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Original Article

PREPARATION AND OPTIMIZATION OF CILOSTAZOL NANOEMULSION ORAL LIQUID DOSAGE FORM

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ABSTRACT

Objective: Cilostazol has poor water solubility and low oral bioavailability. Therefore, the formulation of cilostazol as a nanoemulsion may enhance its solubility and improve oral bioavailability. Hence, the aim of this study was to formulate and characterize an oil-in-water (o/w) nanoemulsion of cilostazol as an oral liquid dosage form.

Methods: Pseudo-ternary phase diagrams were constructed using the aqueous titration method. Formulations of pseudo-ternary phase plots consisting of oil, various weight ratios of S mix (mixture of surfactant and co-surfactant), and deionized water were made. Different characterization studies, droplet size measurement, polydispersity index, drug content, zeta potential measurement, and *in vitro* release have been conducted to choose the optimized formula.

Results: The characterization studies have demonstrated that the optimized formula is (F-6), consisting of 20 % S mix (3:1), 10% ginger oil, and 70% deionized water. This formula had the following characteristics; droplet size (72.9-110 nm), polydispersity index (0.22), percentage of drug content (99.8%), and *in vitro* release of cilostazol nanoemulsion was significantly higher ($P<0.05$) in comparison with other formulations. A Scanning probe microscopy (SPM) study has revealed that the droplet size of F-6 was at the nano-scale.

Conclusion: In conclusion, the optimized cilostazol formula (F-6) is a promising formula which may have the capability of improving the oral bioavailability of cilostazol.

Keywords: Nanoemulsion, Pseudo-ternary phase diagram, Solubility, Cilostazol

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INTRODUCTION

The bioavailability of oral dosage forms is an important parameter for drugs to achieve the desired therapeutic action. Different factors, such as water solubility, dissolution rate, permeability, and first-pass metabolism, have been shown to affect oral bioavailability. The two most important factors that determine the oral bioavailability of drugs are solubility and permeability [1]. Biopharmaceutics Classification System (BCS) classifies drugs into four classes based on their solubility and permeability. Class II drugs include those drugs with low solubility and high permeability. The bioavailability of drugs that belong to class II is low due to their poor solubility in water. Hence, enhancing water solubility can improve the oral bioavailability of these drugs. Several techniques have been developed to enhance the water solubility of poorly water-soluble drugs, such as salt formation, co-solvency, self-emulsification, particle size reduction, and the nanotechnology approach [2]. Cilostazol is a quinolinone derivative and antiplatelet agent with vasodilating properties that has been used in the symptomatic treatment of intermittent claudication in patients with peripheral ischemia. According to the BCS, Cilostazol belongs to Class II, which means it has low water solubility and high membrane permeability. Cilostazol has low oral bioavailability due to poor aqueous solubility and first-pass metabolism in the liver [3]. Nanoemulsion is an advanced drug delivery system with transparent colloidal dispersions of oil and water, which are stabilized by the presence of a surface-active agent (surfactant) and co-surfactant. This drug delivery system has a number of advantages over other drug delivery systems, which may include increasing the rate of absorption, solubilizing lipophilic drugs, and enhancing their bioavailability. Nanoemulsion can be used to deliver products via different routes, like topical, oral, and intravenous, and improve patient compliance because of its liquid dosage form. Halah Hussein Ali and Ahmed Abbas Hussein develop an oral nanoemulsion of candesartan cilexetil, where the release rate and extent for all prepared nanoformulations were significantly higher ($p< 0.05$) than the marketed tablet formulation and the plain drug powder. This result demonstrates the potential use of this system as a perfect technique for improving the solubility and dissolution of candesartan cilexetil [4]. Furthermore, Khani S, Keyhanfar F, and Amani A. developed an oral nanoemulsion of mebudipine in order to increase its oral bioavailability. They demonstrated that the relative bioavailability of the mebudipine nanoemulsion was enhanced by about 2.6, 2.0, and 1.9-fold, respectively, compared with suspension, ethyl oleate solution, and micellar solution [5]. Hence, the aim of this study was to prepare and optimize an oral (o/w) nanoemulsion of cilostazol in order to increase its solubility, which may enhance its oral bioavailability.

