Journal of Pharmaceutical Innovation (2025) 20:11
https://doi.org/10.1007/s12247-024-09911-0

ORIGINAL ARTICLE

Ezetimibe Loaded Nanostructured Lipid Carriers Tablets: Response Surface Methodology, In-vitro Characterization, and Pharmacokinetics Study in Rats

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Accepted: 10 December 2024

Abstract

Purpose The present study aims to overcome the poor oral bioavailability of ezetimibe (EZ), a selective Biopharmaceutics Classification System (BCS) Class II cholesterol absorption inhibitor drug. EZ-loaded nanostructured lipid carriers (EZ-NLCs) were dried by lyophilization and incorporated in a convenient oral solid dosage form to enhance its dissolution, and absorption and increase patient compliance. Response surface methodology (RSM) was employed to systematically optimize formulation variables, improving the efficiency of disintegration and drug release characteristics.

Methods RSM was adopted to study the effects of (A) increasing the amount of the super-disintegrant, crosscarmelose sodium, (CCS), and (B) varying the ratio between the used drying excipients Avicel and mannitol (A: M) on the disintegration time (R1), and the percentage drug released after 24 h (R2). Thirteen EZ-NLCs tablets were prepared and subjected to pre-compression and post-compression evaluation. Furthermore, a bioequivalence study was conducted by administering EZ-NLCs and ezetrol® tablets to Sprague Dawley male rats.

Results The optimized EZ-NLCs tablet (prepared with the ratio of Avicel: mannitol (7.5:0) using 30 mg CCS), revealed a disintegration time of 3.85 ± 0.03 min, and $98 \pm 3.09\%$ of the drug were released at the end of the 24 h. EZ-NLCs tablet displayed a maximum concentration (Cmax) of 3.57 ± 0.27 ng/mL and an area under the curve (AUC_{0-24}) of 22.44 ± 2.68 ng.hr/mL, while those of ezetrol® were 2.79 ± 0.15 ng/mL and 15.36 ± 0.86 ng.hr/mL, respectively.

Conclusion The assessed relative bioavailability demonstrated the superiority of EZ-NLCs tablet over ezetrol® with 1.5 fold improvement which proves that EZ-NLCs tablet could be a good candidate to enhance the oral bioavailability of EZ.

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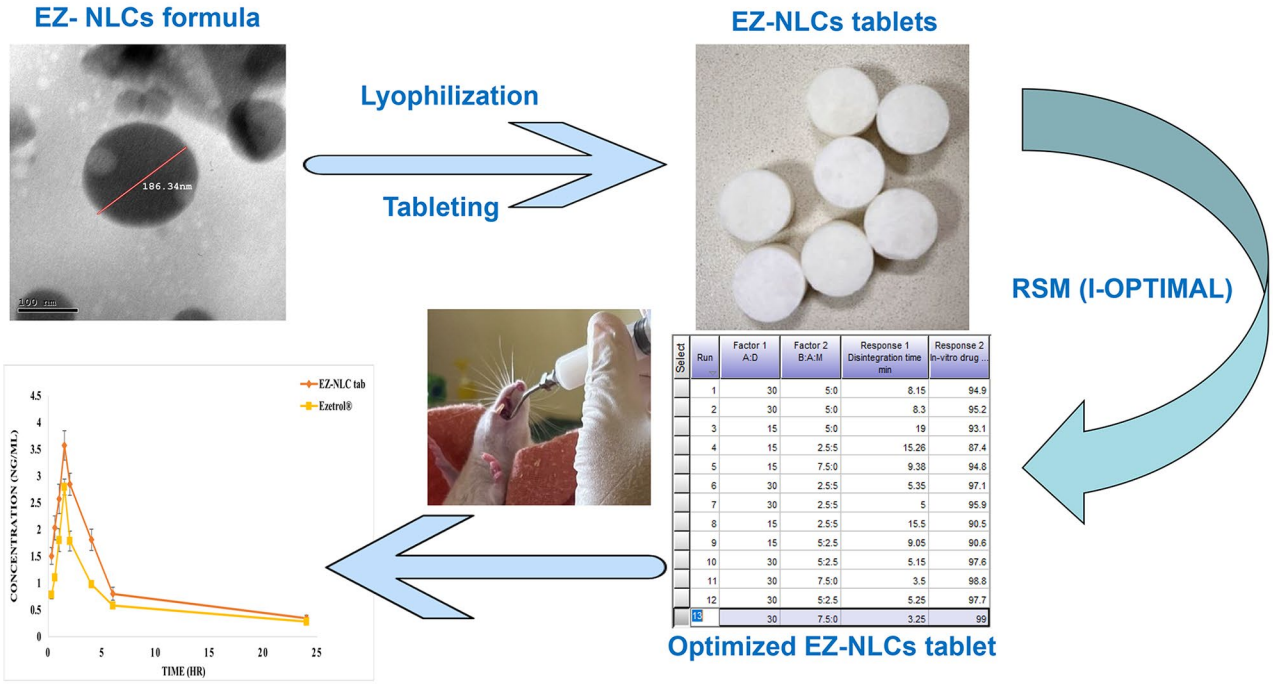
Published online: 09 January 2025

Graphical Abstract



Keywords Ezetimibe · Nanostructured lipid carriers · Pharmacokinetics · Oral bioavailability · I-optimal design

Introduction

Coronary artery disease (CAD), the primary clinical indication of atherosclerosis, is the major cause of death and morbidity worldwide. Hyperlipidemia is responsible for atherosclerosis progression, as evidenced by decreased levels of high-density lipoprotein (HDL) (cholesterol < 40 mg/dL), elevated levels of low-density lipoprotein (LDL) (cholesterol > 190 mg/dL), and triglycerides (TG) (> 200 mg/dL) in plasma. Hyperlipidemia necessitates dietary and lifestyle changes and may require additional lipid-lowering medications [1, 2].

Lipid-lowering medications are classified into statins and non-statins. Although statins are usually well tolerated, adverse drug reactions have been stated in clinical reports, and statin-associated muscle symptoms (SAMS) are the most frequent side effects leading to statin therapy interruption [3].

Non-statin agents include fibrates, which were accompanied by increased serum creatinine levels and decreased glomerular filtration rate (GFR), suggesting that fibrates might have nephrotoxic consequences [4]. Another class of non-statin lipid-lowering drugs is the proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) which are efficient treatments for decreasing LDL plasma levels. However, they may allow blood cholesterol to enter the liver, causing cholesterol redistribution and increasing load on the liver. Moreover, clinical bile acid sequestrants are related to certain adverse effects, such as constipation, and gastrointestinal discomfort, besides they could interfere with the absorption of other nutrients [5].

Ezetimibe (EZ) was selected for our study as a non-statin anti-hyperlipidemic drug to lower plasma cholesterol levels while reducing the side effects of existing lipid-lowering medications.

EZ is a selective inhibitor of dietary and biliary cholesterol absorption in the intestine, it could be utilized for the effective treatment of patients with hypercholesterolemia alone or in combination with statins and fibrates. Unfortunately, EZ is a member of the Biopharmaceutics Classification System (BCS) class II, having low aqueous solubility (0.00846 gm/L), and good tissue permeability (log P:4.56) [6]. It's white solid drug with a molar mass of 409.4 g/mol., it's highly soluble in methanol, ethanol, and acetone. Moreover, it's highly bound to plasma proteins (90%), and mostly excreted in feces (78%) [7]. It undergoes significant hepatic first-pass metabolism following its absorption into the portal venous system, and this results in poor systemic bioavailability (35%). Pre-systemic clearance in the gastrointestinal (GI) mucosa, high lipophilicity, and P-glycoprotein (P-gp) efflux mechanism are further aspects that influence EZ's bioavailability [8].

Owing to the poor solubility of active pharmaceutical ingredients (APIs) in water, various formulation techniques employing nanotechnology have been used to improve drug dissolution and bioavailability through particle size reduction. For example, polymeric micelles, nano-emulsions, nano-suspensions, liposomes, nanoparticles, nanospheres, nanocrystals, and lipid-based nanoparticles (LBNs) such as solid lipid nanoparticles (SLNs) as well as nanostructured lipid carriers (NLCs) [6].

Since SLNs are biocompatible and simple to scale up in mass manufacturing while retaining a cheap cost of materials, they attracted more interest than other lipid-based colloidal carriers. However, this carrier is limited by the crystalline structure of solid lipids at room temperature which only allows the loading of a few number of drug molecules, and this results in particle agglomeration during storage and leakage of drugs out of the carriers [9]. NLCs lipid matrix contains both solid and liquid lipids, increasing the liquid lipid proportion causes the formation of an amorphous structure which may help with better encapsulation of drugs compared to SLNs. NLCs have been utilized since the late 90s, they offer sustained drug release patterns and enhance drug stability and loading capacity, besides reducing drug leakage on storage [8]. They possess excellent biocompatibility, making their scaling-up manufacturing favorable. Moreover, improved oral absorption of drugs encapsulated into NLCs can be attained through their transport via a lymphatic route or Peyer patches [10].

The most outstanding about NLCs is that the surfactants utilized in their formulation can control the P-gp efflux mechanism. Briefly, P-gp returns drugs to the colon as part of their action, which leads to multidrug resistance, and therefore might be a major factor in the insufficient systemic absorption of drugs from the intestine causing a reduction in their bioavailability. However, the surfactants contained within the NLCs can control or hinder this efflux transport, which could change the transcellular paths of these NPs [9].

