Sustained Release Solid Dispersion of a BCS Class II Drug

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The project titled "Sustained Release Solid Dispersion of a BCS Class II Drug" submitted by Fairuza Ahmed (ID 16146002) of Spring, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 1st March 2020.

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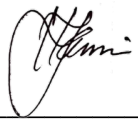

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Ethics Statement

The study does not involve any kind of animal or human trial.

Abstract

The present study aims to develop a sustained-release solid dispersion (SRSD) of a Biopharmaceutical Classification System (BCS) Class II drug by employing a blend of polymers. The SRSD was prepared by kneading technique, followed by solvent evaporation using rotary evaporator. After conducting an equilibrium solubility study, SRSD (F5) was found to be the optimal formulation. The dissolution profiles of SRSD (F5) were examined at different pH values. Since Carvedilol exhibits pH dependent solubility, the drug release from SRSD (F5) was limited at pH 6.8 and increased at pH 1.2. The structural characteristic of SRSD (F5) was examined by using powder X-ray diffraction (PXRD), which shows a change in drug crystallinity to an amorphous form. The results obtained from the dissolution study suggest that SRSD (F5) could be a promising approach for developing sustained-release oral dosage form of Carvedilol, thereby reducing the dosing frequency and improving the oral bioavailability.

Keywords: Sustained release solid dispersion (SRSD); oral bioavailability; dissolution; BCS Class II drug

Dedication

To my grandparents

Acknowledgement

Firstly, I would like to begin by thanking Almighty Allah for keeping me in good health and blessing me with the capability, strength and assistance needed to complete this project. However, this research paper would not have been completed without the support of several individuals and I would like to express my gratitude to all of them.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

CVD	Cardiovascular Disease
CHF	Congestive Heart Failure
NCE	New Chemical Entities
BCS	Biopharmaceutical Classification System
GIT	Gastrointestinal Tract
SD	Solid Dispersion
SRSD	Sustained Release Solid Dispersion
F5	Formulation number 5
CAR	Carvedilol

Chapter 1

Introduction

1.1 Background

Cardiovascular disease (CVD), a major health concern worldwide, has now become one of the leading causes of mortality and morbidity not only in industrialized nations but also in the developing world, with over 18 million deaths per year (Ismaiel & Dumitraşcu, 2019; Thirunavukkarasu & Khader, 2019). Among the different diseases which fall under the umbrella of CVDs, such as angina, arrhythmia, coronary heart disease, heart attack, cardiomyopathy, etc. ischemic heart disease still remains the most common one (Prajnamitra et al., 2019).

According to the analysis of Cappuccio & Miller, 2016, it identifies high salt intake, smoking and obesity as potential risk factors in developed countries and also mentions hypertension (or high blood pressure) as the most common underlying risk factor for CVD. Identifying the risk factors is very crucial, so that preventive measures can be taken in an early stage. Hypertension is a multifactorial disease, which requires adequate control to prevent and reduce premature mortality. CVD patients worldwide now use drugs as a prophylaxis for cardiovascular diseases. Using antihypertensive agents to control blood pressure is a clear example of this type of preventative measure (Alves et al., 2016). Several classes of antihypertensive agents are used in clinical practice, including β-blockers, angiotensin-converting enzyme (ACE) inhibitors, diuretics, angiotensin II receptor blockers and calcium channel blockers (Oliveira-Paula et al., 2019). Among these commonly prescribed drugs, β-blocker is the prime focus in this study. Long-term use of β-blocker helps manage chronic heart failure. In addition to its benefits in the prophylaxis of heart conditions, β-blockers can also be used to treat migraine, anxiety, hyperthyroidism, glaucoma and so on. β-blockers,

although not a first-line therapy for osteoporosis, however it protects the bones from thinning over time. It does so by blocking the release of stress hormones and also preventing the excretion of calcium into urine.

Table 1: List of drugs currently used in the treatment of CVD ("Cardiac Medications | American Heart Association," 2015)

	Type of drug	**Examples**
1.	Anticoagulant	Dabigatran, Rivaroxaban, Heparin, Warfarin
2.	Antiplatelet	Clopidogrel, Dipyridamole, Prasugrel
3.	Angiotensin-Converting Enzyme (ACE) Inhibitors	Captopril, Perindopril, Quinapril, Trandolapril, Ramipril, Fosinopril
4.	Angiotensin II Receptor Blockers (or Inhibitors)	Valsartan, Losartan, Irbesartan, Candesartan
5.	Angiotensin-Receptor Neprilysin Inhibitors (ARNIs)	Valsartan
6.	Beta Blockers	Propranolol, Metoprolol, Atenolol, Sotalol, Acebutolol, Nadolol
7.	Calcium Channel Blockers	Nimodipine, Amlodipine, Nisoldipine, Felodipine, Verapamil, Nifedipine, Diltiazem
8.	Statins	Atorvastatin, Rosuvastatin
9.	Nicotinic Acids	Lovastatin

1.2 β-Blockers

Adverse cardiovascular events like congestive heart failure (CHF), stroke and myocardial infarction are all associated with elevated blood pressure. This is where antihypertensive drugs come to play a vital role, which is to decrease the incidence of such events. Beta adrenergic receptor blockers, also known as beta blockers, fall under the several classes of antihypertensive drugs used in such events (Wong et al., 2015).

Although beta-blockers were initially used to treat angina, overtime they have also proven to be effective in the lowering blood pressure (Wong et al., 2015). Ever since, β-blockers have been used for certain arrhythmias and have been the basis for secondary prevention after a heart attack (Andell et al., 2015).

In pharmacological terms, adrenergic receptors or adrenoreceptors are the main targets of catecholamines (norepinephrine, epinephrine, etc) and are classified into two families- α and β, based on their affinity towards adrenergic agonists norepinephrine, epinephrine and isoproterenol (Harvey et al., 2012). Alpha adrenoreceptors are classified into two subtypes, α1 and α2. Activation of α1 receptors results in vasoconstriction, increased peripheral resistance and increased blood pressure.

Conversely, stimulation of α2 receptors causes inhibition of norepinephrine, acetylcholine and insulin release (Harvey et al., 2012). Beta-receptors are further subdivided into three types, of which β1 and β2 are involved in cardiovascular regulation (Wong et al., 2015). β1 receptors are located in the heart whereas, β2 receptors are found to present in the bronchioles of lungs and the arteries of the skeletal muscles (Harvey et al., 2012). Stimulation of β1 receptors results in an increased heart rate and cardiac output, and in turn leads to cardiac stimulation. Whereas, vasodilation and smooth muscle relaxation occurs after the activation of β2 receptors (Harvey et al., 2012). How β-blockers actually work in lowering blood pressure is yet to be discovered. However, there are many hypothetical mechanisms are proposed which includes: reducing renin production, decreasing cardiac output or a combination of these effects (Wong et al., 2015). All the beta-blockers available for clinical practice are competitive antagonists. The ones that are nonselective act on both β1 and β2, whereas selective β antagonists mainly block β1 receptors. Generally, all beta-blockers end with "-olol", among which Carvedilol is the main drug of interest for this study.

1.2.1 Carvedilol

Carvedilol is a nonselective, third-generation β-blocker primarily used in the prophylaxis of mild to moderate hypertension, angina pectoris and also indicated for congestive heart failure (Kim et al., 2015). It exhibits α1-adrenergic receptor blocking, in addition to β-blocking activities, thereby decreasing the level of intrinsic sympathomimetic activity, making Carvedilol a better tolerable drug in comparison to other β-blockers (Kim et al., 2015). Carvedilol is quite rapidly absorbed after oral administration, with t max (time needed to reach maximum concentration) at 1–2 hours and an elimination half-life of 7–10 hours (Brittain, 2013; Kim et al., 2015). The oral bioavailability of Carvedilol is 20-25%, which indicates that the drug undergoes extensive first-pass metabolism (Brittain, 2013). Carvedilol is highly lipophilic in nature and plasma protein bound (98%, primarily bound to albumin) (Kim et al., 2015). The drug is mainly eliminated via hepatic metabolism, with 16% of its metabolites being excreted renally and over 60% is excreted through the fecal route (Brittain, 2013). Carvedilol is therapeutically used as a racemic drug, with two enantiomers, R (+) and S (-) that differ in terms of their pharmacodynamic activity. Both, R (+) and S (-) show similar α-blocking activity, however S (-) is mainly responsible for β-blocking activity (Alves et al., 2016).

