Solubility Enhancement of Fenofibrate by Solid Dispersion Technique

A project submitted

by

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Dedicated to my parents

Certification Statement

This is to certify that the project titled “Solubility Enhancement of Fenofibrate by Solid Dispersion Technique” submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Dr. Eva Rahman Kabir, Chairperson, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of another.

Signed,

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Counter signed by the supervisor

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Acknowledgement

The blessings and mercy of the Almighty who is the source of our life and strength of our knowledge and wisdom, has helped me to continue my study in full diligence which I hope will reflect in my project.

This research could not also have been completed without the support of many people who are gratefully acknowledged here.

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Abstract

Solubility of drug is an imperative factor in case of oral drug delivery system to acquire intended drug concentration in systemic circulation in order to attain an efficacious therapeutic response. One of the major hindrances with formulation development of new chemical entities is poor aqueous solubility of drug. Despite of many techniques available to improve the solubility of poorly soluble drugs, it is very challenging to overcome all barriers that encounter solubility problems. The aim of the present study was to enhance the solubility of a lipid lowering agent, fenofibrate by solid dispersion which is considered as a potential method of increasing the solubility of poorly soluble drugs. The experiment was designed to obtain nine different formulations of solid dispersed fenofibrate with the combination of β-cyclodextrin and a hydrophilic carrier, PEG 6000 in different ratios to evaluate which ratio yielded better solubility. A comparative *in-vitro* dissolution study of fenofibrate was also performed in pure water and with 0.025M sodium lauryl sulphate in pure water in order to identify the drug release rate. In addition, saturation solubility study of fenofibrate was successfully done to minimize the unnecessary use of solvent. Afterwards, a 3^2 full factorial design was applied to systematically optimize the drug release profile using Design Expert Software to achieve the optimum formulation. The data analysis of the response developed by the software proved that different ratio of excipients has influence on dissolved percentage of drug. Finally, on the basis of desirability criterion and graphical representation, F6 was successfully selected as the optimum formulation, possessing better solubility.

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List of Acronyms

API = Active Pharmaceutical Ingredient

β-CD = Beta-cyclodextrin

CD = Cyclodextrin

CGTase = Cyclodextrin Glucanotransferase

CMC = Critical Micellar Concentration

CMT = Critical Micellar Concentration

DNA = Deoxyribonucleic Acid

EPAS = Evaporative Precipitation in Aqueous Solution

HDDS = Hydrophobic Drug Delivery System

HDL = High Density Lipoprotein

HLB = Hydrophilic Lipophilic Balance

HP-ß-CD = Hydroxy Propyl Beta-cyclodextrin

HPMC = Hydroxy Propyl Methyl Cellulose

GI = Gastrointestinal

IPA = Isopropyl Alcohol

LD = Lethal Dose

LDL = Low Density Lipoprotein

mg = milligram

µg = microgram

ml = millilitre

PBS = Phosphate Buffer System

PEG = Poly Ethylene Glycol

PPARα = Peroxisome Proliferator-Activated Receptor Type Alpha

PRESS = Predicted Residual Sum of Square

PVA = Polyvinylacetate

PVP = Polyvinyl pyrrolidone

R-squared = Multiple Correlation Coefficient

SCF = Super Critical Fluid

SD = Standard Deviation

SDLF = Self Dispersing Lipid Formulation

List of Acronyms

SLS = Sodium Lauryl Sulphate

USP = United State Pharmacopeia

UV = Ultraviolet

VLDL = Very Low Density Lipoprotein

Chapter 1

Introduction:

Oral route of drug administration is one of the preferable methods of drug delivery due to its convenience, ease of administration and a more effective medication system than other drug delivery systems in terms of patient's compliance. However, not all drugs are of same solubility; some poorly soluble hydrophobic drug often generates certain complexities in formulation development as well as other clinical research. As a result, increasing the dose and higher administration frequency of these poorly soluble drugs may not compensate for the therapeutic dose of the drug. Therefore, to effectively utilize the therapeutic efficacy of the drug, enhancement of its solubility and dissolution profile can be a promising approach.

1.1 Techniques Available to Enhance Solubility

The solubility of a solute can be defined as the maximum amount of solute dissolving in a specific quantity of solvent at certain temperature. To enhance the dissolution and solubility of insoluble drugs, numerous methods are available which can be categorized in three basic approaches:

1. Traditional Techniques

2. Solid Dispersion Technique

3. Newer and Novel Techniques

1.1.1. Traditional Techniques:

There are several techniques available which were traditionally employed to improve the solubility of poorly aqueous soluble drugs. Those traditional techniques are discussed below-

1.1.1.1 Use of Co-Solvents

For the purpose of enhancing solubility of a non-polar drug, mostly used and effective way is the incorporation of a water-miscible or partially miscible organic solvent which is termed as co solvency and the solvents used in combination to enhance solubility of the drugs are called co-solvents. Use of co-solvents basically involves the reduction of interfacial tension among predominately aqueous solution and the hydrophobic solute which is referred to as solvent blending. For example- ethanol, propylene glycol, glycerin, sorbitol and polyoxyethylene glycols can be used as co-solvents (Amin, Dannenfelser, Zielinski, & Wang, 2004).

1.1.1.2 Hydrotropy Method

Hydrotropy is a solubilizing method in which amalgamation of a hydrotropic agent increase the aqueous solubility of another hydrotropic agent. The process of increasing the solubility after adding additives or salts in the given solvent is termed as “salt in” the solute whereas decreasing the solubility is referred to as “salt out” the solute. Diverse salts holding overwhelming anions or cations that would themselves exact dissolvable over water brings about “salting in” from claiming non-electrolytes called “hydrotropic salts” a wonder known as “Hydrotropism.”(Ahuja, Katare, & Singh, 2007).

1.1.1.3 Micronization

The solubility and dissolution rate of poorly water soluble drugs can be enhanced by increasing the surface area through the particle size reduction technique. Micronization includes diminishing the extent of the strong medication molecule to 1 to 10 microns fundamentally by shower drying or by utilization of air steady loss strategies, for example, liquid vitality process, stream process, and so forth. The wonder is additionally named as "Micromilling.” The procedure can't be performed for those medications which requires high dosage number since it has no impact on the immersion solvency of the medication. Diminishment of molecule size of medication is not satisfactory as item having less molecule measure has the tendency of agglomeration, which prompts less successful surface zone for disintegration (Chaumeil , 1998).

1.1.1.4 Change in Dielectric Constant of Solvent

The dielectric constant of a substance can be alluded to the vitality expected to separate two oppositely charged bodies. The vitality that requires to isolate two oppositely charged bodies is conversely relative to the dielectric constant of the medium. Solubility of hydrophobic molecules can be enhanced by adding a co-solvent because of the reduction of dielectric constant of the solvent. For polar molecules, water is a good solvent due to the presence of hydrogen bonding as well as having high dielectric constant (Babu, Areefulla, & Mallikarjun, 2010).

1.1.1.5 Amorphous Forms

In undefined shape particles or atoms are haphazardly settled and have higher thermodynamic energy than relating crystalline structures. More noteworthy solvency and also disintegration rates can be accomplished by this procedure.

1.1.1.6 Chemical Modification of Drug

Polar groups like carboxylic acids, ketones and amines when added, solubility increases due to the large number of hydrogen bonds and their interaction with water molecules (Babu et al., 2010).

1.1.1.7 Use of Surfactants

Due to having a polar end (the circular head) and a non-polar end (the tail) surfactants are amphiphilic in nature. At the point when a surfactant, for example, Tween-80, sodium lauryl sulfate is set in water, it will shape into micelles. A non-polar drug will segment into the hydrophobic center of the micelle and the polar tail will solubilize the complex (Bakatselou, Oppenheim, & Dressman, 1991).

1.1.1.8 Inclusion Complex/Clathrates

Considerable increase in solubility and dissolution of the drug can be accomplished by using cyclodextrins. These complexes can be prepared with ß-cyclodextrin (ß-CD) and HP-ß-CD; the required quantity of ß-CD is weighed and water is added to get tough consistency (Challa, Ahuja, Ali, & Khar, 2005). One of the prominent features of cyclodextrin is their tendency to form solid inclusion complexes (host-guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation. Complex formation is a dimensional fit between host cavity and guest molecule (Botella, Castillo, & Martin, 1995). The main driving force of complex formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state. Inclusion in cyclodextrins poses a profound effect on the physicochemical properties of guest molecules as they are temporarily locked or caged within the host cavity giving rise to beneficial modifications of guest molecules, which are not achievable otherwise (Schmid, 1989).

Some of these properties are:

- Solubility improvement of highly insoluble guests,

- Stabilization of labile guests against the degradative effects of oxidation, visible or UV light and heat,
- Control of volatility and sublimation,
- Physical isolation of incompatible compounds,
- Chromatographic separations,
- Taste modification by masking off flavours, unpleasant odours and controlled release of drugs and flavours.

Therefore, the use of cyclodextrins is employed in many sectors like food, pharmaceuticals, cosmetics, packaging and textile industry.

1.1.1.9 Change in pH of Solvents

Diminishing the pH of a solvent can progressively upgrade the solvency. Both pH and complexation processes have synergestic impact on solubilization (Talari, Jaleh, Mostafavi, & Nokhodchi, 2009).

1.1.1.10 Use of Hydrates or Solvates

A stoichiometric or non-stoichiometric adducts, similar to inclusions may include the entrapment of dissolvable particle inside the crystal lattice if there should be an occurrence of a crystalline compound. This stoichiometric adducts are ordinarily referred to as "Solvate," and is an atomic complex which has the inclination of fusing the taking shape dissolvable particles into particular destinations inside the crystal lattice. The complex is called "Hydrate" if the fusing dissolvable is water particle. A compound which does not have any water inside its crystal structure is named as “Anhydrous”. Anhydrous structures will probably be solvent in fluid media in correlation with the hydrate shapes (Leon, Herbert, & L, 1990).

1.1.1.11 Use of Soluble Prodrugs

Bio-reversible chemical alteration is helpful for improving the physicochemical properties of drugs. The most common prodrug strategy involves the incorporation of polar or ionizable moiety into the parent compound in order to enhance aqueous solubility (Hussain & Rytting, 1974).