Oral drug administration is the most favorable method of drug delivery due to its simplicity in handling, patient compliance, and suitability for large-scale manufacturing, in addition to being economical and effective. Direct compression, the most efficient and cheap tableting method, is commonly employed in the pharmaceutical industry for the manufacture of different sorts of tablets for oral delivery [11]. Two of the most fundamental characteristics of immediate-release tablets are disintegration time and dissolution rate, as they determine how quickly the medication can reach the absorption sites, thereby improving bioavailability and efficacy. One of the key steps during the design of a delivery system to guarantee the quality and effectiveness of drugs, is the accurate selection of excipients which play different roles in pharmaceutical formulations [12].

To produce statistically significant investigations with few carried-out experiments, scientists typically employ systematic, effective mathematical models for formulation development and optimization of a wide range of pharmaceutical dosage forms [13]. The optimization procedure has previously been carried out by examining how changes to one parameter affect a response while maintaining the levels of all other parameters constant. The primary drawbacks of this approach are that the full influence of the factors on the response is not clarified, and the interactive effects between the variables are not considered. Furthermore, this approach requires more experiments to complete the study, which raises the expense and duration. Multivariate statistical techniques can be used to conduct optimization studies to address this issue. The most often used multivariate statistical method in the optimization process is response surface methodology (RSM). RSM is a group of mathematical and statistical techniques based on fitting a polynomial model to data that must represent a data set's behavior to generate statistical predictions. The method helps plan, create, and optimize processes when a response or reactions depend on several variables [14].

To the best of our knowledge, several studies were conducted on EZ to improve its oral bioavailability employing SLNs and NLCs as those carried out by Y. O. Agrawal, G. Shevalkar, F.ud Din, R. Kaoud, and K. Borderwala [8, 15–18]. Whereas Gardouh AR, Narayan R, and Yadav P designed a self-nano emulsifying drug delivery system to enhance drug solubility and dissolution [1, 19, 20]. Moreover, Shukr MH prepared chitosan nanoparticles to improve drug efficacy [21]. However, none of these studies considered developing a stable oral solid dosage form that accommodates the drug-loaded nanoformulations to overcome the drawbacks of NLCs dispersion (liquid dosage forms) as content variability, bitter taste, instability, and lack of ability to modify drug release patterns. Accordingly, the novelty of our work is represented in conducting a comprehensive study to enhance the stability and patient compliance of EZ-NLCs dispersion through its incorporation in an oral solid dosage form and optimize different excipients using response surface methodology and employing I-Optimal design by Design-Expert 10.1.1 software to achieve the best in-vitro evaluation results out of the prepared formulations. Furthermore, a bioequivalence study will be conducted on the optimized EZ-NLCs tablet to assess the enhancement in the oral bioavailability of EZ.

Experimental

Materials

The drug ezetimibe was obtained as a gift from Marcyrl Pharmaceutical Co. (ElObour City, Cairo, Egypt). Oleic acid was purchased from Alpha Chemicals (Cairo, Egypt). Stearic acid, tween 80, and sodium lauryl sulphate were purchased from Adwic, El-Nasr Pharmaceutical Co. (Cairo, Egypt). Poloxamer 188 was purchased from Sigma-Aldrich Chemical Co, (St. Louis, USA). Mannitol, Avicel PH 102, magnesium stearate, talc, and crosscarmelose sodium were purchased from Piochem (540 area, Giza, Egypt). Ezetrol® was obtained from MSD Pharmaceutical Co. (Cairo, Egypt). All the other reagents which were used are of analytical grade.

Methods

Preparation of Ezetimibe-Loaded NLCs (EZ-NLCs) Powders

Optimized ezetimibe-loaded nanostructured lipid carriers (EZ-NLCs) formula was prepared and characterized in our previously published work.

Briefly, the oily phase was prepared by melting a mixture of stearic acid and oleic acid (30%) at 348 °K on a hot plate stirrer (MSH-20D, HP20D/30D, and MS-20D Models). Simultaneously, the aqueous phase, which was prepared by dispersing the surfactant mixture of 1% Tween 80 and 2% poloxamer 188 in distilled water, was heated to the same temperature. Then, EZ (10 mg) was added to the molten lipid to form a uniform clear oil phase followed by dropwise addition of the aqueous phase to the oily one under magnetic stirring at 1400 rpm and 333 °K and then continuously stirred for 15 min. A pre-emulsion was obtained and then subjected to ultrasonication using a probe sonicator (SONICS Vibra cell VCX500, USA) at an amplitude of 100% for 5 min [22, 23]. The produced EZ-NLCs dispersion was mixed with the dry excipients (Avicel alone or with mannitol) (5-7.5 w/v%) until a homogeneous paste was formed. Using a Christ freeze drier (Alpha 2.4 LD plus, Germany), the paste was lyophilized after being kept in a freezer at 253 °K overnight. The freeze-drying procedure consisted of pre-freezing at 193 °K for 15 min, followed by a ramping time of 20 min, and then a main and final drying cycle at 3 mbar for 6 h total were applied. To enhance the flowability of the prepared EZ-NLCs powders, 10 mg magnesium stearate and 10 mg talc were added as lubricant and glidant respectively, along with croscarmellose sodium (CCS) as a super-disintegrant to aid the process of disintegration [24].

Table 1 Independent variables and dependent responses for EZ-NLCs powders

Independent variables:	Normalized levels of independent variables	
	Low level	High level
A: Amount of disintegrant (mg)	15	30
B: Avicel: Mannitol (A: M) ratio	2.5:5	5:0
Dependent variables:		Aim
R1: Disintegration time (min)		Minimize
R2: Percentage of drug released after 24 h (%)		Maximize

Design of the Experiments

Design-Expert 10.1.1 software (Stat-Ease Inc., Minneapolis, MN) was used, employing response surface methodology (I-optimal design) to investigate the effects of: (A) various amounts of the super-disintegrant crosscarmelose sodium (CCS) (15 and 30 mg) as well as (B) different ratios between Avicel and mannitol (A: M) (2.5:5, 5:2.5, 7.5:0, and 5:0) on the disintegration time (R1), and the percentage of in-vitro release of EZ after 24 h (R2). The goal of our study was to achieve rapid disintegration to accelerate drug absorption, and hence improve bioavailability. Moreover, drug dissolution is the rate limiting step in case of drugs with low aqueous solubility, so it was demanding to maximize the percentage of in-vitro drug release to aid its dissolution [25]. The selection of the independent variables was done based on the results of preliminary experiments (data not shown). Table 1 illustrates the independent variables (A) and (B) with their levels along with the dependent responses (R1) and (R2) as well as their required goals [26].

The findings of the 13 experiments were fitted to linear, cubic, or quadratic polynomial models. Design-Expert 10.1.1 software selects the best-fit model based on an ANOVA study displaying a high F-value, high adjusted and predicted R2 (difference < 0.2), high correlation coefficient, and non-significant lack of fit. The optimized formula was then selected to attain the goals of responses through numerical optimization using the desirability function. The best formulation composition with the highest desirability was selected and prepared then further characterized [27, 28].

Pre-compression evaluation.

Evaluation of the prepared ezetimibe loaded NLCs (EZ-NLCs) powders

Two different methods namely angle of repose and bulk density were employed to determine the flowability and compressibility of the prepared EZ-NLCs powder blends before compression. The bulk density was calculated through the division of the weight of the 50-gram powder blend by its volume in cm^3 in a 100-mL graduated measuring cylinder. The tapped density was measured by dividing the weight

in grams by the volume in cm^3 acquired after tapping the powder blend into the cylinder when its volume became constant. The following equations were used to determine the Hausner ratio and Carr's index [29, 30].

$$\text{Hausner's ratio} = \rho t / \rho o$$

$$\text{Compressibility Index (C.I.)}\ \% = (\rho t - \rho o)/\rho t \times 100$$

Where, ρt = Tapped density, ρo = Bulk density.

To determine the angle of repose, the funnel method was applied. The powder was poured through a fixed funnel at a distance of 0.06 m from the base, and then the height and the diameter of the powder cone were measured. The following equation was used to calculate the angle of repose [31].

$$\tan\theta = h/r$$

where h is the height and r is the radius.

Formulation of Ezetimibe-Loaded NLCs (EZ-NLCs) Tablets

EZ-NLCs powders were compressed directly into tablets using a tablet compression machine (P.O. BOX 404, MUMBAI, INDIA) with a punch diameter of 0.012 m and a thickness of 0.008 m. The machine was adjusted so that the hardness of the tablets ranged from 30 to 50 N [24]. Each tablet contained 500 mg EZ-NLCs powder containing 10 mg EZ, 500–750 mg drying excipients, 15–30 mg CCS, 10 mg talc, and 10 mg magnesium stearate [32, 33].

Post-compression evaluation.

Evaluation of ezetimibe loaded NLCs (EZ-NLCs) tablets.

Weight Variation

The weight variation test was carried out by weighing 10 EZ-NLCs tablets using an electronic balance and their average weight was calculated. Then each tablet was weighed individually and compared to the average weight of the 10 tablets [34].

