do not cause fat tissue pile. The preparation process is also carried out with high temperatures because VCO is resistant and stable against heating with high temperatures.

Water phase preparations include melting PEG 6000 and mixing it with PEG 400 and tween 80 whose concentrations are varied. The choice of PEG 6000 as a solid lipid matrix is due to the solid PEG 6000 at room temperature similar to stearic acid as the lipid commonly used in SLN preparation. PEG with a molecular weight of $1.500 - 20.000$ is used for the preparation of solid dispersion. PEG is generally soluble in water but will decrease with increasing molecular weight. The relatively low melting point of PEG is useful for the preparation of solid dispersions by the melting method. Solid dispersion drug with PEG 6000 is useful to overcome various problems such as stability, solubility, dissolution, and bioavailability. The melting point of PEG 6000 is between $55 - 63°C$ and has a low hygroscopicity compared to PEG 400 in the form of liquid.PEG 6000 has a solubility in many organic solvents, can easily dissolve many drugs, and increase the wetness of the drug. The solubility of PEG 6000 in gastric acid and intestinal fluids is very easy, thus increasing the solubility of a drug in vitro through the previously mentioned mechanism.

Tween 80 is used as a stabilizer which varies in concentrations of 1.0, 1.2 and 1.4 (mg/mL) to maintain the stability of the SLN preparation. The choice of tween 80 due to other SLN formulations is often used Tween 80 as a stabilizer or surfactant. Tween 80 has an oleic acid content that has a logP of 6.5 so that oleic acid will readily bond to other more lipophilic compounds such as VCO. Tween 80 concentration variations were selected to obtain a more stable SLN preparation. Tween 80 is a non-ionic surfactant that has low toxicity (low molecular or chemical-causing damage to the body) so it is safe to use and also has a higher HLB value so it is more commonly used. The water phase was also carried out by the addition of PEG 400 which was used as a co-surfactant to increase the drug solubility rate.

Preparation of the aqueous phase and the lipid phase is done using a magnetic stirrer with a stirring speed of 1000 rpm for 1 hour at $75°C$. Preparation is done by using a high temperature to facilitate the mixing of the materials used. High temperatures up to $145°C$ will not cause glibenclamide to be degraded for 6 hours, so stable if $75°C$ is used for 1 hour.

3.2 Preparation of Glibenclamide Solid Lipid Nanoparticles

Until now, not so much research to perform the development of glibenclamide formulation. Nevertheless the result of a study regarding the incorporation of glibenclamide to hydrophilic polymer showed that the dissolution of glibenclamide was improved (Price et al., 2020). Formation biopolymeric particles of glibenclamide have also been performed as chitosan-heparin (Maretti et al., 2021). In this research, the authors were evaluation the Preparation of glibenclamide solid lipid nanoparticles using the melt emulsification method and ultrasonic techniques. This technique is often used because commonly needed tools are found in every laboratory. This ultrasonication technique is a dispersion technique, used for the production of solid lipid nanodispersions. This ultrasonication method is based on the cavitation mechanism.

The drug is added to the previously melted solid lipid, then for the heated water phase (heated to the same temperature as $75°C$). The combination between the two phases (emulsification) is done by drop by drop followed by stirring on the magnetic stirrer to obtain spheric particles and uniform and prevent aggregation during manufacture. Stirring using a magnetic stirrer can also help to accelerate the merging of the two phases.

The sonication time impact to the particle size tends to be more homogeneous and smaller that eventually leads to a stable size of nanoparticles and the agglomeration also diminishes on the optimum time. Ultrasonic waves in the sonication method can separate agglomeration and complete dispersion occurs with the addition of surfactants as stabilizers. SLN preparations were formed in a slightly turbid white color that is influenced by yellow Tween 80 materials. Tween 80 was dispersed into water then formed a clear liquid.

