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Formulation and Evaluations of Ezetimibe Nanoparticles prepared by controlled Nanoprecipitation and Transformation into Solid Dosage Form

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Abstract

Amorphous nanoparticles of ezetimibe (EZE), a poorly water-soluble drug, were produced by the controlled nanoprecipitation method without adding any surfactants at room temperature. The effects of different types and quantity of solvent and anti-solvent (water) on the physical state of EZE were investigated. The physical state of the formed nanoparticles was significantly influenced by the nature and quantity of solvent. Decreased particle size (627 nm) was observed in batch containing 5 mL ethanol as a solvent and 40 mL water as a anti-solvent, Morphology of optimized nanoparticles was found to spherical in scanning electron microscopy (SEM) this may be due to introduction of the drug solution to the antisolvent generates high supersaturation. The vacuum dried nanoparticles were transfer in to tablet. The XRD analyses confirmed that the prepared EZE was amorphous nanoparticles. Furthermore, the amorphous EZE nanoparticles exhibited significantly enhanced dissolution property when compared to the marketed product.

Keywords: Ezetimibe, nanoparticles, controlled nanoprecipitation, surfactant.

Introduction

Oral administration is the most convenient, widely utilized, and preferred route of drug delivery for systemic action. The solubility/dissolution behaviour of a drug is key determinant to its oral bioavailability. An improvement of oral bioavailability of poor water-soluble drugs remains one of the most challenging aspects of drug development (Seedher et al. 2003). In recent years, 40% of the newly developed molecules have poor water solubility problems, which lead to poor bioavailability and high dropout rate from the drug discovery and development from industrial scale (Kocbek et al. 2006). These drugs tend to be eliminated from the gastrointestinal tract before they get the opportunity to fully dissolve and be absorbed into the blood circulation. As about 70% of the human body is made up of water, a drug must be water-soluble and thus possess an acceptable bioavailability level (Sharma et al. 2009).

Many procedures have been investigated to enhance dissolution properties and, thus, oral bioavailability of drugs with very low aqueous solubility (Kakran et al. 2010).

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Conventional approaches include use of co-solvents, salt formation, pH adjustment, emulsions and micellar dispersions, micronisation, complexation with cyclodextrin and solid dispersion. However, most of these techniques require a more amount of additives limiting their use from the safety perspective. Moreover, these techniques offer little help in the formulation of molecules that are poorly soluble in both aqueous and organic solvents (Verma et al. 2009, Lai et al. 2009). Over the last 10 years, nanoparticle engineering has been developed and reported for pharmaceutical applications. Nanoparticle engineering processes currently used are precipitation, pearl milling and high pressure homogenization, either in water or in mixtures of water and water-miscible liquids or nonaqueous media (Trotta et al. 2001, Liversidge et al. 1995).

Reducing the particle size of an active pharmaceutical ingredient (API) has opened new formulation opportunities in various dosage forms. Particularly, the bioavailability of relatively insoluble drugs (Kayser et al. 2003), which is often limited by poor dissolution rates, benefits from the development of the 'nanoformulation' techniques (Serajuddin 1999). Nanosuspensions are liquid dispersion consisting of solid drug nanoparticles which are stabilized by polymer and or surfactant. Nanosizing has been proven to be an effective tool for an active moiety considered as "brick dust candidate". Generally, drug nanoparticles can be produced by the "breaking-down" or "building-up" technique (Lee 2003). The former involves the diminution of the coarse large drug particles down to the submicron range by virtue of various milling or high pressure homogenization techniques; the latter is actually to build the drug nanoparticles starting from the molecules, which includes the antisolvent precipitation technique, supercritical fluid technology (rapid expansion of supercritical solution, RESS and gas antisolvent precipitation, GAS) and spray-freezing/evaporation into liquid (Kesisoglou et al. 2007). For this technique, an organic drug solution is introduced to the antisolvent under rapid mixing, which generates high supersaturation and thereby fast nucleation rate leading to production of submicron particles (Rabinow 2004). This technique has the advantage of low cost, effective energy consumption and easy scaling-up (Keck et al. 2006).

