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Research Article

Nanoparticulates of Fenofibrate for Solubility Enhancement: *Ex-Vivo* Evaluation

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ABSTRACT

The aim of present research work was to formulate and evaluate nanosuspension of fenofibrate which is categorized as BCS class II agent. With an intention to increase solubility and dissolution rate of fenofibrate nanosuspension were prepared by high pressure homogenization method, a top down technique. Using poloxamer 188 and Tween 80 as a stabilizer. Formulation scheme was developed by Box Behnken Design. Formulation factor which affect the particle size includes Concentration of surfactant and processing parameters includes Homogenization pressure and Homogenization cycles. In this study practically water insoluble fenofibrate was nanosized and surfactant was added for their stabilizing effect. *In vitro* dissolution study showed that the increase in the release rate of fenofibrate from nanoparticles as compared to pure drug. Scanning electron microscopy study showed that the spherical morphology of nanoparticles. Particle size distribution, zeta potential, crystal form of formulated nanosuspension were studied by using particle size analyzer, and X-ray powder diffraction, *Ex-vivo* study for calculating absorption rate. The result showed that the drug dissolution rate in nanosuspension formulation is depends upon the crystal form, solubility, procedure involved, and stabilizer used.

Keywords: Solubility, Dissolution, high pressure homogenization, lyophilization, nanosuspension.

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INTRODUCTION

Solubility is the most important parameter for a drug to show good bioavailability and hence therapeutic effect[1, 2]. More than 40% of new drug entities are poorly water soluble which frequently create problems in formulating them into conventional dosage forms and result in poor bioavailability[3].

Fenofibrate is a antihyperlipidemic belonging to BCS class II, it is practically insoluble in water (0.3μg/ml at 37°C) and has high lipophilicity (logP 5.3), thus it is evident that the rate limiting step for the absorption of fenofibrate from gastrointestinal tract is the dissolution[4, 5]. The solubility / dissolution of drugs can be improved using various conventional techniques such as micronization, precipitation technology, Salt formation and others like liposome, microemulsion, solid dispersion, and inclusion complexation with cyclodextrin. Various approaches have been reported for solubility improvement of fenofibrate[6, 7, 8]. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanoparticulates offer an efficient method as reduction in particle radius coupled with high energy surfaces contribute to improve saturation solubility[9, 10].

The nanoparticulates of fenofibrate have been reported by methods such as melt emulsification and precipitation[11, 12].

Present work describes the formulation and optimization of nanosuspension of fenofibrate by using a high pressure homogenizer. The formulation and process parameters surfactant concentration, homogenization pressure and homogenization cycle are optimized using Box Behnken Design to obtain lowest particle size[13]. The nanoparticles were evaluated for DSC, PXRD and SEM analysis. These nanoparticles were incorporated into the tablet formulation and subjected to *invitro* dissolution and *exvivo* absorption studies.

MATERIALS AND METHODS

Materials

Fenofibrate was obtained from Medley pharmaceutical Ltd, Research centre, poloxamer 188 (Lutrol F68) was given from

Vishal chem, Mumbai:400 002, Tween 80 was purchased from SD Fine-chem. Pvt. Ltd. Mumbai, SLS (Sodium Lauryl Sulfate)was obtained from LOBA chemicals Mumbai LTD. and all other chemicals were obtained from local sources and were of analytical grade.

Standard Calibration Curve of Fenofibrate

Standard calibration curve of fenofibrate was developed by suitably diluting methanolic stock solution of fenfibrate in 1% SLS solution in distilled water to obtain concentrations between 5 to 30μg/ml. The absorbance of resulting solutions was measured at 290 nm using double beam UV-Visible Spectrophotometer against 1% SLS solution as blank[14].

Determination of drug solubility in the various surfactants

The drug was added in excess amount into 5mL of each surfactant (Tween 80, Tween 60, Tween 20, Span 80, PEG400, PEG 600, and Transcutol) in separate vials and stirred for 24 h. at room temperature on Orbital Shaker. The equilibrated samples were removed from stirrer and centrifuged at 3000 rpm for 15min to remove the excess drug. The supernatant was filtered through a 0.45μm membrane filter. The concentration of drug in supernatant was measured by UV spectrophotometer after appropriate dilution with methanol at 290 nm the drug solubility (mg/mL) was measured spectrophotometrically[15].

Formulation and optimization of nanosuspension

Nanosuspension was prepared by High pressure homogenization technique. Initially coarse suspensions of fenofibrate were formulated by adding solution of fenofibrate in acetone to the solution of surfactant combination (Poloxamer 188 and Tween 80) in different ratios in distilled water. The mixture was then stirred on mechanical stirrer at 5000 rpm for 30 min. The formulated coarse suspension were subjected to high pressure homogenization using (1000 Bar Homogenization Pressure and 9 Homogenization Cycles) Using GEA-Nirosoavi panda plus 2000.

