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PREPARATION AND EVALUATION OF EZETIMIBE LOADED NANOPARTICLES BY SOLVENT EVAPORATION METHOD

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ABSTRACT

The Ezetimibe loaded Eudragit S 100 nanoparticles were prepared by solvent evaporation technique. Nanoparticles of different core: coat ratio were formulated and evaluated for loading efficiency, particle size, zeta potential, solubility studies, *in vitro* drug release, kinetic studies and stability studies. The prepared nanoparticles have a particle diameter ranging approximately from 289-486 nm and a zeta potential of the ideal formulation FS4 is found to be 42 mV. There was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulations. The *in vitro* drug release profile of the formulation was in the range 70.2±1. to 94.8±1 % for 60 min (fig 6). The batch FS4 showed the fastest dissolution rate, with approximately 95% of the drug being released within 60 min. No appreciable difference was observed in the drug content of product which were stored at 5˚±3°C and room temperature (30º± 2ºC/65%RH), and 40± 2ºC/75%RH, (after 3 months storage (as per QA1(R)) of stability studies. According to the data obtained, this Eudragit S 100- based delivery system opens new and interesting perspectives as drug carriers.

KEYWORDS: Nanoparticles; Eudragit S 100; Ezetimibe; Solvent evaporation, Solubility.

INTRODUCTION

Hyperlipidemia has been categorized as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases.[1] Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the main cause of death.[2] Hyperlipidemia is distinguished by elevated serum total cholesterol, low density lipoprotein, very low-density lipoprotein and decreased high density lipoprotein levels. Hyperlipidemia related lipid

disorders are considered to cause atherosclerotic cardiovascular disease.[3] The causes of hyperlipidemia include inherited defects in lipid metabolism and hypercholesterolemia due to diet, drugs or diseases belongs to statin family of cholesterol lowering agents and is widely used to treat coronary heart disease, dyslipidaemia and hypercholesterolemia.[4] Ezetimibe is the first lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol without affecting the absorption of fat-soluble nutrients.[5] FDA has recently recognized ezetimibe as a novel medicine for the treatment of various disease conditions most particularly cardiovascular disease.[6] Ezetimibe is classified as BCS class-II drug, owing to its sparingly water soluble, yet extraordinary permeation capability it has extremely low dissolution in gastrointestinal (GI) fluids, and exceedingly irregular bioavailability owing to its hydrophobic nature.[7]

Hence, the objective of the present work was to formulate Eudragit S100 nanoparticles containing ezetimibe by Solvent evaporation method, evaluate its physicochemical characteristics such as solubility particle, size shape, zeta potential, drug loading capacity, solubility studies and *in vitro* release characteristics.

MATERIALS AND METHODS

The Ezetimibe was received as a gift sample from Recipharm pharma services pvt ltd., Karnataka, India. Potassium dihydrogen phosphate, disodium hydrogen phosphate sodium hydroxide and sodium acetate were purchased from Thermo fisher scientific India Pvt. Ltd., Bangalore, India. The distilled water was produced in our research laboratory with a distillation unit.

Method of preparation

Solvent evaporation technique

In this study, nanoparticles are formulated by emulsification solvent evaporation method using sonication. Organic phase consisting of polymer (Eudragit S100 or RS) and drug (Ezetimibe) dissolved in Methanol (10 mL). This organic phase is added to an aqueous phase containing glycerol to form an O/W type emulsion (Table 1: The volume ratio of oil and aqueous phases was 1: 10. This emulsion is broken down into nanodroplets by applying external energy through a sonicator. These nanodroplets form nanoparticles upon evaporation of the highly volatile organic solvent. The organic solvent evaporates during magnetic stirring at 900 rpm under atmospheric condition for 2 h.[8]

Table 1: Composition of nanoparticles prepared by solvent evaporation technique.