1.1.1.12 Application of Ultrasonic Waves

Ultrasonic vibrators are also used to increase the solubility. Having high frequency of 100-500 KHz oscillator is used in this technique which is called “Pohlman whistle” (Babu et al., 2010).

1.1.1.13 Functional Polymer Technology

The mechanism of this technique is to improve the dissolution rate of insoluble drugs mainly by escaping the core obstacle for instance dissolution in aqueous media, the lattice energy of drug crystals. The polymers contain acidic or basic groups since they are particle trade materials and interface to adjust their versatile particles having same charge of the encompassing medium by reversibly or stoichiometrically. The complex framed in this procedure is called "Resinate" which can be set up as tablet, suspension, or dry powder. The resultant resins do not retain into the body as they are insoluble and subsequently in the wake of going to the contact of physiological liquids, the medication is discharged from the resinate complex (Babu et al., 2010).

1.1.1.14 Controlled Precipitation Technology

In controlled precipitation technology, the drug is dissolved in a water miscible organic solvent and then dissolved into aqueous medium containing stabilizers (HPMC, cellulose ethers, and gelatin). The solvent dissolves in water and causes precipitation of the drug in the form of micro-crystals. The adsorbed stabilizers avert molecule development and enhance the disintegration profile of inadequately dissolvable medication having the substantial surface territory.

1.1.1.15 Evaporative Precipitation in Aqueous Solution (EPAS)

This technique mainly causes rapid separation to nucleate and produce nanoparticles and microparticles of hydrophobic drugs. Initially, the drug is dissolved in an organic solvent having low boiling point followed by pumping through a tube. At that point under pressure to a temperature over the solvent’s breaking point, the tube is warmed and with a fine atomizing spout showered into a warmed watery arrangement. To improve molecule arrangement and solubilization, surfactants are included both in organic and aqueous solution (Voughn, Gao, Yacaman, & Williams, 2005).

1.1.1.16 Use of Precipitation Inhibitors

Drug precipitation or crystallization happens when there is compelling increment in free medication focus above equilibrium solubility coming about into super saturation. By using inert polymers such as HPMC, PVP, PVA, PEG, etc the precipitation or crystallization can be eliminated. The mechanisms by which the polymers act are: by increasing the viscosity of crystallization medium causing less crystal formation of drugs, by growing crystal surfaces through specific intermolecular interaction which inhibits crystallization by giving a stearic

hindrance to drug molecules or by adsorbing onto the faces of host crystals through the production of micro crystals.

1.1.1.17 Solvent Deposition

In solvent deposition technique, the hydrophobic drugs are first dissolved into an organic solvent (alcohol) followed by deposition on an inert, hydrophilic, solid matrix like starch or microcrystalline cellulose and then evaporates the solvent (Spireas, Jarowski, & Rohera 1992).

1.1.1.18 Precipitation

Precipitation process involves the dissolution of poorly aqueous soluble drugs to dissolve in an appropriate organic solvent followed by rapid mixing with a non-solvent in order to precipitate the drug in nanosize particles. The product so prepared is also called “Hydrosol” (Sertsou, Butler, & Rades, 2002). Hydrosols are colloidal watery suspensions containing drug nanoparticles of inadequately water soluble medications for intravenous administration. They are made by a precipitation procedure as the drug solution is blended with a generally high volume of water (96–98% water after blending) within the sight of stabilizing agents, for example, poloxamer and modified gelatins, which act as "short term stabilizers" (Gabmann, List, Schweitzer, & Sucker, 1994). After precipitation, the amorphous hydrosol is stable for approximately 60 minutes because of the stabilizers and the high amount of non-solvent. In this manner, the drug crystallizes. Because of the association of blurring with molecule size, crystallization and molecule development can be seen through the expansion in absorbance at a wavelength not being the region where drug substance absorbs. As a result, the hydrosols are rapidly spray dried with excipients like lactose or mannitol in order to get sustainable stabilized amorphous nanosized drug before crystals form. The preparation are subjected to reconstitute before use. Thus, hydrosols are preferable for parenteral application as the drug particle size is approximately 200nm.

1.1.2. Solid Dispersion Technique:

Bioavailability of drug is significantly affected by the dissolution properties of medication and its discharge rate from the respective dosage form. In pharmaceutical industry, the advancement of any new chemical entity, minimizing its solvency issue is the most difficult stride. Numerous techniques are available but solid dispersion is the most potential method to enhance the solubility of the poorly aqueous soluble drugs (Kapsi & Ayres, 2001).

Solid dispersion basically refers to dispersing one or more active ingredients in an inert carrier in a solid state, frequently prepared by the solvent evaporation method, melting (fusion) method or fusion solvent-method. Bioavailability enhancement of a poorly aqueous soluble drug by solid dispersion compared with conventional tablet or capsule flow chart is illustrated below in Figure 1.1

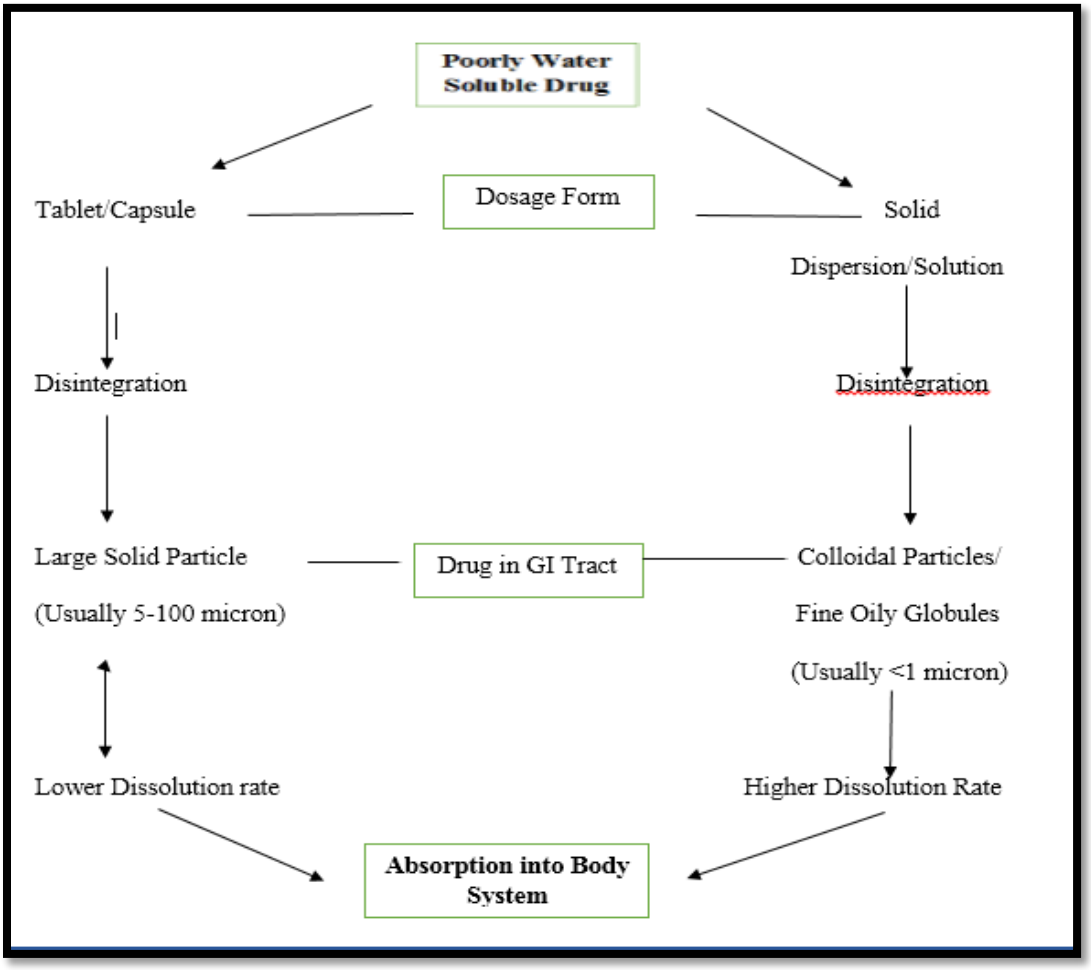

Figure 1.1 A schematic representation of a poorly aqueous soluble drug by solid dispersion compared with conventional tablet or capsule to enhance the bioavailability of drugs.

1.1.2.1 Methods of Solid Dispersion

Numerous methods are available for the preparation of solid dispersion system such as-

☐ Melting method

☐ Solvent method

- Meltevaporation method (melting solvent method)
- Melt extrusion methods
- Lyophilization techniques
- Melt agglomeration process
- The Use of surfactant
- Electrospinnig
- Super Critical Fluid (SCF) technology (Singh, Baghel, & Yadav, 2011)

1.1.2.1.1 Melting Method

The preparation of physical mixture of drug with a water soluble carrier is called melting method. This process involves heating the mixture directly until it is melted. This process is also referred to as fusion method. In an ice-bath the melted mixture is solidified with vigorous stirring and obtained solid mass is then powdered, pulverized and filtered.

1.1.2.1.2 Solvent Method

In this technique, both physical mixture of drug and carrier are dissolved in solvent followed by evaporation until a solvent free film is left. One prominent advantage of this method is that the thermal degradation of the drug and carrier can be prevented as apparently low temperature is applied for the evaporation of organic solvents.

1.1.2.1.3 Melting Solvent Method

This method involves dissolution of the drug in an appropriate liquid solvent and then requires the association of solution directly with a melt of polyethylene glycol. After that the mixture is evaporated in order to attain a clear, solvent free film. This technique incorporates the unique advantages of both the fusion and solvent evaporation methods. However, practically it is only applicable for drugs having minimum therapeutic dose e.g. below 50 mg.

1.1.2.1.4 Melt Extrusion Method

Melt extrusion method is the composition of active ingredient and carrier which is prepared by hot stage extrusion where a co-rotating twin-screw extruder is used. In this technique, the concentration of drug in dispersion medium always needs to be 40% (w/w).

1.1.2.1.5 Lyophilization Technique

In this technique, heat and mass transfer occurs to and from the product under preparation. Lyphilization is an alternative approach to solvent evaporation method. It is basically a molecular mixing process in which the drug and carrier are co-dissolved in a specific solvent, congealed and sublimed in order to get a lyophilized dispersion.