Experimental Design

A Box Behnken Design of 13 experimental runs was used to evaluate three variables at 3 levels viz. Concentration of Tween 80 (0.2%, 0.4%, 0.6%), and the concentration of poloxamer 188 was kept constant. And varying the concentration of tween 80. Homogenization pressure (400, 700, 1000 Bar) and Homogenization cycles (6, 9, 12) in order to determine their effect on two responses particle size, Zeta potential and their interaction therein. The layout of the experimental design is shown in Table 1.

Table 1: Translation of experimental conditions into physical units for preparation of Fenofibrate nanosuspension.

Sr. No.	Factor 1(A): Concentration tween 80 (%)	Factor 2 (B): Homogenization pressure (Bar)	Factor 3 (C): Homogenization cycles (Unit)
-1	0.2	400	6
0	0.4	700	9
+1	0.6	1000	12

Evaluation of Nanosuspension:

Particle size and PDI analysis

The particle size analysis of the prepared Fenofibrate nanosuspension performed using Malvern Zetasizer ZS 90 (Malvern Instruments, Worcestershire, UK), utilizing laser diffraction with beam length 2.40 mm, range lens of 300 RF mm, and at 14.4% obscuration. The sample was diluted in distilled water prior to the analysis. The mean diameter and the poly dispersity index of each batch were recorded. And each sample was analyzed in triplicate.

Zeta potential:

The measurement of zeta potential was carried out using the additional electrode of Malvern Zetasizer that was used for particle size and PDI analysis. A sample of nanosuspension was diluted with distilled water and subjected into disposable sizing cuvette for measurement at temperature of 25°C with setting of dispersant RI at 1.33 and dielectric constant of dispersant at 78.5 in triplicates.

Lyophilization of nanosuspension

The optimized nanosuspension batch was rapidly frozen at -86°C freezer and freeze dried using 2.5 Freezone, Labconco equipments Ltd at a vacuum degree of 200 pas, -30°C for 48 h to yield a dry sample.

Differential Scanning Calorimetry (DSC):

DSC was performed on Mettler-Toledo DSC 823* (Columbus) instrument and an empty standard aluminium pan were used as reference. DSC scans were recorded at heating rate of 10∘C/ min in temperature range 30-300∘C, DSC measurements were carried out on lyophilized fenofibrate nanosuspension.

X-ray diffraction (XRD) study:

X-ray scattering measurements were carried out with an X-ray diffractometer (PW 3710, Philips Ltd). A Cu Ka radiation source was used, and the scanning rate (2h/min) was 5°C per min. X ray diffraction measurements were carried out on unprocessed fenofibrate powder and fenofibrate nanosuspension.

Saturation Solubility Studies:

Nanosuspensions equivalent to 50 mg of Fenofibrate were taken and separately introduced into 25mL stoppered conical flask containing 10mL distilled water. The flasks were sealed and placed in rotary shaker for 24hrs at 37°C and equilibrated for 2days. The samples were collected after the specified time interval and it was filtered and diluted appropriately. The diluted samples were analyzed using UV spectrophotometer at 290 nm.

Scanning Electron Microscopy

SEM was used to verify the uniformity of particle shape and size. The sample was smeared on a small piece of adhesive carbon tape which is fixed on a brass stub. The sample, then s

ubjected to gold coating using sputtering unit for 10sec at 10mA of current. The gold coated sample placed in chamber of SEM and secondary electron/back scattered electron images were recorded.

Formulation of tablet containing Fenofibrate nanoparticles

Fenofibrate nanoparticles, lactose, MCC (Microcrystalline cellulose PH 101), were blended and the powder was moistened using Povidone k30 solution in water was used as a binder. (Table 2) dough was passed through sieve no 10 dried in oven at 50°C. The dried mass was mixed with cross povidone, magnesium stearate and SLS (Sodium Lauryl Sulphate). Compressed using 6 mm punch using B tooling on Rimek tablet machine (Make Karnavati).

Table 2: Formula for tablet containing nanoparticles of fenofibrate

Ingredients	Quantity (mg)
Fenofibrate nanoparticles Equivalent to 45 mg fenofibrate	47
Lactose	32
MCC(PH 101)	10
Cross povidone	5
SLS	2
PVP K 30	3
Magnesium stearte	1
Total	100 mg

***In vitro* dissolution study:**

In vitro dissolution study was carried out using USP dissolution apparatus II.

