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 (hydroxy propyl methyl cellulose composition) and process variables such as homogenization time (min) and ultra-sonication time (min), 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 PN show a significant improvement of dissolution character of Ezetimibe. 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 Ezetimibe to improve its dissolution.

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

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 (PNs) 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 nanoparticles, 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 (hydroxy 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 70, a Schimadzu model equipment, was used to study Ezetimibe and a Polymedexico nanoparticle. The samples were measured at 25 °C, all measurements were made in triplicate. The amplitude of zeta potential was then directly calculated from the equation [23].

\[
\zeta = \frac{\mu}{\eta}E
\]

Where, \(\zeta\) - Zeta Potential, \(\mu\) - Electrophoretic mobility; \(E\) - Electric permittivity of the liquid; \(\eta\) 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 Ezetimibe 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

| Table 1: Design of optimization of the polymeric nanoparticle by 2^3 factorial design |
| Run | Independent variables (Level code) | Independent variables (conc./range) |
| 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 | 5 | 5000 | 5 |
| R 2 | 1 | -1 | 10 | 5000 | 5 |
| R 3 | -1 | 1 | 5 | 10000 | 5 |
| R 4 | 1 | 1 | 10 | 10000 | 5 |
| R 5 | -1 | -1 | 5 | 5000 | 10 |
| R 6 | 1 | 1 | 10 | 5000 | 10 |
| R 7 | -1 | 1 | 5 | 10000 | 10 |
| R 8 | 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 unimodal or uniform monodisperse size distribution. All measurements were done in triplicate (n=3) [21, 22].

Zeta potential (\(\zeta\))

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 >30 mV, indicating the colloid’s durability. Using the Smolochowski equation, the Zeta potential was then directly calculated from the equation [23].

| 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 unimodal or uniform monodisperse size distribution. All measurements were done in triplicate (n=3) [21, 22].

Zeta potential (\(\zeta\))

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 >30 mV, indicating the colloid’s durability. Using the Smolochowski equation, the Zeta potential was then directly calculated from the equation [23].

\[
\zeta = \frac{\mu}{\eta}E
\]

Where, \(\zeta\) - Zeta Potential, \(\mu\) - Electrophoretic mobility; \(E\) - Electric permittivity of the liquid; \(\eta\) 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 Ezetimibe 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 Ezetimibe in the withdrawn sample was measured using a UV Spectrophotometer 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.

\[
\%EE = \frac{X_s - Xt}{Xs} \times 100
\]

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. A donor compartment is formed by a dialysis membrane tied dialysis membrane does not leak polymeric nanoparticle dispersion. A donor compartment was 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 saline after 1 hour. The concentration of Ezetimibe in the withdrawn sample was measured using 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); Peppa’s model (square root of time vs. percent cumulative 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 Ezetamibe 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 823.42 cm⁻¹ respectively. For Optimized Ezetimibe PN drug C -F stretching is 3744.07 cm⁻¹ respectively. From the data it was determined that the drug and excipients included in the formulation of solid lipid nanoparticle. This study used an optimised polymeric nanoparticles dispersion.

Table 2: FTIR spectrum interpretation of ezetimibe formulation

| Functional group | Wavenumber (cm⁻¹) | 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 | 823.42 | 3133.42 |

**Fig. 1: Drug excipients compatibility studies-FTIR 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: Ezetimibe at 164.35 °C; Ezetimibe 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 Ezetimibe drug and fig. 2(b) depicts the DSC endothermic thermogram of optimized Ezetimibe 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 melt 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.
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: polymeric 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              | -2.65±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

>±30 mV

<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
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 ultrasonication time) has a particle size of 87.0±3.64 nm; a zeta potential of -33.4±2.32 mV, and a polydispersity index of 0.477±0.12, 0.480±0.24, 0.488±0.20, respectively, according to the acceptance requirements. 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±2.02 mV to -33.4±2.32 mV, 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±2.32 mV, which meets the approval criteria i.e., for a stable polymeric nanoparticle, it should be ±30 mV. The remaining formulation fell short of the target range i.e., <±30 mV [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 and 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, 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, a 1 level of homogenization speed, and ultrasound time, R7 formulation demonstrated the requisite zeta potential of about 33.4±2.32 mV. With a rise in ultrasound time and homogenization speed, the ZP in mV increased in lockstep with a reduction in polydispersity index of approximately 0.498±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)](image)

**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 Ezetimibe 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. From the regression graph the r^2 value were found to be 0.98±0.02 with good linearity. So it was confirmed that the R7 Ezetimibe 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 42°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±2.32 mV, and 0.488±0.20, and R7 after performing stability investigations, i.e., after 6 mo of storage at 4°C±2°C, was found to be 88.1±6.1 nm, 30.5±1.40 mV, and 0.488±0.42. The PS, ZP, and PI of R7 did not vary 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 Ezetimibe 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 Ezetimibe 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 Ezetimibe to improve its dissolution.

ACKNOWLEDGMENT

The authors are express their gratitude and thank to Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh, India, for providing the labs and other facilities to perform this research in a successful manner.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest associated with this work.

