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Fenofibrate Characterization of Solid Lipid Nanoparticles Using the High Shear Homogenization Method

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A B S T R A C T

Fenofibrate is a drug that can be used to treat hyperlipidemia where the drug is included in the category of Biopharmaceutical classification system II with poor solubility and high permeability. This causes the need to improve the drug delivery system (DDS) made using the solid lipid nanoparticle (SLN) method. SLN fenofibrate can be made using the high shear homogenization method by determining the formula using Factorial 22 Design Expert 12. The formula is made with a concentration of 0.31-1.25% GMS and 1.25-2% Tween-40, then SLN fenofibrate is made by mixing all ingredients until an emulsion is formed and continued with the SLN critical parameter test. From the test results, the critical parameters of SLN fenofibrate for the particle size of 8 formulas 490; 561; 601; 697; 916; 1040; 1818, and 2410 nm. The results obtained for the polydispersity index, respectively, were 0.02; 0.04; 0.08; 0.30; 0.35; 0.48; 0.51, and 0.65. The zeta potential value of the 8 formulas obtained successive values of 2.8; 3.5; 4.2; 4.8; 5.5; 5.8; 8.1, and 8.8 mV. Calculation of the efficiency of the SLN fenofibrate drug obtained successive values of 77.23; 78.53; 79.51; 80.47; 81.17; 87.38; 87.39, and 87.82%. The SLN method can improve drugs that are included in the Biopharmaceutical classification system class II category with the distribution of test results in the particle size range, and the adsorbed drug is more than 70%.

1. Introduction

Fenofibrate is a fibrate hypolipidemic drug, a peroxisome proliferator-activated receptor α (PPAR α) agonist that can downregulate the apoC-III gene and upregulate the apoA-1 and apoA-genes. II, to reduce serum triglyceride levels. Fenofibrate is insoluble in water with a solubility level of 162.5 µg/mL.. Based on the biopharmaceutical classification system (BCS), fenofibrate is included in the BCS class II category, where drugs that fall into this category have poor solubility and high permeability. (Low solubility and permeability drugs). A poorly soluble drug has a low dissolution rate in liquid digestion, thus causing poor bioavailability in the body.[1,2]

Drugs that have poor solubility can be improved by using a drug delivery system (DDS), such as reducing particle size, decreasing crystallinity, making particles amorphous, and increasing drug particle limitations. Solid Lipid Nanoparticles (SLN) can increase drug stability and can provide flexibility in controlling the release of drug substances, and can provide an occlusive effect that can increase the penetration of active ingredients. The method that can be used to make SLN preparations is using ultrasonication or high-speed homogenization method, where the active ingredient is dissolved into solid oil with the addition of a surfactant after the formation of an emulsion, then sonication is carried out.[3] A study showed that fenofibrate nano preparations were able to increase the solubility and dissolution rate of the drug.[4]

SLN fenofibrate was made by the High Shear Homogenization method using solid lipids of the

glyceride group. The advantage of the lipid group of glycerides is that it can provide an increase in the manufacture of stable drugs. With a combination of surfactant tween 40 is used as a stabilizer and added singly with a concentration of 1.33–2% is expected to increase stability and reduce particle size.[5,6] This study aimed to determine the effect of the combination of Glycerin Monostearate and Tween 40 on the characteristics of fenofibrate Solid Lipid Nanoparticles made by the High Shear Homogenization method.

2. Methods

The materials used in this study were fenofibrate, glyceryl monostearate, Tween 40, and aquadest. The tools used in this research are analytical balance (Precisa & B 220A), hot plate magnetic stirrer (Thermo scientific), ultrasonicator, particle size analyzer (Microtrac nanotrac wave II), Polydispersion index (Microtrac nanotrac wave II), zeta potential (Microtrac nanotrac wave II), centrifuge, UV-Vis spectrophotometer, and beaker. Fenofibrate Solid Lipid Nanoparticle (SLN) formulation by determining the concentration of the formula using Design Expert from solid fats and surfactants where each concentration has 2 levels, namely low level (GMS 0.31% and Tween 40 1.33%) and high level (GMS 1,25% and Tween 40 2%).