Formulation code	Drug: polymer ratio	Amount of drug(mg)	Polymer (mg)	Time (Hr)	RPM
FS-1	1:1	50	100	4	900
FS-2	1:2	50	200	4	900
FS-3	1:3	50	300	4	900
FS-4	1:4	50	400	4	900
FS-5	1:5	50	500	4	900

Characterization of prepared nanoparticles

1. Differential scanning calorimetry (DSC)

A DSC study was performed to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC DuPont 9900, differential scanning calorimeter with a thermal analyser.

2. Fourier transform infra-red spectroscopy (FT-IR) Analysis

The FT-IR spectra of pure Ezetimibe and Eudragit S 100 nanoparticles loaded with Ezetimibe were recorded using Shimadzu IR spectrophotometer, Model 840, Japan, to check drug polymer interaction and stability of drug.[9]

3. Practical yield

Nanoparticles were collected and weighed to determine practical yield (PY) from the following equation

$$PY(\%) = \frac{\text{Nanoparticles weight}}{\text{Theoretical mass (polymer+drug+TTP)}} \text{ X } 100$$

4. Drug entrapment efficiency

Nanoparticles equivalent to 5mg Ezetimibe were crushed using a glass mortar and pestle. Then, they were suspended in 25 ml of acetate buffer pH 4.5. After 24 hrs., the solution was filtered and 1 ml of the filtrate was diluted 10 times and analysed for the drug content by UV-visible spectrophotometer at 232nm. The drug entrapment efficiency was calculated using the following formula.[10]

$$\text{Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{X } 100$$

5. Surface morphology study

Scanning electron microscopy (SEM) of the prepared nanoparticles was carried out to examine the particle size and surface morphology. The nanoparticles were arranged on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope under magnification of 7500–20000 ×.

6. Particle size distribution

The particle size distribution of the nanoparticles was determined by photon correlation spectroscopy (PCS, Coulter Counter model N4 MD, Coulter Counter Co. USA). The nanoparticle dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the nanoparticles. The average volume-mean particle size was calculated after performing the experiment in triplicate.

7. Zeta potential

The Zeta-potential of drug loaded nanoparticles were measured by Zeta sizer (Malvern Zetasizer 3000HS, UK). To determine the zeta potential, nanoparticles samples were diluted with KCl (0.1 mM) and placed in electrophoretic cell where an electrical field of 42 V/cm was applied. Each sample was analysed in triplicate.[11]

Table 2: Thumb rule.

Zeta Potential (Mv)	Stability behavior of the colloid
From 0 to ± 5	Rapid coagulation or flocculation
From ± 10 to ± 30	Incipient instability
From ± 30 to ± 40	Moderate stability
From ± 40 to ± 60	Good stability
More than ± 61	Excellent stability

8. Determination of solubility

Solubility was determined by adding excess amounts of pure Ezetimibe and nanoparticles in distilled water at 37 ± 0.5°C, respectively. The solution formed were equilibrated under continuous agitation for 24 h and passed through a 0.8 µm membrane filter to obtain a clear solution. The absorbance of the samples was measured using UV spectrophotometer method (UV 1601 A Shimadzu, Japan) at 232nm and the concentrations in µg/ml were determined. Each sample was determined in triplicate.[12]

9. *In vitro* release studies

In vitro release studies were carried out by using dialysis tubes with an artificial membrane. The prepared ezetimibe nanoparticles were re-dispersed in 5 ml of acetate buffer pH 4.5 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 150 ml of acetate buffer pH 4.5. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37±1˚C. 5ml sample of receptor compartment was taken at various intervals of time over a period of 60min and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrometrically at 232nm.[13]

10. Kinetic modelling

In order to understand the kinetics and mechanism of drug release, the result of *in vitro* drug release study of Nanoparticles was fitted with various kinetic equations like zero-order (cumulative% release vs time), first-order (log% drug remaining vs time), Higuchi's model (cumulative% drug release vs square root of time), Peppas plot (log of cumulative% drug release vs log time). R2 (coefficient of correlation) and k (release rate constant) values were calculated for the linear curve obtained by regression analysis of the above plots.[14]

'n'	**Mechanism**
0.5	Fickian Diffusion (Higuchi matrix)
0.5 < n < 1	Non Fickian Diffusion
>1	Case II Transport

11. Stability studies

Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. A drug formulation is said to be stable if it fulfills the following requirements.