REFERENCES

1. Crucho CCC, Barros MT. Polymeric nanoparticles: a study on the preparation parameters and characterization methods. Mater Sci Eng C Mater Biol Appl. 2017;80:771-84. doi: 10.1016/j.msec.2017.06.004, PMID 28866227.
2. Douglas DA, Pharmacological nanotechnology: a therapeutic revolution. Int J Pharm Sci Dev Res. 2020;6(1):9-11. doi: 10.17352/jpsdr.000027.
3. Mandic B, Savic Radiojevic A, Simic T. Polymeric nanocarriers of drug delivery systems in cancer therapy. Avramović CN. Pharmaceutics. 2020;12:298.
4. Jawahar N, Meyyanathan S. Polymeric nanoparticles for drug delivery and targeting: a comprehensive review. Int J Health Allied Sci. 2021;12(4):217-23. doi: 10.1034/2273-344X.107832.
5. Bennet D, Kim S. Polymer nanoparticles for smart drug delivery. In: Application of nanotechnology in drug delivery. Vol. 8. London, UK: IntechOpen; 2012.
6. Gutierrez SS, Alves MP, Pohlmann AR. Polymeric nanoparticles, nanospheres and nanocapsules, for cutaneous applications. Drug Target Insights. 2007;2:147-57. doi: 10.1177/177982870700200002, PMID 21901071.
7. Kumar S, DiBargi N, Saharan R, Bhanjana G. Nanotechnology as an emerging tool for enhancing solubility of poorly water-soluble drugs. Bio Nano Science 2012;2:227-50.
8. Vasile C. Polymeric nanomaterials in nanotherapeutics. London, UK: Eckvier; 2018.
9. Tulaim UR, Mahmood A, Aslam S, Erum A, Shamshad Malik N, Rashid A, Kausar R, Alqhtani MS. Formulation and evaluation of linum usitatissimum mucilage-based nanoparticles for effective delivery of ezetimibe. Int J Nanomedicine. 2021;16:4579-96. doi: 10.2147/ijn.s308790, PMID 34267514.
10. Din Fu, Zeb A, Shah KU, Zia-ur-Rehman. Development, in vitro and in vivo evaluation of ezetimibe-loaded solid lipid nanoparticles and their comparison with marketed product, Zia-ur-Rehman. J Drug Deliv Sci Technol. 2019;51:583-90. doi: 10.1016/j.jddst.2019.02.026.
11. Mendehe AA, Kharwade RS, Mahajan UN. Dissolution enhancement of poorly water-soluble drug by cyclodextrins inclusion complexation. Int J Appl Pharm. 2016;8:60-5.
12. Escalona Rayo O, Fuentes Vazquez P, Jardon Xicotencatl S, Garcia Tovar CG, Mendoza Evihra S, Quintanar Guerrero D. Rapamycin-loaded polysorbate 80-coated PLGA nanoparticles: optimization of formulation variables and in vitro anti-gloma assessment. J Drug Deliv Sci Technol. 2019;52:488-99. doi: 10.1016/j.jddst.2019.05.026.
13. Kunam V, Suryadevara V, Garikapati DR, Mandava VBR, Sasidhar R. Solubility and dissolution rate enhancement of ezetimibe by solid dispersion and pelletization techniques using soluplus as carrier. Int J App Pharm. 2019;11:57-64. doi: 10.22159/ijapp.2019v11i4.143274.
14. Bohre S, Chourasiya V, Pandey A. Polymeric nanoparticles containing diazepam: preparation, optimization, characterization, in vitro drug release and release kinetic study. Nano Conver, 2016;3(1):3. doi: 10.1186/s40580-016-0061-2, PMID 26191413.
15. Hickey JW, Santos JL, Willford JM, Mao HQ. Control of polymeric nanoparticle size to improve therapeutic delivery. J Control Release. 2015;219:53-67. doi: 10.1016/j.jconrel.2015.10.006, PMID 26450667.
16. Zielinski A, Ferreira NR, Durazzo A, Lucarini M, Cicero N, Mamouni SR, Silva AM, Nowak I, Santini A, Souto EB. Development and optimization of solid lipid nanoparticles (SLN) using experimental factorial design and dispersion analysis. Molecules. 2019;24(15):2683. doi: 10.3390/molecules24152683, PMID 31348802.
17. Ziae A, Afbaradin AB, Padrela L, Femmer T, O'Reilly E, Walker G. Spray drying of pharmaceuticals and biopharmaceuticals: critical parameters and experimental process optimization approaches. Eur J Pharm Sci. 2019;127:300-18. doi: 10.1016/j.ejps.2018.10.026, PMID 30428336.
18. Mouridoudis S, Pallares RM. Thank NTK. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale. 2010;12:12871-934. doi: 10.1039/c0nr02278j, PMID 29926865.
19. Daxon C, Witschger O, Bau S, Fierro V, Llewellyn PL. Nanomaterial identification of powders: comparing volume-specific surface area, X-ray diffraction and scanning electron microscopy methods. Environ Sci.: Nano. 2019;6(1):152-62. doi: 10.1039/C8EN00760H.
20. Jain AK, Thareja S. In vitro and in vivo characterization of pharmaceutical nanocarriers used for drug delivery. Artif Cells Nanomed Biotechnol. 2019;47(1):524-39. doi: 10.1080/21691401.2018.1561457, PMID 30794313.
21. Krishnamoorthy R, Mahalingam M. Selection of a suitable method for the preparation of polymeric nanoparticles: multi-criteria decision-making approach. Adv Pharm Bull. 2015;5(1):57-67. doi: 10.5681/abp.2015.008, PMID 25789220.
22. Cahadas C, Alvarado H, Calpena AC, Silva AM, Souto EB, Garcia M, Abrego G. In vitro, ex vivo and in vivo characterization of PLGA nanoparticles loading pranopopen for ocular administration. Int J Pharm. 2016;511(2):719-27. doi: 10.1016/j.ijpharm.2016.07.055, PMID 27480398.
23. Sanchez Lopez E, Egea MA, Cano A, Espina M, Calpena AC, Etcheto M, Camins A, Souto EB, Silva SA, Garcia ML, Abrego G. In vitro, ex vivo and in vivo characterization of PLGA nanoparticles loading probenecid for ocular administration. Int J Pharm. 2016;511(2):719-27. doi: 10.1016/j.ijpharm.2016.07.055, PMID 27480398.
24. Sanchez Lopez E, Egea MA, Cano A, Espina M, Calpena AC, Etcheto M, Camins A, Souto EB, Silva SA, Garcia ML, Abrego G. In vitro, ex vivo and in vivo characterization of PLGA nanoparticles loading pranopopen for ocular administration. Int J Pharm. 2016;511(2):719-27. doi: 10.1016/j.ijpharm.2016.07.055, PMID 27480398.
25. Sanchez Lopez E, Egea MA, Cano A, Espina M, Calpena AC, Etcheto M, Camins A, Souto EB, Silva SA, Garcia ML, Abrego G. In vitro, ex vivo and in vivo characterization of PLGA nanoparticles loading probenecid for ocular administration. Int J Pharm. 2016;511(2):719-27. doi: 10.1016/j.ijpharm.2016.07.055, PMID 27480398.
26. Sanchez Lopez E, Egea MA, Cano A, Espina M, Calpena AC, Etcheto M, Camins A, Souto EB, Silva SA, Garcia ML, Abrego G. In vitro, ex vivo and in vivo characterization of PLGA nanoparticles loading pranopopen for ocular administration. Int J Pharm. 2016;511(2):719-27. doi: 10.1016/j.ijpharm.2016.07.055, PMID 27480398.
B. N. D. et al.