Table 1. Design factors and concentrations of 2^3 factorial designs.

Formula	Fenofibrate (% w/w)	GMS (% w/w)	Tween 40 (% w/w)	Water (% w/w)
Run 1	0.12	1.25	1.33	97,3
Run 2	0.12	1.25	2	96,63
Run 3	0.12	0.31	2	97,57
Run 4	0.12	1.25	1.33	97,3
Run 5	0.12	0,31	2	97,57
Run 6	0.12	1.25	2	96,63
Run 7	0.12	0.31	1.33	98,24
Run 8	0.12	0.31	1.33	98,24

SLN fenofibrate was prepared with GMS and Tween 40 with various ratios (Table 2) dissolved in water. GMS dissolved with some water and stirred using a hot plate magnetic stirrer at a speed of 700 rpm at a temperature of 80ºC for 15 minutes. After 15 minutes, fenofibrate was added until dissolved. Tween 40 was dissolved with some water, stirred at a speed of 700 rpm using a hot plate magnetic stirrer for 15 minutes at a temperature of 80ºC, then put the Tween 40 solution into the GMS-Fenofibrate solution, stirred with a Hot Plate Magnetic Stirrer at a speed of 700 rpm to form an emulsion. The fenofibrate emulsion preparation was then ultrasonicated for 15 minutes by pulse on-off method for 10 seconds with an amplitude of 55%. The formulation was left at room temperature. The formulations were tested for particle size, polydispersity index, zeta potential (using Microtac wave II), and adsorption efficiency.

Determination of the wavelength was carried out by making a mother liquor, carefully weighed 10.0 mg of fenofibrate dissolved in a 100.0 ml volumetric flask so that a concentration of 100 ppm was obtained. The mother liquor was taken at 1.0 ml, which was dissolved using ethanol solvent into a measuring flask to 10.0 ml so that a concentration of 10 ppm was obtained. Read with a UV-Vis spectrophotometer with a wavelength between 200-400 nm. The maximum wavelength is indicated by the highest absorption value.

The characterization of SLN fenofibrate can be done by testing the particle size, polydispersity index, and zeta potential using the Microtrac nanotrac wave II tools with an RI setting of 3.33 where the sample to be tested should not be bubbly and foamy. The sample is

inserted into the cap, then determine the run and results. The reading will be read on the system connected to the computer. Efficiency test adsorption was carried out with SLN fenofibrate centrifuged at 6000 rpm for 60 minutes. The supernatant (non-absorbed drug) was measured using a UV-Vis spectrophotometer with a maximum wavelength, and the absorbance value obtained was calculated by the formula:

Adsorption efficiency (%) = (Wa-Ws) / Wa x 100%

Information:

Wa: The mass of the drug added to the formula

Ws: Analysis of the weight of the drug in the supernatant.

3. Results and Discussion

Determination of wavelength

The results of screening of pure fenofibrate standard solution were carried out with a concentration of 2 ppm at a wavelength of 200 -400 nm using a UV-Vis spectrophotometer. From the results of the analysis, the wavelength of fenofibrate is 286 nm with an absorption magnitude of 0.5720.

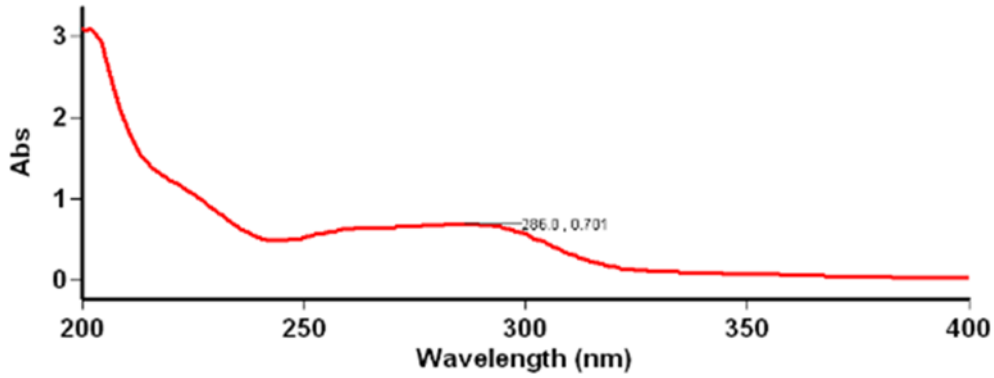

Figure 1. Maximum wavelength.

