Solubility Profile of Linagliptin: Designing of Transdermal Formulation

By

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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 which 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 thesis titled "Solubility Profile of Linagliptin: Designing of Transdermal Formulation" submitted by Md. Kaykobad Hossain (15146065) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on 29th of May, 2019.

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Ethics Statement

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

Abstract

Diabetes mellitus is a common disease in current world and according to a report of 2013, 8.3% of total adult population is suffering from this disease, with diabetes mellitus type two making up 90% of the cases. There are many anti-diabetic agents available which are administered through oral and parenteral routes. One common oral agent is Linagliptin which is a dipeptidyl peptidase-4 (DPP-4) inhibitor. But oral bioavailability of this drug is only 30%, which suggests the introduction of other routes of drug delivery. Transdermal delivery of this drug might be a good choice, but before designing the formulation, solubility profile of this drug should be determined. This needs to be done to determine the solvent or co-solvent to be used and also to get the idea about the concentration of the drug that can be used. One of the most common methods of solubility determination is shake-flask method which was used in this study.

Keywords: diabetes mellitus; linagliptin; solubility profile; shake-flask method; transdermal route; UV-visible spectrometer.

Dedication

Dedicated to my parents and to my supervisor, Professor Dr. Eva Rahman Kabir

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Table of Contents

List of Tables

List of Figures

List of Acronyms

DM	Diabetes Mellitus
GLP-1	Glucagon-like Peptide-1
DPP-4	Dipeptidyl Peptidase-4
T2DM	Type 2 Diabetes Mellitus
PK	Pharmacokinetics
LNG	Linagliptin
USFDA	United States Food and Drug Administration
DD	Drug Delivery
TDDS	Transdermal Drug Delivery System
MN	Microneedles
USP	United States Pharmacopoeia
BP	British Pharmacopoeia
HPLC	High-Performance Liquid Chromatography
UV	Ultraviolet

Chapter 1

Introduction

1.1 Diabetes Mellitus and Associated Complications

Diabetes mellitus (DM) is currently one of the most researched diseases and a key world health problem (Ahad, Al-Saleh, Akhtar, Al-Mohizea, & Al-Jenoobi, 2015). This is defined as a set of metabolic diseases identified by high blood glucose level (hyperglycemia) when our pancreas is unable to produce sufficient amount of the regulatory hormone insulin and/or the insulin that is produced by it is not effectively used (Berná et al., 2014). This endocrine disorder influences glucose metabolism and it has been affecting the humankind for the past two centuries (Akram, Ahmad, Abrar, Sarfraz, & Mahmood, 2018). According to the global statistics of diabetes mellitus from the year 2016, around 422 million people had this disease worldwide (World Health Organization, 2016), with type 2 diabetes making up about 90 % of the cases. This indicates that 8.3% of the total adult population is suffering from diabetes with equal rates in both women and men. It is predicted that, by the year 2035, diabetes will cause deaths of approximately 592 million people around the world (Tao, Shi, & Zhao, 2015). Diabetes mellitus is mainly classified into two types. Type 1 diabetes mellitus is an autoimmune disease where pancreatic beta cells are destroyed and no (or very little amount of) insulin is produced and released from pancreas which causes patients to take exogenous insulin. Major causes of type 1 diabetes mellitus are abnormal beta cells, very strong immune system and environmental and genetic factors (Ahad et al., 2015). Type 2 diabetes mellitus is related to insulin resistance and obesity, along with defects in beta cells function. Children and young adults commonly suffer from type 1 DM, and type 2 DM, on the other hand, develops more frequently in older adults (Guilherme, Virbasius, Puri, & Czech, 2008). Normal or excessive amount of basal insulin levels can be detected in the early phase of

diabetes which is produced to compensate insulin resistance (Skovsø, 2014). Transition from pre-diabetes to actual type 2 diabetes is mainly accelerated by significant loss of functional beta cells (Rivera-Mancía, Lozada-García, & Pedraza-Chaverri, 2015). Additionally, diabetes can affect other major organs in our body, such as liver (Rivera-Mancía et al., 2015), heart (Chiang, Pritchard, & Nagy, 2011), and kidney (Navarro-gonzález, Mora-fernández, & Fuentes, 2011).

Hyperglycemia-induced apoptosis in diabetic patients can cause endothelial dysfunction due to production of reactive oxygen species, super oxide anion and the lack of proper antioxidant enzymes activity (Rivera-Mancía et al., 2015). Hyperglycemia can also closely associated with inflammation and other complications caused by diabetes; for instance, nephropathy and cardio myopathy (Pan et al., 2012). Actually, the main cause of mortality and morbidity in diabetic patients is diabetic nephropathy (Pan et al., 2014). Diabetic nephropathy, caused by the long-standing diabetes mellitus, is a major reason of end-stage kidney disease in older patients (Alsaad & Herzenberg, 2007) (Lv, Chen, Hu, & Li, 2015).

1.2 Treatment Options for Diabetes Mellitus Type 2

There are both pharmacological and non-pharmacological treatment options for type 2 diabetes mellitus (T2DM) (Reusch & Manson, 2017). Physical exercise and dietary intake determine energy balance in a diabetic patients (Kono, 2012) and they are considered as the two most important non-pharmacological bases in diabetes treatment (Reusch & Manson, 2017). Different blood glucose lowering oral and injectable therapeutic agents, on the other hand, are used as pharmacological treatment options for T2DM (Reusch & Manson, 2017). Therapeutic agents that are injected for diabetes treatment include long-acting glucagon-like peptide 1 (GLP-1) (Holst et al., 1996), such as exenatide (Iltz, Baker, Setter, & Keith Campbell, 2006), lixisenatide (Anderson & Trujillo, 2016), and different types of insulin

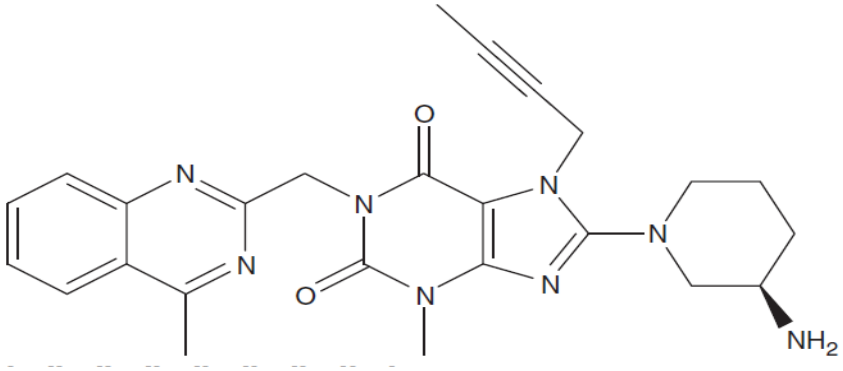
which are categorized as short acting, intermediate acting and long acting based on their duration of action (Meneghini, 2013).

Currently, six classes of oral anti-diabetic agents are available for the treatment of diabetes mellitus type 2 (Akram et al., 2018). These oral anti-diabetic agents encompass second (e.g. glipizide,) and third (e.g. glimepiride) generation sulfonylureas, biguanides (e.g. Metformin), meglitinides (e.g. rapaglinide), thiazolidinedione (e.g. pioglitazone), dipeptidyl peptidase-4 (DPP-4) inhibitors (e.g. linagliptin, sitagliptin) (Mcgill, 2012) and alpha (α) glucosidase inhibitors (e.g. voglibose) (Ahad et al., 2015). Linagliptin, a dipeptidyl peptidase-4 inhibitor, is the drug of choice for the current study.

1.3 Linagliptin

One of the currently developed medicinal classes for the treatment of hyperglycemia in T2DM is dipeptidyl peptidase-4 (DPP-4) inhibitors. Although different therapeutic agents of this class differ in structures, they all act by inhibiting the DPP-4 enzyme. These agents extend the life of incretin hormones which results in increased insulin level and in a glucose-dependent way, they also stop glucagon secretion (Mcgill, 2012). Glucose dependent mechanism of action of this class of drugs comparatively has lower risk of hypoglycemic events than other anti-diabetic agents (Richter, Bandeira-Echtler, Bergerhoff, & Lerch, 2008). Linagliptin (LNG), [BI1356, 8-(3R-amino-piperidin-1-yl)-7- but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2 ylmethyl)- 3,7-dihydro-purine-2,6-dione], is a new significant DPP-4 inhibitor having different pharmacokinetic (PK) properties, when compared with formerly commercialized DPP-4 inhibitors, which may provide some benefits in clinical practice (André J Scheen, 2011). United States Food and Drug Administration (USFDA) approved this drug for the treatment of patients having type 2 diabetes mellitus on May 2, 2011 (P Toth, 2011). A drug summary on Linagliptin is given in the following table (Table 1).