3.3 Evaluation of Glibenclamide SLN

3.3.1 Analysis %EE of Glibenclamide SLN

The percentage analysis of EE glibenclamide solid lipid nanoparticles was performed to determine the amount of encapsulated glibenclamide substance. The higher the EE percent the more encapsulated the drug. Higher %EE revels in the release of an active ingredients. The recent research informed that the release of glibenclamide was enhanced by forming the glibenclamide as proliposomes (Khan et al., 2021). EE percentage determination begins with the purification process using the centrifugation method at 12.000 rpm for 30 minutes. The centrifuged preparation will form two phases: the absorbed phase and the unabsorbed phase. The absorption phase (pellet) is the number of substances/drugs encapsulated in the preparation, usually like a white precipitate. The unabsorbed phase (supernatant) is the amount of unencapsulated substance/drug, usually a separate clear liquid. The supernatant will be taken and analyzed using a UV-Vis spectrophotometer to determine unexploited drug levels. The %EE analysis process includes scanning the maximum wavelength of glibenclamide, determining the calibration curve, and measuring the absorbance of the 3 formulas. According to the glibenclamide literature, it has

two maximum wavelengths or two peaks with ethanol and water (5:1) solvent ie at 230 nm wavelength with high absorption intensity and low absorption intensity at 300 nm wavelength. The result of the scanning wavelength of glibenclamide in 96% ethanol solvent is 229 nm and 301 nm. Based on the results of wavelength scanning can be said suitable from the literature. Determination of glibenclamide calibration curve by making serial concentrations of 50, 100, 150, 200, and 250 ppm from a 1000 ppm glibenclamide parent solution using 96% ethanol solvent. Selection of 96% ethanol solvent due to glibenclamide has a solubility in ethanol of 5 mg / mL. Measurements were made in three replications to get valid and good results. The calibration curve is chosen based on correlation coefficient value (r) which is closest to the value 1 that is at replication to 3 and has the equation y = 0.10374 + 0.0056516x with r^2 value equal to 0.99121. The correlation coefficient shows the linearity level of the relationship between the sample rate and the peak area.
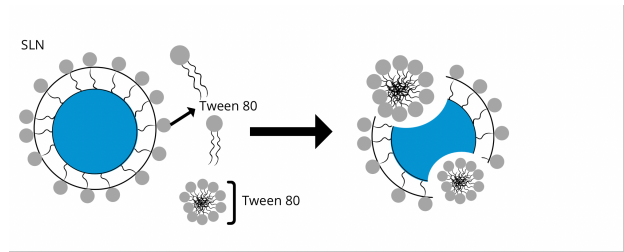
Table 2. The %EE of Glibenclamide SLN

Formula	Tween 80 (g)	Mean (%) ± SD
F1	10	60.6194 ± 0.0184
F2	12	56.3994 ± 0.0503
F3	14	55.0045 ± 0.0975

The percentage of EE as shown in Table 2, the value was obtained in a high amount entrapped in SLN. Formula 1 showed the highest percentage of formula 2 and formula 3. EE percent values obtained in formulas 1, 2, and 3 were 60.6165 ± 0.0184%, respectively; 56.3994 ± 0.0503%; and 55.0045 ± 0.0975% shown in Table 2. Precision is expressed as relative standard deviation (RSD) or the coefficient of variation (CV). CV calculation results show uniform data or accuracy is good if have CV ≤ 5% or not exceed the maximum limit.

Tween 80 is a non-ionic surfactant that can reduce surface tension while maintaining the physical stability of the dosage, thereby increasing the percentage of encapsulation efficiency. Surfactants help with the immediate formation of oil/water droplets and accelerate the dissemination of formulations in aqueous media and also have a natural amphiphilic ability and can dissolve relatively high levels of lipophilic drugs. Tween 80 can create a good layer around the surface of the particles, so agglomeration can be reduced and the efficiency of encapsulation tends to increase. The addition of tween 80 to a hydrophobic active substance can increase the dissolved rate of the insoluble active substance. This is due to the decrease in inter-surface tension thus increasing the surface area, which further provides a faster dissolution rate.