Characterization test of SLN fenofibrate

SLN fenofibrate characterization test was carried out, including particle size, polydispersion index, and zeta potential, which could be measured using a Nanotrac Wave II device with an RI setting of 3.33, and the adsorption could be carried out using a UV-Vis spectrophotometer. The results of the analysis are as follows:

Table 2. SLN fenofibrate characterization test results.

Run	PSA	PDI	Zeta	EE
1	916	0.04	2.8	79.51
2	561	0.0842	4.2	78.53
3	1040	0.652	8.8	87.39
4	1818	0.515	5.5	87.82
5	490	0.486	8.1	81.17
6	2410	0.35	4.8	87.39
7	601	0.01858	3.5	77.23
8	697	0.308	5.8	80.47

Particle size and polydispersity index

Particle size is the most important parameter in the manufacture of SLN preparations. The use of lipids with the heating method makes the viscosity decrease so that it affects the particle size. The fenofibrate trapped in the GMS causes the SLN size to get

smaller.[7,8] Formula making using GMS makes the preparations have unstable particle sizes because GMS can form crystals of orderliness which make the drug enter quickly and also easily come out,[9,10] with the addition of surfactants, it is expected to stabilize the emulsion of the two liquids that do not mix so that can reduce the repulsion and attractive intermolecular forces of each liquid and can increase the formation of particle size.[11,12]

The influence of GMS and Tween 40 with the SLN preparation method can make almost all formulas fall into the nanoparticle size range of 0-1000 nm. The non-uniformity of particle size is caused by the lack of accuracy when making the formula or when preparing measurements. The non-uniformity of particle size can also be seen from the non-uniformity. The higher the polydispersity index uniformity, it indicates that the resulting particle size is increasingly not the same.

With the P-Value Analysis of variance (ANOVA) on Design Expert 12 software with a 95% confidence level (P-Value <0.05), the particle size test and polydispersity index were obtained with values of 0.0201 and 0.0473 where these values <0.05 so the data obtained is significant. Meanwhile, for statistical analysis data, to fulfill the requirements, it must obtain an R2 value close to 1 for the particle size analysis data itself, the R2 value is 0.8946, and the polydispersity index is 0.8365 so that the data meets the requirements, with Adjusted R2 0.8156 for particle size and 0.7138 where the value is above 0.7 which means that the data we get is following the requirements. With the equation value

Y = -280.88(A) -372.88 (B) +393.63 (AB) -> particle size

Y= +0.174545(A) -0.561984(B) -0.022055 (AB) -> polydispersity index

Information: A: GMS, B: Tween 40.

From the above equation, it shows that there is an effect between GMS (A) and Tween 40 (B) in each factor and there is an interaction between SLN preparations. The results of the resulting regression coefficients indicate that GMS and tween 40 with negative results affect the particle size test, while in the Polydispersity index equation, the GMS value is positive, which shows that GMS has the greatest influence compared to tween 40. Based on the equation, it can be seen based on the contour plot particle size and polydispersity index are presented in the figure below:

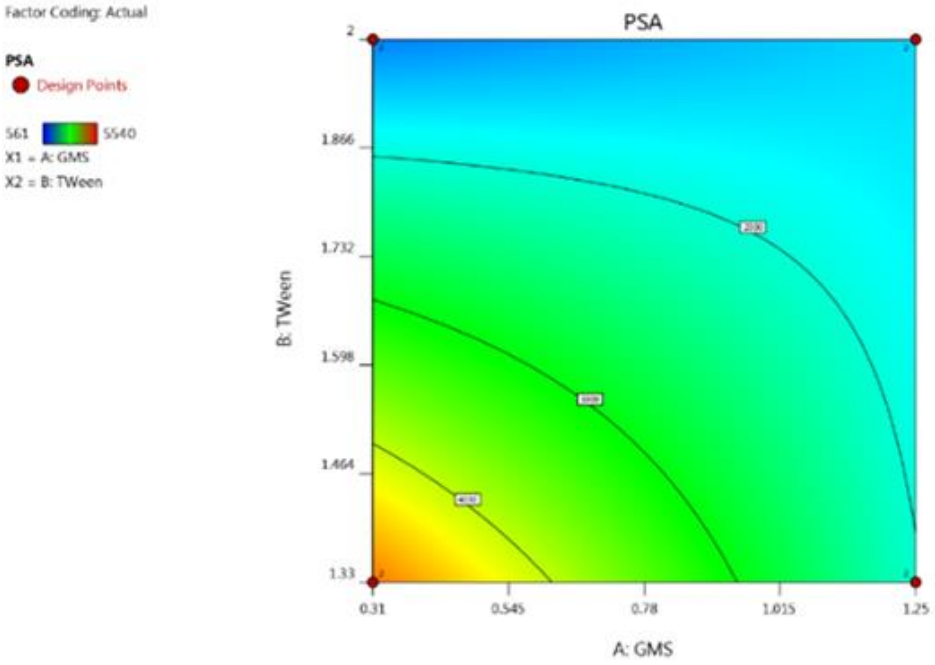

Figure 2. PSA formulation graph.

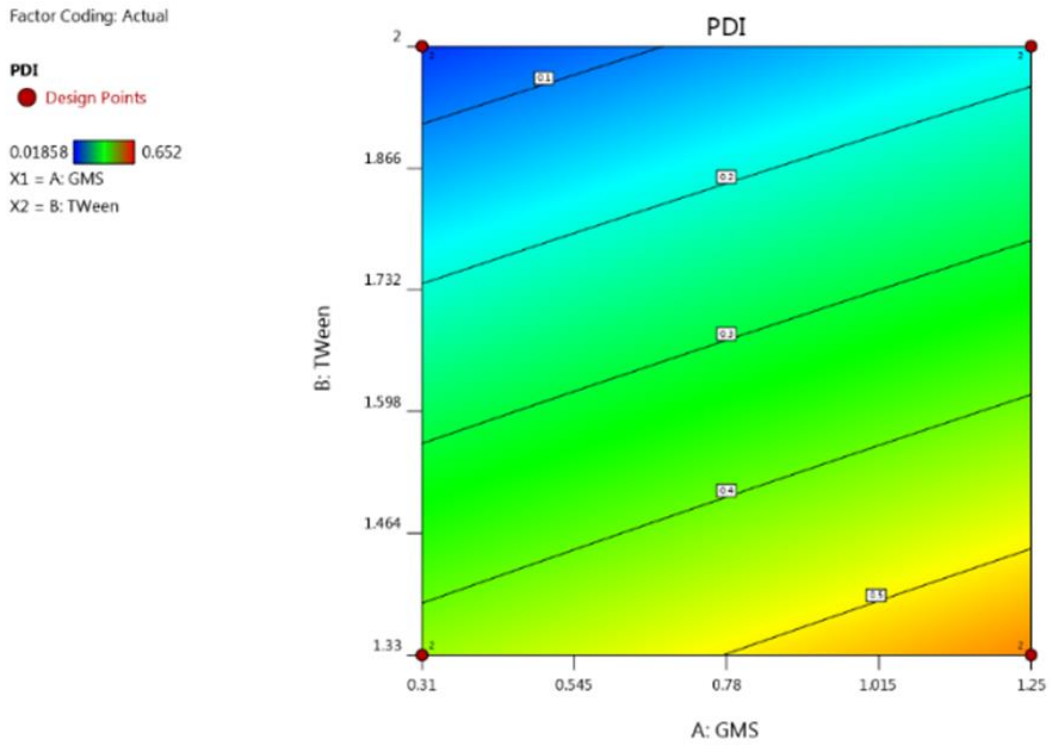

Figure 3. Formulation of PDI graph.