The rotation speed of the paddles was set to 100 rpm. About 900 mL of 1.0% SDS

at 37 ± 0.5°C was used as the dissolution medium. At predetermined time intervals 5 mL samples were withdrawn, filtered through 0.22 μm membrane immediately, and 5 mL blank dissolution medium was added for replenishing of the dissolution medium, respectively.

The amount of dissolved drug was determined at 290 nm using a UV spectrophotometer.

***Ex-vivo* permeation studies using everted rat intestine**

To understand the absorption mechanism of nanosuspension, everted gut sac studies using rat intestinal segments were performed. Intestine was washed carefully with Krebs ringer solution and different segments of small intestine were identified. A length of 8–10 cm was rapidly removed and gently everted over a glass rod. The everted intestine was then slipped off the glass rod and placed in a flat dish containing Krebs–Henseleit bicarbonate (KHB) buffer oxygenated with O_2/CO_2 (95%/5%) at 37°C. The in vitro absorption system consisted of USP dissolution apparatus II operated at 100 rpm containing 1% SDS (1000 ml) as dissolution medium maintained at 37±0.5°C. Modified perfusion apparatus holding isolated everted intestine segment was placed in dissolution vessel.

In this system, drug dissolution from formulation and permeation across everted intestine occurred simultaneously.

The marketed tablet and tablet containing fenofibrate nanoparticles was transferred in separate dissolution vessels. The aliquotes were collected at predetermined time intervals of 5, 10, 15, 30, 45, 60, 90 min and the equal volumes of dissolution and serosal fluids were replaced. The samples were analyzed spectrophotometrically at 290 nm[17].

RESULTS AND DISCUSION

Standard Calibration Curve of fenofibrate:

The absorption maxima of fenofibrate are reported at 290 nm.[13] A linear relationship between the concentration and absorbance of fenofibrate were established over the examined concentration range (5-30 μg/mL). Calibration curve of fenofibrate was established (Fig 1). The equation of line was y = 0.021x + 0.009 and R^2 = 0.998.

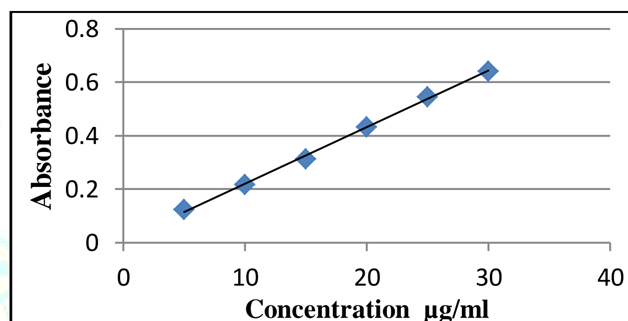

Figure 1: Calibration Curve of fenofibrate in 1% SLS.

Solubility Determination: The solubility of fenofibrate in various surfactants is Given in (Fig 2).

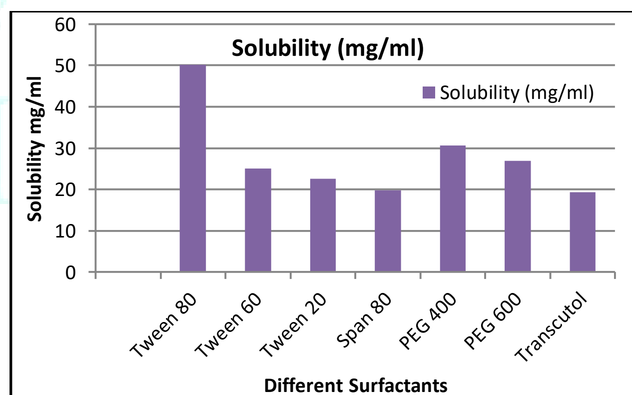

Figure 2: Solubility data of Fenofibrate in various surfactants

Formulation and optimization of nanoparticles:

Table 3: Particle size of nanoparticles at various surfactant Concentrations :

Sr No	Surfactant system	Surfactant Ratio (%)	Particle size(nm)
1	Poloxamer 188: Tween 80	0.2:0.2	750
2	Poloxamer 188: Tween 80	0.2:0.4	420
3	Poloxamer 188: Tween 80	0.2:0.6	300

Tween 80 was selected due to high solubility of fenofibrate (50.10 mg/mL) (Fig 2). While poloxamer 188 was selected as the hydrophobic poly propylene oxide chains can drive the polymer to be adsorbed on the surface of the drug particles, while the hydrophilic PEO chains surround the drug particles and provide a steric hindrance against aggregation. This is evident from particle size in Table 3.

