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Formulation and evaluation of nanosuspension for enhancing the solubility of poorly soluble Antihyperlipidemic Drugs

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Abstract
Gemfibrozil is a lipid regulating agent that decreases serum triglycerides and very low density lipoprotein cholesterol and increases high-density lipoprotein (HDL) cholesterol. According to the Biopharmaceutical Classification System, GEM is classified under class-II drugs.Class-II drugs are the drugs with poor solubility and high permeation in the human body and pose problems in their pharmaceutical product development process. The aim of this work is to prepare GEM nanosuspensions using a precipitation ultrasonication method to increase its water solubility. The prepared nanosuspension was evaluated for Percent transmitance and in vitro dissolution. A Box behnken design was employed to study the effect of the independent variables i.e drug concentration in organic phase (mg/ml) at levels 20, 50 and 80 mg/ml (X1), Polyvinyl alcohol concentration at 0.1, 0.3 and 0.5 % (X2) and Sonication time at levels 10, 20 and 30 minutes (X3) on the dependent variables (i.e., percentage of drug released after 90min).The resulting data were fitted into Design Expert software and analysed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of concentration of Drug , PVA concentration and sonication time on dependent variable.The results show that nanosuspensions prepared with the higher concentrations of drug , the higher quantities of PVA and higher sonication time reduced the particle size and enhanced the dissolution rate of the formulation. The dissolution rate of the optimized nanosuspension Formulation was enhanced (96.2% in 90 min) mainly because of the formation of nanosized particles. The particle size of optimized nanosuspension was 191.0 nm and zeta potential was -12mV which is enough for sufficient electrostatic stabilization of Gemfibrozil nanosuspension. The X-ray powder diffraction and differential scanning calorimetry results indicated that the amorphization of gemfibrozil ra crystal and convert into nanocrystalline form and presence of drug. Conclusively, nanosuspension of gemfibrozil prepared using precipitation ultrasonication method showed improved solubility as compare with pure gemfibrozil.

Keywords: Gemfibrozil, Nanosuspension, Precipitation-ultrasonication method, particle size, Solubility, bioavailability.

1. Introduction

Oral delivery of drug entities is often limited due to poor drug solubility and lower

bioavailability. More than 40 % of newly discovered drugs are poorly soluble in water . As solubility plays a crucial role in drug formulation, poor drug solubility has limited the commercialization of many drugs. Extensive efforts have been made to enhance the solubility of drugs by conventional methods, such as, micronisation, use of surfactants and solubilizers, co-solvency, co-crystallization, solid dispersions, self microemulsifying drug delivery systems, complexation, polymorphism etc. One of the most popular approaches being investigated presently is formulation of nanocrystals or nanosuspension. During formulation of nanosuspension, a drug is reformulated and as per FDA is considered as a new drug that can be patented and is not considered as generic .

Considering the limitations of alternative approaches like, lack of universal applicability to all drugs as in inclusion complexes and microemulsion (Patel et al), nanoparticle engineering remains as a preferable choice for pharmaceutical application and may serve as an effective tool for "brick dust candidates"(singare et al). Nanosuspensions are formulated by two major approaches; top down and bottom up technology (34). Top down approach depends on reduction in size of large crystalline particles to the desired size range. Bottom up approach involves solubilization of drug in solvent and further addition of it to a nonsolvent to obtain precipitated nanocrystals under controlled conditions in presence of stabilizer(35). These technologies have been used to increase the solubility and bioavailability of simvastatin , carvedilol, nitrendipine, efavirenz etc.

Gemfibrozil is a widely used antihyperlipidemic agent classified as fibric acid derivative. It increases the activity of extrahepatic lipoprotein lipase, resulting in lipolysis process. Gemfibrozil activates peroxisome proliferator-activated receptor-alpha transcriptor factor ligand, a receptor that is involved in metabolism of carbohydrates and fats and also in adipose tissue distribution. This results in increased synthesis of lipoprotein lipase thereby increasing the clearance of triglyceride. Gemfibrozil belongs to BCS class II (log P 3.6) with poor solubility and high permeability resulting in variable bioavailability. Poor dissolution rate of Gemfibrozil is responsible for its limited and variable bioavailability.

In the present study, an attempt has been made to formulate and evaluate the nanosuspension of gemfibrozil using nanoprecipitation-ultrasonication method for improved solubility. gemfibrozil nanosuspension was evaluated for particle size, drug content, drug release, zeta potential, morphology, solubility and in vitro drug release. Nanosuspension was characterized using Fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffractometry.

2. Material and Method:

Gemfibrozil was obtained from Aurbindo Lab, Hyderabad as gift sample. Poloxomer 188 was received from BASF Mumbai. Tween 20 & Polyvinyl alcohol were obtained from Lobachemie, Mumbai. All other solvents used were of Analytical grade.

2.1. Preparation of Nanosuspension:

2.1.1 Formulation and optimization of Gemfibrozil Nanosuspension:

Gemfibrozil nanosuspensions were prepared through the antisolvent precipitation–ultrasonication method. Briefly, Gemfibrozil was dissolved completely in ethanol to prepare the organic phase and the solution was then filtered through a 0.45-μm filtered to remove the precipitated impurities. The antisolvent phase was prepared separately by dispersing stabilizer polyvinyl Alcohol in distilled water. At a fixed temperature, 2 ml of organic solution was injected drop wise by syringe into 20ml of anti-solvent using mechanical stirrer (Remi125,51D, Mumbai) at 3600 rpm for 1 h. The resultant nanosuspension samples were ultrasonicated with an probe sonicator (Pci analytics,250, Mumbai) 20–25 kHz for the specified period. During the ultrasonication, the temperature was controlled at 4–8°C using an ice–water bath(10).

2.2 Experimental Design: Box behnken Designs:

2.2.1. Optimization of nanosuspension by Box- Behnken Design :

3^3 randomized response surface Box-Behnken design was used with 17 trials runs to study the impact of three factors on the key response variable**.** Box- Behnken design are simplest three level

designs with three factors each at three levels. The three levels are usually referred as low, intermediate and high levels. These levels are numerically expressed by the digits -1, 0, and +1. One variable was (X1) drug concentration in organic phase (mg/ml) at levels 20, 50 and 80mg/ml. Second variable was (X2) polyvinyl alcohol concentration at 0.1,0.3 & 0.5% and third variable was (X3) Sonication time10,20 & 30 minutes .Total 17 batches of nanosuspension were prepared. The resulting data were fitted into design Expert software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3- D response surface methodology to determin the influence of concentration of drug , PVA and sonication time on dependent variable. Table.1 shows the data for evaluation of Gemfibrozil nanosuspension[7].

