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Original Article

IMPLEMENTATION OF FACTORIAL DESIGN TO OPTIMIZE THE FORMULATION METHOD OF EZETIMIBE POLYMERIC NANOPARTICLE BY HOMOGENIZATION CUM ULTRA-SONICATION METHOD

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ABSTRACT

Objective: The objective of this study is to use a 2^3 factorial design to optimize the formulation factors of Ezetimibe polymeric nanoparticle.

Methods: By varying formulation variables such as polymer concentration (hydroxyl propyl methyl cellulose composition) and process variables such as homogenization time (min) and ultra-sonication time, the formulation of polymeric nanoparticles was designed using a 2^3 factorial design and prepared using the homogenization cum ultra-sonication method (min). Particle size (nm), zeta potential (mV), polydispersity index, entrapment efficiency (%), drug content, *in vitro* drug release, *in vitro* release kinetic studies, and stability studies were used to analyse and optimize polymeric nanoparticles according to ICH criteria.

Results: R7 formulation showed predicted and desired less particle size 87.0±3.64 nm; maximum zeta potential-33.4±2.32 mV; desired polydispersity index 0.488±0.20; maximum entrapment efficiency of 96.45±2.42 % and controlled dissolution release pattern of about 90.42±3.56% in 24h.

Conclusion: The polymeric nanoparticle was formulated and optimized by the parameters like Particle Size (PS in nm), Polydispersity Index (PI), Zeta Potential (ZP in mV), % Entrapment Efficiency and *in vitro* drug release for 24 h were evaluated. These parameters showed significant changes while formulating polymeric nanoparticles along with various formulation and process variables. From the release pattern data it was observed that PNs show a significant improvement of dissolution character of Ezetamibe. According to the findings, PNs have a controlled drug release pattern and can be used as a suitable drug delivery carrier for low solubility and poorly bioavailable drugs like Ezetamibe to improve its dissolution.

Keywords: Optimization, Ezetimibe, Polymeric nanoparticle, 2^3factorial design, Homogenization, Ultrasonication

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INTRODUCTION

Polymer nanotechnology is one of the most potential drug delivery technologies for overcoming problems in drug distribution, such as low solubility and permeability [1]. The creation of innovative polymeric nanoparticle formulations that can change the pharmacological, biopharmaceutical, and pharmacokinetic characteristics of pharmaceuticals has been aided by advances in nanotechnology [2]. Polymeric nanoparticles (PNs) are particulate materials with a one-dimensional size of at least 10–100 nm. Polymeric nanoparticles (NPs) are one of the most commonly employed nanomaterials in nanomedicine because they can deliver a drug to a specific region of an organ with a lower dose, hence increasing drug bioavailability at the desired target [3]. Polymeric NPs are used in drug delivery, such as medicine conjugation and entanglement, prodrugs, stimuli sensitive systems, imaging modalities, and theranostics [4]. Biodegradable polymeric nanostructures have shown exceptional promise in a variety of therapeutic applications, including analysis, imaging, sedative delivery, cosmetic agents, organ embeds, and tissue design [5].

To address drug delivery difficulties such as low solubility, permeability, and bioavailability, polymer nanotechnology, i.e., polymeric nanoparticles, is being recognised as one of the most appropriate drug delivery systems [6]. Many pharmaceutical substances have had their pharmacokinetics and pharmacodynamics modified and improved using particle systems such as nanoparticles [7]. The term "nanoparticle" is used to describe both nanocapsules and nanospheres, which differ in their morphological structure. Polymeric NPs have showed considerable promise in the delivery of medications for a variety of illnesses, including anticholesteremia [8].

Ezetimibe is a BCS class II drug that is used to treat excessive blood cholesterol and other lipid problems. It's usually combined with dietary adjustments and a statin. It is less recommended than a statin on its own [9]. Furthermore, it is taken by mouth. Ezetimibe is a strong and selective inhibitor of cholesterol absorption that has been proven to limit total cholesterol transport to the liver, consequently increasing LDL receptor production and lowering serum LDL-C [10]. When administered alone or in addition with statin therapy, ezetimibe decreases intestinal and biliary cholesterol absorption and can considerably lower LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C, defined as total cholesterol minus high-density lipoprotein cholesterol) [11]. The pharmacokinetics of ezetimibe demonstrate that it has a bioavailability of 35 to 65 percent and an elimination half-life of 19 to 30 h. Ezetimibe's protein binding was determined to be>90%, and it was metabolised in the intestinal wall and liver. 78 percent of the unaltered form of ezetimibe was eliminated in the faeces, and 11 percent was excreted in the kidney [12].

To improve ezetimibe's dissolution profile, it was homogenised and developed into polymeric nanoparticles using the homogenization and ultra-sonication technique by modifying formulation variables such as polymer concentration (hydroxyl propyl methyl cellulose concentration-HPMC) and process variables such as homogenization time (min) and ultra-sonication time (min). Further *in vivo* pharmacokinetic investigations will be conducted using the best optimized formulation.

