T.C. YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

STUDIES ON CYCLODEXTRIN COMPLEXATION OF A POORLY WATER SOLUBLE ANTI-HYPERLIPIDEMIC DRUG, TABLET FORMULATION AND CHARACTERIZATION

MASTER THESIS

NEJVA KAİD, MSc

ISTANBUL-2021

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THESIS APPROVAL FORM

Institute : Yeditepe University Institute of Health Sciences

Program : Master in Drug and Cosmetics Production Technologies

Title of the Thesis : Studies on cyclodextrin complexation of a poorly water-soluble anti-hyperlipidemic drug, tablet formulation and characterization.

Owner of the Thesis : Nejva KAİD, MSc.

Examination Date : 12.07.2021

This study has approved as a Master Thesis in content and quality by the Jury.

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APPROVAL

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgement has been made in the text.

12.07.2021

Nejva KAİD, MSc

DEDICATION

To my precious family…

ACKNOWLEDGEMENTS

I would first like to give my sincerest gratitude to my advisor Dr. Muhammed Abdur Rauf, for his guidance, time, knowledge and support.

Then, I would also like to express my thanks to all my friends and to everyone at the Pharmaceutical Technology Department, Faculty of Pharmacy, Yeditepe University who helped me in many different ways.

Finally, my greatest thanks go to my mother, father, sister, and brothers for their unconditional and never-ending support of all types.

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LIST OF SYMBOLS AND ABBREVIATIONS

α-CD	Alpha-cyclodextrin
β-CD	Beta-cyclodextrin
γ-CD	Gamma-cyclodextrin
µg	Microgram
µg/mL	Microgram per milliliter
µm	Micrometer
apoC-III	Apolipoprotein ciii
AUC	Area under the curve
BBB	Blood–brain barrier
BF	Bezafibrate
C_a	Added concentration
CAR	Chimeric antigen receptor
CDs	Cyclodextrin(s)
C_f	Average found concentration
CGTase	Cyclodextrin glycosyltransferase
CNS	Central nervous system
Coeff.	Coefficient
DI-PAC®	Direct compacting & tableting sugar, SEPPIC, France
DNA	Deoxyribonucleic acid
DSC	Differential scanning calorimetry
e.g.	Exampli gratia = for example
FDA	Food and drug administration
FTIR	Fourier transform infra-red (spectroscopy)
g	Gram
GIT	Gastrointestinal tract
h	Hour
HbA1C	Haemoglobin a1c
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HP-β-CD/ HPβCD	Hydroxypropyl-β-cyclodextrin
HSA	Human serum albumin
IUPAC	International union of pure and applied chemistry
K	Stability constants
L/h	Liter per hour
LDL	Low-density lipoprotein

LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
min	Minute
mL	Milliliter
mM	Millimole per liter
mRNA	Messenger ribonucleic acid
NFκB	Nuclear factor kappa light chain enhancer of activated B cells
NIDDM	Non-insulin-dependent diabetes mellitus
nm	Nanometer
PM	Physical mixture
PPARα	Peroxisome proliferator-activated receptor alpha
RNA	Ribonucleic acid
rpm	Rotation per minute
RXR	Retinoid X receptor
SBE-β-CD	Sulfobutylether-beta-cyclodextrin
SEM	Scanning electron micrograph
TC	Terephthaloyl chloride
UV	Ultra-violet
VLDL	Very low-density lipoprotein
w/v	Weight per volume

ABSTRACT

Kaid N. (2021). Studies on Cyclodextrin Complexation of a Poorly Water Soluble Anti-hyperlipidemic Drug, Tablet Formulation and Characterization. Yeditepe University, Institute of Health Sciences, Department of Pharmaceutical Technology, MSc Thesis, Istanbul. The aims of the present study were to study the complexation of a poorly water-soluble anti-hyperlipidemic drug, bezafibrate (BF), with different cyclodextrins (CDs) and to formulate the complexes as tablet dosage form. Alpha-cyclodextrin (αCD), beta-cyclodextrin (βCD), gamma-cyclodextrin (γCD), and hydroxypropyl-β-cyclodextrin (HPβCD) were used for these purposes. Continuous stirring of drug with CD solutions followed by filtration and lyophilization was performed for the complexation. Initial studies showed BF solubility increase with all the CDs and equilibrium complexation period was 2 days. Experiments with aqueous medium of pH 7.0, 4.0, and 1.5 showed that acidic pH drastically reduced intrinsic solubility of BF but increases complexation efficiency. Experiment at 60˚C increased complexation solubility but reduced the stability of the complexes. Overall, βCD and HPβCD showed better solubilization than αCD and γCD. As solubility of HPβCD is much higher (> 33 g/100 mL water) than 25 mM tested, solubility of BF can presumably be increased proportionally. FTIR, DSC and SEM studies confirmed complexation of BF with all the CDs tested. The complexes of BF with βCD (BFβCD) and HPβCD (BFHPβCD) were formulated with two different directly compressible vehicles, namely, Avicel PH102 (microcrystalline cellulose) and DiPac (compressible sugar). Both types of tablets were of good quality when evaluated by quality attributes like thickness, diameter, hardness, friability, disintegration time and dissolution characteristics. In tablets, cyclodextrin played the dual roles of solubility enhancer and filler reducing the requirement of directly compressible vehicles. Lyophilized BFHPβCD produced very hard and non-friable tablets without negatively affecting disintegration or dissolution. Finally, dissolution test showed that complexation of bezafibrate with βCD and HPβCD exhibited an enhanced dissolution rate in comparison to pure drug. The same observation were also found with tablets; both Avicel and DiPac tablets with complexes showed superior dissolution profiles compared to tablets with pure drug or physical mixture of drug with cyclodextrins. These dissolution profiles were tested with similarity factor, f_2, which showed the similarity of dissolution between βCD and HPβCD complexes or tablets containing these complexes but both were different from those of non-complex powder or tablet formulation.

Key words: Bezafibrate, cyclodextrin, solubility, inclusion complex, tablet

ÖZET

Kaid N. (2021). Suda Çok Az Çözünen Hipolipidemik Bir İlaç ile Siklodekstrin Kompleksasyonu, Tablet Formülasyonu ve Değerlendirmesi Üzerine Çalışmalar. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmasötik Teknoloji ABD, Yüksek Lisans Tezi, İstanbul. Bu çalışmanın amacı, suda az çözünen bir anti-hiperlipidemik ilaç olan bezafibrat'ın (BF) farklı siklodekstrinler (CD'ler) ile kompleksleşmesini incelemek ve kompleksleri tablet dozaj formu olarak formüle etmektir. Bu amaçlar için alfa-siklodekstrin (αCD), beta-siklodekstrin (βCD), gama-siklodekstrin (γCD) ve hidroksipropil-β-siklodekstrin (HPβCD) kullanılmıştır. İlaç, CD çözeltileri ile sürekli karıştırılması, ardından filtrasyon ve liyofilizasyon yapılarak kompleks oluşturması gerçekleştirilmiştir. İlk çalışmalar, tüm CD'ler ile BF çözünürlüğünün arttığını göstermiş ve denge kompleksleşme süresi 2 gün olarak belirlenmiştir. pH 7.0, 4.0 ve 1.5 olan sulu ortam ile yapılan deneyler, asidik pH'ın BF'nin içsel çözünürlüğünü büyük ölçüde azalttığını ancak kompleksleşme verimliliğini arttırdığını göstermiştir. 60°C'deki deney, kompleksleşme çözünürlüğünü arttırmış, ancak komplekslerin stabilitsini azaltmıştır. Genel olarak, βCD ve HPβCD; αCD ve γCD'ye göre daha iyi çözünürlük artışı göstermiştir. HPβCD'nin çözünürlüğü test edilen 25 mM'den çok daha yüksek (> 33 g/100 mL su)) olduğundan, BF'nin çözünürlüğü muhtemelen orantılı olarak arttırılabilir. FTIR, DSC ve SEM çalışmaları, test edilen tüm CD'lerle BF'nin kompleksleştiğini doğrulamıştır. BF'nin βCD (BFβCD) ve HPβCD (BFHPβCD) ile kompleksleri, iki farklı doğrudan sıkıştırılabilir eksipien, yani Avicel PH102 (mikrokristalin selüloz) ve DiPac (sıkıştırılabilir şeker) ile formüle edilmiştir. Her iki tablet türü de kalınlık, çap, sertlik, gevreklik, dağılma süresi ve çözünme özellikleri gibi kalite özellikleriyle değerlendirildiğinde iyi kalitede olduğu görlmütür. Tabletlerde, siklodekstrin, doğrudan sıkıştırılabilir eksipienlerin gereksinimini azaltıp çözünürlük arttırıcı ve dolgu maddesi olarak ikili rol oynamıştır. Liyofilize BFHPβCD, parçalanmayı veya çözünmeyi olumsuz etkilemeden çok sert ve gevrek olmayan tabletler üretmiştir. Son olarak, çözünme testi, bezafibrat'ın βCD ve HPβCD ile kompleksleşmesinin, saf ilaca kıyasla üstün bir çözünme hızı sergilediğini göstermiştir. Aynı gözlem tabletlerde de bulunmuş; kompleksleri içeren hem Avicel hem de DiPac tabletleri, saf ilaç içeren tabletlere veya ilacın siklodekstrinlerle fiziksel karışımına kıyasla üstün çözünme profilleri göstermiştir. Bu çözünme profilleri, benzerlik faktörü, f_2, ile test yaplmış ve bu f_2 testi, βCD ve HPβCD kompleksleri veya bu komplekleri taşıyan tabletleri arasındaki çözünme benzerliğini, ama her ikisi de kompleks olmayan toz veya bu tozları taşıyan tablet formülasyonununkinden farklı olduğunu göstermiştir.

Anahtar Kelimeler: Bezafibrat, siklodekstrin, çözünürlük, inklüzyon kompleksi, tablet

1. INTRODUCTION AND OBJECTIVES

Bezafibrate is a lipid-lowering drug. It is practically insoluble in water. In hypertriglyceridaemia patients, triglyceride is reduced substantially by bezafibrate dosage of 200 mg thrice daily or 400 mg sustained-release preparation once daily. Similarly in patients with hypercholesterolaemia, cholesterol is reduced substantially by the same dosage levels (1).

Cyclodextrins (CDs) are produced from starch or starch derivative by the action of the enzyme CD glycosyltransferase. Structurally CDs are cyclic oligosaccharides in which α -D-glucopyranose units are linked by (α-1-4)-linkages. There are three natural type (non-derivatized) of CDs. They are called alpha-cyclodextrin (α-CD) having 6 glucose unit, beta-cyclodextrin (β-CD) with 7 glucose unit and gamma-cyclodextrin (γ-CD) containing 8 glucose units. They all have a shape which resemble truncated cone. The empty space in the middle of the molecules is hydrophobic in nature whereas outer surface is hydrophilic. With this physical shape, they are able to accept suitable hydrophobic other molecules or part of other molecules inside the central empty space, thus forming a non-covalently bonded host-guest complex. Such complexation offer many advantages including increase in water solubility of the guest molecule, suitable dissolution properties and enhancement of bioavailability. As a result, they have gained the status of pharmaceutical excipients (2).

Oral drug application is the simplest and easiest and so most commonly used way of drug administration. Among the orally administered dosage forms, tablets have advantages over other dosage forms because of the greater stability, lesser bulk, accurate dosage and cheaper cost of production. Most of the recently discovered drugs are poorly water soluble. Such drugs are generally difficult to process. They have dissolution problems and sometimes bioavailability problems, and thus this may lead to decreased inherent efficiency of drugs. Therefore, formulator try different techniques to improve drug solubility and it one of the most challenging aspects of overall R&D operations (3).

In this research work, the objectives are to study the complexation of bezafibrate with three natural cyclodextrin and a hydroxypropyl derivative of beta-cyclodextrin, characterize the complexes, formulate them as tablet dosage form, and evaluate the tablets by different quality control tests, especially the dissolution test.

2. GENERAL INFORMATION

2.1. Bezafibrate

Bezafibrate is a derivative of a known fibrate compound as a class of lipid-lowering drugs (Figure 2.1). Bezafibrate is a white or almost white crystalline powder. It is practically insoluble in water, freely soluble in dimethylformamide, sparingly soluble in acetone and in ethanol 96%. It dissolves in dilute solutions of alkali hydroxide (4,5).

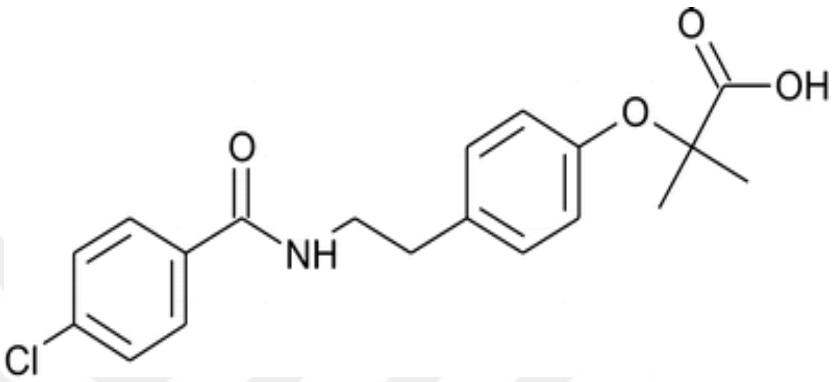


Figure 2.1. Chemical Structure of Bezafibrate (5)

USAN (generic name): Bezafibrate

IUPAC Name: 2-(4-{2-[(4-chlorobenzoyl) amino] ethyl} phenoxy)-2-methylpropanoic acid.

Chemical formula: C19 H20 Cl N O4

Molecular weight: 361.823

Melting Point: 186° C

CAS Registry Number: 41859-67-0

Water Solubility: 0.00155 mg/mL

pKa (Strongest Acidic): 3.83

pKa (Strongest Basic): -0.84

2.1.1. Discovery

The main compound, clofibrate, was discovered during the screening of aryloxy acetic acid derivatives and licensed for human usage in 1962. Since then, the second and third-generation compounds that show higher potency and fewer side effects have been developed. These include: bezafibrate, etofibrate, ciprofibrate, gemfibrozil and

fenofibrate (Figure 2.2) (6). In the mid-1950s the first fibrates were synthesized. Other fibrates were further introduced in the late 1970s and early 1980s, such as bezafibrate and ciprofibrate in Europe and gemfibrozil in the United States (7).

2.1.2. Pharmacological Properties

Bezafibrate significantly reduces triglyceride levels by stimulating lipoprotein lipase activity, and, to a minor extent, hepatic triglyceride lipase. Bezafibrate boosts triglyceride-rich lipoproteins catabolism. Levels of high-density lipoprotein (HDL) cholesterol, particularly the HDL3 fraction, are raised, as well as those of the attendant lipoprotein's apolipoprotein AI and All. However, the effects on other apolipoproteins are less documented. Bezafibrate also reduces low density lipoprotein (LDL) and total cholesterol levels, possibly by enhancing the removal of receptor-bound LDL particles. During the treatment with bezafibrate, Apolipoprotein B associated with LDL cholesterol usually is reduced, but may be elevated in some patients with type lIb or IV dyslipidaemia. In some researches, bezafibrate decreased levels of atherogenic LDL particles and increased resistance of LDL to oxidation in vitro. Bezafibrate lowers lipoprotein (a) [Lp(a)] levels in patients with abnormally high baseline values (8). Moreover, high plasma fibrinogen levels are consistently lowered with bezafibrate and also reduces platelet aggregability and blood viscosity in vitro (9).

Hyperlipidemia and non-insulin-dependent diabetes mellitus (NIDDM) patients receiving bezafibrate showed unchanged or improved metabolic control as evaluated by fasting glucose level, insulin and glycosylated hemoglobin (HbAlc). With this effect, oral hypoglycemic drugs could be reduced in some patients. In few cases, reduction of free fatty acid levels have also been observed (10).

2.1.3. Mechanism of Action

Effects on lipids and lipoproteins

The primary mode of action of the fibrates is by activating the nuclear transcription factor PPARα, which is mostly expressed in tissues that metabolize fatty acids, such as the liver, heart, kidney, and muscle. Fibrates might also interact with other PPAR receptors to differing degrees. Upon activation by binding of the fibrate, PPARα binds as heterodimers with retinoid X receptor (RXR), which then recognizes and binds to specific PPARα response elements, leading to modulation of expression of the target

genes (Figure 2.2) (11). In particular, lipoprotein lipase activity increased and synthesis of apolipoprotein CIII (apoC-III) is decreased, which together improve the clearance of circulating triglyceride-rich lipoproteins. Hepatic fatty acid oxidation also increased leading to reduced triglyceride - rich very low-density lipoprotein (VLDL) production (Table 2.1). Additionally, fibrates promote a shift in the density of LDL particles towards larger, more buoyant particles that are less susceptible to oxidation and have increased affinity for the LDL receptor (12,13).

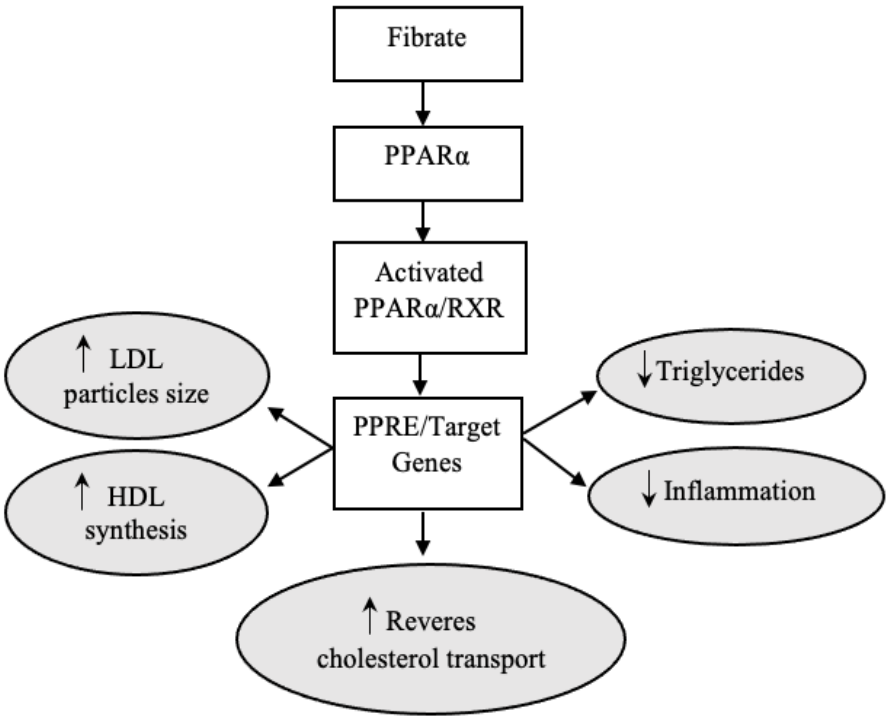


Figure 2.2. Schematic representation of the mechanism of action of fibrates (11)

Moreover, fibrates exert pleiotropic effects in the artery wall. PPARα is implicated in the control of the anti-inflammatory response, through inhibition of the transcription factor nuclear factor kappa light chain enhancer of activated B cells(NFκB) (14). Certainly, fibrates can alleviate the manufacture of pro-inflammatory stimuli such as interleukin6 and various prostaglandins, in addition to the acute phase proteins, including fibrinogen and C-reactive protein (15,16). Fibrates have beneficial effects on fibrinolysis and coagulation. Bezafibrate, Ciprofibrate, and fenofibrate decrease levels

of fibrinogen by up to 20%,(17,18) and ciprofibrate and fenofibrate have been revealed to increase fibrinolysis and attenuate platelet hyperaggregability in hypercholesterolemia subjects (19). Given that atherogenic dyslipidemia are commonly characterized by a prothrombotic state, these effects must reduce thromboembolic complications of atherosclerosis.

Table 2.1. PPARα-mediated gene regulation by fibrates (11)

Target gene	Function of gene product	Gene expression
Lipoprotein lipase	Lipolysis, clearance of triglyceride-rich lipoproteins	Increase
Apolipoprotein CIII	Inhibits VLDL clearance	Decrease
Apolipoprotein AI	HDL-C formation, reverse cholesterol transport	Increase
Apolipoprotein AII	HDL-C formation, reverse cholesterol transport	Increase
Acyl CoA synthetase	Fatty acid activation, acyl CoA esters	Increase
ABCA1	HDL-C formation, cellular cholesterol efflux	Increase
Fatty acid binding protein	Cellular fatty acid uptake	Increase
b-oxidation pathway	Fatty acid oxidation	Increase
Acetyl CoA carboxylase	Fatty acid synthesis	Decrease
Fibrinogen	Blood clotting	Decrease
C-reactive protein	Acute phase reactant	Decrease
Interleukin 6	Acute phase reactant	Decrease
Cyclooxygenase-2	Fatty acid metabolism	Decrease
VCAM-1	Adhesion molecule	Decrease

Abbreviations: ABCA, ATP binding cassette transporter A1; VCAM-1, vascular cell adhesion molecule-1.

2.1.4. Pharmacokinetic of Bezafibrate

Bezafibrate is absorbed quickly and almost fully from the tablets standard formulation. In healthy volunteers, after administration a single dosage of 300mg a peak plasma concentration of approximately 10 mg/L is attained after approximately 2 hours. This drug is protein-bound with 95 percent, and has a clear distribution volume of around 17Litre. The elimination of the drug is rapidly and almost exclusively by renal excretion. 94% of bezafibrate drug with a dose of 300mg was recovered in urine during 24h, 40% and more as an unaltered drug, and 3.4 to 4.3 Liter/hour was the rate of the renal clearance of the drug. Bezafibrate has an elimination half-life of around 2 hours. Bezafibrate drug

is absorbed faster than clofibrate and has a smaller peak plasma concentration and an area under the plasma concentration time (AUC) curve at steady-state. The elimination of the drug bezafibrate is decreased with the patients that have renal insufficiency, and to prevent the bezafibrate accumulation and toxicity, the dose adjustment is required (1,5).