Experimental Design

Table 4: Experimental run and responses for optimization of Fenofibrate nanosuspension formula using Box-Behnken design.(*n=3 i.e. average of three readings)

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A:Concentration of Tween 80 (%)	B:Homogenization Pressure Bar	C:Homogenization Cycles Unit	Particle Size Nm	Zeta potential mV
1	1	0.2	400	9	584	-18.2
9	2	0.4	400	6	452	-21.3
13	3	0.4	700	9	353	-19.2
2	4	06	400	9	338	-24.6
12	5	0.4	1000	12	402	-21.0
4	6	0.6	1000	9	298	-31.2
8	7	0.6	700	12	312	-28.0
6	8	0.6	700	6	332	-29.2
11	9	0.4	400	12	464	-17.1
10	10	0.4	1000	6	442	-18.2
5	11	0.2	700	6	612	-16.0
7	12	0.2	700	12	592	-16.2
3	13	0.2	1000	9	584	-17.0

Determination of Particle Size:

The particle size of prepared nanoparticles ranged between 290-630 nm Table 4. It is evident from the findings that increase in the Stabilizer concentration decreases the particle size. From this we could conclude that the prepared suspension is in nano size. Polydispersity index (PDI) is the measure of size distribution and varies from 0.0 to 1.0. The closer the PDI value to zero, the more homogenous is the nanosuspension, the PDI for prepared nanosuspensions of trial runs were between 0.102-0.302, hence indicating good homogeneity.

Determination of Zeta potential:

Zeta potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). The electric potential at the boundary of the double layer is known as the zeta potential of the particles and is taken as a measure for stability of nanosuspension. Poloxamer 188 and Tween 80 are non-ionic surfactant is used as a stabilizer which provides steric stabilization so negative zeta potential is attributed to nanocrystal. The range of zeta potential was found to be -16 to -31.2 mV.

Optimization Data Analysis:

The statistical model generated for particle size is represented by equation 1

$$\boldsymbol{Particle\ Size} = +353.00 - 136.50A - 14.00B - 8.50C - 10.00AB - 2.50AC - 13.00BC + 60.00A^2 + 38.00B^2 + 49.00C^2 \quad \text{............Equation 1,}$$

Concentration of surfactant (A) is having greater effect on particle size whereas homogenization pressure (B) and homogenization cycles (C) which is having significantly less effect on the particle size. The interaction between A & B i.e. Concentration of Tween 80 and homogenization pressure was more dominant in reducing the particle size compared with that of AC and BC. (Fig 4, 5).

The interaction term AC i.e. the particle size was seen to be decreasing with the increase in the concentration of surfactant but the influence of homogenization cycle on particle size was not so dominant. As tween 80 and poloxamer forms a protective coat around the drug particles thus preventing the aggregation of the particles upon size reduction. Increasing homogenization pressure reduces the particle size, which is due to homogenizing valve under high density fluid dynamic energy conditions. Similar effects were shown in (Fig 3, 4, and 5).

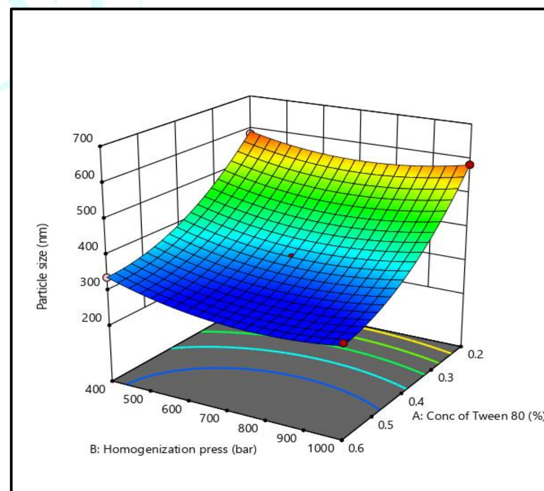

Figure 3: Response surface plot showing influence of Concentration of Tween 80 and Homogenization pressure on Particle size.

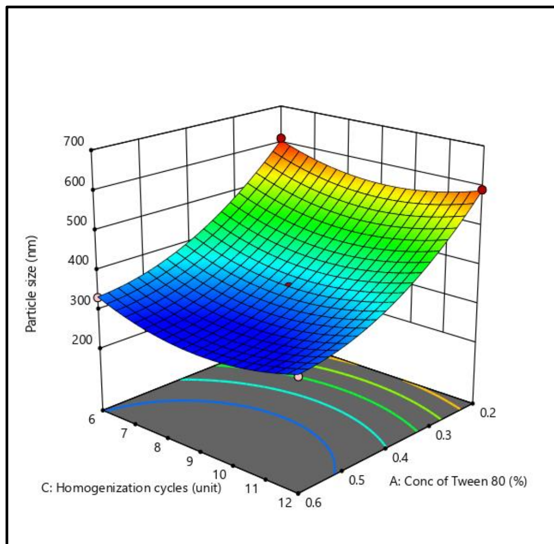

Figure 4: Response surface plot showing influence of Concentration of Tween 80 and Homogenization Cycles on Particle size.