The addition of surfactants at low concentrations can decrease the surface tension and increase the solubility rate of the drug, whereas at higher concentrations surfactants will converge to form aggregates called micelles. The amount of adsorbed surfactant increases with increasing surfactant concentration. Conversely, after reaching critical micelle concentration, the

Figure 2. The Illustration of The Degradation Process of SLN by Aggregation

amount of adsorbed surfactant decreases with increasing surfactant concentration. The concentration of Tween 80 with high concentration can cause an aggregate formation (Geng et al., 2017). The particles that have been formed and protected by Tween 80 will be broken or damaged as they are affected by the aggregate formed, which can decrease the percent value of EE (Figure 2).

3.3.2 pH Measurement of Glibenclamide Solid Lipid Nanoparticles

The measurement of the pH of glibenclamide SLN was done by measuring pH using a pH meter device. Based on previous research it was known that the pH influences the stability of the colloidal suspension (Yokoyama et al., 1988) Use of this tool is expected to produce a valid and calibrated pH compared to pH strips. Based on the measurement results, the outline tends to be weakly acidic with pH 6.01 - 6.46 which can be seen in Table 3. Acidity values are still accepted for oral preparations when approaching neutral (pH 7). The concentration of Tween 80 may affect the pH of the preparation. Tween 80 has a pH between 6 − 8 to 5% w/v in aqueous solutions.

Table 3. The pH of Glibenclamide SLN

Formula	Tween 80 (g)	Mean ± SD
F1	10	6.0167 ± 0.0058
F2	12	6.2267 ± 0.0058
F3	14	6.4633 .0058

3.3.3 Thermodynamic Stability Test and Centrifugation of Glibenclamide Solid Lipid Nanoparticles

Stability testing of glibenclamide SLN preparation is also important to large-scale production. The rapid condition for the test is chosen by varying temperature and mechanics (Tekin et al., 2020). using the heating-cooling cycle and centrifugation method. The test was performed by storing the SLN preparation at 4°C and 40°C for 24 hours for each cycle to identify the physical stability of the preparation by organoleptic and pH testing. This method was chosen to see changes in preparations with extreme temperatures and faster time (Almanassra et al., 2021). The parameters observed were organoleptic, pH, and stability level of preparation.

Table 4. Results of Observation of Heating-Cooling Cycle Test in Glibenclamide SLN

Cycle to-	Formula 1 Organoleptic	pH	% Pp	Formula 2 Organoleptic	pH	% Pp	Formula 3 Organoleptic	pH	% Pp
0	White is clear, sligly turbid, no sediment	6.01	-	White slightly cloudy, no sediment	6.23	-	White slightly cloudy, no sediment	6.47	-
1	White is clear, sligly turbid, no sediment	5.99	0.3339	White slightly cloudy, no sediment	6.14	1.4658	White slightly cloudy, no sediment	6.27	3.1898
2	White is clear, sligly turbid, no sediment	5.95	0.6723	White slightly cloudy, no sediment	6.03	1.8242	White slightly cloudy, no sediment	6.08	3.25
3	White is clear, sligly turbid, no sediment	5.91	0.6728	White slightly cloudy, no sediment	5.94	1.5152	White slightly cloudy, no sediment	6	1.3333
4	White is clear, sligly turbid, no sediment	5.76	2.6042	White slightly cloudy, no sediment	5.87	1.1925	White slightly cloudy, no sediment	5.93	1.1804
5	White is clear, sligly turbid, no sediment	5.51	4.5372	White slightly cloudy, no sediment	5.8	1.2069	White slightly cloudy, no sediment	5.85	1.3675
6	White is clear, sligly turbid, a little sediment	5.45	1.1009	White slightly cloudy, sedimentation, formed two phases	5.76	0.6944	White slightly cloudy, sedimentation, formed two phases	5.79	1.0363

%Pp : Percent decrease of pH

Solid lipid particles which were formed by lipid powder are very stable but difficult to degraded (Freitas and Müller, 1999) than soft matrix carrier system. In this research (using liquid lipid), based on organoleptic observation (Table 4) it has been seen that the preparation was also stable against the varying storage temperature characterized by no change in dye color and there is little precipitate formed but for the last cycles particles were able to degrade. Observations on the pH parameter decreased in each test cycle. This decrease is due to the components in the VCO consisting of fat or triglycerides decomposed into free fatty acids and glycerol due to the hydrolysis process. The acidity constant and the degree of ionization of each fatty acid will affect the pH in the preparation. The hydrolysis reaction can cause a rancid odor that causes VCO changes to ketone compounds.