Table 1 . : Optimization of formulation Variable

Formulations	Drug concentration in organic phase(mg/ml)	PVA concentration (% w/v)	Sonicattion Time
NS1	20	0.1	20
NS2	80	0.1	20
NS3	20	0.5	20
NS4	80	0.5	20
NS5	20	0.3	10
NS6	80	0.3	10
NS7	20	0.3	30
NS8	80	0.3	30
NS9	50	0.1	10
NS10	50	0.5	10
NS11	50	0.1	30
NS12	50	0.5	30
NS13	50	0.3	20
NS14	50	0.3	20
NS15	50	0.3	20
NS16	50	0.3	20
NS17	50	0.3	20

For each formulation 2 mL of ethanol was used and temperature of phase was maintained at 40c. The prepared nanosuspensions were evaluated for percent transmittance, Particle size, Drug content and percent drug release.

2.2.2. Lyophilisation:

Nanosuspension along with cryoprotectant was frozen at – 20 0 C for 24 h and further lyophilized using LabConco USA model 195(A65412906) to get dry sample. Lyophilized nanosuspension was characterised for its solid state characterisation using IR, DSC, and XRD.

3. Evaluation of Gemfibrozil nanosuspension:

All the Gemfibrozil nanosuspension formulations were evaluated for the following parameters.

3.1 Percent transmittance measurement

In order to determine the physical stability of nanosuspension, the optical transmittance was measured at 600 nm using an UV spectrophotometer.

3.2 Particle Size Analysis

The particle size analysis of formulations was performed using particle size analyzer.An aliquot of nanosuspension was diluted(1 to 5 ml) in deionized water prior to measurements. All the measurements were carried out in triplicate at a temperature of 25 °Cand at a fixed angle of 90° to the incident laser beam. Data was analyzed by windows Z type software (Version 1.90) and values of mean particle size and particle size distribution curve were recorded.

3.3. Zeta potential measurement:

For measurement of zeta potential, Zetasizer (HORIBA, SZ100, Japan) was used. Nanosuspension sample (1ml) was taken and dispersed in double distilled water . To prevent the agglomeration, the dispersed solution was placed for 5 minutes in ultrasonicator bath. Then the sample was taken in the glass cuvette and zeta potential was measured by using zetasizer.

3.4 Fourier transform infrared

Gemfibrozil, physical mixture and dried gem nanosupension were diluted with potassium bromide. The FTIR spectra Gemfibrozil, physical mixture and dried gem nanosupension were recorded using an FTIR spectrometer.

3.5. Differential Scanning Calorimetry:

The physical state of Gemfibrozil in solid nanoparticle was characterized by the differential scanning calorimetry thermogram analysis (PerkinElmer 4000, UK). Sample (approximately 1 mg) analysis was performed in an aluminum pan under Nitrogen Purging with Flow rate of 20ml/min and at Heating Range of 30-3000C with 100C/min of heating rate. The endothermic and exothermic transition was studied using obtained thermograph to determine interaction between gemfibrozil and polymer used for nanosuspension.

3.6. X- Ray Diffraction Studies

X- ray diffraction Study was performed in Advanced X-ray diffractometer (Brucker D 8, India) using Cu K 2α rays with a voltage of 40 kV and a current of 25 mA to estimate effect on crystalline structure of lyophilized nanoparticles. Samples were scanned for 2Ɵ from 10 to 80 °. Diffraction pattern for pure Gemfibrozil, physical mixture and solid nanoparticles were analyzed.

3.7 Drug Content :

For determination of drug content from Nanosuspension, the Nanosuspension was centrifuge for 5-10 minutes . then the supernatant 1ml was remove and dilute upto 10 ml with methanol then filter and take absorbance at 276nm.

3.8 Drug Release Studies

The release of gemfibrozil from pure drug and nanosuspension was performed using the dialysis bag previously soaked in dissolution medium.(mole.wt. cutoff – 11000 Da) with modified USP dissolution apparatus type I (Veego, DT60, Mumbai). The dialysis bag was soaked overnight in dissolution medium before dialysis to ensure thorough wetting of the membrane. Dissolution medium used was 7.5 pH phosphate Buffer. Pure drug and equivalent quantity of nanosuspension was placed in dialysis bag respectively and ends tied to basket rod . The bag was then inserted into the dissolution medium containing 100 ml of 7.5 pH phosphate buffer at 37±0.5° C with stirring speed of 50 rpm for 90 minutes. An aliquot of 5 ml were withdrawn at an interval of 15 min,suitably diluted, filtered and analyzed for the content of Gemfibrozil by UV- spectrophotometer (Shimadzu,1700, Japan) at 276. nm. Aliquot was replaced with equivalent volume of fresh dissolution medium to compensate for the loss due to sampling. The % drug release for pure drug and nanosuspension formulation was calculated and compared for 90 minutes with frequency of 15 minutes.

6.9 . Saturation Solubility Study

The saturation solubility of Pure Gemfibrozil and lyophilized Gemfibrozil nanosuspensions was determined in water and phosphate buffer pH 7.5. Excess amounts of samples were added into 10 ml wateer in a capped vial which was then placed in a controlled temperature shaking water bath at 37°C, leaving them to dissolve for 72 h. Then, samples (1 ml) were withdrawn and centrifuged for 20 min. The obtained supernatant sample was assayed using a UV/Vis spectrophotometer

(UV-1700, Shimadzu,) at 276 nm. The experiment was conducted in triplicate.

4. Result and Discussion:

Optimization of process and formulation Variables

Optimization of Gemfibrozil nanosuspension carried out on the basis of effect of different process and formulation variables like type of solvent, type of surfactant and precipitation temperature on percent transmittance and drug content of resultant nanosuspension formulations were studied. Various formulations were prepared and evaluated for pre- optimization investigation. The result showed that formulation variables significantly had an impact on percent transmittance, percent drug content and short term physical stability of nanosuspension. Effect of various formulation variables on evaluation parameters are shown in table

Table 2 : Preliminary Study of formulation of Gemfibrozil nanosuspension

Batch No	% T	% Drug content	Physical Stability after 24 h (visual observation)
1	81.3	83.5	High precipitation
2	91.7	89.9	Low precipitation
3	49.6	98.23	Opalescent
4	83.4	59.2	High precipitation
5	87.5	90.03	High precipitation
6	78.6	85.6	High precipitation
7	92.5	73.2	Low precipitation
8	86.2	80.6	Low precipitation
9	72.3	95.7	Opalescent
10	83.2	84.5	Low precipitation
11	88.3	82.3	Low precipitation
12	90.4	91.32	Low precipitation
13	78.0	81.6	Low precipitation
14	84.7	79.2	High precipitation
15	79.3	80.36	High precipitation
16	86.3	84.32	High precipitation

Effect of solvent:

Various nanosuspension formulations were prepared using different solvents for drug like Ethanol,Acetone and Acetone:ethanol (1:1) . The formulations with Ethanol as the solvent showed highest drug content as compared to other formulations. The precipitation was observed in formulations with Acetone as solvent. This could be due to formation of nanosuspension with larger particle size as a result of faster evaporation of solvent. The formation of large particles resulted in agglomeration. Thus, for further study, Ethanol was selected as solvent.