1.1.2.1.6 Melt Agglomeration Process

Melt agglomeration is a process where the binder acts as a carrier for the preparation of solid dispersion. Apart from this, using a high shear mixer either by heating drug, binder and excipient at a temperature above the melting point of binder or by spraying a dispersion of drug in molten binder on heated excipient, solid dispersion of drugs can be prepared.

1.1.2.1.7 Melt Agglomeration Process

Having some unique characteristics, surfactants have grabbed the attention of researcher for preparation of solid dispersion. In solubilization, use of surfactants is very effective. Adsorption of surfactants on solid surface causes alteration of hydrophobicity, surface charge and other important factors which controls interfacial processes like flocculation/dispersion, wetting, detergency, solubilization, floatation and corrosion inhibition. In addition, solvation/plasticization, glass transition temperature and the combined glass transition temperature and manifestation in decreasing the melting of API of solid dispersion have been observed because of the use of surfactants.

1.1.2.1.8 Electrospinning

Electrospinning refers to the formation of solid fibers from polymeric fluid stream solution or melt delivered through a millimeter-scale nozzle. It is an excellent process for the preparation of nanofibers and effective for controlling the release of biomedicine. In addition, this technique can be utilized widely due to its simplicity and cost effectiveness in future.

1.1.2.1.9 Super Critical Fluid (SCF) Technology

This technique involves the use of carbon dioxide as an antisolvent for solute yet as a solvent with respect to organic solvent. After completion of the process, it is easier to remove the supercritical carbon dioxide from polymeric materials, even though less quantity of carbon dioxide remains entrapped inside the polymer. Carbon dioxide also plasticize and swell polymers and the process can be run in ambient temperature, thus supercritical carbon dioxide is used in this technique. In

addition, to decrease the temperature of melt dispersion process by lowering the melting temperature of dispersed active agent, supercritical fluids are used.

1.1.2.2 Application of Solid Dispersion System:

Some of the applications of solid dispersion system are listed below-

1. Decrease the particle size

2. Increase the rate of dissolution

3. Ensure faster absorption

4. This technique can be used to obtain homogenous distribution of small amount of drug at a solid state, to stabilize unstable drugs, to dispense liquid or gaseous compounds, to formulate release or prolonged-release regimens of soluble drugs by using poorly soluble or insoluble carriers.

1.1.2.3 Limitations of Solid Dispersion System:

There are several limitations in solid dispersion systems although this is very useful technique for poorly soluble drugs. Problems limiting the commercial application of solid dispersion technique involve-

(a) The way it is prepared,

(b) Consistency of both physical and chemical properties,

(c) Different dosage forms formulation,

(d) The scale up of manufacturing processes, and

(e) Stability of the physicochemical parameters of drug and vehicle.

1.1.3. Newer and Novel Techniques

In recent years, due to technological advancement a wide variety of newer and novel techniques evolved to enhance the solubility of poorly soluble drugs that are viewed below-

1.1.3.1 Size Reduction Technologies

It is a standout amongst the most complex procedures in which not just drug particles needs to stay into nanosize but additionally should be steady and formulated rigorously keeping in mind the end

goal to hold the nature and properties of the nanoparticles (*High Pressure Process Technology: Fundamentals and Applications*, 2001).

1.1.3.2 Lipid Based Delivery System

Lipid-based drug delivery system is very effective technique to improve the oral absorption of lipophilic drugs (Pouton, 2000). For formulation design of most of the lipids used in these oral formulation must have a known "required HLB" value, usually given by vendors as a guideline to initiate the design of the formulation. This HLB value must conform the optimal HLB value for the surfactant blend which is required for oil in water emulsification. Emulsifier can be another choice in case of lipid based drug formulation. Some of the emulsifiers are listed below:

Table 1.1 Emulsifiers used in lipid-based formulations (Porter, Trevaskis, & Charman, 2007)

Common name/type	Examples
Phosphatidylcholine and phosphatidylcholine/solvent mixtures	Low HLB (<10) emulsifier
	Phosphatidylcholine, phosphatidylcholine in propylene glycol, phosphatidylcholine in medium chain triglycerides, and phosphatidylcholine in safflower oil/ethanol
Unsaturated polyglycolized glycerides	Oleoyl macrogolglycerides, linoleoyl macrogolglycerides
Sorbitan esters	Sorbitan monooleate, sorbitan monostearate, sorbitan monolaurate, and sorbitan monopalmitate
Polyoxyethylene sorbitan esters	High HLB (>10) emulsifier
	Polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80
Polyoxyl castor oil derivatives	Polyoxyl 35 castor oil, polyoxyl 40 hydrogenated castor oil
Polyoxyethylene polyoxypropylene	Poloxamer 188, poloxamer 407
Saturated polyglycolized glycerides	Lauryl macrogolglycerides, stearyl macrogolglycerides

PEG-8 caprylic/capric glycerides	Caprylocaproyl macrogolglycerides
Vitamin E derivative	Tocopherol PEG succinate

1.1.3.3 Self Dispersing Lipid Formulation (SDLF)

In this technique, the drug is incorporated into the mixture of oil and surfactant. They emulsify when mixed with aqueous environment (Porter, Trevaskis, & Charman, 2007).

1.1.3.4 Micellar Technologies

Mixed micelles which are fit for diminishing the surface tension of a solvent, for example, amphiphilic, ionic, anionic or amphilytic micelles must arrange over a specific critical concentration. Micelle arrangement can just happen over a specific solute concentration, the critical micellar concentration (CMC), and at solution temperatures over the critical micellar temperature (CMT) (Dangi, Vyas, & Dixit, 1998).

1.1.3.5 Polymeric Micelles

Polymeric micelles are groups of amphiphilic polymers that have assembled into nanoscopic supramolecular core-shell structures. There are several block copolymers that are used for the formation of polymeric micelles, such as Pluronics®, poly (ethylene glycol) (PEG)-phospholipid conjugates, PEG-b-poly (ester), and PEG-b-poly (L-amino acids).

1.1.3.6 Porous Microparticle Technology

In this strategy, the insoluble drug must be installed into microparticles having a permeable, fluid dissolvable sponge like matrix. The matrix dissolves upon supply of water which causes the wetting of medication and leaves a suspension of quickly dissolving drug particles. This is the essential innovation applied as Hydrophobic Drug Delivery System (HDDS).

The process flow chart of the present study is shown in Figure 1.2.

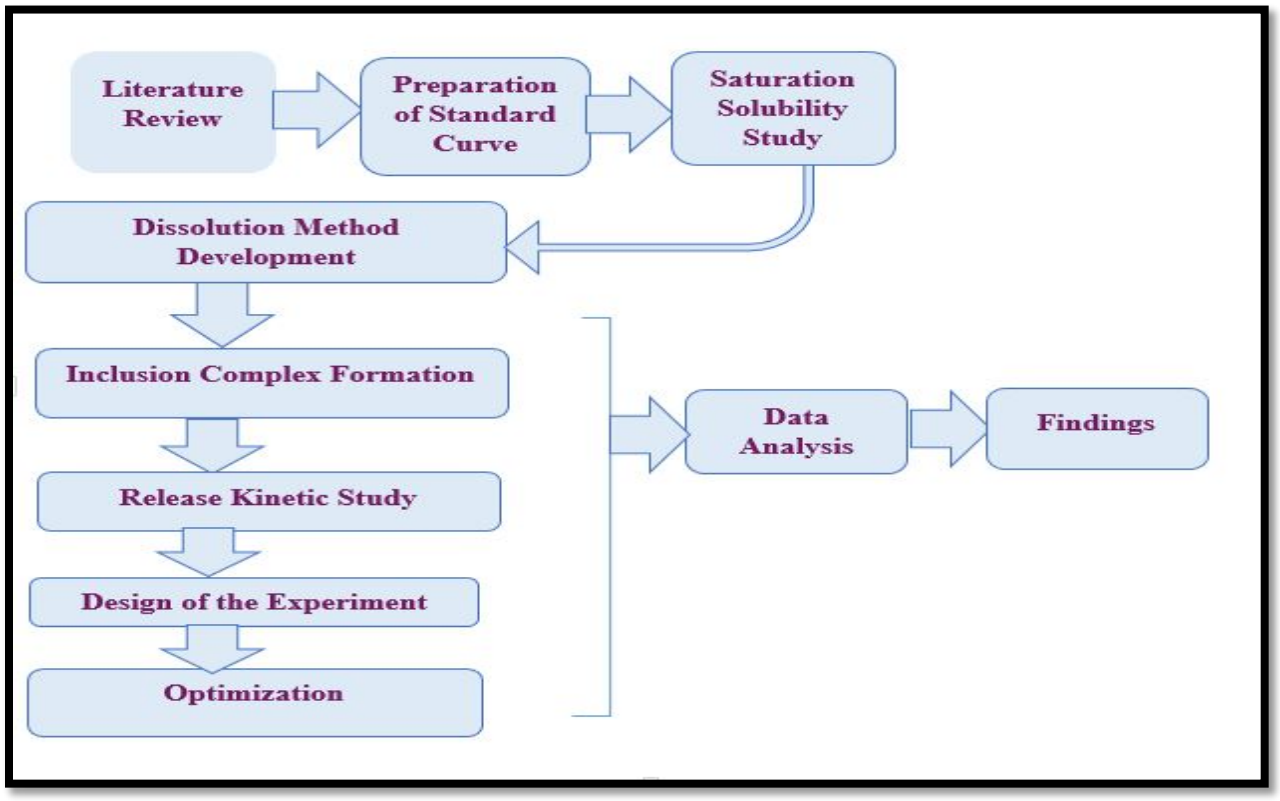

Figure 1.2 Flow chart of the study design of project work

Among the numerous methods available to enhance the solubility, solid dispersion technique is one of the best approaches to get a better formulation by improving its solubility and dissolution profiles, thus confirming greater bioavailability. The present study involves an attempt to enhance the solubility of immediate release tablet, fenofibrate, an anticholesterol agent by solid dispersion technique and hence, leads to the determination of an optimum formulation among all the formulations designed.

