Wet granulated liquisolid drug delivery systems with hydrophobic and hydrophilic drugs

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Abstract of Research

The formulation of hydrophobic drugs into appropriate dosage forms is challenging due to the problems associated with those drugs such as low solubility and poor dissolution. Using a liquisolid system is a promising method to improve the dissolution of hydrophobic drugs and in sustaining the release of hydrophilic drugs, in which solid drugs are dispersed in non-volatile liquid vehicles. The aim of this research was to use the liquisolid technique to enhance the dissolution rate of glibenclamide, a model hydrophobic drug, and to sustain the release of metformin-HCl, as a model hydrophilic drug. The wet granulation process was applied to liquisolid powders with the aim of overcoming issues of poor powder flowability and compressibility, especially using high viscosity liquid vehicles. This process was performed with liquisolid powders prior to compaction into tablets. Different liquisolid formulations were prepared using three liquid vehicles (polyethylene glycol400 (PEG® 400), Synperonic® PE/L44 and Cremophor® ELP), at 10 and 30 % w/w drug concentrations for glibenclamide; and 30% and 60% w/w drug concentrations for metformin-HCl. Avicel®PH102 was used as a carrier, whilst colloidal silicon dioxide was employed as a coating material to convert the wet mixtures into dry powders. Potato starch, 5% w/w, as a disintegrant was blended with the mixtures manually for 10 minutes and then 0.75% of magnesium stearate as a lubricant was added and mixed for 5 minutes. The final powder (depending on its flowability and compactability) was then compacted automatically using a single-punch tableting machine to give tablets with 4 mg for glibenclamide and 40 mg for metformin-HCl. Prepared liquisolid compacts were characterized by using British Pharmacopeia quality control tests: uniformity of weight, friability, disintegration, hardness and drug dissolution.

It was found, for both drugs, that by application of wet granulation to liquisolid powder admixtures, the large-scale production of liquisolid compacts is feasible, which can be easily adapted to the pharmaceutical industry. In addition to enhancing the flowability and compressibility of the powders, the glibenclamide dissolution was also improved due to the enhanced binding of particles and because of the wetting effect of liquid vehicles on the hydrophobic drug, which make the drug more available for dissolution.

For the sustained release preparations of liquisolid metformin-HCl, hydroxyl propyl cellulose (HPC) was used as a novel carrier in liquisolid compacts. The results showed 92% drug release after 12 hours using Cremophor®ELP (with 30% w/w drug concentration) which was the best sustained drug release formulation. Additionally, Eudragit® RL30D and Eudragit® RLPO have been used to study their effects on drug release from liquisolid formulations, examining if they can sustain or give more rapid drug release. Both types of Eudragit revealed immediate release with metformin-HCl rather than sustained drug release, with the tablets disintegrating within seconds. This suggests formulating orodispersible metformin-HCl tablets using Eudragit® RL30D as a liquid vehicle.

In summary, liquisolid technology has led to promising results, not only in enhancing the drug dissolution of hydrophobic drugs, but also in sustaining and promoting the release of hydrophilic drugs.

Research Activities

Research activities from the PhD research:

- Javaheri, H., Carter, Paul and Elkordy, Amal (2014) *Wet granulation to overcome liquisolid technique issues of poor flowability and compactibility: A study to enhance Glibenclamide dissolution.* Journal of Pharmaceutics and Drug Development, 1 (5). pp. 501-512. ISSN 2348-9782

- Javaheri, H., Elkordy, Amal and Carter, Paul (2015) *Dissolution study of liquisolid compacts containing metformin: Overcoming the issue of poor powder flowability and compactibility using wet granulation technique.* In: 6th APS International PharmSci 2015, Innovation in Pharmaceutical Sciences, 7 - 9 Sept 2015, East Midlands Conference Centre, Nottingham, UK. (Unpublished)

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- Javaheri, H, Elkordy, Amal and Carter, Paul (2013) *Preparation and Evaluation of Liquisolid Compacts Containing Salbutamol Base with PEG 400 and Synperonic® PE/L44.* In: UK-PharmSci 2013 conference, 2 - 4 Sep 2013, Edinburgh, UK.

- Javaheri, H, Elkordy, Amal and Carter, Paul (2013) *Preparation and Evaluation of Liquisolid Compacts Containing Griseofulvin with Synperonic® PE/L44 and Cremophor® ELP.* In: 2013 UKICRS Symposium, 16 Apr 2013., University of Reading, Reading.

- Elkordy, Amal, Javaheri, H., Hussain, I. and Essa, E.A. (2011) *Characterisation of liquisolid tablets containing griseofulvin.* In: The Science of Medicines: UK PharmSci 2011, 31 Aug-02 Sept 2011, East Midlands Conference Centre, Nottingham University, Nottingham. (Unpublished)

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Chapter 1 - Introduction

1.1. General introduction

There are several types of routes of drug administration available (Table 1.1) such as: oral, rectal, parenteral, nasal, topical, eye and ear routes of drug delivery. However, before choosing the right dosage form, special attention must be made to relate the drug substance to the clinical complication and factors governing the correct choice of drug administration such as bioavailability and solubility which must be taken into consideration. Amongst the above-mentioned route of administration, oral drug delivery remains the main and most appropriate and commonly employed route of administration due to its safety with lowest microbial related restrictions during the manufacturing process and high patient compliance due to ease of administration. It is mostly intended for systemic effect resulting in drug absorption in the gastrointestinal tract (GIT). The drug gets dissolved in the fluid of the mouth or stomach and then gets absorbed into the systemic circulation (Alderborn, 2013). However, drawbacks are correlated with the high desired route of administration and that is due to the slow onset of action and gastrointestinal enzyme secretion which can affect the absorption of the drug into the blood stream.

In the field of pharmacy or pharmaceutics, a route of drug administration is the path by which a drug is taken into the human body. Additionally, a pure chemical substance must be formulated into an appropriate dosage form to be taken by the patient in the chosen route of administration. Active ingredients are not the only substances that play a role in the manufacturing of medicines, simple solutions to complex drug delivery systems use appropriate additives and excipients in the formulation where they provide specialised

functions. Nevertheless, the main purpose of dosage form design is to reach and achieve a desired therapeutic outcome while maintaining the reproducibility and high quality control in the large batch production for the pharmaceutical industry (York, 2013).

Table 1-1: Routes of administration with different dosage forms (York, 2013)

Route of administration	Dosage forms
Oral	Solutions, tablets, capsules and powders
Topical	Ointments, creams and transdermal patches
Parenteral	Injections
Rectal	Suppositories
Nasal	Solutions and inhalations
Respiratory	Aerosols and inhalations
Eye and Ear	Solutions, suspensions and creams

Tablets are known to be the most popular form; tablets can be manufactured by several stages where the final stage is the compression of the powder held within a confined space. In addition, the use of tablets as dosage forms became of interest to the pharmaceutical industry, thus the formulation of tablets with variety of forms such as modified release, orodispersible, immediate release, chewable and effervescent were formulated. (European Pharmacopoeia , 2017) defines tablets as “solid preparations each containing a single dose of one or more active ingredients and obtained by compressing uniform volumes of particles”. The secret behind the difference between tablets is in the type of excipients used. Pharmaceutical industry uses different excipients, diluents, disintegrants and lubricants to ensure that tablets of specified quality are prepared. However, one excipient can affect the properties of the tablet. Thus, it can be said that

each excipient added has a specific role in the formulation of the oral tablets.

1.2. Common challenges in the manufacturing of oral dosage forms

The pharmaceutical industry has been facing challenges in the manufacturing of oral drug delivery systems. The major obstacle that concerns the industry can vary from dissolution rate to poor aqueous solubility and bioavailability of the active ingredient(s) (Dressman, 2007)This requires special attention for the pharmaceutical scientists because active ingredients in tablets must undergo dissolution before they are available for absorption from the GI tract (Javadzadeh, et al., 2007)Therefore, drugs with poor water solubility will face problems in the dissolution and absorption into the blood stream. About 40% of the newly developed drugs are known to be hydrophobic and thus do not dissolve in water and have poor dissolution profiles (Lipinski, 2002). Moreover, the first site of drug absorption is the stomach where an oral administration can release its active ingredient (Dressman, 2007). Therefore, the dissolution is an essential step before absorption can take place from the GI tract to the systemic circulation. According to the Food and Drug Administration agency, drugs that are hydrophobic and have poor dissolution profiles can cause therapeutic in-equivalence due to low and high variable bioavailability which can be vital for patient's health.

Dissolution rate in the fluid of the absorption site controls the absorption rate of a poorly water-soluble drug. In other words, the rate of absorption is controlled by how fast the drug dissolves in the fluid at the site of absorption. Therefore, it can be documented that the dissolution rate is the rate limiting step in drug absorption (Javadzadeh, et al., 2007). Dissolution rate can be calculated using the Noyes-Whitney equation (Equation 1.1):

$$\frac{dc}{dt} = \frac{D}{h} \cdot A(C_s - C_b) \qquad \textbf{Equation 1.1}$$

where "D" is the diffusion coefficient, "A" the surface area, "C_s" the solubility of the drug, "C_b" the concentration of drug in the bulk solution and "h" is the thickness of the diffusion layer around each drug particle (khorshed and Maghreby 2016). Each term has an influence on drug dissolution and thus absorption. For example, if the surface area increases, the dissolution rate will also increase which then improves drug bioavailability, see Figure 1.1 below:

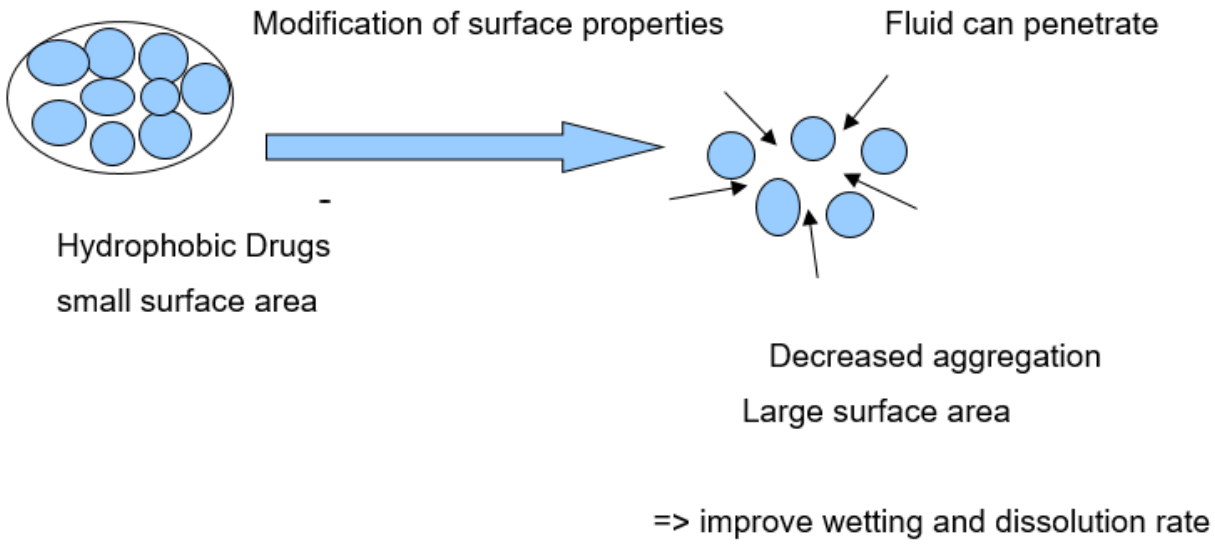


Figure 1.1: Representation of effect of modification of drug surface on particle properties.