Effect of surfactant:

Various surfactants were studied for their effect on formulation of nanosuspension. The formulations were prepared with PVA, Poloxamer 188 and Tween 20 as surfactant.The formulations prepared with tween 20 and poloxamer 188 showed precipitations after 24 hours whereas no precipitation was observed in formulations with PVA. The observed precipitation could be due to low zeta potential values because of steric stabilization by long nonionic chains of tween 20 and Poloxamer 188. The colloidal system with low zeta potential resulted in precipitation or coagulation. Thus, for further study PVA was selected as surfactant. 7, 13)

Effect of precipitation temperature:

In order to study the effect of temperature on nanosuspension, the batch no 17 prepared at two different precipitation temperature, viz. 4±10c and 25±10c. It was observed that at lower temperature drug content of the formulations were higher and precipitation was not observed. At higher temperature the precipitation was observed. This could be due to effect of temperature on particle size in several ways. At higher temperature, the solubility of drug is increased reducing the amount of supersaturation after antisolvent addition. This led to lower rate of nucleation resulting in lower number of crystal nuclei. This could result in crystal overgrowth and formation of large crystals leading to precipitation. Secondly, the evaporation of solvent also plays an important role. Secondly, at higher temperature, the evaporation of solvent was at higher rate leading to formation of large crystals. Hence, considering these results, 4±10C temperature was selected for further study.

4.1 Design of Experiments (DoE)

A 3-factor,3-level Box–Behnken design is used to suitably explore the main,interaction and quadratic terms and construct second order polynomial equation using Design Expert (version
10) This cubic design is characterized by a set of points lying at the mid point of each edge of a multidimensional cube and centre point replicates (n=3) . A design matrix of 17 batches are constructed to generate a non-linear quadratic model equation as –

$$Y = b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X11 + b22X22 + b33 + X32$$

Where Y is the measured response for each factor level combination ;b0 is an intercept; b1 to b33 are regression coefficient figured from the observed experimental values of Y; and X1, X2 and X3 are coded levels of independent variables.

In the present study , for optimization of formulation variables of Gemfibrozil nanosuspension, Box-Behnken design was applied.consisted of three independent variables and 3 levels of each factor. One variable was (X1) drug concentration in organic phase (mg/ml) at levels 20, 50 and 80mg/ml. Second variable was (X2) polyvinyl alcohol concentration at 0.1,0.3 & 0.5% and third variable was (X3) Sonication time10,20 & 30 minutes. Total 17 batches of nanosuspension were prepared and these batches were evaluated for percent drug release after 90 minute (Y1). Table 7.11shows the data for evaluation of Gemfibrozil nanosuspension. The drug content of nanosuspension formulation was found to be in the range of 43.7 % to 96.2%. During optimization, formulations with high drug loading and low precipitation after 24 hrs were considered.

BATCH NO	Drug Conc X1 (mg)	PVA Conc X2 (%)	Sonication Time X3 (Minutes)	Cumulative Drug Release after 90 min (Y1) %	Drug content (%)	Stability after 24 hrs
NS1	-1	-1	0	81.3 ± 3.2	72.2± 1.2	Low precipitation
NS2	1	-1	0	86.2 ±1.0	43.7 ± 2.7	High precipitation
NS3	-1	1	0	89.8 ±2.4	93.5± 0.5	Low precipitation
NS4	1	1	0	96.2 ± 1.9	87.3± 3.1	Opalescent
NS5	-1	0	-1	86.5 ± 2.0	90.7± 4.1	High precipitation
NS6	1	0	-1	85.4 ± 3.6	80.0± 2.6	High precipitation
NS7	-1	0	1	87.2 ±1.9	94.1± 3.8	Low precipitation
NS8	1	0	1	80.1 ±2.6	82.7 ±0.9	Low precipitation

NS9	0	-1	-1	74.2 ± 4.1	63.8± 1.3	High precipitation
NS10	0	1	-1	79.1 ±2.1	76.6± 2.9	High precipitation
NS11	0	1	-1	76.7 ± 3.1	88.8±3.4	Low precipitation
NS12	0	1	1	82.0 ± 2.5	96.2± 1.9	Opalescent
NS13	0	0	0	80.2 ±1.2	87.0± 2.7	Low precipitation
NS14	0	0	0	81.3 ± 2.5	84.2± 1.7	Low precipitation
NS15	0	0	0	80.6 ± 1.5	89.1± 3.3	Low precipitation
NS16	0	0	0	82.3 ± 2.1	87.6± 2.0	Low precipitation
NS17	0	0	0	81.6 ± 2.0	85.9± 3.2	Low precipitation

Mean ±SD(n=3)

The result of dependent variables % drug release after 90 min (Y1) from 17 experiments are shown in Table 7.11 and were used to generate quadratic equation from "Design Expert 10". Mathematical relationship was generated using MLRA for the studied response variables expression (equation 1).

Drug release after hrs (Y1)= 81.26 + 0.39 X1 + 3.58 X2 + 0.084X3 + 0.39X1X2 – 1.53X1X3 + 0.12X2X3 + 6.95X12 + 0.18X22 – 3.38X32 (1)

The significant test for regression coefficients was carried out by applying student t-test. A coefficient is significant if the calculated' value is greater than the critical value of t. If the probe values were greater than 0.05, the coefficients were not considered as significant.The lower value of p (0.042) indicated the significance of applied model.Thus, all the three variables were found to exert significant effect on drug release from nanosuspension.

