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RESEARCH ARTICLE

FORMULATION AND DEVELOPMENT OF FENOFIBRATE LOADED LIPOSPHERE SYSTEM

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ABSTRACT:

Lipospheres offer a new approach to improve an aqueous solubility of BCS class-II drugs. Fenofibrate(FNO) is a third-generation fibric acid derivative belonging to this class , employed clinically as a hypolipidemic agent to lessen the risk caused by atherosclerosis. An attempt was made to improve aqueous solubility of FNO by aid of stearic acid and Paraffin oil. The factorial batches of the FNO lipospheres were formulated by melt dispersion technique using 3^2 factorial design with variables X1- concentration of stearic acid and X2- concentration of paraffin oil and responses Y1 - % Drug Entrapment (%DE) and Y2 - % Drug Release (% DR). From the surface response graphs the optimized batch was formulated and evaluated for saturation solubility, in-vitro and in-vivo animal studies. Significant improvement in the aqueous solubility of the drug in the FNO lipospheres supports the applicability of lipospheres as a tool for improving aqueous solubility of the BCS class-II drugs.

Keyword: Fenofibrate, Melt dispersion Technique, Liposphere.

INTRODUCTION: [1, 2, 3, 4)]

Various techniques have been employed to formulate oral drug delivery system that would enhance the dissolution profile and in turn, the absorption efficiency of water insoluble drug. Solid dispersion, drug micronisation, lyophilisation, microencapsulation, inclusion of the drug solution or liquid drug into soft gelatin capsules are some of the methods that have been used to enhance dissolution characteristics of water insoluble drugs. Among them, lipospheres are amongst the promising particulate drug delivery systems for improving dissolution rate of water insoluble drugs that were initially reported as a particulate dispersion of solid spherical particles between 0.2-100µm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives, stabilized by monolayer of phospholipids. Lipospheres represent a new type of fat based encapsulation system developed for parenteral and topical delivery of bioactive compounds and have been utilized in the delivery of anti-inflammatory compounds, local anaesthetics; antibiotics, anticancer agents, insect repellent, vaccines, proteins and peptides. The lipospheres are distinct from microspheres of uniformly dispersed material in homogenous polymer since they consist of two layers, the inner solid particle that contains the entrapped drug with phospholipids outer layer. The combination of solid inner core with phospholipid exterior confers several advantages on the lipospheres as compared with conventional microspheres and microparticles, including high dispersibility in aqueous medium, and a release rate for the entrapped substance that is controlled by the phospholipid coating and the carrier. Further, the substance to be delivered does not have to be soluble in the vehicle since it can be dispersed in the solid carrier. Lipospheres have a lower risk of drug –excipient interactions due to the solid nature of vehicle. Moreover, the drug release rate can be manipulated by altering either or both the inner solid vehicle or the outer phospholipid layer. The ease of preparation as compared to liposomes that have inherent problem of stability adds an advantage over other lipid systems. Fenofibrate (FNO), a BCS class-II drug is a third-generation fibric acid derivative employed clinically as a hypolipidemic agent to lessen the risk caused by atherosclerosis.

MATERIALS AND METHODS

Materials:

Fenofibrate was procured as gift sample from Alembic Pharma Gujarat; India. Stearic acid , paraffin oil 80 and rest of the raw materials and solvents were purchased from were purchased from Himedia Lab Pvt. Ltd. and Loba Chemicals Pvt. Ltd. Mumbai India.

Method

Preformulation Studies:

Organoleptic properties of Drug: [5, 6, 7]

The drug FNO was analyzed visually for organoleptic properties, like colour, odour and appearance.

Melting point determination

A small amount (2-4mg) of sample was transferred into a small closed-end capillary tube and gently tapped on the bench top until the sample reached to the bottom of the tube avoiding the firm packing of the material in the end of the tube, and was placed vertically with closed end downward in the holes located in the top of the base of Digital Melting Point Apparatus. Speed of knob was adjusted to medium to avoid the formation of bubbles. The dial was adjusted to the appropriate temperature. The sample temperature was gradually raised simultaneously reaching the bath temperature. The melting point determination was carried out using the periscopic viewer

to help monitor the temperature at which the sample melts. The procedure was repeated thrice.

Solubility profile:

An accurately weighed 100mg of FNO was dissolved separately each in 10ml of distilled water and a mixture of phosphate buffer pH6.8 with 1% SLS in the ratio 9:1 and the solubility was observed visually.

Saturation solubility study

Saturation solubility of FNO was determined following standard approach by stirring an excess amount of drug separately in distilled water ,9:1 mixture PBS pH 6.8and 1%SLS in 100ml separate calibrated volumetric flasks at room temperature (25ºC) for 15 hours. The sample were taken manually and filtered through 0.45µm filters and the absorbance was determined by UV-visible spectrophotometer (UV/VIS) spectrophotometer (V-630, Jasco Corporation Tokyo, Japan), at 280 and 290nm respectively for both the solvent system. The procedure was repeated until the maximum concentration of the drug was achieved till the constant concentration in both the solvent.

Calibration curve

Phosphate buffer pH6.8 (PBSpH6.8):

An accurately weighed (100mg) of FNO was dissolved in 100 ml solution containing 90ml PBS pH 6.8 and 10ml of 1% SLS in 100ml calibrated volumetric flask. The dilutions of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg/ml were prepared. Absorbance of each solution was measured at 290nm in triplicate by taking PBSpH6.8 and 1% SLS (9:1) as a reference standard.