Carvedilol plays a very significant part in the preventive treatment of cardiac diseases, however its insolubility in water, dilute acids, gastric and intestinal fluids is its major drawback. Carvedilol exhibits pH-dependent solubility, which means that solubility decreases with increasing pH (Hamed et al., 2016). Its poor aqueous solubility explains why Carvedilol exhibits very low oral bioavailability (Hamed et al., 2016).

1.3 Solubility

For any drug, its solubility in water plays a crucial role in drug absorption after oral administration. Drug solubility also determines the rate at which drug from a particular dosage form passes into solution (Khadka et al., 2014). In simple terms, solubility can be defined as the extent to which a substance dissolves in a solvent to give a homogenous solution (Savjani et al., 2012). It is an important parameter that determines the amount of drug entering the systemic circulation to give the desired therapeutic response (Savjani et al., 2012).

In drug discovery, about 70% of new chemical entities (NCE) that have been discovered, in recent years, show poor water solubility. Since water constitutes about 60% of body weight, it is essential that a drug has certain aqueous solubility. The dissolution rate, aqueous solubility and bioavailability of drugs are important parameters that need to be considered in order to achieve in vivo efficiency (Khadka et al., 2014; Dizaj et al., 2015). Drugs with poor water solubility are directly eliminated from the gastrointestinal tract before they are dissolved in GI fluids, which result in reduced clinical effects (Sarfraz et al., 2017). This is why in- vitro dissolution of drugs is an issue that pharmaceutical scientists need to address because consumers are being deprived of a huge number of beneficial NCEs, which are being rejected due to their inadequate dissolution (Khadka et al., 2014; Sarfraz et al., 2017).

1.3.1 Importance of solubility enhancement

Till date, oral drug delivery is preferred over routes of administration because of high patient compliance, less sterility requirements, flexibility in design, cost effectiveness and so on (Savjani et al., 2012). Hence, pharmaceutical companies dedicate most of its resources in producing bioequivalent oral dosage forms (Savjani et al., 2012). The major challenge lies in developing a proper oral dosage form for drugs with poor bioavailability (Savjani et al.,

2012). Oral bioavailability depends on multiple factors such as first-pass metabolism, drug permeability, dissolution rate, aqueous solubility etc. (Savjani et al., 2012).

The amount of drug being absorbed is also regulated by the physical and chemical properties of the drug, its dosage form, and nature of its absorption site (Dizaj et al., 2015). A drug is usually absorbed by passive diffusion mechanism (Dizaj et al., 2015). Solubility in gastrointestinal fluids is also a factor to be considered in drug absorption (Dizaj et al., 2015). Therefore, solubility of a drug is the main determinant parameter for achieving in vivo efficiency (Dizaj et al., 2015).

A drug that has low aqueous solubility will show limited dissolution rate, which in turn limits its therapeutic activity. Hydrophobic drugs are usually required in higher doses and need high dosage regimen to reach the minimum therapeutic concentration (Savjani et al., 2012). In case of Carvedilol, which is a weak base, has an acidic pKa of 15 and a basic pKa of 7.8 with a log P value of 3.8. Carvedilol exhibits pH-dependent solubility, thus dissolving rapidly in acidic pH of stomach but precipitates in the small intestine (Hamed et al., 2016).

It is essential for any drug to demonstrate adequate solubility in water, so that it can be absorbed from its absorption site (Savjani et al., 2012). Drugs are either weakly basic or weakly acidic in nature. Recent studies have noted that about 40% of NCEs entering the drug pipeline are almost insoluble in water (Savjani et al., 2012). These drugs, due to their slow absorption, eventually show limited or variable bioavailability (Savjani et al., 2012). For any orally administered drug, solubility and permeability are two vital parameters that influence the success of a drug. Hence improving solubility of a drug can thereby improve its oral bioavailability. Thus, researchers are continuously striving to develop new approaches for solubility enhancement.

1.4 Biopharmaceutical Classification System

Depending on the solubility and permeability characteristics, the Biopharmaceutical Classification System (BCS) allocates drugs into four distinct classes, as displayed in Figure 1.

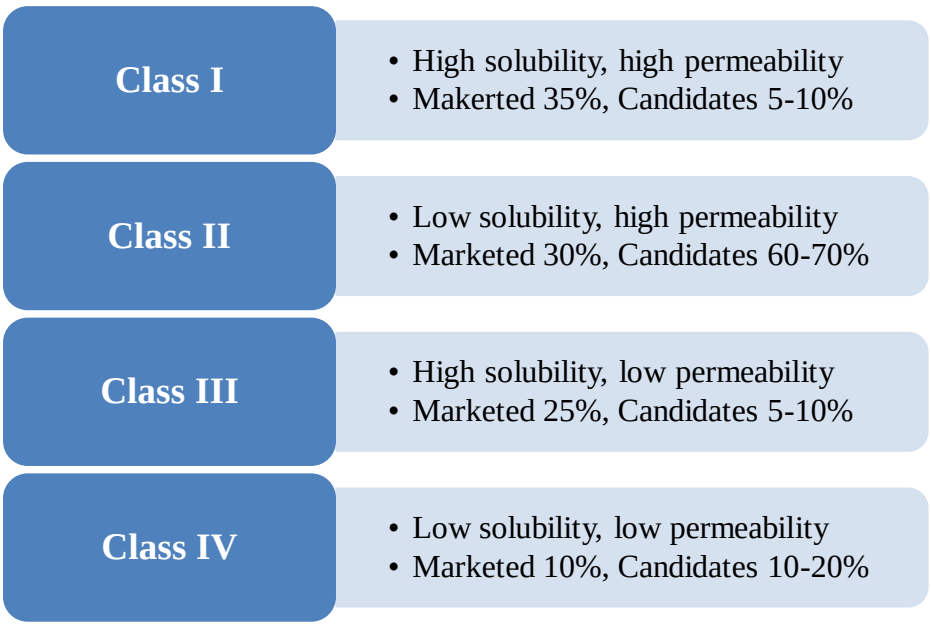

Figure 1: Percent of drug molecules marketed according to the BCS (Nikolakakis & Partheniadis, 2017)

Table 2: Solubility criteria according to USP

Descriptive term (solubility definition)	**Parts of solvent required for one part of solute**	**Solubility range (mg/mL)**	**Solubility assigned (mg/mL)**
Very soluble (vs)	< 1	≥ 1000	1000
Freely soluble (fs)	From 1 to 10	100-1000	100
Soluble (s)	From 10 to 30	33-100	33
Sparingly soluble (sps)	From 30 to 100	10-33	10
Slightly soluble (ss)	From 100 to 1000	1-10	1
Very slightly soluble	From 1000 to 10,000	0.1-1	0.1
Practically insoluble (pi)	≥ 10,000	< 0.1	0.01

In case of BCS class II drugs, their bioavailability is dissolution rate limited. Since these drugs are highly permeable, so formulation scientists continue their search of developing new methods to improve the solubility and oral bioavailability of such drugs (Khadka et al., 2014). Carvedilol is also a well-known BCS Class II drug that shows a complex solubility pattern because of the difference in pH of gastrointestinal (GI) fluid in the stomach and intestine (Hamed et al., 2016).

For drugs of BCS class III, permeability is the rate-limiting factor. Therefore, manufacturing immediate release tablets with absorption enhancers could be a potential solution for these drugs (Khadka et al., 2014).

On the contrary, the bioavailability of BCS class IV compounds is restricted by both dissolution and permeability. As a result, these drugs are often ignored in the drug development process because formulation strategies have very small impact in improving their absorption (Khadka et al., 2014; Fahr & Liu, 2007).