- It should contain at least 90 % of the stated active ingredient.
- It should contain an effective concentration of added preservatives, if any
- It should exhibit neither discoloration nor precipitation, nor develops foul Odour.
- It should not develop irritation or toxicity.

Procedure

Stability studies were performed according to ICH guidelines. The optimized formulation was selected for stability studies. They were subjected for short-term stability studies and accelerated studies. Formulation FS4 was divided into 3 sets of samples and stored at 5^0±

3^0C in refrigerator and stored at room temperature (30 ± 2°C, 65% ± 5% RH) and their % drug content and *in vitro* releases were determined after 3 months. Similarly, an accelerated stability study was carried out by storing the selected formulation at 40° ± 2°C,75% ± 5% RH for a period of 3 months. The % Drug content and *in invitro* release were determined after 3 months.

RESULTS AND DISCUSSION

a. Physicochemical characterization of nanoparticles

The FTIR spectrum shows that there were no significant changes in the chemical integrity of drug and also indicates that the polymer and drug are compatible with each other.

Nanoparticles prepared by Solvent evaporation technique were found to be discrete. The DSC thermogram of pure Ezetimibe shows a sharp endothermic peak at 161°C by Thiel's tube (capillary tube) method which corresponds to its melting point. The thermogram of Ezetimibe with Eudragit S 100 shows sharp endothermic peak at 162°C. Thus, the thermal data shown in Fig.1 and 2, indicates that there is no interaction between the drug and the polymer.

The drug entrapment efficiency of Nanoparticles containing drug: polymer in various ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 was found to be 70.2% ,77.5%,81.74%,94.81%,86.39% (Table 2). Thus, there was a steady increase in the entrapment efficiency on increasing the polymer concentration in the formulation. The maximum entrapment efficiency (94.81%) was observed in formulation FS4. Weak aqueous solubility and high drug binding potential on polymer surfaces may explain the shift in drug entrapment. Through SEM analysis (Fig. 3), their mean size distribution was found to be 289-486 nm. Since the particle size is less than 1000nm, this drug delivery system can be used for parenteral formulations, drugs administered by such routes will achieve direct systemic delivery, thereby avoiding first pass hepatic metabolism and reaching a reduction in the dose delivered. Zeta potential of Ideal formulation FS4 was found to be 42 mV, which indicates that they are Excellent stable. The prepared nanoparticles show increase in solubility compared to pure drug.

b. Differential scanning calorimetry

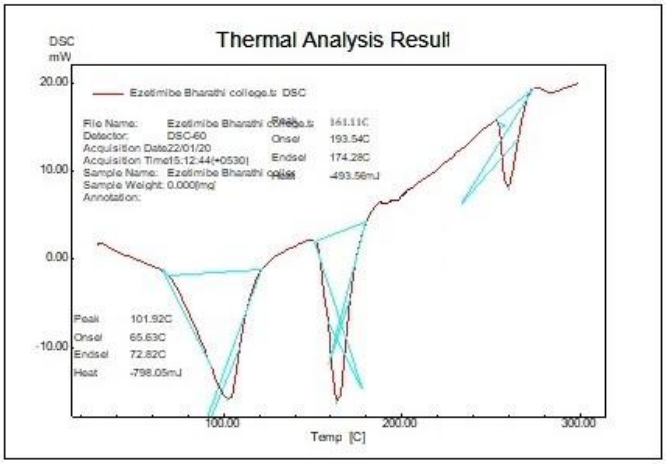

Fig. 1: DSC Thermograph of Ezetimibe.