2.1.5. Dosage and Administration

In patients with type lIa, lIb, III, IV, or V dyslipidemia the recommended dosage of bezafibrate is a sustained release 400 mg tablet as once daily in addition to the dietary management. Alternatively, 200 mg twice or thrice daily can be used. Depending on serum creatinine levels, dosage reduction is required in patients with mild to moderate renal impairment. It is contraindicated in patients with severe renal failure. Patient requiring dialysis could be successfully managed with a 200 mg dose every 3 days. Patients with the impaired renal function must be monitored for symptoms of exacerbation renal dysfunction or myositis/myalgia (8).

2.1.6. Side Effect Profile of Bezafibrate

The most common side effects of fibrate treatment comprise gastrointestinally (diarrhea, epigastric discomfort, constipation, flatulence, queasiness), and the skin symptoms are (pruritus, erythema, urticarial), whereas in the musculoskeletal system (muscular pain, fatigue, muscle spasm) and the neurologic system (headache, virtigo) symptoms are being less prevalent (11). Alterations in liver function tests as well as decreased libido have been noted. The lithogenicity of the bile is increased by all of the fibrates. Bezafibrate, like clofibrate, can cause gallstones to develop (5).

2.2. Cyclodextrins

2.2.1. Definition

Cyclodextrins (cyclomaltooligosaccharides, CDs) are crystalline, Non hygroscopic, cyclic oligosaccharides originated from starch containing of glucopyranose subunits linked by α-1,4-glycosidic bonds. Cyclodextrins chemical structure consist of glucose units ranging from 6 to 8 units, (Figure 2.3) creating a cone shapes of α-cyclodextrin a six glucose units (G6) ring molecule, β-cyclodextrin a seven glucose units (G7) ring molecule, and γ-cyclodextrin an eight glucose units (G8) ring molecule (20).

The interior of CDs is hydrophobic and exterior is hydrophilic. The hydrophobic nature of the interior typically leads to accommodation of relatively hydrophobic guests

within the cavity. This accommodation of a molecule within another molecule is known as complexation and the resulting product is referred to as an inclusion complex. The surrounding compound is referred to as the host and the compound that is included in known as the guest. For this reason, inclusion complexes are often termed host/guest complexes (21). According to the cavity size of cyclodextrins, α-cyclodextrin has the smallest cavity size whereas γ- cyclodextrin has largest cavity size. There are various applications of cyclodextrins such as in food, pharmaceutical industries, and other industrial applications like in cosmetics, and in agriculture chemicals formulation. In general, cyclodextrins have been recognized as safe by the FDA in the United States and have been approved by other global organizations for food and pharmaceutical applications (22).

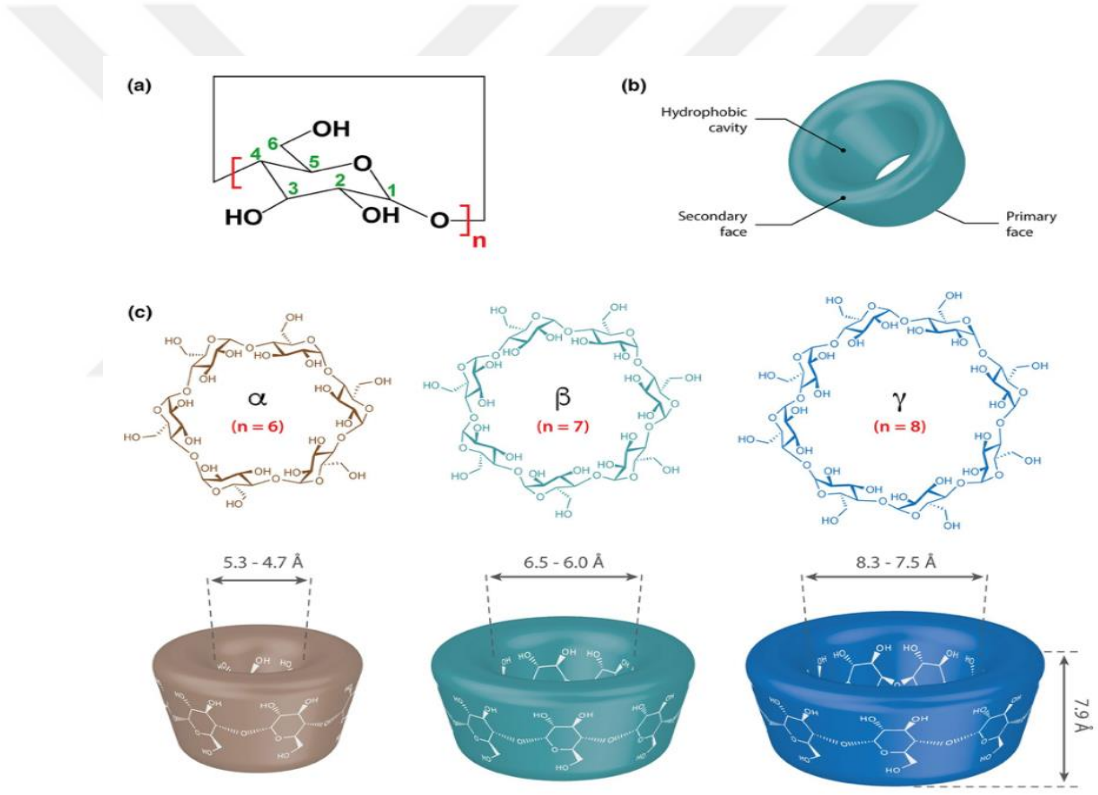


Figure 2.3. Schematic illustrations: (a) General chemical structure. (b) three-dimensional structure of CDs, and (c) chemical structure and dimensions for α-cyclodextrin n=6, β-cyclodextrin n=7 and γ-cyclodextrin n=8 (23)

2.2.2. History

Antoine Villiers published the first paper on cyclodextrins (CDs). He obtained crystalline dextrins called cellulosines by from potato starch by enzymatic digestion with

help of the the micro-organism Bacillus amylobacter. Franz Schardinger revealed the cyclic polysaccharide feature of cellulosines, dextrin A and B. Their structures were elucidated by Freudenberg and his co-workers (24). The term cyclodextrin was used by Friedrich Cramer around the end of the 1940s. He also investigated the procedure for the separation and purification of natural cyclodextrins. Solution and solid state inclusion complexation was also studied by him. Their use in pharmaceutical industries began in 1970s. Prostaglandin E2/βCD (Prostarmon ETM sublingual tablet) was the first product that came into Japanese market in 1976. Since then, numerous publication and patients came out related to the application of cyclodextrin in enhancement of water solubility of poorly soluble drugs, improvement of stability, masking the taste of disagreeable tasting drug, and above all enhancement of bioavailability of many drugs (25).

2.2.3. Properties

Physical Properties

CDs are insoluble in alcohols, ethers, chlorinated hydrocarbons, ketones, aromatic and aliphatic hydrocarbons. Table 2.2 represents the main physical properties of the three parent cyclodextrins.

Table 2.2. Physical properties of α, β and γ cyclodextrins (26)

	α-Cyclodextrin [*10016-20-3*]	β-Cyclodextrin [*7585-39-9*]	γ-Cyclodextrin [*17465-86-0*]
Formula	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Molecular mass	972.85	1135.00	1297.14
Solubility in water (25 ˚C),g/100 ml	14.5	1.85	23.2
Crystal water, wt %	10.2	13.2 – 14.5	8.13 – 17.7
mp, ˚C	> 200 ˚C	> 200 ˚C	> 200 ˚C
Pk_a value (25 ˚C)	12.331	12.202	12.081

Chemical Properties

CDs are non-reducing chiral oligosaccharides. The glucose rings are broken by periodate oxidation, but no formic acid or formaldehyde is generated. In acidic solution, the one and only breakdown product of all CDs is glucose. The hydrolysis rate of cyclodextrins is in the following order: gamma CD > betaCD > alphaCD. Cyclodextrins hydrolyze slower than maltooligosaccharides in acidic environments. The hydrolyzation of the glycosidic bond of the CDs is γ -amylase. The Enzymatic hydrolysis rate of cyclodextrins is fastest by γ cyclodextrin, followed by β cyclodextrin and α cyclodextrin. In alkaline solution (pH > 14), all CDs are relatively stable and very soluble. CDs are stable up to 250 ˚C in a nitrogen environment (27).

2.2.4. Cyclodextrins Production

Enzymatic decomposition of starch with cyclodextrin glycosyltransferase (CGTase) at a temperature of 30-90°C produces cyclodextrins (Figure 2.4). Potato and corn starch are significant in manufacturing production, although tapioca and wheat starch can be utilized too. Several bacteria are capable of CGTases production, such as *Bacillus circulans; Bacillus megaterium; Klebsiella pneumoniae; Bacillus macerans* (26). The main steps in producing cyclodextrins include:

- Cultivation of the bacteria that produces (CGTase) enzyme
- Enzyme segregation and purification
- Starch prehydrolysis with amylases
- Enzymatic starch conversion together with enzyme
- Inhibiting the enzyme action by heat treatment
- cyclodextrins separation from the conversion mixture
- Purification and crystallization from water

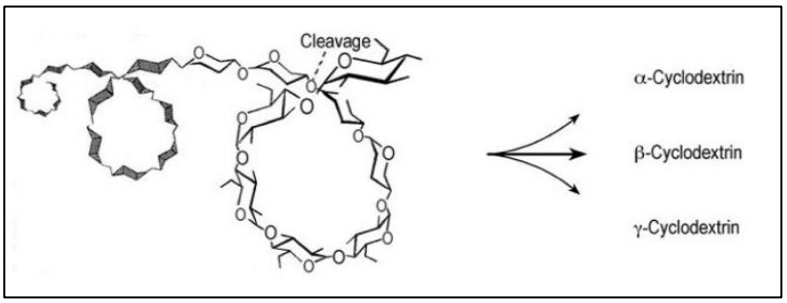


Figure 2.4. Enzymatic degradation of starch with CGTase (26)

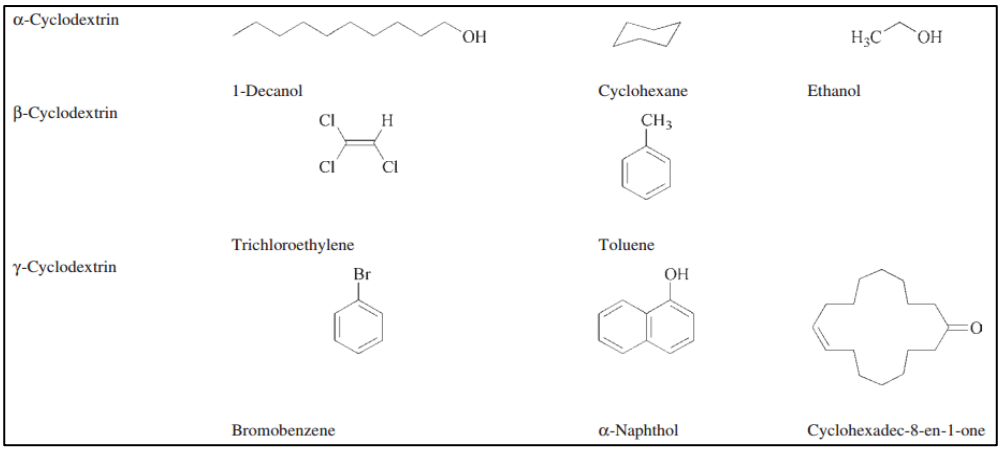
Solvent Process

At temperature of 30–90˚C, an aqueous starch solution transformed into cyclodextrins. In general based on the enzyme microbiological source and the conversion condition, all three kinds of cyclodextrin are produced by CGTase enzymes. Adding complexing agents are done to enhance the yield and change the ratio of the various obtained cyclodextrins. The majority of the complexing agents form solids complexes (insoluble) with a specific cyclodextrin. The formation of the complex shift the reaction equilibrium to the desirable cyclodextrin, and easy to separate the complex from the liquid conversion mixture. For isolation of cyclodextrins, steam distillation is applied to the complex to dissociate it or extraction by the organic solvent of the complexing agent. The use of specified enzymes can improve the yield. In the first phase, producing the α-CGTase by *K. oxytoca* lead to the production of α-cyclodextrin. For large-scale production of γ-cyclodextrin a specified γ-CGTase was developed (26, 28).

Non-solvent Process

In the non-solvent process, the reaction mixture involves just enzyme, starch, and water. This process consists of an extra step for breaking down the non-converted starch by glucoamylase action through hydrolysis into glucose and maltose. In the non-solvent process, it is difficult for the final crystallization of cyclodextrins. β -cyclodextrin only can be economically produced using this technique. The non-solvent process usually has a higher manufacturing cost than the solvent process (29).

Table 2.3. Specific complexing agents used for the production of CDs (26)



2.2.5. Modified Cyclodextrins

The solubility of the parent cyclodextrins (α, β and γ) in water is limited, which limits their use as solubilizing complexing agents. There is a primary and a pair of secondary hydroxyl groups on each glucose ring of the cyclodextrin structure. The solubility of the cyclodextrins can be changed by different groups or moieties on these hydroxyl group. Chemical modification of CDs have also been done to manipulate physicochemical nature of CDs as well as their inclusion capacity. Other purposes of chemical modification of CDs were physical and microbiological stability and decreased parenteral toxicity (25). Some of the modified CDs with the parent CDs and their commonly used abbreviations are given in Table 2.4 (30).

Table 2.4. Parent and Modified Cyclodextrins and their abbreviations (30)

Cyclodextrin (CD)	Abbreviation
α- cyclodextrin	α- CD
β- cyclodextrin	β- CD
γ- cyclodextrin	γ- CD
Hydroxyethyl-β-CD	HE -β-CD
Hydroxypropyl-β-CD	HP-β-CD
Sulfobutylether-β-CD	SBE-β-CD
Methyl-β-CD	M-β-CD
Dimethyl-β-CD	DM-β–CD (DIMEB)
Randomly dimethylated -β-CD	RDM-β-CD
Randomly methylated-β-CD	RM-β–CD (RAMEB)
Carboxymethyl - β -CD	CM-β-CD
Carboxymethyl ethyl- β-CD	CME-β-CD
Diethyl-β-CD	DE-β-CD
Tri-O-methyl-β- CD	TRIMEB
Tri-O-ethyl–β-CD	TE-β-CD
Tri-O-butyryl–β-CD	TB-β-CD
Tri-O-valeryl–β-CD	TV-β-CD
Di-O-hexanoyl–β-CD	DH-β-CD
Glucosyl-β-CD	G1-β-CD
Maltosyl-β-CD	G2 -β-CD
2-hydroxy-3-trimethyl-ammoniopropyl-β-CD	HTMAPCD

Economic Aspects

Cyclodextrin manufacturers and their trade names are listed in table 2.6

Table 2.5. Producers and trade names of CDs (26)

Company	Country	Products	Tradename
Cerestar	USA	β-CD, HP-β-CD, A-CD	Cavitron
Wacker Biochem	USA,	α-CD, β-CD, γ-CD	CAVAMAX
	DE	M-CDs, HP-CDs, A-CDs	CAVASOL
Roquette	FR	β-CD , HP-β-CD	Kleptose
Ensuiko Sugar Refining	JP	β-CD, CD-mixture, branched CD	
Nihon Shokuhin Kako	JP	β-CD, HP-β-CD, CD-mixture	
Janssen Pharmaceuticals	BE	HP-β-CD	Encapsin
Cydex	USA	SBE-β-CD	Captisol Advasep

2.2.6. Mechanism of Cyclodextrin Inclusion-Complexation

Most of the newly developed drugs have poor water solubility. Therefore, they are suitable candidates for complexation with cyclodextrins. Since CD molecules have a somewhat hydrophobic inner cavity, they may accept lipophilic molecules or their part inside the central cavities if their size fit. Intermolecular interaction between the CD (host) and guest molecule can thus be part or total inclusion of the guest molecule inside the CD cavity. The driving force for this host-guest complexation is mainly hydrophobic interaction between the cyclodextrin inner cavity and the accepted molecule of part of the molecule. In addition, van der Waals force and dipole-dipole interaction can also materialize the complexation (31). The hydroxyl groups of CDs forms hydrogen bond with water making them or the complex soluble in water. Figure 2.5 represents the inclusion complexation of the guest molecules and their entrapment inside the cyclodextrin cavity. Inclusion complexation may occur in a variety of stoichiometries; for

example, 1 guest molecule can complex with 1 or 2 CD molecules or 2 guest molecules can complex with 1 CD molecules and so on (32).

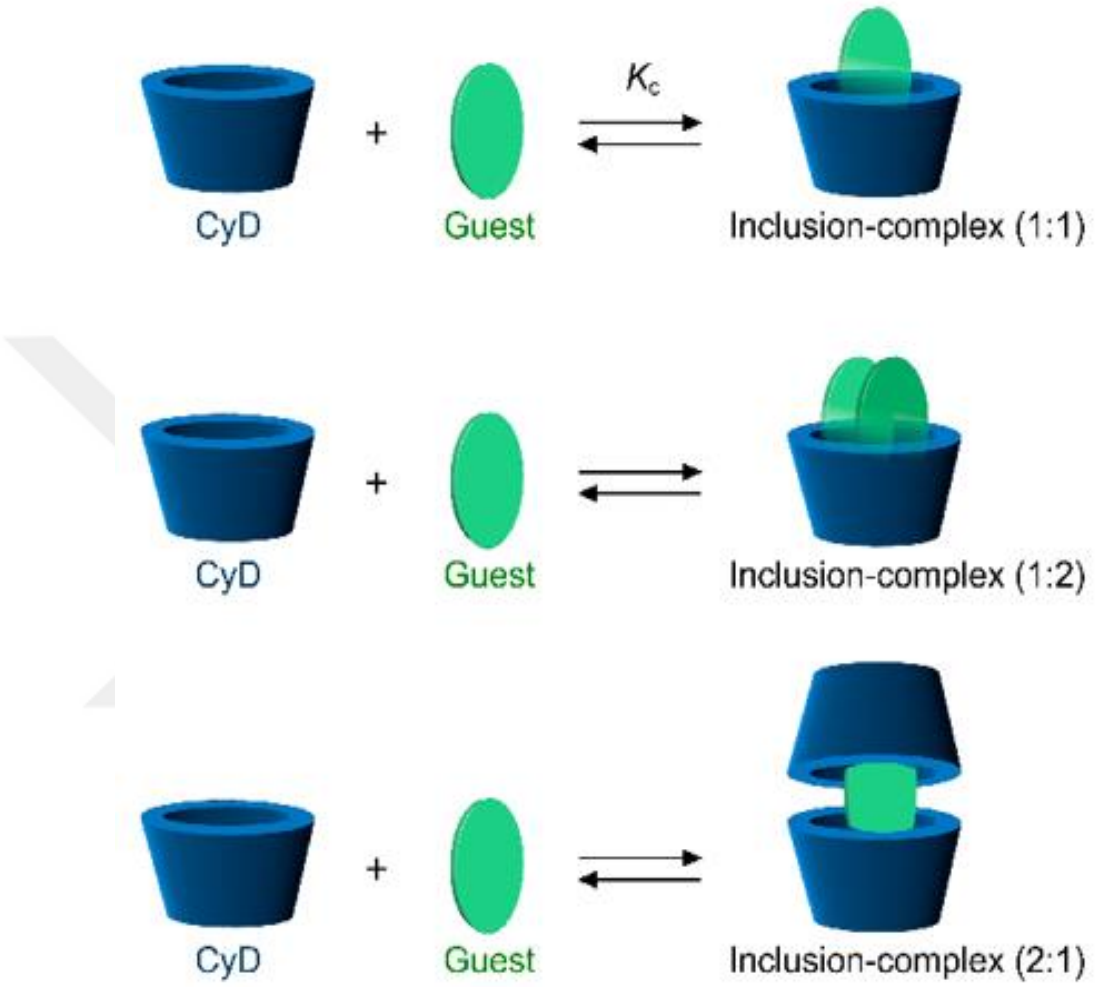


Figure 2.5. The inclusion complex at various stoichiometries (32)

2.2.7. Phase Solubility Analysis

Higuchi and Connor phase solubility analysis method is the most commonly utilized to study the inclusion complexation (33). This method analyzes the impact of the concentration of the CDs (ligand) on the solubilization of the guest molecules (substrate) (Figure 2.6). The phase solubility diagrams may be broadly classified in to type A and type B depending on the solubility of the formed complex. When the drug:CD complex is soluble, as is the case with most soluble chemically modified/derivatized CDs (for

example HPβCD and SBEβCD), the diagram is termed type A. On the other hand, if the formed complex is of limited solubility or insoluble, as is the case with the most natural CDs, the diagram is called type B. With three subtypes in type A and two subtypes in type B, there are actually 5 different type of phase solubility diagrams. If the dissolved guest (drug) concentration linearly increases with CD concentration, the diagram is termed type A_L. If the guest concentration disproportionately increases with a positive deviation from the linearity, this produces A_P type , whereas a negative deviation from the linearity produces A_N type diagram. In some combination of host and guest, there is observed an initial proportional increase in guest solubility up to a maximum followed by a decrease in solubility. The diagram that represent this type of limited solubility of the complexes is termed B_S type. Finally, if the complex is insoluble then we get B_I type diagram (Figure 2.6).