Content Uniformity

To determine the content uniformity of different EZ-NLCs tablets, sample tablets (each containing 10 mg EZ) were crushed, and sonicated in 25 mL methanol using a water bath sonicator at 298 °K for 5 min. Then the obtained solutions were filtered through a 0.22 Millipore filter, and the concentration of EZ was estimated by measuring its absorbance spectrophotometrically at λmax of 231 nm [16, 35].

Hardness

The tablet hardness or tablet tensile strength is defined as the maximum force required for a tablet to be broken. (Mecmesin®, UK) hardness tester was used to measure the breaking force of ten randomly selected EZ-NLCs tablets in Newton (N) [26, 34].

Friability

To determine the friability percentage of the prepared tablets, six EZ-NLCs tablets were randomly selected, de-dusted, and weighed (Wti). Then they were placed into the drum of the friability tester (DR. SCHLEUNIGER®, Pharmaton) which was adjusted to operate at 25 rpm for 4 min (100 rotations) at room temperature. After that, the tablets were removed, brushed, and re-weighed (Wtf) and the percentage friability was calculated according to the below equation [26].

$$\text{Friability}\ (\%) = (\text{Wti} - \text{Wtf}\ /\text{Wti}\ ) \times 100$$

Disintegration Time

The time required for the tablets to disintegrate completely was determined in-vitro by (COPLEY®, Scientific, UK) disintegration tester. Briefly, one EZ-NLCs tablet was placed in each of the six tubes of the basket. Then the tester was operated at 310 °K using 0.1 N HCL pH 1.2 as a disintegration medium [24, 31].

In-Vitro Drug Release Study

The in-vitro drug release of EZ from the prepared EZ-NLCs tablets was performed using the USP standard dissolution tester, Apparatus II. Briefly, one tablet from each formulation and the market product ezetrol® (10 mg), were introduced into the vessels of the dissolution apparatus maintained at 310 °K ± 0.5 and rotated at a speed of 100 rpm. The study was carried out for 24 h in 900 mL of buffer transition system at different pHs to reflect the impact of varying pH conditions on EZ-NLCs tablets dissolution throughout its passage along the human GIT. The formula was exposed to 650 mL of 0.1 N HCl, pH 1.2 as a dissolution medium for 2 h, after which 250 mL of disodium hydrogen orthophosphate solution was added to the vessels for the transition to the buffer of pH 6.8, and the experiment was conducted for another 22 h [20, 36]. 1% sodium lauryl sulphate (SLS) was added to the media to maintain sink conditions due to the poor solubility of EZ in aqueous media [37]. Suitable volumes of samples were withdrawn at predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, and 24 h) and replaced with

equal fresh media to keep the volume constant throughout the study [24]. Test samples performed in triplicates were filtered through a 0.22 Millipore filter and analyzed spectrophotometrically at 231 nm [15, 21].

To evaluate the kinetics of drug release, the mathematical models: zero order, first order, Higuchi, and Peppas–Korsmeyer were fitted to the experimental data utilizing Microsoft Excel software version (2021) [38]. The regression coefficient (R^2) was estimated to determine the best-fit model.

Characterization of the optimized EZ-NLCs tablet.

Differential Scanning Calorimetry (DSC)

A thermoanalytical method known as differential scanning calorimetry (DSC) was used to pinpoint various physical characteristics and thermal transitions of polymeric materials. Shimadzu DSC-50 (Shimadzu Instruments, Japan) was used to perform DSC measurements on the pure drug, the optimized EZ-NLCs tablet, and the blank NLCs tablet. One mg of each sample was hermetically sealed in flat-bottomed aluminum pans and heated to temperatures between 298 and 623 °K, at a rate of 283 °K/min while being purged with dry nitrogen at a rate of 25 mL/min [22, 30].

Fourier Transform Infrared (FTIR) Analysis

Through Fourier transform-infrared (IRAffinity-1 S A219654 SHIMADZU 01660) tests, the optimized EZ-NLCs tablet and its blank as well as pure drug FTIR spectra were analyzed for the interactions between the drug and the added excipients. An accurately weighed 10 mg of each sample was added onto the FTIR's glass window and their FTIR spectra were all obtained at a resolution of 0.04 m^{-1} over a range of 40–4 m^{-1} for 50 scans [18, 27].

Determination of Particle Size (PS), Zeta Potential (ZP), and Polydispersity Index (PDI)

Using a Malvern particle size analyzer, the mean particle size (PS), polydispersity index (PDI), and zeta potential (ZP) were investigated and compared to evaluate the physicochemical characteristics of NLCs before and after lyophilization. The analysis was conducted on optimized EZ-NLCs dispersion and optimized EZ-NLCs tablet (before compression) after its redispersion in water. Samples were diluted 1:100 with double-distilled water, subjected to bath sonication for 5 min, and then examined at 298 °K [28, 39]. The test samples were carried out in triplicates and means ± S.D were estimated.

In-Vivo Pharmacokinetics Study.

The purpose of the pharmacokinetics study was to evaluate and compare the bioavailability of the optimized EZ-NLCs tablet with the market product ezetrol®.

Study Design

Six adult Sprague Dawley male rats weighing between 200 ± 10 g (age: 8–10 weeks) were brought from Nile Co. for the Pharmaceutical and Chemical industries (Cairo, Egypt).

Under standard laboratory conditions of 301 °K, 55% humidity, and alternating 12-hour light and dark cycles, the animals were housed with free access to standard diet pellets and water. The experimental protocol was carried out in compliance with ethical guidelines and was approved by the Research Ethics Committee of the Faculty of Pharmacy, Cairo University, Egypt (PI 1719).

Based on the elimination half-life time of EZ as reported by Nasef A [40], a parallel randomized design was selected for the study where the six animals were allocated into two groups ($n = 3$). Group I received optimized EZ-NLCs tablet (test) and Group II received the market product ezetrol® as standard.

Procedure

A single oral dose was administered to each of the six subjects at a rate of 25 mg/kg [17]. The tablets were crushed, redispersed in water, and administered to the rats using an oral gavage. At predetermined time intervals (0.3, 0.6, 1, 1.5, 2, 4, 6, and 24 h), 1 mL blood sample was withdrawn from the rat's orbital sinus using a heparinized capillary tube and transferred to Eppendorf tubes previously heparinized.

Processing of Samples

Heparinized blood samples were centrifuged using (Sigma Laboratory centrifuge, Germany) at 8000 rpm for 15 min at 298 °K. Then the obtained plasma samples of both test and standard were stored frozen at 253 °K until being investigated adopting the valid method reported by Oliveira PR, with slight modifications (the parameters of the valid method were studied). Briefly, 0.5 mL plasma samples were placed into Eppendorf tubes with 50 µl hydrochlorothiazide (HCTZ) 1 µg/mL and vortexed for 30 s at room temperature. The tubes were further extracted with 4 mL ethyl acetate and centrifuged for 15 min at 4000 rpm. After being separated, the supernatant was dried under nitrogen at 313 °K before observation [41].

Chromatographic separations were conducted on a reversed-phase Waters SunFire C18 column (0.0046 × 0.05 m), the mobile phase was composed of

water: acetonitrile (20:80 v/v) with a flow rate of 0.9 mL/min and an injection volume of 10 µL. The detection was conducted utilizing a triple quadrupole detector MS/MS. The analysis was operated at the MRM (multiple reaction monitoring) mode [41].

Statistical Analysis

Each rat's plasma concentration-time profile data was investigated to determine the pharmacokinetic parameters for both EZ-NLCs and ezetrol® tablets. Utilizing Microsoft Excel software version (2021), the elimination half-life time (T1/2), maximum concentration (Cmax), mean residence time (MRT), the time required to achieve maximum concentration (Tmax), and the area under the plasma concentration-time curve (AUC) were estimated to compare the bioavailabilities of EZ-NLCs and ezetrol® tablets. The significance of each parameter was calculated via an unpaired student *t*-test with Welch's correction using GraphPad Prism V.8.01, USA [17, 42].

Results and Discussion

The optimized EZ-NLCs formula (10 mg EZ in 10% total lipid (3:7) oleic acid: stearic acid), with 1% tween and 2% poloxamer) was selected employing response surface methodology using Design-Expert 10.1.1 software (Stat-Ease Inc., Minneapolis, MN), and designated to complete the study. The selection of this optimized formula was carried out in our previous work based on the desirability function fulfilling the required goals (minimized particle size as well as maximized zeta potential, entrapment efficiency %, and % of in-vitro drug released after 24 h).

Thirteen EZ-NLCs tablets were successfully prepared adopting RSM to investigate the effects of the amount of the super-disintegrant CCS at two levels namely 15 and 30 mg and different ratios between Avicel and mannitol (2.5:5, 5:2.5, 7.5:0, and 5:0), on the disintegration time and the % of in-vitro drug released after 24 h, the results could be given under the following headings.