1. **Influence of drug concentration and PVA concentration on the Drug Release after 90 minutes.**

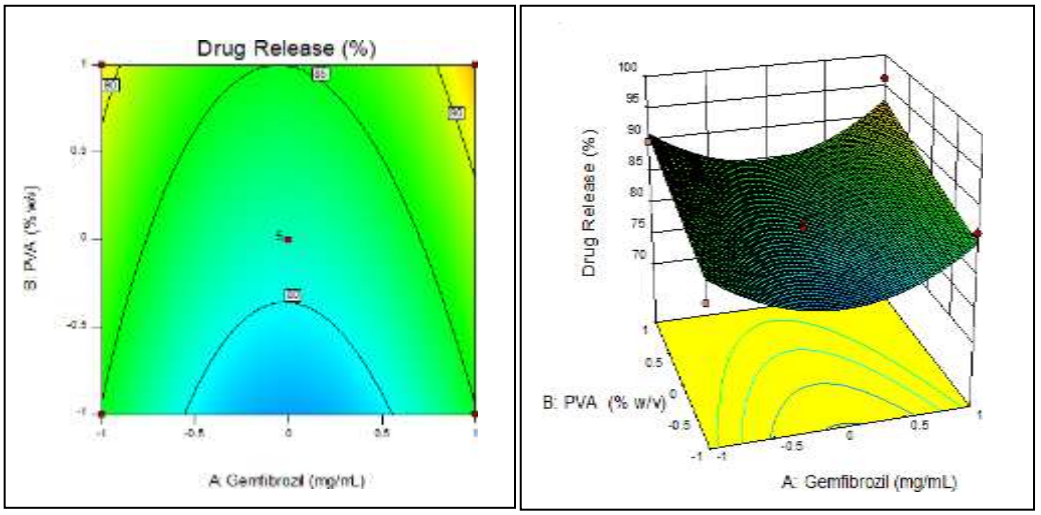


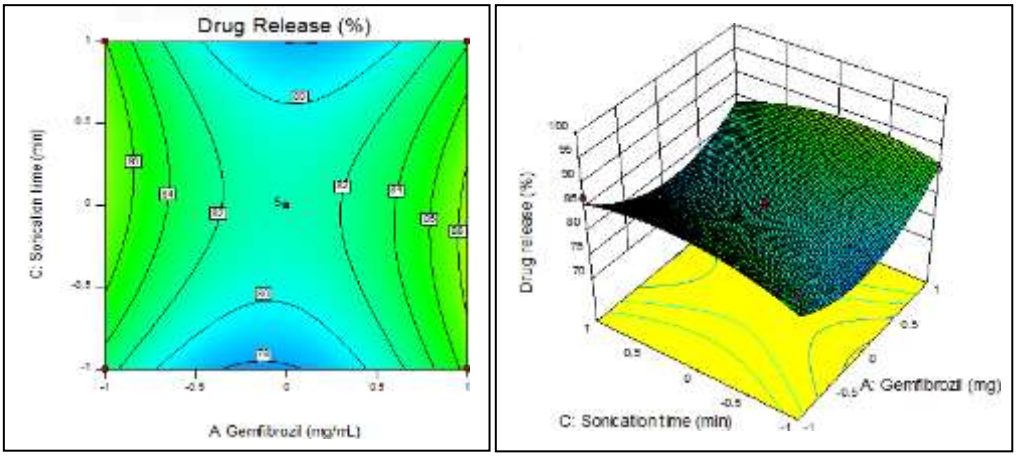
Figure a) Counterplot b)Response surface area showing influence of drug concentration and PVA concentration on the drug release of formulation

To understand the characteristics of drug release from nanosuspension, an in vitro dissolution study was carried out in phosphate buffer pH 7.5 using modified USP tablet dissolution test apparatus I with dialysis bag tied to central shaft.

Fig 7.6 shows effect of PVA concentration and drug concentration on drug release . It was observed that with increase in drug concentration in organic phase from 20 to 50 mg/ml, drug release was found to be decreased. This could be due to increase in particle size with increase in the drug concentration leading to lower surface area and lower drug release. With further increase in drug concentration from 50 to 80 mg/ml drug release was found to be increased. At higher drug concentration the degree of supersaturation is high.This increased the rate of precipitation at faster rate. Drug precipitates out in amorphous form resulting in higher drug release.

Increase in PVA concentration was also found to be increasing the drug release. This effect was pronounced at higher drug concentration. At higher drug concentration when PVA concentration was increased particle size of nanosuspension was decreased leading to increase in drug release.

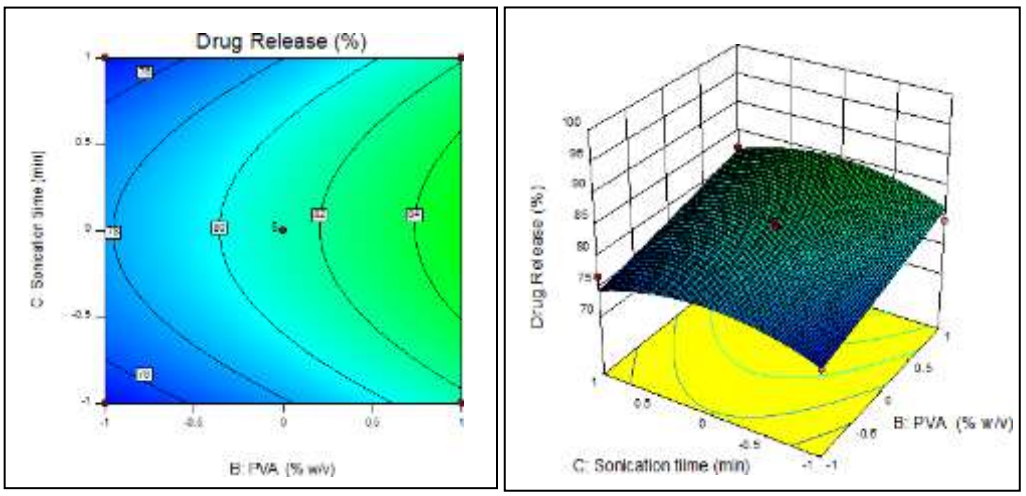
2. **Influence of drug concentration and sonication time on drug release after 90 minutes**



a) Counterplot b)Response surface area showing influence of drug concentration and sonication time on the drug release from formulations.
Change this fig axis should be mg/ml

From figure , it is evident that with increase in drug concentration from 20 to 50 mg/ml at lower sonication time (10 min) drug release was found to be decreased. This could be due to increase in particle size with increase in the drug concentration leading to lower surface area and lower drug release. With further increase in drug concentration from 50 to 80 mg/ml, drug release was found to be increased. With increase in sonication time, drug release from NS was found to be increased. This could be due to decrease in particle size with increased sonication
, that might have led to increased surface area resulting in increased drug release. At higher drug concentration, effect of sonication time was not significant and required lower particle size was not achieved.