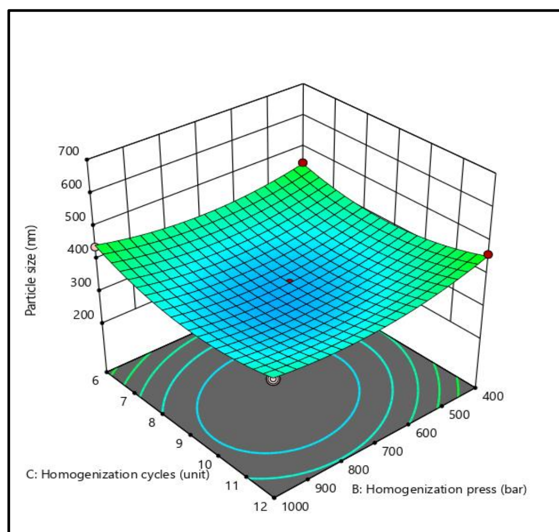

Figure 5: Response surface plot showing influence of Homogenization pressure and Homogenization cycles on Particle size.

Equation for Zeta potential:

$$\boldsymbol{Zeta\ Potential} = -\mathbf{19.20} - \mathbf{5.70}\boldsymbol{A} - \mathbf{0.7750}\boldsymbol{B} + \mathbf{0.3000}\boldsymbol{C} - \mathbf{1.95}\boldsymbol{AB} + \mathbf{0.3500}\boldsymbol{AC} - \mathbf{1.75}\boldsymbol{BC} - \mathbf{3.25}\boldsymbol{A}^2 - \mathbf{0.3000}\boldsymbol{B}^2 + \mathbf{0.1000}\boldsymbol{C}^2$$ .... Equation 2,

The model indicates that as the concentration of Surfactant goes on increasing the Zeta potential .Tween 80 and poloxamer 188 is a non-ionic surfactant which is used as a stabilizer, which provides steric stabilization so the Negative zeta potential attributed to the drug nanocrystal. The interaction term BC and AC did not have dominant effect on Zeta potential as these contributing merely to reduce the particle size. (Fig 7 and 8).

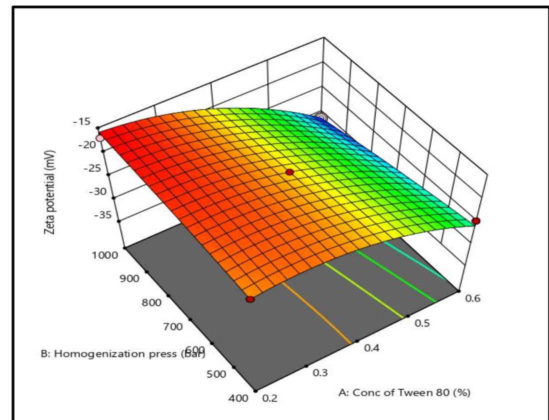

Figure 6: Response surface plot showing influence of Concentration of Tween 80 and Homogenization pressure on Zeta potential.

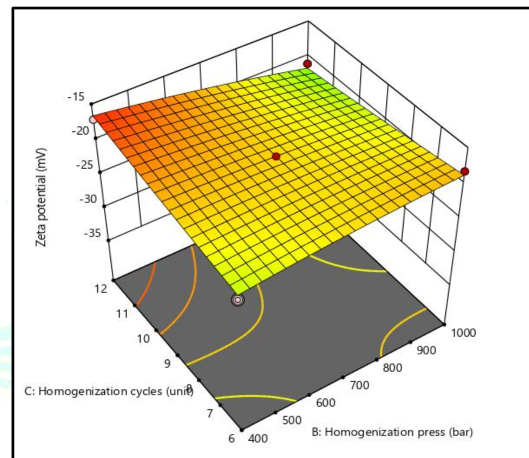

Figure 7: Response surface plot showing influence of Homogenization pressure and Homogenization Cycles on Zeta potential.