MATERIALS AND METHODS

Materials

Aurobindo Pvt. Ltd. in India provided Ezetimibe. Himedia Labs Ltd in Chennai provided the hydroxyl propyl methylcellulose. High-Speed Homogenizer, Ultra Sonicator, Brukers FT-IR Spectrophotometer, Horiba Nanoparticles Size Analyzer, and Zeiss Scanning Electron Microscopy are some instruments utilized in the creation and

evaluation of polymeric nanoparticles. Excipients and solvents of analytical grade are employed in the production and evaluation of polymeric nanoparticles.

Methodology

Drug and excipients compatibility studies

FTIR studies

The chemical interactions between the medications (ezetimibe) and other constituents in the composition, such as polymer and surfactants, were determined using FTIR analyses. Ezetimibe and a physical combination were studied using the potassium bromide (KBr) pelletization process. The drugs (0.2%) were ground with the KBr, and the combination was then squeezed using a tiny KBr pellet press at a pressure of around 7 tonnes by repeatedly rotating the press handle. In the FTIR instrument (Bruker, Germany) equipped with the OPUS Spectrum software, prepared KBr pellets are scanned throughout a wave number range of 4000 to 500 cm–1 with a resolution of 4 cm–1. Samples were placed on the sample stage using a force gauge of 100 N, ensuring regular contact between both the specimen and the crystal holder for scanning [13, 14].

Differential scanning calorimetry (DSC) studies

The melting point of samples was determined using DSC tests. It aids in the reporting of drug purity, drug-excipient compatibility, and the crystalline quality of polymeric nanoparticle formulations. The DSC-70, a Schimadzu model equipment, was used to study Ezetimibe and drug-loaded polymeric nanoparticles. The samples were measured at 5 mg and cooked in aluminium pans at a rate of 20 °C/min with dry nitrogen as the effluent gas at a temperature of 20-200 °C. The melting point was measured as an exothermic or endothermic peak [15, 16].

High-speed homogenization followed by ultrasonication method-preparation of polymeric nanoparticles (PNS)

The required amount of ezetamibe was homogeneously dispersed in various concentrations of polymeric solution (ranging from 80 to 5%), which was made by dissolving different concentrations of surfactant and co-surfactant in deionized water and heating if necessary. The aqueous phase was homogenised for 10 min at 15000 RPM in a High-Speed Homogenizer before slowly dispersing the medication into the aqueous phase. As an outcome, polymeric nanoparticles precipitated in the form of an emulsion. Using a Probe Ultrasonicator, the resulting emulsion was ultrasonicated for 5 min at a 2 sec pulse rate to create uniformly dispersed stable polymeric nanoparticles. Continue the lyophilisation procedure while keeping the nanoemulsion at ambient temperature. 2^3 statistical factorial designs were used to improve the above formulation process. Its eight formulation runs were created by adjusting the constraints and increasing the level by three (low, medium and high). As product and process variables, polymer concentration (A in mg), homogenization time (in rpm) for 10 min, and ultrasonication time (C in min) are all fixed. 8 PNs formulations are prepared and analysed for response parameters such as particle size (Y1), zeta potential (Y2), and polydispersity index (Y3) using this design (Y3). The major result of the independent variable over the dependent variable is clarified by these designs. Table 1 [17-20] shows the formulation design.

Table 1: Design of optimization of the polymeric nanoparticle by 2^3 factorial design

Run	Independent variables (Level code)			Independent variables (conc./range)		
	Product variable	Process variable		Product variable	Process variable	
	Factor A: Polymer (HPMC) Conc. (mg)	Factor B: homogenization time (min)	Factor C: ultra sonication time (min)	Factor A: Polymer Concentration (mg)	Factor B: homogenization time (rpm)	Factor C: ultra sonication time (min)
R 1	-1	-1	-1	5	5000	5
R 2	1	-1	-1	10	5000	5
R 3	-1	1	-1	5	10000	5
R 4	1	1	-1	10	10000	5
R 5	-1	-1	1	5	5000	10
R 6	1	-1	1	10	5000	10
R 7	-1	1	1	5	10000	10
R 8	1	1	1	10	10000	10

Evaluation parameters of PNs

Particle size and particle size distribution

A Horiba Nanoparticle size analyzer was used to determine the particle size distribution, mean particle size (PS-Z average in nm), and Polydispersity Index (PI) of polymeric nanoparticles (SZ-100 Nanopartica series). The samples were made with the necessary dilution of polymeric nanoparticles and distilled water twice deionized. Filtering the aforesaid solution using a 0.45 membrane filter was used for the analysis. The equipment automatically adjusted the dynamic light scattering intensity dependent on the viscosity of the medium, with 90o light scattering for low viscous samples and 170o light scattering for high viscous samples. Polymeric nanoparticles should have a particle size of 10 to 100 nm and a PI of less than 0.5, indicating a unimodel or uniform monodisperse size distribution. All measurements were done in triplicate (n=3) [21, 22].