(Mosharraf & Nystrom, 1995) have reported that "poorly water-soluble drugs can be identified as small as 100µg/L solubility". In addition to that, the biopharmaceutical classification system has provided a mechanistic framework to help understand the concept of drug absorption by means of solubility across the gastrointestinal (GI) pH

range 1-7.5 at 37°C and permeability across the GI mucosa. The drugs can be classified into four categories or classes (Figure 1.2) in which the drug's dose, permeability and solubility must be known (Helga, 2002)

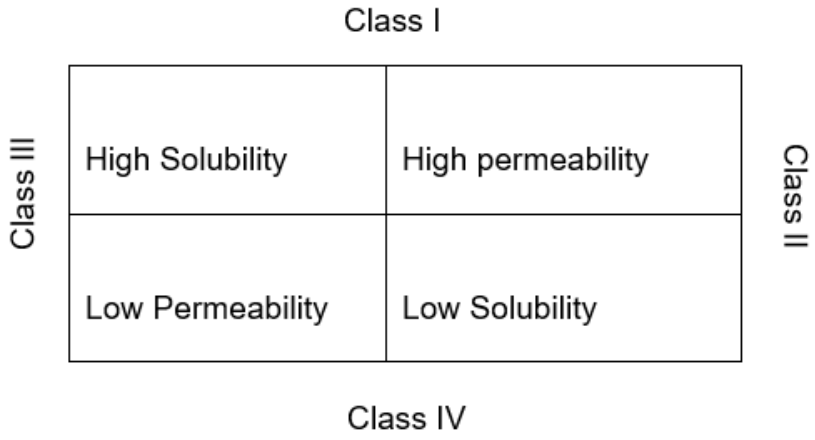


Figure 1.2: Four classes of Biopharmaceutics Classification Systems (BCS)

1.3. Strategies in overcoming poor solubility and dissolution

One of the major roles of pharmaceutical industries is to enhance oral bioavailability by improving drug solubility and dissolution. High doses of hydrophobic drugs being involved to increase plasma concentration to a therapeutic level will result in poor absorption into the blood stream, which can lead to accumulation of the drug in the GI tract and as a result can lead to toxicity (Savjani, et al., 2012). Consequently, these drawbacks have delayed several potential drugs in pre-clinical development. Since hydrophobic drugs are difficult to formulate using a conventional tableting method, different techniques have been reported in the literature to achieve enhanced drug dissolution rates. Some of these methods are: a) Solvent evaporation method (its limitations include: difficulty in completely removing the liquid solvent and higher cost of preparation) (Serajuddin, 1999),

b) Reducing particle size to increase surface area (particle size reduction using jet mill, rotor stator colloidal mill and ball mill. Limitations include: degradation due to thermal and physical stress, Takano et al 2004), c) Self-emulsifying drug delivery systems, SEDDS, (limitations include: administration in lipid-filled gelatin capsules due to the liquid nature of the system in which interaction between the drug and the capsule shell should be taken into consideration). Additionally, SEDDS' administration is not intended for long-term use due to possibility of diarrhea effect (Shah, et al., 1994). Also, other attempts have been made such as: d) Solid dispersion (Scale up problems, decomposition of the solvent during process and dissolution reduction with ageing, Gowardhane et al.2014) e) Nanosuspension (limitation includes: the drug needs to soluble in at least one solvent in which this solvent must be miscible with the other non-solvents used (Muller, et al., 2000)). Among the mentioned techniques to overcome poor dissolution, scientists have reported in several articles a new and promising method to enhance aqueous solubility of hydrophobic drugs known as "liquisolid technology" (Javazadeh , et al., 2005). Therefore, the aim of this current work is to introduce and to apply the concept of the liquisolid technique.

1.4. Liquisolid compact technology and its mathematical principles

The information on liquisolid technique was first introduced by (Spireas & Bolton, 1999), several research papers have shown successful results in the use of liquisolid compacts, yet, there are currently no liquisolid dosages available in the market. The concept of "liquisolid tablets" was evolved from the powdered solution technology where liquid medication can be used (Tiong & Elkordy, 2009), (Spireas & Sadu, 1998). The term

“liquisolid medication” implies to solid drugs dispersed in non-volatile solvent systems with the addition of appropriate powder excipients such as carriers, coatings and disintegrants. The incorporation of liquid surfactants into the oral hydrophobic drug allows the formation of drug-liquid interaction that helps in the solubility of the nonpolar drug molecules. Liquisolid compacts possess acceptable flowability and compactibility properties depending on the non-volatile liquid vehicle used. Flowchart, Figure 1.3 shows the summary for liquisolid compacts.

Table 1-2: Liquisolid mathematical expression and its definition

Symbol	Definitions
W_{liquid}	Weight of liquid medication
W_{solid}	Weight of carrier and coating material
Q	Weight of carrier powder
R	Ration between carrier and coating
Q	Weight of coating powder
Lf	Liquid load factor
Φ_{Ca}	Flowable liquid retention potential of the carrier powder
Φ_{Co}	Flowable liquid retention potential of the coating powder

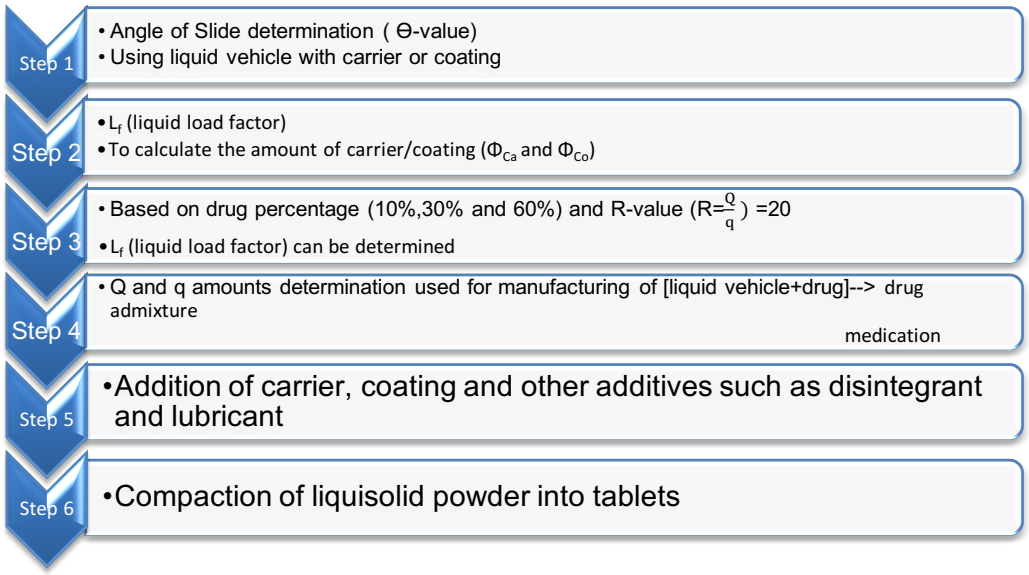


Figure 1.3: Flowchart summary of liquisolid powder preparation.

Before using equations (1.3-1.5) and formulating the liquisolid compacts, determination of the optimal flowable liquid-retention potential for the carrier and coating used is essential, which is the Φ value required for Equation (1.3). This is usually the first step in the formulation. This measurement is termed as “angle of slide”, where it evaluates the flowability of carrier and coating materials. Then, a graph of angle of slide (Θ) versus the

measured Φ value is plotted and hence the load factor is then calculated from the results obtained (Figure 1.3; Table 1.2). The Φ value is defined as “the maximum weight of liquid (W_{liquid}) that can be retained per unit weight of the sorbent (W_{solid}) yielding a mixture with acceptable flowability” (Spireas , et al., 1992)

$$\Phi \text{ value} \quad \frac{W_{liquid}}{W_{solid}} \qquad \textbf{Equation 1.2}$$

Therefore, several equations are used to calculate the right amount of carrier, coating and liquid medication needed for the formulation of the liquisolid compact:

$$L_f = \Phi_{CA} + \Phi_{CO}\left(\frac{1}{R}\right) \quad \textbf{Equation 1.3}$$

Where Φ_{CA} is the flowable liquid-retention potential of the carrier and Φ_{CO} is the flowable liquid-retention potential of the coating material.

$$L_f = \frac{W}{Q} \qquad \textbf{Equation 1.4}$$

Where L_f is the “loading factor” where the maximum amount of drug liquid is loaded onto the carrier material. Q is the amount of carrier (such as microcrystalline cellulose, Avicel® PH102) and W is the liquid medication (such as: drug in PEG® 400 or Synperonic®PE/L44 or Cremophor® ELP or Eudragit® RL30D)

$$R = \frac{Q}{q} \qquad (R=20) \qquad \textbf{Equation 1.5}$$

The amount of Q can be calculated (Equation 1.4) and applied to equation 1.5 to calculate the amount of coating (for example: Silica, Cab-o-Sil® M-5 (q)) required. Hence, after the

calculation of Q and q, the formulation of the liquisolid dosage can be initiated. In addition, the R value is the ratio between the carrier and the coating and it has been suggested that a ratio of 20 will produce a flowable and compactible admixture (Tiong & Elkordy, 2009).

The liquisolid tablets that contain the hydrophobic drug are expected to show significant increase in the wetting properties and surface area of the drug availability and thus, enhance drug dissolution. Also, it is expected to show enhanced drug released characterisation profiles. Nevertheless, this new dosage form will benefit the pharmaceutical industry in offering the most efficient drugs that can then be released to the market to improve patient compliance. With the new dosage form, the patient would need a decreased dose that is administered less frequently due to the ability of the hydrophobic liquisolid drug to release its contents faster allowing for maximum bioavailability.

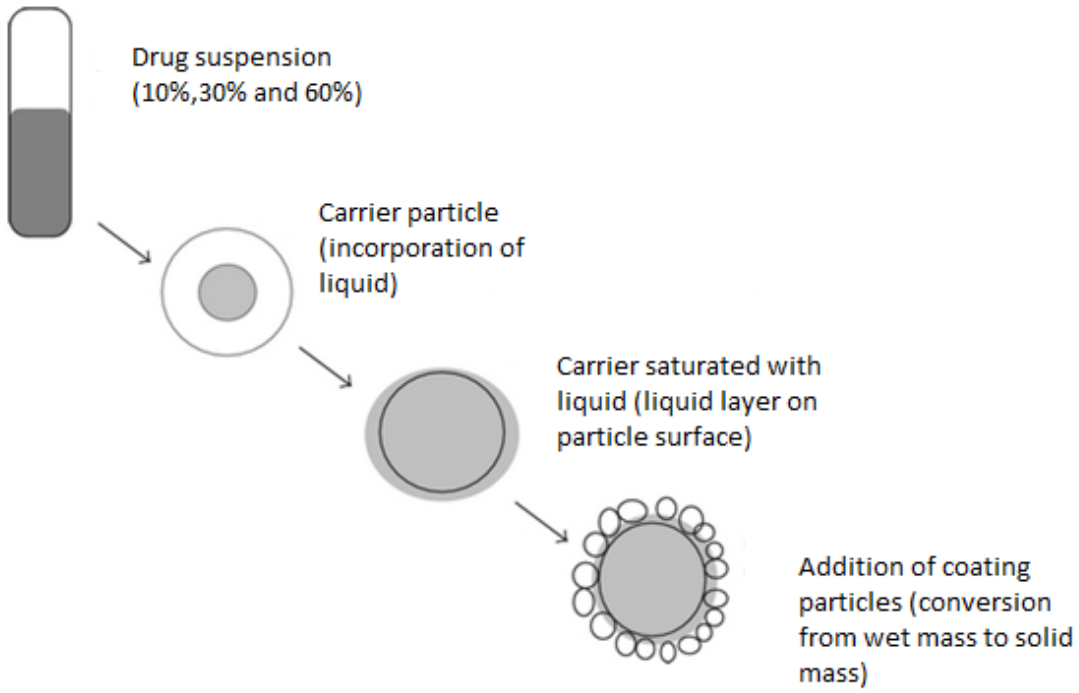


Figure 1.4: Theoretical model of liquisolid system adapted from (Spireas , et al., 1992)

Figure 1.4 summarises liquisolid tablet formulation. The lipophilic drug gets dissolved in a selected non-volatile liquid vehicle. Selected suitable fillers such as microcrystalline cellulose act as carrier which carries the drug- liquid admixture. The liquid will then be absorbed in the interior of the particles. The addition of coating material such as silica powder converts the wet surface to a dry free flowing powder with acceptable compactibility. Depending on the amount of coating added, either mono or multilayer can be formed (Spireas , et al., 1992).