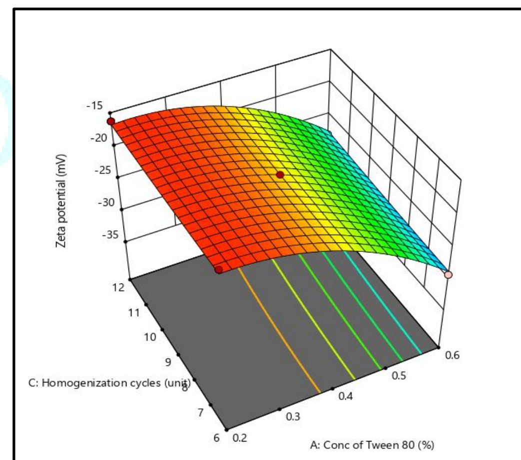

Figure 8: Response surface plot showing influence of Concentration of Tween 80 and Homogenization Cycles on Zeta potential.

The formulations prepared as per the experimental design were evaluated and the analysis of experimental results was done by using Stat-Ease Design Expert. The ANOVA, P-value and Model F-value for particle size and Zeta potential were obtained Table 5.

Table 5: ANOVA output of the Box-Behnken Design for Optimization of Fenofibrate nanosuspension

Sr. No.	Outcomes	Particle size	Zeta potential
1	Models	Quadratic	Quadratic
2	R^2 VALUE	0.9985	0.9846
3	F – VALUE	221.47	21.31
4	P – VALUE	0.0005	0.0144
5	ADEQUATE PRECISION	40.0139	13.3992

F value for both models was found to be high which indicated that the models were significant. P value less than 0.05 indicated that the model terms were significant. Adequate precision indicates signal to noise ratio, its value higher than 4 indicates minimum noise. Higher R^2 value indicated good agreement between formulation variables and response parameters. Thus both models can be used to predict the values of the response parameters at selected values of formulation variables within the design space.

Validation of the Response Surface Methodology (RSM):

Thus, the formulation batch giving minimum particle size and maximum Zeta potential was chosen as the optimized batch based on desirability function (1). Thus, the optimized batch consisted of concentration of tween 80 (0.6%) Homogenization pressure (1000 Bar) and (9) homogenization cycles. To evaluate the findings of the RSM, verification run was carried out and no significant difference was found between the theoretical and the actual values of particle size and Zeta potential is given in Table 6. Thus the model is seen to have good prognostic ability.

Table 6: Check Points for optimization, actual, predicted value and % error (n=3, Mean± SD)

Formulation Code	Composition of optimized formulation			Response	Predicted Value	Actual Value	% Error
	X1	X2	X3				
Optimized batch	0.6%	1000 Bar	9	Y1	294.37 nm	298 nm	1.21
				Y2	-31.16 mV	-31.2 mV	0.769

Evaluation of Nanosuspension

Differential Scanning Calorimetry (DSC)

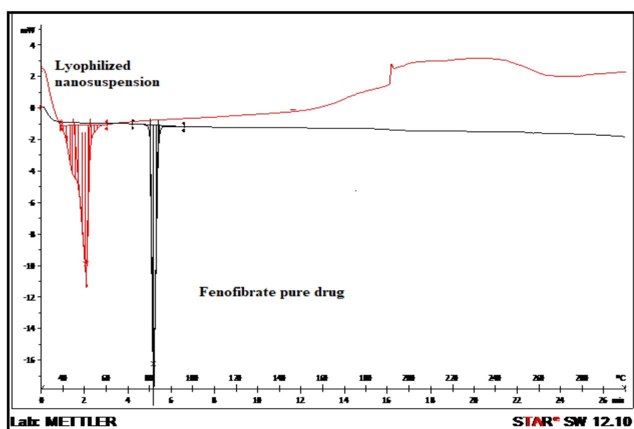

Figure 9: A. DSC Thermogram of Unprocessed Fenofibrate , B. DSC Thermogram of Lyophilized fenofibrate nanosuspension.

DSC thermogram of fenofibrate and lyophilized fenofibrate nanosuspension shown in (Fig 9). The sharp endothermic peak ascribed to the melting point was obtained for the formulation. However slightly lower melting observed for formulation than that of unprocessed fenofibrate is might be due to smaller particle size and subsequent amorphisation[17].

X-ray diffraction (XRD) study

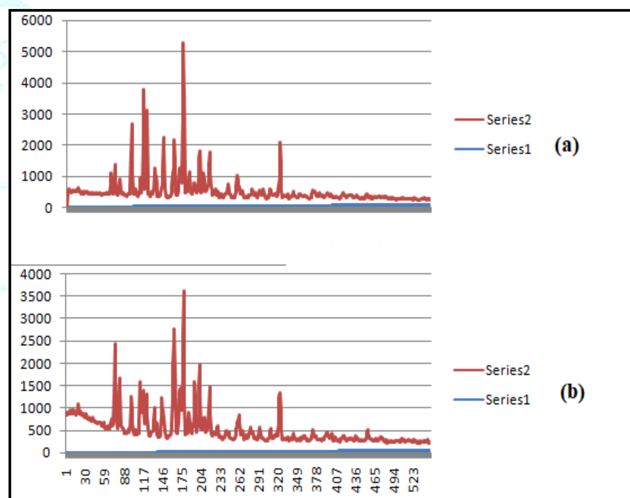

Figure 10: XRD data of a) Unprocessed fenofibrate powder b) Lyophilized fenofibrate nanosuspension.