1.2 Rationale of the Study

Several problems are associated with the poorly soluble nature of the traditional oral tablet dosage form of fenofibrate including poor bioavailability due to its low aqueous solubility and hepatic metabolism. Therefore, to increase the solubility profile, solid dispersion can be an effective approach. The main focus is to improve the already existing formulation in order to attain patient compliance in a cost effective manner by reducing the frequency of drug administration. In addition, it will be helpful to attain the desired concentration of drug in systemic circulation, thereby, providing effective pharmacological response.

1.3 Drug Profile

Fenofibrate

Fenofibrate is a lipid regulating small molecule usually indicated for the treatment of hypercholesterolemia, hyperglyceridemia. Fenofibrate is used with a low-fat diet, exercise, and sometimes with other medications to reduce the amounts of fatty substances such as cholesterol and triglycerides in the blood and to increase the amount of HDL (high-density lipoprotein; a type of fatty substance that decreases the risk of heart disease) in the blood. It is official in British Pharmacopoeia.

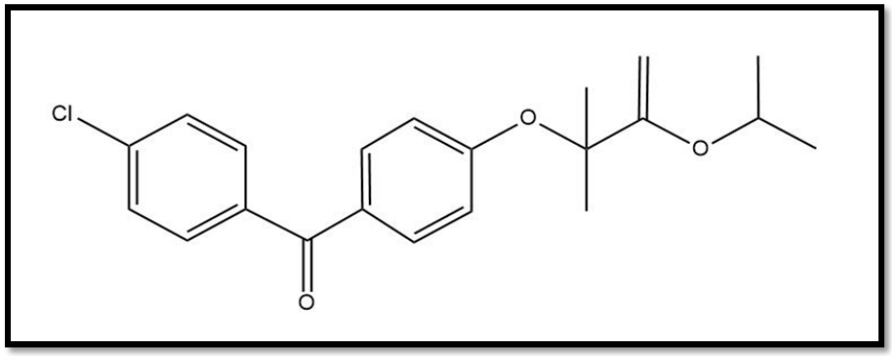

Figure 1.3 Chemical structure of Fenofibrate.

1.4 Excipients

1.4.1 Beta-cyclodextrin:

Cyclodextrin (CD) is a group of structurally- related oligosaccharides that have a polar cavity and a hydrophilic external surface. It consists of six α-cyclodextrin, seven β-cyclodextrin, eight γ-cyclodextrin or more glucopyranose units linked by α-(1,4) bonds. They are also known as cycloamyloses, cyclomaltoses and schardingerdextrin. Formation of cyclodextrin results from intramolecular transglycosylation reaction through degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme. Cyclodextrins are pretentious molecular chelating agent. They are basically of three types- α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, referred to as first generation or parent cyclodextrins. Among the three types β-cyclodextrin is most widely used because of its easy availability and low cost.

Table 1.2 Properties of β-Cyclodextrin (Janisse Crestani de Miranda & Ferraz, 2011)

Property	β-Cyclodextrin
Number of glucopyranose units	7
Molecular weight (g/mol)	1135
Solubility in water at 25 ◦C (%, w/v)	1.85

Beta-cyclodextrin possesses several other properties such as-

- Less irritating than α–cyclodextrin and after intramuscular injection binds with cholesterol;
- Very small amount (1–2%) absorbed in the upper intestinal tract after oral administration;
- No metabolism in the upper intestinal tract;
- Metabolised by bacteria in cecum and colon;

Currently, β-cyclodextrin is the most commonly used cyclodextrin in pharmaceutical formulations and, thus, probably the best studied cyclodextrin in humans. Application of high doses may be harmful and is not recommended; bacterial degradation and fermentation in the colon may lead to gas production and diarrhea (Valle, 2003).

1.4.2 PEG 6000

Polyethylene glycol, referred to as PEG, is used as an inactive ingredient in the pharmaceutical industry as a solvent, plasticizer, surfactant, and lubricant in ointments, suppository base, tablets and capsules. PEG has low toxicity with systemic absorption less than 0.5%. It is a white or slightly yellowish flake like substance that has a molecular weight of 5400-6600 and a melting point of 60°C.

1.5 Literature Review

Initially, thorough literature review was performed to gather relevant information regarding drug, solvent, excipients, polymers, and suitable methods of identification and for other necessary parameters required for the release kinetic study and optimization process of fenofibrate. List of the journals which were looked for present study are as follows:

1. International Research Journal of Pharmacy
2. Biomacromolecules, ACS publication
3. International Journal of Molecular Sciences
4. International Journal of Pharmaceutics

5. Journal of Validation Technology
6. Tropical Journal of Pharmaceutical Research
7. International Journal of Pharmacy & Life Sciences
8. International Journal of Pharmaceutical Sciences and Drug Research
9. Journal of Drug Delivery & Therapeutics
10. Pelagia Research Library
11. Drug Development and Industrial Pharmacy
12. Journal of Microencapsulation
13. Pharmaceutical Development and Technology

Chapter 2

Methodology:

The research methodology of this project has been designed based on the proposed method to enhance the solubility of fenofibrate. In the study, fenofibrate has been combined with β-cyclodextrin and PEG 6000 in different ratios to form an inclusion complex to determine their effect in terms of solubility. The proposed formula for enhancing solubility of fenofibrate is given in Table 2.1.

Table 2.1 Proposed formula of the drug

Active Pharmaceutical Ingredient	**Amount**
Fenofibrate	10mg
Excipients	**Justification (of use)**
β-cyclodextrin	Inclusion complex former
PEG 6000	Hydrophilic carrier

2.1 Standardization of Fenofibrate by UV-Visible spectrophotometer

10mg of fenofibrate was precisely weighed and dissolved in 100ml of methanol to get a concentration of 100µg/ml. From this mixture, 5ml of sample was withdrawn and the volume adjusted to get a concentration of 10µg/ml. Then, 2ml, 4ml, 6ml, 8ml solution was withdrawn from the mixture of 10µg/ml and diluted to 10ml to get the concentrations in the range 2ml to 8ml respectively. With the help of UV-Visible Spectrophotometer absorbance was determined for each concentration at a wavelength of 286nm.

2.2 Method Development for Dissolution

For the assessment of bioavailability of a drug, dissolution criteria is an important parameter. Dissolution can be defined as the amount of drug entering into solution from solid state per unit time. Assessing the dissolution profile is one of the best approaches to establish the *in vitro-in vivo* relationships between the release of drug from the dosage form and absorption rate of drug. Dissolution study gives an overview about the release of drug used in a particular formulation. Dissolution of fenofibrate was carried out in USP dissolution apparatus Type II (Paddle method)

at a temperature 37ºC with a fixed rpm of 50. Two different dissolution media were used to evaluate the dissolution profile of fenofibrate separately, of which one was pure water (pH 5.5-5.7) and the other was 0.025M sodium lauryl sulphate in pure water. All the studies were carried out in triplicate and sampling time was 5, 10, 20, 30 and 45 minutes for pure water and sampling time was 5, 10, 15, 20 and 30 minutes for 0.025M SLS media.

2.2.1 Preparation of 0.025M Sodium Lauryl Sulphate Media

Approximately 3.5L of 0.025M SLS media was required for the dissolution study. For the preparation of 3.5L of 0.025M solution, 25.23gm sodium lauryl sulphate was accurately weighed and dissolved in a small amount of distilled water and the volume adjusted to 3.5L.

2.2.2 Preparation of Sample for Dissolution

Initially, dissolution vessels were filled with 1000ml of 0.025M SLS medium and the media was heated to a temperature of 37°C ± 0.5°C. 75mg of fenofibrate was weighed and poured into the media when the temperature reached 37ºC. The apparatus was operated for 30 minutes at 50 rpm. At a time interval of 5, 10, 15, 20 and 30 minutes, samples of 5ml was withdrawn from the vessel and replaced with the same quantity of fresh dissolution medium. Further, the stored samples were filtered with cotton and individual absorbance values were noted by using UV-Visible spectrophotometer at 286nm. The percentage of drug release was then calculated by using the following standard curve equation:

$$Y = 0.049X - 0.016$$

where, Y= Absorbance

X= Concentration (µg/ml)

Formula for percent of drug release calculation:

$$\% \text{ Release} = \frac{X\ (\mu g/ml) \times 1000ml \times 100}{\text{Weight of API } (\mu g)}$$

Similarly, samples were prepared and the dissolution rate of fenofibrate in pure water was analyzed.

2.3 Saturation Solubility Studies of Fenofibrate in Different Solvents

Quantitatively, solubility can be defined as the amount of solute in a saturated solution at a certain temperature. The bioavailability of a drug, rate of drug release into the dissolution medium and

thus the efficacy of the pharmaceutical product are largely dependent on its solubility (Wisher, 2002).

The common solvents used for the study are:

- PBS 6.2 pH
- PBS 7.2 pH
- Methanol
- Ethanol
- Distilled water

2.3.1 Preparation of Saturated Solution

Different solvents can dissolve different amount of solutes. To minimize the unnecessary use of solvent, the saturation solubility of drug was determined. Using minimum quantity of solvent, drug powder was dissolved into selected different solvents until it reached its undissolved state to assess the solubility of the drug powder in the selected solvents.

2.4 Solubility Study of Fenofibrate & Beta-cyclodextrin in Different Ratios Using Ethanol by Solid Dispersion

To assess the effect of β-cyclodextrin for enhancing the solubility of fenofibrate, β-cyclodextrin was incorporated into the drug at different ratios ranging from 0.5 to 2. Minimum amount of drug and excipient were taken in order to eliminate the wastage of both drug and β-cyclodextrin. A combined formulation of drug and β-cyclodextrin were prepared in the ratios 1:0.5, 1:1, 1:1.5 and 1:2 respectively. Required amount of drug and β-cyclodextrin were weighed accurately and dissolved in 10ml of ethanol. Then the solution was kept into water bath at 70ºC until the solvent evaporates. After complete evaporation the solid residue was dissolved in 10ml of distilled water followed by cotton filtration. Finally, at a wavelength of 286 nm absorbance was determined by using UV-Visible spectrophotometer. However, it was found that the drug particles dissolved in ethanol but β-cyclodextrin remains in suspension form that is not desired in solid dispersion technique which was the reason for not considering ethanol as the solvent in this study.