Int J App Pharm, Vol 14, Issue 2, 2022, 151-159

27. Mourdikoudis S, Pallares RM, Thanh NTK. Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale. 2018;10(27):12871-934. doi: 10.1039/c8nr02278j, PMID 29926865.

28. Yang Y, Jiang Y, Xu J, Yu J. Conducting polymeric nanoparticles synthesized in reverse micelles and their gas sensitivity based on quartz crystal microbalance. Polymer. 2007;48(15):4459-65. doi: 10.1016/j.polymer.2007.06.005.

29. Baer DR, Engelhard MH, Johnson GE, Laskin J, Lai J, Mueller K, Munusamy P, Thevuthasan S, Wang H, Washton N, Elder A, Baisch BL, Karakoti A, Kuchibhatla SV, Moon D. Surface characterization of nanomaterials and nanoparticles: important needs and challenging opportunities. J Vac Sci Technol A. 2013;31(5):50820. doi: 10.1116/1.4818423, PMID 24482557.

30. Krishnamoorthy K, Mahalingam M. Selection of a suitable method for the preparation of polymeric nanoparticles: multi-criteria decision-making approach. Adv Pharm Bull. 2015;5(1):57-67. doi: 10.5681/apb.2015.008, PMID 25789220.

31. Fu Y, Kao WJ. Drug release kinetics and transport mechanisms of non-degradable and degradable polymeric delivery systems. Expert Opin Drug Deliv. 2010;7(4):429-44. doi: 10.1517/17425241003602259, PMID 20331353.

32. Palanikumar L, Al-Hosani S, Kalmouni M, Nguyen VP, Ali L, Pasricha R, Barrera FN, Magzoub M. pH-responsive high stability polymeric nanoparticles for targeted delivery of anticancer therapeutics. Commun Biol. 2020;3(1):95. doi: 10.1038/s42003-020-0817-4, PMID 32127636.

33. Zielińska A, Ferreira NR, Feliczak Guzik A, Nowak I, Souto EB. Loading, release profile and accelerated stability assessment of monoterpenes-loaded solid lipid nanoparticles (SLN). Pharm Dev Technol. 2020;25(7):1-13832-44. doi: 10.1080/10837450.2020.1744008, PMID 32204628.