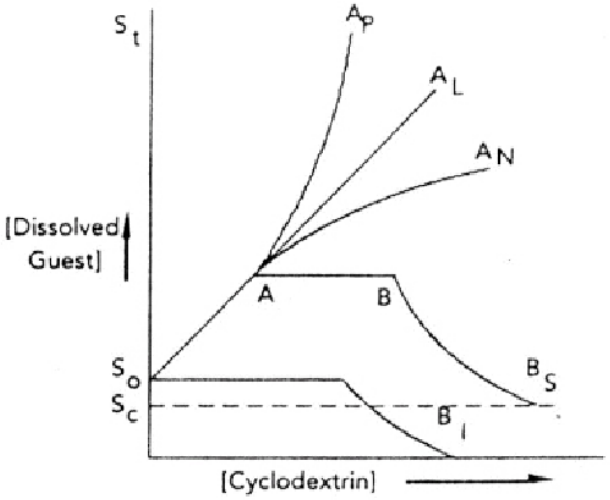


Figure 2.6. Theoretical phase solubility diagram (33)

The equilibrium binding of guest and CD to produce a 1:1 complexation can be represented by:

Drug + CD ⇌ Drug:CD Complex

In such cases of a 1:1 complexation stoichiometry, Higuchi and Connors suggested the following equation to calculate the equilibrium-binding constant, $K_{1:1}$. This is also called equilibrium association constant.

$$K_{1:1} = \frac{Slope}{S_0(1 - Slope)}$$

Here, the Slope is the slope of the linear portion of the diagram/curve and S_0 is the intrinsic solubility of the drug, which is determined either from the y-axis intercept or experimentally without adding any CD to the drug water suspension. Binding constant values for many drug/CD complexes range from 100 to 20000M^{-1} (30).

2.2.8. Factors Influencing Inclusion Complex Formation

Type of CD, cavity size, pH and ionization state, and temperature are factors affecting inclusion complexation with various specific drugs and CDs (34) and main observations during these complexations are summarized in Table 2.6

Table 2.6. Factors Affecting Inclusion Complexation (30)

Factor	Drug	CDs Studied	Observation
Type of CD	Albendazole, Mebendazole, Ricobendazole	β-, HP-β-, M-β-CDs	More effective enhancement of solubility with substituted CDs.
	Fenoprofen	α-, β-, γ-, HP-β-CDs	Better stability constant values of pharmaceutical interest with only β-CD and HP-β-CD complexes.
	Ketoprofen	M-β-, β-CDs	Better dissolution performance of M-β-CD complex.
	Cocaine	α-, β-, γ- CDs	Drug binding with reasonable affinity only to β-CD in aqueous solution.
Cavity size	Gliclazide	β-, α- CDs	Cavity size of β-CD was suitable for complexation while that of α-CD was insufficient to include GL rings.
	Digitoxin	δ- CD	Enhanced solubility due to partial inclusion of the drug in CD cavity.
	Macrocyclic compounds (MCCs)	α-, β-, γ-, δ-CDs	Complexes of smaller MCCs with α- and β-CDs and those of larger MCCs with γ- and δ-CDs were relatively stable.
	Ibuproxam	α-, β-, γ- CDs	Effective enhancement of dissolution rate with only with β- and γ-CDs but the cavity of α-CD was less suitable.
	Prochloro-methazine	β-, HP-β-, DM-β-CDs	Decreased solubility due to failure of the CD cavities to include phenothiazine ring.
pH and ionization state	DY– 9760e	SBE-β-CD	Strong drug/CD interaction in acidic region, at pH 4.
	NSC-639829	SBE-β-CD	Increased solubility of the cationic drug at pH 1.
	ETH–615	HP- β-, RM-β-, CM-β-, SBE-β-HTMAP-β-CDs	Increased solubility with uncharged RM-β-CD. Complex stability constants were low with the highly polar drug at pH 5 due to its lesser ability to enter the CD cavity but were high with anionic less polar form at pH 10.
	Piroxicam	β-CD	Effective complexation at low pH
	Levemopamil HCl	HP-β-CD	Enhancement of solubility (mg/mL) was 3-fold with the charged drug (by 7.88 to 25.62 at pH 4) and 525-fold with the neutral form (0.0026 to 1.37 at pH 10.6).
	Ziprasdone mesylate	SBE-β-CD	Complexation was more favored with the ion pair over the dissociated ionic form.
	Sulindac	β-CD	Complexation was easier with nonionized form.
	Mebendazole	HP-β-CD	Un-ionized form was less included than the ionized form.
Temperature	DY–9760e	SBE-β-CD	Temperature change showed negligible effect on the stability constant.
	Sulindac	β-CD	Increasing the temperature decreased the apparent stability constant.
	Phenolphthalein	β-CD	Increasing the temperature decreased association constant for binding.
	Danazol	SBE-β-CD	Increasing the temperature decreased the complex stability constant.

2.2.9. Advantages of Cyclodextrins in Pharmaceutical Formulations

The CDs are used mainly as complexing agents to increase the water solubility of hydrophobic drugs. They also increase bioavailability and stability of labile drugs. Prevention of reduction of gastrointestinal or eye irritation, reduction or elimination of unpleasant odors or tastes, prevention of drug interactions are some other purposes of their application in pharmaceutical formulation. They even can convert liquid oils and drugs into fine crystalline or amorphous powder (35).

Improvement of water solubility

Hydrophobic molecules or a part of the hydrophobic molecules are taken inside the central cavity of the cyclodextrin. As a result interaction of hydrophobic guest molecule with water is minimized. The outer surface hydroxyl group of CDs help the complex molecule in water. In certain cases multiple CD molecule cover different parts of the hydrophobic molecule. Thus, CDs increase the overall water solubility of poorly aqueous soluble drugs (36).

Improvement of bioavailability

Poor bioavailability occurs due to low solubility, and here CDs are of maximum value. The basic requirement for absorption of an orally administered drug is its release from the formulation in a soluble form. When the drug is complexed with a CD, the dissolution rate and, accordingly, absorption improves. Also, reducing the hydrophobicity of the drugs by CD complexation improves their absorption through the skin or rectum. additionally, to improving solubility, CDs also prevent crystallization of the active ingredients by complexing individual drug molecules so that they cannot self-assemble into a crystal lattice (37).

Enhancement of stability

The physiochemical properties and the thermal stability of medicines can be improved by the cyclodextrins complexation. The active molecule of the medicine are trapped and protected inside the cyclodextrin cavity, therefore, it is difficult to decompose while exposing to chemical reactions such as oxygen, H2O, temperature, and radiation because the reactants can’t diffuse and interact with the active molecules inside the CD cavity (35,38).

Irritation Reduction

Medicines encapsulated inside the cavity of the CD aimed to decrease the irritatations that occurs in the stomach, eyes, or skin. The free drugs concentration reduced by complexation with cyclodextrins, beneath the range of irritation. When the complex progressively separates, the releasing of the free drug occurs then absorbed by the body, and the concentration of the free drug always seems to be lower than the level which might irritate the mucosa (39).

Prevention of incompatibility

Often the drugs in the formulation are incompatible with one other nor with another inactive component. Encapsulation of an incompatible component inside the molecule of cyclodextrin make the formula stable by components separating physically to avoid the drug interaction or drug additive interactions (38).

Masking Taste and Odur

The complexation of the drugs with cyclodextrins can mask the bitter taste and unpleasant odor of medications (40). The functional groups that make the bad smell and bitter taste can be masked by the encapsulation inside the cyclodextrin cavity from their sensory receptors. The Complexes that arise are tasteless, odorless, or slightly tasteless and hence more agreeable to the patient (39).

Advantages of Material handling

Materials at room temperature, such as oils or, liquids may be tough to handle and formulate into constant solid dose form. Complex formation with CDs can transform these substances into amorphous or microcrystalline powders that can be easily handled and formulated to solid dose form by using standard manufacturing techniques and equipment (41).

2.2.10. Application in Drug Delivery

Oral Drug Delivery

Drug release from the oral delivery system can be in the form of dissolution control, osmosis control, diffusion control, density control, or pH control. There are various applications of cyclodextrins in oral drug delivery as enhancing drug bioavailability due to increased solubility of the drug, improving dissolution rate,

stabilizing drug at the site of absorption, reducing drug irritation, and masking the unpleasant taste. Cyclodextrins primarily increase the mucosal drug permeability by increasing the availability of the free drug at the absorbing surface (25). Moreover, tablets for sublingual and buccal administration have been developed and formulated by rapid dissolving complexation with cyclodextrins (42).

Parenteral Drug Delivery

CDs applications are used in parenteral delivery to dissolve medication, reduce drug irritation at the site of administration, and stabilize unstable drugs in the aqueous environment. CDs are solubilizing complexing agents that are being used in various marketed parenteral solutions and usually at high concentrations. The water-soluble βCD derivatives, SBEβCD and HPβCD are quickly excreted unmetabolized with the urine and utmost cases, CDs do not affect the drug pharmacokinetics. Due to their widely favorable toxicological and pharmacokinetic profiles their usage in parenteral drug formulations is often preferred above other solubilizing excipients such as organic solvents and surfactants (43).

Ocular Delivery

CDs applications in the ophthalmic drug delivery system offer an attractive way to enhance the solubility of poorly soluble and wetted drugs, and to increase retention and penetration into the ocular surfaces. Additionally, CDs are growingly being reported for their drug stabilizing properties and safety profiles. the CD has the potential to enhance the conventional eye drops to offer preferable permeation, efficacy, stability and safety in the ocular topical delivery of the anterior and posterior segments. An accurate selection of process parameters, and concentrations of CDs and other excipients can enable the development of suitable ocular delivery platforms in terms of nanotechnology, inserts, in situ gels and contact lens (44).

Nasal Drug Delivery

Due to the significant gastrointestinal collapse and high hepatic first-pass impact, using nasal mucosa to transfer the drugs is a new strategy for systemic delivery of highly potent drugs having poor oral bioavailability. Cyclodextrins can enhance nasal drug delivery thru biological barriers without compromising their barrier function, making them excellent intranasal drug delivery enhancers. CDs microsphere assisting the drug

adhere to the mucosa of the nose by increasing its stability and providing excellent adhesive qualities. In both thermo reversible gels and situ nose insertion, the synthesis of cyclodextrins and drugs complex cause a rise in viscosity and progressive drug absorption from the formulations (45). In addition, cyclodextrins have been studied to improve the transport of insulin across the mucosa of the nose, with high content of insulin loading, as well as in vivo lowering the concentration of blood glucose (46).

Rectal Drug Delivery

Recent studies have shown that the rectal mucosa could be used as a potential drug delivery site. However, the rectal mucosa provides very limited space for drug absorption resulting in an irregular release of the drug. To overcome these problems, it has been reported that CDs applications are useful in enhancing the absorption of the drug from the base of the suppository either by enhancing drug release from the base or by increasing drug mucosal permeability, providing sustained drug release, increasing drug stability in the base or at the absorption site, and relieve irritation caused by drugs (47).

Dermal and Transdermal Delivery

Natural cyclodextrins and their many derivatives have been utilized to improve skin delivery of local and systemic drugs. The Applications of cyclodextrins in the transdermal drug delivery involve improving drug release and or permeability, stabilize the drug in formulation or at the absorption site, reducing drug-induced local irritations, maintaining the drug release from carriers, and changing drug biotransformation in the viable dermis (48).

Controlled and targeted drug delivery system

CDs recently have been studied as carriers in controlled-release drug delivery systems. Hydrophobic cyclodextrins, like the alkylated and acylated derivatives, were utilized to extend the drug release rate, whereas hydrophilic derivatives were employed to improve the drug release rate. (49).

Peptide and protein delivery

Enzymatic and chemical instability, malabsorption thru biological membrane, quick plasma clearance, exotic dosage response curves, and immunogenicity are among the different problems that are related to the practical application of therapeutic peptide

and protein. Due to the cyclodextrins bio adaptability in pharmaceutical usage and their ability to interact with cellular membranes, they are promising carriers for the transport of proteins, peptides, and oligonucleotide medicines (39).

Gene delivery

Cyclodextrins have been widely studied as carriers for many types of gene therapy. They utilized in delivery systems for improving gene edit, replacements, and modification inside the cells by DNA and RNA delivery. Since they haven't yet demonstrated any uses in T cell engineering CD-based (ex-vivo), this might alter if the technology proceeds in-vivo and CAR antigens transitory expression through mRNA transport becomes more prevalent. Despite the existence of (pure) CD carriers for gene therapy, CDs are more generally one of several constituents in gene delivery systems. Cyclodextrin addition can transmit much of its beneficial chemical and physical features to the system, like hosting drugs small molecule, serving as a link or modular component, decreasing immunity, and damaging membranes. In some instances, CDs interact immediately together with the genetic cargo. In another case, genetic cargo is retained by other system components however, CD as yet plays a critical role like stabilizing the vehicle or ensure endosomal escape. Gene delivery vectors that lack these characteristics are unable to deliver cargo effectively to its intended location. By transferring these capacities to the broader system, CD's addition allows for improving the delivery of gene therapeutics applications (50).

Brain drug delivery

Natural CDs and their derivatives are important as both excipients and active pharmaceutical ingredients in the treatment of neurological diseases. They are present as a solubilizing agent in numerous centrally acting marketed drugs like antiepileptics. HP-β-CD received orphan drug designation for the treatment of Niemann-Pick type C disease, which prompted more research to discover the potential therapeutic use of CDs in lysosomal storage diseases, stroke, neuro infections, neurodegenerative diseases and brain tumors. simultaneously, new promising CD derivatives and CD nanoparticles are being developed for drug delivery to the CNS. The BBB is a key player in both drug delivery to the CNS and pathomechanism of many neurological diseases (51).

Novel Delivery Systems

Nanotechnology based on cyclodextrin appears to have a greater therapeutic effect and maintain the long life of healthy and healed cells. Encapsulation of cyclodextrin complexed drug into carriers such as liposomes, nanoparticles, millirods, micelles, and niosomes will improve the capacity of drug loading, confinement efficacy, extend the drug existence in systemic circulation, lowering the toxicity and provide the drug release to be controlled, sustained or targeted. The addition of cyclodextrin increases the product value. The distinctive characteristics of optimized drug-cyclodextrin complex comprises lower aggregation, better administration, distribution, metabolism, excretion properties (ADME), rare poisoning, and patient acceptance (52).

Examples of marketed cyclodextrins formulated drugs are given in Table 2.7

Table 2.7. Approved & Marketed Drugs Formulated with Cyclodextrins (53)

Drug/CD	Trade name	Formulation	Company (region)
Alprostadil/αCD	Caverject Dual®	Intravenous solution	Pfizer (Europe)
Aripiprazole/SBEβCD	Abilify®	Intramuscular solution	Bristol-Myers Squibb (USA); Otsuka America (USA)
Cisapride/HPβCD	Propulsid®	Suppository	Janssen (Europe)
Diclofenac sodium/ HPγCD	Voltaren Ophtha®	Eye drop solution	Novartis (Europe)
17β-Estradiol/RMβCD	Aerodiol®	Nasal spray	Servier (Europe)
Ethinylestradiol and drospirenone/βCD	Yaz®	Tablet	Bayer (Europe, USA)
Mitomycin	MitoExtra®, Mitozytrex®	Intravenous infusion	Novartis (Europe)
Nicotine/βCD	Nicorette®	Sublingual tablet	Pfizer (Europe)
PGE1/αCD	Prostavastin®	Parenteral solution	Ono (Japan); Schwarz (Europe)

3. MATERIALS AND METHODS

3.1. Materials and Equipements

3.1.1. Materials

The materials used in this study are given in Table 3.1.

Table 3.1. List of Materials

Materials	Company
(2-Hydroxypropyl)-beta-cyclodextrin	CycloLab, Hungary
Alpha-Cyclodextrin	CycloLab, Hungary
Avicel PH-102	FMC Corporation, USA
Bezafibrate	AfaAesar, ThermoFisher
beta-cyclodextrin	CycloLab, Hungary
DiPac	SEPPIC, France
Gamma-cyclodextrin	CycloLab, Hungary
Magnesium stearate	Pharmaceutical grade
Orthophosphoric acid	Analytical grade
Potassium dihydrogen orthophosphate	Sigma-Aldrich
Sodium hydroxide	Analytical grade

3.1.2. Equipements

The equipment used in this study are listed in Table 3.2.

Table 3.2. List of Equipements

Equipment Names	**Company and Models**
Differential Scanning Calorimeter	Perkin Elmer, DSC 6000
Disintegration	PharmaTest PTZ AUTO EZ, Germany
Dissolution Apparatus	PharmaTest, PTWS 620, Germany
Friability tester	PharmaTest, PTF 300, Germany
FTIR spectrophotometer	Spectrum one, Perkin Elmer, USA
Hardness tester	PharmaTest, PTB 111E, Germany
Lyophilizer	L 2-4, Christ, Germany
Magnetic stirrer	Isolab, Germany
Scanning Electron Microscopy	Zeiss EVO 40, Carl Zeiss AG, Germany
Single punch tablet machine	Minipress MII, Riva SA, Argentina
Sputter Coater	BAL-TEC SCD 005
UV-Spectrophotometer	Agilent 8453, USA

3.2. Methods

3.2.1. Identification of Bezafibrate

UV Spectrum

Stock solution of bezafibrate in methanol was diluted to a bezafibrate solution of 10 µg/mL. This was used to measure the UV spectrum of bezafibrate in the range of 200 - 800 nm and λ_{max} was determined. Later, aqueous solution of the same concentration was also prepared and spectrum taken in the same manner.

Differential Scanning Calorimetry Thermogram

Differential scanning calorimetry (DSC) was determined on bezafibrate sample weighing ~8 mg. The sample was heated in hermetically sealed aluminum pans at a rate of 5ºC/min up over 25 - 300ºC in a dynamic nitrogen atmosphere. Deepest endothermic peak was noted from the thermogram and taken as melting point of bezafibrate after comparison with previously reported theoretical values.

Fourier Transform Infrared (FTIR) Spectrum

Fourier transform infrared (FTIR) spectrum of bezafibrate was taken with a PerkinElmer SpectrumOne FTIR Spectrometer using previously prepared discs of bezafibrate and potassium bromide containing 0.01 g of bezafibrate in 0.1 g of potassium bromide between the wave number of 400 and 4000 cm^{-1}. The spectrum was compared to that of bezafibrate from British Pharmacopoeia, 2013.

3.2.2. Quantitative Determination of Bezafibrate by UV-VIS Spectrophotometry

Construction of Calibration Curve

UV-VIS spectrophotometric analytical method was used to analyze and quantify of bezafibrate (54). Bezafibrate stock solution of 10 µg/mL was prepared in deionized water. A serial dilution was performed and absorbaces measured. Then, calibration curve was constructed using 0.5, 1, 2, 4, 6, 8 and 10 µg/mL of bezafibrate in deionized water. For each point, 6 replicates were used. Absorbance was measured at the bezafibrate λ_{max} of 228 nm.

Validation of Analytical Method for Bezafibrate

The method was validated by determining the following validation characteristics: limit of detection and quantification (sensitivity), specificity, linearity, accuracy, and precision (54,55).

Limit of Detection and Quantification/ Sensitivity

Sensitivity of the method was determined by finding the detection and quantitation limit. The detection limit is determined by the analysis of bezafibrate samples with known concentrations and by establishing the minimum level at which the bezafibrate can be reliably detected. Determination of the signal-to-noise ratio was performed by comparing measured signals from samples with known low concentrations of bezafibrate with those of blank samples (deionized water and/or cyclodextrin solution). Three times the noise level was considered as the limit of detection. Ten times the noise level is considered as the limit of quantification.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present in the course of analyzing a sample. Bezafibrate alone and with different cyclodextrins were dissolved in deionized water; and then spectra were taken and compared to see whether λ_{max} of bezafibrate is affected or not. Similarly, bezafibrate was measured in matrix of tablet formulation.

Linearity

The linearity of an analytical procedure is the range of concentration in which concentration and method response (absorbance) is directly proportional. Linearity was demonstrated using a seven-points (0.5, 1, 2, 4, 6, 8 and 10 µg/mL calibration curve. Average absorbance for each concentration point was plotted as a function of bezafibrate concentrations. Linearity was observed at first by visual inspection of the plot and then by calculation of a regression line by the method of least squares.

Accuracy and Precision

The accuracy of an analytical method is indicated by the closeness of the found value to the reference value, which is often the added known concentration. Three known concentrations; 1, 4, and 8 µg/mL on the calibration curve were analyzed with 3 replicates

(n = 3) for each of these three concentrations. The measurement were done at three different times in a day (intra-day) and on three different days (inter-day). Average of the three measurement was used by the regression equation to calculate the found concentration. Accuracy was calculated by the following equation:

$$Accuracy = \frac{C_f - C_a}{C_a} \times 100$$

where,

C_f = Average found concentration calculated by regression equation

C_a= Added known concentration.

Precision of an analytical method means lower degree of scatter among a series of measurements on multiple sampling. Precision may be considered at the levels of repeatability (intra-assay precision), intermediate precision (within-laboratories: inter-days, same or different analysts etc.) and reproducibility (between laboratories). The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Intra-day and inter-day precision was calculated on the same measurement values as found in accuracy measurement. The precision was calculated by the following equation:

$$Precision = \frac{\sigma}{\mu} \times 100$$

where

σ = standard deviation of the three measurement

μ = Average of these three measurement

3.2.3. Bezafibrate-Cyclodextrin Complexation: Complexation Method, Equilibrium Period, pH and Temperature Effect, and Stability

Complexation Method

Initial experiment with kneading method of complexation (56) was not successful. FTIR spectra of kneaded sample (bezafibrate with each cyclodextrin separately) did not show any difference from physical mixtures of bezafibrate with cyclodextrins. Therefore, bezafibrate fine powder was magnetically stirring with αCD, βCD, HPβCD and γ-CD solutions in water separately for prolonged period of time at different conditions.