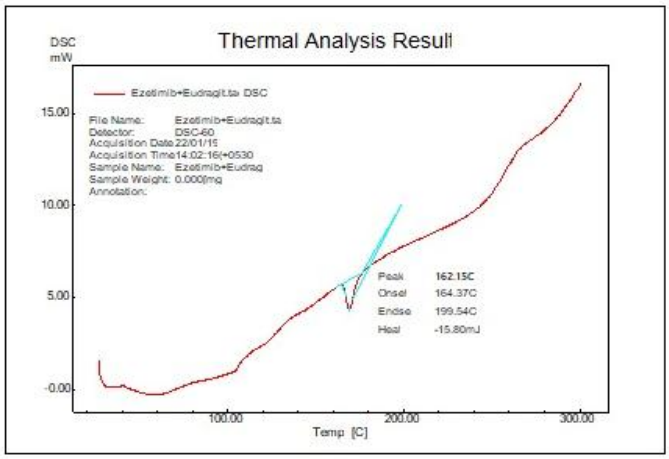

Fig. 2: DSC Thermograph of Ezetimibe and Eudragit S 100.

c. Percentage yield, Drug content and Entrapment efficiency

Table 3: Percentage yield, drug Content and Entrapment efficiency ofFormulations by solvent evaporation technique.

Formulation code	%Yield	%Drug content	%Entrapment efficiency
FS-1	74.09	74.52	75.87
FS-2	78.99	79.85	81.25
FS-3	82.47	83.67	85.76
FS-4	96.59	95.76	97.02
FS-5	92.86	89.04	90.98

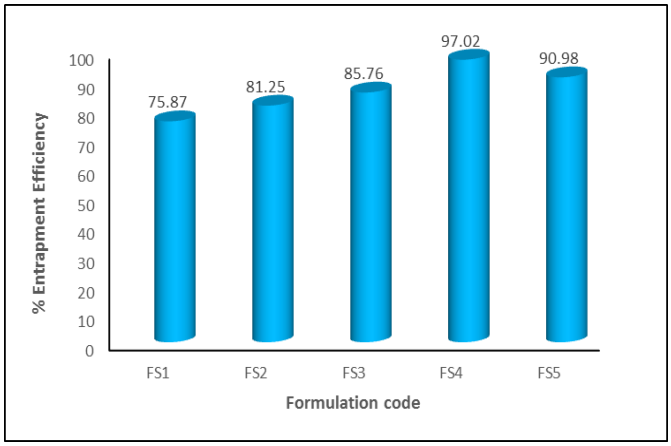

Fig. 3: Percentage Entrapment Efficiency of Formulations FS1-FS5.

d. Particles size distribution

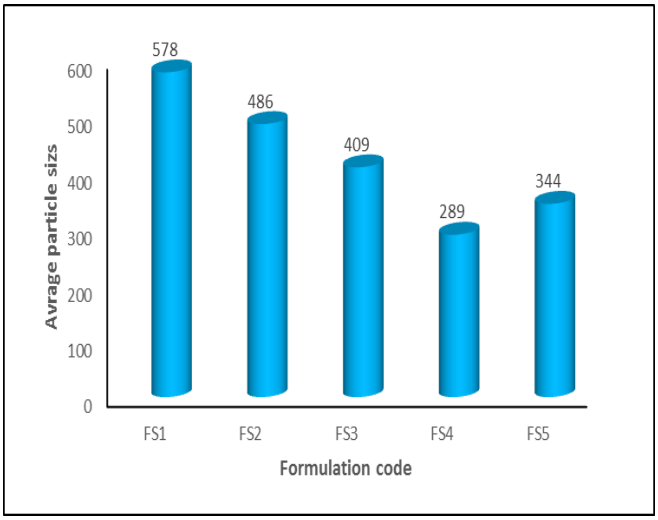

Fig. 4: Average Particle Size range of Formulations FS1-FS5.

e. Surface Morphology (SEM)