Table 1: Summary of Linagliptin including its Pharmacokinetic Properties

Drug name	Linagliptin (André J Scheen, 2011)
Dose	5 mg tablet once daily (Aletti & Cheng-Lai, 2012)
Phase	Launched (André J Scheen, 2011)
Indication	Type 2 diabetes mellitus (André J Scheen, 2011)
Chemical Structure	 Source: (André J Scheen, 2011)
Pharmacology Description	DPP-4 inhibitor Protease inhibitor Dipeptide hydrolase inhibitor Insulin secretagogue (André J Scheen, 2011)
Route of administration	Alimentary, p.o. (André J Scheen, 2011)
Peak Plasma Concentration	8.9 nmol/L which occurs at 1.5 hours postdose (Aletti & Cheng-Lai, 2012)
Bioavailability	30% (Aletti & Cheng-Lai, 2012)
Major Elimination System	Enterohepatic system (Aletti & Cheng-Lai, 2012)
Renal Excretion	Less than 7% (A. J. Scheen, 2010)
Elimination half-life ($t_{1/2}$)	69.7 hours (Sortino, Sinagra, & Canonico, 2013)

Linagliptin is taken orally as 5 mg dose once daily (Aletti & Cheng-Lai, 2012) by a patient suffering from type two diabetes mellitus (André J Scheen, 2011). Peak plasma concentration of this drug after administering 5 mg dose is 8.9 nmol/L, which occurs after 1.5 hours of administration and the elimination half-life of this drug is 69.7 hours (Sortino et al., 2013) (Aletti & Cheng-Lai, 2012). The oral bioavailability of this drug is 30% and the major elimination happens through enterohepatic pathway, where renal excretion is less than 7% (Aletti & Cheng-Lai, 2012) (A. J. Scheen, 2010).

Since Linagliptin is mainly eliminated unchanged via enterohepatic system (Blech, Ludwig-Schwellinger, Gräfe-Mody, Withopf, & Wagner, 2010), one of the major advantages of this drug is that, dose adjustment is not required for the patients having hepatic or renal impairment (Gallwitz, 2013). Moreover, the mechanism, duration of action, potency and selectivity of Linagliptin in-vitro and in-vivo were shown to be better than other DPP-4 inhibitors which were already available in the market (Himmelsbach et al., 2008).

1.4 Proposed Drug Delivery System for Linagliptin

To ensure that drugs are administered in a way that leads to proper therapeutic efficacy, drug delivery (DD) has been very important factor from the beginning of history (Cheung & Das, 2016). Currently, various forms of drug delivery routes are being employed, which include oral administration (colonic, gastric, enteric and so on), inhalation, hypodermic injections (e.g. intra-muscular, intra-venous, intra-cranial, subcutaneous etc.) and transdermal route of drug delivery (Li et al., 2013)(Nelson & Guyer, 2012). Among these routes, oral administration covers approximately 80% of total drugs and the parenteral route being the second most common route of drug administration. However, degradation of drugs due to change in pH, enzymatic activity, side effects, variable transit time and first pass metabolism are typical difficulties that are associated with oral administration of drugs (Cheung & Das, 2016). For the delivery of vaccines, insulin, small molecules, chemotherapeutic agents and

other liquid formulations, parenteral routes are widely accepted route of drug delivery. Although this drug delivery system is economical and a direct method to deliver medicinal agents, it can cause patient non-compliance and injection phobia, especially in case of self-administration (Rejinold, Shin, Seok, & Kim, 2016). In addition, this painful procedure of drug administration can cause hypersensitivity, discomfort, bruising, bleeding at the area of administration and, in few cases, is associated with chance of contamination (Ita, 2015). A superior alternative that can be applied to overcome such problems is delivery of drugs through the transdermal route (Cheung & Das, 2016). In case of transdermal drug delivery system (TDDS), drugs are administered topically in the form of patches which are delivered across a patient's skin for systemic action at a controlled and predetermined rate (Shah, Prabhu, & Gundad, 2011). This drug delivery system has been a popular way of drug administration via the skin for both local (therapeutic action on diseased skin) and systemic delivery of medicines (Prajapati, Patel, & Patel, 2011). Transdermal route provides continuous input of medicines with short biological half-lives, avoids pulsed entry of drugs into systemic circulation (eliminate undesirable side effects) and most importantly provides constant and controlled administration of drugs (Shah et al., 2011). Transdermal drug delivery system has numerous important advantages over many other systems of drug administration, which include, ability to avoid the problems of gastric irritation, gastric emptying rate and pH change effects and first-pass metabolism through liver thereby increasing the bioavailability of drug. This route also reduces the chance of systemic side effects by decreasing plasma drug concentrations compared to oral drug delivery, provides sustained release of drug at the administration area, facilitates fast termination of therapy by removal of the formulation or device and avoids pain associated with injections (Prajapati et al., 2011).

This type of drug delivery requires penetration of lipophilic stratum corneum and also reabsorption of drugs into the aqueous main compartment of the systemic circulation (Wiedersberg & Guy, 2014) (Mccrudden et al., 2013). The outermost lipophilic stratum corneum layer is responsible for the limited permeability of drugs into systemic circulation (Park, Park, Seo, & Lee, 2014). A number of physical and chemical methods have been designed to enhance permeability of drug across the skin (Wiedersberg & Guy, 2014) (Schoellhammer, C.M.; Blankschtein, D.; Langer, 2016). In recent years, microneedles (MNs) are widely used physical method of enhancing drug delivery through skin (Alkilani, McCrudden, & Donnelly, 2015). Currently, researchers are trying to deliver several active substances, including vaccines, hydrophilic and/or high molecular weight drugs (Dillon, Hughes, O'Reilly, & McLoughlin, 2017), RNA and DNA (Pere et al., 2018), , anti-cancer drugs, proteins and oligonucleotides (Uddin et al., 2015) using transdermal microneedles. Microneedles are composed of a collection of micron sized needles which can penetrate the skin in a painless manner, when applied to the skin surface, and through the piercing of the stratum corneum by creating micro-channels, drug substances are released into the dermis (Dillon et al., 2017) (Uddin et al., 2015) (Pere et al., 2018). The minimally invasive administration of microneedles has shown to improve systemic drug absorption and thus bioavailability (Wermeling et al., 2008). Since Linagliptin has a low oral bioavailability (30%), we can consider designing the transdermal formulation of this drug using microneedles.

1.5 Solubility Study

Solubility can be defined as the phenomenon of the dissolution of one or more solutes in a solvent which results in a homogenous system (Savjani, Gajjar, & Savjani, 2012). Solubility also can be defined as the characteristic of solid, liquid and gaseous chemical entity called solute to be dissolved in a solid, gaseous or liquid solvent to make a homogenous mixture of

solute in the solvent. It plays a very important role when we want to achieve required concentration of any drug in our systemic circulation in order to get the desired pharmacological response of the drug (Savjani et al., 2012). The primary factors that affect the solubility of a solute include the type of solvent used, temperature and pressure. The maximum amount of solute that is dissolved in a solvent, forms a saturated solution of that solute in that particular solvent, where addition of more solute does not increase the concentration of the solute in that solvent (Lachman, Lieberman, & Kanig, 1987). In most of the cases, the solvent is a liquid, which can be in its pure form or in combination with one or more solvents. There might also be solution in a solid solvent, but solution in a gas is uncommon. The extent to which a solute is dissolved in a solvent ranges widely and based on this, they are classified as very soluble, such as ethanol in water, to poorly soluble, such as silver chloride (AgCl) in water. There is also a term called insoluble which is frequently applied to very poorly soluble compounds. (Savjani et al., 2012). Table 2 (below) shows the solubility criteria used by United States Pharmacopoeia (USP) and British Pharmacopoeia (BP).

Table 2: USP and BP Solubility Criteria (Savjani et al., 2012)

Descriptive term	**Part of solvent required per part of solute**
Practically insoluble	10,000 and over
Very slightly soluble	From 1000 to 10,000
Slightly soluble	From 100 to 1000
Sparingly soluble	From 30 to 100
Soluble	From 10 to 30
Freely soluble	From 1 to 10
Very soluble	Less than 1

1.5.1 Importance of Solubility Study

Solubility plays important role in all the dosage forms of drug delivery. Most common dosage form incorporates oral routes and the major challenge while designing oral dosage forms is their low bioavailability. Oral bioavailability of a drug depends on many factors, among which aqueous solubility is most important. This is why solubility study should be performed before designing the formulation of any drug. Solubility study is also of same importance for other dosage forms, such as parenteral and transdermal delivery (Kerns & Di, 2008).