Based on literature, liquisolid technology has been widely used to improve the dissolution rate of hydrophobic drugs. In liquisolid systems, the wetting properties and surface of the

drug available for dissolution is significantly increased. This occurs when the drug is in a solubilized, almost molecularly dispersed, state or when held within the powder substrate in a solution form. Thus, liquisolid compacts of water- insoluble drug is expected to display enhanced drug release properties and instantaneously improves bioavailability (Fahmy & Kassem, 2008). Initially the liquisolid technique was implemented in 1998 by Spireas and Sadu, whose first formulated liquisolid drug was prednisolone, which was then compared to directly compressed tablets (Spireas & Sadu, 1998)As a result, prednisolone liquisolid compacts demonstrated significantly higher drug release rates.

(Javazadeh , et al., 2005) improved the dissolution rate of piroxicam by using liquisolid technology. In this study, the development of several liquisolid tablet formulations of piroxicam and tween 80 were prepared using different concentrations of tween 80. As a result, liquisolid compacts showed significantly higher drug release rates than conventional formulations.

Furthermore, in 2007, Javadzadeh *et al.* further studied the effects of liquisolid technology on carbamazepine. According to the BCS, Carbamazepine belongs to class II category, where its bioavailability is limited by its poor dissolution rate in the gastrointestinal tract. Different liquisolid formulations of carbamazepine were prepared. In this study, the loading factor was increased by the addition of some namely additives such as: polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC) and polyethylene glycol (PEG 35000) to the liquid vehicle. As a result, compared to directly compressed tablets, liquisolid formulations containing PVP, exhibited higher dissolution rates. Additionally, increased PVP concentration in liquid medication showed significant

increase in the drug dissolution rate in the first 30 min of dissolution. Therefore, these additives were realized to have the capability to increase the liquid absorption capacity of carrier and coating materials.

In 2008, Javadzadeh *et al.* utilized the liquisolid technique as a new approach to sustain the release of propranolol hydrochloride from tablet matrices. In this study, propranolol was dispersed in polysorbate 80, followed by the addition of a binary mixture of carrier–coating materials Eudragit RL or RS (carrier) and silica (coating material) to the liquid vehicle. In comparison with conventional matrix tablets, propranolol liquisolid tablets exhibited a greater retardation property. Furthermore, (Gubbi & Jarag, 2009), have reported the use of liquisolid technique to study the dissolution rate of bromhexine hydrochloride and compared it with conventional bromhexidine. Furthermore, using Avicel PH102 (carrier), Aerosil® 200 (coating material) and Explotab (disintegrant), different liquisolid formulations were developed. The drug release rates of liquisolid formulations were significantly ($p<0.05$) higher as compared to conventional tablets, proving the great effect of liquisolid technique on dissolution properties.

In 2009, Javadzadeh *et al.* examined the effect of several commercial grades of microcrystalline cellulose on flowability, compressibility, and dissolution rates of piroxicam liquisolid tablets. Thus, several formulations were prepared using different MCC grades (as carrier); propylene glycol as a non-volatile liquid, silica as a coating material, and sodium starch glycolate as a disintegrant. Formulations containing MCC PH 101 and PH102 resulted in better tablet properties, while enhanced flowability was observed with MCC PH 101 grade.

In 2009, liquisolid technology was also practiced by Tiong and Elkordy, to study the effects of liquisolid formulations on the dissolution rate of naproxen. The liquisolid tablets were formulated with three different liquid vehicles - PEG® 400, Synperonic® PE/L64 and Cremophor® EL, at 20%w/w and 40%w/w drug concentrations. Furthermore, Avicel® PH102 was used as a carrier, Cab-o-sil® M-5 (silica) as a coating material and maize starch was used as a disintegrant. The dissolution study was performed in simulated gastric fluid (SGF), pH 1.2, and simulated intestinal fluid (SIF), pH 7.2, and was further compared with conventional naproxen tablets. The results exhibited that naproxen liquisolid tablets formulated with Cremophor®EL at 20%w/w (drug concentration) produced the best dissolution profile with high release and improved tablet properties. Additionally, stability studies showed that formulation prepared with Cremophor® EL was negligibly affected by aging; while Differential Scanning Calorimetry revealed that drug particles in liquisolid formulations were completely in a solubilised state.

Furthermore, liquisolid tablets of glipizide was applied by (Mahajan, et al., 2011). Liquisolid tablets of glipizide were prepared by using Avicel® PH102 as a carrier, Aerosil® 200, as a coating material and gellan gum as a disintegrant to increase dissolution rate of glipizide. Glipizide liquisolid tablets dissolution profiles were compared with the commercial counterparts which exhibited higher dissolution rates of glipizide liquisolid tablets in comparison to the marketed tablets. The study also showed that dissolution rates increased with an increase in the concentration of non-volatile liquid vehicles and the best drug release was achieved by formulations containing polyethylene glycol 400 (PEG® 400).

(Saeedi, et al., 2011) investigated a water insoluble drug, indomethacin, for enhancement of drug dissolution rates. They applied liquisolid technology and found that in comparison to conventional indomethacin, the liquisolid formulations exhibited significantly higher drug dissolution rates. Enhanced dissolution rates of liquisolid indomethacin tablets was due to an increase in the wetting properties and surface area of drug particles that are available for dissolution. This indicates that, the fraction of molecularly dispersed drug in the liquid medication of liquisolid systems is directly proportional to the indomethacin dissolution rate.

In 2016, (Pezzini, et al., 2016)performed a study on the practicality of liquisolid technology, not on compact form, but on pellets formation of a model drug, felodipine. The study was conducted to record the effects of Kollidon®CL-SF (as a coating material and as a disintegrating material) and the type of non-volatile solvents, PEG 400 or Cremophor®EL, on felodipine dissolution behaviors. As a result, a higher drug release was found in formulations using Cremophor®EL which were more effective in comparison to PEG® 400. This is due to the formation of softer and more porous structures. Remarkable positive effects were also seen in the amount of Kollidon®CL-SF used. The study consists of an innovation of the current liquisolid technology of the development of liquisolid pellets and the use of Kollidon®CL-SF as a coating material in liquisolid formulations. The promising results revealed that it is feasible to adopt liquisolid pellets as novel drug delivery systems to improve the dissolution rate of poorly water- soluble drugs.

1.5. Liquisolid preparation methodology

Liquisolid preparation is commenced by blending the calculated amount of hydrophobic drug with the non-volatile liquid vehicle. With the use of pestle and mortar, the drug is satisfactorily mixed with the liquid to allow the liquid to penetrate between the hydrophobic drug particles. The process takes around 5 to 10 min of continuous mixing. The admixture is then allowed to rest. This is vital to allow the non-volatile liquid particles to be fully absorbed into the interior of the powder particles. Gradually, and in small batches, the carrier (usually microcrystalline cellulose) gets added to the admixture. The carrier's role is to carry the drug along with the liquid vehicle. The coating (Silica Cab-o-Sil ® M-5) is then added to coat any excess liquid particles present in the admixture (Figure 1.3) that have not been absorbed into the internal structure of the carrier particles. At this stage, the liquisolid system is formed with suitable flowability and compressibility profile. As a final stage, 5% potato starch and 1% magnesium stearate (calculated according to the weight formation) are added as a disintegrant and lubricant respectively, where starch is required in the formulation to break up the tablets into primary powder particles in order to increase the surface area of the drug in the GI fluid, thus promoting the dissolution rate (Fahmy & Kassem, 2008). Magnesium stearate is added to reduce the friction between the punch of the tableting machine and the powder. Additionally, as seen in Figure 1-5, before the compaction stage, which is a novel step in this research, a wet granulation step was introduced to enhance flowability and compressibility of the liquisolid powder especially using highly viscous liquid vehicle such as Cremophor®.

Evidence suggests that liquisolid tablets using hydrophobic drugs have shown enhanced dissolution profile due to an increase in the surface area and wetting properties of the

water-insoluble drug particles (Spireas & Bolton, 1999); (Nokhodchi, et al., 2005). During the process of liquisolid preparation, heating or drying does not take place; therefore, the drug remains embraced by the non-volatile liquid vehicle. Hence, the chosen non-volatile solvent must have a high boiling point with inert properties and preferably less viscous to maintain even distribution of the drug inside the admixture. In addition, a highly viscous liquid vehicle can affect the flowability and compactibility of the final liquisolid powder. Therefore it is crucial to select a non-volatile liquid vehicle with good property characteristics. Several studies have been conducted using different liquid vehicles such as Polyethylene glycol, Synperonic®PE/L44, Cremophor®ELP and Tween®80. Each liquid vehicle possesses its own characteristics and behaviours when combined with a specific hydrophobic drug, therefore, there is no common single liquid vehicle suitable for use in liquisolid preparation for a wide range of non-polar drugs. Additionally, the carrier must have a porous nature to be able to absorb more liquid and the coating must have adsorptive properties to coat the admixture and form a thorough dry powder (Elkordy, et al., 2013).