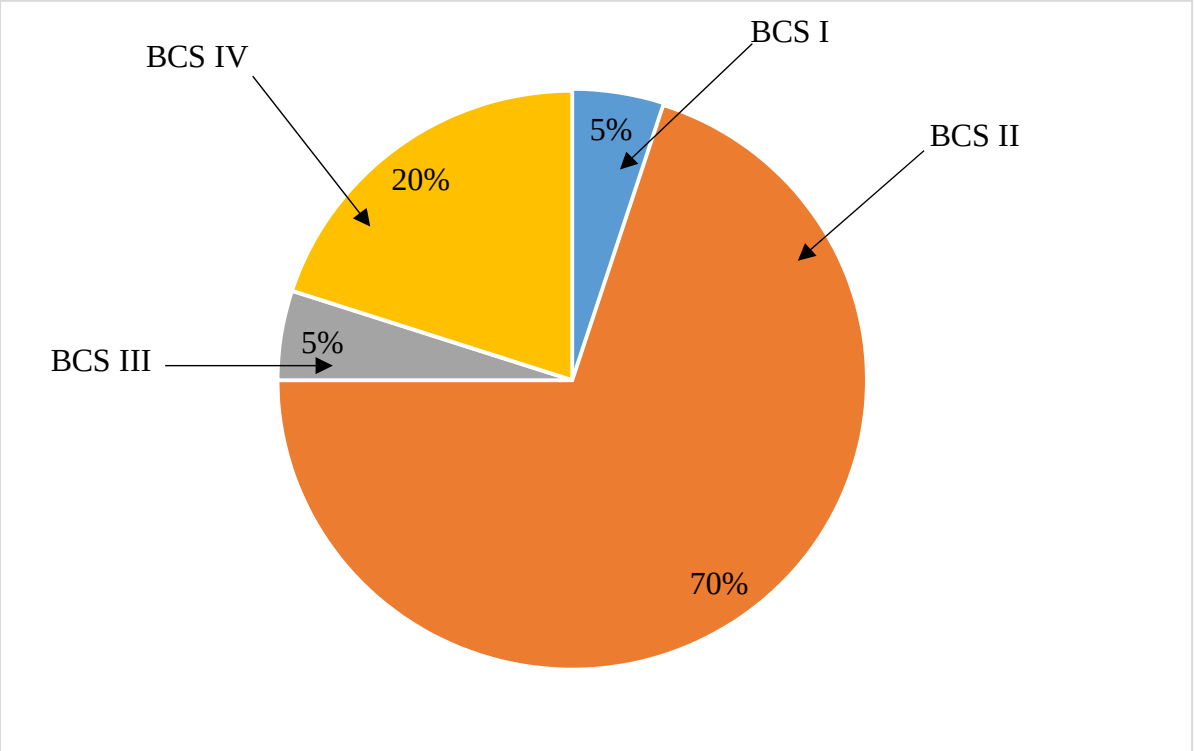

Figure 2: Recently developed drugs (Evonik, retrieved on January, 2020)

Formulation has a vital role to play when it comes to the absorption of drugs from the GI tract. As water-solubility of these drugs is below 1 μg/ml (Figure 2), which is quite unacceptable, so various techniques are adopted to enhance the dissolution rate and get sustained solubilisation of such drugs (Fahr & Liu, 2007). Despite having low and variable oral bioavailability, majority of the formulation development approaches are greatly focused on drugs belonging to BCS class II (Khadka et al., 2014; Sarfraz et al., 2017).

1.5 Techniques for Solubility Enhancement

Various techniques have been established for solubility improvement and these can be divided into physical modifications, chemical modifications and other techniques (Savjani et al., 2012; Sarfraz et al., 2017).

1.5.1 Physical Modifications

Particle size reduction is by far the most safe and ancient method employed to improve the solubility of drugs, with no chemical modifications. As particle size decreases, surface area to volume ratio is increases. A larger surface area means more exposure to solvent, which contributes to increased solubility (Khadka et al., 2014; Savjani et al., 2012). Milling, spray drying and grinding are examples of the conventional methods that utilize mechanical stress to break the active compound. This may lead to product degradation, which can be unsuitable for thermolabile drugs. Although particle size reduction is an economically viable technology, but its use is very limited due to low success rates in enhancing the solubility up to the desired level (Sarfraz et al., 2017).

1. Micronization

Micronization is a traditional technique of particle size reduction, used for improving the solubility of BCS Class II drugs (Khadka et al., 2014). Micronization increases the

dissolution rate by an increase in surface area, which is accomplished by ball mills, jet mills and high-pressure homogenization (Khadka et al., 2014). Nanosizing of drugs is also a promising new approach to overcome the problem of poor aqueous solubility (Dizaj et al., 2015).

2. Solid Dispersion

According to Zhang et al., 2018, solid dispersions are a mixture of one or more drugs in an inert carrier. Solid dispersion technique is extremely useful for increasing the absorption, dissolution and therapeutic efficacy of a drug (Savjani et al., 2012). A solid dispersion is comprised of two parts: the hydrophilic matrix and the hydrophobic drug. Solid dispersions can be prepared in the following methods:

- Melting Method: This method was discovered by Sekiguchi and Obi in 1961 and is considered to be very advantageous because of its simplicity and economic feasibility (Zhang et al., 2018; Savjani et al., 2012). In this technique, a uniform mixture of the drug and water-soluble carrier is prepared and is subjected to direct heat till the components are molten (Zhang et al., 2018). The resulting mixture is cooled and solidified in an ice bath, with continuous stirring (Savjani et al., 2012). The remaining solid mass is crushed and sieved, and finally compressed into a solid dosage form (Savjani et al., 2012).

- Solvent Evaporation Method: This technique is mainly used for thermo sensitive compounds because it requires a low temperature. Both the drug and the hydrophilic carrier are dissolved in a common organic solvent. By application of a rotary evaporator the solvent is evaporated and the resulting solid mass is ground, sieved and dried (Zhang et al., 2018; Savjani et al., 2012).

- Hot-melt extrusion: This process uses intense pressure and heat to mix all the components. It is somewhat similar to the melting method however it allows simultaneous

melting and homogenization. This continuous process is beneficial for large-scale production (Zhang et al., 2018; Savjani et al., 2012).

3. Co-crystallization, solid solutions and cryogenic techniques can also be used to improve aqueous solubility of drugs (Savjani et al., 2012).

Table 3: FDA-Approved Solid Dispersion Products (Baghel et al., 2016)

Product Name	**Drug**	**Polymers used**	**SD Preparation Method**	**Maximum Drug Loaded in a Tablet/ Capsule (mg)**	**Dosage Form**
Kalydeco	Ivacaftor	HPMCAS	Spray drying	150	Tablet
Zelboraf	Vemurafenib	HPMCAS	Coprecipitation	240	Tablet
Incivek	Telaprevir	HPMCAS	Spray drying	375	Tablet
Intelence	Etravirine	HPMC	Spray drying	200	Tablet
Novir	Ritonavir	PVP	Melt extrusion	100	Tablet
Kaletra	Lopinavir	PVP	Melt extrusion	200	Tablet

1.5.2 Chemical Modifications

I. Microemulsions: Microemulsion is combination of oil, surfactant and solvent that is used to dissolve a drug. The drug is uniformly dispersed in small, uniform oil droplets. This system is also known as self-microemulsifying drug delivery system (SMEDDS) and is often used in solubility enhancement (Sarfraz et al., 2017).

II. Salt formation, complexation, use of buffer and change of pH can also improve solubility of drugs (Savjani et al., 2012).

1.5.3 Miscellaneous Methods

I. Cosolvency: In this method one or more water miscible solvents (cosolvents) are added in combination with water to improve the solubility of poorly water-soluble drugs

(Sarfraz et al., 2017). PEG 300, ethanol or propylene glycol are typically used to prepare the cosolvent mixtures.

II. Supercritical Fluid (SCF): Fluids whose temperature and pressure are higher than its critical pressure (Tp) and critical temperature (Tc) are called supercritical fluids, which have the properties of both a liquid and a gas (Sarfraz et al., 2017). SCFs are highly compressible near to its critical temperature. So, any slight change in pressure can greatly alter the density of the fluid. When the drug is solubilized in the SCF (e.g. carbon dioxide), it is recrystallized at greatly reduced particle sizes (Sarfraz et al., 2017). This new technique is widely used in solubility improvement of poorly water-soluble drugs (Sarfraz et al., 2017).

1.6 Purpose of this study

Solubility has a significant impact on the bioavailability of a drug. The success of any formulation depends on how effectively and efficiently the drug reaches its site of action. Among the various challenges of pharmaceutical formulation development, low aqueous solubility of hydrophobic drugs is a major problem that formulation scientists are struggling to overcome. A large portion of drugs available in the market is hydrophobic or water insoluble. Many drugs are not formulated in any dosage form because they fail to reach their full potential and hence are screened out. Despite having beneficial effects, drugs are restricted from entering the market because of their poor aqueous solubility. A drug that has the potential to save lives is not always manufactured into a suitable dosage form because of its low bioavailability. This explains why scientists are putting tremendous efforts in discovering new strategies to address this aspect of formulation development. BCS class II drugs are the primary focus of researchers because drug solubility is the only issue that needs to be worked on. Whereas, both permeability and solubility are the rate limiting factors that affect the oral bioavailability of BCS class IV drugs. So, a potential solution could be

structural modification of the drug itself. Moreover, it is easier to work with existing drugs rather than new chemical entities because of the vast availability of information. Drugs currently in the market have already gone through preformulation studies, accelerated stability tests and other necessary evaluation criteria. In contrast, NCEs are still under clinical trial and their therapeutic effects are questionable. After the discovery of a lead compound, it takes approximately ten years for a drug to be commercially available. The clinical trial step alone takes about seven years, so it quite unreasonable to work with a drug that is still under investigation. Therefore, investing resources in the development of an established drug is a more feasible option for pharmaceutical companies.