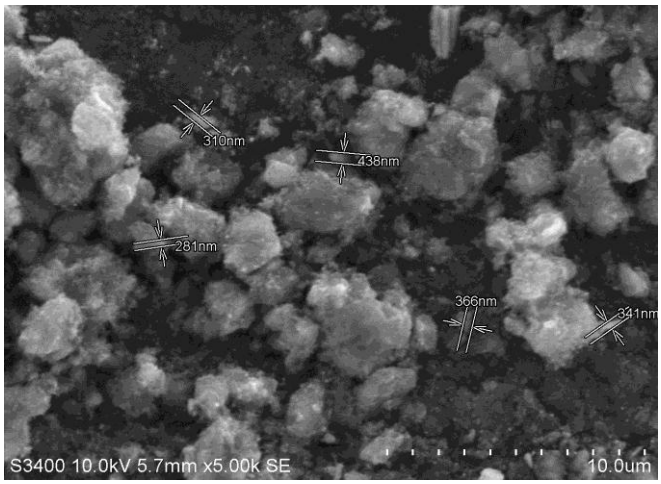

Fig. 5: SEM images of Formulation FS-4.

f. Solubility of prepared nanoparticles in water

Table 4: Solubility of prepared nanoparticles by solvent evaporation technique.

Formulation code polymer: drug ratio (w/w)	Solubility (µg/ml)
Pure drug	7.5183
FS-1	9.011
FS-2	9.421
FS-3	9.865
FS-4	10.769
FS-5	9.712

Results indicated that solubility of all batches of the nanoparticles increased as compared to pure drug.

g. *In vitro* release of nanoparticles

The *in vitro* drug release profile of the formulation was in the range 70.2±1. to 94.8±1 % for 60 min (fig 6). The batch FS4 showed the fastest dissolution rate, with approximately 95% of the drug being released within 60 min.

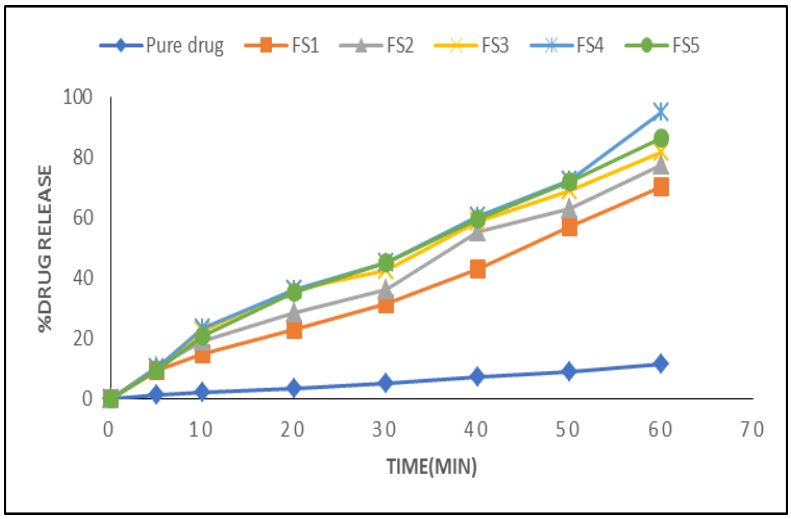

Fig. 6: Cumulative release of ezetimibe loaded nanoparticles (mean ±SD, n=3).

h. Kinetic studies

In order to describe the release kinetics of all five formulations the corresponding dissolution data were fitted in various kinetic dissolution models like zero order, first order, and Higuchi, respectively (Table 5). As indicated by higher R^2 (coefficient of correlation) values, the drug release from all formulations follows Zero order release and Higuchi model. Since it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is Fickian

diffusion, non-Fickian diffusion. 'n' (release exponent of Korsemeyer- Peppas model) value could be used to characterize different release mechanisms. The 'n' values for all formulations were found to be more than 0.50. This indicates that the release approximates non-Fickian diffusion mechanism.

Table 5: Regression co-efficient (r^2) values of different kinetic models and diffusion exponent (n) of Peppas model for Ezetimibe Nanoparticles FS1-FS5.

Formulation code	%Cumulative drug release	Zero Order	First Order	Higuchi	Peppas	'n' values
FS-1	70.21	0.993	0.9649	0.9188	0.9757	0.9634
FS-2	77.58	0.987	0.9782	0.9403	0.9682	09915
FS-3	81.74	0.9813	0.9848	0.9558	0.968	1.0217
FS-4	94.81	0.9904	0.9123	0.9399	0.9718	1.042
FS-5	86.39	0.9911	0.9709	0.9482	0.9777	1.0413

i. Stability studies

The results of drug content of ideal formulation FS4 after 3 month of stability testing at different storage conditions were shown in Fig. 7. *In vitro* release profiles for the same formulation stored at different storage conditions were also showed in Fig. 8.