Ezetimibe (EZE) is the first member of a novel class of lipid lowering agents that selectively inhibits the absorption of biliary and dietary cholesterol as well as related phytosterols from the intestine without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids. Ezetimibe is a class II molecule as per Biopharmaceutics Classification System (BCS). Due to its very high hydrophobic character, EZE exhibits highly erratic and very low dissolution profile in gastrointestinal fluids. Together with permeability, the solubility and/or dissolution rate of a drug are key determinants of its oral bioavailability. It is generally considered that compounds with very low aqueous solubility will show dissolution rate-limiting absorption and hence poor absorption, distribution and target organ delivery. Improvement of aqueous solubility in such a case is a valuable goal to improve therapeutic efficassy (Patel et al. 2008).

The objective of this study was to recover nanoparticles of ezetimibe produced by antisolvent precipitation at room temperatures by vacuum drying and transfer these nanoparticles in to solid dosage form.

Materials and Methods

Ezetimibe was obtained as a gift sample from Torrent Pharmaceutical Ltd., Ahmedabad, India. Acetonitrile, Ethanol, Methanol, Acetone,Ethyl Acetate as a gift sample from S.D.Fine chemicals. Bidistilled water was prepared in laboratory for study. All materials used for study conformed to USP-24 standards.

Preparation of EZE nanoparticles by controlled nanoprecipitation technique

Ezetimibe was dissolved in the solvent at definite concentration as shown in Table 1, The solution was filtrated through 0.45 µm pore size membranes to remove the possible particulate impurities. The EZE nanoparticles were then prepared by the controlled nanoprecipitation. Briefly, EZE solution was quickly poured into the anti-solvent under magnetic stirring and the precipitation was formed immediately upon mixing (Patel et al. 2010). The freshly formed nanoparticles were then dried under vacuum at 50°C for 12 hrs. The dried particles were then analyzed by particle size, SEM, XRD and dissolution testing measurements.

Table 1. Formulation of ezetimibe nanosuspension

Ingredients/Batch	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Ezetimibe (mg)	10	10	10	10	10	10	10	10	10	10	10
Ethanol (mL)	1	2.5	5	-	-	-	-	-	-	-	-
Methanol (mL)	-	-	-	2.5	5	-	-	-	-	-	-
Acetone (mL)	-	-	-	-	-	2.5	5	-	-	-	-
Acetonitrile (mL)	-	-	-	-	-	-	-	2.5	5	-	-
Ethyl acetate (mL)	-	-	-	-	-	-	-	-	-	2.5	2.5
Water (mL)	40	40	40	40	40	40	40	40	40	40	40
Particle size (nm)	1211	712	627	841	796	1114	1045	1240	1120	1324	1214

Evaluations of prepared nanoparticles

Prepared nanoparticles were evaluated for its yields, different physical parameters like Angle of repose, bulk density, Tapped density, car's index and Hausener's ratio as shown in Table 2.

Table 2. Evaluation of physical parameters

Evaluation parameters	Results
% Yield	62.82
Bulk density (mg/mL)	103.33
Tapped density (mg/mL)	124
Carr's index	17

Tabletting of vacuum dried nanosuspension

Tablet of nanoparticles containing ezetimibe was prepared by direct compression method using a rotary tablet machine. Dicalcium phosphate (DCP) was used as filler, sodium starch glycolate (SSG) as a disintegrant and magnesium stearate as a lubricant were used to prepare tablets containing ezetimibe nano- sized particles as shown in Table 3. The ezetimibe nanoparticles, DCP and SSG were carefully mixed using a pestle and mortar. 2% of magnesium stearate was added to compositions. The mixture was compacted using a rotary tablet machine to prepare tablet. For each tablet 300 mg of powder mixture was weighed into the die and compressed manually.