This study aims to develop Carvedilol in Sustained Release Solid Dispersion (SRSD) to control its release and improve its oral bioavailability for better management of hypertension and other related cardiovascular diseases. Sustained-release dosage form can help decrease the dosing frequency and simultaneously maintain the effective drug concentration over a prolong period and eventually improve patient compliance. In addition, SRSD would also help lower costs, improve efficacy, and prevent adverse effects (Lee et al., 2017).

1.7 Literature review

In order to select the drug, polymer, materials and experimental conditions for this study, extensive literature review was performed. Following is the list of journals and databases that were used for this study:

1. American Association of Pharmaceutical Scientists
2. Pharmaceutical Development and Technology
3. Drug Design, Development and Therapy
4. American Journal of Cardiology

5. Journal of the American Heart Association

6. Cochrane Database of Systematic Reviews

7. International Journal of Pharmaceutics

8. Frontiers in Cellular and Infection Microbiology

9. PubMed

10. Elsevier

Chapter 2

Methodology

2.1 Materials

Carvedilol was purchased from Arabinda Pharma (India). Polymer Y was a kindly provided by BASF Bangladesh Co. Ltd (Germany) and Polymer X by Evonik, Bangladesh. All other chemicals and apparatus were procured from commercial sources.

2.2 Standardization of Carvedilol

Preparation of the stock solution involved dissolving 100 mg of Carvedilol (API) in sufficient quantity of pure methanol in a volumetric flask to make the final volume 100 ml, thus the concentration of stock solution was 1mg/ml. Then 10 ml of stock solution was diluted using 50% methanol to form the primary solution of 0.1mg/ml concentration. This primary solution was further diluted to prepare solutions of varying concentrations in the range of 0.25-5 μg/ml. 50% methanol was used as a blank.

2.3 Preparation of sustained release solid dispersions

SRSD-Carvedilol was prepared by kneading method. 125 mg of Carvedilol was mixed with 2 ml of water, using a mortar, to prepare slurry. To this mixture, required amount of Polymer Y was added and triturated properly. 3 ml of water was added drop wise, which was followed by the addition of Polymer X to get a uniform mixture. The mixture was taken in a round bottom flask to evaporate the solvent using a rotary evaporator at 100 rpm. After drying the solid dispersion at 50°C, the product was crushed using mortar and pestle to ensure the drug was uniformly distributed throughout the formulation. The resulting solid dispersions were stored in a glass vial sealed with parafilm and kept at an ambient temperature, away from sunlight.

2.4 Polymer Ratio

Different combinations of the polymers Y and X (1:1, 1:2, 1:3, 2:1 and 3:1) were used to prepare solid dispersions in order to study their effect on solubility enhancement.

Table 4: Amounts of drug-polymer for the preparation of sustained release solid dispersion of Carvedilol

Formulation Number	Carvedilol (mg)	Polymer X (mg)	Polymer Y (mg)	Polymer ratio	Total (mg)
01	125	187.5	187.5	1:1	500
02	125	93.75	281.25	1:3	500
03	125	281.25	93.75	3:1	500
04	125	125	250	1:2	500
05	125	250	125	2:1	500

2.5 Equilibrium Solubility

The equilibrium solubility of the prepared SSRD was determined in water (Halder et al., 2018). The samples were prepared by using varying ratios of the two polymers. Sample containing 12.5 mg equivalent weight of Carvedilol was taken in a test tube containing 10 ml of distilled water. The test tube was wrapped and sealed with parafilm and placed in an oscillation incubator at 37°C at 50 rpm for 6 hours. Once the shaking process was completed, the suspension was allowed to stand for some time. Using the 10 ml pipette the supernatant was collected and the filtered. The filtrate was centrifuged at 3500 rpm for 5 minutes. The resulting supernatant was diluted (10, 20 and 40 times) using 50% methanol and the aqueous solubility of the sample was determined using UV spectrophotometer. The entire process was carried out for the five formulations as well as Carvedilol.

2.6 Sustained-Release in pH 6.8 Phosphate Buffer Solution

2.6.1 Preparation of Phosphate Buffer

To prepare 0.05M-phosphate buffer (pH 6.8), 65.62g of disodium hydrogen phosphate and 35.217 g of sodium hydrogen phosphate was dissolved in sufficient water to produce 10000 ml buffer solution (0.1N HCL was used to adjust the pH of the solution).

2.6.2 Procedure of Dissolution study

Among the SRSDs, the one with the most satisfactory result in equilibrium solubility study was selected for in-vitro dissolution study. The SRSD (containing approximately 100 mg Carvedilol) was added to a dissolution vessel equipped with a water bath. The dissolution study was carried out at 37 ± 0.5 °C using the USP paddle method, where 900 ml of phosphate buffer (pH 6.8) was poured in each vessel rotating at 75 rpm. The time at which the paddles started to rotate was noted down. After 15 minutes, 10ml of the dissolution medium was withdrawn from each vessel and filtered. Simultaneously, an equivalent volume (10 ml) of phosphate buffer was added in each vessel to restore the original volume. 1 ml of the filtered sample was taken and diluted 10 times using the same dissolution medium. The concentration of Carvedilol in the medium was analyzed by UV spectrophotometer. Three replicates were performed for each analysis (Lee et al., 2013).

2.7 Dissolution study with 0.1N HCl (pH 1.2)

Drug release from SRSD was evaluated, in comparison to Carvedilol, by carrying out in vitro dissolution test. The experiment employs USP II dissolution apparatus that includes 900 ml dissolution vessel, each containing 0.1N HCl (at pH 1.2) as the dissolution medium. Samples of SRSD containing 100 mg equivalent weight of Carvedilol were added to each vessel at 37 ±0.5◦C and 75 rpm (Halder et al., 2018). 10 ml of dissolution media was withdrawn from

each vessel, at certain time intervals of 15, 30, 45, 60, 90 and 120 minutes, and simultaneously replaced with 10 ml of new dissolution medium. The amount of drug released was determined spectrophotometrically at 240 nm. The study was carried out in triplicates (Planinšek et al., 2011).

2.8 Physical Characterization

2.8.1 Powder X-ray diffraction (PXRD)

PXRD analysis of Carvedilol, SRSD-Carvedilol, Polymer Y and Polymer X were obtained using the X-ray Diffractometer (Bruker AXS GmbH, Karlsrube, Germany) equipped with Cu-Kα radiation generated at 40 mA and 35 kV. The samples were analyzed in the range between 10 and 30° 2θ (degree) with a scanning speed of 4° per minute.

Chapter 3

Results

3.1 Standardization of Carvedilol

A linear calibration curve was obtained when the absorbance was plotted against the concentration, with a correlation coefficient (R^2) of 0.98849 as shown in Figure 3.1.