In case of transdermal delivery of drugs, therapeutic response depends on the drug action and also the type and amount of solvents and other vehicles that are used (Hamishehkar, Khoshbakht, Jouyban, & Ghanbarzadeh, 2015). This is because for a drug to be absorbed through skin, drug must be in aqueous solution at the site (under stratum corneum) of absorption (Savjani et al., 2012). Therefore, when we design a drug to be administered through transdermal route, the appropriate solvent must be chosen and solvent-drug properties must be examined. In addition, to get homogenous mixture of drug and solvent we are using, appropriate amount of drug must be soluble in small amount of solvent in case of the transdermal formulation. To determine the amount of drug that will be dissolved in the solvent, solubility of the drug in that solvent should be known. To formulate transdermal dosage from, very few solvents have been frequently used, such a glycerin, ethanol, propylene glycol (PG) (Hamishehkar et al., 2015).

1.5.2 Methods of Solubility Determination

There are various theoretical and experimental approaches currently available to determine solubility of a substance in a solvent or combination of solvents. Theoretical approaches include many non-computerized and computerized methods, such as Yalkowsky equation (modified version of Hansch equation), WSKOWWIN program, the ACD/Solubility DB

algorithm (Advanced Chemistry Development Inc.), HYBOT, ABSOLV (Sirius Analytical Instruments Ltd.) and Artificial Neural Networks (Dressman & Glomme, 2005).

Following are the various theoretical approaches employed in the determination of solubility.

i. One of the most significant early techniques to solubility calculation is the **Hansch equation** (eq. 1) which is applicable to many organic lipids.

$\log S = A \log K_{OW} + B$ ……...…….……...(1)

Here, $\log K_{OW}$ is the octanol/water distribution coefficient and A and B are constants which depend on the lipids that are considered (Hansch, Quinlan, & Lawrence, 1968).

ii. **Yalkowsky** derived a new equation (eq. 2) from Hansch which can be applied to all nonelectrolytes.

logS = 0:8 - logP - 0:01(mp -25) ……..…….…..(2)

Where, melting point (25^0 C for liquids) is indicated by mp (Samuel H. Yalkowsky, 2000).

iii. The Syracuse Research Corporation introduced a program called **WSKOWWIN** by adding different correction factors ($\sum$ fi) for the changeable group fragments to the Yalkowsky. By this program, we can calculate melting points and logP values that are necessary and this program can also use experimental values that are known (Meylan & Howard, 2000).

iv. Advanced Chemistry Development Inc. also has derived an algorithm system called **The ACD/Solubility DB Algorithm** from the Yalkowsky equation. Using this system, we can calculate the aqueous solubility of most of the organic compounds at 25^0C. Solubility is measured in different pH ranging from 0 to 14 in case of acids and bases (Dressman & Glomme, 2005).

v. **HYBOT** calculates the solubility of drug from the power of the hydrogen bond acceptors (HA) and donors (HD), and also from the polarizability. The semi-empirical equation that is given below is valid.

Log $(1/S_w)$ = -0:42 + 0.17*Polarizability + 0:13*HA + 0:08*HD……(3)

(Faller & Wohnsland, 2007).

vi. **ABSOLV**, developed by Sirius Analytical Instruments Ltd., uses similar variables for the solubility algorithm like the HYBOT program.

$\log S_w = 0{:}518 - 1{:}004\ R_2 + 0{:}771\ \pi^H_2 + 2{:}168\ \sum\alpha^H_2 + 4{:}238\ \sum\beta^H_2 - 3{:}362\ \sum\alpha^H_2\ \sum\beta^H_2 - 3{:}99V_x$ ……(4)

where, R_2 represents the excess molecular refraction, π^H_2 polarizability/dipolarity, $\sum\alpha^H_2$ acidity of the hydrogen bond, $\sum\beta^H_2$ basicity of the hydrogen bond, and V_x the McGowan characteristic volume (Abraham & Le, 1999).

vii. Along with these algorithm approaches, **neural networks** is also used for theoretically predicting the aqueous solubility of any substance (Huuskonen, Salo, & Taskinen, 1998).

On the other hand, the different experimental methods for determining solubility of drug (or other substances) are potentiometric titration, shake-flask method and turbidimetry (Dressman & Glomme, 2005). A specific shift that is seen in the middle of the titration curve caused by precipitation is the basis of **potentiometric acid-base titration** for the solubility measurements (Alex Avdeef, 1998). In **turbidimetry** technique, the compound is dissolved in an organic solvent and the resulting solution is added to a pH 7 buffered solution in intervals of 1 min. Some additional aliquots of the solution are added after the first detection of turbidity by light scattering. The added volume can be plotted against the turbidity

afterwards. Back extrapolation estimates the solubility to the point where precipitation started (A. Avdeef & Testa, 2002).

1.5.3 Shake-Flask Method

The experimental solubility determination method used in the current study was shake-flask method which is briefly described below.

In shake flask method, excess amount of substance (drug) is added to a solvent in a flask/vial that can be tightly capped. The mixture is then kept in shaker for a predetermined period of time at a certain temperature to get a saturated solution (equilibrium condition) of the substance in that solvent and then filtered to get rid of any remaining undissolved drug. Filtered solution is then diluted to get a concentration of the substance that will fall within validated linear calibration curve that is found from the High-Performance Liquid Chromatography (HPLC) analysis of standard solution of that drug in the same solvent using different concentrations. From the chromatogram found for the sample, the concentration of the diluted solution is calculated using the linear equation that is gained from the calibration curve and by back calculation method, concentration of the saturated solution is calculated. The concentration of the substance in the saturated solution is actually the solubility of that substance in that solvent. (Dressman & Glomme, 2005) (Hofs, Dressman, & Pl, 2018).

There is a modified technique derived from shake-flask method which is called **miniaturized shake-flask method**. In this technique, solubility of the drug is first calculated by using one of the theoretical methods that were described above (see 1.5.2) so that the amount of substance that will be added in the solvent can be determined. Normally, twice amount of the theoretically calculated substance is added to the solvent to prepare the saturated solution. (Dressman & Glomme, 2005). Further steps of this technique are same as shake-flask method. Shake-flask or miniature shake-flask method is the best technique to determine the

equilibrium solubility of a substance. These methods also require fixed amount of time unlike potentiometric titration and turbidimetry methods (Hofs et al., 2018) (Dressman & Glomme, 2005). Since maximum solubility of the drug was determined, this method was used in the current study.

1.6 Rationale of the Study

The aim of this study is to determine the solubility profile of Linagliptin in different solvents prior to designing transdermal delivery of this drug using microneedles. In our study, we are determining the solubility profile of Linagliptin in different solvents for the first time which will help us select appropriate solvent (or co-solvent) to be used and also to determine the concentration of the drug that we can use in the transdermal formulation we are going to design.

Chapter 2

Materials and Method

2.1 Materials:

2.1.1 Chemicals:

Linagliptin was gifted by Beximco Pharmaceuticals Limited which is our active pharmaceutical ingredient and the solubility profile of this drug was determined. Other chemicals that were used for determining solubility of Linagliptin are ethanol (Merck, Germany), methanol (Active Fine Chemicals Limited, Bangladesh), acetonitrile (Active Fine Chemicals Limited, Bangladesh), sodium hydroxide, NaOH (Merck, India), potassium dihydrogen orthophosphate anhydrous (Active Fine Chemicals Limited, Bangladesh) and ortho-phosphoric acid (RCI Labscan Limited, Thailand).

2.1.2 List of Equipment and Apparatus:

1. UV Spectrophotometer, Model: UV-1800 (Shimadzu Corporation, Japan)
2. pH meter (Mettler Toledo, United States of America)
3. Electronic Balance, Model: ATY 224 (Shimadzu Philippines Manufacturing Inc.)
4. Digital Shaking Incubator, Model: I10-OE+OL30-ME **(**Suministros Grupo Esper, S.L., Spain)
5. Syringe filter, 0.22 µm PTFE 100pK White, Luer Lock Inlet (Restek, France)
6. Test tube
7. Glass rod
8. Spatula
9. Wax paper
10. Foil paper and so on.

2.2 Methodology

Following figure (Figure 1) contains the flowchart of the whole methodology of the study in short.