Zeta potential (ζ)

The Horiba Nanoparticle size analyzer was used to measure the Zeta Potential, or surface charge potential (SZ-100 nanopartica series). An electrophoretic cell with an 80 mV electric field was used to transport the diluted polymeric nanoparticles into the probe. At 25 °C, all measurements were made in triplicate. The amplitude of zeta potential polymeric nanoparticles should be>30mV, indicating the colloid's durability. Using the Smolochowski equation, the Zeta potential was then directly calculated from the equation [23].

$$\zeta = \varepsilon\mu/\eta$$

Where, ζ-Zeta Potential, μ-Electrophoretic mobility; Ɛ-Electric permittivity of the liquid; η is the viscosity of the liquid

Surface morphology studies-scanning electron microscope (SEM) studies

The Scanning Electron Microscope was used to examine the surface morphology of the Polymeric nanoparticles for the selected optimum Ezetamibe polymeric nanoparticles (Hitachi S-3000 N). Lyophilized Polymeric nanoparticles powder sections were stained with 600 platinum using a sputter coater and analysed using a scanning electron microscope (SEM). After that, the polymeric nanoparticles were put on a sample holder and scanned with an electron beam. The surface morphology picture of polymeric nanoparticles is created when an electron beam contacts the polymeric nanoparticles particles and releases secondary electrons dependent on the nature of the surface. Then consider the average particle size of polymeric nanoparticles acquired by SEM with the average particle size of polymeric nanoparticles obtained by Horiba Nanoparticle size analyzer [24, 25].

Encapsulation efficiency studies

The centrifugation method was used to determine encapsulation efficiency. In this investigation, 1 ml of polymeric nanoparticles dispersion with a molecular weight of 12,000–14,000 Daltons and a pore size of 2.4 nm was placed in dialysis bags (Himedia). The dialysis

membrane bag was placed in the centrifuge tube once it had been prepared. To extract the free drug from the polymeric nanoparticles carrier, this centrifuge tube was previously filled with 9 ml of pH 7.4 phosphate buffer and centrifuged at 15,000 rpm for 1 hour in a REMI centrifuge. 5 cc of the sample was taken from the phosphate buffer saline after 1 hour. The concentration of Ezetamibe in the withdrawn sample was measured using a UV Spectrophomotometer set to 234 nm. The blank solution was made using the same method and ingredients as the medication solution but without the drug. The experiment was repeated three times (n=3). The below equation was used to calculate percentage entrapment efficiency.

$$\%EE = \frac{Xs - Xt}{Xs} X\ 100$$

Where, Xs-Total amount of drug used for formulation; Xt-Amount of drug in 5 ml saline [26, 27].

In vitro drug release studies

The percentage amount of the drug released from polymeric nanoparticles dispersion performed out using the dialysis membrane technique is referred to as *in vitro* drug release. 1 ml of polymeric nanoparticles dispersion was put into the dialysis membrane with 0.45 m pore size after one end of the dialysis membrane was closed or tied firmly. Both ends of the dialysis membrane were tightly knotted after it was filled. Ascertain that the tied dialysis membrane does not leak polymeric nanoparticle dispersion. A donor compartment is formed by a dialysis membrane that has been filled. The dialysis membrane was then immersed in a 100 ml pH 7.4 Phosphate Buffer Solution, which was maintained at 100 rpm in a magnetic stirrer. At regular intervals of 0, 1, 2, 4, 8, 12, 16, 20, 24 h, 5 ml of the sample was taken from the phosphate buffer solution phase. To establish a sink state, the same 5 ml of fresh PBS solution was replenished in the receptor compartment. A UV spectrophotometer set to 234 nm was used to detect the released drug absorbance at each sampling span. The experiment was performed in triplicate (n=3) [28, 29].

In vitro release kinetic study

The drug release survey of PNs was fixed in various release kinetic parameters such as first order (time vs. log percent drug remaining); zero order (time vs. percent cumulative release); Higuchi's model (square root of time vs. cumulative percent drug release); Peppa's model (Time Vs. log of drug concentration) and their regression (r2) and k values were determined in order to acquire a linear regression analysis to verify the impact and process of release over time.

Stability studies

This study used an optimised polymeric nanoparticles dispersion. Each formulation was split into two batches for testing. Three lots of samples were collected in test tubes for each batch. Each test tube was labelled with the months 3rd, 6th, and 12th. An aluminium foil layer is carefully covered and placed over these test tubes to shield them from light deterioration. One batch was kept at 2–6 °C in the refrigerator. Another batch was kept at room temperature for 60 percent of Relative humidity at 25 °C±2 °C. Particle size (nm), zeta potential, polydispersity index (PI), and entrapment efficiency were assessed in each sample from both storage conditions over a period of time (percent). The findings of each formulation were examined for consistency [32, 33].