Table 3. Composition of ezetimibe nanoparticles tablet

Ingredients	Quantity
Ezetimibe (Eq.to.10mg)	63
Di calcium phosphate (mg)	229.5
Mg.Stearate (mg)	6
SodiumStarch Glycolate (mg)	1.5
Total weight (mg)	300

Evaluation of physical parameters of prepared tablets

Mean hardness and Percentage friability of the tablets was found to be 4.2 kg/cm^2 and 0.89 respectively. Thickness and diameter were found 8.6 mm and 8 mm respectively. Disintegration time and % assay were found 15 sec and 97.15 ± 0.85 respectively.

In vitro dissolution study

In vitro drug release studies were performed in USP apparatus-Type II using paddle method at rotation speed of 50 rpm. Dissolution was carried out in phosphate buffer pH 7.8 as a dissolution medium. The volume and temperature of the dissolution medium were 900ml and 37.0 ± 0.2°C. 5 ml of sample was withdrawn periodically (after 10 min) and replaced with an equal volume of fresh distilled water up to 60min. Samples were diluted suitably and filtered through a filter paper. The dialyzate was then subject to the UV analysis against the blank (distilled water). Percent cumulative release of EZE was calculated based on the standard UV calibration curve at 232nm (Systronic 2203, Japan).

Characterization of ezetimibe nanoparticles

Particle size determination

Particle size of vacuum dried particles was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range: 20–1000 nm). All the data presented are the mean of average values of three independent samples produced under identical production conditions.

Scanning electron microscopy

The surface morphology of the pure ezetimibe and optimized formulated nanosuspension was visualized by scanning electron microscopy (SEM). Particle morphology was investigated using a Hitachi (S-4700, Japan) scanning electron microscope with an acceleration voltage of 30 kV. Samples were prepared by drying suspension droplets on clean SEM sample stages, followed by coating with Pt-Pd for 2 min.

Powder X-ray diffraction (PXRD) analysis

The physical state of raw material and the formed particles were characterized by X-ray powder diffraction measurements. Powder X-ray diffraction patterns of all samples were determined using Phillips PW 3710 scanner, IW 1830 generator with a CuK α anode at 40 kV and 30 mA and at a scan rate of 1° min-1 from 2θ range from 1° to 40°C.

Comparison of prepared tablet with marketed tablets

The developed tablet formulation was compared with conventional market tablet for in vitro drug release profile and Mean Dissolution Time (MDT). Percentage of drug dissolved in 10 min (Q10) and Mean Dissolution Time were considered for comparison.

Similarity factor (f_2)

A value of 100% for the similarity factor (f2) suggest that the test and reference profiles are identical. Value between 50 and 100 indicates that the dissolution profiles are similar while smaller value implies an increase in dissimilarity between release profiles.

Result and Discussion

Ezetimibe nanoparticles were prepared by the controlled nanoprecipitation method. Nanosuspension of EZE was prepared as formulation shown in Table 1. S1-S3 formulation were containing ethanol as a solvent, S4-S5 formulation were containing methanol as a solvent, S6-S7 formulation were containing acetone as a solvent, S8-S9 formulation were containing acetonitrile as a solvent and S10-S11 formulation were containing ethyl acetate as a solvent. The amount of anti-solvent (water) was kept constant for all the prepared batches. The effects of different types and quantity of solvent and anti-solvent (water) on the physical state of EZ were investigated. The physical state of the formed particles was significantly influenced by the nature and quantity of solvent. Decreased particle size (627nm) was observed in batch S3 containing ethanol as a solvent this may be due to introduction of the drug solution to the antisolvent generates high supersaturation. This results in high nucleation rate and produces a large number of nuclei, mixing efficiency provides the mass transfer and the rate of evaporation of organic solvent between the multiphase, which induced homogenous supersaturation in and thus rapid nucleation to produce smaller drug particles. Amount of organic solvent contribute much towards the change in particle size in nanosuspension preparation. Furthermore vacuum drying was used to get the dried powder product. The yield of dried powder was 62.82%, the powder was evaluated for preformulation study, from the Table 2 shows that the vacuum dried power having excellent flow property and good compressibility. So further this powder was transformed in to table dosage form.