Fourier Transform Infrared Spectrophotometer (FTIR):

The IR absorption spectra of FNO were recorded by potassium bromide dispersion technique. Dry sample of drugs and potassium bromide were mixed uniformly and filled into the die cavity of sample holder and an IR spectrum was recorded separately using FTIR spectrophotometer (JASCO– FT/IR- 4100) in the range 500-4000. The resultant spectrum of the FNO was compared with reference spectrum of FNO respectively.

Differential Scanning Calorimetry(DSC):

The thermal behavior of FNO, were studied using Shimadzu D.S.C TA60 WS Thermal Analyzer. Accurately weighed samples of FNO (8.00mg), was run at the scanning rate of 15°C/min and 20˚C/min over a temperature range of 100°C to 300°C respectively. The system was calibrated with a high purity sample of Indium. Peak transitions and enthalpy of fusion were determined for the samples using TA 60 integration software

Drug excipients compatibility study:

The accurately weighed one gm FNO and excipients in the ratio of 1:1 were subjected to storage at room temperature and elevated temperature of 40^0C for one month in sealed amber colored twelve vials. Sampling was performed at a predetermined time intervals of 7, 14, 21 and 30days. The samples were analyzed for any interaction between drug and excipient by FTIR studies.

Preparation of Liposphere by Melt Dispersion Technique: [8]

A mixture of paraffin oil and stearic acid was melted to obtain a one phase melt on thermostate hot plate (2 MLH, Remi Equipment Ltd.India). FNO was dispersed in the molten solution and the temperature of the resulting oil phase was maintained at 70°C. The surfactant solution comprising 1%(v/v) of Tween 80 in water was maintained at a temperature of 80°C under continuous stirring using propeller stirrer (RQ 121 D Remi Equipment Ltd.). The molten oily phase was emulsified in the aqueous surfactant solution maintained at 28000rpm on ultra turrax (IKA® T25). Hardening of the oily internal phase resulting in encapsulation of the drug was accomplished by pouring twice the emulsion volume of ice cold water maintained at 4°C. The resulted lipospheres were separated by filtration, washed with ice cold water and dried at room temperature (24°C) for 24hours.

EVALUATION OF LIPOSPHERES

Bulk Characterization:

Angle of Repose

The angle of repose for the lipospheres of each batch was determined by the funnel method. Accurately weighed 10gm of lipospheres were allowed to flow out of the funnel orifice fixed at a height of 2cm from the surface on a plane paper kept on the horizontal platform. The gradual addition of the lipospheres from the funnel mouth formed a pile of granules on the surface this was continued until the pile touches the stem tip of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured. Angle of repose was calculated by using the following formula:

$$\theta = \tan^{-1}\frac{h}{r} \qquad (1)$$

Where,

Θ = angle of repose

h = height of the pile

r = Average radius of the powder cone

Bulk Density

Accurately weighed 10gm of lipospheres were placed into a 50ml cylinder of the bulk densitometer (CL100). The volume occupied by the sample was recorded. The bulk density was calculated as following formula:

$$\text{Bulk Density} = \frac{\text{Weight of Sample in gm}}{\text{Volume Occupied by Sample}} \qquad (2)$$

Tapped Density

Accurately weighed 10gm of lipospheres were poured gently through a glass funnel into a cylinder of bulk densitometer (CL100). The cylinder was tapped from a height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated as follows by formula.

$$\text{Tapped Density} = \frac{\text{Weigh t of Sample in gm}}{\text{Volume Occupied by Sample after tapping}} \qquad (3)$$

Carr's Index (CI)

One of the important measures that can be obtained from bulk and tapped density determinations is the percent compressibility or the Carr's index, I, which is determined by the following formula

$$\text{Carr's Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \quad (4)$$

% Yield (%Y)(9)

Dried lipospheres were accurately weighed, and considering the total amount of drug and excipient used for preparing the feed solution, the % yield of liposphere was calculated using the following formula

$$\% \text{ Yield} = \frac{\text{Total Weight of Liposphere}}{\text{Total Solid Material Amount used before Emulsification}} \times 100 \quad (5)$$

% Drug Entrapment (%DE)[9]

Accurately weighed 100mg of Lipospheres were dissolved in 100ml of PBSpH6.8.The solution was kept overnight and was filtered through whatman filter 0.45µm. The drug concentration was determined by UV spectrometer at maximum wavelength of 290nm. The following equation was used to calculate % drug entrapment:

$$\%\text{Drug Entrapment} = \frac{\text{Calculated Drug Content}}{\text{Therotical Drug Content}} \times 100 \quad (6)$$

Morphology [10]

The morphology of the optimized batch of lipospheres was investigated using Scanning Electron Microscopy (SEM). The samples were prepared by sprinkling the formulation on double-adhesive tape stuck to aluminum stub. The stub was placed in high-vacuum evaporator. The samples were then randomly scanned and photomicrographs were taken with a Scanning Electron Microscope (Joel JSM-630A).

DSC

The thermal behavior of the samples were determined with a DSC-823[e], Mettler Toledo, cell using aluminum crucibles with about 2mg of the lipospheres, under dynamic N_2 atmosphere (40ml min−1) at a heating rate of 10 °C min^{-1} in the temperature range of 25 to 300°C. The DSC cell was calibrated with indium (mp 156.6°C; ΔH_{fuss} =28.54 J g−1) and zinc (mp 419.6°C).