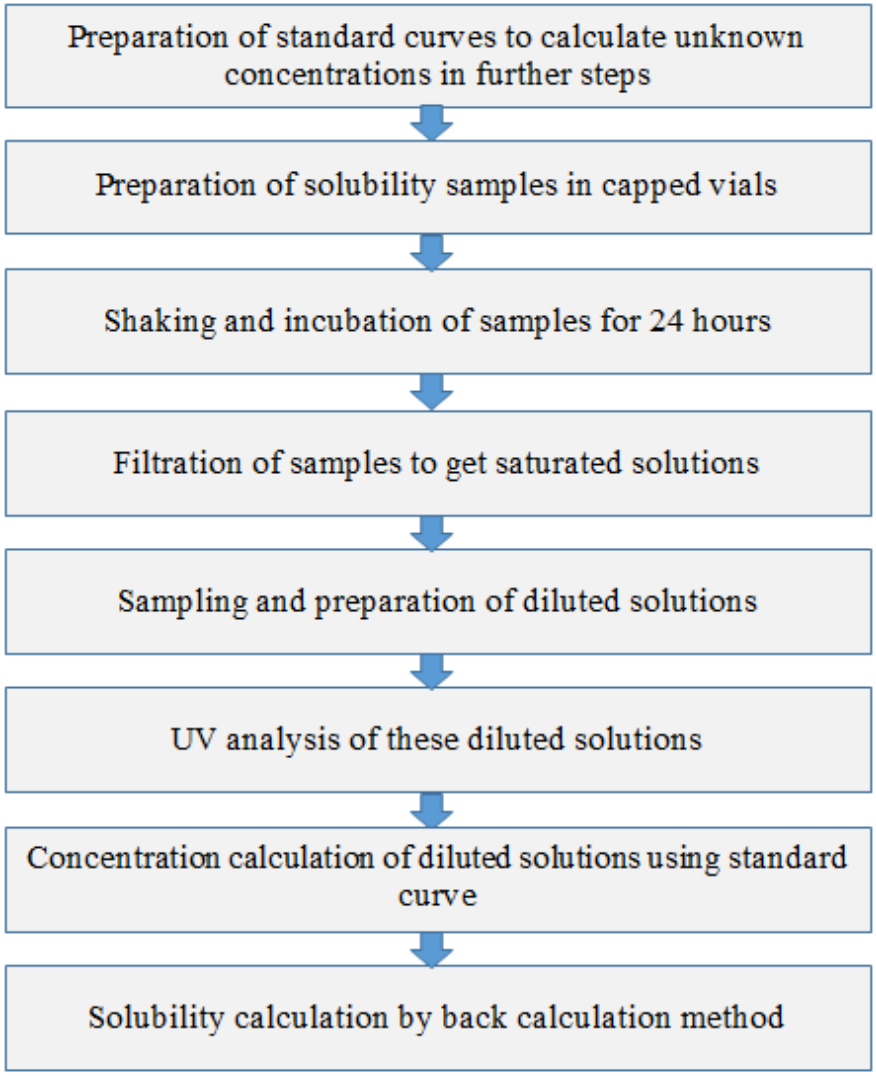


Figure 1: Flowchart of the Methodology of the Study

2.2.1 Standardization of Linagliptin

10 mg of Linagliptin was weighed and taken in a 100 mL volumetric flask and a solvent was added to prepare a solution of 100µg/mL concentration. This solution was used to prepare the solutions of Linagliptin of different concentrations, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL and 10

µg/mL. Absorbance of these solutions was then taken by UV-visible spectrophotometer and then these absorbance values were put in a scatter diagram using excel to find out the linear relationship between two variables, which are concentration and absorbance, and to get the standard curve. Value of regression coefficient showed the extent of linearity between these two variables and the equation that was found from the standard curve was used to calculate the concentrations of diluted solutions of Linagliptin in different solvents in further steps.

In the current study, standard curves were drawn for ten solvent systems, which include 100% ethanol, 75% ethanol, 50% ethanol, 100% acetonitrile, 75% acetonitrile, 50% acetonitrile, 100% methanol, 75% methanol, 50% methanol and a phosphate buffer (pH 7.4).

2.2.2 Sample Preparation and Quantification by UV-Visible Spectrophotometer

The method that was used to determine the solubility profile of Linagliptin is shake-flask method which gave us the equilibrium solubility of this drug (Hofs et al., 2018). In this method, excess amount of Linagliptin was taken in a test tube in triplicate which can be capped tightly. 3 mL of solvent was added to the test tube and mixed well with the drug. This mixture of the drug and the solvent was then kept in a shaker-incubator for 24 hours at 37 ± 0.5^0 C temperature and at a rotational speed of 45 rpm. After 24 hours, the saturated solution of the drug was filtered by injection filter to eliminate undissolved drug particles. Then the filtered saturated solution was diluted several times and absorbance was taken in UV-visible spectrophotometer at 299 nm. The saturated solution was diluted enough so that the absorbance of the solution fell within the range of absorbance values that were used to get the standard curve of Linagliptin in the same solvent. Using the equation found from the standard curve prepared before for the same solvent, the concentration of the diluted solution of the drug was calculated. From the concentration of the diluted solution, concentration of

the saturated solution was calculated, and the concentration of the saturated solution indicated the solubility of the drug in that solvent. The process was repeated for ten solvent systems which are listed in the following table (Table 3).

Table 3: Names of the Solvents used with the Amount of Drug Added to the Solvent

Solvent (3 mL)	**Amount of drug added to the solvent**
100% Ethanol	0.25 gm
100% Methanol	0.5 gm
100% Acetonitrile	0.4 gm
75% Ethanol	0.35 gm
75% Methanol	1.0 gm
75% Acetonitrile	1.5 gm
50% Ethanol	0.6 gm
50% Methanol	1.2 gm
50% Acetonitrile	1.5 gm
Buffer [KH2PO4+NaOH (pH 7.4)]	0.25 gm

2.2.3 Solubility Calculation Using the Equation Derived from the Standard Curve

In this portion, concentration (and solubility) calculation method is given below for 100% ethanol as solvent system. Concentrations of other diluted solutions were calculated using the same method. Filtered saturated solution of Linagliptin in 100% ethanol was diluted in following method (Table 4).

Table 4: Dilution of Saturated Solution of Linagliptin in 100% Ethanol

Dilution process	**Diluted solution number**	**Absorbance**
0.5 mL of saturated solution was taken to make 10 mL solution	Solution 1	4
1 mL was taken from solution 1 to make it 10 mL	Solution 2	4
1 mL of solution 2 was taken to make it 10 mL	Solution 3	0.389
1 mL of solution 3 was taken to make it 5 mL	Solution 4	0.077

The absorbance of the solution 4 was considered for the calculation because it had fallen within the range of absorbance values that were used to draw the standard curve. Concentration of the diluted solution was calculated using the equation that was found from the standard curve. For Linagliptin in 100% ethanol, the equation is given below (eq. 5).

$y = 0.0313x - 0.0137$……..... (5)

Here, y is the absorbance of the diluted solution and x is the concentration of the diluted solution.

For sample 1 (total 3 samples) of Linagliptin solution in 100% ethanol, the absorbance of the diluted solution was 0.077. Using this value, concentration of the diluted solution was calculated in following way.

Concentration, $x = \frac{y+0.0137}{0.0313} = \frac{0.077+0.0137}{0.0313}$ = 2.8977 µg/mL

From the concentration of diluted solution, concentration of the saturated solution was calculated which is the solubility of the drug in the solvent system. This process was repeated for all the solvent systems.

Chapter 3

Results

3.1 Solubility in 100% Ethanol

Absorbance values of the solutions of standard Linagliptin in different concentrations found are given in the following table (Table 5).

Table 5: Absorbance of Different Concentrations of Standard Linagliptin in 100% Ethanol

Concentration (µg/mL)	**Absorbance**
1.25	0.035
2.5	0.065
5	0.126
10	0.307

The standard curve prepared by using these four different concentrations of Linagliptin in 100% ethanol is given below (Figure 2).