RESULTS AND DISCUSSION

Drug excipients compatibility studies-FTIR studies

On comparing pure Ezetamibe and Ezetamibe PNs data collected from FTIR spectra, as shown in fig. 1 and table 2. The main functional groups with their wave number for Ezetimibe drug like C-F Stretching Aromatic C-H in ring structure is 823.42 cm-1, C=O Stretching is 1110.56 cm-1, C-H Bending is 1517.31 cm-1, Aromatics, C=O Stretching is 1899.59 cm-1, C=C is 2356.16 cm-1, C-OH is 3744.07 cm^{-1} respectively. For Optimized Ezetimibe PN drug C-F Stretching Aromatic C-H in ring structure is 821.79 cm-1, C=O Stretching is 1157.09 cm-1, C-H Bending is 1507.80 cm-1, Aromatics, C=O Stretching is 1849.75 cm-1, C=C is 2433.30 cm-1, C-OH is 3743.16 cm^{-1} in the respectively. From the data it was determined that the appropriate frequencies of fingerprint regions of Ezetamibe drug were replicable in Ezetamibe PNs i.e., there is no any vibrational changes when the Ezetamibe mixed with the formulation excipients and also there was no any change in amorphous nature of the drug. It was determined that the drug and excipients included in the formulations were compatible with one another and suitable for the formulation of solid lipid nanoparticle.

Table 2: FTIR spectrum interpretation of ezetimibe formulation

Functional group	Wavenumber (cm^{-1})	
	Ezetimibe	Optimized ezetimibe PN
C-F Stretching Aromatic, C-H in ring structure	823.42	821.79
C=O Stretching	1110.56	1157.09
C-H Bending	1517.31	1507.80
Aromatics, C=O Stretching	1899.59	1849.75
C=C	2356.16	2433.30
C-OH	3744.07	3743.16
C-H Stretching		3133.42

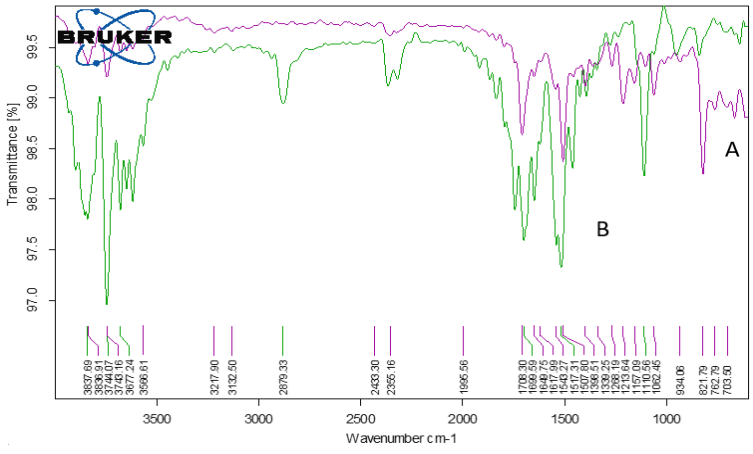

Fig. 1: Drug excipients compatibility studies-FTIR studies of (A) Ezetimibe pure drug and (B) Optimized ezetimibe PN

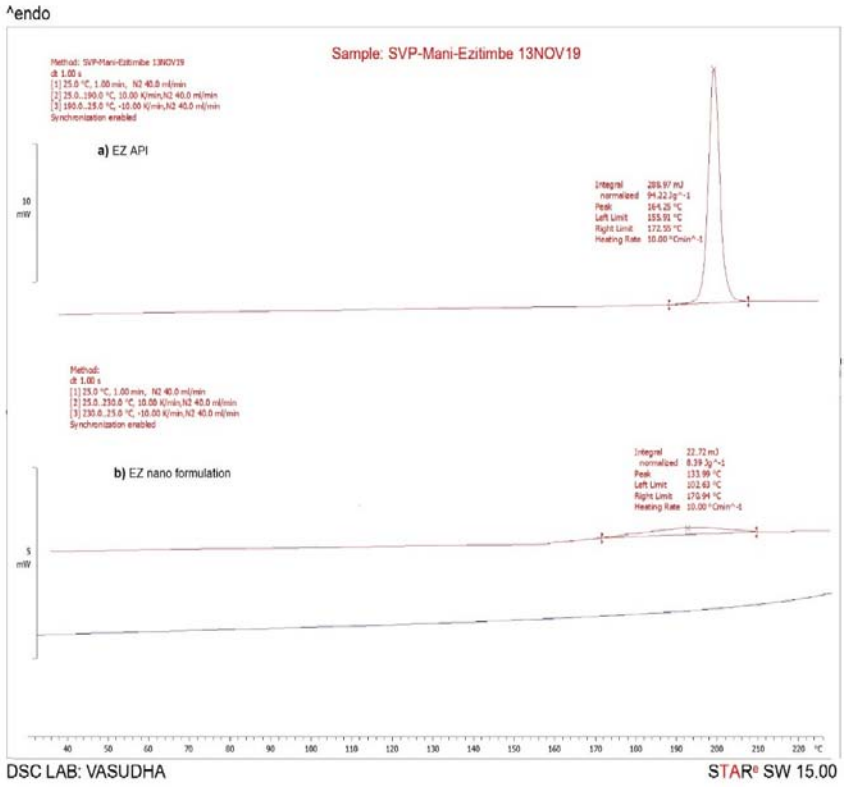

Fig. 2: Drug excipients compatibility studies-DSC studies of (A) Ezetimibe pure drug and (B) Optimized Ezetimibe PN

Drug excipients compatibility studies-DSC studies

As endothermic peak values in a DSC thermogram, the relevant melting points were observed: Ezetamibe at 164.35 °C; Ezetamibe polymeric nanoparticle at 133.4 °C. The polymer melted first, followed by the drug, ensuring that the drug was successfully encapsulated within the polymer during the formulation of the nanoparticle. Fig. 2(a) depicts the DSC endothermic thermogram of Ezetamibe drug and fig. 2(b) depicts the DSC endothermic thermogram of optimized Ezetamibe polymeric nanoparticle. From the data it was confirmed that the drug are amorphous or molecularly dispersed in nature. And also from DSC studies, it was confirmed that the lipid first started to melted followed by the drug, which ensures that the drug was effectively encapsulated within the lipid. This thermal behaviour confirms that the drug exists in an amorphous form or is molecularly dispersed in nature in the formulation.