Evaluation of the Prepared Ezetimibe Loaded NLCs (EZ-NLCs) Powders

Increasing both the chemical and physical stability of nanoparticle formulations can be achieved through the removal of the surrounding aqueous media followed by the transformation of liquid nanoparticles into dried solid form. This modification supports the medication to redisperse easily in GIT fluids after oral administration [24]. The powder's flowability directly impacts the die-filling capacity, and the ability of the particles in the powders to stay together after the applied compression force is withdrawn is directly related to the success of tablet preparation. Porosity, tensile strength, and dissolution performance are only a few of the important tablet qualities that are defined by the overall balance between these phenomena. Poor comprehension of these events may result in inadequate bioavailability, higher production costs, decreased yield, and lower-quality products, making flowability a critical factor in ensuring tablet compression efficiency [43]. Table 2 shows the results of Hausner's factor that ranged from 1.21 ± 0.004 to 1.24 ± 0.029, Carr's index which was found to be from 17.19 ± 1.455% to 22.52 ± 1.297%, and the angle of repose

Table 2 Pre-compression and post-compression evaluation results of different EZ-NLCs tablets

[a] Run	[b]A	[c]B	*Hausner's Factor	*Carr's index (%)	*Angle of repose (°)	*Disintegration time (min) ($n=6$)	*In-vitro release % after 24 h (%) ($n=3$)	*Mean weight (mg) ($n=10$)	*Content uniformity (%) ($n=3$)	*Hardness (N) ($n=10$)	Friability (%)
1	30	5:0	1.23 ± 0.05	19.38 ± 3.09	27.53 ± 2.16	8.15 ± 0.08	94.9 ± 2.42	890 ± 4.51	91.48 ± 2.91	37.86 ± 2.61	0.17
2	30	5:0	1.23 ± 0.06	18.99 ± 2.17	27.89 ± 1.89	8.3 ± 0.01	95.2 ± 1.61	900 ± 2.39	91.25 ± 3.65	38.15 ± 2.24	0.17
3	15	5:0	1.21 ± 0.04	18.31 ± 0.29	26.43 ± 2.35	19 ± 0.08	93.1 ± 1.17	1012 ± 1.99	87.11 ± 3.78	44.13 ± 1.99	0.10
4	15	2.5:5	1.23 ± 0.02	18.57 ± 1.43	25.23 ± 0.67	15.2 ± 0.02	87.4 ± 1.01	1052 ± 4.08	89.42 ± 5.14	40.21 ± 3.05	0.12
5	15	7.5:0	1.23 ± 0.03	16.65 ± 1.63	24.33 ± 1.53	9.38 ± 0.03	94.8 ± 1.71	1047 ± 3.75	90.23 ± 2.21	38.74 ± 2.67	0.15
6	30	2.5:5	1.21 ± 0.02	17.19 ± 1.46	27.05 ± 2.87	5.35 ± 0.02	97.1 ± 2.22	1082 ± 5.15	91.68 ± 3.19	37.11 ± 2.55	0.18
7	30	2.5:5	1.22 ± 0.04	16.89 ± 1.79	27.52 ± 2.55	5 ± 0.01	95.9 ± 1.50	1090 ± 2.92	86.96 ± 4.42	36.68 ± 3.17	0.19
8	15	2.5:5	1.23 ± 0.03	18.19 ± 2.06	25.89 ± 1.76	15.5 ± 0.03	90.5 ± 1.12	1061 ± 3.45	87.38 ± 5.25	41.19 ± 2.05	0.11
9	15	5:2.5	1.23 ± 0.01	18.98 ± 0.93	27.5 ± 1.32	9.05 ± 0.02	90.6 ± 1.17	1055 ± 5.81	89.81 ± 1.94	38.54 ± 1.55	0.16
10	30	5:2.5	1.22 ± 0.03	17.97 ± 1.78	25.87 ± 1.80	5.15 ± 0.03	97.6 ± 1.67	1088 ± 4.38	86.81 ± 5.21	36.29 ± 1.08	0.22
11	30	7.5:0	1.24 ± 0.02	17.41 ± 1.56	29.93 ± 1.90	3.5 ± 0.02	98.8 ± 1.21	1110 ± 2.65	93.18 ± 3.42	35.21 ± 1.95	0.32
12	30	5:2.5	1.22 ± 0.04	18.25 ± 1.87	26.21 ± 2.54	5.25 ± 0.02	97.7 ± 2.51	1095 ± 3.59	91.59 ± 2.39	35.99 ± 2.09	0.25
13	30	7.5:0	1.24 ± 0.03	17.89 ± 1.29	29.23 ± 1.98	3.25 ± 0.06	99 ± 1.84	1093 ± 5.02	92.89 ± 2.56	35.59 ± 2.12	0.28

[a]Each tablet contains 500 mg ezetimibe-loaded nanostructured lipid carriers equivalent to 10 mg ezetimibe, 10 mg magnesium stearate, and 10 mg talc. [b]A: disintegrant (CCS) amount. [c]B: Avicel to mannitol ratio. * Data presented as mean ± SD

which were between 22.67°±1.102 and 29.93°±1.901. ANOVA analysis revealed that no significant difference was observed between different EZ-NLCs powder formulations concerning Hausner's factor, Carr's index, and angle of repose. According to the USP 39/NF 34 classification system, the flow properties measured suggested good flowability for all EZ-NLCs powder formulations, and guaranteed uniform die filling during tablet compression, which led to consistent tablet weight and content uniformity.

Evaluation of Ezetimibe Loaded NLCs (EZ-NLCs) Tablets

The produced EZ-NLCs tablets were subjected to different characterization tests, all their findings complied with the pharmacopeial standards and are displayed in Table 2.

Statistical Analysis of the Experimental Design

Design-Expert 10.1.1 software was utilized to investigate the effects of (A) the amount of the super-disintegrant CCS, and (B) the ratio of Avicel to mannitol on the disintegration time and % of in-vitro drug released after 24 h. The selection of the independent variables as well as their levels was done based on preliminary trials and determined to be 15 and 30 mg for A, and (2.5:5, 5:2.5, 7.5:0, and 5:0) for B. While the ratios (0:7.5 and 0:5) were excluded from the study due to their poor compressibility. The two responses that were examined for the produced EZ-NLCs tablets were fitted to various models. In terms of R1 (disintegration time), and R2 (% of in-vitro drug released after 24 h), it was discovered that the 2FI was the best-fit model for both R1 and R2 as displayed in Table 3. The effects of these independent variables on the responses are indicated by the coefficient values of A, B1, B2, and B3. A positive coefficient sign denotes a synergistic effect, while a negative coefficient indicates an antagonistic one upon the reaction. The stronger the influence of the independent variable on the response, the higher the coefficient's value. The significance at the 5% level was determined using ANOVA [27, 30, 44].

Effect of Formulation and Process Variables on Disintegration Time (R1)

For a super-disintegrant to be ideal, it should induce tablet disintegration within short time intervals (not exceeding three minutes) without residuals left. As observed from Table 2, the disintegration time of the prepared EZ-NLCs tablets ranged from 3:35 ± 0.02 to 19:00 ± 0.08 min. ANOVA analysis demonstrated the significance of both independent variables on the disintegration time with p value < 0.0001. The negative coefficient of A (amount of CCS) in the equation displayed in Table 3 reflects the inverse relationship between the amount of the super-disintegrant (CCS) added and the time required for the tablet to become fully disintegrated. This means that increasing the amount of CCS caused a decrease in the disintegration time as shown in Fig. 1a. This finding came in agreement with previously carried out experiments performed by Maclean N, Berardi A, and Ghumman S.A [43, 45, 46].

For the ratio between Avicel and Mannitol added in the tablet preparations, we can conclude from Fig. 1b that the ratio of Avicel to mannitol which showed the least disintegration time was (7.5:0), followed by (5:2.5) and (2.5:5), while (5:0) was found to cause an increase in the time required for tablet disintegration. This means that the presence of mannitol in EZ-NLCs tablet preparations influenced the disintegration time negatively, likely due to its lower porosity compared to Avicel, which reduces water absorption and tablet swelling. Moreover, increasing the amount of Avicel from 500 to 750 mg contributed to the faster disintegration of EZ-NLCs tablets proved by the delayed disintegration observed with the ratio (5:0). Briefly, studies have shown that tablets made of insoluble excipients (Avicel) dissolve more quickly than those made of soluble excipients (Mannitol). Filler particles will dissolve as a result of liquid penetration if the tablet matrix is made of a soluble substance, whereas, only few amount of water is required to moisten the filler particles in tablets made of an insoluble filler, freeing up more water for the disintegrant [43]. The intrinsic dissolution rate of the excipients must be considered in addition to the solubility of the tablet's constituent parts to differentiate between those that will dissolve more slowly and those that will dissolve quickly when in contact

Table 3 Analysis of responses results using design expert 10 software

Response	Model	R^2	Adjusted R^2	Equation
R1	2FI	0.9995	0.9989	R1 = + 9.35–3.85*A + 0.93*B1-2.22*B2-2.97*B3-1.25*AB1 + 1.93*AB2 + 0.85*AB3.
R2	2FI	0.9628	0.9107	R2 = + 94.44 + 2.58*A-1.72*B1-0.32*B2 + 2.41*B3 + 1.19*AB1 + 0.92*AB2-0.53*AB3.