The decrease in glibenclamide amount (Table 5) at the end of the smallest test cycle occurred in F1 with the highest percentage of EE that was 60.6194 ± 0.0184% compared with the decrease in levels of F2 and F3 having a low EE percentage of 56.3994 ± 0.0503%, and 55.0045 ± 0.0975%. %EE affects the degradation process characterized by many encapsulated particles that will reduce degradation as well as a decrease drug concentrations and vice versa.

Stability testing was also performed mechanically using centrifugation. The basic principle of the centrifugation test is the centrifugal force or the circular motion force away from the center of the circle. The suspension/emulsion preparation was used to qualify if there was no phase separation in the preparation. High-speed centrifugation tends to alter the shape of particles (internal phase) that were dispersed and trigger coalescence which was related to the force. VCO and PEG-6000 as on the outer layer have the capability for physical interacting by hydroxyl and carbonyl groups. The test results indicated the separation phase between the supernatant and pellet. The phase separation was caused by the pressure and heat generated by the centrifugation process which was known to increase the degradation rate of drug particles in the preparation.

The result of the pH measurement of each cycle was statistical analysis using SPSS®24 software. The pH stability data is the first tested requirement that is normality test which aims to see whether the data is a normal distribution or not. The Shapiro-Wilk column section views the sig value. >0.05 indicating that the pH data of stability is normally distributed for all formulas. The test was continued by hypothesis test by One way ANOVA (Analysis of Variance) method along with post hoc Tukey HSD and LSD test. The sig value. in the ANOVA test table shows that the sig value Ie >0.05 which means there is no significant difference in pH values between formulas. Tukey HSD and LSD post hoc test tables between groups of data, can be seen there is a significant difference between F1 and F3 LSD section is indicated by sig value. <0.05; while the Tukey HSD section showed that there was no significant difference

Table 5. Stability Level of Glibenclamide SLN at Previous and Last Cycles

Formula	Early content (cycle to-0)		Final content (cycle to-6)		% Decrease
	Mean (%) ± SD	CV (%)	Mean (%) ± SD	CV (%)	
F1	95.4845 ± 0.0265	0.0278	51.1590 ± 0.0088	0.0173	46.4216
F2	93.6531 ± 0.0265	0.0283	33.9573 ± 0.0135	0.0397	63.7414
F3	92.9631 ± 0.0265	0.0286	33.9131 ± 0.0135	0.0398	63.5198

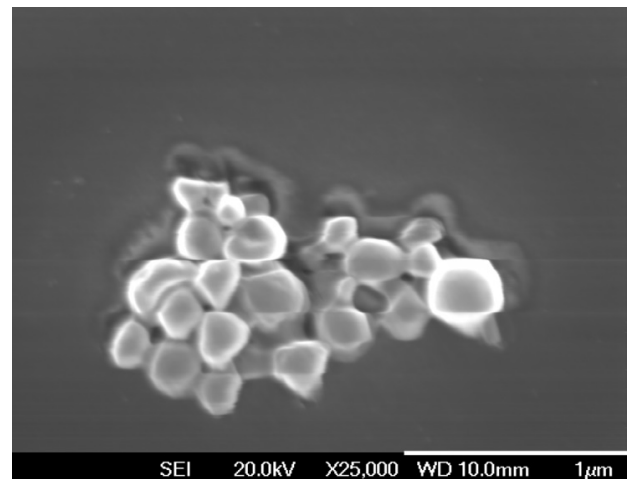between the pH data stability of F1 and F2, F2 and F3 which were sig values >0.05.

3.3.4 Diameter, Particle Size Distribution, and Zeta Potential

Analysis of diameter, particle size distribution, and potential zeta can be determined using the particle size analyzer (PSA) tool. The working principle of PSA was based on DLS (dynamic light scattering) method that utilizes infrared ray scattering. The refracted light at an angle of $173°$ will be captured by the detector to produce a potential zeta. The light dissipated at an angle of $90°$ was captured by the detector to produce diameter, molecular weight, and particle size distribution. The smaller particle size has an impact on the surface area and dissolution rate to increase the bioavailability of the compound.