3. **Influence of PVA concentration and Sonication time on drug release after 90 minutes**



a) Counterplot b) Response surface area showing influence of PVA concentration and Sonication time on the Drug Release of formulation

From figure , it was observed that with increase in PVA concentration, drug release was found to be increased at all levels of sonication time. At all levels of PVA concentration, increase in sonication time from 10 min to 20 min, increased the drug release, however, with further increase in sonication time to 30 min, decreased the drug release. This could be due to agglomeration of particles due to excessive sonication. Increase in sonication increases the free energy of system drastically, thus resulting in thermodynamic instability in nanosuspension. Cumulative % drug release from nanosuspension formulation is shown in table 7.12 and fig 7.9.

4.3 Particle size determination:

The particle size of nanosuspension formulation was found to be 191.0 nm as shown in table . The lower particle size is suitable as it increases the physical stability of nanosuspension. The lower particle size, increases the surface area of particle leading to increased drug dissolution. The PDI of nanosuspension formulation was found to be 0.28 which indicated narrow particle size distribution. Narrow size distribution is required to reduce Ostwald ripening which otherwise may lead to physical instability and precipitation.

Table 7.14.Particle size, Zeta potential and PDI for optimized formulations.

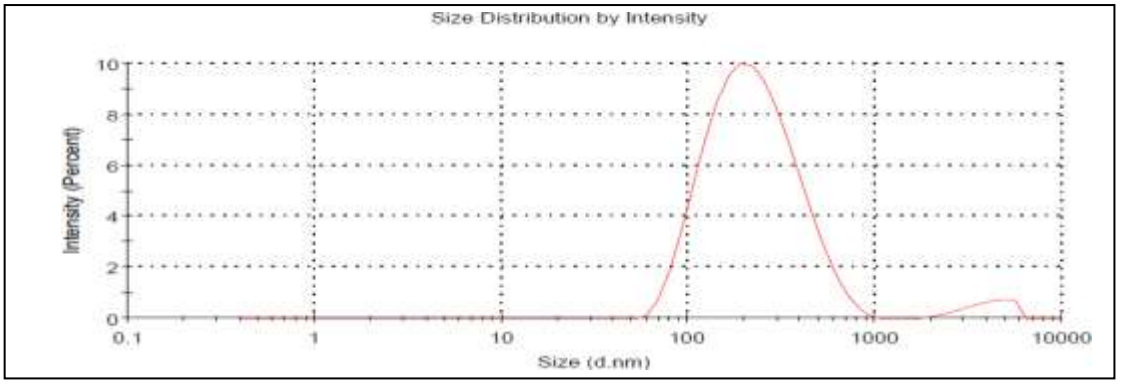


Figure : Particle size analysis graph of NS04 batch

4.4. Zeta potential :

The surface charge on the particle is measured as zeta potential. The stability of colloidal dispersion depends on zeta potential value. Higher zeta potential indicates greater charge on the particle surface resulting in repulsion of particles, reducing the agglomeration or aggregation of particles.Thus physical stability of nanosuspension is indicated by high zeta potential value. The zeta potential of nanosuspension formulation was -12.0 mV,shown in table & fig which was sufficient to keep the particles separate.

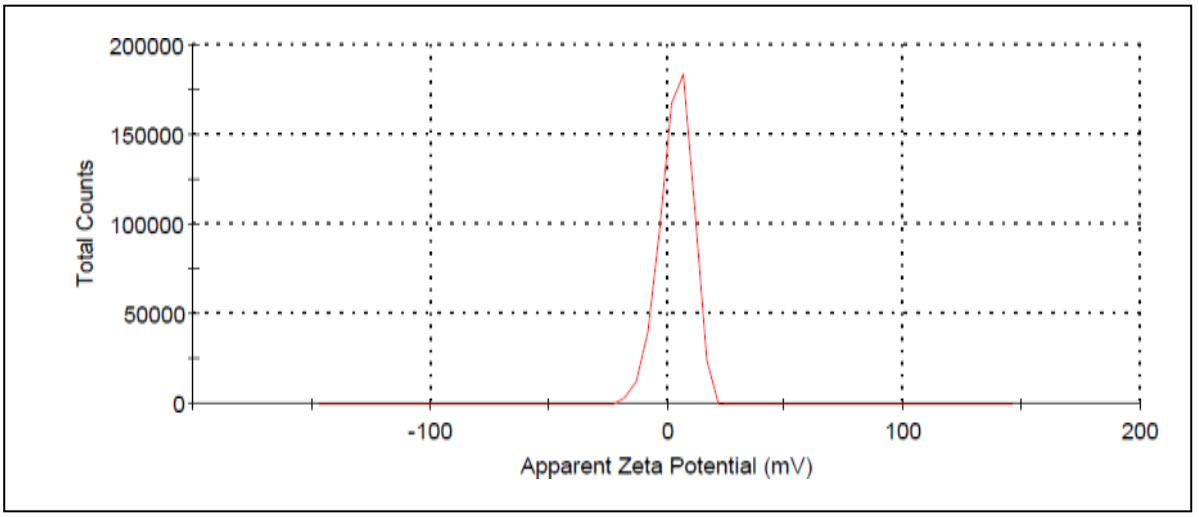


Figure :Zeta potential analysis graph of NS 04 batch

4.5 Infra Red Spectrum

In order to observe any major chemical change after formulation of nanosuspension, FTIR analysis of Gemfibrozil, its physical mixture with PVA as stabilizer and nanosuspension formulation was carried out. The characteristic absorption peak for Gemfibrozil were observed as –C-H stretch at 3045.70 cm-1, -C=O stretch at 1836.28cm-1, -C-O stretch at 1049.31 cm-1 respectively. There was no major shift observed at these wavelengths in IR of physical mixture or nanosuspension. This indicated that no chemical change was occurred in the formulation during nanosuspension formulation.