2.5 Solubility Assessment of Fenofibrate & Beta-cyclodextrin Using Isopropyl Alcohol & Water by Solid Dispersion

Since β-cyclodextrin did not dissolve in ethanol, water and isopropyl alcohol were taken to see if it was possible to eliminate any error associated with the suspension form of β-cyclodextrin in ethanol. In this study, drug and β-cyclodextrin were taken in ratios of 1:2 and 1:3 and analyzed.

10mg drug was weighed accurately and dissolved in minimum amount of isopropyl alcohol for each preparation. 20 and 30mg of β-cyclodextrin were taken and dissolved in minimum amount of water. When dissolved, both the solutions were mixed accordingly and dried well the mixtures using a vacuum drying oven. At 60º C temperature both the mixtures were kept in the oven for approximately 24hr and the solid powder obtained was dissolved in 20ml of distilled water. This was then filtered and absorbance measured at a wavelength of 286 nm using UV-Visible spectrophotometer (Table 3.6).

2.6 Solubility Study after adding Different Ratios of PEG 6000 to Drug and Beta-cyclodextrin

This was done to find the amount of PEG 6000 to improve solubility. In this process, similar conditions were followed, with the exception of the incorporation of a hydrophilic carrier named PEG 6000. Different ratios of drug, β-cyclodextrin and PEG 6000 were prepared by dissolving required amount of drug in isopropyl alcohol. The β-cyclodextrin was first added in minimum quantity of water and then PEG 6000 was added accordingly. Both the mixture were added together and dried at 60ºC under vacuum drying oven. The solid mixtures were then dissolved in 20ml of distilled water and filtered, followed by recording of absorbance under UV-Visible spectrophotometer at 286 nm (Table 3.7).

2.7 Optimization of Solid Dispersed Fenofibrate with Beta-cyclodextrin & PEG 6000

Optimization is considered as establishment of most efficacious and economic approach to maximize desired and minimize undesired effects under certain constraints and to visualize the relationship between independent and response variable (Bushra, Shoaib, Aslam, Hashmat, & Rehman, 2008). A 3^2 full factorial design promotes the identification of the effects of all the variables at a time. By this design, influence of the factors and their possible interactions were analyzed. The study involves the use of two independent factors namely β-cyclodextrin and PEG 6000, where three factorial levels were coded as -1, 0, +1 denoting as low, mid and high respectively and 9 possible formulations were selected for experimental trial. In the study, the amounts of β-cyclodextrin (A) and PEG 6000 (B) was chosen as independent factors and the percentage dissolved as the response factor. A 3^2 full factorial design layout and proposed formulation (F1-F9) for experimental trial batch is given in Table 2.2 and Table 2.3.

Table 2.2 3^2 full factorial design layout

Coded value	Actual values	
	A (mg)	B (mg)
-1 (low level)	20	10
0 (mid-level)	30	20
+1 (high level)	40	30

Table 2.3 Proposed formulation of drug, β-cyclodextrin and the carrier PEG 6000

Formulations	Coded value		Proposed ratios	Amount of Drug	Amount of β-cyclodextrin	Amount of PEG 6000
	A	B				
F1	-1	-1	1:2:1	10mg	20mg	10mg
F2	-1	0	1:2:2	10mg	20mg	20mg
F3	-1	+1	1:2:3	10mg	20mg	30mg
F4	0	-1	1:3:1	10mg	30mg	10mg
F5	0	0	1:3:2	10mg	30mg	20mg
F6	0	+1	1:3:3	10mg	30mg	30mg
F7	+1	-1	1:4:1	10mg	40mg	10mg
F8	+1	0	1:4:2	10mg	40mg	20mg
F9	+1	+1	1:4:3	10mg	40mg	30mg

By using Design Expert Software, the relationship between response and independent variables of all the proposed formulations were analyzed. Furthermore, statistical evaluation was performed along with the illustration of 2D contour and 3D surface plots claim the influence of independent variables on response variables and also optimum formulation was selected based on desirability criteria and graphical presentation.

Chapter 3

Result & Discussion:

3.1 Standardization of Fenofibrate

Concentration of 2-10 µg/ml samples were analyzed to record absorbance values under UV-Visible Spectrophotometer at a wavelength of 286nm (Table 3.1) The calibration curve of fenofibrate obtained from the plot of absorbance *versus* concentration was seen to exhibit a linear relationship with a correlation coefficient (R^2) of 0.9904 (Figure 3.1).

Table 3.1: Standardization of Fenofibrate

Concentration (µg/ml)	Absorbance (λ_{max}=286nm)
2	0.104
4	0.214
6	0.333
8	0.426
10	0.495

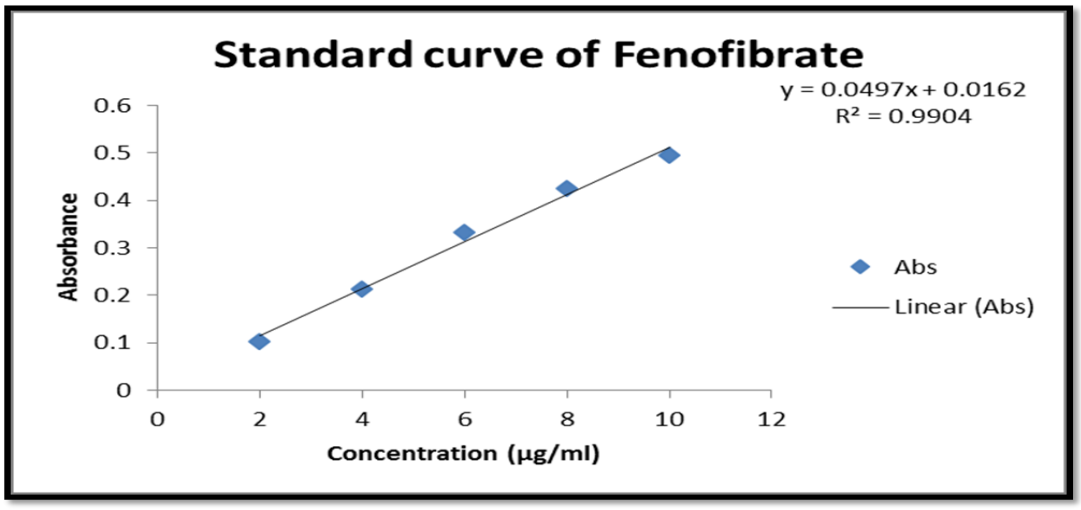

Figure 3.1: Standard curve of Fenofibrate

3.2 Dissolution Study of Fenofibrate (75mg) in Pure Water

In the study, 75mg fenofibrate API was taken and poured into 1000ml of dissolution media. At a time interval of 5, 10, 20, 30 and 45 minutes; 5ml sample was withdrawn and filtered with cotton and then absorbance was determined in UV- Visible Spectrophotometer at a wavelength of 286 nm. By putting the absorbance value in standard curve equation of fenofibrate, concentration and percentage release were calculated as shown in Table 3.2.

Table 3.2 Dissolution Study of Fenofibrate (75mg) in Pure Water

Time	Absorbance	Concentration	% Release
5 min	0.072	1.14 µg/ml	1.52
10 min	0.103	1.77 µg/ml	2.36
20 min	0.086	1.42 µg/ml	1.9
30 min	0.059	0.87 µg/ml	1.17
45 min	0.088	1.46 µg/ml	1.95

From Table 3.2 it was observed that with the increment of time, dissolution of 75mg fenofibrate in pure water showed erratic release rate as the drug is very poorly soluble in aqueous media. As a result, it yields anomalies in the result which depicted the unequal distribution of drug powder within the dissolution medium.

3.3 Dissolution Study of Fenofibrate (75mg) in 0.025M SLS in Pure Water

Again, 75mg fenofibrate API was taken and poured into 1000ml of 0.025M SLS media. 5ml of sample was collected each time at an interval of 5, 10, 15, 20 and 30 minutes and filtered with cotton followed by the determination of absorbance at a fixed wavelength of 286 nm in UV-Visible spectrophotometer. Percentage release was calculated by putting the absorbance value in standard curve equation. As the absorbance value was more than 1, 10 times dilution was carried out for each sample and percentage release was determined as given in Table 3.3.

Table 3.3 Dissolution Study of Fenofibrate (75mg) in 0.025M SLS in Pure Water

Time	Absorbance	Concentration	% Release of drug
5 min	0.111	1.93 µg/ml	25.85
10 min	0.123	2.18 µg/ml	29.11
15 min	0.143	2.59 µg/ml	34.55

20 min	0.178	3.30 µg/ml	44.08
30 min	0.203	3.81 µg/ml	50.88

From the above table it can be observed that 0.025M SLS in pure water media generates an increase in the rate of release of drug with the increase of time which reflects that the use of 0.025M SLS successively increase the rate of release of poorly aqueous soluble drug, fenofibrate.

3.4 Analysis of Saturated Solutions

By using UV-Visible Spectrophotometer at a wavelength of 286 nm, absorbance was measured after filtering the solution with cotton plug.

Table 3.4: Saturation solubility study of Fenofibrate in different solvents

Solvent	Solubility (µg/ml)
PBS 6.2 pH	5.489
PBS 7.2 pH	6.57
Methanol	6.26 (By diluting the solution for 60 times)
Ethanol	5.83 (By diluting the solution for 80 times)
Distilled water	3.87

3.5 Solubility Study of Solid Dispersed Fenofibrate & Beta-cyclodextrin (Ethanol) Dissolved in Pure Water

To analyze the influence of β-cyclodextrin for the purpose of enhancing the solubility of fenofibrate, different ratios of drug and β-cyclodextrin were prepared and listed solubility profile for different ratios indicate that if the amount of excipient increases there is an increment in the solubility of drug. However, remaining in the suspension mood of β-cyclodextrin in ethanol while drug and β-cyclodextrin were mixed together may be one of the possible conflict for further analysis.