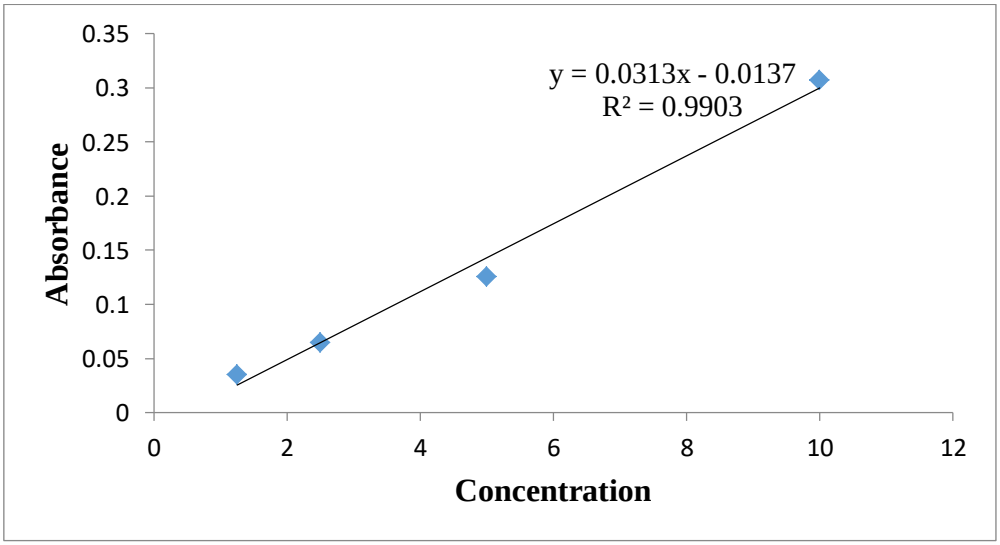


Figure 2: Standard Curve of Linagliptin in 100% Ethanol

Three samples were studied to find out the solubility of Linagliptin in 100% ethanol. Results of the experiment are given in following table (Table 6).

Table 6: Solubility Calculation of Linagliptin in 100% Ethanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 100% ethanol (mg/mL)
1	0.077	2.8977	28.977	
2	0.070	2.6741	26.741	28.848
3	0.083	3.0894	30.894	

So, the solubility of Linagliptin in 100% ethanol is 28.848 mg/mL.

3.2 Solubility in 75% Ethanol

Absorbance values of solutions of the standard Linagliptin in different concentrations in 75% ethanol are listed in the following table (Table 7).

Table 7: Absorbance of Different Concentrations of Standard Linagliptin in 75% Ethanol

Concentration (µg/mL)	**Absorbance**
1.25	0.056
2.5	0.110
5	0.220
10	0.447

The standard curve that was similarly prepared by using these four different concentrations of Linagliptin in 75% ethanol is given below (Figure 3) along with the regression coefficient value and the equation.

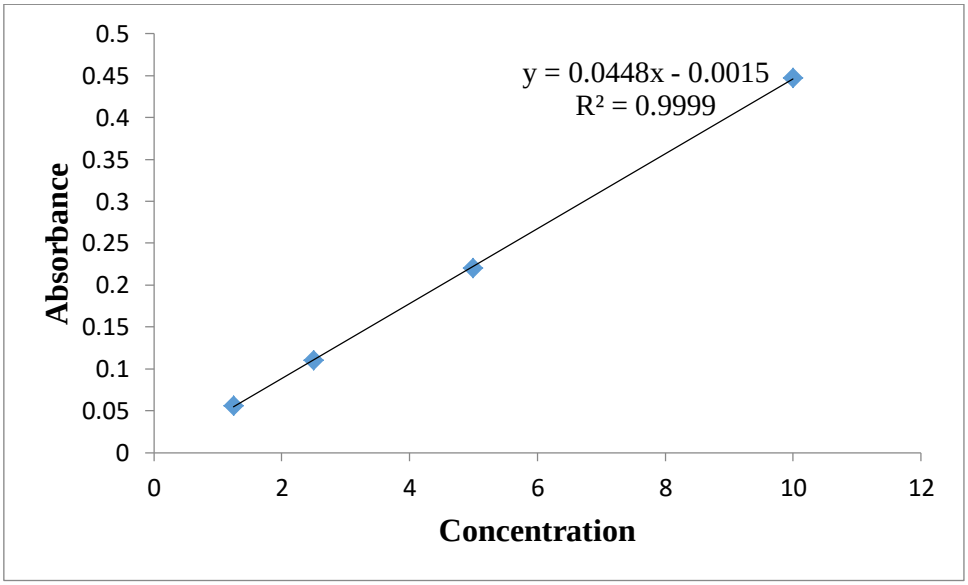


Figure 3: Standard Curve of Linagliptin in 75% Ethanol

The equation in the standard curve was used to calculate the concentration of the drug in diluted solutions. Results of solubility study of Linagliptin in 75% ethanol are given in following table (Table 8).

Table 8: Solubility Calculation of Linagliptin in 75% Ethanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 75% ethanol (mg/mL)
1	0.292	6.551	65.51	
2	0.306	6.863	68.63	67.963
3	0.311	6.975	69.75	

So, the solubility of Linagliptin in 75% ethanol is 67.963 mg/mL.

3.3 Solubility in 50% Ethanol

Absorbance of different solutions of the standard Linagliptin in different concentrations using 50% ethanol as solvent is listed in following table (Table 9).

Table 9: Absorbance of Different Concentrations of Standard Linagliptin in 50% Ethanol

Concentration (µg/mL)	**Absorbance**
1.25	0.060
2.5	0.111
5	0.241
10	0.430

The standard curve of Linagliptin in 50% ethanol is given in the following figure (Figure 4), along with the value of regression coefficient and the equation.

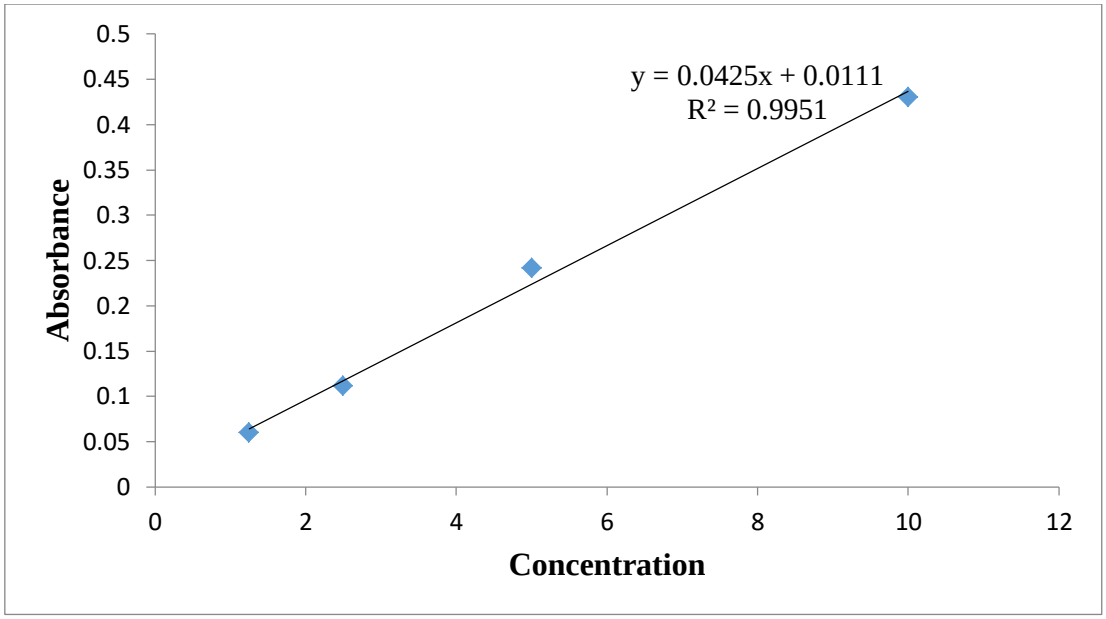


Figure 4: Standard Curve of Linagliptin in 50% Ethanol

Concentrations of the diluted solutions of three samples were calculated using the equation in the standard curve and the solubility of Linagliptin in 50% ethanol was calculated too. Results of the experiment for 50% ethanol as solvent system are given in following table (Table 10).

Table 10: Solubility Calculation of Linagliptin in 50% Ethanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 50% ethanol (mg/mL)
1	0.254	5.7152	114.304	
2	0.231	5.1741	103.482	108.971
3	0.243	5.4564	109.128	

So, the solubility of Linagliptin in 50% ethanol is 108.971 mg/mL.

3.4 Solubility in 100% Acetonitrile

Different absorbance values of standard Linagliptin solutions of different concentrations using 100% acetonitrile as solvent system are given in the following table (Table 11).

Table 11: Absorbance of Different Concentrations of Standard Linagliptin in 100% Acetonitrile

Concentration (µg/mL)	**Absorbance**
1.25	0.055
2.5	0.101
5	0.213
10	0.448

Using these absorbance values, a standard curve of Linagliptin in 100% acetonitrile was drawn which is given in following figure (Figure 5).