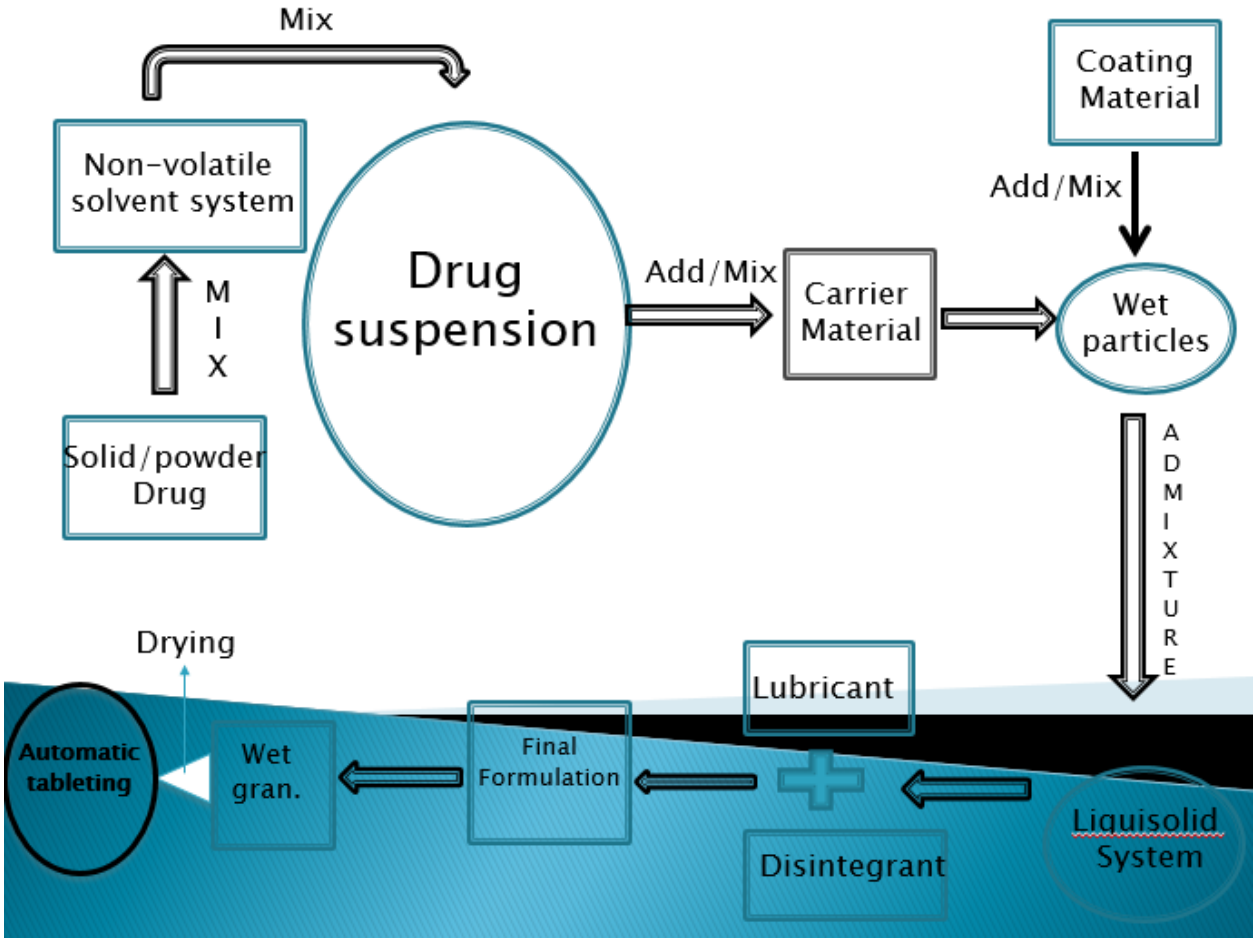


Figure 1.5: Schematic diagram of the preparation of liquisolid tablets.

1.6. Mechanism of enhanced drug release from the liquisolid system: the advantages and limitations of such system:

Numerous mechanisms of enhanced drug release have been developed for liquisolid technology to understand the dissolution theory of such a system. Improved wetting properties, enhanced aqueous solubility and increased surface area of the hydrophobic drug are the three principal mechanisms which have been suggested by several studies (or groups or researchers). (Nagabandi, et al., 2011) , (Spireas & Sadu, 1998), (Darwish & El-Kamel, 2001), (Akinlade, et al., 2010). Besides, the liquid vehicle acts as a surface

active and surface tension lowering agent. In return, the wettability of the liquisolid particles can be enhanced. Such a theory is demonstrated by the determination of lower contact angles and shorter water rising time resulting in an increase in aqueous solubility. In addition to the above-mentioned mechanism of drug release enhancement, it is expected that the solubility of the hydrophobic drug can be increased with liquisolid systems. The increased drug solubility phenomenon of liquisolid system suggests that a small amount of liquid vehicle in a conventional manner is not enough to solubilize the total amount of drug; however, if the liquid vehicle acts as a co-solvent, it is possible that this small amount of liquid vehicle diffusing from the total amount out of a single liquisolid particle together with the drug molecules is sufficient to increase the aqueous solubility of the drug at the solid liquid interface. Lastly, as the surface area of the drug increases, the dissolution rate of the drug with the liquid vehicle also increases (Balaji, et al., 2014).

1.6.1. Advantages of liquisolid technique include:

- Numerous hydrophobic solid drugs can be formulated into liquisolid systems.
- Bioavailability improvement can be achieved as compared to conventional tablets.
- Liquisolid preparation is a straightforward method and similar to that of conventional tablets preparation and industrial production is applicable.
- When compared to soft gel capsules, the production of liquisolid compacts are considerably economical and affordable.
- Liquisolid system is not limited to the dissolution enhancement of hydrophobic drugs, but also can be used to modify the release of highly water-soluble drugs with the addition of certain surfactants known as sustaining agents.

1.6.2. Limitations of liquisolid technique includes:

- Small amount of active ingredient used in the liquisolid compacts, because as the weight of the drug increases, this leads to an increase in the weight of the liquid vehicle, carrier, coating materials and will yield a large tablet which can be difficult to swallow and causes reduction in patient compliance. To solve this problem, several suggested theories have been made in literature by which to select a suitable liquid vehicle to which its loading factor would be high. Increase in loading factor means a decrease in tablet weight (Elkordy, et al., 2013); (Nagabandi, et al., 2011), (Spireas , et al., 1992) (karmarkar, et al., 2009).
- High specific surface area and adsorption properties are required to be shown in the used surfactants.
- Increasing the amount of silica Cab-o-Sil®M-5 (coating material) in the formulation can affect negatively on drug release, due to the hydrophobicity of silica Cab-o-Sil®M-5 (coating material), which can delay drug release if added in high quantities (Spireas & Bolton, 1999).
- Depending on the liquid vehicle used, the liquid being squeezed out of the tablet is possible during the compression process resulting in improper hardness and even loss of shape (Javaheri et al 2014)
- Due to the use of highly viscous liquid vehicles, desired tablets cannot be achieved into a suitable compression force, hardness and shape. However, wet granulation as used in this research can overcome some of this limitation.

1.7. Pharmaceutical wet granulation:

Most pharmaceutical industries recognize wet granulation as a widely-used method of tablet processing. It is no longer required to depend on the intrinsic properties of the drug and the excipients used because via wet granulation process it has become feasible to produce well-formed tablets. The granulation method is divided into two parts: dry granulation, in which no liquid is used, and wet granulation, which incorporates the use of liquid binder. The granulation mechanism can be defined as the process in which primary powder particles agglomerate. In other words, the particles in the powder form adhere in a way that they form larger multi-particle entities called granules. These pharmaceutical granules have different sizes that can range between 0.2mm to 0.4mm (Summers & Aulton, 2013). The reasons behind the use of granulation technique in pharmaceutical industry are as follows:

- Segregation prevention of the constituents of the powder mix. Pharmaceutical industries look for the ideal granule which contains all the constituents of the mix in a proportional manner.
- Improvement of powder flow properties. Poor flowability will result in wide weight variation in the final product due to the irregular shape and small size of the powder. Granulation process with aid of liquid binder adds weight to the particle and enhances the flowability.
- In addition to the above advantages, granulation is used to improve the compaction characteristics of the powder mix. This is due to the adhesive properties within the granules. Additionally, as the particle size gets evenly distributed using the

granulation process, this leads to increased density of the powder and enhancement of its compactibility and compressibility.

- Toxic materials can be turned into granules using non-friable and suitable mechanical strength granulation processes, reducing the hazard associated with handling of toxic powders.
- Wet granulation can also be used to modify and improve drug release.

In the wet granulation process, the liquid solution known as the “liquid binder” gets added to the dry primary powder to produce bonded particles via capillary forces that remain adherent and strong enough to lock particles together (Summers & Aulton, 2013). The liquid binder or the granulating fluid, must be non-toxic and volatile in nature in order to be evaporated or removed by drying. Examples of such fluids include ethanol, water and isopropanol. Water is widely used for its economic and ecological benefits. However, the disadvantage of using water as a granulating fluid is that it may affect drug stability and show adverse effects. Additionally, water requires longer drying time which can affect the stability of the drug due to the extended exposure to heat.

Granulation process is known to be a simple method depending on the characteristics of the powder and equipment used. Using traditional wet granulation method, the liquid added to the dry powder gets distributed evenly through the powder particles using mechanical agitation, which yields to particle adherence. Furthermore, wet granules are formed using pharmaceutical grade sieves with desired sizes, by wet powder mass being forced through which are then dried subsequently. A screening stage is then performed to break granules agglomerates. There are five primary particle bonding mechanisms in

granulation process which include the following: (Kienkens, et al., 2000)

- Adhesion and cohesion forces between individual particles of the powder. As the contact area between particles increases, this causes a decrease in their interparticulate distance strengthening and increasing the bond among those particles. This can happen only when sufficient liquid is added to the powder to form a thin and immobile layer.
- Solid bridges formation upon solvent evaporation. This can be achieved using three steps: crystallization of dissolved substances, partial melting and hardening binders.
- Interfacial forces in mobile liquid films within the granules. The added liquid during wet granulation gets distributed as films among and across particles of the powder mix to form mobile films.
- Mechanical interlocking adhesion can occur when an adhesive enters into the pores of the adhered surface of a substrate, and locks to the surface mechanically.
- Attractive forces between solid particles. Solid bridges get formed using binding agents in the absence of liquid. Electrostatic forces and Van der Waals forces are the two main kinds of forces that run between particles.

1.7.1. Mechanism of granule formation:

The suggested granulation mechanism can be recognized in the following three stages: (Shanmugam, 2015)

- Nucleation: This primary step of granulation process commences with particle-particle adhesion due to liquid bridges. The even distribution of the liquid binder inside the dry powder bed leads to the formation of nuclei granule.

- Transition: The presence of various number of small granules with a broad distribution size is what describes the transition stage. Moreover, nuclei can grow by either the addition of single particles to the nuclei by pendular bridge, or by the combination of several nuclei.

- Ball growth: Depending upon the amount of liquid added and the characteristics of the powder being granulated, granule coalescence will continue upon agitation which produces an unfeasible and over massed system. The ball growth mechanism is illustrated in Figure 1.6 which states the four possible stages or mechanism of granule growth (Mirza, et al., 2007); (Summers & Aulton, 2013).

1) Coalescence: Is when several granules join to form a larger granule.
2) Breakage: The breakage of granules into fragments that can attached to other granules. As a result, a layer of material over the surviving granule can be formed.
3) Abrasion transfer. At this stage, the granule beds get agitated can promote to the attrition of material from granules. As a result, due adhesion to other granules, their sizes will be increased.
4) Layering. Layering occurs when an extra batch of powder mix is being added to a bed of granules, the powder then coheres to the granules and forms a layer over the surface in which as a result, a rise in their size will occur.