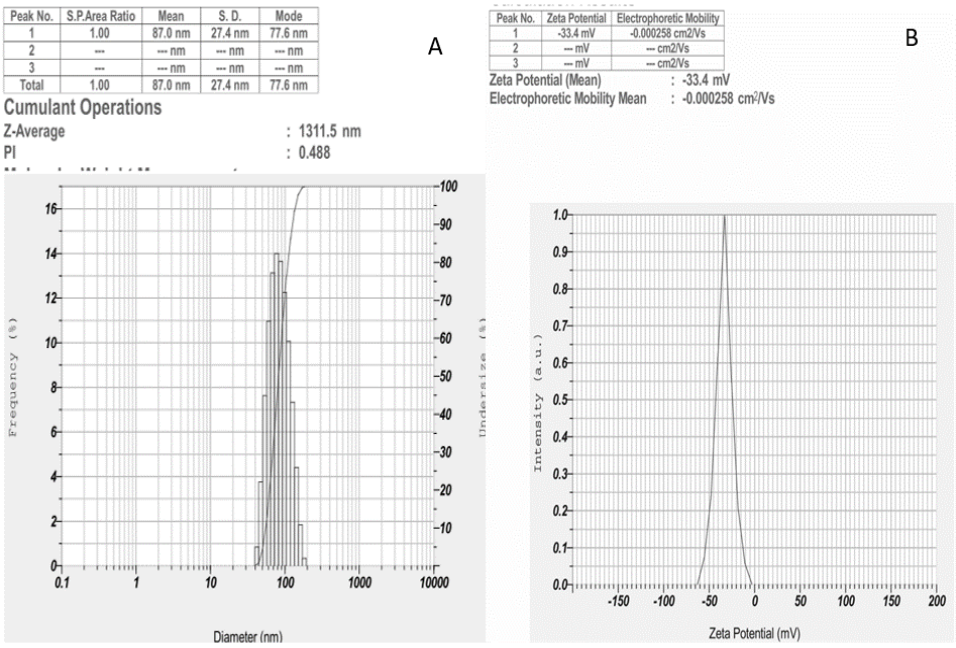

Fig. 3: Ezetamibe polymeric nanoparticle (A) Particle size and polydispersity index report; (B) Zeta potential report

Table 3: Optimization design showing the effect of independent variables on the dependent variable in the formulation of polymeric nanoparticle

Formulation Run	Independent variables			Dependent variables		
	Factor A: polymer conc. (mg)	Factor B: homogenization time (min)	Factor C: ultra sonication Time (min)	Particle size (PS) (Y1)	Zeta potential (ZP) (Y2)	Polydispersity index (PI) (Y3)
R1	-1	-1	-1	511.0±6.06	-13.1±2.02	0.579±0.20
R2	1	-1	-1	667.0±7.86	-16.5±1.72	0.700±0.24
R3	-1	1	-1	207.1±2.36	-21.7±2.68	0.650±0.40
R4	1	1	-1	319.3±5.94	-19.4±1.08	0.477±0.12
R5	-1	-1	1	308.4±4.86	-26.5±2.12	0.515±0.16
R6	1	-1	1	125.4±2.66	-30.7±2.68	0.480±0.24
R7	-1	1	1	87.0±3.64	-33.4±2.32	0.488±0.20
R8	1	1	1	145.4±2.46	-22.4±1.74	0.358±0.22
Approval criteria				10–100 nm	>±30mV	<0.5 for PI

All values for dependent variables shown in table are measured as mean±SD, n=3

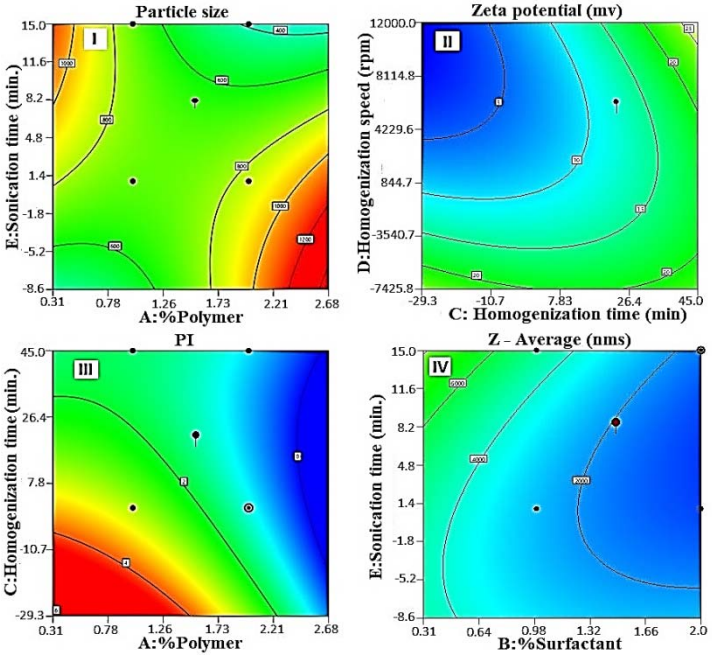

Fig. 4: Contour profile graph showing the response of independent variable on the dependent variable