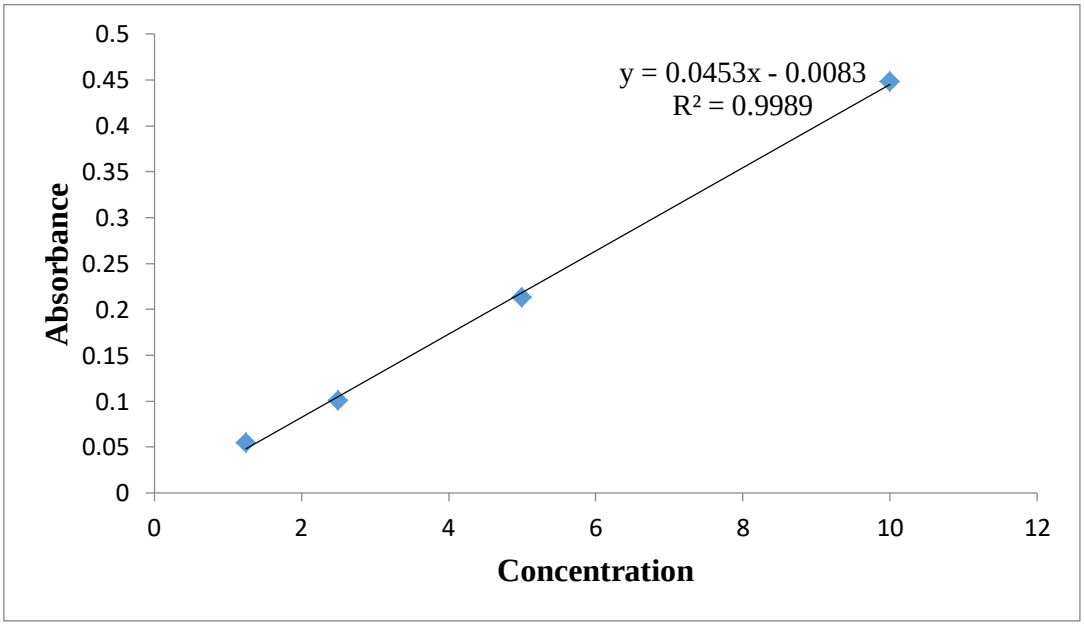


Figure 5: Standard Curve of Linagliptin in 100% Acetonitrile

Solubility of Linagliptin in 100% acetonitrile was calculated for three samples using the equation found from the standard curve and by back calculation method. The results are listed in the following table (Table 12).

Table 12: Solubility Calculation of Linagliptin in 100% Acetonitrile

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 100% acetonitrile (mg/mL)
1	0.125	2.9426	29.426	
2	0.131	3.0750	30.750	30.529
3	0.134	3.1412	31.412	

So, the solubility of Linagliptin in 100% acetonitrile is 30.529 mg/mL.

3.5 Solubility in 75% Acetonitrile

Absorbance values for four different concentrations of standard Linagliptin in 75% ethanol are given in the following table (Table 13).

Table 13: Absorbance of Different Concentrations of Standard Linagliptin in 75% Acetonitrile

Concentration (µg/mL)	**Absorbance**
1.25	0.080
2.5	0.153
5	0.226
10	0.452

Using these absorbance values, a standard curve of Linagliptin in 75% acetonitrile was drawn which is given in the following figure (Figure 6) including the value of regression coefficient and the equation derived from the curve.

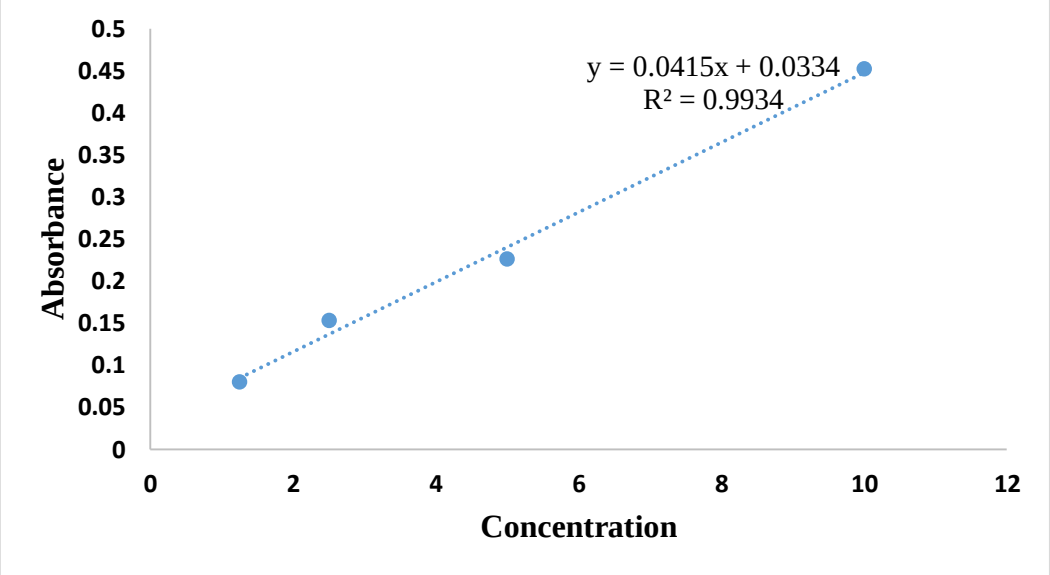


Figure 6: Standard Curve of Linagliptin in 75% Acetonitrile

Solubility of Linagliptin in 75% acetonitrile was calculated for three samples using the equation and back calculation method. The results are listed in the following table (Table 14).

Table 14: Solubility Calculation of Linagliptin in 75% Acetonitrile

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 75% acetonitrile (mg/mL)
1	0.251	5.2433	209.734	
2	0.223	4.5686	182.744	195.275
3	0.234	4.8337	193.348	

So, the solubility of Linagliptin in 75% acetonitrile is 195.275 mg/mL.

3.6 Solubility in 50% Acetonitrile

Absorbance values for four different concentrations of standard Linagliptin in 50% acetonitrile are given in the following table (Table 15).

Table 15: Absorbance of Different Concentrations of Standard Linagliptin in 50% Acetonitrile

Concentration (µg/mL)	**Absorbance**
1.25	0.054
2.5	0.101
5	0.201
10	0.392

Using these absorbance values, a standard curve of Linagliptin in 50% acetonitrile was drawn which is given in the following figure (Figure 7) including the value of regression coefficient and the equation derived from the curve.

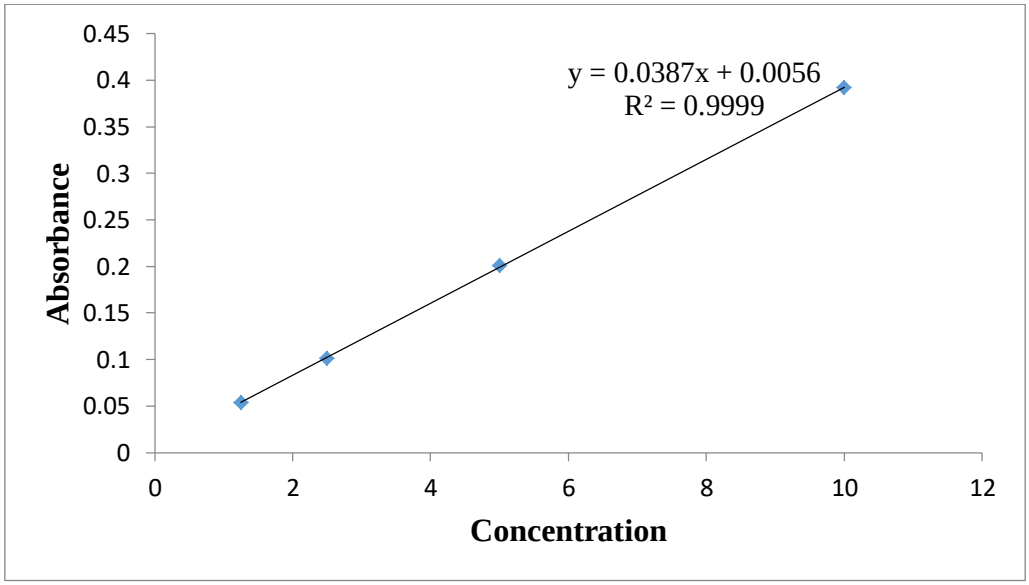


Figure 7: Standard Curve of Linagliptin in 50% Acetonitrile

Solubility of Linagliptin in 50% acetonitrile was calculated for 3 samples using the equation found from the standard curve. the results are given in the following table (Table 16).