I) IR spectrum of Gemfibrozil

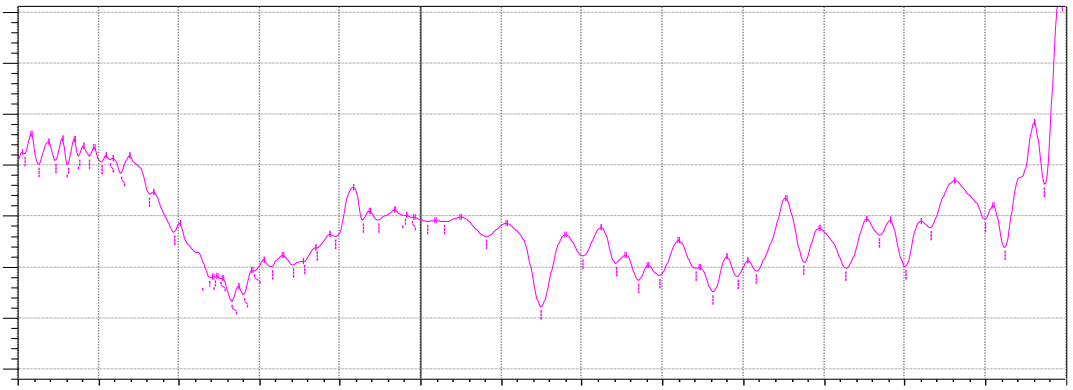


Fig IR spectrum of Gemfibrozil

Table 7.16 Details of FTIR spectrum of Gemfibrozil

Wave number (cm^{-1})	**Functional groups**
3045.70	Aromatic C-H
2991.69	Asymmetric CH_3
2877.89	Symmetric CH_3
1836.29	COOH
1049.31	C-O
798.56	Para substituted Aromatic bending vibration

Fig IR spectrum of optimized NS formulation Table Details of FTIR spectrum of optimized formulation

Wave number (cm^{-1})	**Functional groups**
3045.70	Aromatic -C-H
1703.20	-COOH
1039.67	-C-O Single bond Stretch
808.20	Aromatic –C-H

4.6 Differential Scanning calorimetry

Figure 7.16, 7.17 and 7.18 indicate DSC of Gemfibrozil, its physical mixture with PVA and lyophilized nanosuspension formulation (NS4). DSC studies exhibited a sharp peak at 60.80C

which indicates the melting point of Gemfibrozil. The presence of sharp peak indicates crystalline nature of drug. DSC of physical mixture and Nanosuspension formulation showed an endothermic peak at 221.90 C indicating presence of PVA. DSC of physical mixture retained the endotherm of drug. DSC of formulation indicated a very small peak at 55.4 0 C representing a major change in crystallization of gemfibrozil during nanosuspension formulation. The presence of small and wide endothermic confirms conversion of gemfibrozil to amorphous form.

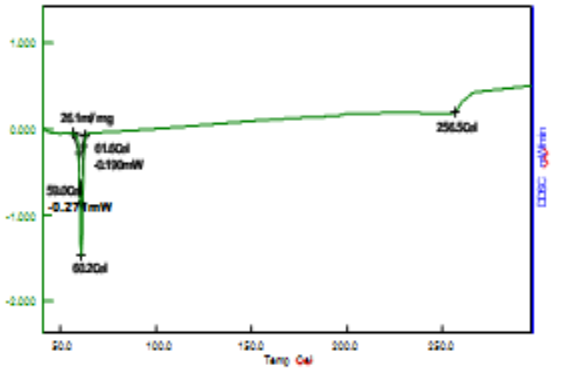


Figure 7.17: DSC of Gemfibrozil

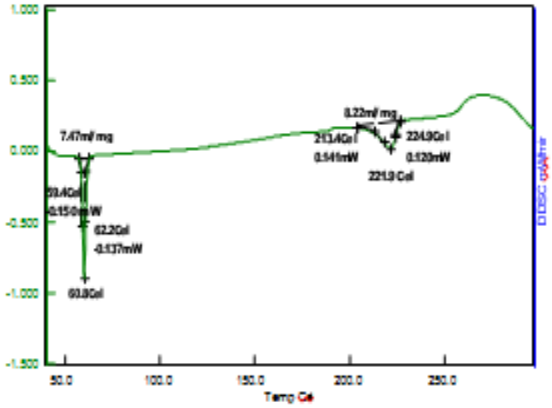


Figure 7.18: DSC of Physical Mixture

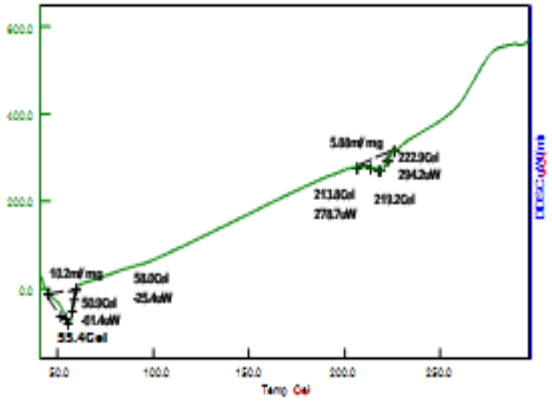
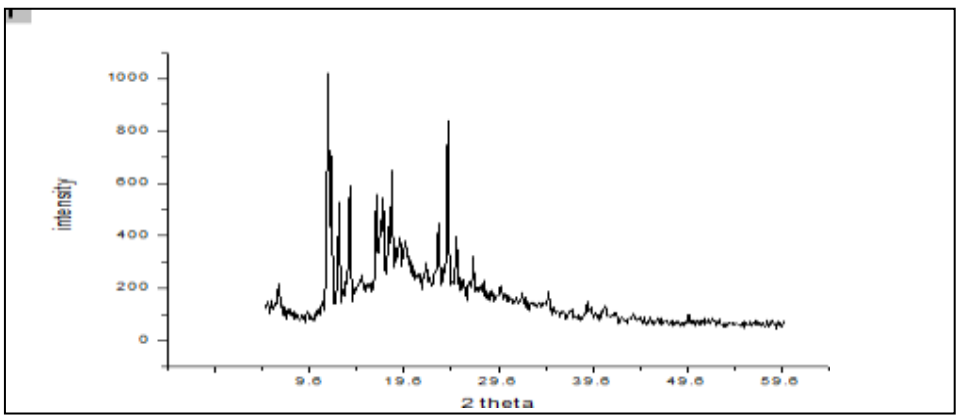


Figure 7.19: DSC of Gemfibrozil Formulation

4.7.X-Ray diffraction Studies

In order to study the changes occurred in crystallinity of drug during formulation of



nanosuspension, XRD study was carried out.Figure 7.19, 7.20, 7.21 indicate XRD pattern of Gemfibrozil, physical mixture of drug , PVA and nanosuspension formulation NS04. XRD pattern of pure Gemfibrozil indicated intense peaks at 11.5, 11.6, 11.9 which were characteristic of pure drug Gemfibrozil. These intense peaks indicated crystalline nature of drug.XRD of physical mixture retained intense peak of Gemfibrozil and also showed additional intense peaks at 18.3, 24.2 and 24.30 representing presence of PVA. XRD of formulation indicated significant reduction in the intensity of Gemfibrozil peaks at 11.5, 11.5, 11.9. This could be due to partial amorphization of drug during process of nanosuspension. As the intensity of peak was not completely masked, there was possibility of presence of drug in nanocrystalline form.