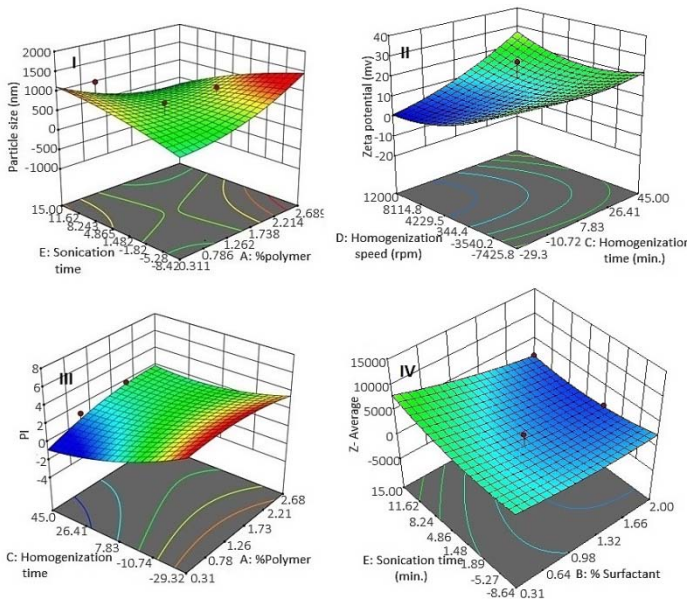

Fig. 5: 3D surface response graph showing the response of independent variable on dependent variable

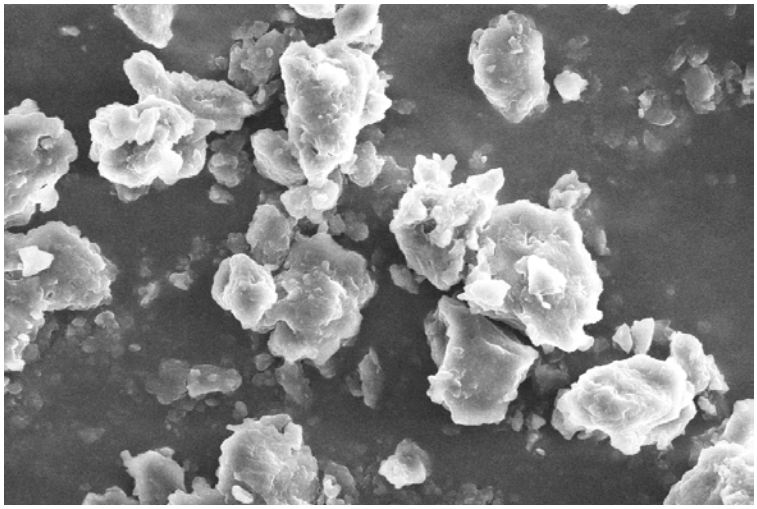

Fig. 6: SEM studies of optimized ezetimibe polymeric nanoparticle

Table 4: Evaluation of the effect of independent variables on other dependent variable in the formulation of polymeric nanoparticle

Formulation Run	Independent variables			Dependent variables		
	Factor A: polymer conc. (mg)	Factor B: Homogenization time (min)	**Factor C: ultra-sonication time (min)**	**%EE***	**%Yield***	**% drug release at 24 h**
R1	-1	-1	-1	68.44±2.68	72.88±2.92	59.66±6.28
R2	1	-1	-1	64.42±3.44	56.82±2.84	56.46±3.48
R3	-1	1	-1	84.86±3.26	82.68±3.26	80.46±4.28
R4	1	1	-1	76.42±3.64	73.84±5.08	72.26±3.84
R5	-1	-1	1	80.38±2.64	84.90±3.64	76.64±3.64
R6	1	-1	1	92.82±2.46	89.46±3.86	84.38±3.76
R7	-1	1	1	96.45±2.42	94.28±2.56	90.42±3.56
R8	1	1	1	90.32±3.69	86.24±2.68	81.44±3.76
Approval criteria				>85%	>85%	85–115% at 24 h

All values for dependent variables shown in table are measured as mean±SD, n=3

Particle size

The particle size was measured by the Horiba particle size analyzer and it was reported in table 3 and fig. 3, which shows the average particle sizes for all formulations. Based on the impact of the independent variable in the formulation process, particle sizes for all Ezetimibe PNs formulations were found to be in the range of 87.0±3.64 to 667.0±7.86 nm. However, the particle size of polymeric nanoparticles should be 60-100 nm to meet the approval standards. The formulation R7 (5 mg polymer concentration, 10000 rpm homogenization time, 10 min ultrasonicator time) has a particle size of 87.0±3.64 nm; a zeta potential of-33.4±2.32mV, and a polydispersity index of around 0.4880±0.20, according to the approval criteria. The remaining formulations from R1 to R8 showed a particle size of more than 100 nm, which was deemed to be outside of the intended range. Hence it was inferred that the particle size are in desired acceptable criteria limit and shows a significant effect independent variable [9, 10].