Table 16: Solubility Calculation of Linagliptin in 50% Acetonitrile

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 50% acetonitrile (mg/mL)
1	0.081	1.9483	389.66	
2	0.086	2.0775	415.50	403.44
3	0.0.84	2.0258	405.16	

So, the solubility of Linagliptin in 50% acetonitrile is 403.44 mg/mL.

3.7 Solubility in 100% Methanol

Absorbance values for four different concentrations of standard Linagliptin in 100% methanol are given in the following table (Table 17).

Table 17: Absorbance of Different Concentrations of Standard Linagliptin in 100% Methanol

Concentration(µg/mL)	**Absorbance**
1.25	0.066
2.5	0.132
5	0.261
10	0.464

Using these absorbance values, a standard curve of Linagliptin in 100% methanol was drawn which is given in the following figure (Figure 8).

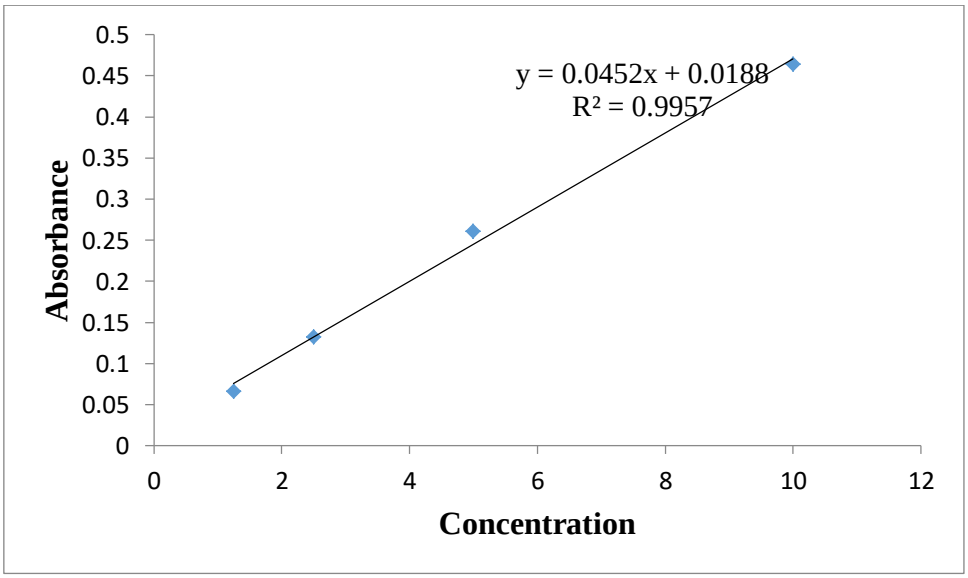


Figure 8: Standard Curve of Linagliptin in 100% Methanol

Solubility of Linagliptin in 100% methanol was calculated for three samples using the equation that was found from the standard curve and back calculation method. The results are given in following table (Table 18).

Table 18: Solubility Calculation of Linagliptin in 100% Methanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 100% methanol (mg/mL)
1	0.134	2.5486	101.944	
2	0.129	2.4380	97.522	101.945
3	0.139	2.6592	106.371	

So, the solubility of Linagliptin in 100% methanol is 101.945 mg/mL.

3.8 Solubility in 75% Methanol

Absorbance values for four different concentrations of standard Linagliptin in 75% methanol are given in the following table (Table 19).

Table 19: Absorbance of Different Concentrations of Standard Linagliptin in 75% Methanol

Concentration (µg/mL)	**Absorbance**
1.25	0.061
2.5	0.120
5	0.231
10	0.443

Using these absorbance values, a standard curve of Linagliptin in 75% methanol was drawn which is given in the following figure (Figure 9) including the value of regression coefficient and the equation derived from the curve.

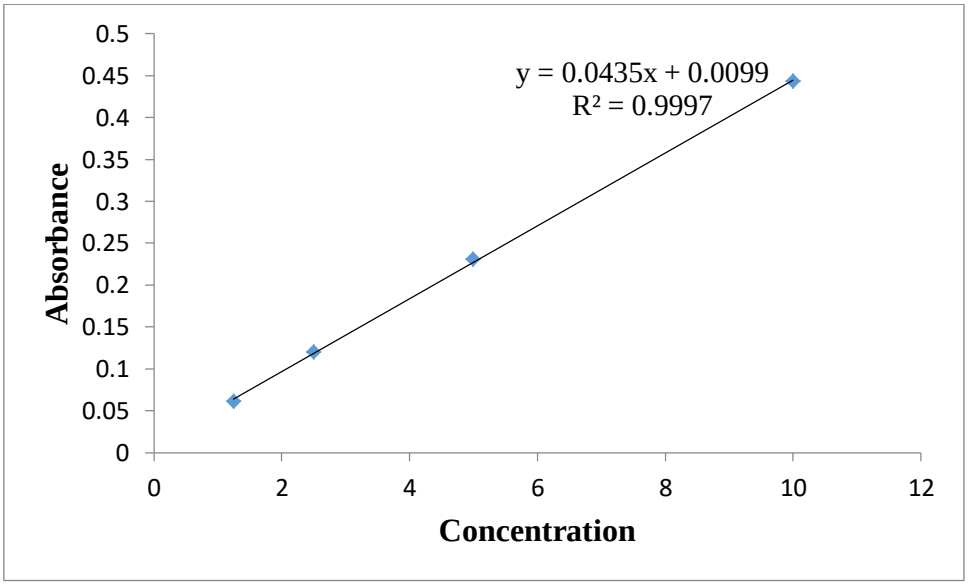


Figure 9: Standard Curve of Linagliptin in 75% Methanol

Solubility of Linagliptin in 75% methanol was calculated for three samples using the equation and back calculation method. The results are given in the following table (Table 20).

Table 20: Solubility Calculation of Linagliptin in 75% Methanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 75% methanol (mg/mL)
1	0.313	6.9678	278.712	
2	0.297	6.60	264	271.969
3	0.307	6.8298	273.195	

So, the solubility of Linagliptin in 75% methanol is 271.969 mg/mL.

3.9 Solubility in 50% Methanol

Absorbance values for four different concentrations of standard Linagliptin in 50% methanol are given in the following table (Table 21).

Table 21: Absorbance of Different Concentrations of Standard Linagliptin in 50% Methanol

Concentration (µg/mL)	**Absorbance**
1.25	0.065
2.5	0.116
5	0.222
10	0.435

Using these absorbance values, a standard curve of Linagliptin in 50% methanol was drawn which is given in the following figure (Figure 10).

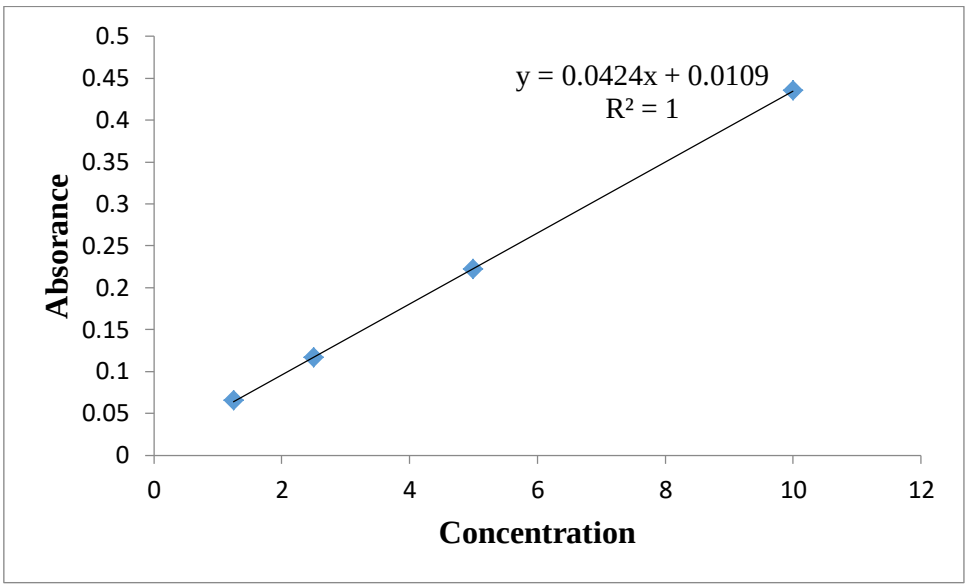


Figure 10: Standard Curve of Linagliptin in 50% Methanol

Solubility of Linagliptin in 50% methanol was calculated for three samples using the equation found from the standard curve and back calculation method. The results are given in the following table (Table 22).