X- ray diffraction pattern of unprocessed fenofibrate powder and lyophilized fenofibrate nanosuspension are shown in (Fig 10). It was found that no crystalline changes were found in the lyophilized fenofibrate nanosuspension, because their powder X-ray diffraction pattern was consistent with the pattern of raw crystals and spherical crystals. However the differences in the relative intensities of their peaks might be attributed to the differences in the crystallinity of the sample.

Saturation Solubility Studies:

Table 7: Saturation solubility study

Media	Solubility at 37±1°C (mg/ml) [n=3, mean ±S.D]	
	Unprocessed Fenofibrate	Nanosuspension
PH 6.8	2.2±0.12	9.12±0.23
PH 7.4	3.4±0.16	11.34±0.32
Distilled water	1.7±0.11	9.52±0.15

Saturation solubility for unprocessed fenofibrate and lyophilized nanosuspension is given in Table 7. Approximately 4 times increase in saturation solubility of lyophilized nanosuspension was observed than that of unprocessed drug.

The explanation for increase in saturation solubility can be given with Ostwald-Freundlich's equation which is described as follows.

$$\boldsymbol{S} = \boldsymbol{S}\infty\ \mathbf{exp}\left(\frac{\mathbf{2'\Upsilon M}}{\mathbf{2\rho RT}}\right)$$

Where *S* is the saturation solubility,

S∞ is the solubility of the solid consisting of infinitely large particles,

γ is the interfacial tension of substance,

M is the compound molecular weight,

R is the gas constant,

T is the absolute temperature,

Þ is the density of the solid and

r is the radius.

This equation is significant below 1 µm. This makes nanosizing is more efficient than

Micronization. Another possible explanation for the increased saturation solubility is the creation of high-energy surfaces when disrupting the more or less ideal drug microcrystal's to nanoparticles. Lyophobic surfaces from the inside of the crystal are exposed to the aqueous dispersion medium during nanosizing. According to Ostwal-Freundlich's equation, *S* is dependent on the interfacial tension γ and subsequently on the interfacial energy *G* (G=γA). Differences in interfacial energy have a profound effect on the saturation solubilities of polymorphic forms of the drug; the same explanation might be valid for the nanosuspension (high energy form = polymorph II = higher *S*) compared to microparticulate suspensions (low energy form = stable polymorph I = lower *S*

Scanning Electron Microscopy:

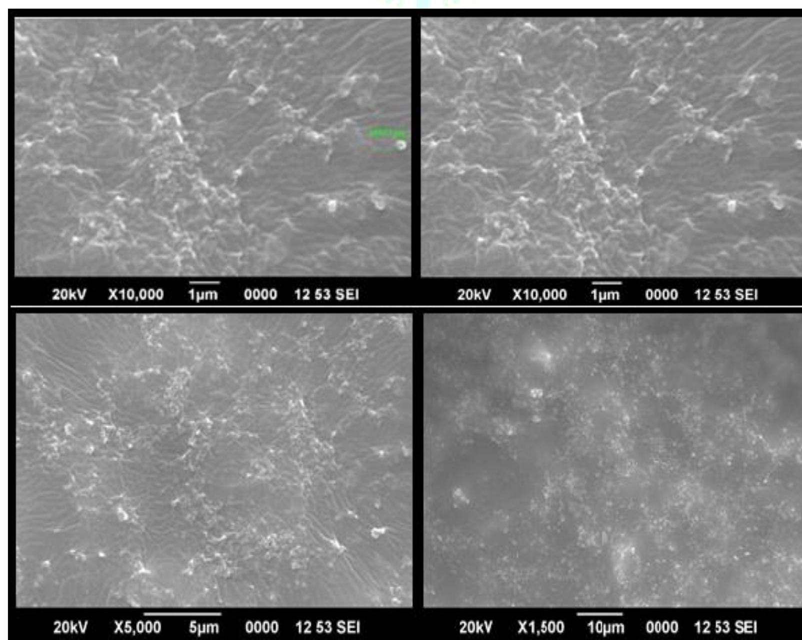

Figure 11: SEM photomicrograph of Fenofibrate nanosuspension

Morphology of precipitated drug particles in the suspension after air drying followed by oven-drying is shown in (Fig 11). The drug particles precipitated with the Poloxamer188 as stabilizer were oval in shape and the size ranges from 290 nm. The particles were discrete and uniform in size and there was no sign of agglomerations.