In-vitro cumulative % drug release study [11]

The FNO release from the lipospheres was evaluated by using the US Pharmacopoeia Dissolution Apparatus-II Paddle (XVIII) in 900ml mixture of PBSpH6.8 with 1% SLS in 9:1 at 37°C ± 0.5 temperature. The rotational speed of dissolution apparatus was maintained at 100rpm. Each run was carried out in triplicates. Accurately weighed 243mg of lipospheres were filled in a "0" size capsule to get the final weight of 475mg each of the capsules was transformed into dissolution media. The 5ml samples were withdrawn at predetermined time intervals with dissolution media replacement and were filtered through 0.45µm whatman filter paper. The drug content was determined spectrophotometrically at 290nm on Jasco-630 UV/ Vis-spectrophotometer.

Preparation of optimized batch Q1

The optimized batch Q1was formulated using the predicted values of the concentration of stearic acid and paraffin oil from the surface response graph of xi,x2 for reponses y1() , y2 () obtained from software design expert version 8.

Evaluation of optimized batch Q1

Saturation Solubility:

Saturation solubility of the optimized batch Q1was determined by stirring excess amount of samples of lipospheres , physical mixture of drug, stearic acid and paraffin oil in the ratio of 1:1:0.2 in distilled water and 9:1 mixture of PBS pH 6.8 with 1%SLS in 100ml separate calibrated volumetric flasks at room temperature (25ºC) for 15 hours. The samples were taken manually and filtered through 0.45µm filters and the absorbance was noted at 290nm UV-visible spectrophotometer (UV/VIS) spectrophotometer (V-630, Jasco Corporation Tokyo, Japan). The procedure was repeated until the maximum concentration of the drug was achieved till the constant concentration in both the solvent.

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In vivo study:

The in vivo study was performed on normal healthy Wistar albino rats. The animal experiments were conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations, per the spirit of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International's expectations for animal care and use/ethics committees. The approval (CPCSEA/IAEC02/02/2012) of the Institutional Animal Ethics Committee was obtained before starting the study.

Animals

Two groups of male Wistar albino rats weighing 150 to 200g each (4 in each group) under fasting condition prior to experiment were used for the study.

Induction of Hyperlipidemia [9]

Hypercholesterolemia was induced by adding 500mg/kg cholesterol powder, 250mg/kg of cholic acid and 10ml/kg of groundnut oil to the food. The animals were divided into control and experimental group. Two groups each of three rats each were fed on the following diet for 2 weeks. Blood samples were collected after 2 weeks and blood glucose levels were estimated. The suspensions of FNO lipospheres were administered orally at a dose equivalent to 800g/kg to respective group using stomach intubation. Blood samples were withdrawn at predetermined time intervals of 0, 1, 2, 4, 6.8, 10, 12 and 24hours by retro

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orbital puncture. The blood glucose level of the control and test samples was determined using the glucose measuring instrument auto analyzer (IKA® T25) .The percentage reduction in the blood glucose level was plotted against time.

Organoleptic properties of drug:

The FNO was checked visually for color, odor, appearance melting point and solubility. The results have been depicted in the Table I.

RESULTS

Table I: Organoleptic properties of FNO

Characterization	Parameter				
	Organoleptic Properties			Melting Point	Solubility
	Colour	Odour	Appearance		
FNO	Red orange	Characteristics	Amorphous	79 to86°C	Very slightly soluble

The melting point of FNO, was determined by capillary method using Digital Melting Point apparatus was found to be 82°C. The value was comparable with the literature value of 69 TO 86°C.given in Table I. The melting point as obtained practically matched the literature value confirms the purity of the sample

The solutions were checked visually for their clarity. The phosphate buffer water system (9:1) found to be turbid.

Saturation solubility:

Table II enlists the saturation solubility of FNO and physical mixture of FNO, stearic acid and paraffin oil in ratio 1:1.5:0.2.in different media.The solubility of FNO was improved when mixed with stearic acid and paraffin oil.

Table II: Saturation solubility of FNO in different solvent

Solvent	FNO	Physical mixture
Water	98.54µg/ml	170.21µg/ml
PBS 6.8 with 1% SLS	167.74µg/ml	261.94µg/ml

Calibration Curve:

Calibration curve of FNO was carried out in PBS pH6.8 system (9:1) as shown in the Figure I. The Beer-Lambert's law was obeyed in PBS pH6.8 system in the range of 2 to 20µg/ml at wavelength 290nm.

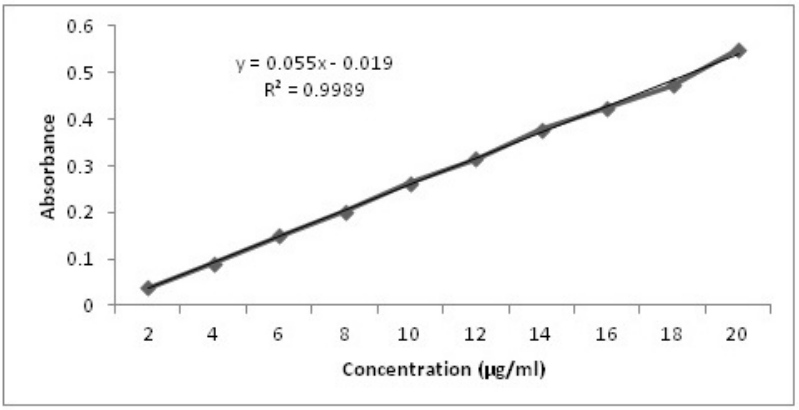

Figure I: Calibration curve in 6.8 pH phosphate buffer with 1%M SLS

FTIR study of drug excipients:

The FTIR of FNO was determined by using Jasco software as shown in the Figure II. The result has been presented in the Table III. The FT-IR finger prints of FNO demonstrated characteristic peak at 3211cm^{-1} due to monosubstituted benzene ring. The peak at 3439cm^{-1} indicates the presence of phenol moiety. Characteristic peaks were obtained at 1796 and 1225cm^{-1} due to aromatic ester moieties and peak at 1625cm^{-1} indicate the presence of carboxylic group. Presence of CH_2Cl group is indicated by peak at 1225cm^{-1}.Thus the FT-IR spectra confirm the purity of the FNO.