MATERIALS AND METHODS

Reagents

Cilostazol (pure powder) was purchased from Hyperchem company (China); Tween 80, Tween 20, and Tween 60 were from Thomas Baker Chemicals (India); Castor oil, Peppermint oil, Ginger oil, and Basil oil were from Al-Ameer company for plants oil (Iraq); Soybean oil was from Genuine Chemicals (India); Olive oil was from Pomace Olive |(Spain); Poly ethylene glycol 300, Poly ethylene glycol 400, and Propylene glycol were from M/s Provizer Pharma (India); Methanol was from Avantor performance materials (Norway), and deionized water was from Al-Hayat company for chemical and laboratory materials (Iraq).

Methods

Differential scanning calorimetry (DSC)

DSC was conducted by placing 5 mg of cilostazol in the aluminum pan of the DSC-60 Shimadzu. An analysis of this technique was made by using nitrogen at a rate of 10 °C/min as inflow gas with a heating range of 50–250 °C [6]. DSC thermogram of cilostazol was recorded.

Screening of components by solubility study

Different oils, including peppermint oil, castor oil, ginger oil, basil oil, olive oil, and soya bean oil, were utilized to solubilize cilostazol. Tween 80, tween 60, and tween 20 were used as surfactants, while PEG 300, PEG 400, and propylene glycol were used as co-surfactants. A solubility study was performed by adding an excess amount of cilostazol powder to 5 ml of each oil, surfactant, and co-surfactant. Samples were placed in an isothermal shaker water bath for 72 h at 25±0.5 °C, then the samples were centrifuged at 2000 rpm for 10 min and the supernatant layer for each sample was filtered using 0.45 µm filters. The supernatant layer for each sample was filtered using 0.45 µm filters. Then, samples were diluted with methanol, and the solubility was determined at the maximum wavelength using a UV-visible spectrophotometer [7].

Pseudo-ternary phase diagrams construction

Oil, Smix (a mixture of surfactant (S) and co-surfactant (CoS),) and double-distilled water were the components of the pseudo-ternary phase diagram. The construction of the ternary phase diagram was conducted using a low-energy method of emulsification (aqueous titration method). Surfactant and co-surfactant were mixed in different weight ratios (1:1, 2:1, and 3:1). Different combinations of oil and Smix were prepared and slowly titrated with the aqueous phase (double-distilled water). The titration of water was terminated when a transparent, clear, and o/w nanoemulsion was produced. The pseudo-ternary phase diagram was plotted using Pro Sim ternary phase diagram software (version 1.0). Nanoemulsions were then subjected to thermodynamic stability tests, centrifugation tests, freezing-thawing tests, and heating–cooling tests to know the most stable formulations [8].

Preparation of cilostazol nanoemulsion

Cilostazol (0.05 g) was dissolved in oil. Then, a different amount of surfactant and co-surfactant mixture (Smix) was added for the oil-loaded drug, and the whole mixture was mixed using a vortex mixer. The aqueous phase was then titrated drop by drop to obtain a transparent, clear (o/w) nanoemulsion. Nanoemulsions were stored in tightly closed glass containers at 25 °C for a characterization study.

Characterization of nanoemulsion

Droplet size measurement

Samples of nanoemulsions were placed in the sonicator for 30 min at 35 °C. Then the measurement was performed using the particle size analyzer ABT-9000 (Angstrom Advanced lnc. USA). The droplet size and distribution plot of droplets were recorded.

Polydispersity index (PDI) measurement

PDI is used to investigate the uniformity of droplet distribution within the prepared nanoemulsions. The higher the value of the PDI, the lower the uniformity of droplet distribution within the formulation. In the herein study, the measurement was made utilizing the particle size analyzer ABT-9000 (Angstrom Advanced lnc. USA) [9].

Zeta potential (ZP) measurement

Zeta potential is used to investigate the stability of colloidal dispersions and gives an indication of the charge on the droplet surface. A Zeta sizer instrument (nano brook zetaplus. Holtsville. USA) was used for assaying the ZP of the nanoemulsions [10].