Table 3.5 Solubility Assessment of solid dispersed Fenofibrate and Beta-Cyclodextrin

Drug: Beta-cyclodextrin	Absorbance (λ_{max}=286nm)	Solubility (µg/ml)
1:0.5	0.493	9.73
1:1	0.733	14.63

1:1.5	0.87	17.4
1:2	0.967	19.4

3.6 Analysis of Solubilty of Solid Dispersed Fenofibrate and Beta-cyclodextrin (Water + Isopropyl Alcohol) dissolved in pure water

Solid dispersed powder of both drug and β-cyclodextrin of 1:2 and 1:3 ratios show per ml solution contains 12µg and 17µg of drug which renders that with the increasing proportion of β-cyclodextrin solubility is also increased. There exists a minor change in solubility under acidic condition which indicates that changing the pH of the sample does not influence the solubility rate.

Table 3.6 Solubility Analysis of Solid Dispersed Fenofibrate and Beta-cyclodextrin

Drug: Beta cyclodextrin	Absorbance (λ_{max}=286nm)	Solubility	Absorbance in Acidic pH	Solubility in Acidic pH
1:2	0.622	12.36 µg/ml	0.603	12.08 µg/ml
1:3	0.833	17.69 µg/ml	0.802	16.04g/ml

3.7 Assessment of Solubility of Drug, Beta-cyclodextrin & PEG 6000 of Different Ratios

In the study, variation in the amount of PEG 6000 was analyzed in order to identify the effect of hydrophilic carrier, PEG 6000 in solid dispersion of fenofibrate. The percent of drug release positively increased with the increasing proportion of PEG 6000 as given in Table 3.7.

Table 3.7 Solubility Analysis of Drug, Beta-cyclodextrin & PEG 6000 of Different Ratios

Formulation	Coded value		Drug: β-cyclodextrin: PEG 6000	Absorbance (λ_{max}=286nm)	Concentration (µg/ml)	% of Drug Release
	A	B				
F4	0	-1	1:3:1	0.778 (After 5 times dilution of stock solution)	15.55	15.55

F5	0	0	1:3:2	0.662 (After 10 times dilution of stock solution)	13.18	26.36
F6	0	+1	1:3:3	0.684 (After 10 times dilution of stock solution)	13.63	27.26

3.8 Optimization of Formulation Using 3^2 Full Factorial Design

3.8.1 Summary of the Design

Using a 3^2 full factorial through Design Expert Software where Factor 1 and Factor 2 namely β-cyclodextrin and PEG 6000 respectively were taken as independent variables. Levels were set from the range of -1 to 1 where -1 denoted the lower, 0 denoted medium and 1 denoted higher level followed by the input of percentage of drug dissolved to analyze the response due to the independent variables.

Table 3.8 The Actual design of the experiment:

Std	Run	Factor 1 A: Beta-cyclodextrin %	Factor 2 B: PEG 6000 %	Response 1 Dissolved percentage %
8	1	0	1	17.44
3	2	1	-1	7.12
4	3	-1	0	17.12
5	4	0	0	14.26
6	5	1	0	8.26
10	6	0	0	14.26
9	7	1	1	13.34
2	8	0	-1	8.1
1	9	-1	-1	7.04
7	10	-1	1	19.73

3.8.1.1 Graph Column

The Graph column involves determination of correlation among percentage of drug release with respect to β-cyclodextrin and PEG 6000. Figure 3.2 represents the plot of Factor 1 (β-cyclodextrin) *versus* dissolved percentage of drug which yielded a correlation value of -0.429 indicating that β-cyclodextrin is weakly correlated with the dissolved percentage of drug. Alternatively, Figure 3.3

reflects that Factor 2 (PEG 6000) *versus* dissolved percentage of drug, giving a correlation value of 0.821 which indicates a strong correlation of PEG 6000 with the dissolved percentage of drug.

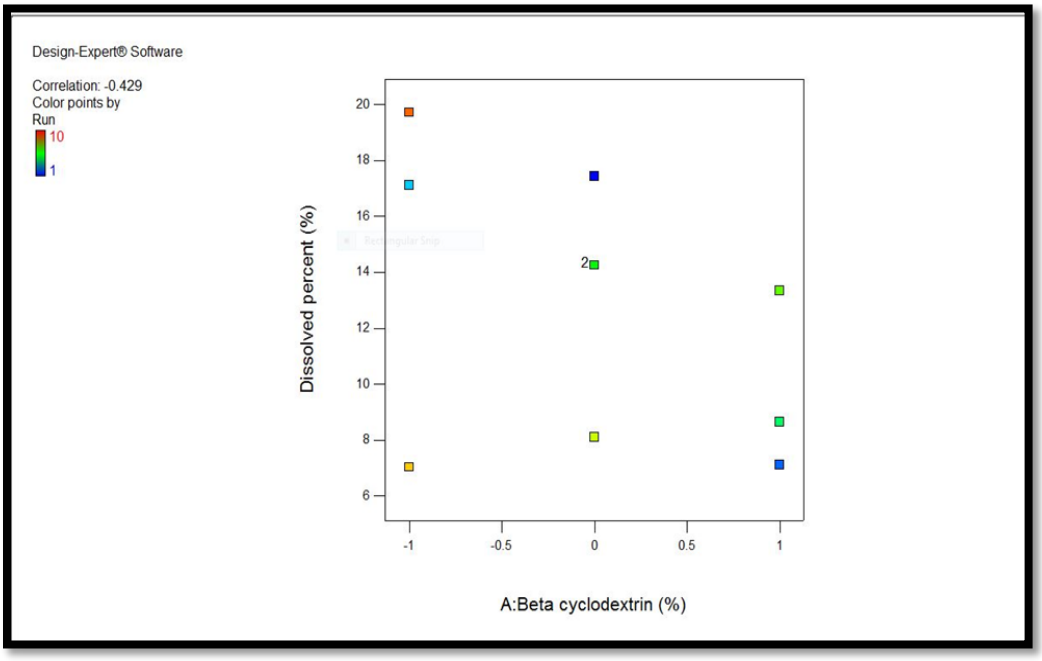

Figure 3.2 The Graph column of Beta-cyclodextrin

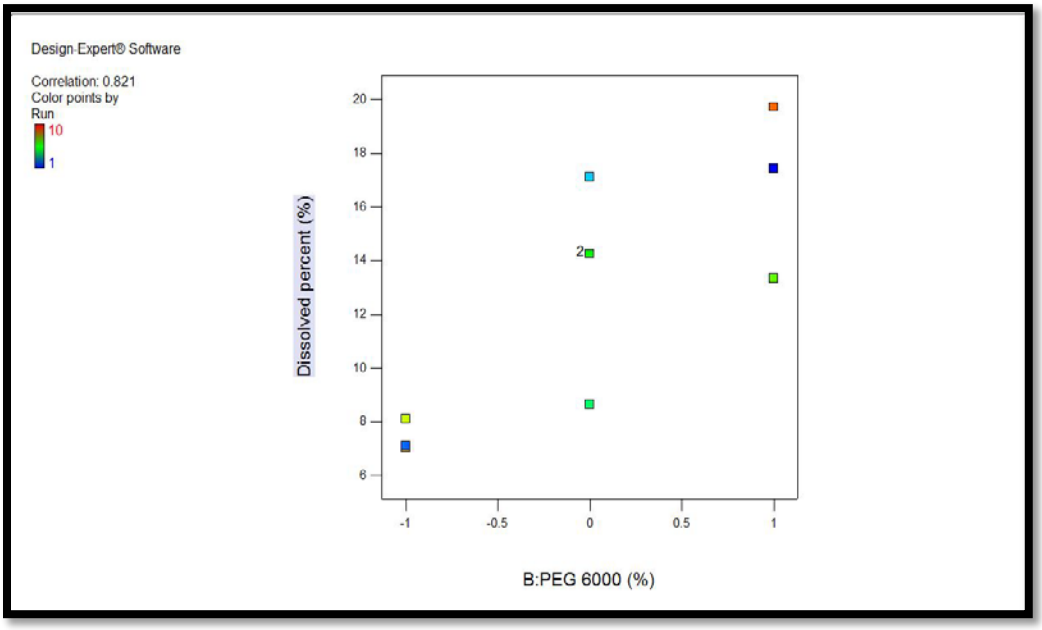

Figure 3.3 The Graph column of PEG 6000

3.8.2 Analysis of the Full Factorial Design

Statistically the targeted parameters were analyzed and polynomial terms were used to evaluate the response through the following equation-

$$Y = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$$

where,

Y is the dependent variable,

A, B are the coded levels of the independent variable,

b_0 is the intercept representing the arithmetic mean response of the 9 runs,

b_1 to b_5 are the estimated coefficients computed from the observed experimental response values of Y.

The primary impacts (A and B: Linear) indicates to the average effect of transforming one variable at once from its low to high values. The collaboration terms (AB: 2 FI) demonstrate how the response changes when two factors are simultaneously changed. The polynomial terms (A^2 and B^2: Quadratic) are incorporated to examine nonlinearity of the model.

3.8.2.1 Response of Dissolved Percent of Drug

A. Statistical Model Analysis:

In order to determine the statistical significance of the influence of β-cyclodextrin and PEG 6000 on percentage the of drug dissolved, the probability value (p-value) was evaluated for each source of terms (intercept, A, B, AB, A^2, B^2). The Design Expert Software depicted that the response, dissolved percentage of drug followed a linear model (Figure 3.4). Moreover, the lack of fit test performed by the software points out less lack of fitness for the linear model (Figure 3.5). The analysis of variance (ANOVA) at a significant level of 5% was also carried out to analyze the response of dissolved percentage of drug statistically. From the ANOVA for response surface linear model, the p-value was observed to be 0.0011 suggesting that the model is significant and there will be less chance of getting erratic results (Figure 3.6).