In vitro drug release study

The in vitro dissolution of a drug is an indirect method to predict its bioavailability from a formulation. Dissolution behavior was investigated for optimized formulation and pure drug. For that DP10 min (percent drug dissolved within 10 min), mean dissolution time (MDT) and t50% (time to dissolve 50% drug) values calculated from release profile are reported in Table 4, from this data, it was evident that onset of dissolution of pure EZ was very low (DP10 min value 1.21% and t50% >>3 h) as compared to nanosuspension tablet (DP10 min value 36.15% and t50% 18min).

Table 4. % Drug dissolved within 10 minutes (DP10 min), time to dissolve 50% drug ($t_{50\%}$) & mean dissolution time (MDT) from pure EZ and nanosuspension.

Parameters	Pure Drug	S3
$DP_{10\ min}$	1.21	36.15
MDT	26.41	12.87
$t_{50\%}$ (min)	> ı80	18

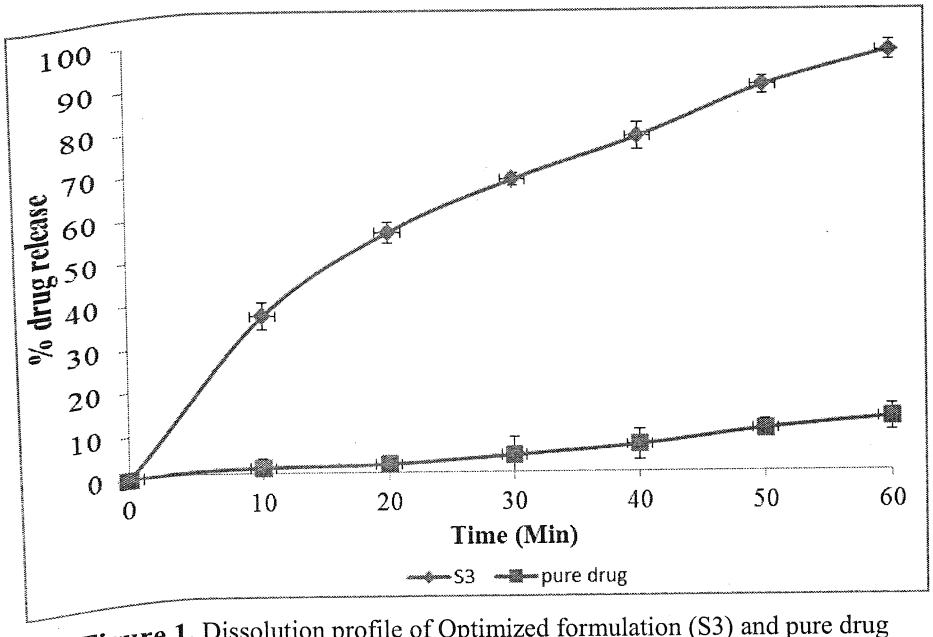

Figure 1. Dissolution profile of Optimized formulation (S3) and pure drug

As shown in Figure 1, nano-sized ezetimibe showed a dramatic increase of rate and extent of dissolution compared to pure drug ezetimibe. The slope of dissolution profile is especially different for nanoparticles in the initial stage (first 10 min) and maintained throughout the experiment compared to pure drug ezetimibe.