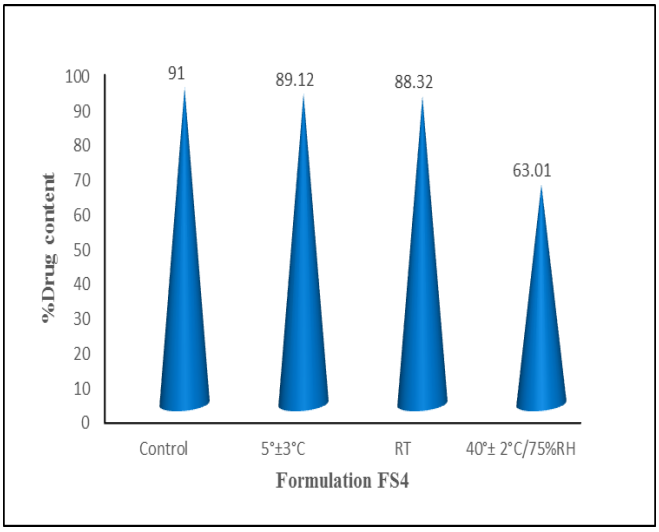

Fig. 7: Comparison of percentage drug content of formulation FS-4 stored at 5°± 3°C, RT and 40°± 2°C/75% RH, (after 3 months storage as per QA1(R)).

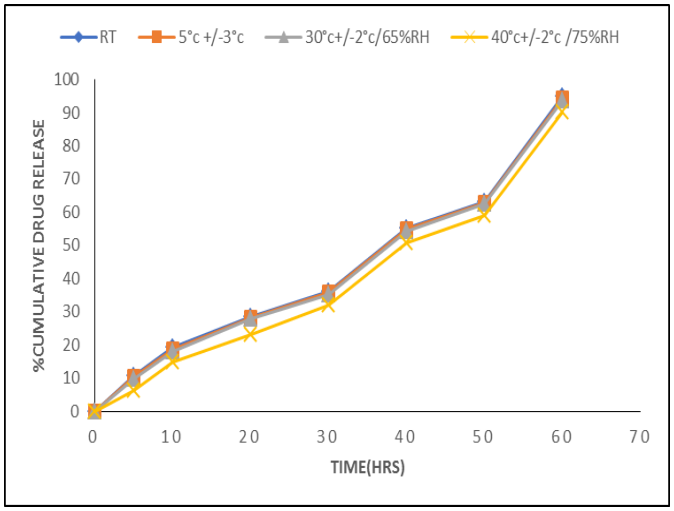

Fig. 8: Comparison of *in vitro* drug release profile for formulation FS-4 at 5º± 3ºC, 30º± 2ºC/65%RH and 40±2ºC/75%RH, (after 3 months storage as per QA1(R)).

CONCLUSION

Based on drug content, drug entrapment efficiency, particle size morphology, zeta potential and *in vitro* release, formulation FS4 was selected as an optimum formulation. Ezetimibe improvement in saturation solubility by 2-3-fold respectively. The *in vitro* dissolution study shows nearly 90% of Ezetimibe releases within 60 min. The stability studies showed that maximum drug content and closest *in vitro* release to previous data was found for FS4 stored at 5˚±3°C and room temperature. Thus, Ezetimibe nanoparticles (FS4) with core: coat ratio 1:4 was found to be spherical, discrete and free flowing with high solubility and able to release the drug effectively. From the above result it can be concluded that preparation of nanoparticles by precipitation method appeared to be a unique technology, which provided improved solubility and consequently increased bioavailability of BCS class II drug that could prove to increase therapeutic effectiveness. solvent evaporation technique is useful technique to improve the solubility and dissolution of poorly water-soluble drug like Ezetimibe.

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