Figure 7.20:X-Ray diffraction of pure Gemfibrozil drug

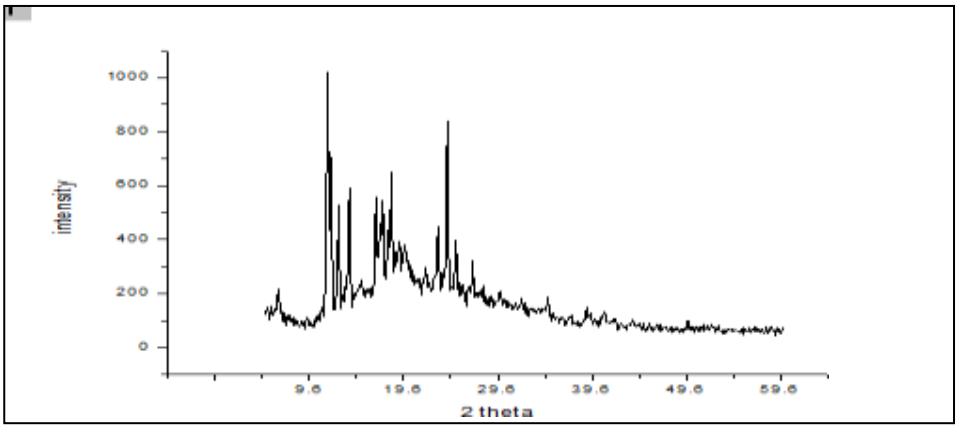


Figure 7.21:X-Ray diffraction of physical mixture

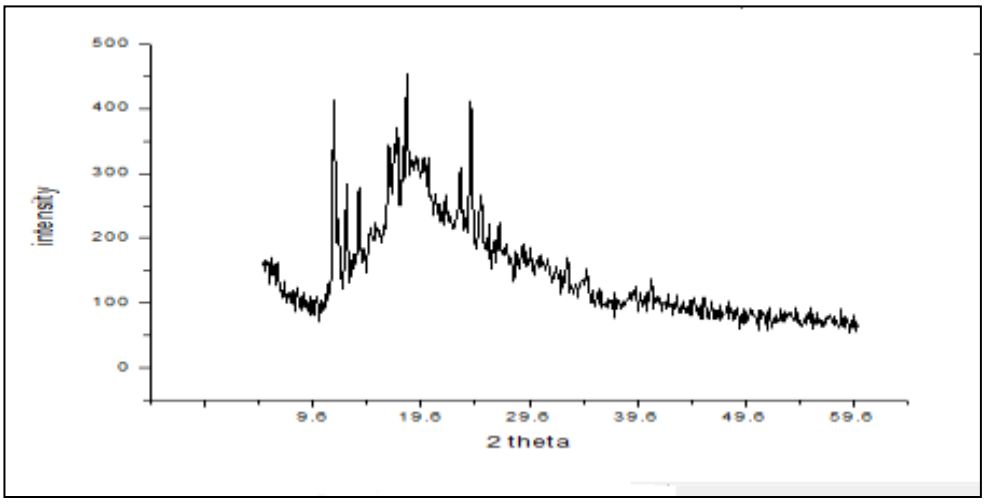


Figure: 7.22:X-Ray diffraction of Nanosuspension formulation

Cumulative percent drug release of Gemfibrozil Nanosuspension

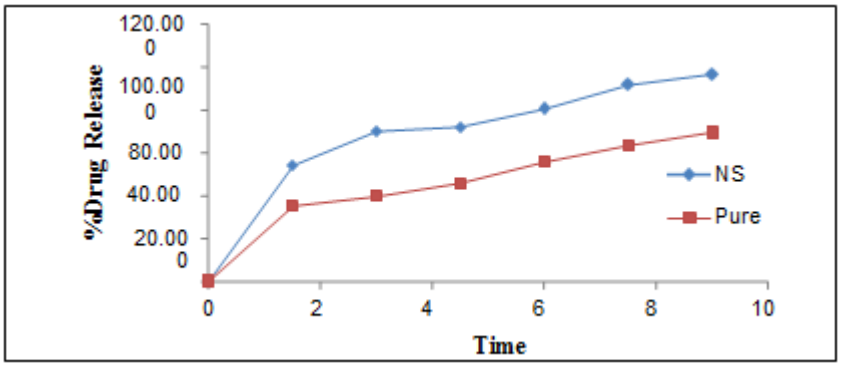
Batch code	Time					
	15	**30**	**45**	**60**	**75**	**90**
NS1	39.23 ± 1.23	59.46 ±2.36	62.76 ±1.36	65.03 ±2.12	71.13 ± 1.96	81.35 ±1.77
NS2	41.21 ±2.91	50.67 ±0.52	55.71 ±2.25	67.18 ±1.66	75.98 ± 2.33	86.2 ±1 .35