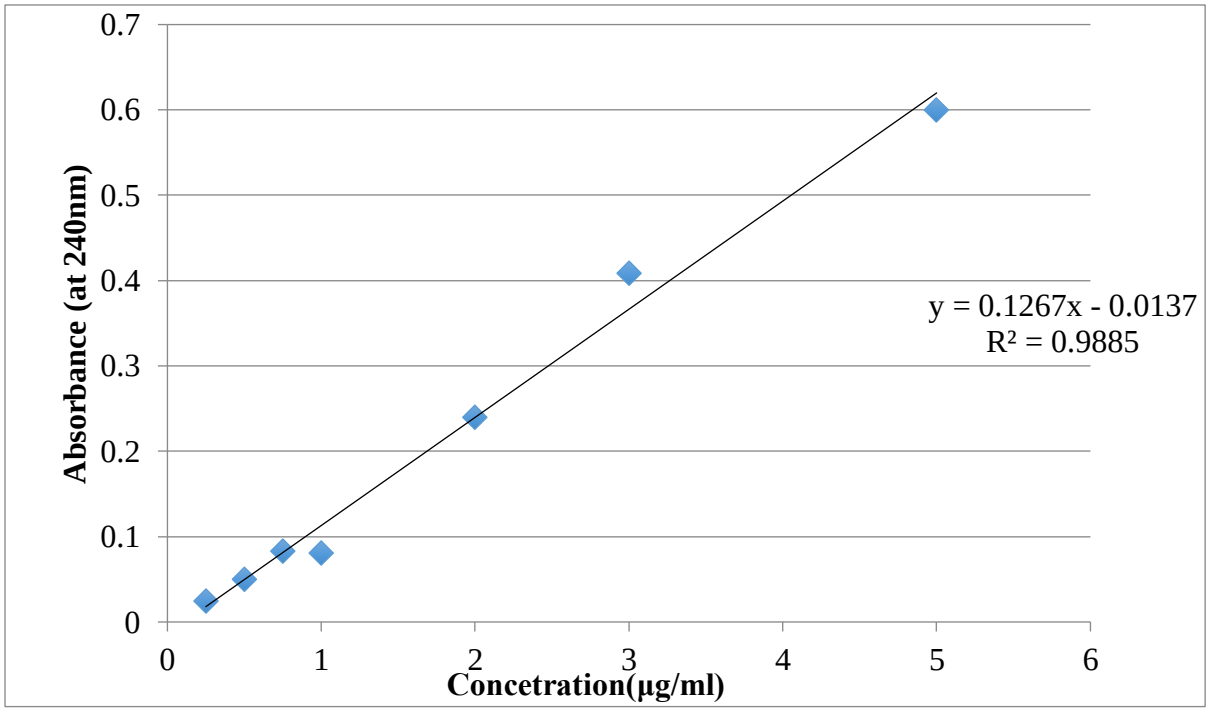

Figure 3: Standard curve of Carvedilol in 50% Methanol

3.2 Equilibrium solubility study of prepared formulations and Carvedilol in water

Solubility of Carvedilol in water was calculated using the equation derived from the standard curve and back calculation method. The results are listed below:

Table 5: Solubility calculation of the prepared formulations and Carvedilol in water

Formulation	F1	F2	F3	F4	F5	CAR
Final Concentration (Mean)	19.9	49.37	33.26	26.64	60.84	11.17
	15.05	36.31	29.73	23	45.62	8.63
	14.55	38.05	28.84	19.81	43.28	7.13
	1.2	19.5	1.59	1.56	17.86	0.96
	2.6	0.71	0.33	0.27	0.73	1.23
Standard Deviation (SD)	0.67	0.43	0.82	0.48	0.57	1.18
Average Solubility in water	16.5	41.24	30.61	23.15	49.91	8.98

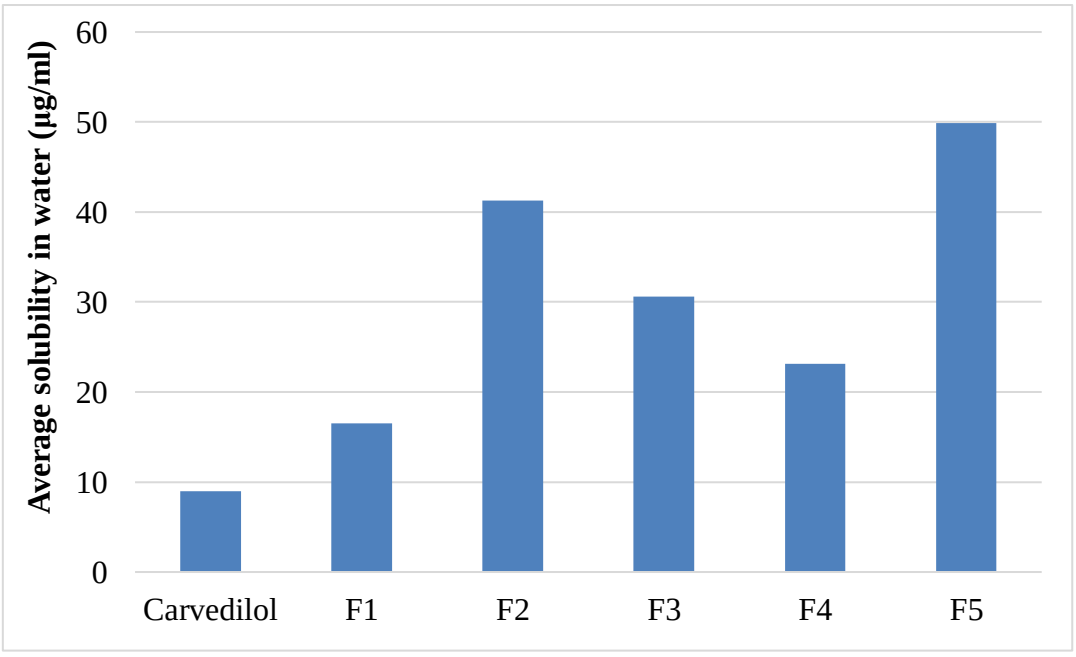

Figure 4: Comparison between the solubility of different formulations with Carvedilol

3.3 Dissolution study

Table 6: Dissolution study of F5 in 0.1N HCl (at pH 1.2)

Time (minutes)	Average % Drug Released	SD
15	64.382794	2.870802441
30	64.2644041	0.837477786
45	61.02052092	3.344132171
60	67.48460931	1.810088226
90	66.86898185	1.047198631
120	69.26045777	3.822875596

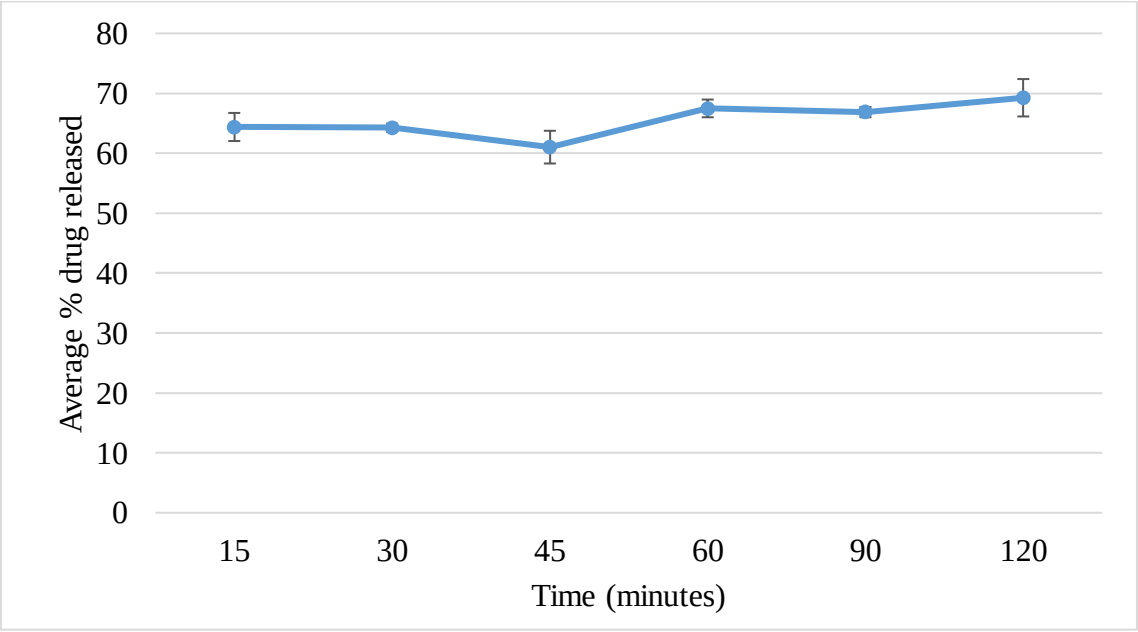

Figure 5: Dissolution profile of F5 in 0.1N HCl (pH 1.2)

Table 7: Dissolution study of Carvedilol in 0.1N HCl (at pH 1.2)

Time (minutes)	Average % Drug Released	SD
15	25.07734807	2.53774482
30	26.1902131	5.27806541
45	42.55169692	2.770627147
60	43.21468035	3.132212766
90	43.33307025	3.588678816
120	45.17995265	3.961588497