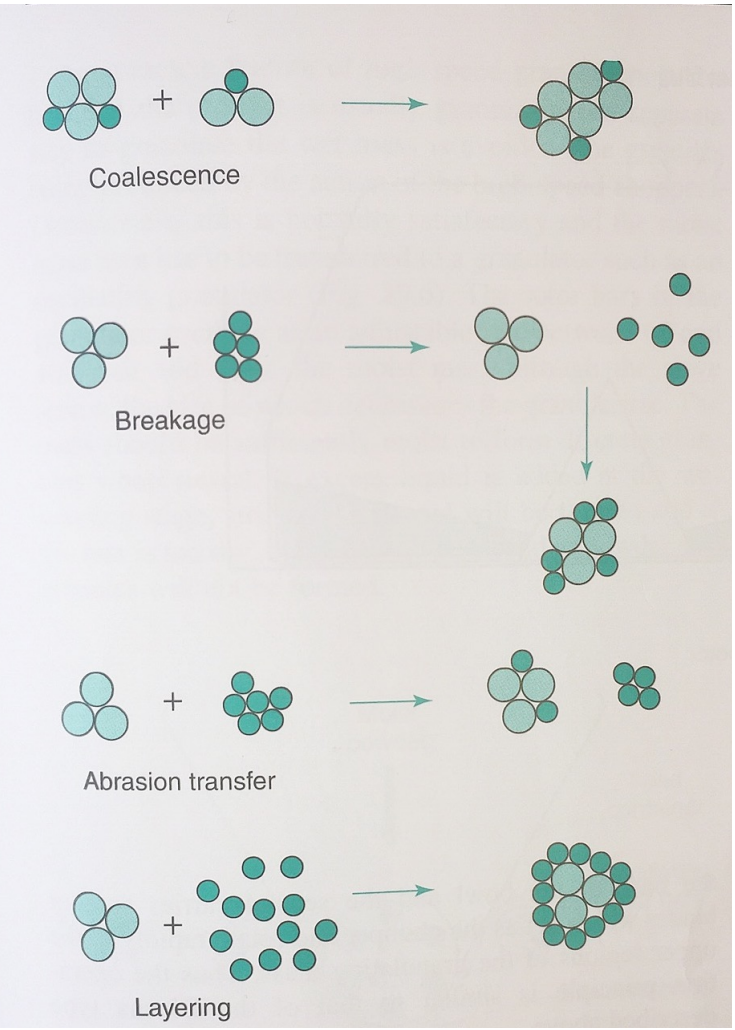


Figure 1.6: Ball growth mechanisms during granulation process (Summers & Aulton, 2013).

1.8. Poly vinyl pyrrolidone (PVP) as a liquid binder in the wet granulation process:

Soluble polyvinylpyrrolidone or poly-[1-(2-oxo-1-pyrrolidinyl) (see Figure 1.7) was the first polymerisation product of N-vinylpyrrolidone in water using hydrogen peroxide or using an organic peroxide as initiator (Bühler, 2005). Moreover, Polyvinylpyrrolidone is a free-flowing white or yellowish-white powder that comes in different particle sizes. The method of synthesis determines the odour of individual product. Table 1.3 below lists

the official names and abbreviations of PVP that are specific to the pharmaceutical industries.

Table 1-3: Soluble polyvinylpyrrolidone official names and abbreviations (Bühler, 2005).

Povidone	Current valid Pharmacopoeias (e.g. USP 26, Ph.Eur. 5, JP 14)
Polyvidon(e)	Former editions of Pharmacopoeias (e.g. Ph.Fr. IX)
Povidonum	Pharmacopoeias (e.g. Ph.Eur. 5)
Polyvidonum solubile	Former edition of the DAC (1986)
Poly(1-vinyl-2-pyrrolidon)	Deutsches Arzneimittelgesetz 1984
PVP	General abbreviation, commercial name for cosmetics/tech- nical grade

In addition to that, PVP polymers are obtainable in different viscosity grades and can range from low to high molecular weight. The viscosity of aqueous solutions of PVP depends on its average molecular weight. Additionally, polyvinylpyrrolidone is a stable polymer which can be safely used in liquid and solid dosage forms. PVP possess very useful application in the field of pharmaceutical industry. The feature that distinguishes PVP is its excellent solubility in water. This is an advantageous property in almost all dosage forms such as in wet granulation (for tablet production), oral solutions, and injectable medications. Nevertheless, its fundamental adhesive and binding influence allows it to be distinctive in the wet granulation and direct compression of tablet manufacturing making it a good material to use in this research as a liquid binder. The other feature that makes PVP a unique polymer is its wettability enhancement and these important characteristics can improve the rate of dissolution of hydrophobic drugs if

tablets being formulated using PVP. Research article by (Javaheri, et al., 2014), has shown dissolution enhancement of poorly water soluble, glibenclamide, when PVP has been used as a liquid binder in wet granulation of liquisolid tablets. Nevertheless, as the molecular weight of PVP increases, its dissolution rate decreases while the viscosity and adhesive power still increases. Therefore, according to the properties of the molecular weight, the optimum grade for individual dosage form can be provided in order to acquire the best outcome. Other studies have also shown tablet quality enhancement after the use of PVP, where wet granulation using PVP showed better flow properties, harder granules, lower friability and enhanced dissolution results when compared with cellulose derivatives (Bühler, 2005). Moreover, a study performed by (HIlton & Summers, 1986) was conducted on a poorly water-soluble drug known as Indomethacin in different solid dispersion formulations to test the drugs dissolution profile using PVP with different chain length. Consequently, it was noticed that dissolution rate was slower in tablets containing longer chain length of PVP and was higher in shorter chain ones.

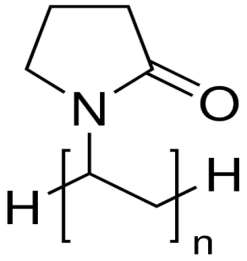


Figure 1.7: Poly vinyl pyrrolidone (PVP) Struc ture (Sigma Aldrich)

1.9. Wet granulation and liquisolid tablets:

Several scientists in the field of pharmaceutics, specifically in the formulation of high

quality tablets with enhanced dissolution, have challenged the use of liquisolid technique in their studies. Additionally, many have implemented new ideas in the manufacturing of liquisolid tablets. Amongst the newer generation ideas was the use of wet granulation in liquisolid preparation (Javaheri, et al., 2014). Wet granulation using PVP was applied on liquisolid system using glibenclamide (hydrophobic drug) with three different non-volatile liquid vehicles PEG®400, Synperonic®PE/L44 or Cremophor® ELP at 10 %w/w. The study proved the advantages of using wet granulation when used in liquisolid preparation not only enhances the solubility of glibenclamide, but also improves the flowability and compactibility of the liquisolid powder. Another study by (Javadzadeh, et al., 2007), has included polyvinylpyrrolidone (PVP) as an extra additive to the liquisolid systems to enhance the release of the hydrophobic carbamazepine in dissolution media. As a result, it was shown that the liquisolid tablets prepared with PVP improved dissolution rate in comparison with other formulations. The reason behind the success in drug dissolution enhancement is the ability of PVP to prevent drug retention in the spaces of porous surfactant, thereby enhancing the release of the hydrophobic drug into the dissolution medium.

1.10. Liquisolid technique based sustained release formulations

Development of sustained-release oral delivery systems are beneficial to achieve therapeutically effective concentrations of the desired drug at a constant and predetermined rate over a period of time which can be an optimal therapy in terms of patient compliance, efficacy and possible side effects reduction. (Wadher, et al., 2011). The most commonly used method of modifying the drug release is to use hydrophilic

matrix systems. A research on sustaining nicorandil release was performed by (Reddy, et al., 2003). Hydroxypropyl methylcellulose (HPMC) was used as a hydrophilic matrix agent. However, it was concluded that HPMC alone could not control the release of nicorandil release over 24 hours effectively. Therefore, several different techniques of sustaining the release of the drug over a desired time to deliver a constant concentration of drug have been performed. Nevertheless, it has been suggested by several researches that liquisolid technique has the potential to produce sustained release tablets by the reduction of drug dissolution rate of water soluble drugs such as trimetazidine. (Pavani, et al., 2013) investigated the release of trimetazidine dihydrochloride in polysorbate 80 and it was demonstrated that the use of polysorbate 80 can prolong the release of the used hydrophilic drug. In 2008, Javadzadeh et al. successfully used liquisolid technique to sustain the release of the water-soluble propranolol hydrochloride. Additionally, polysorbate 80, Eudragit® RL and RS grades were used as non-volatile liquid vehicles along with carrier and coating materials to prepare a liquisolid system with prolonged release mechanism. The importance of liquisolid technology on the ability to improve the dissolution of the hydrophobic drug or lower the rate according to the excipients used was understood. (Gonjari, et al., 2009) declared that the use of hydrophobic carriers such as Eudragit® RL or RS is likely to be a more efficient in the formulation of liquisolid prolonged release tablets. These hydrophobic carriers can lead the liquisolid compact to poor wettability, resulting in slower disintegration and slower drug release. In the case of hydrophobic carriers being ineligible for use, it is suggested to use hydrophilic carriers with the caveat that the hydrophilic carrier can be used only by the incorporation of a retarding agent such as hydroxy propylmethyl cellulose (HPMC). Depending on its

molecular weight, HPMC can either form a hydrated matrix layer or swells as it gets in contact with the gastrointestinal fluid which then allows the drug to erode and slowly dissolve. The dissolution of such systems is controlled by diffusion mechanism and the gel type formation of the HPMC acts as a protective barrier to the influx of water and efflux of the drug (Wadher, et al., 2011).

Till date, a limited number of drugs and sustaining agents have been formulated into liquisolid sustained release tablets. For instance, hydroxy propyl cellulose (HPC-H) has not been used in the any liquisolid research study as an agent to prolong the release of the water-soluble drug. Therefore, one aim of this work was to use liquisolid system with HPC to sustain metformin-HCl (a model drug) release. Glibenclamdie and metformin-HCl are used in this research as model drugs to be formulated into liquisolid tablets.

1.11. Glibenclamide properties and characteristics

Glibenclamide is an oral antidiabetic drug of the second-generation group known as “sulfonylurea”. It is used to treat non-insulin dependent patients with type II diabetes mellitus. It works by inhibiting the ATP-dependent potassium channels. It lowers blood glucose level by stimulating the insulin release from pancreatic β-cells and by enhancing the sensitivity to insulin by peripheral tissues (Manimaran, et al., 2010). In the β-cells of the plasma membrane, the K_{ATP} channels have receptors that give high affinity to glibenclamide to bind on to and stimulates the release of insulin. The drug is manufactured into low doses of 2.5mg and 5 mg and is excreted readily from the body. Also, due to glibenclamide’s active metabolites, hypoglycemic effects can be one of its adverse effects. Moreover, several research papers have witnessed low dissolution rate

behavior of glibenclamide due to the poor solubility and wettability characteristics. As a result, this can cause a reduction in its bioavailability. As seen in Figure 1-8, glibenclamide is a weak acid with a pK_a of 5.3 and thus it is a pH dependent of the medium (Gianotto, et al., 2007). Several methods have been reported to enhance the poor solubility properties of glibenclamide and in this work liquisolid technique was used to formulate glibenclamide tablets into 4 mg dose. By this way any possible adverse effects may be minimised, yet maintaining the enhanced dissolution properties by the aid of the liquisolid system.