Table 22: Solubility Calculation of Linagliptin in 50% Methanol

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in 50% methanol (mg/mL)
1	0.337	7.6910	307.640	
2	0.379	8.6816	347.264	325.879
3	0.353	8.0683	322.735	

So, the solubility of Linagliptin in 50% methanol is 325.879 mg/mL.

3.10 Solubility in Buffer (pH:7.4)

Absorbance values for four different concentrations of standard Linagliptin in buffer are given in the following table (Table 23).

Table 23: Absorbance of Different Concentrations of Standard Linagliptin in Buffer

Concentration (µg/mL)	**Absorbance**
1.25	0.059
2.5	0.110
5	0.219
10	0.424

Using these absorbance values, a standard curve of Linagliptin in buffer was drawn which is given in in the following figure (Figure 11) including the value of regression coefficient and the equation derived from the curve.

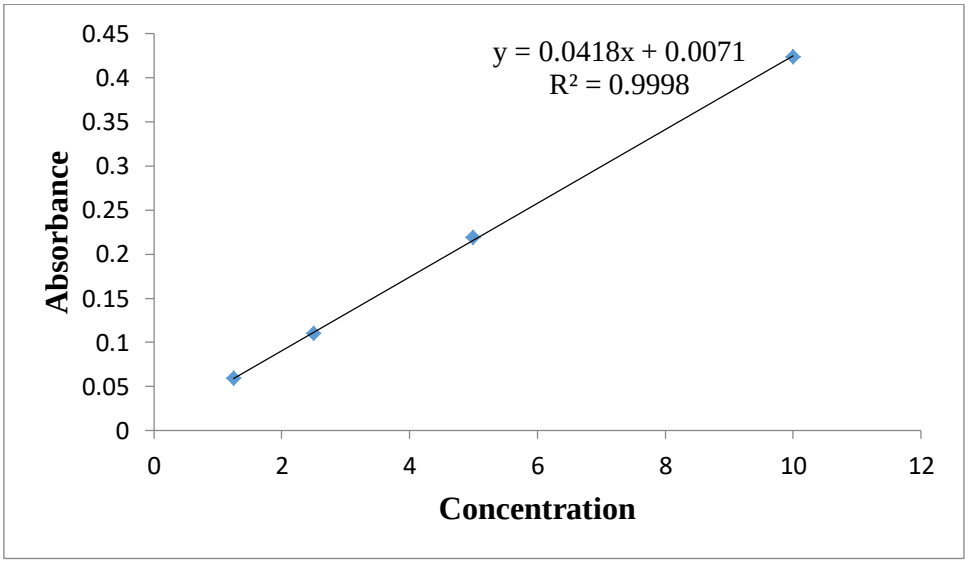


Figure 11: Standard Curve of Linagliptin in Buffer

Solubility of Linagliptin in buffer was calculated for three samples using the equation e and back calculation method. The results are given in the following table (Table 24).

Table 24: Solubility Calculation of Linagliptin in Buffer

Sample	Absorbance that falls in range	Concentration using the equation in standard curve (µg/mL)	Concentration of the saturated solution (solubility) (mg/mL)	Average solubility in buffer (mg/mL)
1	0.092	2.0311	10.155	
2	0.087	1.9114	9.557	9.7963
3	0.088	1.9354	9.677	

So, the solubility of Linagliptin in buffer (pH 7.4) is 9.7963 mg/mL.

Chapter 4

Discussion

4.1 Solubility Classification in Different Solvent Systems

Solubility values of Lingliptin in 100% ethanol, 75% ethanol, 50% ethanol, 100% acetonitrile, 75% acetonitrile, 50% acetonitrile, 100% methanol, 75% methanol, 50% methanol and buffer (pH 7.4) were 28.848 mg/mL, 67.963 mg/mL, 108.971 mg/mL, 30.529 mg/mL, 195.275 mg/mL, 403.440 mg/mL, 101.945 mg/mL, 271.969 mg/mL, 325.879 mg/mL and 9.7963 mg/ml, respectively. Solubility of Linagliptin in these ten solvent systems was classified into different classes based on USP and BP solubility criteria that were mentioned in Table 2 (see 1.5). Solubility values of the drug in different solvent systems are mentioned in the following table (Table 25) with the solubility classification. In this solubility classification, volume (in mL) of solvent required for 1 gm of Linagliptin to be dissolved was considered.

Table 25: Solubility Classifications of Linagliptin in Different Solvent Systems

Solvent name	**Solubility (mg/mL)**	**Solubility classification**
100% ethanol	28.848	Sparingly soluble
100% acetonitrile	30.529	Sparingly soluble
100% methanol	101.945	Freely soluble
75% ethanol	67.963	Soluble
75% acetonitrile	195.275	Freely soluble
75% methanol	271.969	Freely soluble
50% ethanol	108.971	Freely soluble
50% acetonitrile	403.440	Freely soluble

50% methanol	325.879	Freely soluble
Buffer	9.7963	Slightly soluble

From the table above, it can be said that the drug is sparingly soluble in two solvent systems, freely soluble in six solvent systems, soluble in one solvent system and slightly soluble in one solvent system. The solubility values of Linagliptin in methanol and ethanol that have been determined are higher than the solubility values that are available in literature because we have determined the equilibrium solubility of the drug. Normally, solubility of Lingliptin in methanol is 60 mg/mL and in ethanol, it is 10 mg/mL.

From the solubility results, it is found that solubility of the drug increases as the percentage of water used with ethanol, acetonitrile and methanol was increased. This might be due to the co-solvent effect of these chemicals when used with water.

The solubility values that were determined suggest that ethanol and acetonitrile in different combination with water can be used as the solvent system for the transdermal formulation of Linagliptin using microneedles. Methanol is usually not used in transdermal formulation and Linagliptin is poorly soluble in buffer. This is why; these two solvent systems will not be used as solvents for transdermal formulation.

4.2 Choice of Solvents in the Current Study

The solvent systems were mainly different combinations of ethanol, methanol and acetonitrile with water. This is because ethanol and acetonitrile have been used in transdermal formulation before (Uddin et al., 2015) (Gill & Prausnitz, 2007) and methanol and buffer have been solvents of choice in different solubility profile determination studies (Hofs et al., 2018) (Teychene, Autret, & Biscans, 2006). pH of the buffer was kept 7.4 because our blood has the pH of 7.4 (Aoi & Marunaka, 2014).

4.3 Choice of Experimental Conditions and Challenges

For the solubility studies using shake-flask or miniature shake-flask method, Whatman^{TM} Uniprep^{TM} vials are usually used (Hofs et al., 2018), but in the current study, test tubes with caps were used which were available in the research lab. As the equilibrium solubility of the drug was determined in aforementioned solvents, the drug-solvent mixtures were kept in the shaker-incubator for 24 hours because most drug achieve their equilibrium solubility within this time period (Hofs et al., 2018). Drug-solvent mixtures were kept in shaker for 24 hours also because formulation for transdermal delivery is kept for 24 hours for complete dissolution of drug and other excipients in the solvent that is used (Uddin et al., 2015).

The reference research papers that were followed for the determination of solubility of the drug in several solvents have used High Performance Liquid Chromatography (HPLC) for the quantification of the amount of drug that was dissolved (Hofs et al., 2018) (Hofs et al., 2018), whereas UV-visible spectrophotometer was used in the current study as the HPLC machine in the research lab was not working properly.

Chapter 5

Conclusion

Linagliptin is a widely used DPP-4 inhibitor indicated in type 2 diabetes mellitus. Oral bioavailability of this drug is 30%, which suggests the introduction of a new drug delivery system. In the current study, transdermal route of drug delivery has been proposed. However, before designing the transdermal formulation, solubility profile of this drug will need to be determined. Ten different solvent systems were taken for the determination of solubility profile of Linagliptin using shake-flask method. Among the different solvent systems that were used, Linagliptin was found to be sparingly soluble in two solvent systems, freely soluble in six solvent systems, soluble in one solvent system and slightly soluble in one solvent system. These solubility classifications were made based on the solubility criteria of BP and USP. From the solubility profile study, it can be concluded that the solvent systems of ethanol and acetonitrile can be used in the development of the transdermal formulation of Linagliptin, which will be an alternative drug delivery system to the existing oral system.

Chapter 6

Future Work

To design transdermal formulation of Linagliptin using microneedles which will possibly increase the bioavailability of the drug.

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