Drug content estimation

To measure the drug content of each nanoemulsion formula, 0.25 ml of each was diluted with methanol (1:10). Then, absorbance was measured using a UV-visible spectrophotometer at its maximum wavelength. The percentage of drug content was estimated using the following equation:

$$\text{Drug content} = (\text{Measured content})/(\text{Theoretical content}) \times 100\%$$

***In vitro* release study**

The *in vitro* release study of cilostazol nanoemulsion and pure cilostazol drug was made by dissolution apparatus USP-II (Copley dissolution tester DIS 8000, UK) using the dialysis bag technique [11]. A dialysis bag containing 50 mg of pure cilostazol solution or cilostazol nanoemulsion was immersed in 900 ml of phosphate buffer at pH (6.8). The dissolution apparatus was set at 37±0.5 °C and at a rotation velocity of 50 rpm for two hours. Samples (5 ml each) were taken every 15 min for two hours, and 5 ml of fresh medium was added to maintain the sink condition. Samples were filtered using 0.45 µm filter units and analyzed by a UV-visible spectrophotometer to measure cilostazol content.

Kinetics and mechanisms of drug release

The dissolution data were fitted to various kinetic models, which are the zero-order kinetic, first-order kinetic, Higuchi model, Korsmeyer model, and Peppa's model. The regression coefficient (R^2) was used to determine the kinetic release. The higher value of R^2 indicates the kinetics of drug release. To determine the mechanism of drug release, the dissolution data were fitted to Korsmeyer and Peppa's model, in which the value of the diffusion exponent (n) will determine the best mechanism that is compatible with the release of formulations [12].

Scanning probe microscopy (SPM) study

SPM (triple probe microscope) was used to investigate the morphology of droplets and droplet distribution within the prepared system. A drop of cilostazol nanoemulsion was placed on a glass slide and tested [13].

Statistical analysis

Data from at least three independent experiments were analysed using IBM SPSS software (version 23) and Excel 2016. All means are reported with standard deviation. A one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey, multiple comparisons post-test was performed as appropriate. Statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Differential scanning calorimetry

A sharp endothermic peak of cilostazol powder appeared at 163 °C (fig. 1) [14]. This reading is similar to the melting point of cilostazol powder, which gives an indication of the purity of the drug powder.

Screening of components by solubility study

The main components used in the preparation of nanoemulsion are oil, surfactant, and co-surfactant), which were selected on the basis of the solubility study. The selection of suitable components can aid in the production of stable nanoemulsions. The result of the solubility of cilostazol in various oils in this study was; ginger oil>olive oil>peppermint oil>basil oil>soyabean oil>castor oil. Cilostazol solubility was the highest in tween 80 and the least in tween 60. Additionally, higher solubility was observed in PEG 300, followed by PEG 400 and propylene glycol (table 1). Based on the above results, ginger oil as the oil phase, tween 80 as a surfactant, and PEG300 as a co-surfactant were selected to prepare the cilostazol nanoemulsion [15].

Pseudo-ternary phase diagram construction

The components that were included in the plotting of the pseudo-ternary phase diagram were oil, double distilled water, and Smix. Smix was presented in different weight ratios, such as 1:1, 2:1, and 3:1 (fig. 2). The colored region in the plot represents the region of nanoemulsion, and the larger colored region indicates a good nanoemulsifying activity [16].

Thermodynamic stability tests

The thermodynamic stability study of the prepared nanoemulsion was carried out to analyze the ability of the nanoemulsion to stand out throughout the study period. All the selected cilostazol nanoemulsions loaded were thermodynamically stable when exposed to different tests, including centrifugation, freeze-thawing, and heat-cooling (table 2). These nanoemulsions have not revealed any signs of breaking or separation on visual inspection. Based on the thermodynamic stability tests and taking low percentages of Smix, a high percentage of water, and different Smix ratios, six formulas were selected for further characterization studies [17].