Separately bezafibrate was stirred only with water for comparison. The suspension were separately filtered through 0.20 μm syringe filter. Samples of the filtrate were then analyzed by UV spectrophotometer after appropriate dilution. After observing an increase in solubility of bezafibrate with CD solutions in comparison to only-water mixture, this was used as a method of complexation. The samples were then freeze-dried which completes the method of complxation by what is reported as Freeze Drying Method (57).

Equilibrium Period

Initially, 5 and 15 mM of each CD was stirred separately with excess bezafibrate for 1, 2, 3 days to determine the time period required for equilibrium in the complexation. Solubility of bezafibrate after these periods are given in Table 4.3 in section 4.3.

pH and Temperature

For observing the effect of pH on the complexation, deionized water (pH 7.0), pH 4.0 and pH 1.5 buffers were used. The buffers were made by dissolving 1.36 g of potassium dihydrogen orthophosphate in sufficient deionized water to produce 480 mL and adjusting the desired pH using ortho-phosphoric acid and making the volume finally with deionized water to 500 mL. The complexation was carried out with all the four CDs (αCD, βCD, HPβCD and γCD). Observing relatively poor effect of αCD and γCD, they were excluded from temperature experiment which were carried out at 25 and 60˚C with βCD and HPβCD. Results are presented in Table 4.4. and Table 4.5.

Stability

An experiment was also done to understand the effect of the higher complexation temperature (60˚C) on the stability of bezafibrate. An amount bezafibrate stock solution was mixed with deionized water, pH 4.5 and pH 1.5 buffers in three separate conical flask. Concentration was measured immediately after mixing which was the initial, 0 h concentration. All the three solutions were then placed on 60˚C hot plate for three days with continuous stirring with magnetic bar. Samples were taken at different time and analyzed to observe the changes in concentration over time.

Preparation of Complexes in Large Quantity

In order to produce enough complex for subsequent characterization experiments, large amount of complexation was carried out in deionized water and filtrates were frozen

at -80˚C and lyophilized. Lyophilized solid samples were preserved for subsequent characterization experiments or in the preparation of tablets.

3.2.4. Phase Solubility Analysis

Phase solubility analysis of bezafibrate was performed as follows: A series of 20 mL vials were taken containing 0, 1, 2, 5, 10, 15 and 15 mM (close to maximum solubility) of βCD solutions; 0, 1, 2, 5, 10, 15, 25 mM of solutions of αCD, HPβCD and γCD solution in water or buffers of pH 1.5 and 4. Excess of bezafibrate was added so that a solid phase was always maintained. The mixtures of bezafibrate and CD solutions were stirred at room temperature or at 60˚C for two days. Then, 2 mL cyclodextrin drug suspensions from each vial were filtered through 0.20 µm syringe filter. The filtrates, diluted with deionized water or buffers if required, were then measured spectrophotometrically. Phase solubility diagrams were prepared by plotting cyclodextrin concentration versus dissolved bezafibrate concentration found by analysis. The apparent stability constants (K) were calculated by the equation of Higuchi and Connors (33) using the slope of the 1:1 type complexation:

$$K_{1:1} = \frac{Slope}{S_0(1 - Slope)}$$

where,

S_0 is the intrinsic solubility of drug in the medium (water or buffer)

3.2.5. Characterization by Fourier Transform Infra-Red (FTIR)

Fourier transform infrared **(**FTIR) spectra of the following samples were taken between the wave number of 400 and 4000$cm^{-1}$with SpectrumOne (Perkin Elmer): pure bezafibrate, αCD, βCD, HPβCD and γCD; their corresponding complexes, namely, BFαCD, BFβCD, BFHPβCD and BFγCD; and their corresponding physical mixtures, namely, BF+αCD, BF+βCD, BF+HPβCD and BF+γCD. KBr crystal were first triturated. Then each sample was mixed with it and lightly triturated to avoid possible disruption in the complex structure. The mixture was then compressed to make a thin disc which was used to take the spectra. The amount of sample and potassium bromide was taken in such a ratio that transmittance was not blocked below 30%; approximately 0.01 g of sample in 0.1 g of KBr was enough for most of the measurements. The related FTIR spectra were stacked to facilitate comparison.

3.2.6. Characterization by Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) analyses were performed with PerkinElmer DSC 6000 on the following samples: pure bezafibrate, αCD, βCD, HPβCD and γCD; their corresponding complexes, namely, BFαCD, BFβCD, BFHPβCD and BFγCD. Each sample weighing ~6-8 mg was heated in hermetically sealed aluminum pans at a rate of 10ºC/min over 25 - 350ºC in a dynamic nitrogen atmosphere. The related spectra were then grouped in an overlay position to facilitate comparison.

3.2.7. Characterization by Scanning Electron Micrograph (SEM)

Morphological aspects of particles such as shape, surface and size were studied using a scanning electron microscope (Zeiss EVO 40, Carl Zeiss AG, Germany). The dry particles of pure bezafibrate, βCD, lyophilized βCD, HPβCD, lyophilized HPβCD and two complexes, namely, BFβCD and BFHPβCD were fixed to the metallic stub using a double-side adhesive tape. Then the particles were sputter coated (BAL-TEC SCD 005) with Au-Pd before observation in the electron microscope.

3.2.8. Dissolution Study on Bezafibrate and Its CD Complexes

Dissolution experiments were carried out with a PharmaTest dissolution test apparatus (PTWS) in dissolution medium that was prepared as follow: 0.608 g of sodium hydroxide and 6.805g of potassium dihydrogen orthophosphate in sufficient deionized water to produce 900 mL and adjusting the pH to 6.5 ± 0.05 using ortho phosphoric acid and making the volume finally with deionized water to 1000 mL (British Pharmacopoeia, 2013). Dissolution Apparatus 2 (paddle method) was used with a rotation speed of 75 rpm. The test was carried out with 900 mL of the above dissolution media maintained at at 37°C. Bezafibrate alone, BFβCD and BFHPβCD were tested, others were not included due to poor performance in other characterization studies. Each powder samples equivalent to approximately 1800 µg (1.8 mg) of bezafibrate were added to the dissolution medium. At different time intervals, 5 mL of the dissolution medium were withdrawn and filtered through a 0.20 µm syringe filter before analysis with UV-Spectrophotometer. Dissolution medium was used as the blank as both βCD and HPβCD was tested before to show no effect on the measurement. Fresh 5 mL of dissolution medium was added to the dissolution vessel after each sampling. Sampling times were 5, 10, 20, 30, 45, 60 minutes or until 100% dissolution.

The dissolution profiles were evaluated for similarity by the factor, f_2, as proposed by Moore and Flanner (58). The equation for f_2 is as follows:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{n-1}^{n} (R_t - T_t)^2 \right]^{-0.5} * 100 \right\}$$

3.2.9. Preparation of Tablets with Complexes

Tablets were prepared separately with two directly compressible vehicles. They were microcrystalline cellulose (Avicel PH102) and co-crystallized sugar consisting of 97% sucrose and 3% modified dextrin (DiPac). The formulation of different tablets are presented in Table 3.3. The total weight of each tablet was targeted to be 620 mg. Ingredients are mixed thoroughly with geometric dilution technique. Tablets were made by compressing the powder/granule mixtures in a single punch tablet machine. The die and punches were 12 mm in diameter. The amount of complexes used was close to the equivalent 1.80 mg pure bezafibrate, considering the possibility of maintaining sink condition in the dissolution test.

3.2.10. Characterization of Tablets with Complexes

Thickness, Diameter and Hardness Test

Tablet thickness, diameter and hardness were measured in a single operation with a PharmaTest 3-in-1 Tablet Testing Instrument (PTB). For thickness measurement the tablets were placed in vertical position and for diameter in diametric horizontal position. The instrument applied forces to the tablets diametrically to determine hardnesses.

Weight, Friability and Disintegration Test

Tablet weight variations were tested by weighing individual tablets with an analytical balance.

Friability was tested with a PharmaTest Tablet Friability Test Instrument (PTF 300). Pre-weighed tablets were put in the rotating drum. The instrument was set to rotate the drum 100 time. After this, the tablets were visibly checked for any physical deformation like crack, peeling, and erosion; lightly wiped with a soft brush to remove any dust particles from the tablet surfaces and weighed again. The difference in weight expressed as percentage of pre-weights was taken as the friability.

Table 3.3. Tablet formulations

Tablets	Bezafibrate, mg	Complex, mg	Vehicle, mg	Magnesium stearate, mg
A(PH102)	-	-	613.8	6.2
A-BF	1.8	-	612.0	6.2
A-BFβCD	-	258.3	355.5	6.2
A-BFHPβCD	-	310.0	303.8	6.2
D(DiPac)	-	-	613.8	6.2
D-BF	1.8	-	612.0	6.2
D-BFβCD	-	258.3	355.5	6.2
D-BFHPβCD	-	310.0	303.8	6.2

A(PH102): placebo (no API) tablet with Avicel, A-BF: Tablet with bezafibrate in Avicel, A-BFβCD: Tablet with bezafibrate βCD complex in Avicel, A-BFHPβCD: Tablet with bezafibrate HPβCD complex in Avicel, D(DiPac): placebo (no API) tablet with DiPac, D-BF: Tablet with bezafibrate in DiPac, D-BFβCD: Tablet with bezafibrate βCD complex in DiPac, D-BFHPβCD: Tablet with bezafibrate HPβCD complex in DiPac.

Tablet disintegration time was determined with PharmaTest Three Basket Tablet Disintegration Tester (PTZ). One tablets was placed into each glass tube of the basket-rack assembly without disk above tablet. The instrument moved the basket-rack assembly up and down over a distance of 55 mm (each way), 30 times per minute in 1 L water kept at 37˚C. The machine was set to run for 30 min to see weather tablets meet the common disintegration time of 30 min. In addition, time was noted when all the disintegrated particle passed out through the bottom wire mesh (2 mm aperture) on which each glass tubes rest.

Dissolution Test on Tablets

Dissolution studies on the tablets were performed in a similar way as described in 3.2.8. for complexes with some exception. Along with other API containing tablets in

their respective vessel, one A(PH102, placebo) tablet was put to a separate vessel to produce the blank samples against which Avicel tablet samples were measured spectrophotometrically. Similarly D(DiPac, placebo) tablet was used for other DiPac tablets. These blank/placebo tablets were run because it was seen that UV measurement against dissolution medium blank was completely different than with placebo tablet blanks. Sampling times were 5, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180 minutes or until 100% release.

4. RESULTS

4.1. Results of Bezafibrate Identification Studies

4.1.1. UV Spectrum

The UV spectrum of bezafibrate is given in Figure 4.1. λ_{max} was found to be 228 nm. Neither the spectrum nor the λ_{max} differed in the methanol or water.

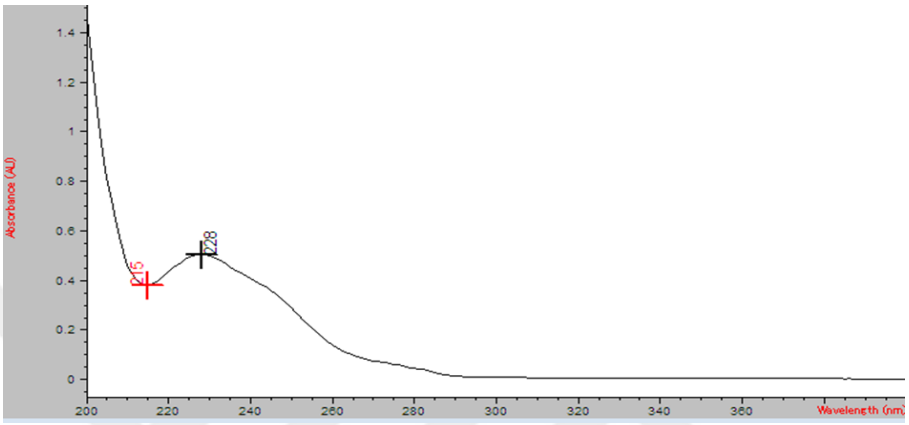


Figure 4.1. UV spectrum of bezafibrate (10 µg/mL)

4.1.2. DSC Thermograms of Bezafibrate

The differential scanning calorimetry thermogram of bezafibrate is given in Figure 4.2. The starting point of the endothermic peak corresponds to the melting point of bezafibrate is at 186ºC.

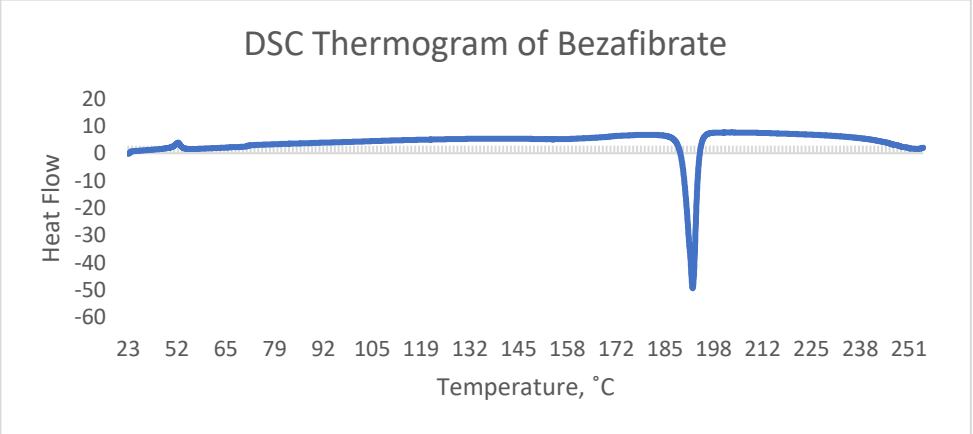


Figure 4.2. DSC thermogram of bezafibrate

4.1.3. FTIR Spectrum

The FTIR spectrum of bezafibrate that was obtained between wavelength of 4000 and 450 cm^{-1} in our studies is given in the Figure 4.3.

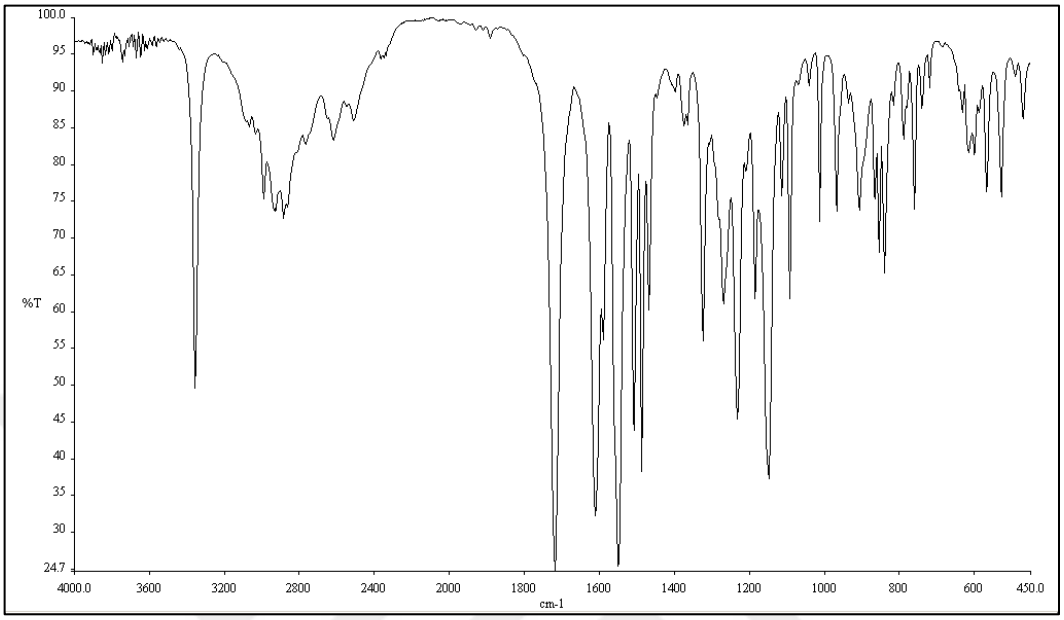


Figure 4.3. FTIR spectrum of bezafibrate, between 4000-450 cm^{-1}

The related reference spectrum from British Pharmacopoeia is given in Figure 4.4. It is to be noted that this spectrum shows the transmission peaks between 2000 and 400 cm^{-1}.

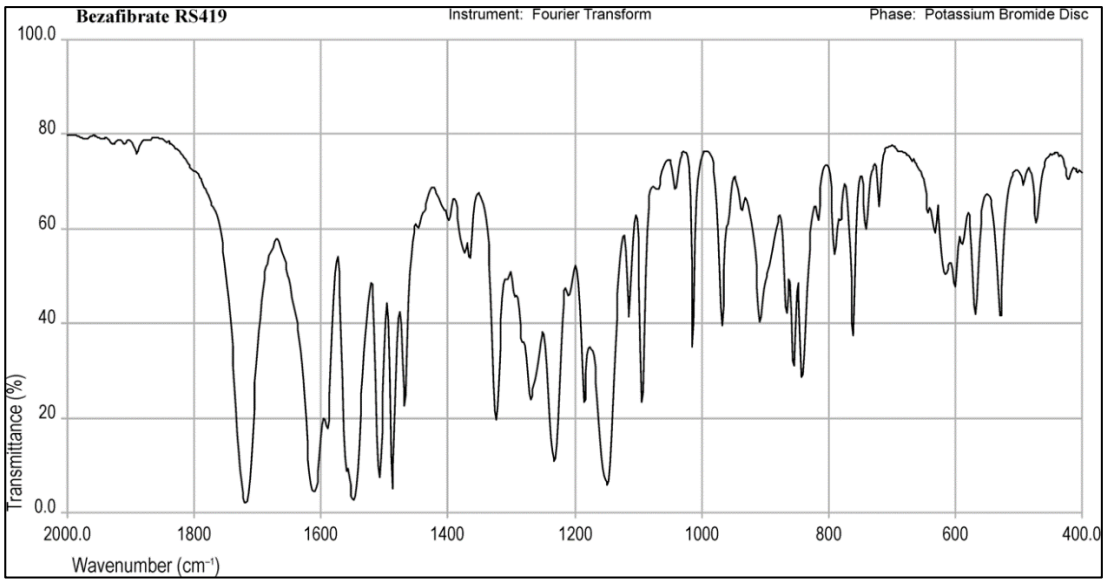


Figure 4.4. Bezafibrate IR spectrum from British Pharmacopoeia 2013, 2000-400 cm^{-1}

4.2. Quantitative Determination of Bezafibrate

4.2.1. Bezafibrate Calibration Curve

Bezafibrate calibration curve was constructed according to the method described in the section 3.3.2. The calibration curve is given in Figure 4.5. The values of the slopes and intercepts, along with other analytical data, are given in the Table 4.1.

4.2.2. Validation of Analytical Method for Bezafibrate

Specificity and Sensitivity

There was no other peaks observed nearby bezafibrate λ_{max} when absorbances were measured in solutions containing possible other substances, specially different cyclodextrins. However, appropriate blank was used in subsequent measuremnets as precaution. For example, placebo tablet dissolved or mixed with medium/water during dissolution studies.

Against water blank, multiple measurement of water as sample gave a noise absorbance of about 0.001. Therefore, concentration that gave 3 and 10 time this 0.001, that is, 0.003 and 0.01 were considered limit of detection and limit of quantification, respectively. With cyclodextrin solutions, the noise level was 0.002 giving a limit of detection and limit of quantification of 0.006 and 0.02, respectively.

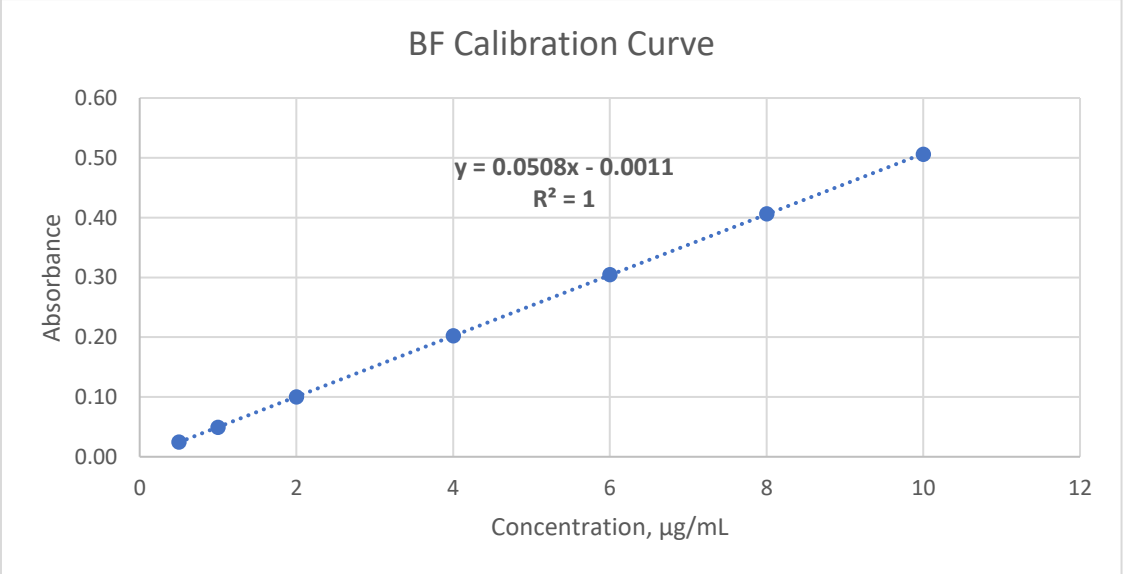


Figure 4.5. Calibration curve of bezafibrate in deionized water and regression equation (n = 6)

Table 4.1. Linear regression data (n=3) obtained in UV analysis of bezafibrate.