Table III: Interpretation of IR spectra

Frequency (cm^{-1})	Interpretation
3039	Alkanes
3211	Monosubsituted benzene ring
3439	Phenols
3514	1,4-Disustituted phenol
1417	-CH_3 group
1625	Carboxylic group
1796,1225	Aromatic ester
1225	-CH_2Cl

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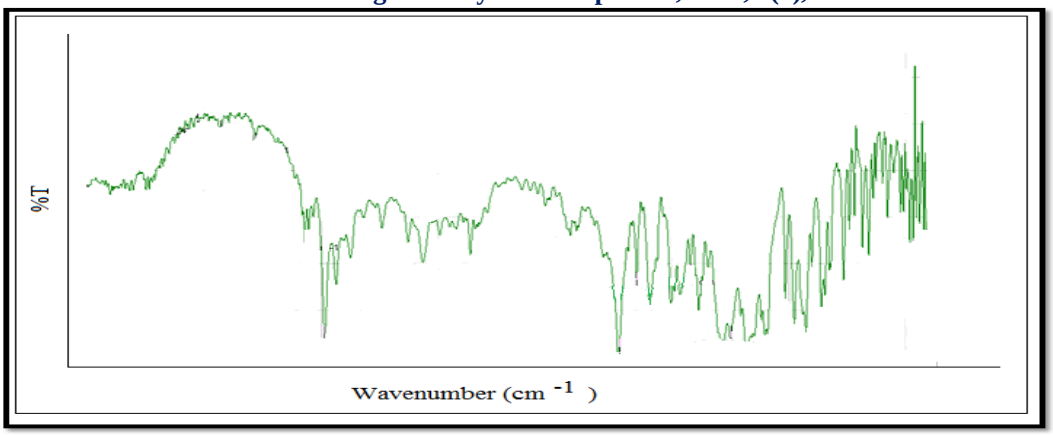

Figure II: FTIR Spectra of Fenofibrate

Differential Scanning Calorimetry(DSC):

The DSC thermogram of FNO and stearic acid has been depicted in Figure III. The DSC of FNO exhibited broad endothermic peak at 82°C indicates melting point of FNO. Lack of sharp melting peak in the DSC of a FNO in the Figure III indicates that the FNO is present in an amorphous rather than crystalline form of FNO. The sharp endothermic peak at 68°C represent melting point of stearic acid.

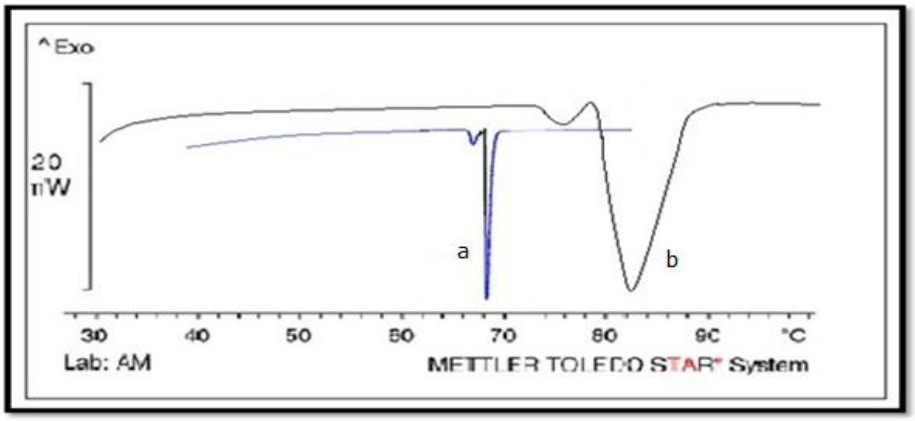

Figure III: DSC of (a) Stearic acid and (b) FNO

Drug excipients compatibility study:

The IR spectra of FNO and excipient Figure IV were compared with the standard spectrum of FNO and excipient. The interaction between the drug and the excipient often leads to identifiable changes in the IR profile of spectrum. IR spectra of drug excipients mixture showed no interaction with the FNO and prominent peaks of FNO were not affected. There was neither shift and nor disappearance of characteristic peaks suggesting that there is no interaction between FNO and other excipients or no degradation in drug molecule. Hence drug excipients compatibility was established.