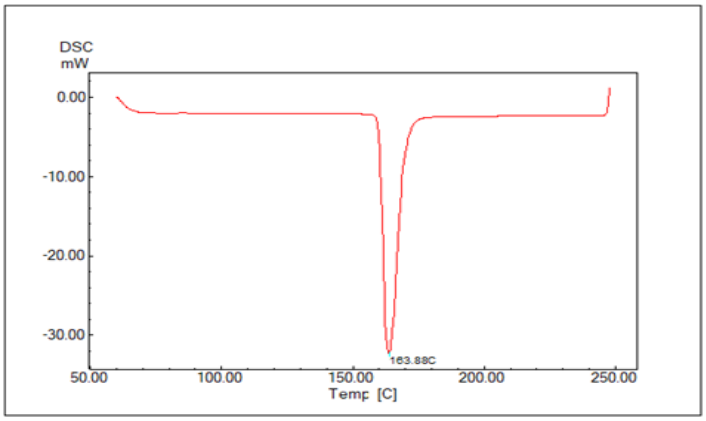


Fig. 1: DSC thermogram of pure cilostazol

Table 1: Cilostazol solubility in various oils, surfactants, and co-surfactants

No.	Components	Solubility (mg/ml)*
1	Olive oil	64.12±2.0
2	Ginger oil	73.37±3.4
3	Peppermint oil	49.42±2.6
4	Basil oil	39.78±3.3
5	Soya bean oil	24.56±2.1
6	Castor oil	12.83±3.0
7	Tween 80	64.22±4.2
8	Tween 20	44.23±2.4
9	Tween 60	21.14±1.2
10	PEG300	53.72±3.7
11	PEG400	33.55±2.0
12	Propylene glycol	9.13±1.3

*Results of solubility (mean±SD, n= 3)

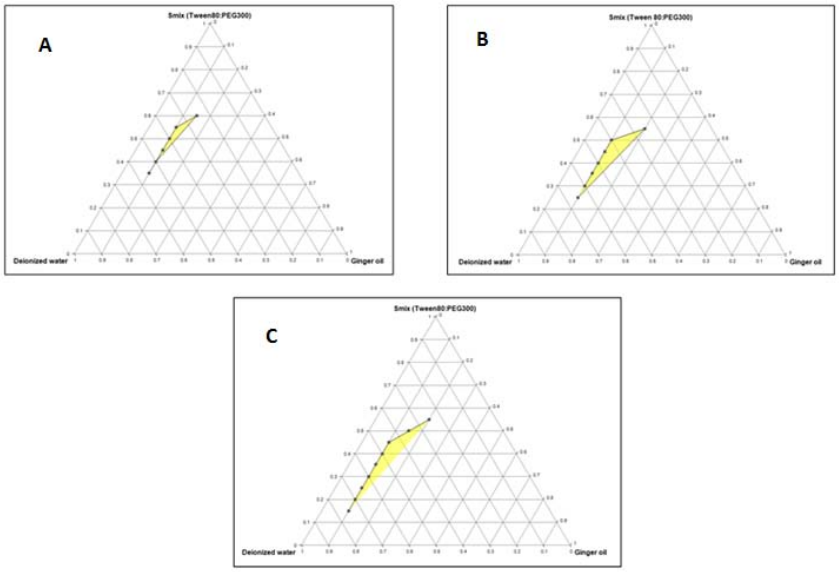


Fig. 2: Pseudo–ternary phase diagram with different S mix (A 1:1, B 2:1, C 3:1)

Table 2: Thermodynamic stability tests of cilostazol nanoemulsions

S mix ratio	Formula no.	%W/W component of nanoemulsions			Thermodynamic stability tests			Results
		S mix (S: CoS)	Ginger oil	Deionized water	Centrifuge	Freeze-thawing	Heating–cooling	
1:1	F-1	35 (17.5:17.5)	10	55	√	√	√	Pass
	F-2	40 (20:20)	10	50	√	√	√	Pass
2:1	F-3	25(16.66:8.33)	10	65	√	√	√	Pass
	F-4	30 (20:10)	10	60	√	√	√	Pass
3:1	F-5	15 (11.25:3.75)	10	75	√	√	√	Pass
	F-6	20 (15:5)	10	70	√	√	√	Pass

Table 3: Composition of cilostazol nanoemulsions

Smix ratio	Formula no.	S mix (S: CoS) w/w %	Ginger oil w/w %	Cilostazol Gm/100 Gm	Deionized water w/w %
1:1	F-1	35 (17.5:17.5)	10	1.0	54
	F-2	40 (20:20)	10	1.0	49
2:1	F-3	25 (16.66:8.33)	10	1.0	64
	F-4	30 (20:10)	10	1.0	59
3:1	F-5	15 (11.25:3.75)	10	1.0	74
	F-6	20 (15:5)	10	1.0	69

Preparation of cilostazol nanoemulsions

Cilostazol nanoemulsions were prepared by dissolving 0.05 g of cilostazol in the determined quantities of oil and Smix to prepare a formula of 5 g (table 3). These nanoemulsions were stored in tight glass containers for characterization studies.