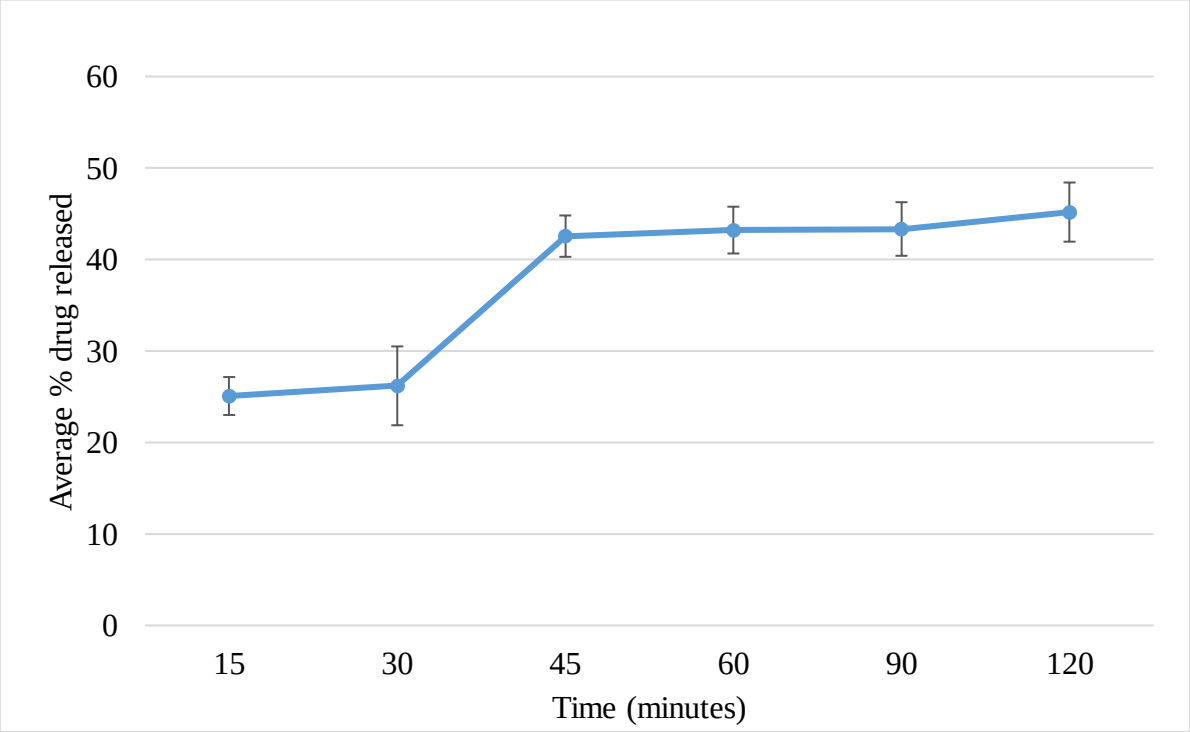

Figure 6: Dissolution profile of Carvedilol in 0.1N HCl (pH 1.2)

Table 8: Dissolution study of F5 in 0.05M phosphate buffer (at pH 6.8)

Time (minutes)	Average % Drug Released	SD
15	10.18389897	0.503957298
30	10.61010261	0.472967269
60	14.63535912	0.178764823
120	15.20363063	0.287080245
180	16.93212312	0.320309771
240	17.90292028	0.217012266
300	19.34727703	0.59147574
360	20.0812944	0.741616091

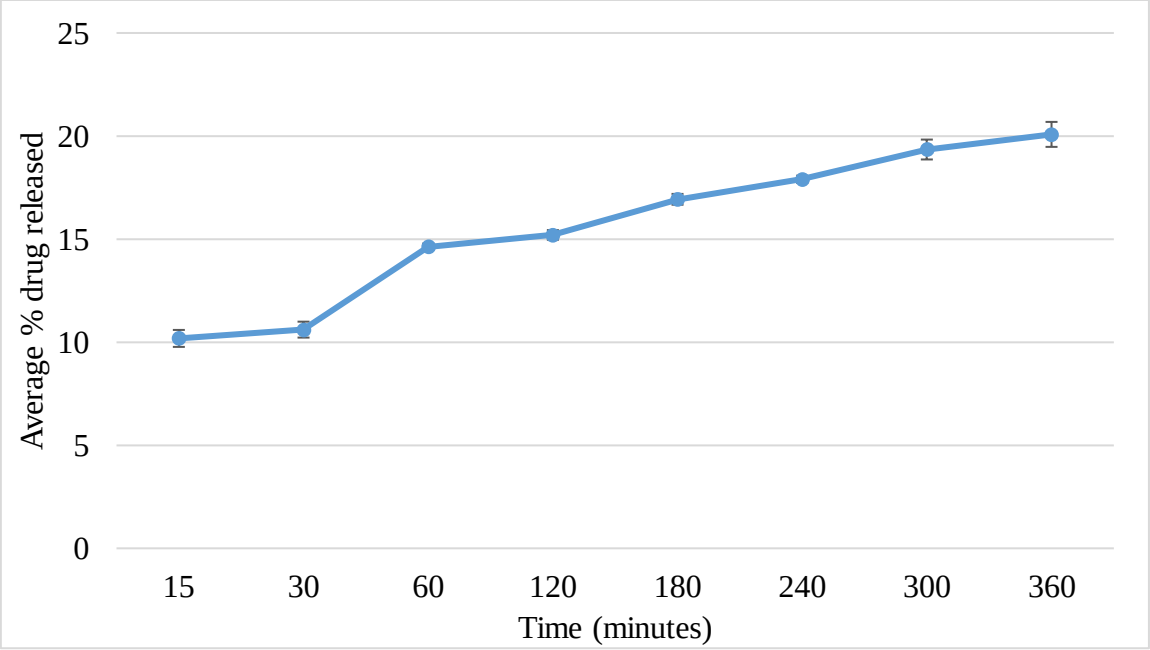

Figure 7: Dissolution profile of F5 in 0.05M phosphate buffer (pH 6.8)

Table 9: Dissolution study of Carvedilol in 0.05M phosphate buffer (at pH 6.8)

Time (minutes)	Average % Drug Released	SD
15	2.677979479	0.994475138
30	3.009471192	0.391224427
60	4.430149961	1.752010487
120	7.342541437	0.082022926
180	3.861878453	0.746138181
240	6.75059195	0.228342165
300	9.047355959	3.529845965
360	7.247829519	1.027744329

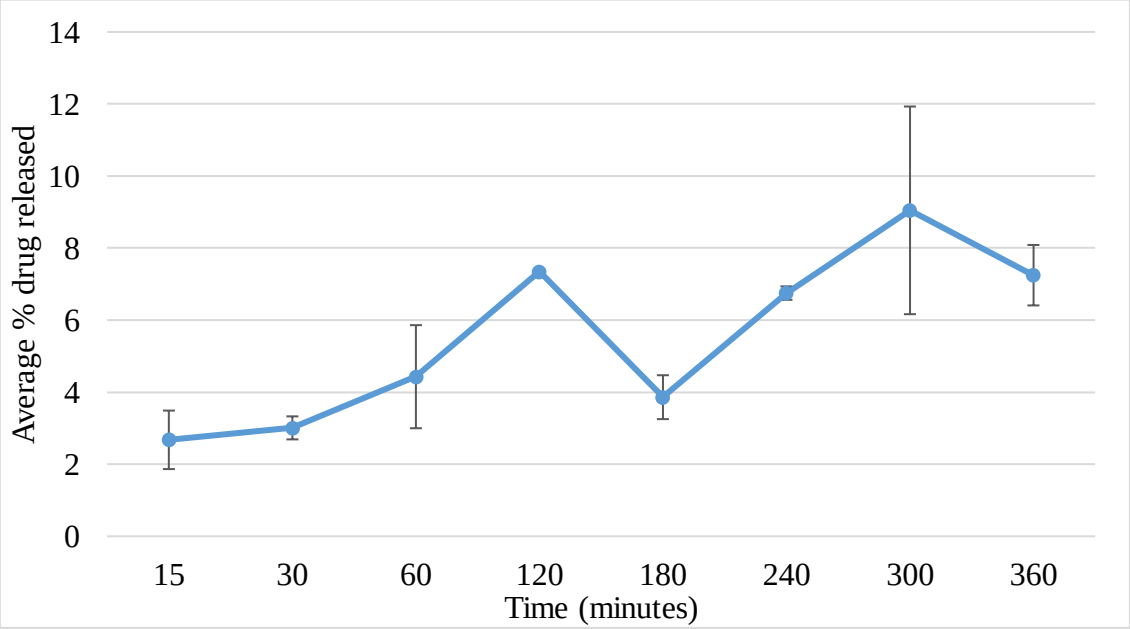

Figure 8: Dissolution profile of Carvedilol in 0.05M phosphate buffer (pH 6.8)