NS3	56.58 ±2.42	66.25 ±1.29	68.52 ±0.51	79.73 ±3.42	82.86 ±2.82	89.81 ±2.59
NS4	57.26 ±1.50	64.98 ±2.29	73.68 ±1.50	79.39 ±2.88	89.18 ±0.84	96.2 ±1.87
NS5	53.94 ±1.08	61.82 ±0.78	69.35 ± 0.87	72.47 ± 1.5	74.25 ± 2.29	86.5 ±2.31
NS6	46.57 ± 2.32	56.39 ± 1.36	63.91 ± 2.36	67.95 ± 1.36	72.38 ± 2.37	85.48 ± 1.69
NS7	57.70 ± 2.45	64.98 ± 1.56	72.14 ±2.71	77.9 ± 2.25	82.3 ± 3.62	87.23 ± 1.47
NS8	49.11 ± 4.20	60.23 ± 3.25	61.88 ± 1.69	66.59 ± 2.45	74.18 ± 1.79	80.10 ± 4.12
NS9	35.32 ± 2.60	45.79 ± 1.27	57.19 ± 2.34	64.17 ± 3.65	69.87 ± 1.36	74.29 ± 2.87
NS10	37.80 ± 1.65	45.36 ± 3.24	58.31 ± 0.36	65.98 ±1.87	71.21 ±0.89	79.17 ± 1.58
NS11	24.71 ± 2.69	38.67 ± 3.78	47.32 ± 4.13	56.32 ± 2.71	67.24 ± 1.09	76.72 ± 2.34
NS12	48.95 ± 1.04	58.79 ± 0.56	67.36 ± 1.34	72.42 ± 2.09	79.21 ± 1.72	82.06 ±0.79
NS13	25.39 ± 2.32	34.71 ± 1.54	47.27 ± 2.01	64.36 ± 1.23	73.68 ± 2.08	80.27 ±3.64
NS14	34.81 ± 0.98	49.22 ± 1.87	62.39 ± 2.87	71.28 ± 1.65	76.30 ± 2.05	81.36 ± 3.12
NS15	36.21 ± 0.39	41.08 ± 1.65	52.36 ± 2.65	60.89 ± 1.06	73.39 ± 1.97	80.68 ± 2.98
NS16	31.25	38.36	46.78	67.25	71.02 ±	82.35

	± 1.74	± 2.41	± 1.65	± 2.65	3.21	± 1.03
NS17	29.37 ± 2.34	37.89 ± 1.52	57.69 ± 3.21	64.26 ± 1.25	75.80 ± 4.01	81.63 ± 2.31
Pure Drug	23.41 ±1.7	31.78 ±0.74	40.98 ±2.35	45.57 ±1.08	56.23 ±1.5	62.36 ±2.77

The drug release was found to follow biphasic release pattern, initial release burst release followed by sustained drug release over 90 minute of dissolution study.initial burst release is due to small sized amorphous particles and crystal nuclei. Slow drug release could be due to agglomerated particles found during process.

Drug release patterns for nanosuspension Optimized formulation and Pure gemfibrozil



Drug release from NS 4 formulation was found to be highest, NS 4 formulation released 57.2 % ± 1.50 after 15 minutes of dissolution and 96.2 % ± 1.72 after 90 minute of dissolution. Hence, these formulation was selected as optimized for further study. The drug content and physical stability of these formulation was high. when dissolution profile of pure drug was compared with NS4 formulation fig it was observed that 2.5 fold increase in drug release after 15 minutes of dissolution whereas after 90 minutes of dissolution there was 1.54 fold increase in drug release. These could be due to change in crystallity of drug during NS process.during the NS formulaton, gemfibrozil was dissolved in ethanol and was further precipitate out in antisolvent in process of stabilizer which could have changed the drug from crystalline to amorphous form. These was further confirmed from DSC and XRD study.

4.8 Saturation solubility of optimised formulation and pure drug

The solubility of optimized nanosuspension formulation (NS4) and pure gemfibrozil was carried out in water and in phosphate buffer pH (7.5), result are shown in Table . formulated gemfibrozil nanosuspension showed high solubility in water as compared to pure drug. The increase in solubility was 5 fold and 9 fold in water and phosphate buffer respectively. These was attributes to reduction in particle size and transition to amorphous state of drug during NS formulation.

Table no 7.13 : saturation solubility of Optimized formulation

Sr. No	Solvent System	Solubility of pure drug (µg/ml)	Solubility of NS 04 at 25±1^{0}c (µg/ml)
1	Water	27.8 ± 1.3	139 ± 3.2 µg/ml
2	Phosphate buffer pH 7.5	2.3 ± 0.3	11.3 ± 2.7 µg/ ml

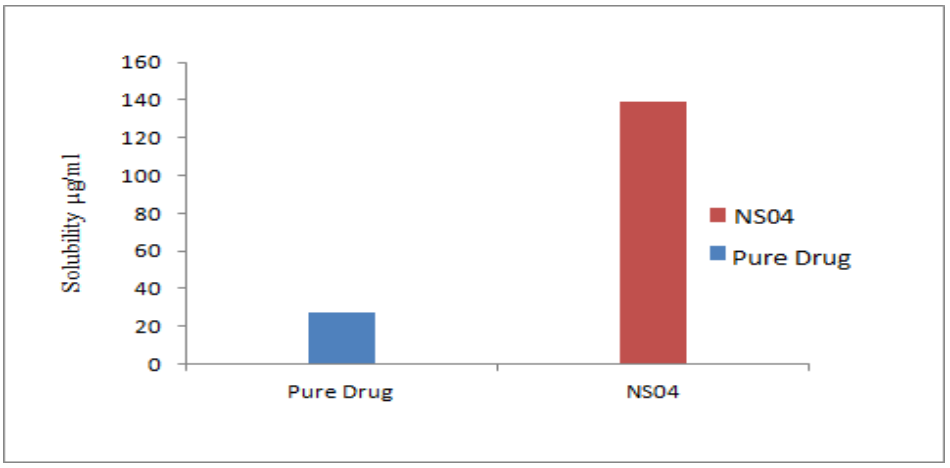


Fig. 7.10 : Saturation Solubility of optimized nanosuspension formulation & Pure gemfibrozil in water

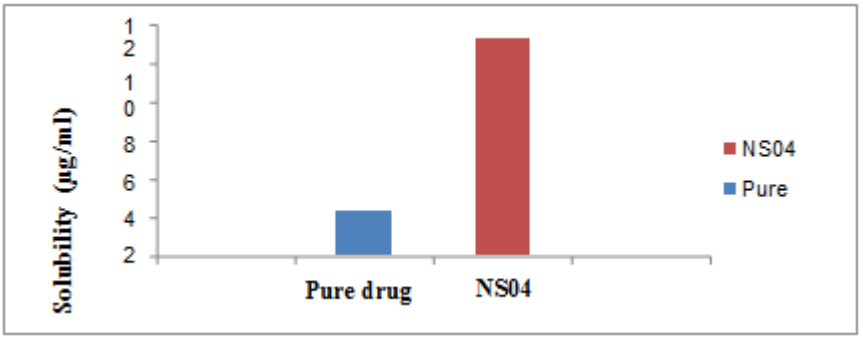


Fig . 7.11: Saturation Solubility of Optimised nanosuspension formulation & Pure gemfibrozil in Phosphate buffer pH 7.5

Conclusion:

An optimized formulation of Gemfibrozil nanosuspension were successfully prepared with particle size less than 200nm with higher zeta potential. Gemfibrozil nanosuspensions exhibited markedly enhanced solubility & dissolution rate compared to gemfibrozil drug.

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