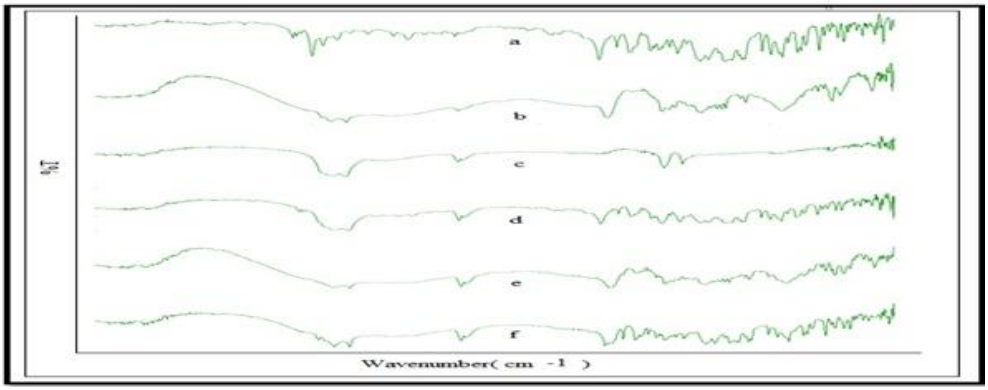

Figure IV: FTIR Spectra of (a) FNO (b) Stearic acid (c) Paraffin oil (d) FNO: Stearic acid (e) FNO: Paraffin oil and (f) FNO: Stearic acid: Paraffin

Preparation of Liposphere by Melt Technique: [11, 12]

The lipospheres contain lipid core that consists of physiological naturally occurring biodegradable lipids, thus minimizing the danger of acute and chronic toxicity. The lipid that constitutes the core component of the lipospheres is solid at room temperature, and might melt,

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or stay solid at body temperature, depending on the particle design. By utilizing solid lipid as a core, several setbacks associated with the usage of liquid or semi-liquid lipid core might be reduced or avoided, i.e. inherent instability and irreversible drug/excipient precipitation. Usually, the oil, which has a maximum solubilizing potential for the drug under investigation, is initially selected with the intention of achieving the maximal drug loading in the lipospheres. Paraffin oil added in the solubilization of drug. The neutral lipids that are usually utilized for the hydrophobic core of the liposphere formulations are tricaprin, trilaurin, tristearin, stearic acid, ethyl stearate, and hydrogenated vegetable oil. In the present study stearic acid was selected as it posses polar carboxylic acid group, which made the matrix more susceptible to hydration and thereby created a hydrophilic pathway for water molecules to access drug. This decreases the resistance to diffusion of the dissolution fluid through the wax matrix and increases the drug dissolution. The lower melting point and density of stearic acid also reported to be contributing factor to enhancing the drug release from the heterogeneous wax matrix system. The choice of an appropriate surfactant for the liposphere formulations is often dictated by safety considerations. Emulsifiers of natural origin are preferred since they are considered to be safer than the synthetic surfactants. Non-ionic surfactants are less toxic than ionic surfactants but they may lead to reversible changes in the permeability of the intestinal lumen. The usage of organic solvent was avoided as discolouration of drug in the capsule due to volatility.

EVALUATION OF LIPOSPHERES

Bulk Characterization:

Angle of Repose

As presented in the Table IV the angle of repose of the factorial batches of lipospheres were in the passable range of 33.01 to 34.69. The presence of aerosil improved the flow property of the lipospheres.

Bulk Density and Tapped Density

The bulk density as shown in the Table IV depends upon the particle size, shape and the cohesiveness between the particles. The values of bulk and tap densities of the factorial batches were found in the range of 0.2021to 0.2586 and 0.3041 to 0.3865 respectively.

Table IV: Micromeretics properties of factorial batches

Formulation code	Bulk Density	Tapped Density	Carr's Index	Angle of Repose
F1	0.2021	0.3041	33.54	32.63
F2	0.2109	0.3238	34.86	32.18
F3	0.2210	0.3546	37.67	33.01
F4	0.2347	0.3548	33.85	34..69
F5	0.2401	0.3854	37.7	36.27
F6	0.2504	0.3481	33.77	32.5
F7	0.2586	0.3865	33.09	33.81
F8	0.2216	0.3455	35.86	34.31
F9	0.2117	0.3352	36.84	33.25

Carr's Index (CI)

The values of Carr's index of the factorial batches were in the range from 33.09 to 37.7as have been shown in the Table IV supporting the fact that were out of acceptable range with poor property of compression.

% Yield:

% Yield has been exhibited in the Table V. The presence of stearic acid showed a drastic change in % yield, %DE and hence in %DR.

% Drug Entrapment: [13]

% Drug entrapment of drug entrapped within the polymer matrices were in the range of 70-97 % as has been shown in the Table V. An entrapment efficiency depends on the drug solubility in the solvent system used for processing. Various co-solvents such as ethanol, di methyl sulfoxide and dimethylformamide been often used in the formulation of lipospheres since they aid in the higher drug entrapment.

Table V: % Yield %DE and cumulative % DR of factorial batches

Formulation code	% Yield	% Drug Entrapment	% Drug Release
F1	38.00	80.00 ± 0.105	63.051 ± 0.061
F2	42.81	79.75 ± 0.109	87.870 ± 0.620
F3	47.44	77.50 ± 0.102	60.250 ± 0.058
F4	44.22	84.08 ± 0.119	67.791 ± 0.517
F5	46.81	97.89 ± 0.151	90.690 ± 0.032
F6	42.14	82.12 ± 0.133	73.560 ± 0.567
F7	45.10	81.12 ± 0.157	68.460 ± 0.319
F8	41.76	86.92 ±0.146	79.890 ± 0.092
F9	44.32	70.92 ± 0.120	65.670 ± 0.168

Data represents n=3, mean± S.D.

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Morphology:

The surface morphology of the lipospheres prepared by melt technique was studied and has been depicted in Figure V. The SEM showed uniformly sized spherical lipospheres with minimum evidence of the crystals of FNO suggesting presence of FNO as solid solution in the carrier.