3.4 Physicochemical Characterization

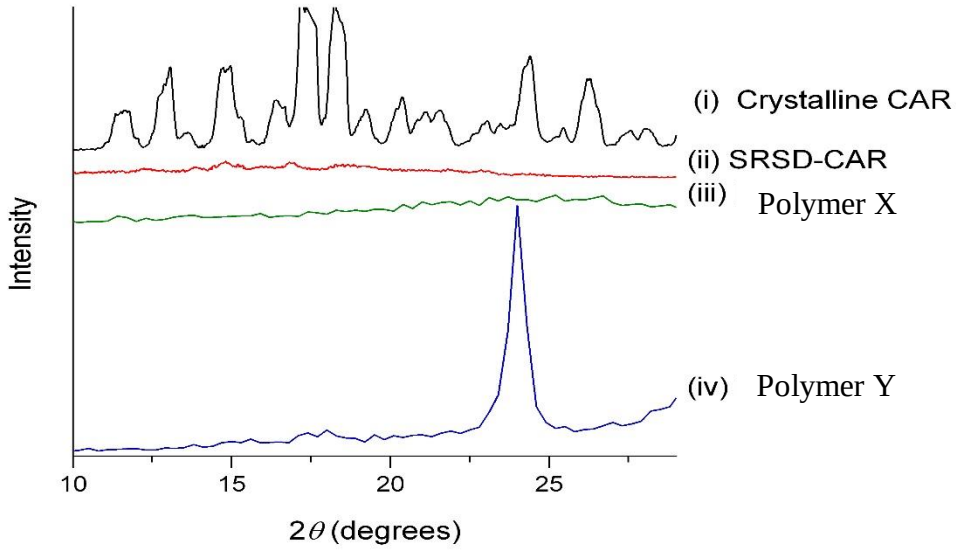

Figure 9: X-ray powder diffraction (XRPD) of F5

Chapter 4

Discussion

During the pre-formulation studies of any pharmaceutical dosage form, solubility and dissolution rate both are very important. Solubility enhancement allows the development of a more dose-efficient formulation for drugs that exhibit poor aqueous solubility. The solubility behavior of Carvedilol was studied by equilibrium solubility and in vitro dissolution methods. To optimize the drug: polymer ratio, various combinations of SRSD-Carvedilol (F1-F5) were prepared (Table 4).

4.1 Equilibrium Solubility Study

The shake flask method for equilibrium solubility study employs a mechanical agitation device, which maintains the ideal temperature of 37 ± 1 °C as well as the agitation speed needed for particle contact with diluents (WHO, 2018).

From the results of solubility study, it is quite evident that solubility is increased for all the SDs in comparison to Carvedilol. This increase in solubility is mainly due to the polymer Y. It is a poloxamer, a class of synthetic block copolymers, which has hydrophobic poly (propylene oxide) (PPO) and hydrophilic poly (ethylene oxide) (PEO) arranged in such a way that enables the poloxamer to form micelles when it comes in contact with any aqueous solution (BASF, retrieved on January, 2020). Polymer Y is often used as a wetting and dispersing agent in the preparation of SDs (BASF, retrieved on January, 2020).

As illustrated in Figure 3.2.1, the results show a five-fold increase in solubility of SRSD (F5) in comparison to crystalline Carvedilol. At the end of this six-hour study the average solubility of F5 in water was found to be 49.91µg/ml, whereas in case of untreated Carvedilol it was only 8.98µg/ml. This suggests that the blend of the two polymers Y and X has modified the solubility characteristics.

4.2 Dissolution Study

From the data obtained after equilibrium solubility study, F5 was chosen as the optimal SRSD formulation and its drug release profile was determined at pH 1.2 and 6.8 (Lee et al., 2017). The drug release profiles of F5 was examined in simulated gastric pH by employing the USP paddle method at 75 rpm and 37 ± 0.5 °C (Lee et al., 2017). The concentration of drug was determined using the equation obtained from the standard curve (Figure 3):

$y = 0.1267x - 0.0137$

The percentage of drug released was calculated using the following formula:

(Concentration X Dilution Factor X 900) / 1000

The formulation F5 achieved 67% of drug dissolution within the first hour and 70% in 2 hours, which indicates initial burst release (Lee et al., 2017). The sustained release characteristic of the formulation can be attributed to the Polymer X. It is insoluble and capable of pH-independent swelling, so when exposed to the dissolution medium it forms a very thick gelatinous barrier, which controls the amount of drug released and the amount of aqueous medium that penetrates into the matrix (Lee et al., 2017). Polymer X serves as a carrier polymer for SRSD due to their excellent solubilizing and stabilizing capabilities (Evonik). As the ratio of Polymer X is increased from F4 (1:2) to F5 (2:1), the formulation was found to demonstrate a sustained drug release over time.

In contrast, the dissolution study of Carvedilol in gastric pH shows a gradual increase in the amount of drug released, as summarized in Figure 3.3.2. This indicates that Polymer X in combination with Polymer Y is a promising drug delivery technology in order to achieve a controlled release. It can be concluded that SRSD (F5) exhibited a high dissolution rate with at least 1.5-fold increase in the level of drug dissolution after two hours than crystalline Carvedilol (Halder et al., 2018).

Similar findings were obtained when dissolution study of F5 was carried out in a buffer system (intestinal pH) (Lee et al., 2017). Carvedilol demonstrates pH- dependent solubility which means it dissolves very quickly in acidic media, because of its ionized form, but precipitates in basic media (Hamed et al., 2016). The percentage of Carvedilol released in simulated intestinal fluids was significantly less in comparison to the dissolution of SRSD (F5) in pH 1.2. About 15% of drug was released within the first hour in 0.05 M phosphate buffer. At the end of six hours, only 20% of Carvedilol entered the dissolution media (Hamed et al., 2016). In general, weak bases like Carvedilol have a high dissolution rate in stomach, but tend to precipitate when they enter the small intestine (Hamed et al., 2016). This explains the pH dependency of SRSD-Carvedilol (F5) in dissolution as it shows very limited dissolution in basic condition (pH 6.8) (Lee et al., 2017).

Whereas, the dissolution study of pure Carvedilol clearly indicates that the drug is only slightly soluble in the intestinal pH. As illustrated in Figure 8, 4% of Carvedilol was released into the media in the first hour and around 7% at the end of the six-hour study. The results obtained after dissolution of untreated Carvedilol in 0.1N HCl shows slight deviation. A possibility could be that the drug was not uniformly distributed in the dissolution vessel, which caused the sample extracted at different time intervals to be of different concentrations. Moreover, the depth from which the sample was taken may also have affected the result. According to Hamed et al., 2016, the absorption of Carvedilol could be rate-limited because of the gastrointestinal pH conditions. Similar observation was made with the dissolution of untreated Carvedilol in phosphate buffer. The results lack uniformity because the drug may have exhibited limited solubility in the dissolution media.

4.3 Physicochemical characterization

Overall, among all the tested SRSD formulations, F5 displayed the desirable sustained drug release profile and hence was selected for further characterization (Lee et al., 2017). To identify the crystallinity of Carvedilol in the SRSD (F5), power X-ray diffraction was carried out. As demonstrated in Figure 9, the PXRD pattern of untreated Carvedilol shows multiple peaks at 2θ angles, in the range 10–30°. The Polymer Y also shows a sharp peak at 2θ angles, in the range 22.5– 25°. These characteristic peaks of Carvedilol are indicative of the fact that the drug is in crystalline form (Lee et al., 2017). On the contrary, SRSD (F5) shows no distinct peak of Carvedilol (with approximately 95% of crystallinity removed), which indicates that Carvedilol is dispersed in the polymer matrix and is possibly in an amorphous form. A similar observation was found by Lee et al. in 2017 with the drug Pelubiprofen.

Chapter 5

Conclusion

In the emerging field of drug discovery, a large portion of drugs in the pipeline are not water soluble and thus becomes a challenge for formulation scientists (Baghel et al., 2016). The application of SRSD is mainly intended for solubility enhancement of poorly water-soluble drugs such as Carvedilol (Nikolakakis & Partheniadis, 2017). The present work used SRSD-Carvedilol, where a blend of polymers was used. This new approach provides a sustained release action, which is quite desirable in case of drugs with short half-life that require frequent dosing (Nikolakakis & Partheniadis, 2017). The prepared SRSD-Carvedilol (F5) was found to demonstrate a five-fold increase in solubility and at least 1.5 times better dissolution, in comparison to untreated Carvedilol. Furthermore, the SRSDs were also characterized by X-ray powder diffraction. Preparation of SRSD can be a potential strategy to overcome solubility limitations because it involves molecular engineering of the drug using carrier polymers. To conclude, SRSD-Carvedilol (F5) formulation could lead to a delivery system that has the desirable properties and maybe be a feasible option to solubilize drugs.