MDT reflects the time for the drug to dissolve and is the first statistical moment for the cumulative dissolution process that provides an accurate drug release rate. It is accurate expression for drug release rate. A lower MDT value indicates faster dissolution rate.

$$MDT_{In\ vitro} = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M}$$

MDT value of pure EZ is very high (26.41min). This value decreased to a greater extent after preparing its nanoparticles. Nanoparticles showed lowest MDT value (12.87 min). MDT was considerably lower for the nanoparticle formulation, indicating a faster dissolution rate compared to the pure drug (Table 4).

In addition, the time to dissolve 50% of the drug (t50%) was strongly reduced in optimized batch (S3). DP10min values increased nanosuspension< pure drug; while MDT and t50% values decreased in the same order. Moreover, dissolution parameters demonstrated that optimized batch (S3) had better dissolution properties than pure drug.

Particle size

The optimized batch (S3) had a Z-average particle size of 672 nm with 0.202 poly-dispersivity index which indicate the particles are in uniform distribution. The particle size distribution pattern of the optimized nanosuspension formulation is given in Figure 2.

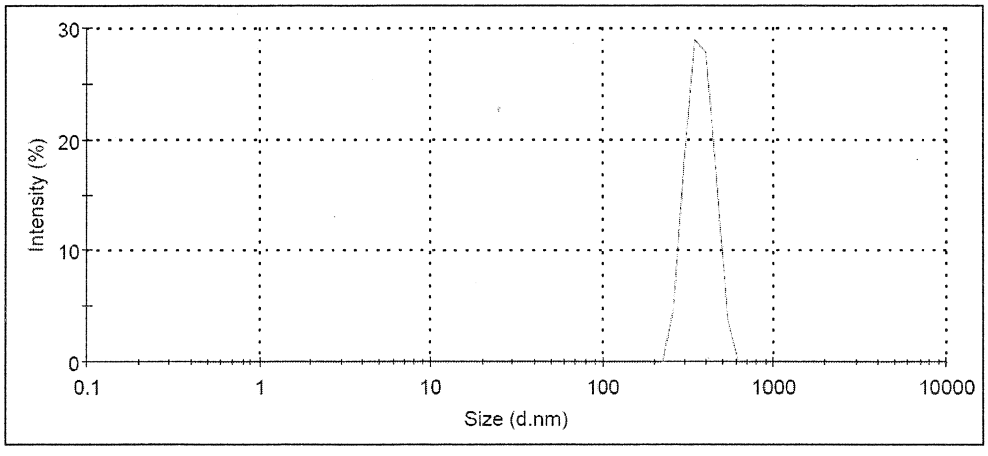

Figure 2. Particle size analysis of Batch S3

Scanning electron microscopy

Figure 3 and 4 shows SEM micrograph of the EZE powder particles and optimized EZE nanosuspension (S3) respectively. Micronized EZE powder showed irregular shapes with particle size generally larger (5-50 μm), where as EZE nanoparticles had the different morphology and spherical in shape with 516-616 nm diameters range. The EZE prepared nanoparticles were more uniform and the uniformity was more prominent.