Characterization of nanoemulsions

Droplet size measurement

The results of the average droplet size measurement of the six cilostazol nanoemulsions were in a nano-scale range (table 4). It was found that an increase in the Smix ratio results in a decrease in the droplet size. This may be attributed to increasing the quantity of surfactant (tween 80), which may lead to lowering the interfacial tension and hence decreasing droplet size [18, 19]. Hence, a higher amount of Smix ratio results in the production of nanoemulsion with lower droplet size, as in Smix ratio of 3:1 (F-5 and F-6, which had a droplet size of 75-133 nm, and 72.9-110 nm, respectively). Using ANOVA, there was a significant correlation between the S mix ratio and the droplet size ($P<0.05$).

Polydispersity index (PDI) measurement

PDI results of all six nanoemulsions were less than one (table 4). A PDI value<1 indicates a good uniformity in droplet size distribution within the formulations [20]. Hence, all the prepared nanoemulsions are monodispersed. Formula F-6 had the lower PDI value (0.22±0.13). PDI results are means±SD (n=3).

Zeta potential measurement

Zeta potential is one of the most important indicators of colloidal dispersion stability. The effect of zeta potential on the stability of nanoparticles in a dispersion medium has been demonstrated by the rule of thumb. A zeta potential in the range of-5 mV to+5 mV indicates fast aggregation, and a value of-20 mV to+20 mV indicates short-term stability. While a zeta potential value less than-30mV or above+30 mV indicates good stability and a value less than-60mV or above+60 mV indicates excellent stability in the formulations [21]. All six cilostazol nanoemulsion formulas had a zeta potential in the range of (-17 mV to–28 mV), which means the stability of nanoemulsions was in the range of short-term to good stability.

Drug content estimation

The percent of drug content for cilostazol nanoemulsions was found to be in the range of (91.3%±0.27) and (99.8%±0.31). Formula (F-6) with S mix (3:1) had a higher percentage of drug content (99.8%±0.31), while Formula (F-2) with S mix (1:1) had a lower percentage of drug content (91.3%±0.27) (table 4). Increasing surfactant concentration causes a high solubility of the drug and, therefore, good entrapment of the drug within oil droplets as in F-6 [22]. The small surface of the droplet size makes it unable to adsorb all particles of emulsifier on the surface, which may lead to forming a micellar solution of the drug, increasing its solubility in the aqueous phase [23, 24]. This explains the higher drug content in F-5 and F-6 (97 %±0.22, 99.8%±0.31, respectively). Drug content results are means±SD (n=3).

Table 4: Characterizations of cilostazol nanoemulsions

Formula	Average droplet size (nm)	PDI*	Zeta potential (mV)	Drug content* %
F-1	223-315	0.34±0.18	--28	93±0.33
F-2	199-281	0.26±0.11	-26	91.3%±0.27
F-3	111-166	0.29±0.16	-24	95 %±0.26
F-4	81-140	0.30±0.12	-20	93 %±0.37
F-5	75-133	0.25±0.17	-17	97 %±0.22
F-6	72.9-110	0.22±0.13	-21.2	99.8%±0.31

*PDI and drug content as mean±SD (n=3)