A: amount of the super-disintegrant (CCS)

B1: 2.5:5

B2: 5:2.5

B3: 7.5:0

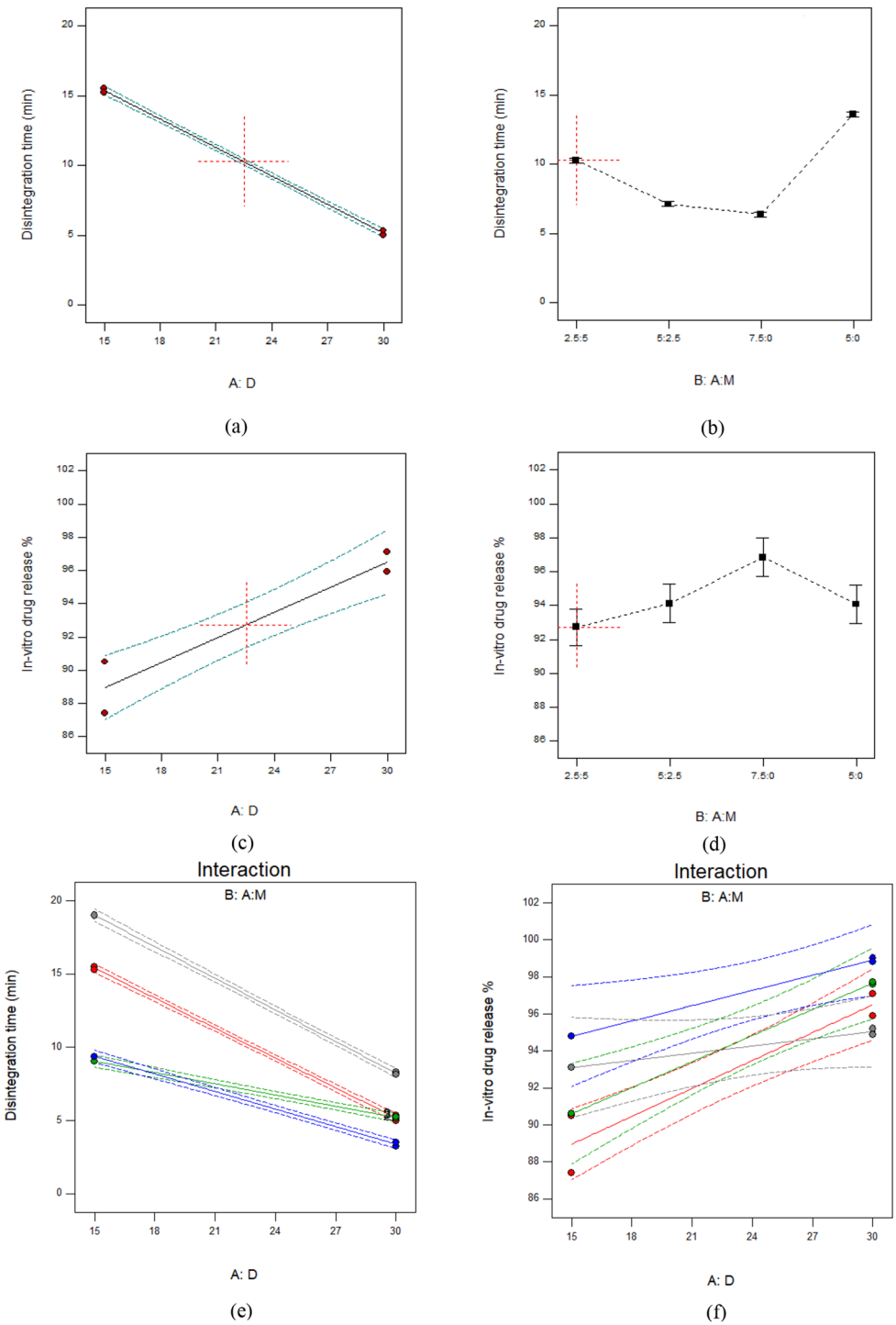


Fig. 1 Perturbation model graphs for the effects of the independent variables on the investigated responses: **a**. effect of A on disintegration time, **b**. effect of B on disintegration time, **c**. effect of A on the percentage of drug released after 24 h, **d**. effect of B on the percentage of drug released after 24 h, **e**. interaction plot for the effect of A and B on disintegration time, and **f**. interaction plot for the effect of A and B on the percentage drug released after 24 h

with fluids. These particles will quickly dissolve from the matrix when mannitol is added as a filler, increasing the apparent pore space and enhancing liquid penetration. The dissolution of particles from the matrix drives disintegration if the main tablet components have high solubility and intrinsic dissolution rates, which subsequently improves liquid penetration. Thus, the disintegration mechanism is controlled by dissolution [47].

Regarding the interaction, ANOVA analysis revealed that there was a significant interaction between the variables A, and B on disintegration time (p value < 0.0001). Where an antagonistic effect was observed with mannitol while a synergistic effect was devoted to Avicel as shown in Fig. 1e. These findings can be explained based on excipients' porosity, as Avicel has higher porosity and water absorption capacity than mannitol [24, 48, 49].

Effect of Formulation and Process Variables on the % of In-Vitro Drug Released After 24 h (R2)

Generally, drug release is considered a routine quality control test to guarantee the final uniformity of the finished product and is a fundamental step during the development stages of a new formulation. The in-vitro release experiment was carried out under sink conditions, 1% SLS was added to the dissolution medium to increase the solubility of poorly water soluble molecules, and thereofre improve their dissolution and maintain sink conditions [50]. The release profiles of EZ from the prepared EZ-NLCs tablets over 24 h showed a biphasic drug release pattern as shown in Fig. 2. A faster drug release was observed in the first 6 h (2 h in pH 1.2 and 4 h in pH 6.8) followed by a slower and sustained release over the remaining 18 h. The rapid release initially observed can be related to the free EZ percentage that was weakly bound or adsorbed to the large surface area created by the nanocarrier formation. Whereas the second slower phase describes the pathway of the entrapped drug molecules through the formed nanostructured lipid carriers from the core matrix to the surface [51].

The percentage of drug release during the first 2 h in pH 1.2 ranged from 53.84 ± 1.11% to 90.6 ± 1.11%, while the market product ezetrol® showed 48.3 ± 1.53% of drug release in the same pH. The tablets prepared with 15 mg CCS exhibited higher release percentages that ranged from

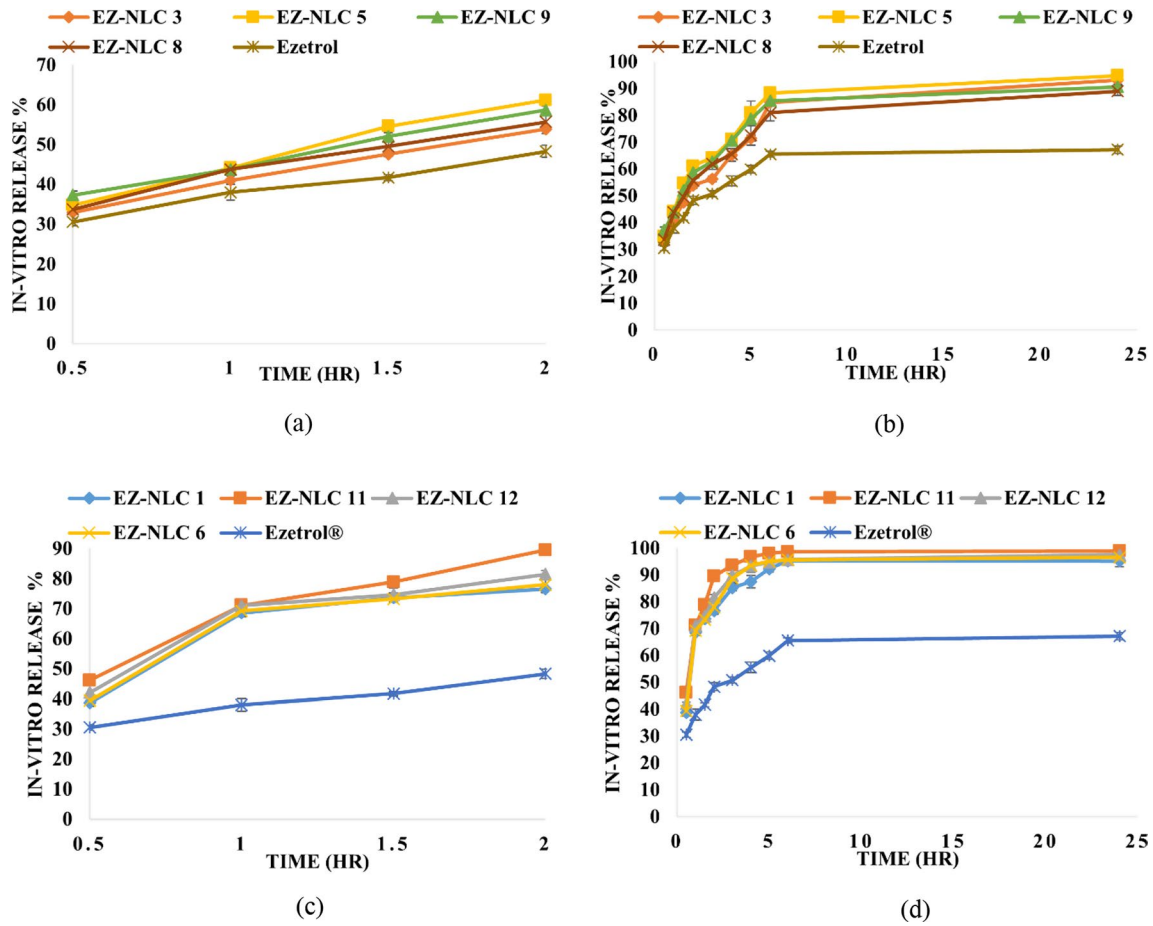


Fig. 2 I*n-vitro* release profiles of EZ from different EZ-NLCs and ezetrol® tablets showing biphasic drug release patterns: **a**. Tablets with 15 mg CCS in pH 1.2, **b**. Tablets with 15 mg CCS over 24 h, **c**. Tablets with 30 mg CCS in pH 1.2, **d**. Tablets with 30 mg CCS over 24 h

53.84 ± 1.11% to 61.13 ± 1.45% as shown in Fig. 2a, while those prepared with 30 mg CCS revealed release percentages between 76.58 ± 0.51% and 90.6 ± 1.11% as displayed in Fig. 2c. At the end of the 24 h the percentages ranged from 87.4 ± 1.01% to 99 ± 1.84% and a percentage of 67.17 ± 1.39% was observed for ezetrol® as shown in Fig. 2b and d. The higher release percentage observed in pH 1.2 in comparison with the remaining 22 h in pH 6.8 may be attributed to the ionization of EZ as a weak base ($pK_a = 9.7$) in pH 1.2 which aided its solubility in this acidic medium, while upon transition to pH 6.8 EZ's solubility decreased due to the transformation of EZ to its free base [24, 52].