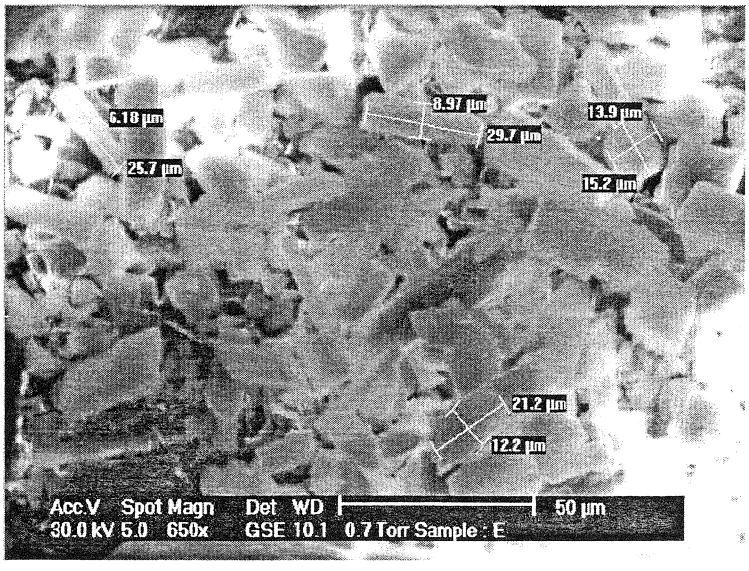

Figure 3. SEM image of ezetimibe powder

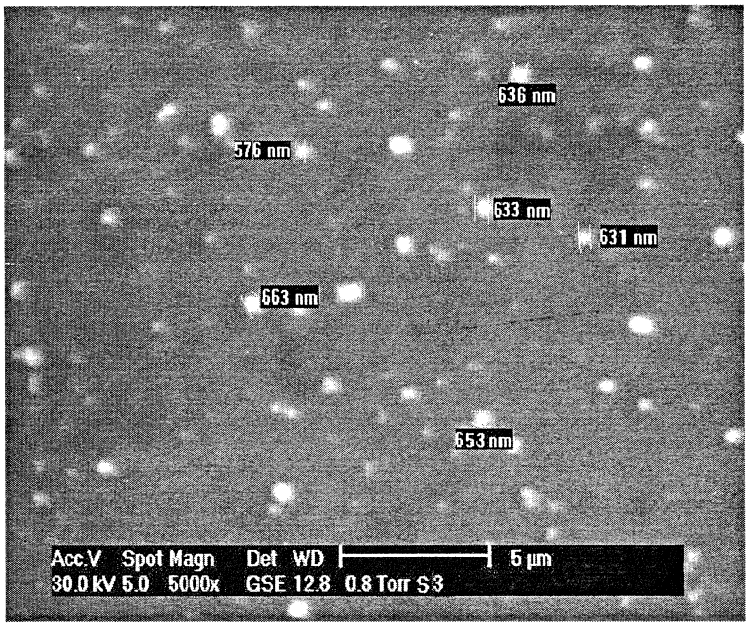

Figure 4. SEM image of EZ nanoparticles of optimized formulation S3

Powder X-ray diffraction (PXRD) analysis

X-ray diffraction spectroscopy (XRD) has been used to assess the degree of crystalinity of EZE in the controlled nanoprecipitation process. The XRD patterns are shown in Figure 5. it was shown that the pattern of the raw EZE exhibited some intense crystalline peaks at 2θ value, which proved that the raw EZE was crystalline form. However, instead of those intense crystalline peaks, only one broad and diffuse maxima peak was detected in the patterns of the nanosized EZE, which indicated that the nanosized EZE was amorphous form.