Parameter	Result
Linear Concentration Range	0.5µg/mL - 10 µg/mL
Slope of the regression line	0.0508
Intercept	-0.0011
Correlation Coefficient	1
Detection Limit	0.003 (water), 0.006 (CD)
Quantification Limit	0.01 (water), 0.02 (CD)

Linearity

Visual inspection of the bezafibrate calibration curve clearly shows a linearity over the range of 0.5-10 µg /mL. Data fitness coefficient, R^2 value found to be the maximum possible, 1. This also proves the linearity.

Accuracy and Precision

As described in section 3.3.2, three standard concentrations (1, 4, and 8µg /mL) of bezafibrate on the calibration curve were prepared and analyzed with 3 replicates (n = 3) for assessing intra-day and inter-day accuracy and precision. The accuracy and precision date are presented in Table 4.2 and 4.3.

Table 4.2. Intra-day accuracy and precision

	Added concentra-tion, µg/mL	**Found concentration µg/mL**	**Accuracy, %**	**Precision (coeff.of variation), %)**
Intra-day	1	1.005	0.50	4.15
	4	4.019	0.49	1.79
	8	8.070	0.88	2.22

Table 4.3. Inter-day accuracy and precision

	Added concentra-tion, µg/mL	**Found concentration µg/mL**	**Accuracy, %**	**Precision (coeff.of variation), %)**
Inter-day	1	0.964	-3.59	3.00
	4	3.991	-0.22	1.44
	8	7.958	-0.51	1.02

The intra-day and inter-day accuracy and precision data are presented in Figure 4.6 and 4.7, respectively.

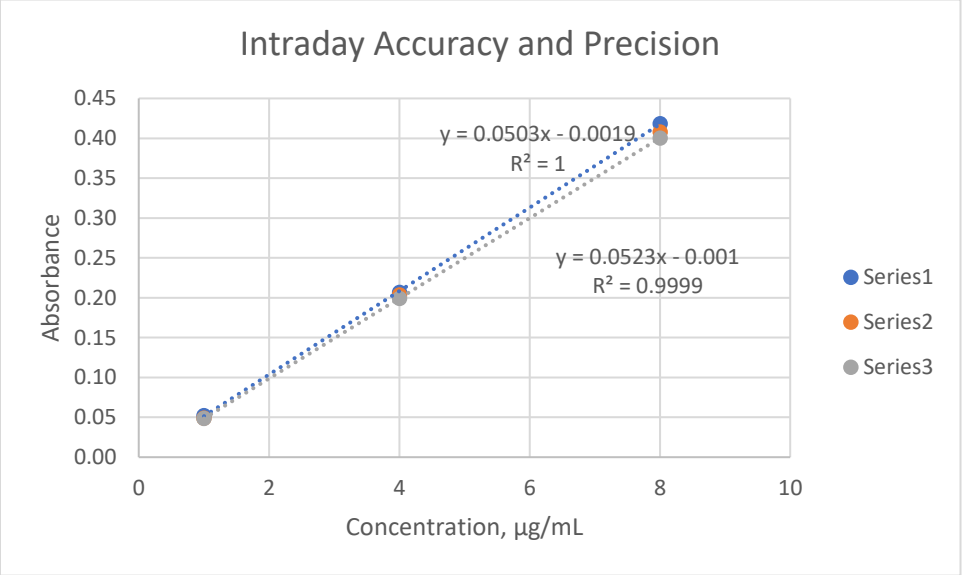


Figure 4.6. Intra-day accuracy curves (n=3) for bezafibrate

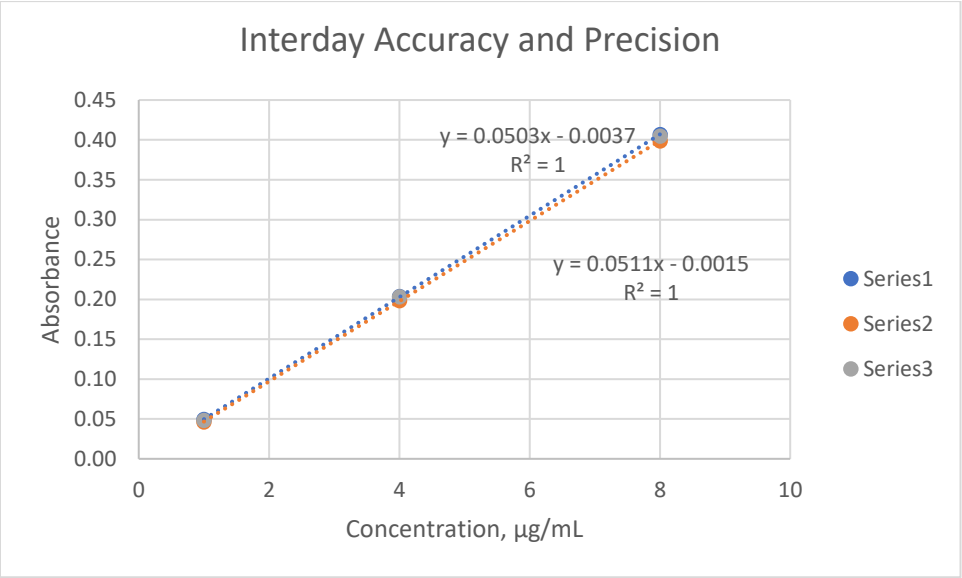


Figure 4.7. Inter-day accuracy curves (n=3) for bezafibrate

4.3. Complexation Period, pH and Temperature Effect, and Stability

4.3.1. Complexation Period

Data for the determination of equilibrium period are presented in Table 4.4. After 3rd day the bezafibrate solubility remained almost the same as after the 2nd day for all cyclodextrin types.

Table 4.4. Solubility of bezafibrate after different period of complexation

	αCD, mM		**βCD, mM**		**HPβCD, mM**		**γCD, mM**	
Days	5	15	5	15	5	15	5	15
	Concentration of bezafibrate , µg/mL							
1	58.63	81.52	78.77	160.91	84.89	148.17	60.78	87.56
2	65.40	86.22	93.94	168.36	93.34	147.39	71.42	91.21
3	66.04	86.09	92.02	169.31	93.75	148.08	71.79	92.39

4.3.2. Effect of pH on Complexation

The effect of pH on the complexation with all the four CDs are presented in Table 4.5. A parallel experiment (No CD) was done without addition of CD for comparison purpose. All of this experiments were done at room temperature.

Table 4.5. Effect of pH on the complexation of bezafibrate with different cyclodextrins (5 mM each, room temperature)

	pH 7.0	**pH 4.0**	**pH 1.5**	**pH 7.0**	**pH 4.0**	**pH 1.5**
	Concentration of BF, µg/mL			Increase in BF Solubility, x		
No CD	50.09	23.96	5.24	-	-	-
αCD	65.17	32.38	8.88	1.30	1.35	1.70
βCD	93.71	58.73	27.10	1.87	2.45	5.17
HPβCD	93.10	51.29	21.97	1.86	2.14	4.19
γCD	71.19	34.45	7.95	1.42	1.44	1.52

4.3.3. Effect of Temperature on Complexation

The effect of temperature was measured at all the 3 pH level with βCD and HPβCD. The results are presented in Table 4.6. A parallel experiment (No CD) was done without addition of CD for comparison purpose.

Table 4.6. Effect of temperature on the complexation of bezafibrate with βCD and HPβCD (5 mM each)

	pH 7.0		**pH 4.0**		**pH 1.5**	
	25˚C	60˚C	25˚C	60˚C	25˚C	60˚C
	Concentration of BF, µg/mL					
No CD	50.28	113.35	24.16	65.37	5.47	14.66
βCD	93.90	182.30	58.93	131.18	21.35	33.14
HPβCD	93.30	203.51	51.49	67.32	20.32	35.92

4.3.4. Stability of Bezafibrate at 60˚C

The changes in concentration at 60˚C and at different pH are shown in Table 4.7. The date are presented in graphical form in Figure 4.8.

Table 4.7. Stability of bezafibrate at 60˚C

	pH 7.0	**pH 4.0**	**pH 1.5**
Hours	Concentration of bezafibrate, µg/mL		
0	10.23	9.44	8.72
2	10.66	10.17	8.99
4	10.90	10.01	8.92
6	10.87	10.06	8.79
8	11.16	10.37	8.85
24	10.96	10.26	9.18
30	10.89	10.17	9.13
48	11.19	10.43	9.67
72	11.46	9.51	8.72

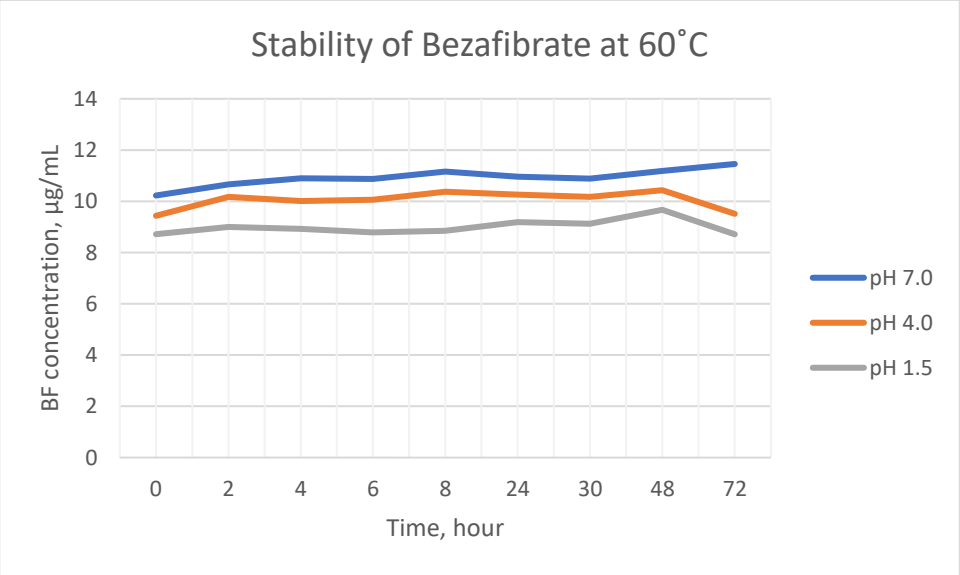


Figure 4.8. Stability of bezafibrate at 60˚C in aqueous solutions at pH 7.0, 4.0 and1.5

4.4. Results of Phase Solubility Analysis

4.4.1. Bezafibrate with αCD

Phase solubility analysis data complexation of bezafibrate with αCD are presented in Table 4.8 and Figure 4.9.

Table 4.8. Solubility of bezafibrate with αCD at 25˚C in aqueous solutions at different pH values

25˚C	**Concentration of BF, mM**		
αCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.139	0.025	0.016
1	0.156	0.058	0.022
2	0.181	0.056	0.019
5	0.186	0.090	0.027
10	0.197	0.085	0.027
15	0.238	0.093	0.037
25	0.239	0.158	0.041

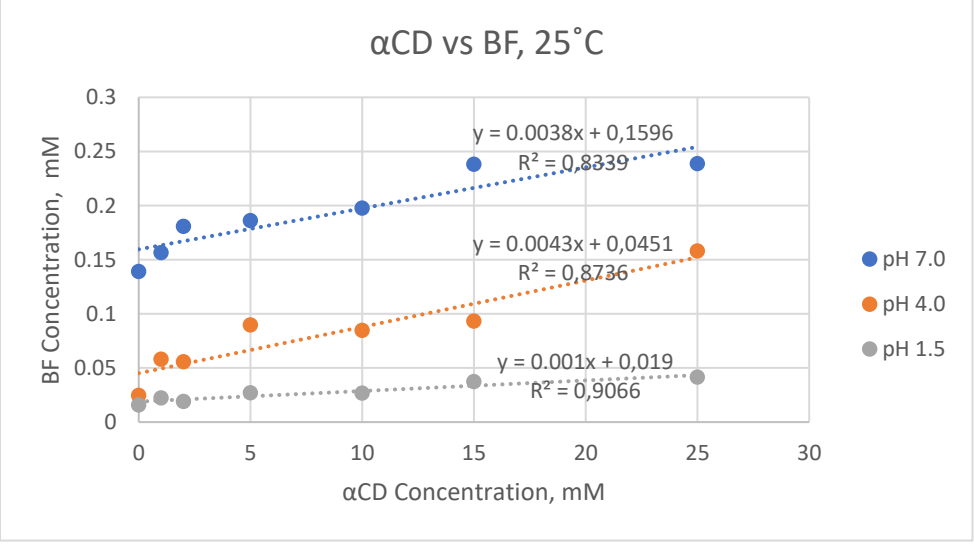


Figure 4.9. Phase solubility diagram of bezafibrate with α-cyclodextrin in aqueous solutions at PH 7.0, 4.0 and 1.5 at 25˚C

4.4.2 Bezafibrate with βCD

Phase solubility analysis data complexation of bezafibrate with βCD are presented in Table 4.9-4.10 and Figure 4.10-4.11.

Table 4.9. Solubility of bezafibrate with βCD at 25˚C in aqueous solutions at different pH values

25˚C	**Concentration of BF, mM**		
βCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.139	0.025	0.014
1	0.162	0.061	0.026
2	0.191	0.077	0.034
5	0.260	0.163	0.059
10	0.347	0.243	0.119
15	0.397	0.333	0.187

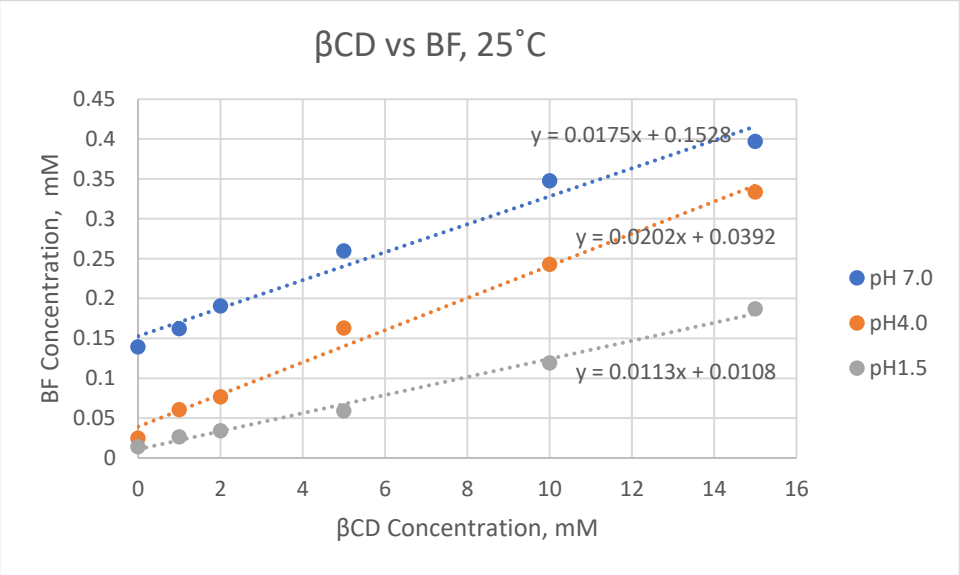


Figure 4.10. Phase solubility diagram of bezafibrate with β-cyclodextrin in aqueous solutions at PH 7.0, 4.0 and 1.5 at 25˚C

Table 4.10. Solubility of bezafibrate with βCD at 60˚C in aqueous solutions at different pH values

60˚C	**Concentration of BF, mM**		
βCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.313	0.181	0.065
1	0.385	0.189	0.056
2	0.439	0.276	0.072
5	0.504	0.363	0.092
10	0.800	0.464	0.198
15	0.997	0.661	0.218

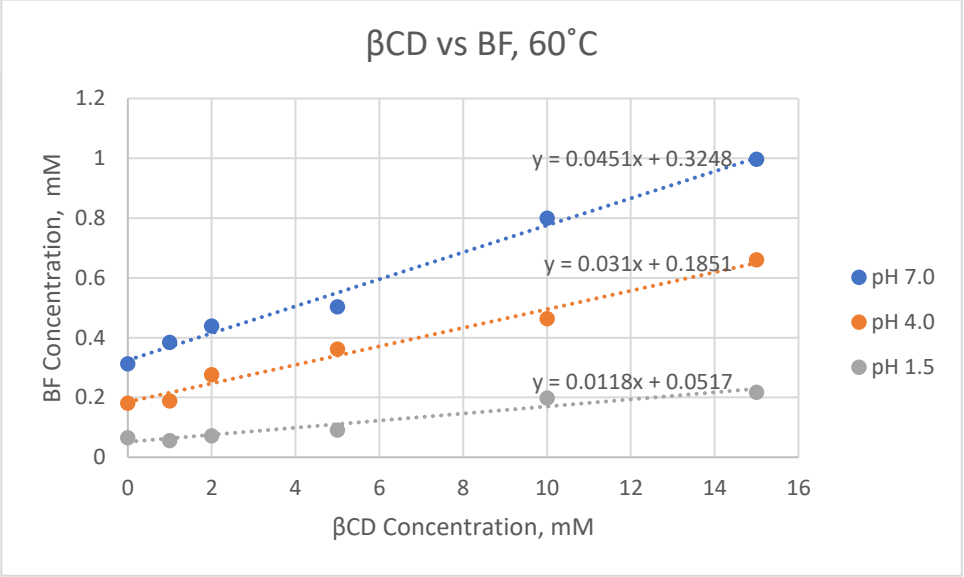


Figure 4.11. Phase solubility diagram of bezafibrate with β-cyclodextrin in aqueous solutions at PH 7.0, 4.0 and 1.5 at 60˚C

4.4.3. Bezafibrate with HPβCD

Phase solubility analysis data complexation of bezafibrate with HPβCD are presented in Table 4.11-4.12 and Figure 4.12-4.13.

Table 4. 11. Solubility of bezafibrate with HPβCD at 25˚C in aqueous solutions at different pH values

25˚C	**Concentration of BF, mM**		
HPβCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.139	0.025	0.014
1	0.168	0.076	0.026
2	0.215	0.088	0.035
5	0.258	0.142	0.056
10	0.326	0.186	0.101
15	0.408	0.273	0.170
25	0.638	0.314	0.290

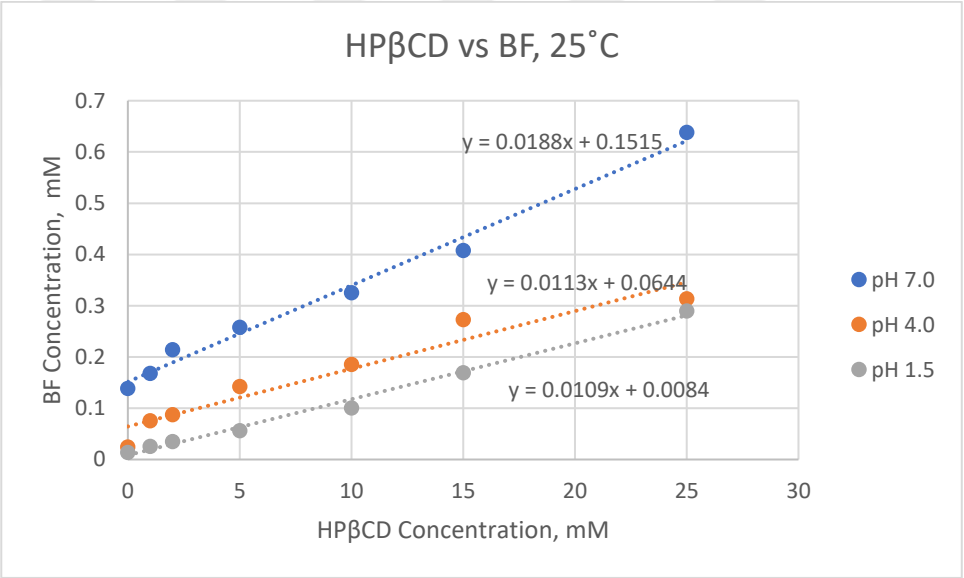


Figure 4.12. Phase solubility diagram of bezafibrate with HPβ-cyclodextrin in aqueous solutions at PH 7.0, 4.0 and 1.5 at 25˚C

Table 4. 12. Solubility of bezafibrate with HPβCD at 60˚C in aqueous solutions at different pH values

60˚C	**Concentration of BF, mM**		
HPβCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.313	0.181	0.065
1	0.359	0.183	0.056
2	0.444	0.199	0.070
5	0.562	0.284	0.099
10	0.749	0.559	0.140
15	0.976	0.748	0.212
25	1.365	0.944	0.200

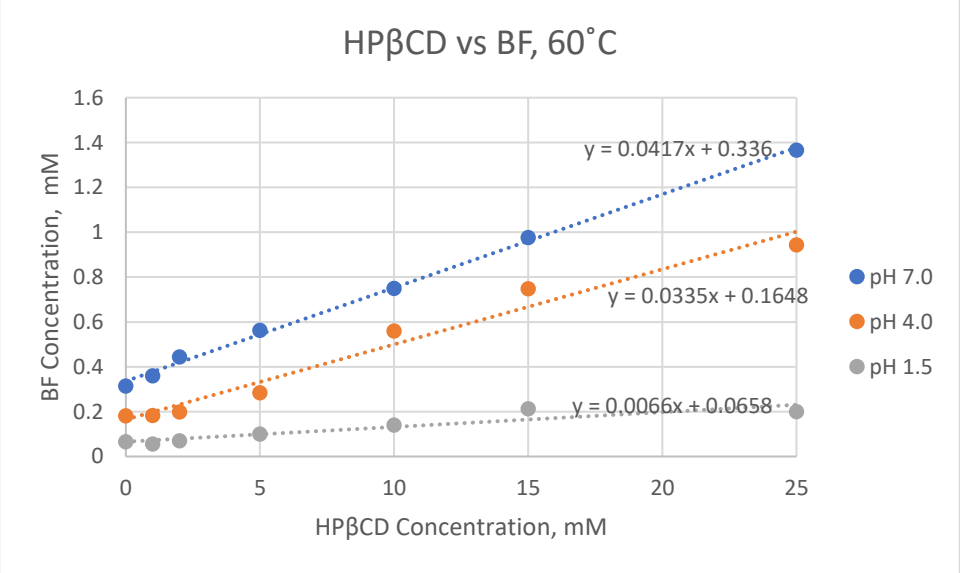


Figure 4.13. Phase solubility diagram of bezafibrate with HPβ-cyclodextrin in aqueous solutions at PH 7.0, 4.0 and 1.5 at 60˚C

4.4.4. Bezafibrate with γCD

Phase solubility analysis data complexation of bezafibrate with γCD are presented in Table 4.13 and Figure 4.14.