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	1614.42	1	1614.42			
Linear vs Mear	169.42	2	84.71	21.16	0.0011	Suggested
2FI vs Linear	10.47	1	10.47	3.58	0.1074	
Quadratic vs 2	8.03	2	4.02	1.69	0.2941	
Cubic vs Quad	9.42	2	4.71	96.89	0.0102	Aliased
Residual	0.097	2	0.049			
Total	1811.86	10	181.19			

Figure 3.4 Suggested statistical model

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Linear	28.02	6	4.67		
2FI	17.55	5	3.51		
Quadratic	9.52	3	3.17		
Cubic	0.097	1	0.097		
Pure Error	0.000	1	0.000		

"Lack of Fit Tests": Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.00	0.8581	0.8175	0.6444	70.20	Suggested
2FI	1.71	0.9111	0.8666	0.5882	81.29	
Quadratic	1.54	0.9518	0.8915	0.4681	105.02	
Cubic	0.22	0.9995	0.9978	0.9307	13.68	Aliased

Figure 3.5 Fit summary test

Response 1 Dissolved percent

ANOVA for Response Surface Linear model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	169.42	2	84.71	21.16	0.0011	significant
A-Beta cyclo	*36.41*	*1*	*36.41*	*9.10*	*0.0195*	
B-PEG 6000	*133.01*	*1*	*133.01*	*33.23*	*0.0007*	
Residual	28.02	7	4.00			
Lack of Fit	*28.02*	*6*	*4.67*			
Pure Error	*0.000*	*1*	*0.000*			
Cor Total	197.44	9				

Figure 3.6 ANOVA statistical model

B. Mathematical Model Analysis:

The polynomial equation demonstrates the quantitative impact of factors (A and B) upon the response. Coefficients of the factors (A or B) indicates the impact of that specific factor on the response. A positive sign before the terms demonstrates quickening impacts while the negative sign shows hindering impacts of the factor.

The predicted equation for Y in terms of coded factors is shown below:

$$Y = 12.706 - 2.4633A + 4.70833B$$

The above equation is a linear equation and indicates that coefficient b_1 bears a negative sign which implies that "A" diminished the response by 2.4 times. Therefore, expanding the measure of β-cyclodextrin will take longer time frame to discharge medication and in this manner retarded the release rate. On the contrary, coefficient b_2 bear positive sign showing that "B" expanded the response by 4.7 times demonstrating a more prominent effect on drug release compared to Factor "A".

C. Response Surface Analysis:

The 2D contour plot and 3D surface plot (Figure 3.7A and 3.7B) also supported the linear statistical and mathematical model suggested by the software. From the bar beside the 2D contour plot, it can be seen that red indicates highest percentage of drug release and blue indicates the lowest

percentage of drug release. It is evident from the 2D and 3D plot that with decrease in amount of β-cyclodextrin and with increase in amount of PEG 6000, the blue zone moves towards red zone. This indicates that PEG 6000 has relatively a greater impact in enhancing solubility of fenofibrate than β-cyclodextrin and that the solubility is increased in a linear manner.

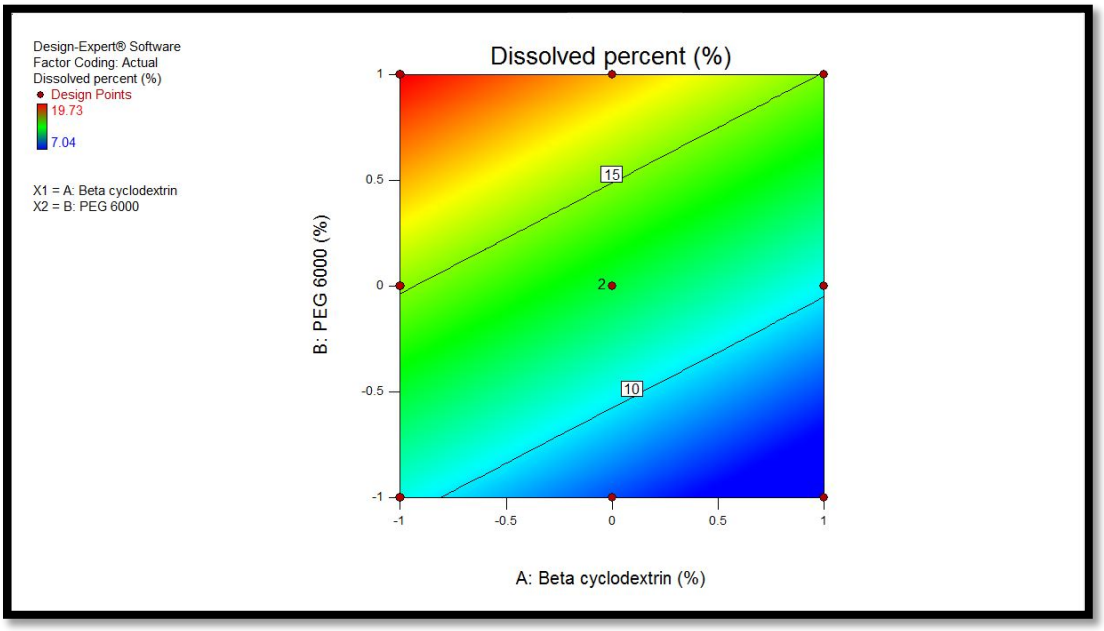

Figure 3.7A: 2D-Contour plot of drug release

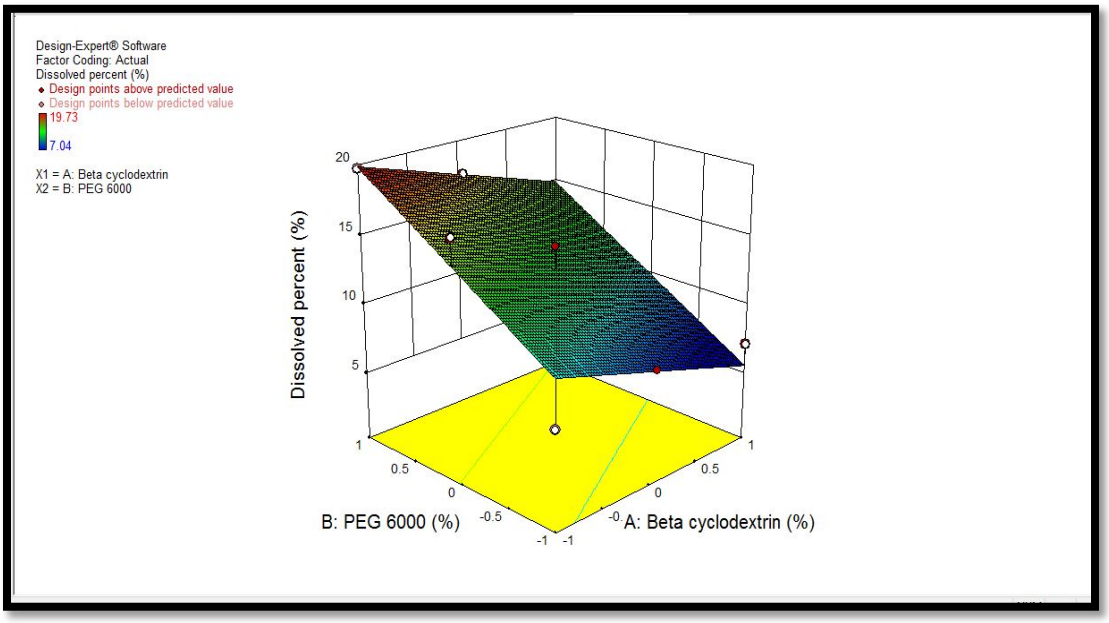

Figure 3.7B: 3D Surface plot of drug release

D. Normal Plot Analysis:

The diagnostic- normal plot of residuals determines the linearity of data points and the practical response values of all the formulations were found in close proximity with the best fit line drawn, thereby, indicating linearity in response values at different ratios of β-cyclodextrin and PEG 6000 (Figure 3.8).

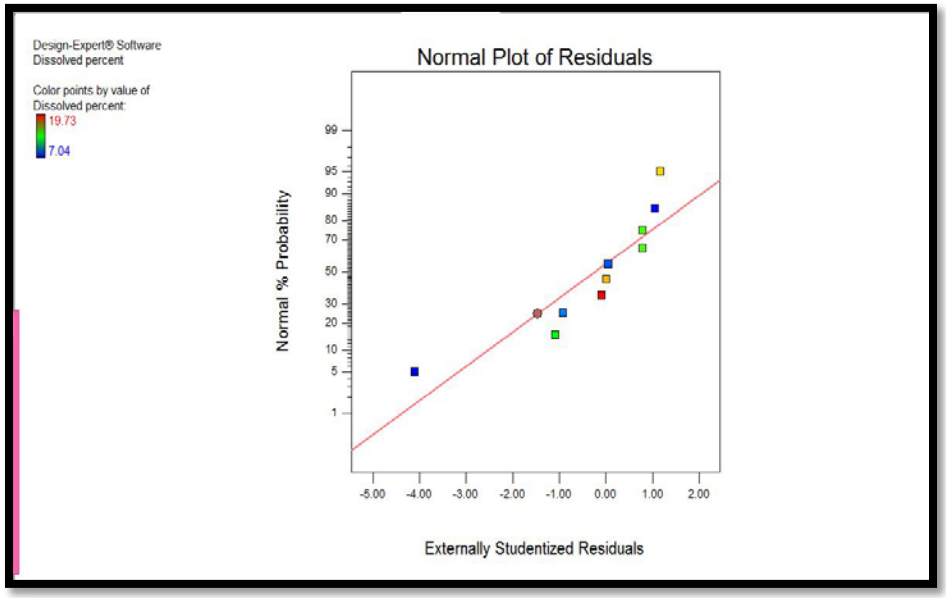

Figure 3.8 Normal plot of residuals

Response	Intercept	A	B		
Dissolved per...	12.706	-2.46333	4.70833		
p=		0.0195	0.0007		
Legend		p <.01	.01<= p <.05	.05<= p <.10	p >=.10

Figure 3.9 Summary of the response

3.8.3 Optimization of the Formulation

To obtain an optimum formulation, analysis of the response was done numerically and from that an optimum formulation was chosen with desired response. The optimum coded levels of each independent variable relied on desirability criterion and graphical examination. The overlay plot which was acquired showed a yellow area for possible optimum formulations (Figure 3.11). Depending on the desirability criterion and graphical analysis of the formulation

(Figure 3.10 & Figure 3.11), the optimum formulation was chosen to be F3 in which the observed responses were found to be in close agreement with the predicted values for the optimized formulation (Table 3.9).