***In-Vitro* Drug release and *Ex vivo* Permeation study:** [17]

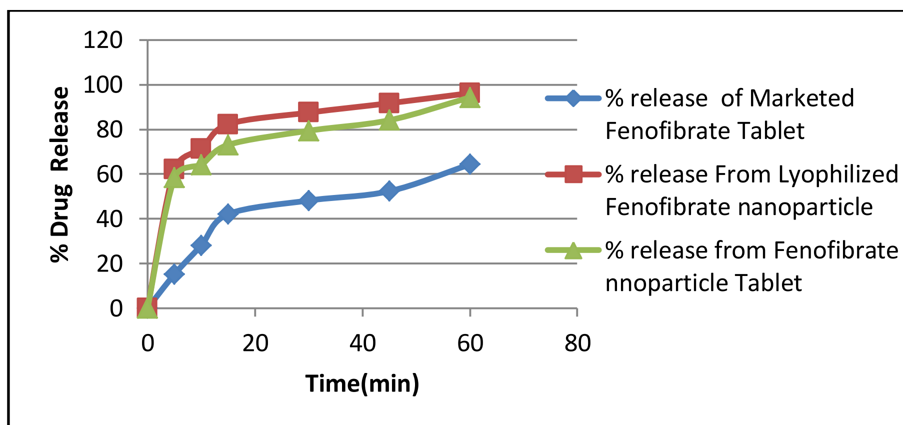

Figure 12: *In vitro* drug release from fenofibrate nanopartiulates, tablet containing nanoparticulates and conventional tablet.

***Ex-vivo* permeation studies using everted rat intestine:**

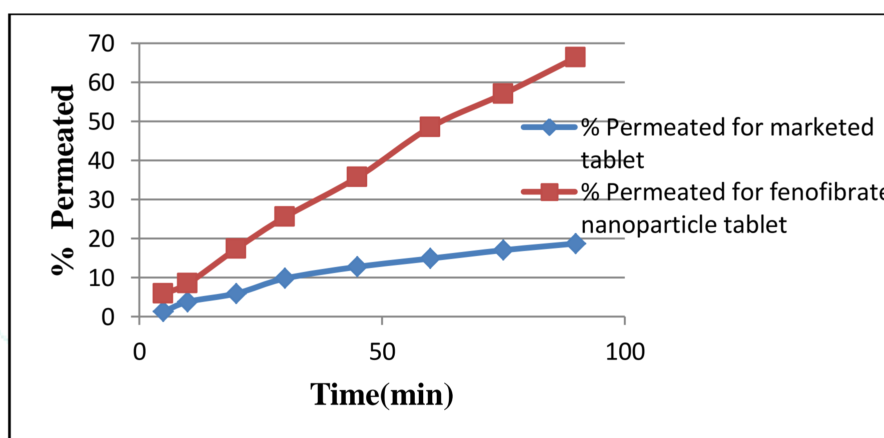

Figure 13: Ex-Vivo Permeation Study

Table 8: Comparison of *In-vitro* % cumulative release against *Ex-vivo* % permeation

Time(min)	*invitro* % cumulative Release		*Exvivo* % cumulative Permeated	
	Marketed Tablet	Fenofibrate nanoparticles incorporate in tablet	Marketed Tablet	Fenofibrate nanoparticles incorporate in tablet
0	0	0	0	0
5	15.23	58.3	1.27	5.95
10	28.2	64.2	3.82	8.91
15	42.1	73.1	5.95	17.4
30	48.21	79.9	9.78	25.5
45	52.36	84.2	12.76	35.74
60	64.3	94.7	14.89	48.51
75	-	-	17.02	57.02
90	-	-	18.72	66.38

Marketed fenofibrate tablet shows 64.3% release within 60 min. While Freeze dried fenofibrate nanoparticles shows 96.2% release within 60 min. And these freeze dried nanoparticles incorporate into a tablet dosage form that shows the 94.7% release within 60 min. Marketed fenofibrate tablet showed 18.72% absorption with absorption rate 0.122 while Fenofibrate nanoparticle tablet showed 66.38% absorption with absorption rate of 0.234. Increase in the absorption rate might be due to the increase in solubility and dissolution rate. Increase in dissolution rate results into increased concentration gradient which cause increase in flux.

CONCLUSION

High pressure homogenization method can be successfully employed to produce a stable fenofibrate nanosuspension. By this method showed significant improvement in aqueous solubility as well as dissolution characteristics which may improve its oral bioavailability.

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