Table 4.13. Solubility of bezafibrate with γCD at 25˚C in aqueous solutions at different pH values

25˚C	**Concentration of BF, mM**		
γCD, mM	pH 7.0	pH 4.0	pH 1.5
0	0.139	0.025	0.016
1	0.150	0.026	0.011
2	0.173	0.047	0.015
5	0.197	0.052	0.015
10	0.229	0.048	0.027
15	0.252	0.059	0.031
25	0.308	0.096	0.043

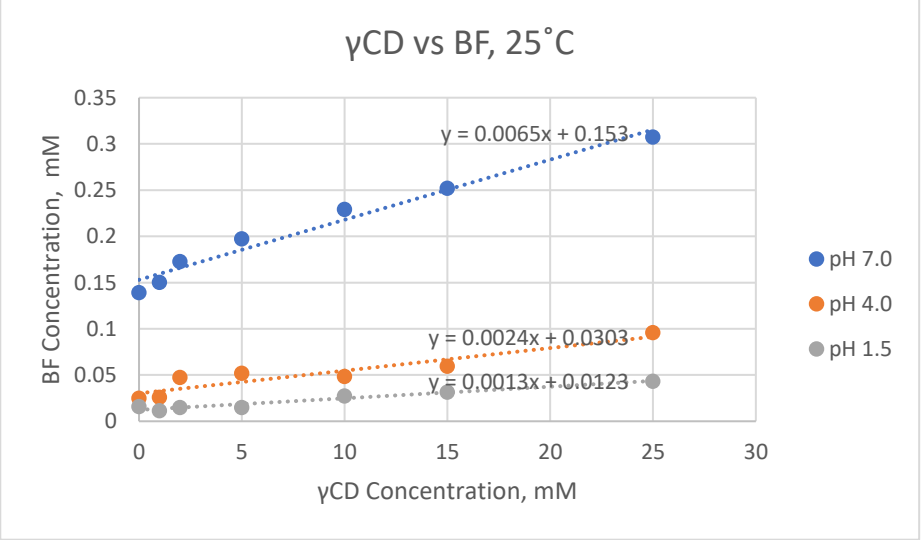


Figure 4.14. Phase solubility diagram of bezafibrate with γCD in aqueous solutions at PH 7.0, 4.0 and 1.5 at 25˚C

4.4.5. Phase Solubility of with Different CDs and Temperature at a Specific pH

Phase solubility data for bezafibrate at a specific pH are presented in the following Figure 4.15 to 4.17 to show comparison with different CDs and temperature.

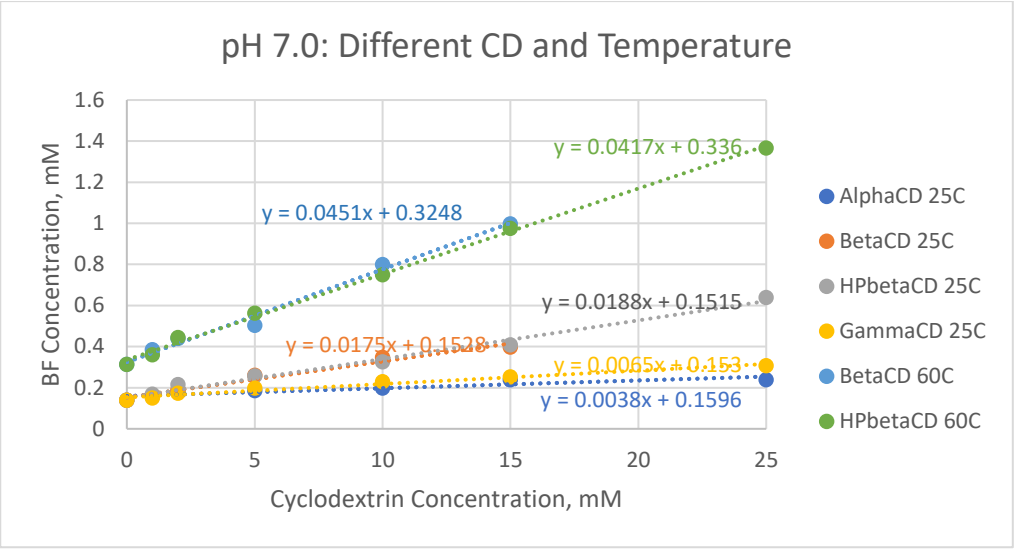


Figure 4.15. Phase solubility diagram of bezafibrate with different cyclodextrins at different temperature in aqueous solution at pH 7.0

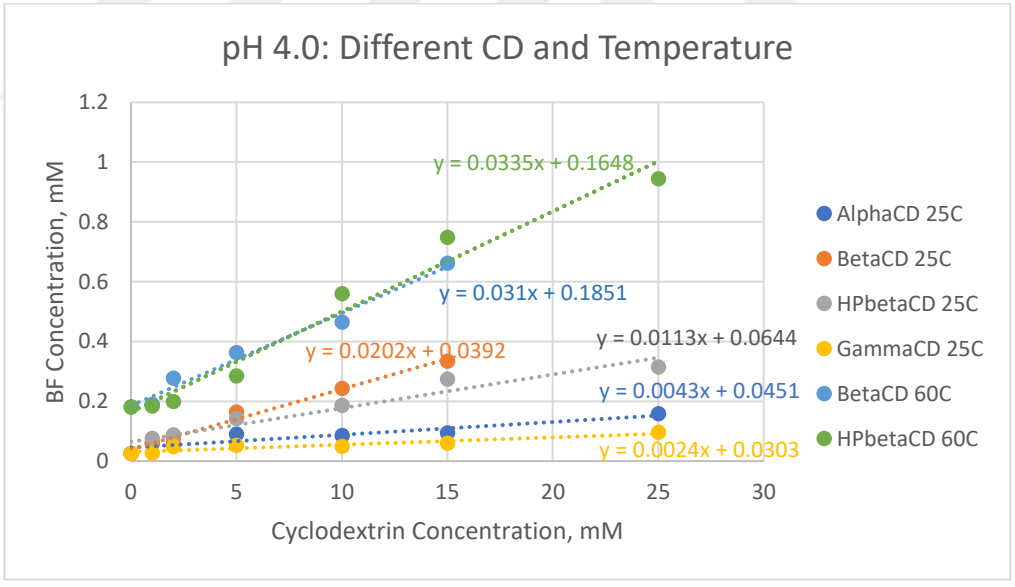


Figure 4.16. Phase solubility diagram of bezafibrate with different cyclodextrins at different temperature in aqueous solution at PH 4.0

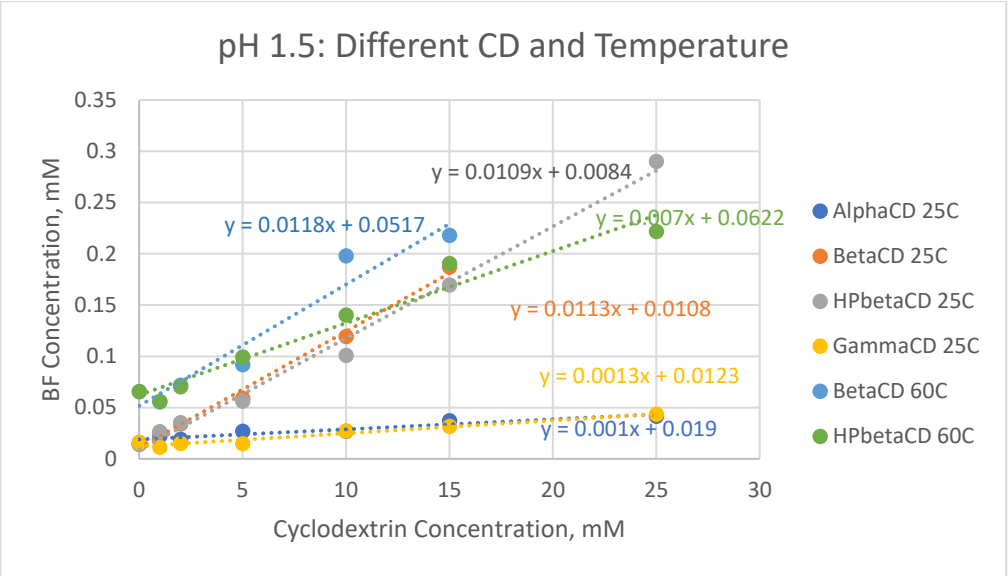


Figure 4.17. Phase solubility diagram of bezafibrate with different cyclodextrins at different temperature in aqueous solution at PH 1.5

4.4.6. Stability Constants for the Complexes

The 1:1 equilibrium constants for different bezafibrate complexes are shown below in Table 4.14.

Table 4.14. Stability constant values for different bezafibrate complexes

pH7.0	
CDs, ˚C	**K, /M**
αCD, 25˚C	27
βCD, 25˚C	128
βCD, 60˚C	151
HPβCD, 25˚C	138
HPβCD, 60˚C	139
γCD, 25˚C	49
pH4.0	
αCD, 25˚C	174
βCD, 25˚C	367

βCD, 60˚C	177
HPβCD, 25˚C	203
HPβCD, 60˚C	192
γCD, 25˚C	43
pH 1.5	
αCD, 25˚C	67
βCD, 25˚C	767
βCD, 60C	182
HPβCD, 25˚C	739
HPβCD, 60˚C	108
γCD, 25˚C	87

4.5. Fourier Transform Infrared Spectroscopy Results

Fourier transform spectra for the four group of samples, namely, αCD, βCD, HPβCD and γCD group are presented in Figure 4.18 through 4.21. In each group, BF and pure cyclodextrin were included for comparison purpose. In the same way, a physical mixture of Bf and corresponding CD was also included.

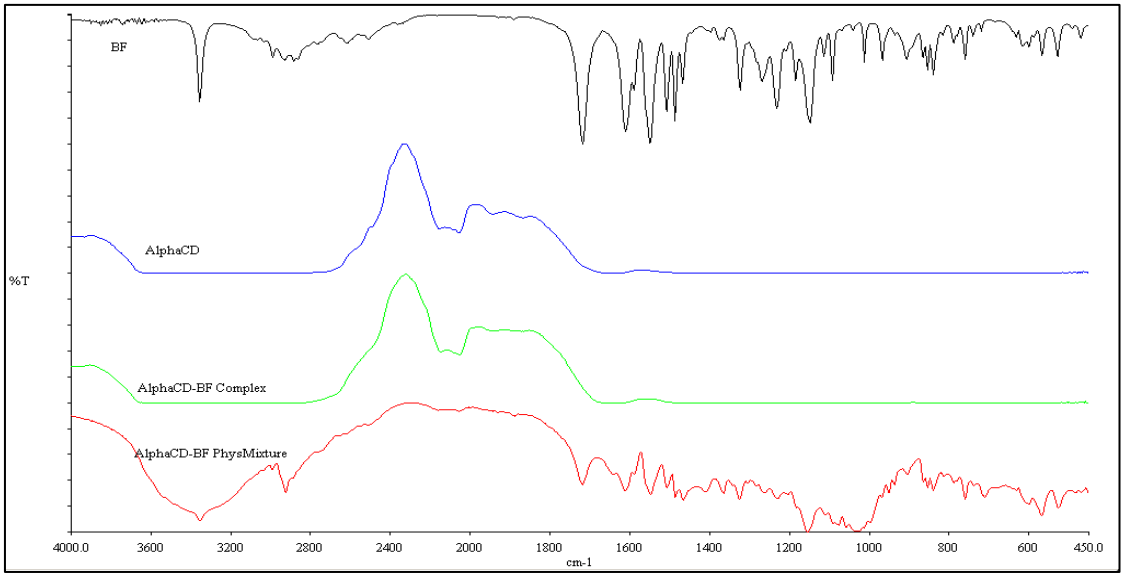


Figure 4.18. FTIR spectrum of bezafibrate, αCD, BFαCD complex and physical mixture of BF and αCD; top to bottom

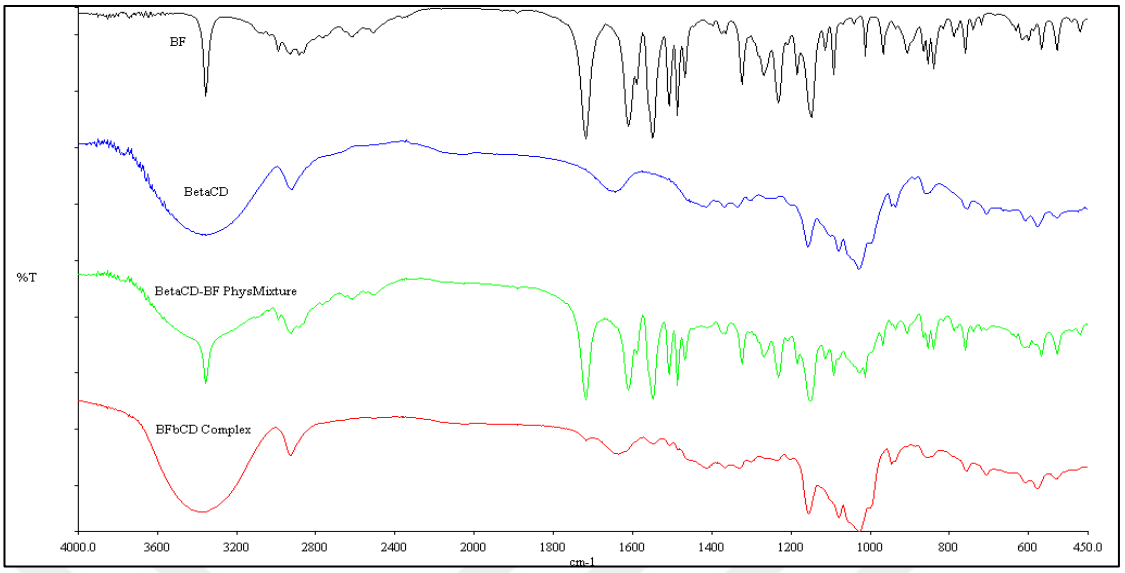


Figure 4.19. FTIR spectrum of bezafibrate, βCD, BF and βCD physical mixture and BFβCD complex; top to bottom

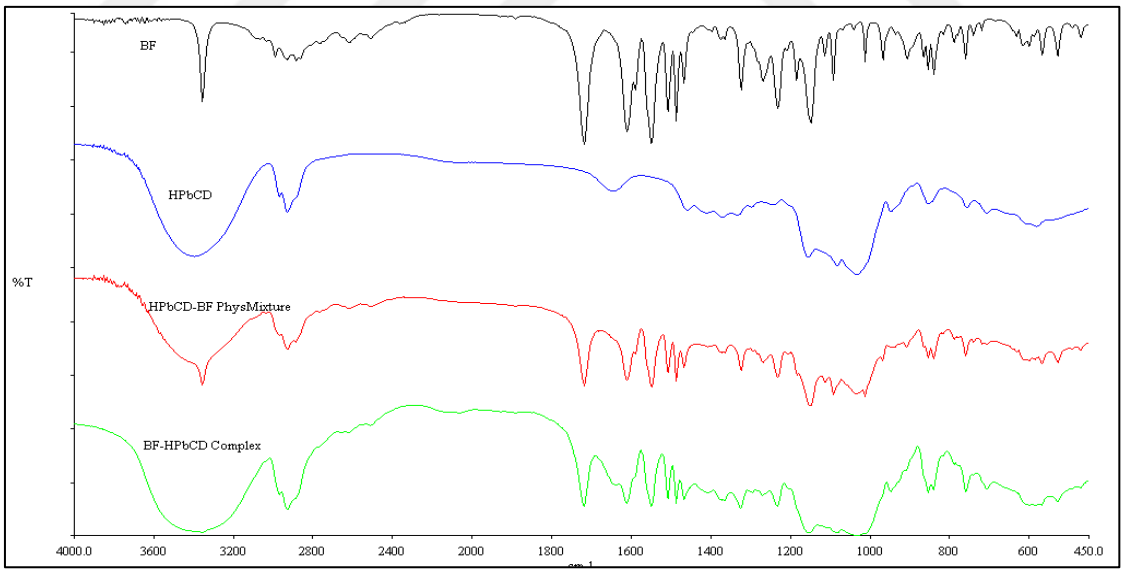


Figure 4.20. FTIR spectrum of bezafibrate, HPβCD, BF and HPβCD physical mixture and BFHPβCD complex; top to bottom

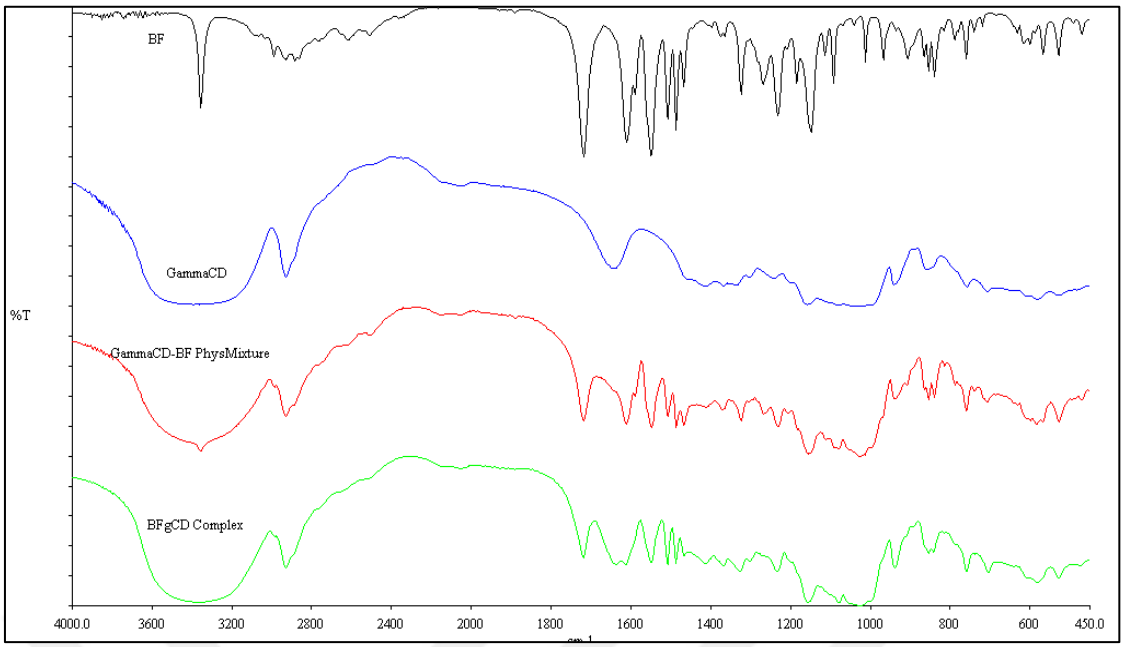


Figure 4.21. FTIR spectrum of bezafibrate, γCD, BF and γCD physical mixture and BFγCD complex; top to bottom

4.6. Differential Scanning Calorimetry Results

The DSC thermograms of different group of samples are presented in Figure 4.22 through 4.25.