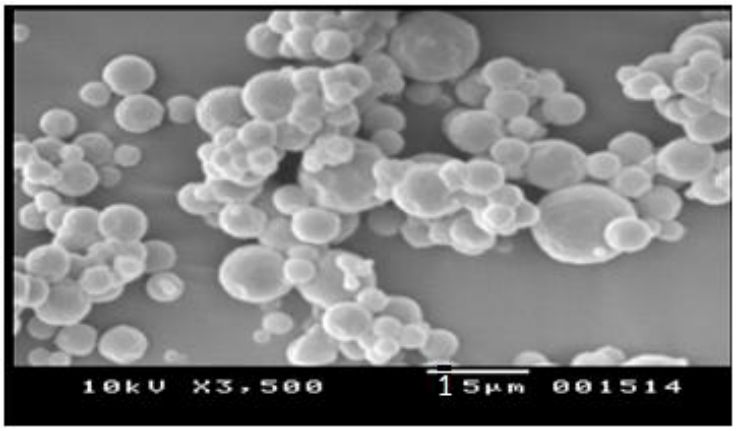

Figure V: SEM of lipospheres

Differential Scanning Calorimetry (DSC) study:

Differential Scanning Calorimetry enables the quantitative detection of all processes in which energy is required or produced (i.e. endothermic and exothermic phase transformations) Thermogram lipospheres have been depicted in Figure VI. In the case of pure FNO, a sharp endothermic peak was observed at 82°C, corresponding to the melting point of FNO. Stearic acid thermogram (bulk material) also displayed an endothermic peak at 68°C corresponding to melting point of stearic acid. No endothermic peak corresponding to fusion of FNO was observed in the thermogram of drug loaded liposphere.

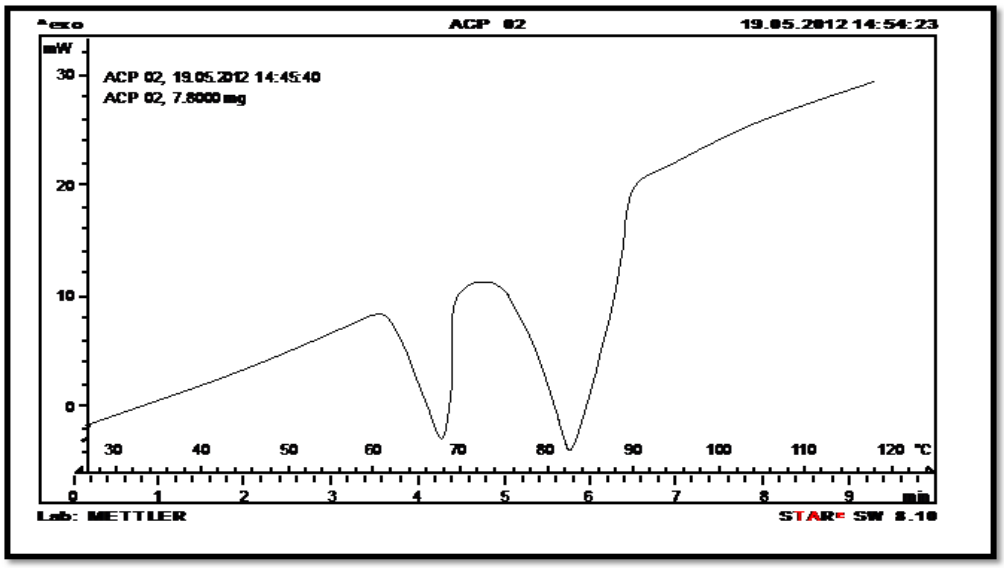

Figure VI: DCS spectra of lipospheres

In-vitro drug release study:

The drug release from lipospheres in phosphate buffer pH 6.8 has been shown in Figure VII. Cumulative % drug release from F1, F3, F4, F7 and F9 were in the range of 60-68% within 12hours. Drug released from F6, and F8 were73-79 % within 12 hr. Drug release from F2 and F5 were 87-90% within 12hours. No formulation is showing burst release which indicates the absence of free particles on the surface of lipospheres which further confirmed by SEM study. The trail revealed that low level of stearic acid (25%) failed to produce liposphere with acceptable physical characteristic where as high level of stearic acid (100%) resulted in liposphere that exhibited high percentage of drug release. As the level of paraffin oil increases the particle size of liposphere increases due to tackiness which in turn decrease the % drug release from the liposphere.

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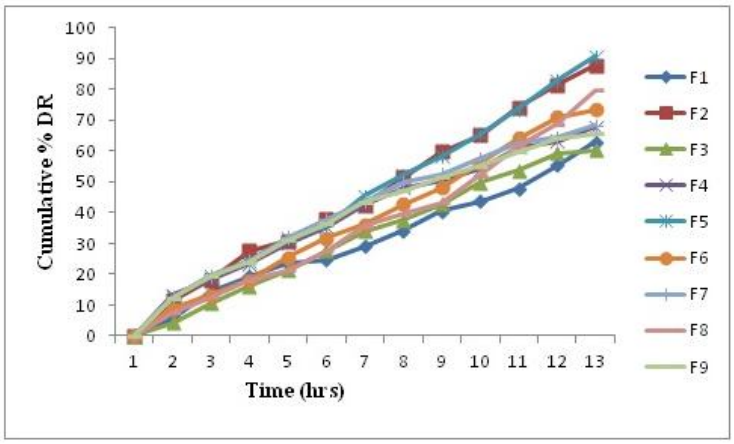

Figure VII: Cumulative % drug release profile of factorial batches (F1-F9)

Optimized formulation

Evaluation of Dependent Variables and Mathematical Modeling:

The values of dependent variables of liposphere formulations are necessary to get polynomial equations from Design Expert software for the respective dependent variable. Mathematical relationships generated using multiple line regression analysis (MLRA) for the studied response variables are expressed in Equation VII, IX.