Chapter 6

Future work

Future work would include preparation of more SRSDs with various polymer ratios and comparison of the prepared formulations with the commercially available Carvedilol tablets. *In-vivo* pharmacokinetic studies could be carried out to prove the *in-vitro* hypothesis of Carvedilol in improvement of oral bioavailability and sustained release.

References

Alves, J. M. V., Prado, L. D., & Rocha, H. V. A. (2016). Evaluation and correlation of the physicochemical properties of carvedilol. *Pharmaceutical Development and Technology*. https://doi.org/10.3109/10837450.2015.1073740

Andell, P., Erlinge, D., Smith, J. G., Sundström, J., Lindahl, B., James, S., & Koul, S. (2015). B-Blocker use and mortality in copd patients after myocardial infarction: A swedish nationwide observational study. *Journal of the American Heart Association*. https://doi.org/10.1161/JAHA.114.001611

Baghel, S., Cathcart, H., & O'Reilly, N. J. (2016, September 1). Polymeric Amorphous Solid Dispersions: A Review of Amorphization, Crystallization, Stabilization, Solid-State Characterization, and Aqueous Solubilization of Biopharmaceutical Classification System Class II Drugs. *Journal of Pharmaceutical Sciences*. https://doi.org/10.1016/j.xphs.2015.10.008

BASF. Kolliphor® P188. Retrieved January 14, 2020, from https://pharmaceutical.basf.com/en/Drug-Formulation/Kolliphor-P188.html

Brittain, H. G. (2013). Profiles of Drug Substances, Excipients, and Related Methodology - Google Books. In *Academic Press*. Retrieved from https://books.google.com.bd/books?id=0PPw_pZdBq4C&pg=PA113&lpg=PA113&dq=profile+of+drug+substances+excipients+and+related+methodology+chapter+4+carvedilol&source=bl&ots=wgfB75JGIn&sig=ACfU3U12CuSM9AaWvsmPRSFQo9grlJOc0Q&hl=en&sa=X&ved=2ahUKEwiOm-mb--zmAhWV

Cappuccio, F. P., & Miller, M. A. (2016, April 1). Cardiovascular disease and hypertension in sub-Saharan Africa: burden, risk and interventions. *Internal and Emergency Medicine*. https://doi.org/10.1007/s11739-016-1423-9

Cardiac Medications | American Heart Association. (2015). Retrieved January 7, 2020, from American Heart Association website: https://www.heart.org/en/health-topics/heart-attack/treatment-of-a-heart-attack/cardiac-medications

Dizaj, S., Vazifehasl, Z., Salatin, S., Adibkia, K., & Javadzadeh, Y. (2015). Nanosizing of drugs: effect on dissolution rate. Research in pharmaceutical sciences. *Research in Pharmaceutical Sciences*. http://www.ncbi.nlm.nih.gov/pubmed/26487886

Evonik. (n.d.). Enteric release solutions – Evonik Health Care. Retrieved January 14, 2020, from https://healthcare.evonik.com/product/health-care/en/products/pharmaceutical-excipients/delayed-release/

Fahr, A., & Liu, X. (2007, July). Drug delivery strategies for poorly water-soluble drugs. *Expert Opinion on Drug Delivery*. https://doi.org/10.1517/17425247.4.4.403

Halder, S., Tabata, A., Seto, Y., Sato, H., & Onoue, S. (2018). Amorphous solid dispersions of carvedilol along with pH-modifiers improved pharmacokinetic properties under hypochlorhydoria. *Biopharmaceutics and Drug Disposition*. https://doi.org/10.1002/bdd.2129

Hamed, R., Awadallah, A., Sunoqrot, S., Tarawneh, O., Nazzal, S., AlBaraghthi, T., … Abbas, A. (2016). pH-Dependent Solubility and Dissolution Behavior of Carvedilol—Case Example of a Weakly Basic BCS Class II Drug. *AAPS PharmSciTech*. https://doi.org/10.1208/s12249-015-0365-2

Harvey, Richard A., Clark, Michelle A., Richard Finkel, Rey, Jose A., Whalen, K. (2012). Lippincott's Pharmacology 5th ed. *Lippincott Williams & Wilkins On*. https://doi.org/10.1017/CBO9781107415324.004

Ismaiel, A., & Dumitraşcu, D. L. (2019, September 13). Cardiovascular Risk in Fatty Liver

Disease: The Liver-Heart Axis—Literature Review. *Frontiers in Medicine*. https://doi.org/10.3389/fmed.2019.00202

Khadka, P., Ro, J., Kim, H., Kim, I., Kim, J. T., Kim, H., … Lee, J. (2014). Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian Journal of Pharmaceutical Sciences*. https://doi.org/10.1016/j.ajps.2014.05.005

Kim, Y. H., Choi, H. Y., Noh, Y. H., Lee, S. H., Lim, H. S., Kim, C., & Bae, K. S. (2015). Dose proportionality and pharmacokinetics of carvedilol sustained-release formulation: A single dose-ascending 10-sequence incomplete block study. *Drug Design, Development and Therapy*. https://doi.org/10.2147/DDDT.S86168

Lee, S. N., Poudel, B. K., Tran, T. H., Marasini, N., Pradhan, R., Lee, Y. I., … Kim, J. O. (2013). A novel surface-attached carvedilol solid dispersion with enhanced solubility and dissolution. *Archives of Pharmacal Research*. https://doi.org/10.1007/s12272-013-0008-7

Lee, Y.-S., Song, J. G., Lee, S. H., & Han, H.-K. (2017). Sustained-release solid dispersion of pelubiprofen using the blended mixture of aminoclay and pH independent polymers: preparation and in vitro/in vivo characterization. *Drug Delivery*. https://doi.org/10.1080/10717544.2017.1399304

Muhammad Sarfraz, R., Bashir, S., Mahmood, A., Ahsan, H., Riaz, H., Raza, H., … Yasmeen, T. (2017). Application of various polymers and polymers based techniques used to improve solubility of poorly water soluble drugs: a review. *Acta Poloniae Pharmaceutica*. http://www.ncbi.nlm.nih.gov/pubmed/29624239

Nikolakakis, I., & Partheniadis, I. (2017, December 1). Self-Emulsifying granules and pellets: Composition and formation mechanisms for instant or controlled release.

Pharmaceutics. https://doi.org/10.3390/pharmaceutics9040050

Oliveira-Paula, G. H., Pereira, S. C., Tanus-Santos, J. E., & Lacchini, R. (2019). Pharmacogenomics and hypertension: Current insights. *Pharmacogenomics and Personalized Medicine*. https://doi.org/10.2147/PGPM.S230201

Planinšek, O., Kovačič, B., & Vrečer, F. (2011). Carvedilol dissolution improvement by preparation of solid dispersions with porous silica. *International Journal of Pharmaceutics*. https://doi.org/10.1016/j.ijpharm.2010.12.035

Prajnamitra, R. P., Chen, H. C., Lin, C. J., Chen, L. L., & Hsieh, P. C. H. (2019). Nanotechnology approaches in tackling cardiovascular diseases. *Molecules*. https://doi.org/10.3390/molecules24102017

Savjani, K. T., Gajjar, A. K., & Savjani, J. K. (2012). Drug Solubility: Importance and Enhancement Techniques. *ISRN Pharmaceutics*, *2012*. https://doi.org/10.5402/2012/195727

Thirunavukkarasu, S., & Khader, S. A. (2019). Advances in cardiovascular disease lipid research can provide novel insights into mycobacterial pathogenesis. *Frontiers in Cellular and Infection Microbiology*. https://doi.org/10.3389/fcimb.2019.00116

WHO. (2018). Protocol to conduct equilibrium solubility experiments for the purpose of 2 biopharmaceutics classification system-based classification of active pharmaceutical 4 ingredients for biowaiver *5*. Retrieved from http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/

Wong, G. W. K., Laugerotte, A., & Wright, J. M. (2015, August 26). Blood pressure lowering efficacy of dual alpha and beta blockers for primary hypertension. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD007449.pub2

Zhang, X., Xing, H., Zhao, Y., & Ma, Z. (2018). Pharmaceutical Dispersion Techniques for Dissolution and Bioavailability Enhancement of Poorly Water-Soluble Drugs. *Pharmaceutics*. https://doi.org/10.3390/pharmaceutics10030074