Zeta potential

The table 3 and fig. 3 show the zeta potential for prepared nanoparticles. The zeta potential of all Ezetimibe PNs was determined to be in the range of-13.1±2.02mV to-33.4±2.32mV, owing to the influence of surfactant during the formulation process. However, the ZP of polymeric nanoparticle acceptability criteria must be determined between 30 and 60 mV. The formulation R7 (5 mg polymer concentration, 10000 rpm homogenization time, 10 min ultrasonicator duration) has a maximum ZP of-33.4±2.32mV, which meets the approval criteria i.e., for a stable polymeric nanoparticle, it should be>±30mV. The remaining formulation fell short of the target range i.e.,<±30mV [9].

Polydispersity index

The polydispersity index for all formulations were shown in table 3 and fig. 3. The polydispersity index for Ezetimibe PNs was reported to be between 0.358±0.22 to 0.700±0.24, owing to the effect of homogenization speed or ultrasonication time in the formulation process. However, for monodisperse nanoparticles, the PI acceptance requirement should be less than 0.7. The formulations R4, R6-R8 have good polydispersity indexes of 0.477±0.12, 0.480±0.24, 0.488±0.20, 0.358±0.22, respectively, according to the acceptance requirements. The other formulations were discovered to have a value larger than 0.5 [10].

Optimization of polymeric nanoparticle

The results of independent variables on dependent variables on Ezetimibe PNs were shown by the 2^3 optimization design table 4 and fig. 3-6. Based on the foregoing data, it was determined that there was a strong link between particle size and polymer concentration, i.e., increasing the polymer concentration increased the particle size of PNs. At low-1 level polymer, R7 formulation showed a required particle size of around 87.0±3.64 nm between all formulations (R1-R8) (5 mg). The reduction in particle size was achieved by combining a low polymer content with a high homogenization rpm and ultrasonication period (table 1). Particle size reduction was also achieved as a result of increased homogenization speed and ultrasonication time, which separated large particles and particle aggregates into small dispersed particles, resulting in particle size reduction. In the preparation of PNs, increasing the homogenization speed and ultrasonication time resulted in a concomitant increase in the zeta potential with a decrease in the particle size, confirming the good phase stability of PNs and achieving the highest conductance of the particle. The charge distribution will be dispersed evenly on split

tiny particles when the surfactant concentration increased, which may lead to a rise in zeta potential or surface charge potential, high nanoparticle stability, and particle mobility without sedimentation. At a high-1 level of surfactant concentration,+1 level of homogenization speed, and ultrasonication time, R7 formulation demonstrated the requisite zeta potential of about-33.4±2.32 mV. With a rise in ultrasonication time and homogenization speed, the ZP in mV increased in lockstep with a reduction in polydispersity index of approximately 0.488±0.20. The surface morphology of the Optimized Ezetimibe PNs, R7 was studied using SEM, as illustrated in fig. 6, where the PNs were observed as smooth spherical surfaced particles. Due to its spherical smooth nanometric surface, it was discovered that it will boost drug loading efficiency, entrapment efficiency, and simple diffusion of the drug into physiological barriers. The greatest percent yield and percent entrapment efficiency for the Ezetimibe PNs (R7) formulation were 96.45±2.42 and 94.28±2.56 percent, respectively. It is also possible to conclude from the above-mentioned findings that the medication concentration was distributed uniformly in the PNs [12-25].

Percentage entrapment efficiency and percentage yield

For polymeric nanoparticles, the required percentage entrapment efficiency and yield should be greater than 85%. The effectiveness of entrapment was found to be 64.42±3.44 percent to 96.45±2.42 percent, and the percent yield was found to be 56.82±2.84 to 94.28±2.56 percent, according to the results provided in table 4. R7 displays the estimated amount of percentage entrapment efficiency and percentage yield by comparing all of the formulations [22-26].

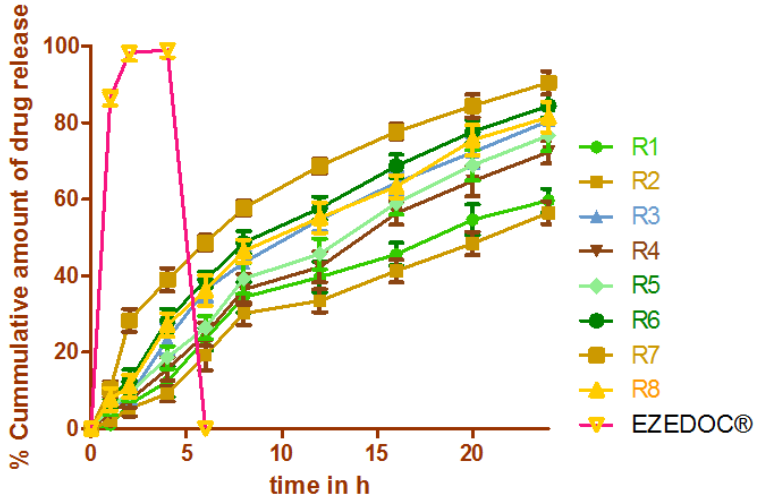

Fig. 7: Comparative *In vitro* drug release studies between polymeric nanoparticle vs. marketed Exedoc ® tablet (All values shown in graph are measured as mean±SD, n=3)