% Drug Entrapment (Y1):=+82.255556 + 6.74500*X (16)

% Release within 12hr

(Y2): =+81.41000–8.23333*X+0.69967*Y 12.5733*Y^2 (17)

The response surface diagrams, known to facilitate an understanding of the contribution of the variables and their interactions, and their respective contour maps are shown for all the responses in Figure VIII, IX. In the Figure VIII response surface plot reveals that for 97% of % drug entrapment the concentration of stearic acid and paraffin oil should be in the range of100-400mg and 40-60mg respectively. Thus it reveals that concentration of stearic acid & concentration of paraffin oil have significant effects on the % drug entrapment of lipospheres.

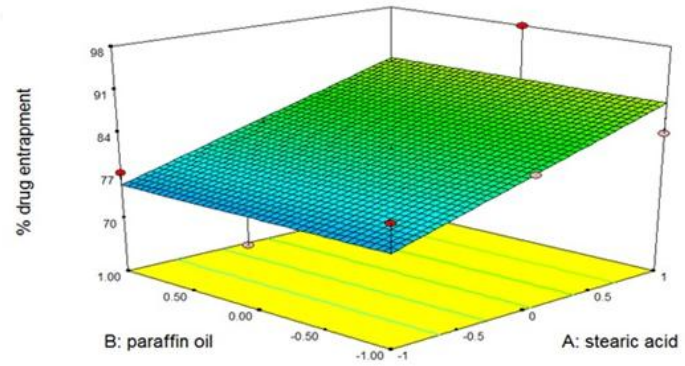

Figure VIII: A contour plot showing relationship between various levels of polymers to attain fixed values of % Drug entrapment

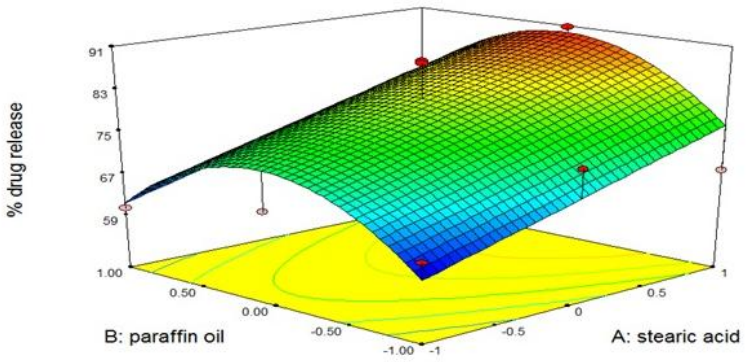

Figure IX: A contour plot showing relationship between various levels of polymers to attain fixed values of % drug release within 12 hours

Figure IX reveals an improved drug release in 12hours with an increased concentration of stearic acid. This may be due to the hydrophobic nature of stearic acid that improves wetting of hydrophobic material by reducing angle of contact between dissolution medium and material and helps in diffusion of drug. The decline in the drug release with an increase in paraffin oil concentration may be attributed to the slower rate of diffusion medium into the liposphere due to an increase in thickness of polymer matrix. For % drug entrapment, the F and P values were found to be 11.92 & 0.0107 respectively, implying the significance of model. For % drug release within 12 hours, the F and P values were found to be 5.53 & 0.0480 respectively, implying the significance of model. Since the values of r^2 were 0.6300 & 0.7684 for % drug entrapment and cumulative % drug release respectively for 12hours, the polynomial equations fits excellently to the experimental data.

Saturation Solubility:

Table VI summarizes the experimentally determined saturation solubility of FNO liposphere in phosphate buffer with 1% SLS. The solubility of FNO (API) was found to be 79.56µg/ml. The liposphere with hydrophobic polymers and surfactants improved the drug solubility, probably due to the solubilization of FNO in the liposphere due to an adsorption of hydrophobic polymer and surfactant.

Table VI: Saturation solubility of FNO lipospheres

FNO	79.56µg/ml
Physical mixture	184.31µg/ml
Lipospheres	352.67 µg/m

In vitro drug release of optimized batch:

The optimized batch Q1 shown in the Figure X and also in Table VII similar release profile compared to factorial batches F5 Release shown was 89.069% in 12 hours and the best fit model was Korsmeyer-Peppas. Its n value was found to be 0.7683. FNO loaded liposphere showed the fastest dissolution rate, with approximately 90% of the drug being released within 12 hours. Addition of aerosil has been shown to reduce the aggregation tendencies of lipospheres, reduced aggregation leads to improved wetting and thereby increased dissolution rate. FNO liposphere with nonionic surfactant exhibited a faster dissolution rate. Once the gelatine capsule had dissolved, the FNO liposphere dispersed rapidly within the dissolution medium, in contrast to the plane FNO which showed some aggregation and floating of a portion of the drug on the surface of the dissolution medium throughout the experiment.