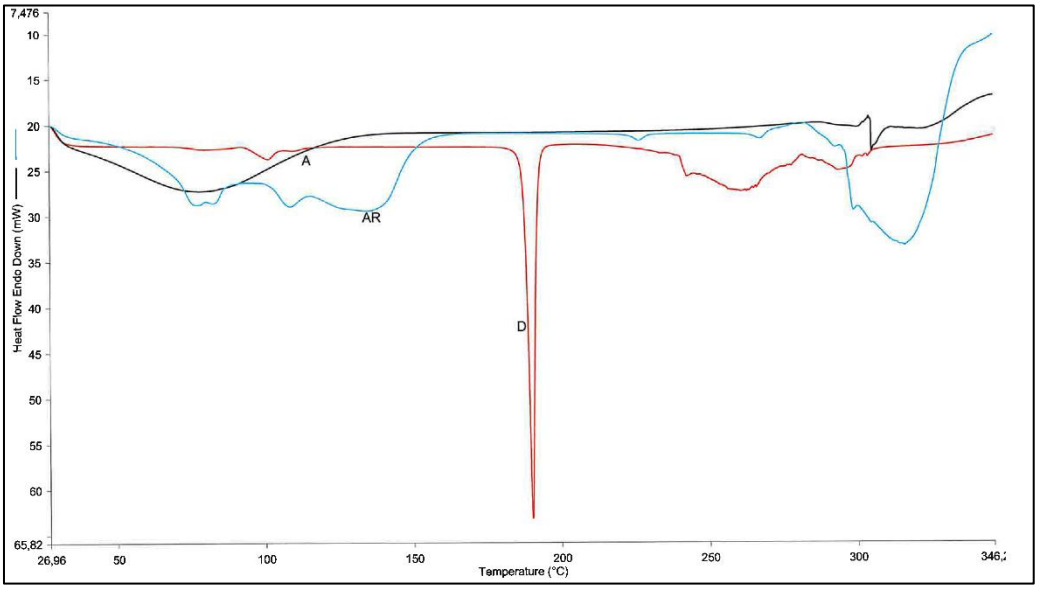


Figure 4.22. DSC thermogram of bezafibrate (D), αCD (AR), and BFαCD complex (A)

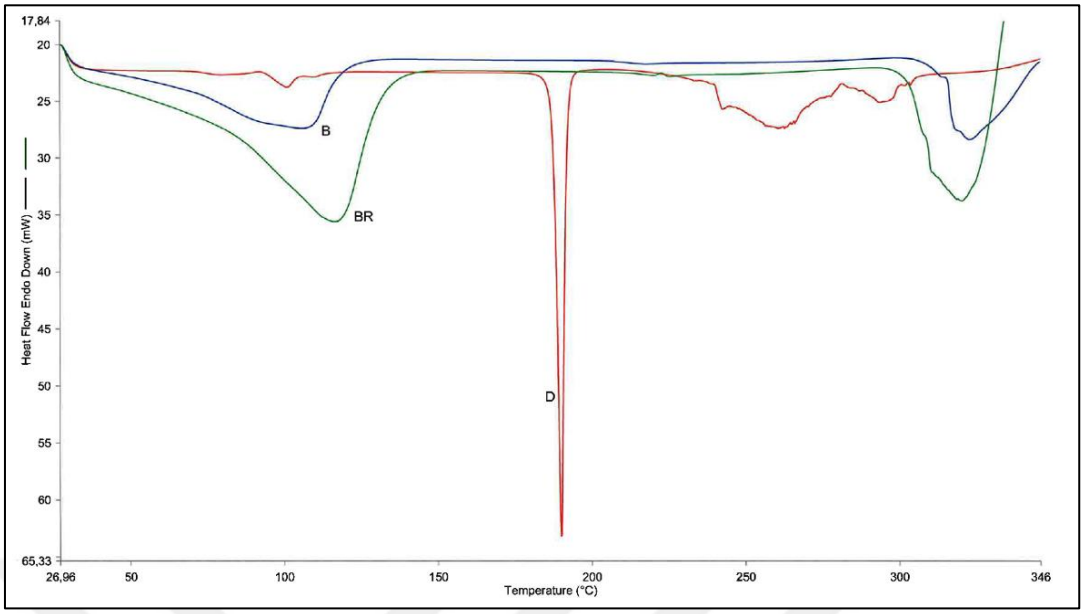


Figure 4.23. DSC thermogram of bezafibrate (D), βCD (BR) and BF βCD complex (B)

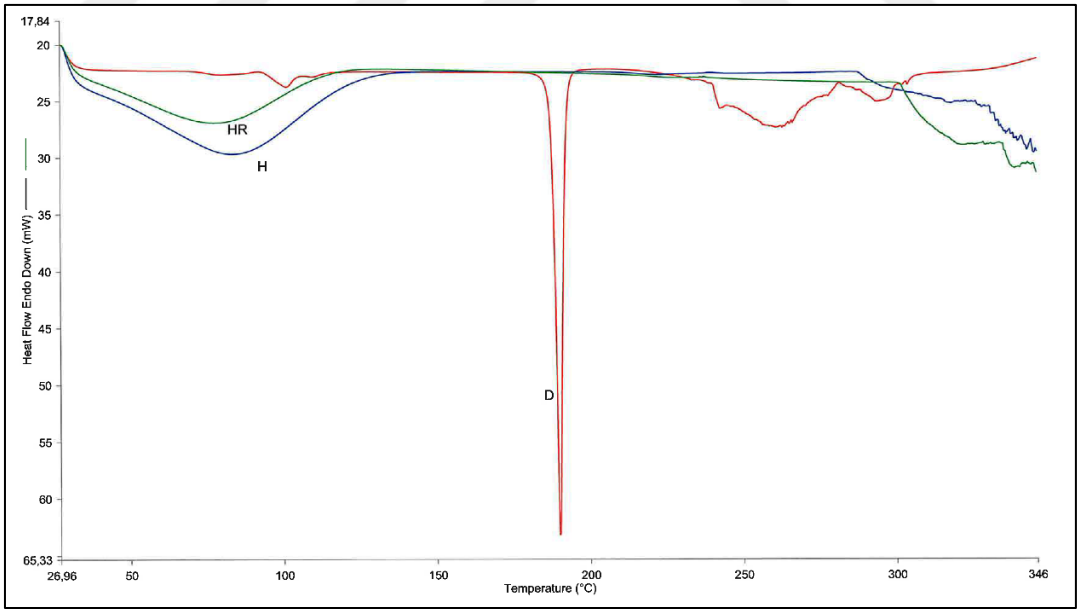


Figure 4.24. DSC thermogram of bezafibrate (D), HPβCD (HR) and BF-HPβCD complex (H)

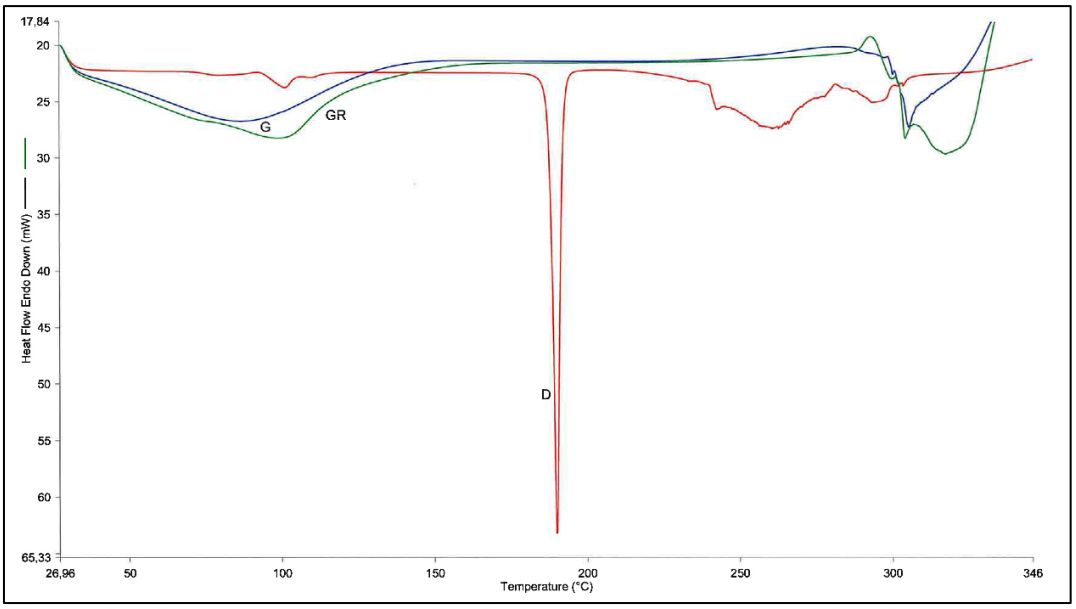


Figure 4.25. DSC thermogram of bezafibrate (D), γCD (GR) and BFγCD complex (G)

4.7. Scanning Electron Microscopic Study

Figure 4.26 shows the scanning electron photomicrograph. The top image (a) belongs to bezafibrate powder which looks like rod shaped long crystal. Three image on the left hand side (b, c, d) belong to un-lyophilized βCD powder, lyophilized βCD, and lyophilized BFβCD complex, respectively. The remaining three images on the right hand side (e, f, g) belong to un-lyophilized HPβCD powder, lyophilized HPβCD, and lyophilized BFHPβCD complex. Related samples of γCD and αCD were not investigated yet due to the limited access to the electron microscope. Therefore, SEM studies were performed on βCD and HPβCD related sample as they showed better complexation in previous studies.

As can be seen from the Figure 4.26, bezafibrate particles were long rod shaped. Both un-lyophilized and lyophilized βCD were irregular but compact particles. While un-lyophilized HPβCD were mostly spongy spherical, lyophilized HPβCD were perforated continuous sheet like structure. BFβCD and BFHPβCD complexes were completely different from their respective CD. They were light flaky or sheet like structures respectively.

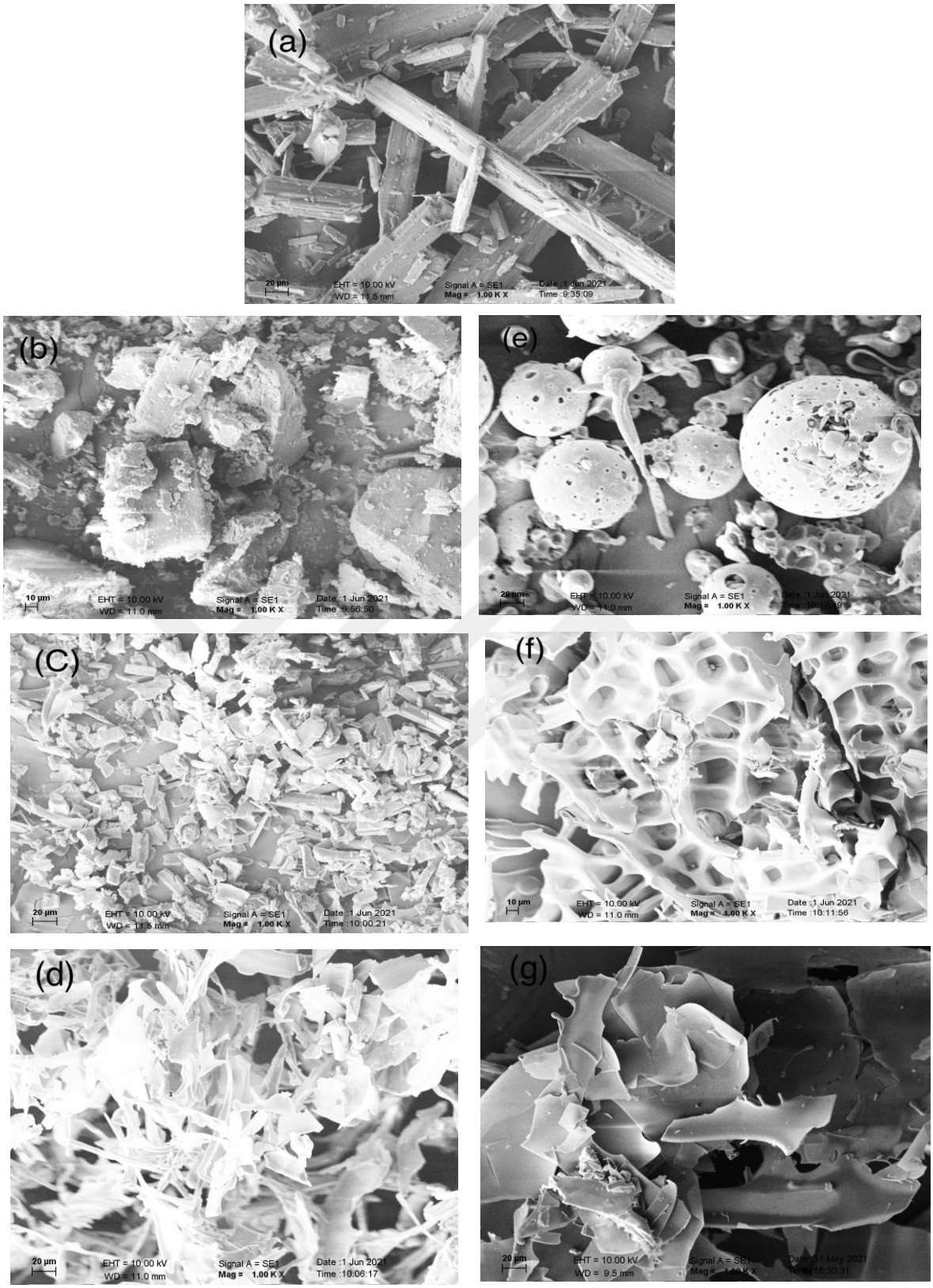


Figure 4.26. SEM photomicrographs of (a) bezafibrate, (b) βCD, (c) lyophilized βCD, (d) BFβCD complex, (e) HPβCD, (f) lyophilized HPβCD, and (g) BFHPβCD complex

4.8. Dissolution Test Results for Bezafibrate and Its CD Complexes

The dissolution study profiles for bezafibrate, its βCD and HPβCD complexes are shown in Figure 4.27

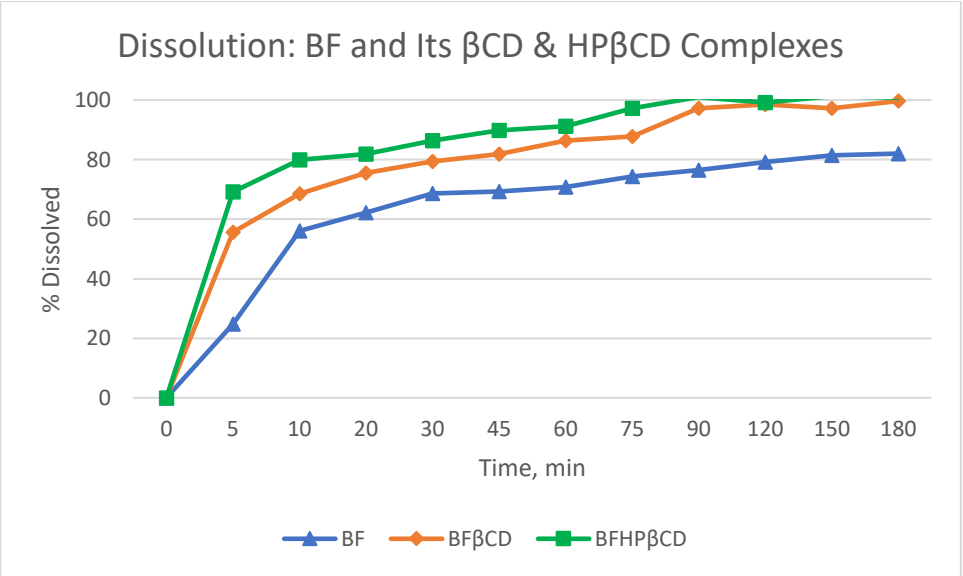


Figure 4.27. Dissolution profiles of bezafibrate (BF), βCD (BFβCD) and HPβCD complexes (BFHPβCD)

The results of comparison between pairs by similarity factors are shown below in Table 4.15.

Table 4.15. Comparison by similarity factor between dissolution of bezafibrate and complexes

Comparison Pairs		**f_2**
BF	BFβCD	37.86
BF	BFHPβCD	30.93
BFβCD	BFHPβCD	**56.18**

4.9. Characterization Studies on Tablets with Complexes

4.9.1. Thickness, Diameter and Hardness Test Results

Average thickness, diameter and hardness of different tablet formulations are presented in Table 4.16.

Table 4.16. Average thickness, diameter and hardness (n = 3)

Tablets	Thickness, mm	Diameter, mm	Hardness, N
A-BF	5.20	11.98	143.10
A-BFβCD	5.31	12.02	171.53
A-BFHPβCD	5.47	12.03	195.57
D-BF	4.81	11.99	80.80
D-BFβCD	4.89	11.98	86.23
D-BFHPβCD	5.14	11.99	106.07

A-BF: Tablet with bezafibrate in Avicel, A-BFβCD: Tablet with bezafibrate βCD complex in Avicel, A-BFHPβCD: Tablet with bezafibrate HPβCD complex in Avicel, D-BF: Tablet with bezafibrate in DiPac, D-BFβCD: Tablet with bezafibrate βCD complex in DiPac, D-BFHPβCD: Tablet with bezafibrate HPβCD complex in DiPac.

4.9.2. Weight, Friability and Disintegration Test Results

Average weight, friability and disintegration time of the same tablets are presented in Table 4.17.

Table 4.17. Average weight, friability and disintegration time (n = 3)

Tablets	Weight, mg	Friability,%	Disintegration time, min
A-BF	612.85	0.12	9.97
A-BFβCD	613.75	-0.31	13.97
A-BFHPβCD	610.83	-0.19	16.03
D-BF	615.50	-0.20	9.65
D-BFβCD	614.33	0.08	9.67
D-BFHPβCD	612.20	-0.03	10.90

A-BF: Tablet with bezafibrate in Avicel, A-BFβCD: Tablet with bezafibrate βCD complex in Avicel, A-BFHPβCD: Tablet with bezafibrate HPβCD complex in Avicel, D-BF: Tablet with bezafibrate in DiPac, D-BFβCD: Tablet with bezafibrate βCD complex in DiPac, D-BFHPβCD: Tablet with bezafibrate HPβCD complex in DiPac.

4.9.3. Dissolution Test Results for Tablets

The dissolution profiles for Avicel PH102 tablets with bezafibrate, its βCD and HPβCD complexes are shown in Figure 4.28. A tablet formulation with a physical mixture of bezafibrate and HPβCD with Avicel is also included.

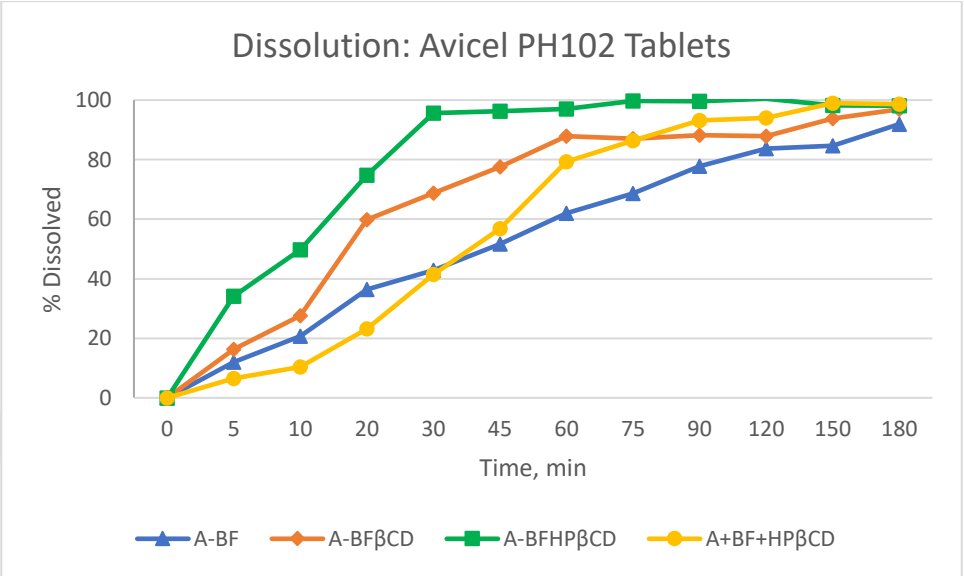


Figure 4.28. Dissolution profiles of Avicel tablets with bezafibrate (A-BF), βCD (A-BFβCD) HPβCD complexes (A-BFHPβCD) and physical mixture of bezafibrate and BFβCD in Avicel (A+BF+HPβCD)

The dissolution profiles for DiPac tablets with bezafibrate, its βCD and HPβCD complexes are shown in Figure 4.29.

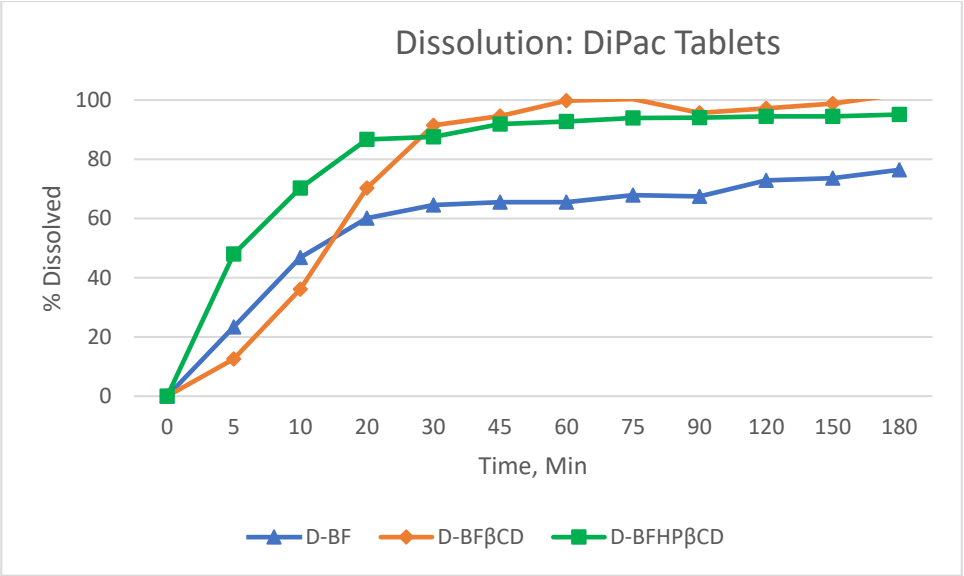


Figure 4.29. Dissolution profiles of DiPac tablets with bezafibrate (D-BF), βCD (D-BFβCD) HPβCD complexes (D-BFHPβCD)

The calculated similarity factor between different pairs of tablet are presented in Table 4.18.

Table 4.18. Comparison by similarity factor between dissolution of different tablet formulations with bezafibrate and complexes

Comparison Pairs		**f_2**
Avicel Tablets		
A-BF	A-BFβCD	38.38
A-BF	A-BFHPβCD	25.24
A-BFβCD	A-BFHPβCD	40.38
Against Physical Mixtutre		
A+BF+HPβCD	A-BF	**46.25**
A+BF+HPβCD	A-BFβCD	38.78
A+BF+HPβCD	A-BFHPβCD	25.97
DiPac Tablets		
D-BF	D-BFβCD	30.21
D-BF	D-BFHPβCD	30.76
D-BFβCD	D-BFHPβCD	39.50

5. DISCUSSION AND CONCLUSION

5.1. Discussion

5.1.1. Identification Studies

The UV spectrum obtained in our study is in agreement with the spectra reported before (54). Strong absorbance was noticed at the λ_{max} of 228 nm with as low concentration as 1-10 µg/mL in methanol. Even though bezafibrate is poorly water soluble (59), such low level of aqueous solution was easily possible. The reason for preference of water is obvious. Spectrum was determined with aqueous solution. The spectra obtained from both water and methanol solution were identical in all respect. Therefore, subsequent analytical method was validated in aqueous solution.