***In vitro* drug release studies**

The *In vitro* drug release studies for all the polymeric nanoparticle formulation was carried out in pH 7.4 phosphate buffer by using dialysis membrane technique. The percentage amount of drug released through dialysis membrane for each formulation was found to be in the range of 54.46±3.04% to 90.42±3.12% in 24 h as shown in fig. 7. By comparing all the formulations, the formulation R7 shows desired drug release based upon the concentration of drug, polymer and enhanced entrapment efficiency due to reduction in particle size. As demonstrated in fig. 7, *in vitro* drug release studies for the Ezetimibe PNs (R7) formulation revealed a better-controlled drug release i.e., 90.42±3.56 percent in 24 h when compared to all other formulation and marketed Ezetamibe tablet (EZEDOC® 20). From the results it was observed that, R7 polymeric nanoparticle showed better control of drug release in a cumulative release pattern. In general the drug release from R7 formulation showed a predetermined controlled release which obeys zero-order drug release pattern [27-31].

***In vitro* release kinetics studies**

The *in vitro* release kinetics of ezetimibe-loaded polymeric nanoparticle were evaluated by fitting the drug release data into various kinetic models like First order, Zero-order, Higuchi, Hixson Crowell and Korsmeyer Peppas equations. The percentage amount of drug release was substituted in various release kinetic model formula like Zero order, First order, Higuchi, Hixson crowell and Korsmeyer Peppas models regression values was calculated. From the linear regression graph the r^2 value were found to be 0.982±0.02, 0.702±0.02, 0.966±0.04, 0.842±0.02 and 0.981±0.04 for the respective kinetic models. The drug release kinetic data for an optimized polymeric nanoparticle formulation R7 followed the Zero-order release kinetic model in which the regression r^2 values were found to be 0.982±0.02 with good linearity. So it was confirmed that the R7 Ezetamibe polymeric nanoparticle formulation followed zero-order kinetics, which release the same amount of drug at unit time intervals in a controlled and predetermined manner. It was an ideal formulation for the release of the drug in order to achieve desired pharmacological action with reduced side effects. When fitting the drug release pattern to Higuchi, it showed a regression value (r^2) as 0.986, indicating that the drug was released by diffusion mechanism. It meant that the drug release from PNs was governed by a non-fickian diffusion process, in which the drug was discharged from the polymer by polymer relaxation and diffusion mechanism. From the Peppas equation fittings, the release exponent value (n) of the drug release for R7 formulation was found to be 0.502, which lied within the range of n = 0.45-0.89. It implied that the release of the drug from polymeric nanoparticle followed the Non-fickian diffusion mechanism. It was validated as the best model for releasing the drug in order to achieve the desired therapeutic effect without causing any side effects [31].

Stability studies

The comparative stability study data for R7 polymeric nanoparticle before and after conducting stability experiments performed. The stability data of optimized polymeric nanoparticles (R7) are tested for short-term stability at 4°C±2°C for 6 mo. At three-month intervals, the parameters like PS nm, ZP mV, and PI were assessed. R7's PS nm, ZP mV, and PI during preparation were found to be 87.0±3.64 nm,-33.4±2.32mV, 0.488±0.20, and R7 after performing stability investigations, i.e. after 6 mo of storage at 4°±2°C, was found to be 88.1±16.1 nm,-30.5±1.40mV, 0.808±0.42. The PS, ZP, and PI of R7 did not very much, according to the results of stability experiments. The drug-loaded R7 polymeric nanoparticle was verified to be stable at 4°C±2°C storage temperature based on the results [32, 33].

CONCLUSION

The objective of this research is to enhance the dissolution of low soluble BCS class II drugs such as Ezetamibe in the form of the polymeric nanoparticle. The polymeric nanoparticle was formulated and optimized by the parameters like Particle Size (PS in nm), Polydispersity Index (PI), Zeta Potential (ZP in mV), % Entrapment Efficiency and *in vitro* drug release for 24 h were evaluated. These parameters showed significant changes while formulating polymeric nanoparticle along with various formulation and process variables. This developed technique i.e. homogenization followed by ultrasonication technique, will be effective and reproducible for the formulation of the polymeric nanoparticle. From the release pattern data it was observed that PNs show a significant improvement of dissolution character of Ezetamibe by reducing dose-dependent unfavourable side effects. According to the findings, PNs have a controlled drug release pattern and can be used as a suitable drug delivery carrier for low solubility and poorly bioavailable drugs like Ezetamibe to improve its dissolution.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest associated with this work.

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