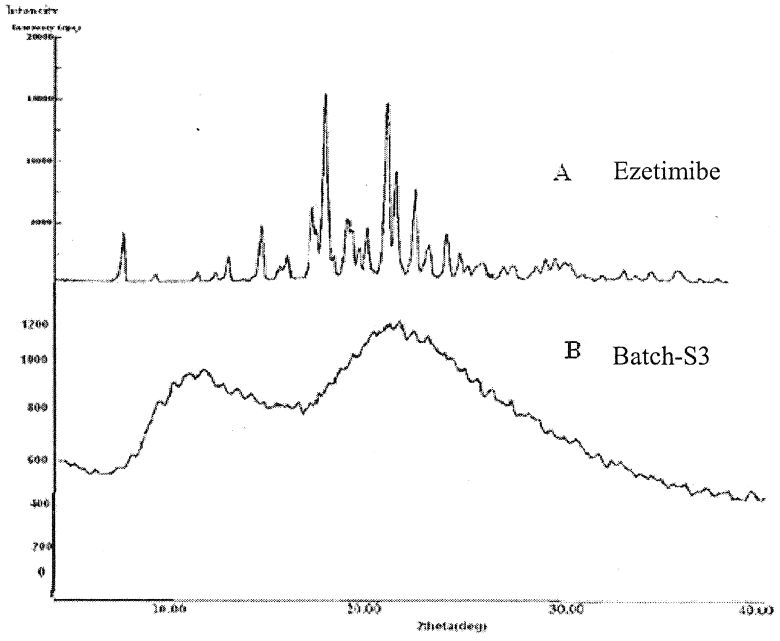

Figure 5. X-ray diffraction patterns of Ezetimibe and Batch S3

Comparison of optimized batch tablets with marketed tablets

The developed tablet formulation (formulation S3) was compared with conventional market formulation tablet (MF-A), (MF-B), (MF-C) and pure drug tablet for in vitro drug release profile, % drug release in 10 min (Q10) and Mean dissolution Time (MDT). The Q10 values of pure drug, (MF-A), (MF- B), (MF- C) and optimized formulation S3 were 1.21, 6.8, 7.5, 6.4, and 36.15 respectively as shown in Figure 6 and Table 5. The MDT values were 26.41, 20.41, 19.45, 19.22, and 12.87 respectively as shown in Figure 7 and Table V.

Comparison of optimized batch tablets with marketed tablets for similarity factor (f_2)

The in vitro release profile of formulation S3 was compared with conventional market tablet (MF- A), (MF B), and (MF- C) for similarity factor (f2). The values for f2 for (MF- A), (MF B), and (MF- C) are 15.50, 15.12 and 15.49 (Table 6) indicating there were no similarity between in vitro dissolution of formulation S3 and (MF- A), (MF B), and (MF- C) .There for formulation S3 was considered better cost effective formulation with higher and rapid in vitro dissolution due to its less mean dissolution time and higher Q10 values.

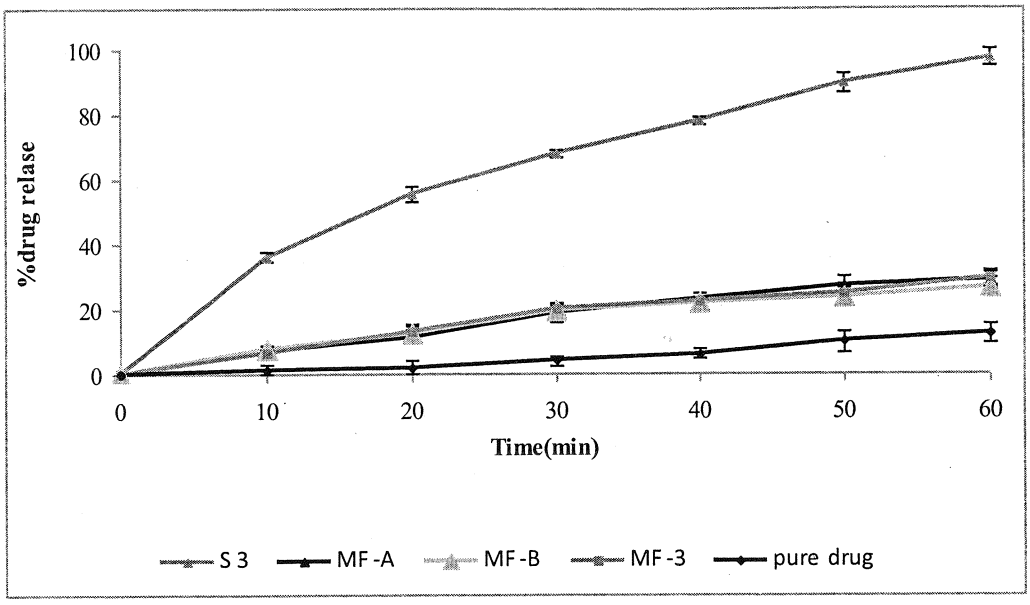

Figure 6. Comparisons of dissolution profile of S3, marketed formulation-A (Torrent), marketed formulation-B (Glenmark), marketed formulation-C (Zydus cadila) and pure drug