This single sharp and deep endothermic peak in the DSC thermogram is in conformity to reported melting point of bezafibrate (59).

The FTIR spectrum in the wave length range of 2000-400 cm^{-1} is peak-to-peak in agreement with that from Pharmacopoeia. This is the range given in Pharmacopoeia. The 4000-2000 cm^{-1} region in our spectrum is an additional information.

Thus the UV, DSC and FTIR study results which were supported by the published literature positively identified bezafibrate.

The peaks in the spectrum of bezafibrate are evaluated with previously reported interpretation (60, 61).

5.1.2. Quantitative Determination of Bezafibrate

The UV absorption spectrum of bezafibrate was found to be 228 nm in our laboratory settings which was the same as that reported literature (62). Spectra of bezafibrate in water, methanol, and along with cyclodextrins and other tablet excipient (Avicel, DiPac and magnesium stearate) were found to be similar. There was no drift of λ_{max} indicating good specificity of the analytical method. As seen in Figure 4.5, seven point calibration curve, with six replicates for each point, was clearly linear with correlation coefficient (R^2) value of 1 between absorbance and concentration. The concentration range selected was 0.5-10 µg/ml between which our expected measurement lied. The limit of detection was 0.003 and limit of quantitation 0.01 with water background whereas these values were 0.006 and 0.02 with CD solution as background. 0.5 µg/mL of bezafibrate, the lowest point of our calibration curve corresponded to this quantification limit. Six more point were selected to cover a range where most of our

sample were expected to fall within. Any sample above this range were appropriately diluted to bring within this range.

Using 3 different concentration of 1, 4 and 8 µg/mL, intra-day and inter-day accuracy and precision were measured (Table 4.2-4.3). Inter-day precision varied between 1.02 and 3.0 % whereas intra-day precision varied between 1.78 and 4.15%. Inter-day accuracy varied between -3.59 and -0.22%; intra-day accuracy varied between 0.49 and 0.88. Thus the maximum accuracy and precision value was -3.59 and 4.15, respectively, with all other values much less than ±2%. When both intra-day and inter-day accuracy and precision data were plotted (Figure 4.6 and 4.7), they were very close, and so they mostly superimpose on each other.

5.1.3. Complexation Period, pH and Temperature Effect, and Stability

Bezafibrate is practically in soluble in water. One of the methods to increase solubility of lipophilic drugs is complexation with cyclodextrins. In this study initial experiment with kneading method of complexation was done with 4 different CDs (αCD, βCD, HPβCD and γCD) and it was not successful as described in section 3.2.3. Stirring CD solution with bezafibrate fine powder was done and it showed good complexation results.

As seen in Table 4.4, bezafibrate concentrations after 2-day and 3-day of continuous stirring were seen to be very similar. This was true for both 5 and 15 mM CD concentrations. Therefore, all subsequent complexation was carried out for a period of 2 days.

The effect of pH on complexation with αCD, βCD, HPβCD, and γCD is reported in Table 4.5 with all the experiment done at 5 mM CD concentration. Three column on right hand side of the table showed how many time the concentration changed. Solubility of bezafibrate without any CD (No CD) showed a drastic reduction in solubility when pH changes from 7 (50.09 µg/mL) to 1.5 (5.24 µg/mL). Presence of CDs increased the solubility but this trend of solubility reduction was all the same when pH turned acidic. That is, all CDs showed highest solubility of bezafibrate at pH 7.0, and least at pH 1.5 with pH 4.5 values in between these two extreme. βCD increased the solubility by a maximum 1.87 time at pH 7.0; by a maximum 2.45 times at pH 4.5, again at pH 1.5, this increase in solubility was 5.17 time. This final high increase was due to the very poor inherent bezafibrate solubility of only 5.24 µg/mL at pH 1.5 when there was no CD. This

observation indicates that reduction in inherent solubility (more bezafibrate in solid state) reduces the chances of molecule to molecule interaction with CD (in solution state). Overall the solubility increase by αCD and γCD was very poor; βCD and HPβCD showed higher increase in solubility. Therefore, αCD and γCD were excluded from the subsequent temperature experiments.

As seen from Table 4.6, with the increase in temperature from 25 to 60˚C, inherent solubility increased. Both βCD and HPβCD also increased the bezafibrate solubility in the same way. This observation was true at all the pH levels. However, absolute concentration remained higher at pH 7.0. An insight into the pH 7 solubility values presents the temperature effect more clearly. With βCD, at 25 ˚C, 93.90-50.28 = 43.62 µg/mL can be supposed to remain in complexed state, at 60˚C this increased to 182.3-113.35 = 68.95 µg/mL. So, the net temperature effect was 68.95-43.62 = 25.33 µg/mL. This value for HPβCD was far greater at 47.14 µg/mL. This indicated that complexation of poorly soluble drug at elevated temperature can be more efficient. However, question arises regarding the stability of the compound.

Sixty degree Celsius is a moderately high temperature, still it occurred to us that a check of bezafibrate stability at this temperature would make sense to dispel the doubt that complexation at this temperature may be associated with bezafibrate decomposition. The stability result can be seen in Table 4.7 and Figure 4.8, bezafibrate concentration remained almost constant throughout the three days period with no sign of instability. This was done with completely dissolved amount from stock solution unlike the complexation experiment where undissolved excess suspended drug particle remains present all the time obscuring the stability issues.

5.1.4. Phase Solubility Analysis

The solubilization ability of CDs can be quantitatively evaluated by the phase solubility analysis where concentration of guest molecule is plotted against cyclodextrin concentration. The slopes of the phase solubility lines are used to calculate the stability constant of the complexes as proposed by Higuchi and Connors (33). The phase solubility data for αCD, βCD, HPβCD and γCD in aqueous solutions of different pHs (7.0, 4.0 and 1.5) and at temperatures of 25 °C and 60°C (βCD, HPβCD) are presented in Table 4.8 to Table 4.13 and their corresponding solubility diagrams are presented in Figure 4.9-4.14. The concentration range of cyclodextrins was use up to 15 mM for βCD (limited by its maximum solubility of 16 mM) and up to 25 mM for other three CDs. The apparent

solubility of bezafibrate increased to different degree but linearly as a function of all CDs. The following Table 5.1 shows the increase in solubility of bezafibrate with 15 mM βCD and 25 mM other CDs. βCD and HPβCD consistently performed better than αCD and γCD over the pH ranges. While βCD increased solubility 2.86 to 13.36 times at 25°C. The higher values at acidic pH, though important itself, was due to the reduction of BF solubility without CD. Because a slight increase in solubility by CDs divided by a smaller number (intrinsic solubility, at 0 mM CD) gave a higher number. The corresponding values at 60°C were 3.18-3.66 indicating that intrinsic solubility was much increased at higher temperature. Such line of logic can be equally applicable to other cases in the Table 5.1. The increase in BF solubilities by αCD and γCD were the least among the CDs. One important consideration is the CD concentration. βCD was used in concentration close to its maximum solubility of 16 mM. So further increase in solubility by increasing CD concentration is not possible. However, other CDs are much more soluble in comparison to βCD. Therefore, increase in BF solubility can be further increased by increasing their concentration, especially HPβCD which can produce even 50% w/v aqueous solution (63).

Table 5. 1. Increase in bezafibrate solubility: different CDs*, temperatures and pHs

Temperature	**CDs**	**pH 7.0**	**pH 4.0**	**pH 1.5**
25°C	αCD	1.72	6.37	2.62
	βCD	2.86	13.44	13.36
	HPβCD	4.59	12.66	20.75
	γCD	2.21	3.87	2.74
60°C	βCD	3.18	3.66	3.33
	HPβCD	3.80	5.15	3.97

*βCD 15 mM, other CDs 25 mM

The linear relation between bezafibrate solubility and CD concentration and indicateed (most of the R^2 values were around 0.95) an AL-type phase characteristic of 1:1 complexation, defined by Higuchi and Connors (64). As such, Higuchi and Connor

method was used to calculate stability constants, K, for complexes. The calculated K values in per M are presented in Table 4.14. Low values K values of all the complexes indicated that complexation of bezafibrate is not generally favored. Among the CDs, βCD and HPβCD again gave relatively higher equilibrium constant at all pH levels. High temperature decreased the constant values as expected. Thus, strengthens the idea that higher temperature disfavor complexation even though there was an initial increase in solubility. On the other hand, dependence of complexation of ionizable drug on pH was reported earlier (65). with a complicated situation with drugs having multiple pKa values. Bezafibrate has two pKa values 3.83 (Strongest Acidic) and -0.84 (Strongest Basic) (4). At lower pH, bezafibrate showed increased stability of the complexes; βCD, 0.367 and 0.767 ate pH 4 and 1.5, respectively, at 25˚C. This can be explained by pH effect on the ionization of the drug; cyclodextrins being neutral nonionizable molecules. Bezafibrate has an ionizable carboxylic acid group at one end and a secondary amino group in the middle of the structure. At lower pH, the carboxylic group become unionized which must be the reason for its reduction of solubility. While this should favor complexation, the amino group which become ionized at these low pH, might have hindered complexation.

5.1.5. FTIR Studies

FTIR is a very sensitive method which reveals the structural features of molecules. The sensitivity and speed of measurement is achieved by the mathematical transformation, called “Fourier transform”, of raw absorption or emission data. This is commonly used technique to generate evidence of complex formation between cyclodextrins and it substrate. In our study, FT-IR spectra of pure bezafibrate, αCD, βCD, HPβCD, γCD, physical mixtures of the drug with each CD, and four inclusion complexes are depicted in Figure 4.18 to 4.21.

The following IR responsive bonds are present in the structure of bezafibrate (Figure 2.1). Corresponding IR absorption wave number are presented alongside the bonds from reliable IR Spectrum Table & Chart (61): Halo C-Cl (stretching 850-550 cm-1), aromatic C=C (conjugated alkene C=C stretching 1650-1600), aromatic C-H (bending, 2000-1650), secondary amide C=O (stretching 1680), secondary amine N-H (stretching 3350-3310), amine C-N (stretching 1250-1020), alkane C-H (stretching 3000-2840), aryl ether C-O (stretching1275-1200), methyl group C-H (bending 1450), carboxylic acid C=O (stretching 1760), carboxylic acid O-H (stretching 3300-2500, bending 1440-1395). Similarly, the cyclodextrin bonds and corresponding wave numbers

are alcoholic O-H (stretching 3700-3584, bending 1420-1330), intermolecularly bonded alcoholic O-H (stretching 3550-3200), primary alcoholic C-O (stretching 1085-1050), secondary alcoholic C-O (stretching 1124-1087), aliphatic ether C-O (stretching 1150-1085). In regard to bezafibrate IR absorption, Shen and Zhou (66) 3400 cm-1 band for possible hydrogen bonds, 1719 cm-1 for C=O stretching vibrations of carboxylic acid, 1613 cm-1 for C=O stretching vibrations, 1551 cm-1 for N–H bending vibrations of amide, 1236 cm-1 and 1159 cm-1 for C–O stretching vibrations of aryl alkyl ethers and the bands located at 1090 and 845 cm-1 for p-substituted phenyl of Ar–Cl.

The FTIR spectra obtained in our experiments were in conformity to these data. In all of the presented four groups, bezafibrate and physical mixtures of bezafibrate with different cyclodextrin showed the characteristic peaks of the related compounds. However, such peaks either disappeared or shifted in the complexes. This observation give some evidence that complexation took place. However, one technique of characterization does not confirm such complexation with complete certainty. Therefore, other techniques like differential scanning calorimetry and scanning electron microscopy were used to further investigate the interaction.

5.1.6. DSC Studies

Differential scanning calorimetry explores physicochemical and thermal behavior of substances (67). Pure bezafibrate, pure cyclodextrins and bezafibrate cyclodextrin complexes were examined in this study with DSC. The results are represented in Figure 4.22-4.25 as group-wise superimposition state. In all the thermograms, the sharp and deep endothermic peak at 186˚C indicated the melting point of the drug. This peak disappeared from all the four thermograms belonging to the complexes. The little endothermic peak at around 100˚C did not appear on dry sample unexposed to atmosphere. This can be attributed to the moisture content of the drug powder which evaporated at 100˚C.

Pure αCD (AR) gave two early peaks, one at 76 and other at 96˚C. These turned to a very broad endothermic starting from 28˚C continued until near 150 ˚C with peaking at 77˚C (Figure 4.22) in the complex (A). The deep 317 ˚C in peak αCD also got shortened in the complex as well. As seen in (Figure 4.23), βCD (BR) broad peak at the 116˚C shifted to the left at 106˚C in BFβCD complex (B) thermogram. HPβCD peak at 78.95˚C shifted to the right at 83.88˚C in BFHPβCD complex (Figure 4.24). In case of γCD and BFγCD, in addition to such changes from 97.90˚C (γCD) to 86.93˚C (BFγCD), there was observed a complete disappearance of γCD peak between 308 and 324 ˚C.

All these difference between thermogram clearly indicated complexation between bezafibrate and all the four cyclodextrin investigated.

5.1.7. SEM Studies

Complexation between bezafibrate & βCD and between bezafibrate & HPβCD were also supported by SEM studies. Un-lyophilized βCD powder (Figure 4.26 b) and lyophilized βCD powder (Figure 4.26 c) both were seen irregular compact shaped but lyophilization made it more uniform in sizes. The difference between un-lyophilized and lyophilized HPβCD is obvious. Before lyophilization the particles were regular perforated/spongy spherical in shape (Figure 4.26 e, f). Lyophilization completely destroyed the regular spherical structures, turned them into long range continuous perforated sheet structure.

The SEM image of BFβCD complex (Figure 4.26 d) is strikingly different from that of βCD (Figure 4.26 c); both lyophilized. The compact irregular βCD particles turned into flaky structure with lot of space among the particles. There was such a large difference between these two images when only difference in their composition was the presence of bezafibrate in the BFβCD complex sample. A physical mixture of βCD and bezafibrate would simply look like a mixture of image (a) (Figure 4.26) and image (b) with the individual particle maintaining their identity. Such physical mixture is not anything near to the completely different flake image of (d) (Figure 4.26). This strongly supported the complexation between bezafibrate and βCD.

A parallel analysis can be done for the images of HPβCD (f) and BFHPβCD complex (g) both of which were lyophilized samples (Figure 4.26). The perforated structure of HPβCD was converted to thin sheet like structure in the BFHPβCD. Again, the only difference between them was presence or absence of bezafibrate. The image (g) is nothing near to the physical mixture of the shapes in image (a) and image (e). Thus, this also gave strong evidence of complexation between bezafibrate and HPβCD.

The observed morphological changes of CD particles are in conformity to myriad previous studies which reported such the effect of lyophilization and complexation on the morphology of cyclodextrin particles (68).

5.1.8. Dissolution Studies on Complexes

The rate of dissolution of pure drug, bezafibrate, is slow. As seen from Figure 4.27, both the βCD and HPβCD complexes dissolved at a faster rate and to a higher extent. The similarity factor values (Table 4.15) of both the complexes are below 50 indicating

that these two dissolution profiles are really different from that of bezafibrate. An f_2 value of 50-100 is considered similar, below 50, dissimilar (69). On the other hand, the βCD and HPβCD complexes were seen to be similar with an f_2 value of 56.18. This can be attributed to the fact that βCD and HPβCD both possess the same 7 membered glucopyranose ring structure responsible for complexation.

5.1.9. Characterization of Tablets

Size, Mechanical Strength, Weight Variation and Disintegration

The formulation weight of the tablets was set at 620 mg. After compressing they were measured individually for take into consideration in the subsequent experiments. They were slightly less than the targeted weight due to the loss of some mixture during compression. The average weights were in the range of 610.83 – 615.50 mg. Avicel formulations were slightly thicker (5.20-5.47 mm) than those of DiPac tablets (4.81-5.14 mm) indicating the more compact nature of directly compressible sugar, DiPac. On the other hand, bezafibrate complex with HPβCD created thicker tablet in both formulations; 5.47 with Avicel and 5.14 with DiPac. After lyophilization, HPβCD complex was really light and voluminous which remained as such even after crushing to convert them into suitable size granules for compression purpose. However, the diameter of all the tablets were almost the same (11.98-12.03 mm) as was determined by the punch and die dimensions (12 mm). This indicated there was no change in tablet diameter after removal of compression force. In the same way, there was no significant loss of materials (generally accepted level is <1%) from tablet when they were subjected to friability test. Even some tablet showed nominal gain in weight which may be attributed to their collection of dust from the friability drum or to normal variation in the analytical balance readings. There was no visible crack or otherwise deformation on the tablets after friability test.

Mechanical strengths of tablets are measured by hardness and friability. The tablet showed dramatic differences in hardness. As seen in Table 4.16, Avicel tablets were harder (143-195 N) than the DiPac tablets (80-106 N). Both βCD and HPβCD complex increased the hardness in both formulationswith HPβCD complex producing the hardest tablets. Harder tablets possess mechanical strength to withstand the stresses to which they are generally exposed during packaging, transport or unpacking by the patients. Excessive hardness can, however, negatively affect tablet performance if they prolong disintegration

time or dissolution rate in vivo. This was not the case as seen in disintegration time column of Table 4.17. All the tablets or tablet particles disappeared relatively quickly out of the basket-rack assembly, well within the generally accepted time of <30 minutes for immediate release tablets, the range of the average time being 9.65-16.03 min. The hardest tablet, A-BFHPβCD, showed the maximum disintegration time of 16.03 minutes. The nature of the material played the dominant role. In case of Avicel PH102, the polymer drew water rapidly inside. In case of DiPac tablets, the tablet actually did not disintegrate in the usual sense, rather they underwent gradual surface erosion by dissolution of sugar molecules.

Dissolution Tests

Tablets consisting of pure bezafibrate with Avicel (A-BF) was taken as the base formulation in the dissolution tests on tablets. Two tables compared to this base formulation were Avicel tablets consisting of βCD complex with bezafibrate (A-BFβCD) and of HPβCD complex with bezafibrate (A-BFHPβCD). The complex containing tablets are seen above the A-BF dissolution profile in Figure 4.28. In conformity to the faster dissolution of HPβCD complex (Figure 4.27), A-BFHPβCD tablet showed faster dissolution. This was immediately followed by A-BFβCD, the βCD complex tablets.

In the same way tablets consisting of pure bezafibrate with DiPac (D-BF) was taken as the base formulation. Two tables compared to this base formulation were DiPac tablets consisting of βCD complex with bezafibrate (D-BFβCD) and of HPβCD complex with bezafibrate (D-BFHPβCD). Among these DiPac formulations, as shown in Figure 4.29, D-BFβCD started slower but caught D-BFHPβCD at around 30 min after which both the profile ran parallel. As with Avicel tablets, complexes showed better dissolution in comparison to drug only tablet (D-BF) although D-BFβCD was slightly slower in the first 10 minutes. One difference observed between Avicel and DiPac tablets was in the pattern of disintegration. Unlike Avicel tablets, DiPac tablets did not disintegrate, rather they underwent gradual surface erosion. However, dissolution rate seemed not to be affected by this difference in disintegration pattern. This might be due to the fact that all the erosion and disintegration were completed within relative short period of 10-15 minutes (Table 4.17).

The fourth dissolution profile in Figure 4.28. belongs to a tablet formulation of a physical mixture of bezafibrate and HPβCD with Avicel. This mixture was included to investigate the cyclodextrin effect on dissolution. Because HPβCD constituted 50 % (310

mg in 620 mg tablet) of the formulation, (Table 3.3 in section 3.2.9) but A-BF tablet contained only drug and Avicel without any water soluble cyclodextrin. The profile belonging to this physical mixture (A+BF+HPβCD) still remained below the profiles of the complexes clearly showing that the faster dissolution by complexes was not due to the mere presence of cyclodextrin in the formulation, rather due to the complexation. Further studies with physical mixtures in the both Avicel and DiPac formulations were not conducted anticipating the same outcome as with A+BF+HPβCD.

An evaluation of the dissolution profiles by the similarity factor (Tablel 4.17) shows that all the Avicel Tablets were different from each other. The same can be observed for the DiPac tablets. All of the f_2 values were below 50 which is considered the lowest value for any two curves to be similar. When compared against the physical mixture, A+BF+HPβCD, both the A-BFβCD and A-BFHPβCD were clearly different. However, the f_2 value for the pair A-BF and A+BF+HPβCD was 46.25. Though it is still signifying difference but just short of 50. This pair of tablets contained the drug in the same pure state, the only difference between them being the presence of HPβCD which might have affected the dissolution through disintegration.

5.2. Conclusion

The aim of this work was to study the complexation of the poorly water-soluble drug bezafibrate with three natural cyclodextrin and a hydroxypropyl derivative of beta-cyclodextrin, formulate them as tablet dosage form. DSC, FTIR, SEM studies gave evidence of complexation. Phase solubility studies revealed 2 times to twenty times of increase in bezafibrate solubility. Temperature and pH were observed to affect the solubility of both pure drug and cyclodextrin complexes. Stability constant values for the complexes showed a moderately stable complexes. Of the double ionizable group in the structure of the bezafibrate molecule, carboxylic acid group ionize higher pH and amine group at lower pH. This seemed to have a complicated effect on complexation as was reported in the literature. Of the four complexes, beta- and hydroxypropyl-beta-cyclodextrin complexes were, having shown better solubility, were chosen to be formulated as tablets with two directly compressible vehicle, namely, Avicel PH 102 and DiPac. Cyclodextrin in the complexes acted both as complexing agent and as tablet filler which lessened the amount of expensive directly compressible vehicle required for tableting. Both complexes showed superior tableting and dissolution profiles.

Comparison of dissolution profiles, by similarity factor, f_2, clearly showed positive effect of complexation different from the tablets of similar composition without complexation. Thus, in conclusion, the inclusion complexes produced between bezafibrate with βCD and with HP βCD hold promise to formulate better pharmaceutical dosage form.

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