Statistical analysis of the % of drug released for different EZ-NLCs tablets demonstrated significant impacts of A (amount of CCS), and B (ratio of Avicel to mannitol) with p value = 0.0027 and 0.0003 respectively upon the total percentage of drug released at the end of the 24 h.

The produced EZ-NLCs tablets exhibited an excipient type-reliant release profile in which the drug release behavior is controlled by the characteristics of excipients. The equation in Table 3 displays the direct relationship between the amount of disintegrant A and the drug release percentage after 24 h, reflected by the positive coefficient of A and confirmed in Fig. 2c. The highest drug release percentage in pH 1.2 and pH 6.8 media together was achieved by the ratio of Avicel: mannitol (7.5:0) followed by both (5:0) and (5:2.5) and lastly (2.5:5). This observation reflects that the addition of mannitol in EZ-NLCs tablet preparations resulted in a fall in the release % from EZ-NLCs tablets. These findings were well correlated with the disintegration results demonstrated in Table 2 and could be accredited to the superiority of Avicel, which has an inherent disintegrating action, in improving the disintegration process and release profiles of solid dosage forms. Another possible justification for these results is that they are most likely the product of a more pertinent porosity property, which is believed to be critical to the redispersion of NLCs tablets after dilution. On the word of reports, relatively large voids tend to be formed in solids with low bulk densities due to the action of adhesive forces between the small particles. This additionally enhances water permeation and body fluid propagation into the tablet by capillary action, resulting in consequent rapid tablet disintegration and in-vitro release as well [24]. In a word, it was obvious from our results that Avicel was far better than mannitol as a drying excipient concerning EZ-NLCs tablets' disintegration and in-vitro drug release. To conclude, EZ-NLCs tablets demonstrated superiority concerning drug disintegration and consequently in-vitro release % which in turn proposed improved oral bioavailability, in comparison with other traditional marketed formulations such as ezetrol®.

Drug Release Kinetics

The observed profile for the release of EZ from EZ-NLCs tablets was analyzed by fitting various kinetic models such as zero order, first order, Higuchi, and Krosmeyer-Peppas using Microsoft Excel software version (2021). The results displayed in Table 4 reveal that EZ-NLCs tablets containing 30 mg CCS followed first-order kinetics with the highest R^2 values amongst other models. While EZ-NLCs tablets prepared with 15 mg CCS were fitted to a diffusion-controlled mechanism (Higuchi model), which may be attributed to the slower disintegration of these tablets owing to the reduced amount of the super-disintegrant CCS. According to literature the lyophilization of EZ-NLCs dispersion has no impact on the drug release kinetics [53].

To offer additional information on drug release mechanisms, the Korsmeyer-Peppas model was employed to determine whether it is Fickian (diffusion), non-Fickian (anomalous), or erosion-mediated (zero order) release [51]. The observed diffusional release exponent values for all EZ-NLCs tablets were < 0.45 which shows that the quasi-Fickian mechanism is the one that best describes the drug release from all EZ-NLCs tablets this means that a mix of diffusion and other processes control EZ release from NLCs [54].

Table 4 Release kinetic models and parameters of EZ-NLCs tablets

Tablet id	Correlation coefficient (R^2)				[a]K		[b]*N*-value
	Zero-order	First order	Higuchi	Krosmeyer-Peppas	Zero-order	First order	
EZ-NLCs tablet 1	0.7303	0.9631	0.8365	0.8519	7.82	0.41	0.31
EZ-NLCs tablet 3	0.9711	0.9231	0.9755	0.9695	8.39	0.23	0.35
EZ-NLCs tablet 5	0.9503	0.9617	0.9851	0.9803	8.86	0.26	0.36
EZ-NLCs tablet 6	0.7272	0.9509	0.8407	0.8659	8.21	0.48	0.32
EZ-NLCs tablet 8	0.9561	0.9739	0.9923	0.9868	7.67	0.22	0.33
EZ-NLCs tablet 9	0.9735	0.9809	0.9918	0.9884	8.33	0.25	0.33
EZ-NLCs tablet 11	0.6769	0.9723	0.8045	0.8653	7.58	0.66	0.28
EZ-NLCs tablet 12	0.7019	0.9468	0.8209	0.8576	7.65	0.45	0.29

First-order equation: $\text{Log } C_t = \text{Log } C_0 - Kt/2.303$
C_t: remaining concentration,
C_0: initial concentration

Higuchi equation: $\%\ \text{released} = K_H \times t^{1/2}$
H: Higuchi constant

[a]K: Elimination rate constant. [b]N-value: Krosmeyer-Peppas coefficient

Table 5 Predicted and observed values for dependent responses of the optimized EZ-NLCs tablet and the prediction error for each response

	Disintegration time (min)	In-vitro drug release % after 24 h
Predicted values	3.375	98.9
Observed values	3.85 ± 0.03	98.3 ± 3.95
Prediction error (%)	14.07	0.6

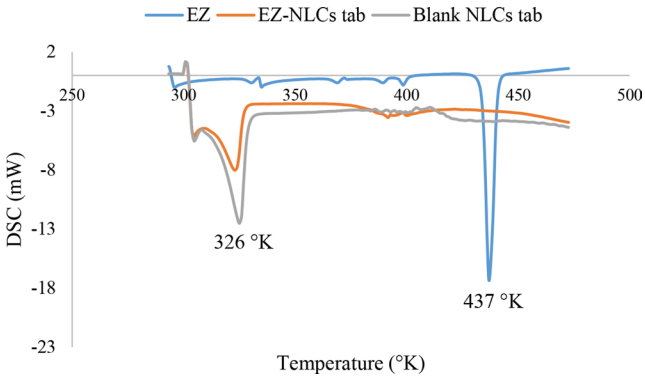


Fig. 3 DSC thermograms of EZ, optimized EZ-NLCs, and blank NLCs tablets

Optimization of EZ-NLCs Tablet

In general, the goal of optimization of pharmaceutical formulations is to determine the levels of variables required to produce a high-quality product. Desirability was calculated using Design-Expert software and considered to optimize the studied responses depending on the provided results. EZ-NLCs tablets were optimized for the responses, disintegration time (R1), and % of in-vitro drug released after 24 h (R2). The aim was to minimize the disintegration time and maximize the % of in-vitro drug released after 24 h as displayed in Table 1. The highest desirability value obtained was 0.967 and it was devoted to the following composition: 30 mg crosscarmelose sodium and (7.5:0) Avicel to mannitol ratio. The predicted and observed values for the dependent responses of the optimized EZ-NLCs tablet as well as the prediction error for each response are demonstrated in Table 5.

Characterization of the Optimized EZ-NLCs Tablet

Differential Scanning Calorimetry (DSC)

To characterize the raw materials used in lipid-based drug delivery systems and to evaluate the compatibility between the drugs and the excipients added in the formulations, differential scanning calorimetry (DSC) was adopted [22]. In the present study, DSC was performed on pure EZ, optimized EZ-NLCs, and blank NLCs tablets. The obtained results displayed in Fig. 3 show a sharp endothermic peak at 437 °K which matches ezetimibe's melting point and pinpoints its crystalline state. The disappearance of this peak may be attributed to the alteration of ezetimibe to its amorphous polymorph and its solubilization in the NLCs tablet [15, 16, 55]. The thermograms of the EZ-NLCs tablet and blank NLCs tablet showed a clear endothermic peak occurring at 326 °K which might be related to the poloxamer 188 melting range.

Fourier Transform Infrared (FTIR) analysis

The aim of conducting FTIR analysis was to ensure the incorporation of EZ into EZ-NLCs tablet, and evaluate the interaction between EZ and the excipients added in the formulations. We can observe from the FTIR spectra in Fig. 4 that EZ exhibits characteristic peaks at 3266.51 cm^{-1} for O-H stretching in alcohols, 1359.84 cm^{-1} for C-F stretching in alkyl halide, 1102.34 cm^{-1} for C-N stretching in amine, 1727.28 cm^{-1} for C = O stretching in β-lactam ring, 2916.42 cm^{-1} for C-H stretching in alkanes, and 1511 cm^{-1} for C = C stretching in benzene ring [21]. These peaks were also seen in EZ-NLCs tablet, and disappeared in blank NLCs tablet, which confirms the encapsulation of EZ in NLCs, and proves the lack of interaction between EZ and the excipients used in NLCs formulation as well as those added to form tablet formulations [21].