The result of measurement of the particles using particles size analyzer (PSA) regarding diameter on optimum formula (F1) was 175.5 nm. The result was bigger than the range of nanoscale. According to the range of nanoscale 1 to 100 nm, it is more relevant to metallic particles. PSA has recorded the hydrodynamic size that is bigger size than measurement without water. The expected particle size for the SLN preparation is $50-200$ nm (Hanafy et al., 2007). The optimum formula has a particle size indicating that the particle size also involves the desired size range. In pharmaceutical application, fewer toxic particles which have 100 up to 500 nm in diameter more suitable for medication. The morphology of particles was determined by scanning electron microscopy (SEM) in Figure 3 revealed the mean size of 109 nm. Differences in measurement results using tools and manuals differ considerably because the accuracy of using the tool was more valid and also the environment of particles such water on PSA measurement.

The particle size distribution was determined by the value of the polydispersity index (PDI). The PDI value <0.5 indicates that the uniformity of the particle size in the preparation. The optimum PSA formula yields a PI value of 0.127 indicating that about 87.3% of the particle size of the preparation has a uniform particle size. The potential zeta value over +25 mV and -25 mV indicates that one particle with another particle does not collide and thus decreases the process of aggregate formation. Aggregates are formed due to Van der Waals bonds due to the pull of charge on the particles (das Neves et al., 2013).

The results in the optimum formula have a small potential zeta value of +5.9 mV. Potential zeta values do not fit within the range of requirements, so small zeta potentials make the

Figure 3. SEM Image of Glibenclamide SLN

partially aggregate due to the weak repelling force. The effect of positively charged N atoms on glibenclamide allegedly causes the charge of particles to be positive.

3.3.5 Correlation Analysis

Correlation analysis was done by using the Minitab®17 program to see whether or not the correlation between two or more variables. Data of each variable is tested normality first to see normally distributed data or not. Based on normality test results indicate that the data of each variable is normally distributed by looking at p-value >0.05. The analytical method used is Pearson correlation because the data is normally distributed (Hardoon et al., 2004). The correlation relationship level can be seen in Table 6.

Table 6. Interpretation of Correlation Coefficient Level

Coefficient interval	Level of relationship
$0.00-0.19$	Very low
$0.20-0.30$	Low
$0.40-0.59$	Medium
$0.60-0.79$	Strong
$0.80-1.00$	Very strong

The correlation result shows that there is a negative linear correlation between EE percent to pH and a positive linear relationship to stability level, whereas correlation value is shown

Table 7. Results of Pearson Correlation Analysis

Relationship	Pearson Correlation	*p-value*
%EE-pH	-0.789	0.421
EE-stability level	0.972	0.152
pH-stability level	-0.622	0.573

between pH and stability level of negative linear relationship which can be seen in Table 7. Pearson correlation value is 0.972 between percent EE to the level of stability that shows that the relationship is very strong means the higher the percent EE then the higher the resulting stability level. EE percentage of pH and stability level, and between pH and pH value of p-value > 0.05 indicated that there was no significant relationship. The p-value value indicates the relation of the variable to the data population, the amount of data is not enough to represent the data population, so the correlation value is considered more influential.

4. CONCLUSIONS

The percent value of the efficiency of the encapsulation of the optimum formula is 60.6194 ± 0.0184%. The higher the concentration of tween 80, the percentage decreased EE. The optimum formula of glibenclamide solid lipid nanoparticles (tween 80 with 1 mg/mL concentration) resulted in particle diameter of 175.5 ± 10.07 nm with a polydispersity index (PDI) of 0.127 and a potential zeta of + 5.9 mV. Stability testing by heating-cooling cycle method of three formulas showed that storage of doses under extreme temperatures leads to a decrease in contents of glibenclamide and phase separation in centrifugation mechanical test. Solubility and dissolution test to glibenclamide SLN is required for further research.

5. ACKNOWLEDGEMENT

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