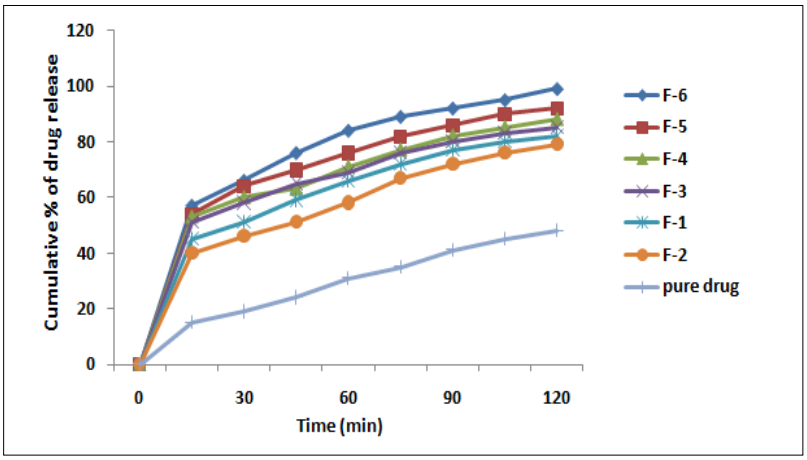


Fig. 3: *In vitro* release profile of cilostazol nanoemulsions and pure drug

In vitro release study

The *in vitro* release of cilostazol nanoemulsions (F-1, F-6) and of the pure drug was made by dialysis bag technique in dissolution medium phosphate buffer (pH 6.8) for two hours. The result of cilostazol release from nanoemulsions and the pure drug was in the following order; F-6>F-5>F-4>F-3>F-1>F-2>pure drug (fig. 3). There was a significant difference (P<0.05) between the release of the drug and time. The release of the drug in the dissolution medium reflects the effect of the concentration of surfactant. It means that as the concentration of tween 80 increases, the release of the drug from the formula will increase up to a certain concentration [23]. Therefore, in a high concentration of surfactant, the drug diffuses from the dialysis bag to the dissolution medium, leading to a lower release of the drug [25]. This has been noticed in a lower release of cilostazol from the nanoemulsion formula with S mix (1:1), and a higher release from the nanoemulsion formula with S mix (1:3).

Kinetic and mechanisms of drug release

Fitting the dissolution data to different kinetic models has indicated that the higher values of the regression coefficient (R^2) of the six cilostazol nanoemulsions and pure drugs were in the Higuchi model (table 5). Hence, the kinetics of the drug release of nanoemulsions and pure drugs was Higuchi. The values of diffusion exponent (n) of nanoemulsions and the pure drug were significantly lower than 0.43 (P<0.05); this indicates that the release mechanism of nanoemulsions and the pure drug was Fickian release (diffusion) [26].

Table 5: The values of the regression coefficient (R^2) and diffusion exponent (n)

NE-code	Zero order model	First order model	Higuchi-model	Korsemyer-peppas model	Diffusion exponent
	R2	R2	R2	R2	N
F-1	0.786	0.902	0.969	0.948	0.19
F-2	0.840	0.948	0.984	0.961	0.24
F-3	0.724	0.932	0.971	0.930	0.35
F-4	0.736	0.970	0.988	0.970	0.22
F-5	0.727	0.915	0.966	0.904	0.27
F-6	0.730	0.902	0.943	0.901	0.33
Pure drug	0.964	0.963	0.992	0.960	0.31

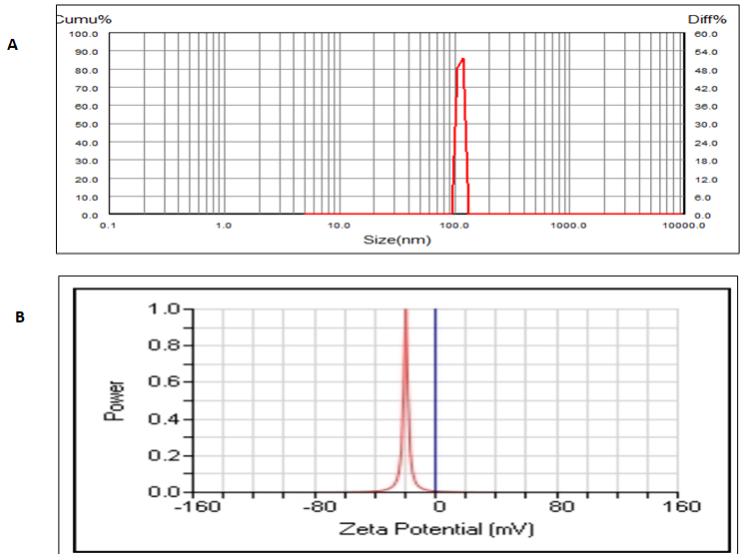


Fig. 4: Droplet size (A) and zeta potential (B) of formula 6 (F-6)