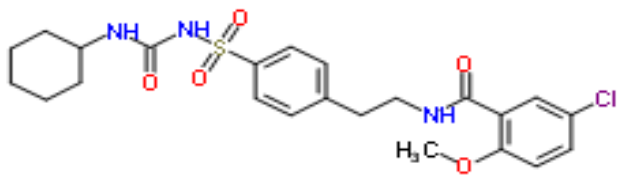


Figure 1.8: Glibenclamide structure (Royal Society of Chemistry-ChemSpider

1.12. Properties of Metformin-HCl

Metformin hydrochloride (dimethylbiguanide) has an empirical formula of $C_4H_{11}N_5$•HCl and its molecular weight is 165.63. Additionally, its structural formula can be seen in Figure 1.9. Metformin-HCl belongs to the biguanide class of oral antidiabetics. For over 15 years, metformin-HCl has been the first line of treatment for the treatment of non-insulin dependent type II diabetes mellitus. Prolong use of metformin to control blood glucose decreases myocardial infarction and coronary diseases seen in overweight diabetic patients (Block, et al., 2008). Metformin-HCl works by inhibiting the glucose

absorption from the intestine and suppressing the hepatic gluconeogenesis. Metformin-HCl is associated with gastrointestinal side effects such as nausea, diarrhea and abdominal discomfort which make a small percentage of patients intolerable to it. The bioavailability of metformin-HCl is about 40% to 60% (approximately 30% unchanged metformin-HCl excreted in urine) with a complete ingestion of approximately 6 hours and a plasma half-life of 1.5-4.9 hours (Nanjwade, et al., 2011). It is absorbed in the small intestine and partially in the stomach and negligible absorption is seen in the large intestine. The therapeutic dose that is currently available in market is 500 mg and up to 1.5 g, which is a large tablet to formulate and it is sometimes difficult to swallow, thereby discouraging patient compliance. Multiple dosing and big tablets can be a potential problem to the pharmaceutical market and pharmacy world. Pharmaceutical industries face formulation challenges when formulating metformin-HCl due to its poor compressibility and high dose. According to the Biopharmaceutical Classification System (BCS), metformin-HCl falls into the Class III of the BCS. It is a highly water-soluble drug with low permeability (Nicklin, et al., 1996) resulting in the need to administer high therapeutic doses to reach the desired plasma level. The other challenge that industries face in metformin-HCl formulation is direct compression because of poor characteristics of the drug, which can be a great problem on large-scale production. Metformin hydrochloride is a white crystalline powder with PKa of 12.4. It is practically insoluble in acetone, chloroform and ether. Nevertheless, the pH of a 1% aqueous solution of metformin hydrochloride is 6.68.

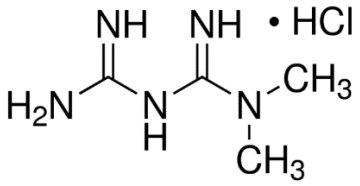


Figure 1.9: Metformin.HCl Structure (Sigma Aldrich)

The above mentioned challenges make metformin-HCl a good drug candidate for liquisolid system for enhancement of its properties. Therefore, not only highly water-soluble drug to be formulated as sustained release formulations, but also liquisolid can be useful to overcome problems such as low permeability.

1.13. Aims and objectives:

The primary aim of this research project is to use the liquisolid technique to enhance the dissolution rate of glibenclamide tablets, model hydrophobic drug. The second aim is to study the release and behavior of the water-soluble drug, metformin-HCl from immediate and sustained release tablets in a liquisolid system to confirm the different application of this technique and to evaluate its importance in powder technology. Furthermore, a novel application of “Wet Granulation” conjointly with liquisolid technique was achieved. It is used to overcome issues of poor powder flowability and compactibility especially with using high viscosity liquid vehicles. Thus, this new technique was performed as a last step upon preparation of liquisolid powders and before the automatic compaction into tablets. The third aim of this research is to enhance the permeability of metformin-HCl using liquisolid technique. Due to poor compactibility problem of metformin-HCl, it was thought to improve and overcome the challenge using liquisolid systems with and without granulation to observe the results.

The objectives of this research are as follows:

- Preparation of ungranulated and granulated liquisolid tablets of Glibenclamide (4mg unit dose) as a hydrophobic model drug using three liquid vehicles PEG®400, Synperonic®PE/L44 and Cremophor®ELP, at 10 %and 30 %w/w drug concentrations.
- Characterisation of tablets via B.P. quality control tests. The tests include uniformity of weight, friability, disintegration, drug content uniformity and dissolution tests.
- Preparation of liquisolid tablets of metformin-HCl (40mg unit dose) as a hydrophilic model drug using three liquid vehicles PEG®400, Synperonic®PE/L44 and Cremophor®ELP, at 30% w/w and 60 %w/w concentrations.
- Investigating the effect of wet granulation process in combination with liquisolid systems, in terms of flowability, compactibility and dissolution of drugs.
- Using Eudragit®RL30D as a non-volatile liquid vehicle with metformin-HCl to study its influence on drug release behaviour (either sustain or immediate release) thereby adding a new non-volatile liquid vehicle to literature.

- Sustaining liquisolid metformin-HCl formulations using hydroxylpropyl cellulose (HPC) as a carrier.

- Studying the permeability of immediate release metformin-HCl using Franz cell diffusion with male Wistar rat biological gut membrane.

For sustained release preparations of liquisolid metformin-HCl, hydroxypropyle cellulose (HPC) was used as a carrier instead of normal carrier. To our best knowledge, preparation of sustained release liquisolid metformin-HCl using HPC is a new study and no publications have been found on the uses of HPC in liquisolid technology. Therefore, many challenges were experienced in using this new excipient. Additionally, Eudragit®RL30D, as non-volatile liquid vehicle and Eudragit®RLPO as a carrier, were used to prepare liquisolid metformin-HCl as immediate release tablets. However, this study has showed that not all excipients work similarly on all drugs. Several publications suggest the usage of Eudragit®RL as sustaining agent, however, when Eudragit®RL30D and Eudragit®RLPO were used in this study to prepare liquisolid metformin-HCl, results showed immediate release with fast disintegration time (within seconds), which can be a new promising discovery for metformin-HCl as orodispersible tablets.

1.14. Structure of the Thesis

Chapter One: Introduction and Literature review to give a background for the application and importance of liquisolid technology to improve drug performance either to enhance or sustain the drug release characteristics.

Chapter Two: Materials and methodology applied in this current research.

Chapter Three: Glibenclamide liquisolid preparation with the application of wet granulation technique. Glibenclamide was used as a model hydrophobic drugs with the aim to enhance its release profiles.

Chapter Four: Application of liquisolid technology using wet granulation on metformin-HCl: Immediate release and permeability studies. Based on the results of liquisolid technology on glibencalmide release, another model drug - but it is water soluble – metformin-HCl was chosen to compare and contrast the effects of liquisolid technology on release behaviors of different drugs.

Chapter Five: Preparation and characterisation of sustained release liquisolid metformin-HCl formulations using Hydroxypropyl Cellulose (HPC-H).

Chapter Six: Orodispersible liquisolid preparation of metformin-HCl immediate release using Eudragit®RL-30D as a non-volatile liquid vehicle: A Novel discovery.

Chapter Seven: Conclusion and future work.

Chapter Eight: References.

Figure 1-10 shows the liquisolid preparations in this research.

1.15 Novelty of the current research work

The novelty of this work can be summarised in the following points:

- Liquisolid technique with the application of wet granulation prior to compaction stage.
- Effect of liquisolid technique on metformin-HCl permeability with Cremophor®ELP as a non-volatile liquid vehicle.
- Liquisolid compacts using Eudragit®RL30D as a non-volatile liquid vehicle to produce orodispersible metformin-HCl tablets.
- Sustained release preparation of metformin HCl using hydroxypropyl cellulose (HPC).

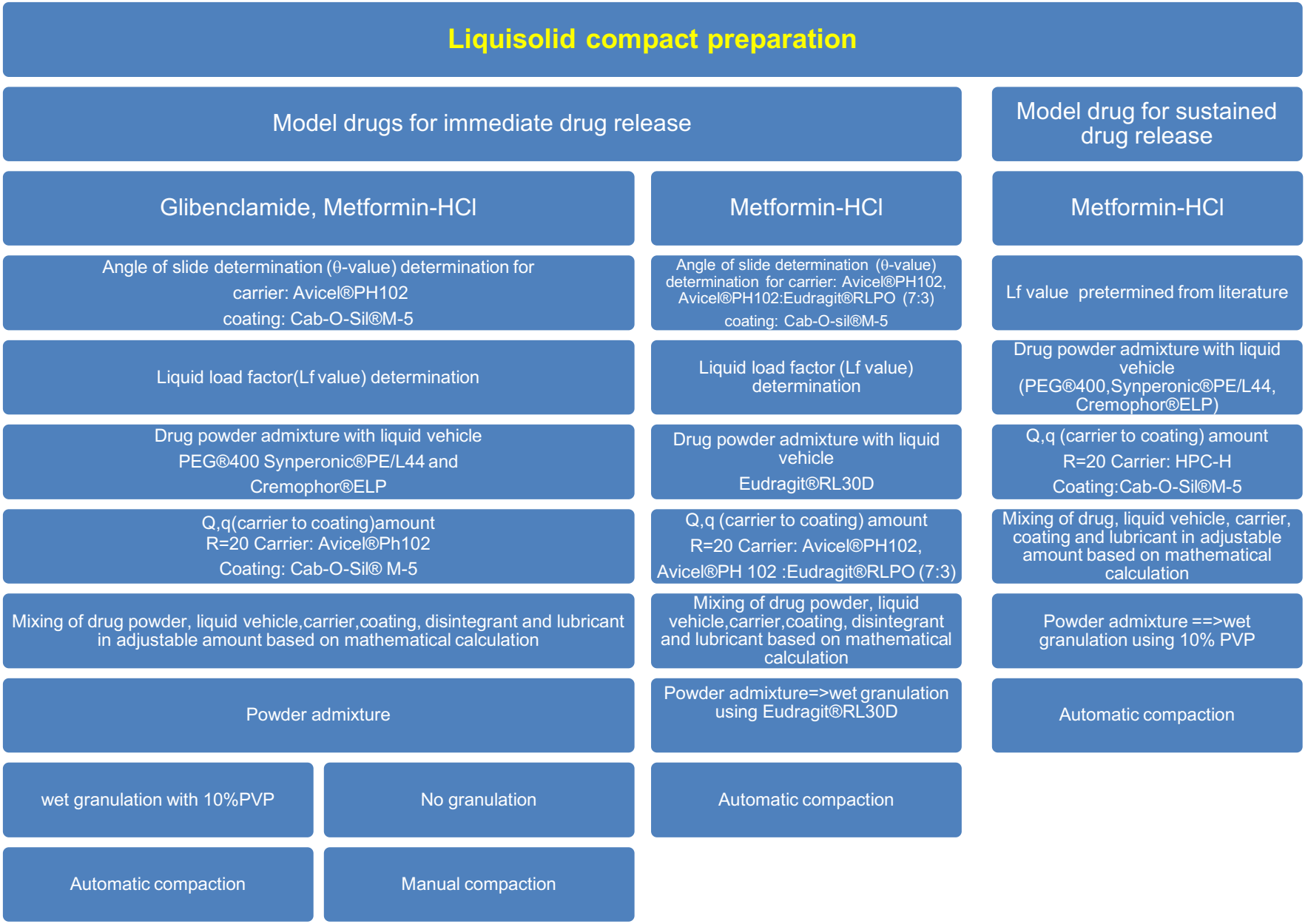


Figure 1.10: Flowchart of liquisolid preparation work

Chapter 2 - Materials and Methods

2.1. Materials

Pure glibenclamide and metformin-HCl hydrochloride were obtained from Sigma-Aldrich UK.

The following excipients were provided by different companies: Microcrystalline cellulose (Avicel® PH102) (FMC Corp., Philadelphia, USA), colloidal silicon dioxide (Cab-o-sil® M-5) (cabot Corporation, Werk Rheinfelden, Germany), potato starch (BDH laboratory supplies, Poole England), Polyethylene glycol 400(PEG®400)(Sigma- Aldrich, Poole, UK), (Synperonic®PE/L44) (ICI surfactants, Everberg, Belgium), polyoxyl 35 castor oil (Cremophor®ELP) (BASF Aktiengesselschaft, Ludwigshafen, Germany), Polyvinylpyrrolidone (Sigma Aldrich, used for wet granulation) and magnesium stearate (MEDEX, Morthants). Sodium Hydroxide pellets (Sigma-Aldrich, UK) were used. In addition, buffer tablets pH= 4.0(BDH Chemical Ltd., England) and Buffer tablets pH=7.0 (Asons Laboratory reagent, UK) are used for calibrating Jenway 3505 pH meter.