Determination of Particle Size (PS), Zeta Potential (ZP), and Polydispersity Index (PDI)

To evaluate the effect of lyophilization on the physicochemical characteristics of NLCs and guarantee their stability, the PS, PDI, and ZP were determined and compared for optimized EZ-NLCs dispersion and optimized EZ-NLCs tablet (before compression) following redispersion in double distilled water. The results displayed in Fig. 5 revealed a PS of 228.5 ± 5.87 nm, and 261.9 ± 5.27 nm for EZ-NLCs dispersion and EZ-NLCs tablet respectively. The observed PDI of EZ-NLCs dispersion was 0.36 ± 0.02, while that of EZ-NLCs tablet was 0.41 ± 0.03. Concerning the ZP, a value of -28.8 ± 0.71 mV was recorded by EZ-NLCs dispersion, whereas − 21.2 ± 0.97 was observed for EZ-NLCs tablet. The results demonstrate that there was a minimal increase in the PS and PDI of EZ-NLCs after lyophilization, which indicates the significant repulsion interaction and lack of aggregation between NLCs particles as well as their sufficient homogeneity. Moreover, a little decrease in ZP value was observed referring to the high long-term stability of EZ-NLCs after lyophilization. These observations may be attributed to the addition of the cryoprotectant (Avicel) which is crucial to get an elegant fast reconstituted

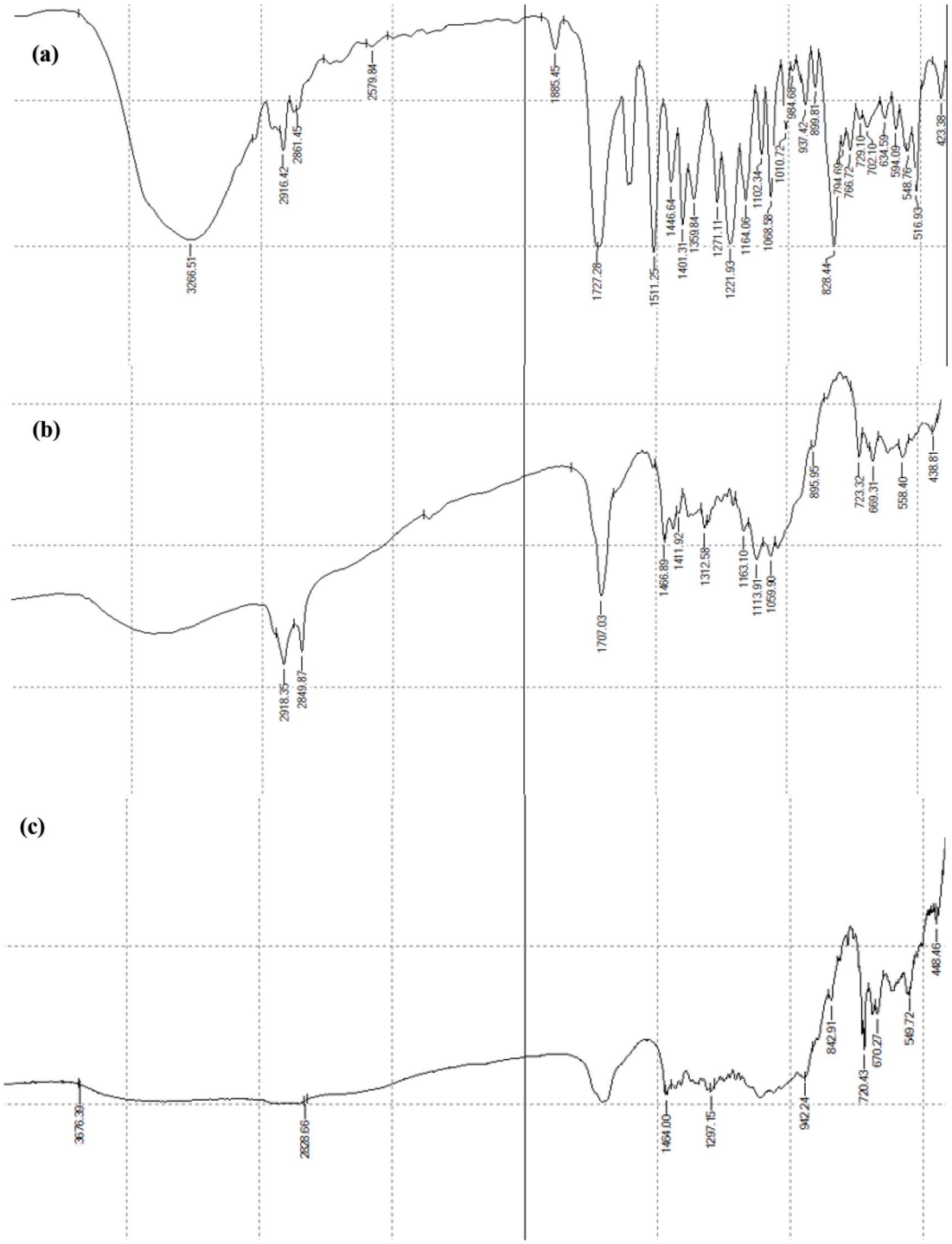


Fig. 4 FTIR Spectra of **a**. ezetimibe, **b**. optimized EZ-NLCs tablet, and **c**. blank NLCs tablet

lyophilized powder with the conservation of the physico-chemical properties of the prepared NLCs. Similar studies that examined the effect of lyophilization on NLCs reported that the addition of different cryoprotectants with various concentrations contributed to the outcomes observed in our study as those conducted by Khan AA, Emami J, and Safwat S [53, 56, 57].

According to the conducted in-vitro disintegration and drug release % studies, and following optimization of EZ-NLCs tablets as well as characterization of the optimized EZ-NLCs tablet, it was demanding to correlate the in-vitro outcomes to in-vivo ones and to evaluate our formulation through a bioequivalence study.

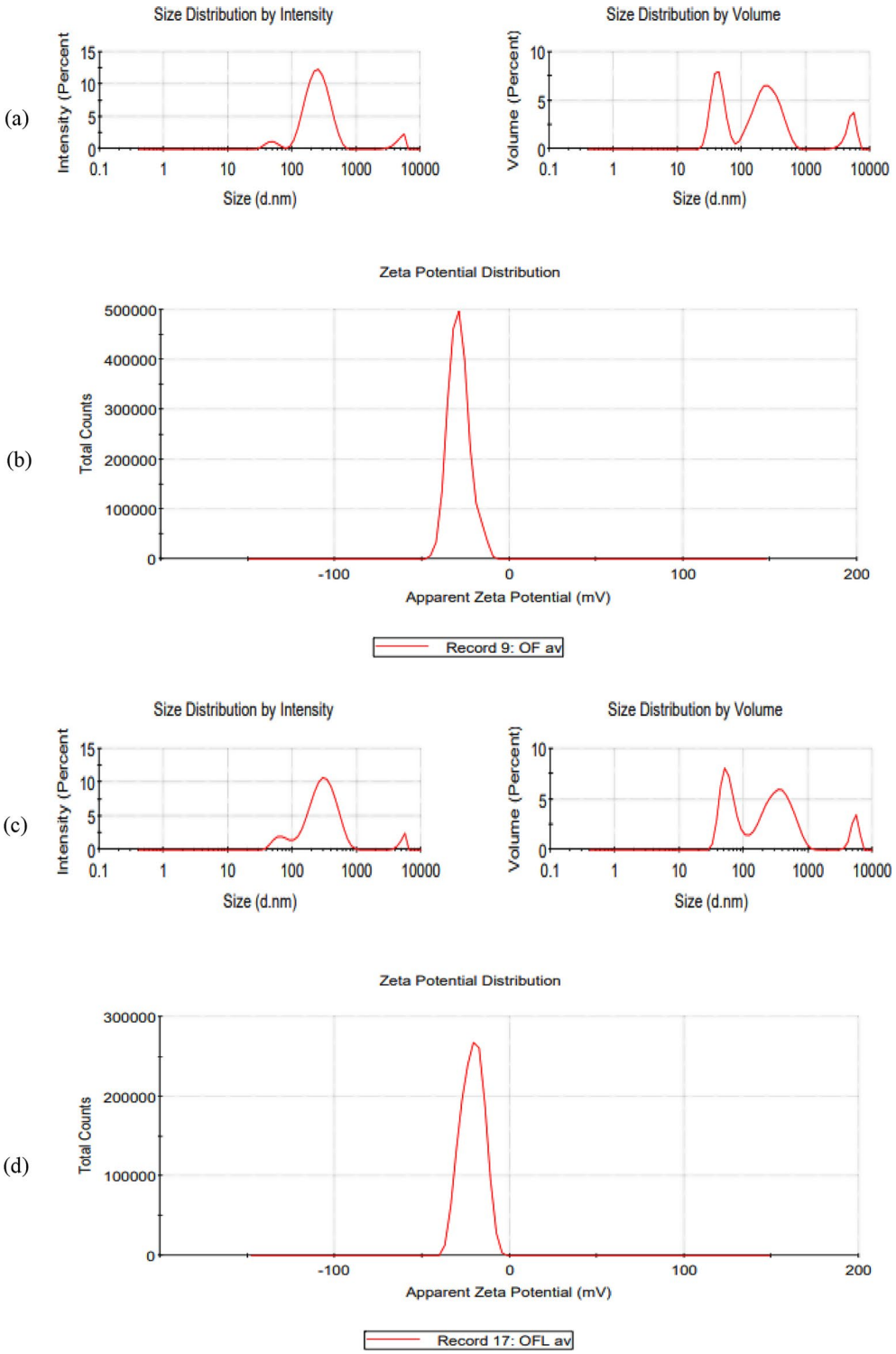


Fig. 5 Particle size distribution and zeta potential curves: (**a**) PS distribution for EZ-NLCs dispersion, (**b**) ZP distribution for EZ-NLCs dispersion, (**c**) PS distribution for EZ-NLCs tablet, and (**d**) ZP distribution for EZ-NLCs tablet