From the figure above, it can be explained that there is a movement in the counterplot of GMS concentration 0.31-1.25% and tween 40 1.25 -2%, which is indicated by the movement of color changes, where orange, yellow, and green are the highest concentrations and blue is the lowest concentration that affects the polydispersity index. The results indicate that there is an interaction between the components of the Polydispersion index and one another. This is indicated by the presence of color gradations on the contour plot.

Zeta potential

Based on the results of the zeta potential test above +/- 30 mV has shown stability, whereas a surface charge prevents particle aggregation. The potential results obtained show that the results are quite stable with a negative particle charge which is influenced by the particles dispersed in water because they tend to adsorb hydroxyl ions. Besides that, it can also be influenced by anionic surfactants.[13]

With the P-Value Analysis of variance (ANOVA) on Design Expert 12 software with a 95% confidence level (P-Value <0.05), the polydispersity index test was 0.0498nm with an R-value of0.8321 and Adjusted R2 of 0.7062 where the value meets the requirements. With the equation value

$$Y= +0.9375 (A) -1.36(B) -0.7125 (AB)$$

Information: A: GMS, B: Tween.

From the above equation, it can be concluded that there is an interaction between the components of GMS and tween 40 where each of these components can affect the potential Zeta test results. The regression coefficient shows a negative result. It shows that the addition of surfactant will affect the low zeta potential value, which can be seen from the figure below.

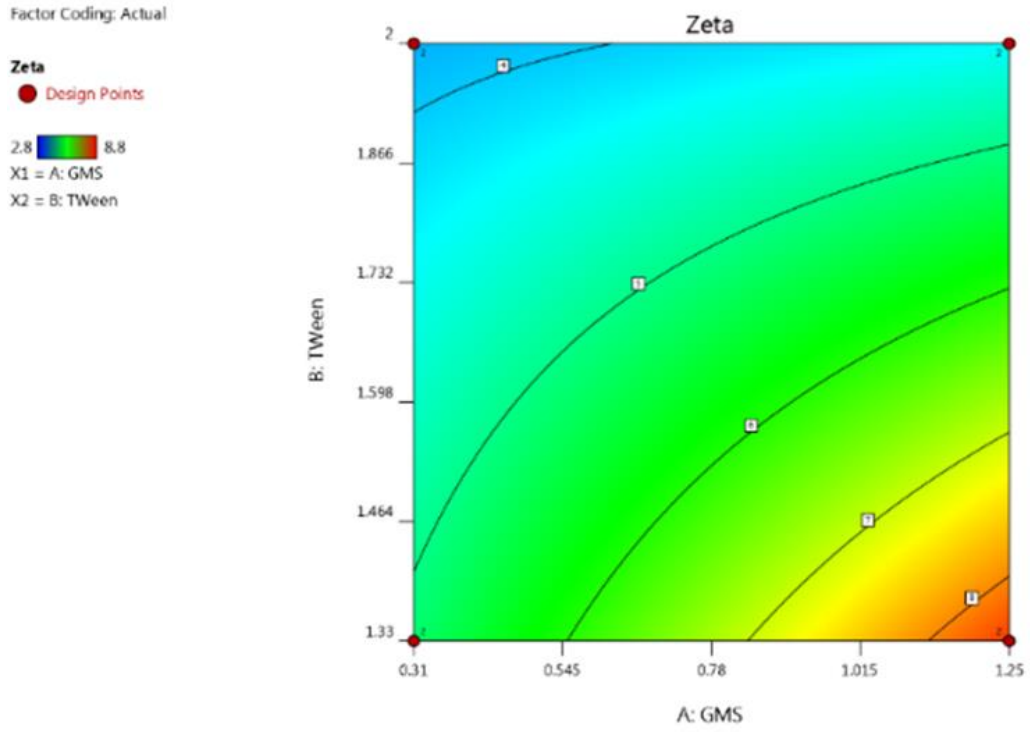

Figure 4. Graph of zeta potential formulation.