For the purpose of sustaining metformin-HCl hydroxypropyl cellulose HPC-H (high grade) was used and was obtained from NISSO Japan. Also, Eudragit®RL30D and Eudragit®RLPO were obtained from Evonik for use in metformin-HCl formulations.

For metformin-HCl permeability study; 28 male Wistar rat stomach were used (Charles River Laboratory, UK), there was no need for the ethical approval (Appendix for Chapter 4). Hydrochloric acid (0.2 M HCl) from Fisher Scientific, UK. In addition, Tyrode Buffer was prepared fresh daily and ingredients were obtained from Fisher Scientific,UK. An American pharmacologist “Maurice Tyrode” invented an isotonic with interstitial fluid

which is used in physiological experiments called Tyrode's solution (buffer). It contains the following ingredients:

Sodium chloride(NaCl), Glucose, Sodium Hydrogen carbonate ($NaHCO_3$), Calcium Chloride ($CaCl_2$), Potassium chloride (KCl), Magnesium Chloride ($MgCl_2$) and Sodium dihydrogen phosphate (NaH_2PO_4). All materials used were either analytical or pharmacological grade. In addition to that, the equipment used were calibrated.

2.2. Pre-formulation studies

2.2.1. Calibration curve of glibenclamide

A calibration curve is needed to calculate the concentration of the drug in the liquisolid formulations in the *in vitro* dissolution study.

2.2.1.1. Solvent preparation (0.2 M Sodium hydroxide) Mw=40

Pure sodium hydroxide pellets were weighed (8.0 g) in a beaker and then dissolved in 1L distilled water to make 0.2M sodium hydroxide solution (which is used for complete dissolving of the drug). The pH meter was calibrated using two different solutions at pH= 7.0 and pH=4.0 before each measurement.

2.2.1.2. Preparation of stock solutions

Glibenclamide was weighed (5.0 mg) using a digital weighing balance (PJ Precisa Junior, Swiss quality, Switzerland) and dissolved in 100 ml of 0.2 M NaOH solution. The stock solution was mixed until the drug completely dissolved. By applying a suitable

dilution, the solution was scanned between 200 nm and 300 nm wavelengths with a bandwidth equals to 1.5 nm by a double beam UV/Vis spectrophotometer (Nicolet Evolution 300, Thermo electron corporation with Vision pro version 2.03 software) to indicate the suitable drug wavelength using the stock solution. The glibenclamide peak wavelength was 229nm.

2.2.1.3. Serial dilution preparation

Furthermore, Equation (2.1) was used to calculate the exact volume required to produce the needed concentrations:

$$C_1V_1 = C_2V_2 \qquad \textbf{Equation 2.1}$$

Serial dilutions were prepared by taking 0.5 ml, 1ml, 3 ml, 5 ml, 7ml and 10 ml and 12 ml from the stock solution and added them to 50 ml volumetric flask and was made up to 50ml using 0.2M NaOH solution. The following were the concentrations of the resulting serial dilutions: 0.5 mg/l, 1 mg/l, 3 mg/l, 5 mg/l, 7 mg/l and 10 mg/l and 12 mg/l respectively. The calibration curve construction was repeated five times (Figure 2.1). The same line equation was obtained for the calibration plot of the drug in phosphate buffer pH 7.6.

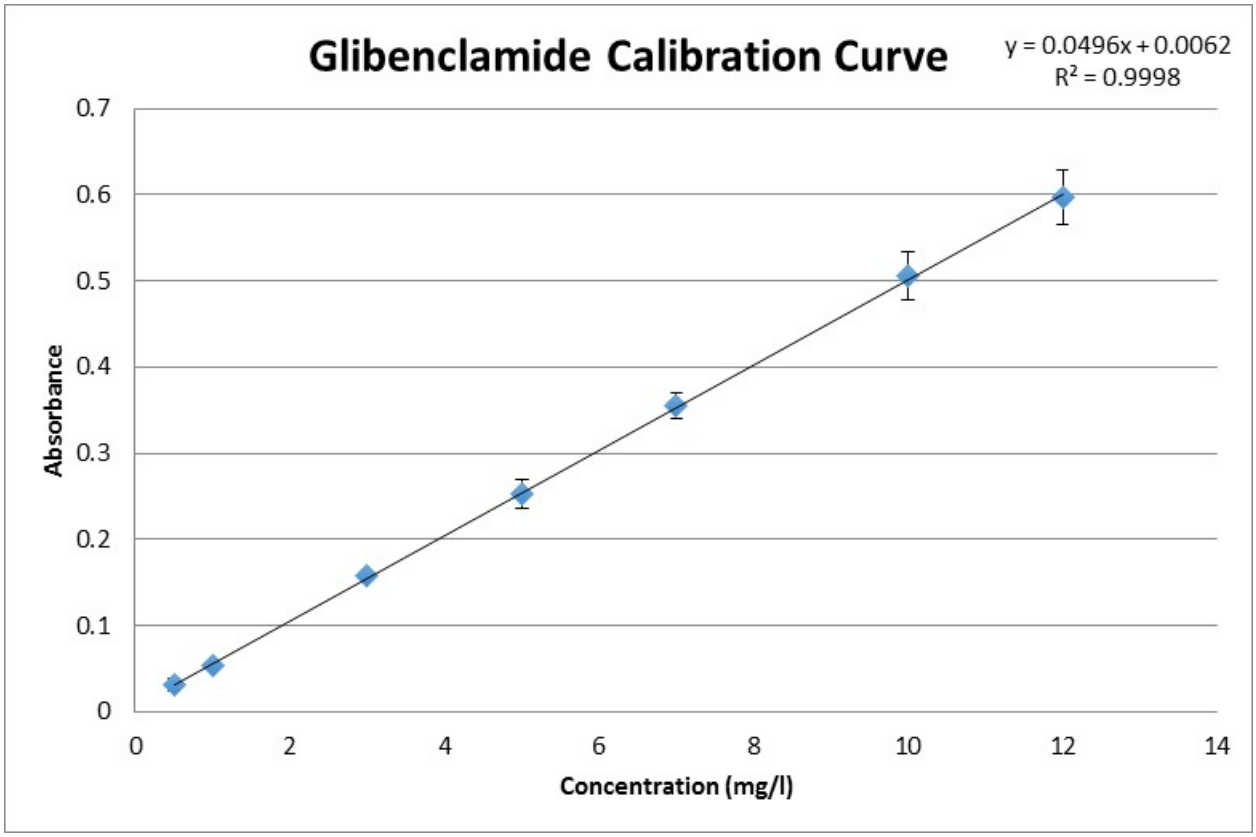


Figure 2.1: Calibration curve of glibenclamide

2.2.2. Calibration curve of metformin-HCl

2.2.2.1. Preparation of stock solution

Pure metformin-HCl (500 mg) was weighed and dissolved in 500 ml of distilled water. The solution was then scanned between 200 nm and 400 nm wavelengths by double beam UV/Vis spectrophotometer (Model M501, Camspec Ltd., Cambridge, UK) to determine the maximum wavelength peaks for the stock solution. The maximum peak wavelength was at 233 nm, hence the absorbance of all metformin-HCl samples was measured at 233nm.

2.2.2.2. Serial dilution preparation

Serial dilutions were prepared by taking 0.2 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7ml and 0.8 ml from the stock solutions and add them to 100 ml volumetric flask and was made up to 100 ml with distilled water. The resulting concentrations were: 2mg/l, 4 mg/l, 5 mg/l, 6 mg/l, 7 mg/l and 8 mg/l, respectively. The calibration curve construction was repeated five times (Figure 2.2).

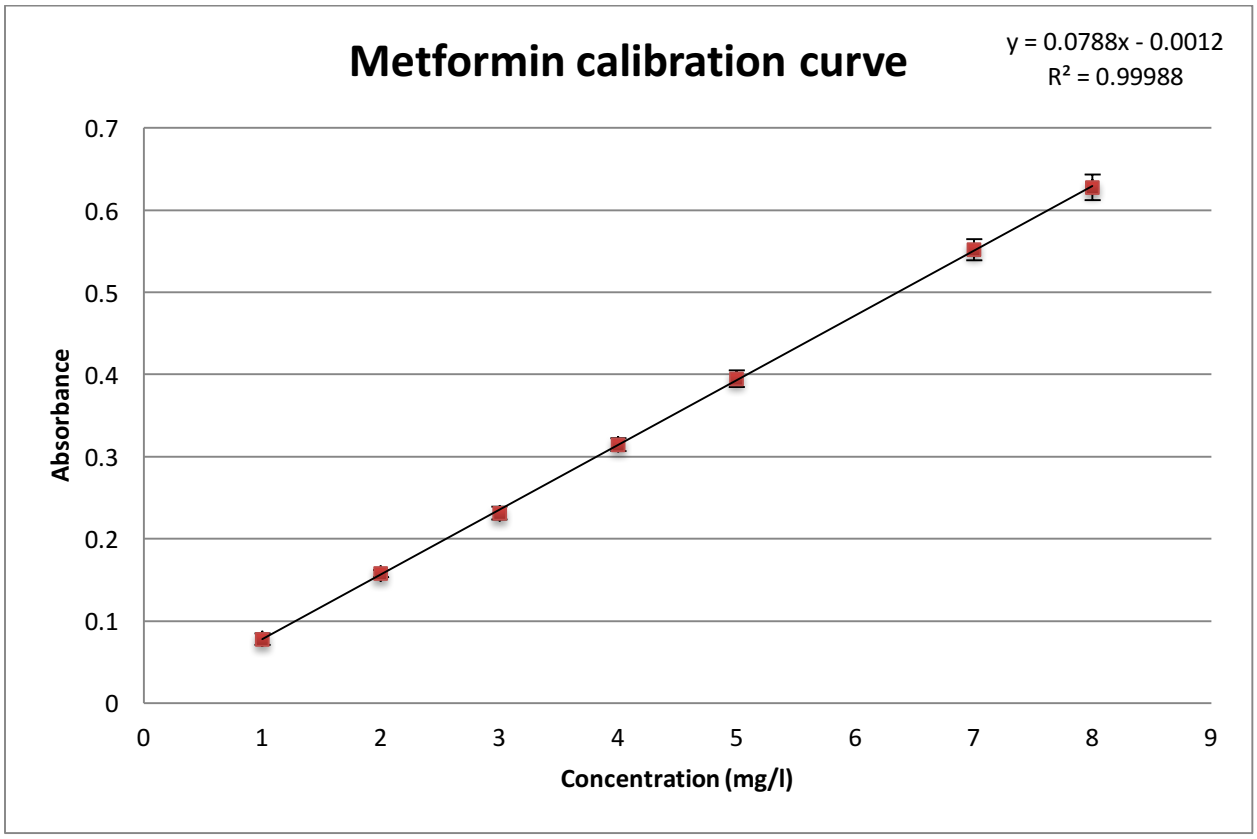


Figure 2.2: Calibration curve of metformin-HCl, standard deviation values are included