Table 5. Comparison of formulation S3 with marketed ezetimibe tablets

Product	% Drug release after 10 min (Q10)	Mean Dissolution Time (MDT)
Pure drug	1.21	26.41
Market product (MF- A)	6.8	20.41
Market product (MF- B)	7.5	19.45
Market product (MF- C)	6.4	19.22
Formulation S3	36.15	12.87

MF- A: is a conventional market tablet from Torrent pharmaceutical Ltd.; MF- B: is a conventional market tablet from Glenmark pharmaceutical Ltd; MF- C: is a conventional market tablet from Zydus cadila healthcare Ltd.

Table 6. Comparison of formulation S3 with marketed ezetimibe tablets for similarity factor (f_2)

Product	Similarity Factor (f_2)
Pure drug	10.42
Market product (MF- A)	15.5
Market product (MF- B)	15.12
Market product (MF- C)	15.49
Formulation S3	-

MF- A: is a conventional market tablet from Torrent pharmaceutical Ltd.; MF- B: is a conventional market tablet from Glenmark pharmaceutical Ltd; MF- C: is a conventional market tablet from Zydus cadila healthcare Ltd.

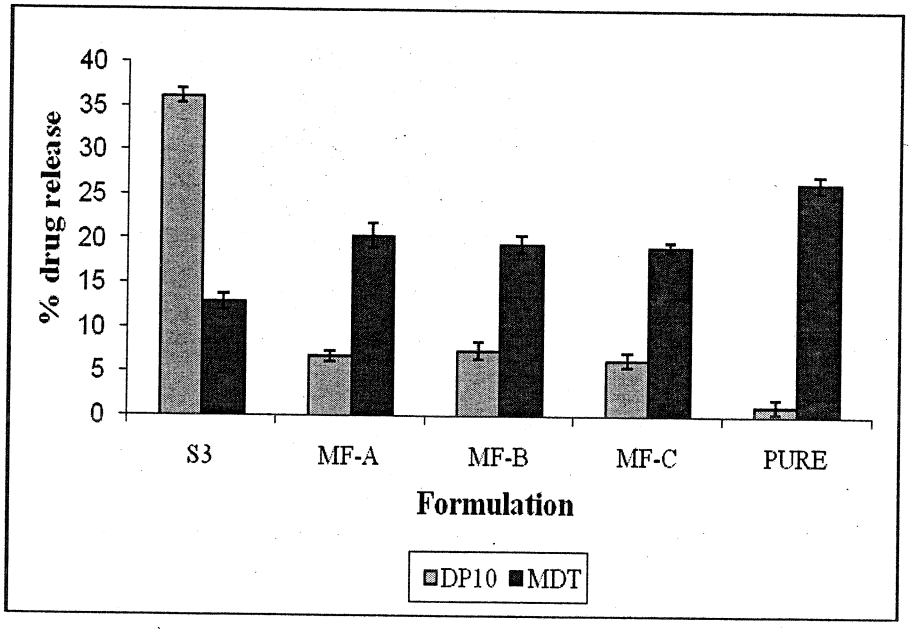

Figure 7. Comparisons of DP $_{10\ min}$ and MDT of optimized batch (S3) marketed formulation-A (Torrent), marketed formulation-B (Glenmark), marketed formulation-C (Zydus cadila) and pure drug

Conclusion

Dry nanoparticles of poorly water soluble drugs can be prepared using controlled nanoprecipitation method at laboratory scale. The prepared nanoparticles were transferred in to tablet and compare with marketed formulation. It was concluded that the drug release in 10 min and Mean dissolution time was much lesser for the tablet prepared with nanoparticles than that of the marketed formulation and no similarity were seen between optimized formulation and marketed formulations which indicated that the controlled nanoprecipitation method was direct process to obtain drug nanoparticles of controllable size, amenable for continuous and consistent production.

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