The counterplot figure above shows that there is a movement in the contours of both GMS concentrations of 0.31-1.25% and tween 40 of 1.25-2%, where the highest concentrations are shown in orange, yellow, and yellow. Green and blue are the lowest concentrations that can affect the zeta potential test. The results indicate that there is an interaction between the adsorption efficiency components with each other. This is indicated by the color gradation on the contour plot.

Adsorption efficiency

The adsorption efficiency is carried out by using a UV-Vis spectrophotometer to determine the percentage of the active substance that is adsorbed in the SLN system. The larger the compound trapped in the carrier, the faster the penetration because the greater the concentration gradient that drives the passive diffusion process in penetration. Formula 8 has a higher adsorption efficiency value than the other 7 formulas. This can be influenced by several factors, such as the solubility of the drug in melted fat, where the lower concentration of a lipid used will affect the ability to load drug compounds will be smaller.[14-20]

With the P-Value Analysis of variance (ANOVA) on Design Expert 12 software with a 95% confidence level (P-Value > 0.05), 52 0.039 and the data are significant. Meanwhile, for statistical analysis data to meet the requirements, it must obtain an R2 value of more than 0.7 or 70%. For data analysis, the sorption efficiency itself has an R2 value of 0.8457, so the data meets the requirements, with Adjusted R2 0.7300, with the equation.

Y = -15,01477 (A) -17,19014 (B) +8,62972 (AB)

Information: A: GMS, B: Tween 40.

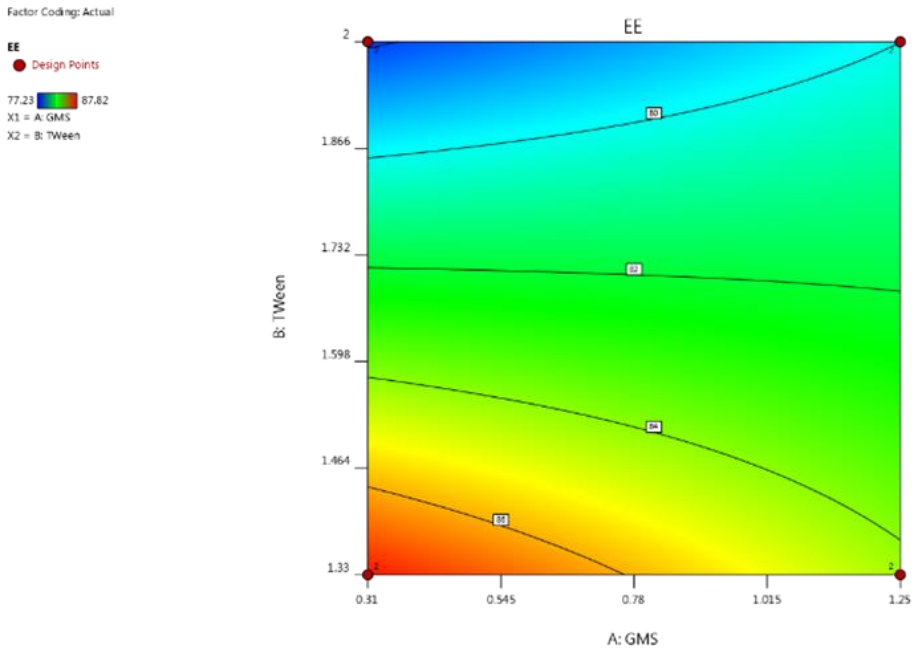

Figure 5. Graph of formulation adsorption efficiency.

The counterplot above with a GMS concentration of 0.31 -1.25% and tween 40 1.25-2% showed a movement of color change from red, yellow, and green which was the highest concentration, and blue was the lowest concentration that could affect the results of the adsorption efficiency test.

4. Conclusion

The SLN method can improve drugs that are included in the Biopharmaceutical classification system class II category with the distribution of test results in the particle size range and the adsorbed drug is more than 70%.

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