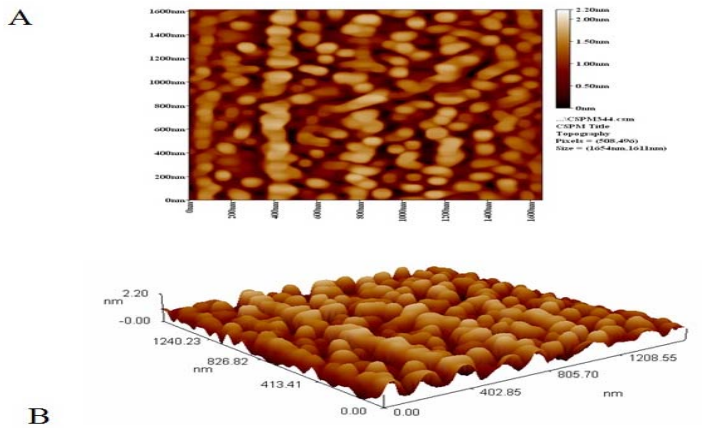
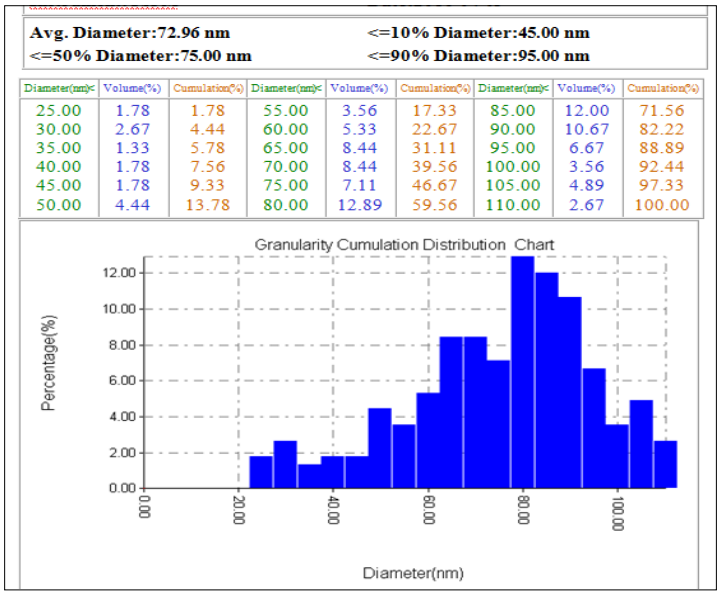


Fig. 5: SPM image of cilostazol nanoemulsion (F-6), A is a 2D image and B is a 3D image



Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)
25.00	1.78	1.78	55.00	3.56	17.33	85.00	12.00	71.56
30.00	2.67	4.44	60.00	5.33	22.67	90.00	10.67	82.22
35.00	1.33	5.78	65.00	8.44	31.11	95.00	6.67	88.89
40.00	1.78	7.56	70.00	8.44	39.56	100.00	3.56	92.44
45.00	1.78	9.33	75.00	7.11	46.67	105.00	4.89	97.33
50.00	4.44	13.78	80.00	12.89	59.56	110.00	2.67	100.00

Fig. 6: Distribution chart of the droplets size range of formula 6 (F-6)

Selection of cilostazol-optimized nanoemulsion formula

Characterization studies of cilostazol nanoemulsions have revealed that the F-6 nanoemulsion was the optimized formula. F-6 nanoemulsion optimized formula has the following characteristics; good droplet size range (72.9-110 nm) (fig. 4), low PDI (0.22), a higher percentage of drug content (99.8%), zeta-potential (-21.2 mV) (fig. 4), and a higher cumulative percent release of drug from the formula (fig. 3).

SPM study

SPM study of cilostazol nanoemulsion (F-6) showed that the droplets were spherical in shape (fig. 5). Droplet size was in nanoscale and similar to the range obtained by particle size analyzer ABT-9000 nanolaser (fig. 6).

CONCLUSION

In conclusion, a nanoemulsion delivery system is a modern approach for improving the solubility of poorly water-soluble drugs belonging to class II of the Biopharmaceutics Classification System (BCS), thereby enhancing their bioavailability. In this study, a method of nanoemulsion preparation has employed a low-energy of emulsification (aqueous titration method) to prepare a nanoemulsion of cilostazol in an attempt to improve its water solubility. The optimized formula (F-6), which contains 50 mg of cilostazol and consists of 20 % S mix (3:1), 10% ginger oil, and 70% deionized water, has a droplet size range (72.9-110 nm), polydispersity index (0.22), high % of drug content (99.8%) can be considered as the promising nanoemulsion formula to improve cilostazol solubility and hence may enhance its bioavailability. This formula has led to an increase in the water solubility of cilostazol, where 97.8% of the drug in the prepared nanoemulsion (F-6) would be released as compared to pure cilostazol, which would release only 41.3%.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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