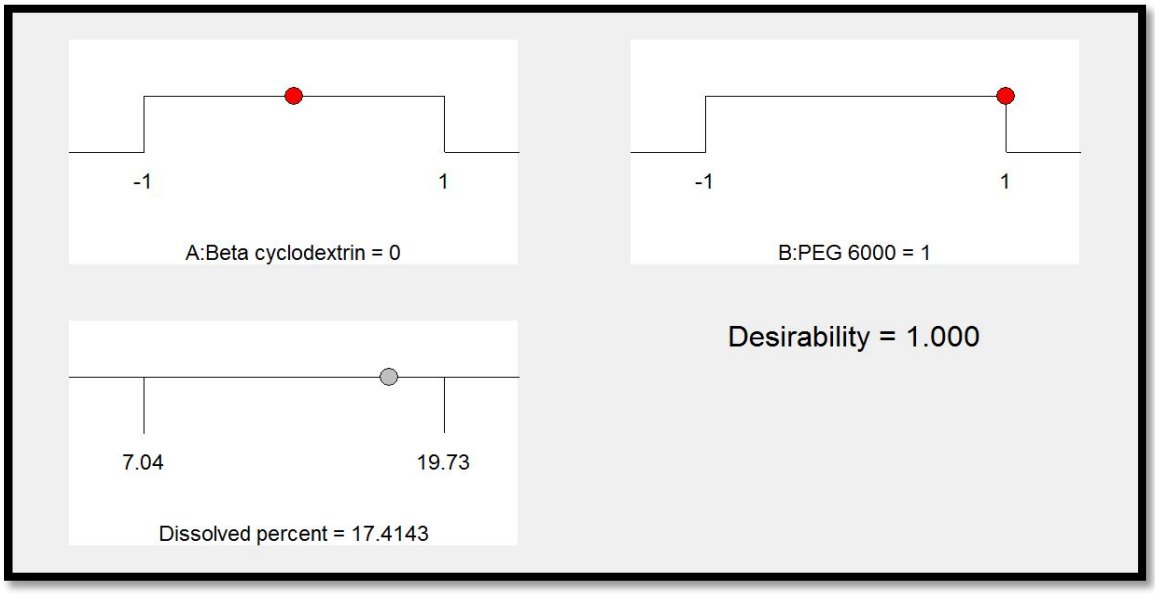

Figure 3.10 Optimum formulation with predicted drug release

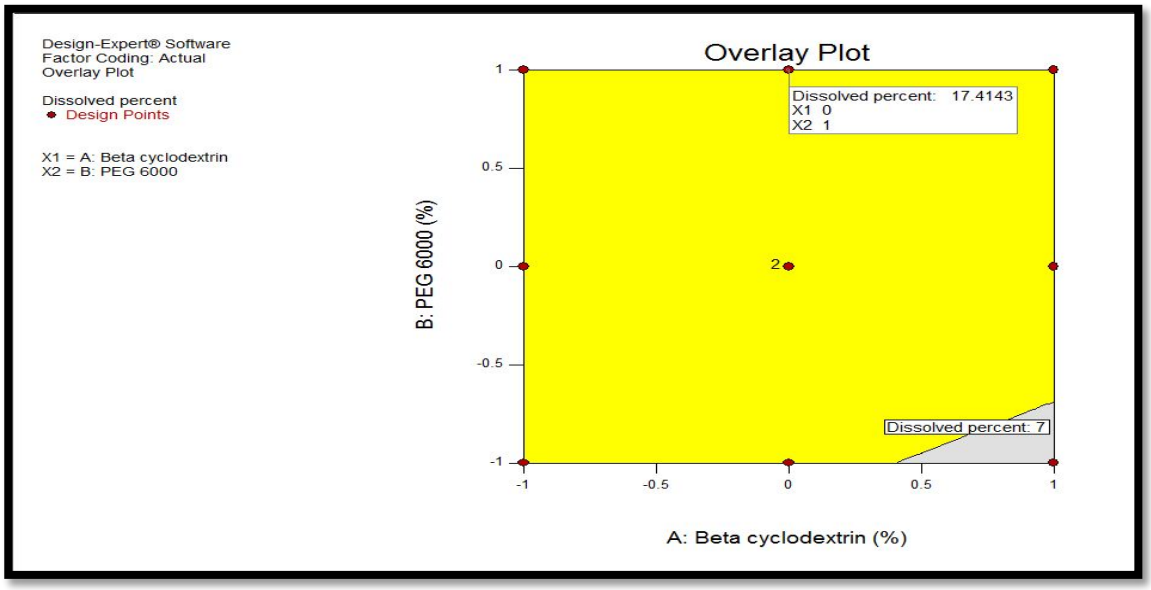

Figure 3.11 The Overlay plot of optimum area

Table 3.9 Composition of the optimum formulation with the predicted and experimental values of response variables

Composition of drug : Beta-cyclodextrin : PEG 6000	Response Variables	Experimental Value (%)	Predicted Value (%)	Prediction Error (%)
F6= 1:30:30	Dissolved Percentage	16.85	17.41	3.36

Chapter 4

Conclusion:

The main aim of the present study was to improve the solubility of fenofibrate which was successfully performed. The design of the experiment was first to establish a calibration curve followed by saturation solubility study of fenofibrate in different solvents, followed by the formation of inclusion complex and incorporation of a hydrophilic carrier, PEG 6000. The study also included the evaluation of release rates of the formulation. Finally, optimization process was employed in order to achieve an optimum formulation by applying the data in Design Expert Software. The solubility of the optimum formulation of fenofibrate (F6) was found to have been significantly enhanced.

Appendix 1

Fenofibrate

Mechanism of Action

Fenofibrate is a lipid regulating agent which exerts its therapeutic action via the activation of peroxisome proliferator-activated receptor type alpha (PPARα). It enhances the lipolysis and elimination of triglyceride- rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein CIII. The resulting fall in triglycerides produces an alteration in the size and composition of LDL from small, dense particles, to large buoyant particles. These larger particles possess greater affinity for cholesterol receptor, thus faster the catabolism.

Pharmacodynamics

Fenofibrate is indicated as adjunctive therapy to diet to reduce elevated LDL-C, Total-C, Triglycerides and Apo B and to increase HDL-C in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson Types IIa and IIb). In addition, it is used in adult patients with hyperglyceridemia (Fredrickson Types IVand V hyperlipidemia). The active metabolite of fenofibrate named fenofibric acid causes reduction in total cholesterol, LDL cholesterol, apolipoprotein B, total triglycerides and triglyceride rich lipoprotein (VLDL) in treated patients. Furthermore, treatment with fenofibrate results in increases in high density lipoprotein (HDL) and apoproteinsapo AI and apo AII.

Clinical Pharmacology

Absorption: Being insoluble in aqueous media, the absolute bioavailability of fenofibrate cannot be determined. However, fenofibrate is well absorbed from the gastrointestinal tract.

Distribution: Steady-state plasma levels of fenofibric acid were achieved within a week of dosing and did not demonstrate accumulation across time following multiple dose administration in healthy volunteers. Serum proteinbinding was approximately 99% in normal and hyperlipidemic subjects.

Metabolism: Following oral administration, fenofibrate is rapidly hydrolyzed by esterases to the active metabolite, Fenofibric acid; no unchanged Fenofibrate is detected in plasma.

Excretion: After absorption, fenofibrate is mainly excreted in the urine in the form of metabolites, primarily fenofibric acid and fenofibric acid glucuronide. After administration of radiolabelled

fenofibrate, approximately 60% of the dose appeared in the urine and 25% was excreted in the feces.

Adverse Effects of Fenofibrate

Fenofibrate is associated with rare but some dangerous adverse effects including Abdominal Pain, Back Pain, Headache, Asthenia, Flu Syndrome, Diarrhea, Constipation, Liver Function Tests Abnormal Respiratory Disorder.

Appendix 2

Cyclodextrin

Applications of Cyclodextrins

Since each guest molecule is individually surrounded by a cyclodextrin (derivative) the molecule is micro-encapsulated from a microscopical point of view. This can lead to several advantageous changes in the chemical and physical properties of the guest molecules such as-

• Improvement of solubility of substances

• Modification of the chemical reactivity of guest molecules.

• Fixation of very volatile substances.

• Stabilisation of light- or oxygen-sensitive substances

• Modification of liquid substances to powders.

• Protection against degradation of substances by microorganisms.

• Masking of ill smell and taste.

• Masking pigments or the colour of substances.

• Catalytic activity of cyclodextrins with guest molecules.

These characteristics of cyclodextrins or their derivatives make them suitable for applications in analytical chemistry, the pharmaceutical field and many more (Singh, Sharma, & Benerjee, 2002).

Appendix 3

PEG 6000

Application of PEG 6000

Some of the applications of PEG 6000 are represented below (Brummer, 2011)

• Used as a lubricating coating for various surfaces in aqueous and non-aqueous conditions

• Enhances hybridization rate of nucleic acids

• Used to create very high osmotic pressure

• Used in ligation of blunt ended DNA

• Precipitates bacteriophage ± particles and plasmid DNA

• Used in preparing single-stranded M13 DNA

• For industrial purpose it is used as polymer

• Promotes cell fusion

Appendix 4

Actual Design and Summary of the Experiment

Select	Std	Run	Factor 1 A:Beta cyclo... %	Factor 2 B:PEG 6000 %	Response 1 Dissolved pe... %
	8	1	0	1	17.44
	3	2	1	-1	7.12
	4	3	-1	0	17.12
	5	4	0	0	14.26
	6	5	1	0	8.65
	10	6	0	0	14.26
	9	7	1	1	13.34
	2	8	0	-1	8.1
	1	9	-1	-1	7.04
	7	10	-1	1	19.73

Figure 3.12 The Actual Design of the experiment

Design Summary

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Study Type Response Surface Subtype Randomized

Design Type 3 Level Factorial Runs 10

Design Mode Quadratic Blocks No Blocks Build Time (r 48.00

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Coded	Values	Mean	Std. Dev.
A	Beta cyclodex	%	Numeric	Continuous	-1	1	-1.000=-1	1.000=1	0	0.816497
B	PEG 6000	%	Numeric	Continuous	-1	1	-1.000=-1	1.000=1	0	0.816497

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
R1	Dissolved perc	%	10	Polynomial	7.04	19.73	12.706	4.68373	2.80256	None	Linear

Figure 3.13 Summary of the Design

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