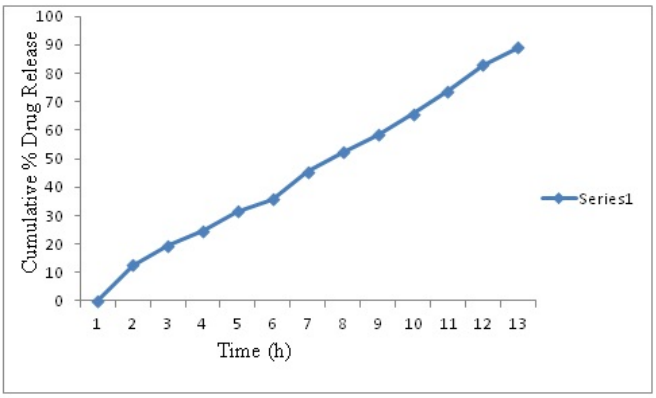

Figure X: Cumulative % drug release of optimize batch Q1

Table VII: Cumulative% drug release of optimize batch Q1

Dependent variables	Optimized formulation F1	
	Experimented value	Predicted value
%Drug Release	89.653±0.673	89.069
%Drug Entrapment	89.006±0.413	89.001

In vivo Study of optimized batch:

In vivo efficiency of the best found formulation of FNO lipospheres (Q1) was performed on healthy normal Wistar albino rats by measuring the lipid profile after an oral administration. The lipospheres were administered at a dose equivalent to 800µg/ kg in suspension form. As depicted in the Figure XI, XII, XII and Table VIII rapid reduction in cholesterol, triglyceride and low density lipoprotein levels was observed and maximum reduction of 45.41%, 49.58%, and 45.67% was observed within 8hours after oral administration respectively.

Table VIII: Reduction in lipid profile levels at different time intervals

Sr. No	Time (h)	Parameters					
		Cholesterol		Triglyceride		Low Density Lipoprotein	
		Control	Liposphere Treated	Control	Liposphere Treated	Control	Liposphere Treated
1	0	98.45±0.10	98.45±0.49	97.21±0.96	96.52±0.78	96.82±0.07	97.87±0.09
2	1	98.31±0.35	85.89±0.01	98.35±0.05	83.51±0.19	98.69±0.43	84.69±0.36
3	2	97.47±0.40	72.22±0.07	96.41±0.47	72.59±0.63	98.20±0.09	75.53±0.74
4	5	94.53±0.15	56.41±0.09	93.25±0.79	61.89±0.89	93.61±0.12	62.15±0.61
5	8	96.63±0.23	45.41±0.41	95.61±0.61	49.58±0.06	94.59±0.60	45.67±0.34
6	12	97.18±059	84.23±0.57	96.83±0.33	82.53±0.04	93.75±0.71	84.19±0.07
7	24	97.47±0.05	97.91±0.39	93.52±0.49	96.28±0.09	95.45±0.57	94.21±0.91

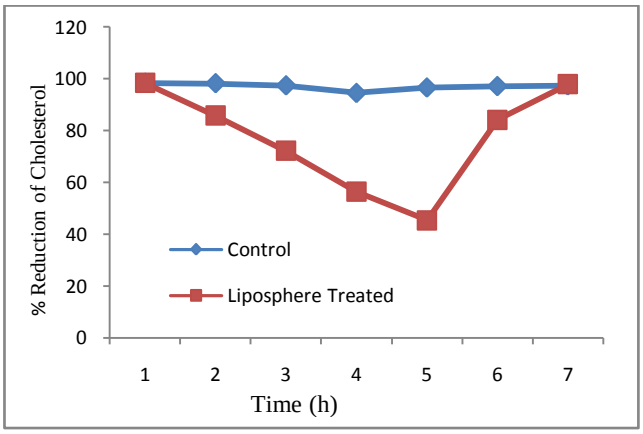

Figure XI: % Reduction of cholesterol in albino rats

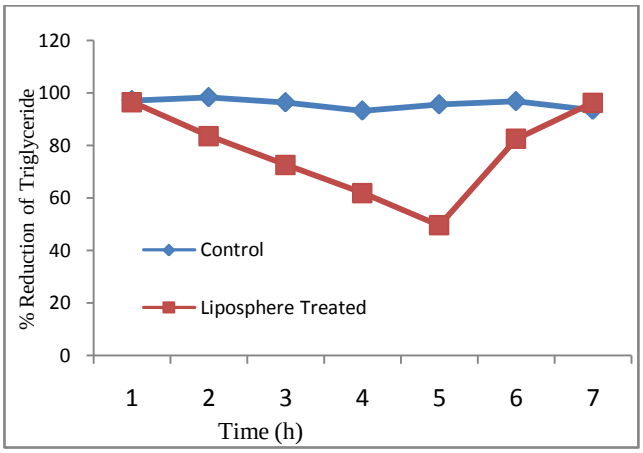

Figure XII: Reduction of triglyceride in albino rats

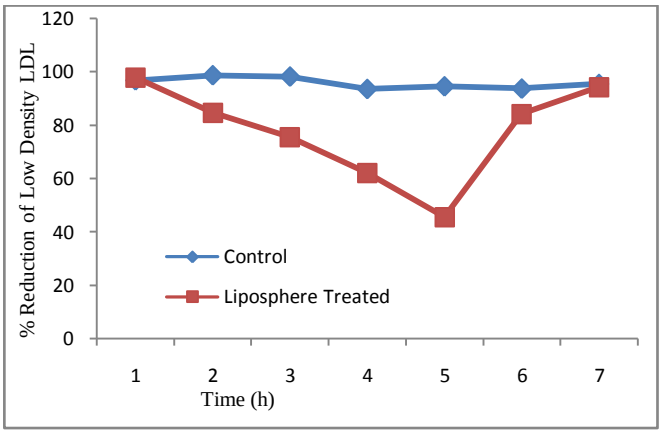

Figure XIII: % Reduction of low density lipoprotein

CONCLUSION:

The present study focused on the development of lipospheres of Fenofibrate by using stearic acid and Paraffin oil as a release retarding polymer using melt dispersion technique. The Invivo data supports the retardation of lipid profile of single dose.

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