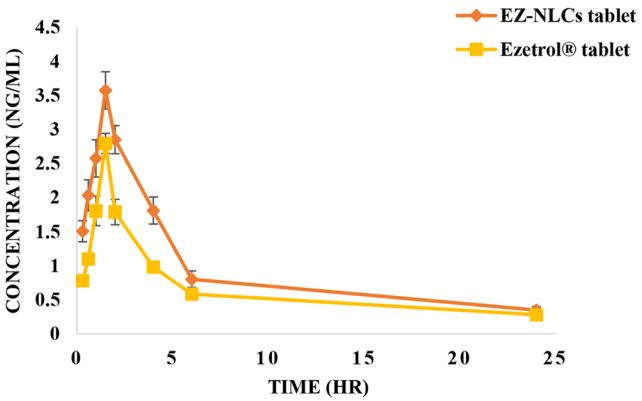


Fig. 6 Average plasma concentration-time curve after oral single dose (25 mg/kg) of EZ-NLCs and ezetrol® tablets in rats, Results are expressed as mean ± SD, $n = 3$

In-Vivo Pharmacokinetics Study

The influence of EZ-NLCs tablet to improve the oral bioavailability of EZ was investigated through a pharmacokinetics study. According to the mean plasma levels of the 6 rats involved in the study, and as observed from Fig. 6, the relative bioavailability was found to be 155% for the tested EZ-NLCs based on the average (AUC_{0-24}) and ($AUC_{0-inf.}$) compared to the market product ezetrol®. The result indicates that there was about 1.5 fold enhancement in the oral bioavailability of EZ achieved by EZ-NLCs tablet relative to ezetrol®. The statistical analysis performed demonstrated that there was a significant difference ($p < 0.05$) between EZ-NLCs and ezetrol® regarding the maximum plasma concentration (Cmax), the area under the plasma concentration curve (AUC_{0-24}) and ($AUC_{0-inf.}$) with p values 0.0214, 0.0345 and 0.0133 respectively. Briefly, EZ-NLCs tablet achieved a (Cmax) of 3.57 ± 0.27 ng/mL, while that of ezetrol® reached 2.79 ± 0.15 ng/mL, an AUC_{0-24} of 22.44 ± 2.68 ng. hr/mL and $AUC_{0-inf.}$ of 18.66 ± 2.02 ng. hr/mL were observed for EZ-NLCs tablet, while ezetrol® showed 15.36 ± 0.86 ng. hr/mL and 11.99 ± 1.19 ng. hr/mL for AUC_{0-24} and $AUC_{0-inf.}$ respectively. However, there was no significant difference observed between both treatments concerning the time needed to attain maximum concentration (Tmax), both treatments showed a Tmax of 1.5 h which was well correlated with that reported by Kaoud R [17]. The elimination half-life time (t1/2) of EZ-NLCs and ezetrol® were 7.49 ± 0.26 h and 8.32 ± 0.578 h respectively which came in a good correlation with that reported by Nasef A [40], and displayed no significant difference according to the statistical analysis performed (p value = 0.1179). The mean residence time (MRT) of the EZ-NLCs tablet and ezetrol® were 5.33 ± 0.14 h and 5.92 ± 0.37 h respectively and showed no significant difference (p value = 0.0945). Table 6 displays the detailed results of the pharmacokinetic parameters with their calculation equations.

These in-vivo observations are in good correlation with the in-vitro dissolution studies conducted, and there was a significant enhancement in the extent of absorption of EZ from EZ-NLCs tablet as compared to ezetrol®. Several scenarios can explain the improved bioavailability of EZ-NLCs tablet, the first proposal might be related to increasing the surface area available for drug release with the decrease in EZ-NLCs particle size to the nano range, besides the transformation of EZ from crystalline to amorphous structure which aided its solubility [17]. Furthermore, Because NLCs are lipid-based carriers, they encourage the secretion of bile salts from the bile duct and pancreas, which in turn leads to emulsion generation in the gastrointestinal tract (GIT) and consequently the drug remains in a solubilized form ready for absorption [15]. Moreover, the enhancement in the bioavailability observed could be related to the absorption of EZ-NLCs into the lymphatic system through their selective uptake either via the lacteals or Peyer's patches, as described in previous studies conducted by P. Pandya, R.S Managuli, and D. Wang [58–60].

The lymphatic uptake of EZ-NLCs can also be explained due to the presence of long-chain triglycerides (LCT) as stearic acid and oleic acid in their structure as reported by G. Shevalkar [15].

Table 6 Unpaired student t-test analysis results of pharmacokinetic parameters of EZ following a single oral dose of EZ-NLCs and ezetrol® tablets to Sprague Dawley rats with the calculation equations

Pharmacokinetic parameter		EZ-NLCs	Ezetrol®	*P* value
*Cmax (ng/mL)	$[^{b}F^{c}D^{d}Ka/^{e}V(Ka-K)]\times(e^{-Keltmax}-e^{-Katmax})$	3.57 ± 0.27	2.79 ± 0.15	**0.0214
*Tmax (hr)	Ln (**Ka/K) / Ka-K	1.5 ± 0	1.5 ± 0	
*AUC (0-t) (ng.hr/mL)	$AUC_{0-inf.}[1-(Kt+1)\times e^{-Kt}]$	22.44 ± 2.68	15.36 ± 0.86	**0.0345
*AUC (0-inf.) (ng.hr/mL)	FD/KV	18.66 ± 2.02	11.99 ± 1.19	**0.0133
*MRT (hr)	(Ka + K) / (Ka×K)	5.33 ± 0.14	5.92 ± 0.37	0.0945
*T1/2 (hr)	$0.693/^{a}K$	7.49 ± 0.26	8.32 ± 0.578	0.1179
*Relative bioavailability (%)	$(AUC_{test}/AUC_{standard})*100$	155.5%		

[a]K: elimination rate constant. [b]F: relative bioavailability. [c]D: administered dose. [d]Ka: absorption rate constant. [e]V: apparent volume of distribution. *Results are expressed as mean ± SD, $n = 3$. *Significant at p value < 0.05

In addition, the surfactants added to the structure of EZ-NLCs might have contributed to the enhanced bioavailability of EZ by overcoming the p-gp efflux mechanism as reported by V.H. Nguyen, G. Shevalkar, and H.M. Ellaithy [9, 15, 24]. For example, tween 80 (0.02 w/v%) was reported in previous studies by G. Cornaire, to be a fluidizer to lipid bilayer [61, 62]. Moreover, this pump translocation may be avoided by mixing weakly soluble P-gp substrates into NLCs themselves. Consequently, it was suggested that NLCs formulation may avoid this outflow and take advantage of transcellular routes [9].

Accordingly, the conducted bioequivalence study suggests that this approach could be promising to overcome the poor variable bioavailability of EZ.

Conclusion

The Elucidated study successfully achieved the transformation of EZ-NLCs to dry EZ-NLCs tablets possessing the potential to enhance EZ's solubility and bioavailability. EZ-NLCs were dried through lyophilization using different percentages and types of drying excipients and then subjected to pre-compression evaluation. EZ-NLCs tablets were directly compressed, and pharmaceutically evaluated for hardness, content uniformity, friability %, disintegration time, and in-vitro drug release %, then further optimized utilizing response surface methodology (I-Optimal design). The optimized EZ-NLCs tablet was produced using the cheap, frequently used excipient Avicel along with 30 mg of the super-disintegrant CCS. The result of the in-vitro release study displayed a 1.5-fold increase for EZ-NLCs tablets over ezetrol®. The bioequivalence study confirmed the enhancement in the bioavailability and encouraged the incorporation of EZ into nanostructured lipid carriers tablet which might decrease drug dosing frequency, and hence enhance patient compliance. Moreover, it could be considered a promising approach to the industrial field to guarantee a high quality, low cost, & more stable NLCs drug products for commercialization. Consequently, a long-term stability study is recommended during future work to ensure EZ-NLCs tablet stability over prolonged periods and investigate its capability for scale-up production.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12247-024-09911-0.

Acknowledgements The authors would like to express their deep gratitude to October University for Modern Sciences and Arts for their contribution to the completion of the study's experimental work.

Author Contributions Dalia Elkhayat: Investigation, and writing. Nevine Abdelmalak: Review and editing. Reham Amer: Editing, and methodology. Heba Awad: Pharmacology part analyst.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).
The submitted work was fully funded by the authors and did not receive financial support from any organization.

Data Availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Cairo University (Date: 30.05.2016/PI(1719)).

Competing Interests The authors declare no competing financial interests.

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