2.2.3. Flowability studies and determination of flowable liquid retention

2.2.3.1. Determination of the angle of slide for Avicel® PH 102 and Cab-o-sil® M-5 (fumed silica)

Flowchart figure 2.4 reveals the steps for liquisolid compacts preparation. Angle of slide is also referred to as the "optimal flowable liquid-retention potential" was measured (to evaluate the flow properties of the excipients used): the angle of slide was obtained using different liquid vehicles with Avicel®PH102 and Cab-o-sil® M-5 (fumed silica). Initially, 10g of the carrier and the coating (separately weighed) were weighed and then 2g of the liquid vehicle were added slowly in increasing intervals. This was then mixed which is known as the admixture and placed on a polished metal plate. The plate was tilted gradually until the admixture powder was about to slide. Then, the angle (Figure 2.3) that was formed between the plate and the horizontal surface was measured "angle of slide (θ)". This was repeated several times with different amount of liquids to observe the flow properties of the powder with the liquid vehicle. This then showed that the flow properties differed from one excipient to the other, which was due to difference in adsorption of the liquid vehicle (PEG®400, Synperonic® PE/L44 and Cremophor®ELP). From there, Equation 2.2 was used to calculate the flowable liquid- retention potential (Φ- value) of each liquid/powder admixture:

Φ- value = weight of liquid/weight of solid (Avicel® & Silica®) **Equation 2.2**

Eg: Weight of liquid= 4 g Weight of Solid= 10g Φ= 4/10= 0.4

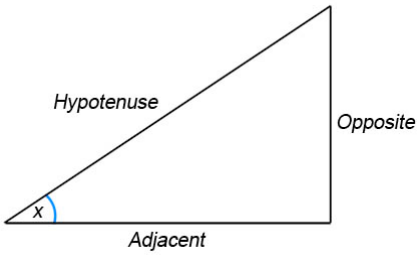


Figure 2.3: Diagrammatic representation of angle of slide

(Tan x = Opp/adj x = inverse Tan; x= θ. Thus θ represents the angle of slide of the admixture in the plate)

With the aid of graphing, the liquid load factor of the vehicle was determined, by plotting the θ values on a graph against the corresponding flowable liquid-retention values (Figure 2.5). Thus, the Φ value, which corresponds to an angle of slide of 33°, represents the flowable liquid-retention potential of Avicel®PH102 (ΦCA) and Cab-O-Sil®M-5 (fumed silica, (ΦCO). An angle of slide of 33° represents optimum flow (Javadzadeh, et al., 2007).

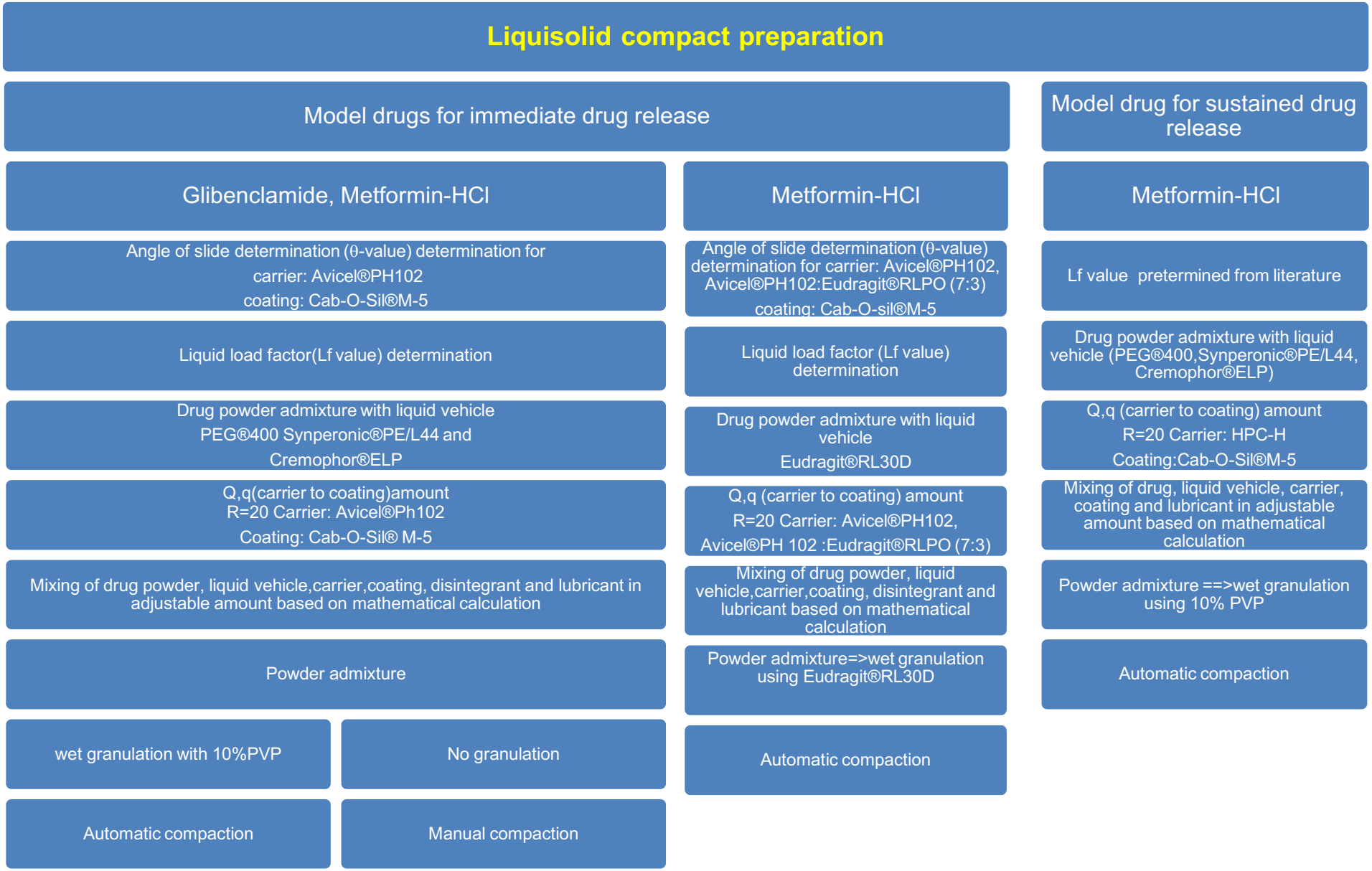
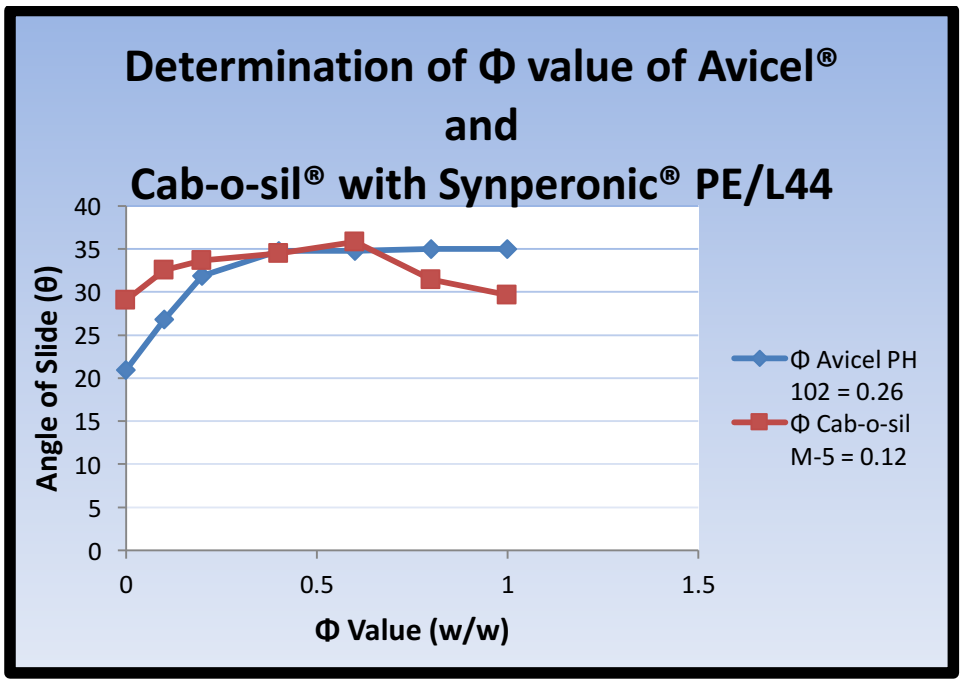


Figure 2.4: Summary of liquisolid preparation work



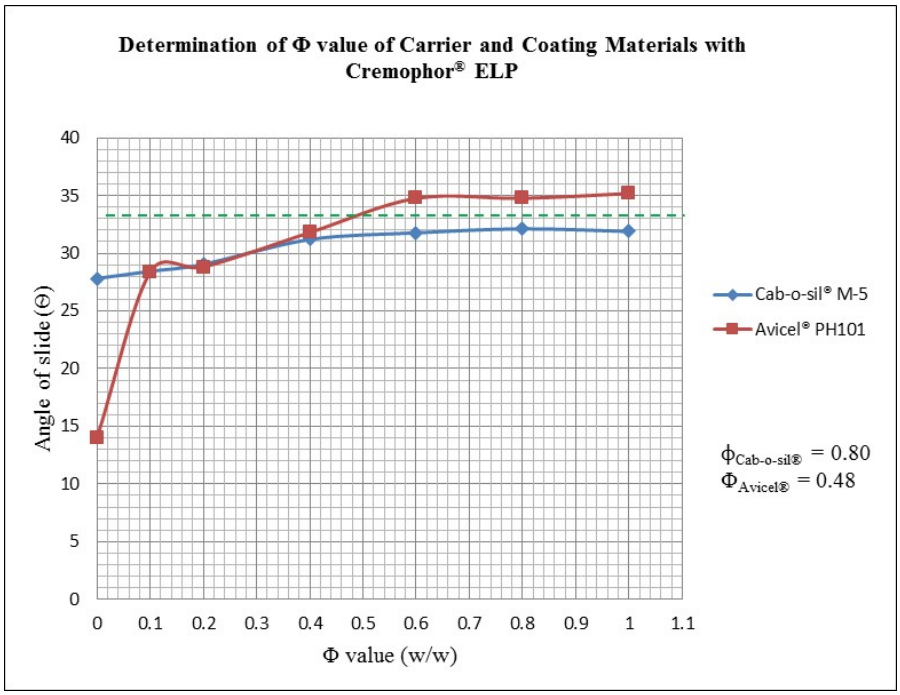


Figure 2.5: The angle of slide of various mixtures of powder excipients (i.e. Avicel®PH 102 and Cab-o-Sil®M-5) with Synperonic®PE/L44 and Cremophor® ELP

Then conventional glibenclamide and metformin-HCLI liquisolid powders were prepared

by following those steps.

$$Lf = \Phi CA + \Phi CO\ (1/R)$$ **Equation 2.3**

Where Φ_{CA} is the flowable liquid-retention potential of the carrier

and Φ_{CO} is the flowable liquid-retention potential of the coating material.

$$Lf = W/Q$$ **Equation 2.4**

Where L_f is the "loading factor" where the maximum amount of drug liquid loads on the carrier material.

Q is the amount of carrier (Avicel®PH102)

and W is the liquid medication (PEG®400/Synperonic®PE/L44/Cremophor®ELP)

$$R = Q/q \qquad (R = 20)$$ **Equation 2.5**

L_f can be calculated by substituting the flowable liquid retention potential of the carrier ($\Phi_{CA,}$) and flowable liquid retention potential of the coating material (Φ_{CO}), see below for an example calculation, into Equation 2.3. As it was mentioned before the R is the ratio between the carrier and the coating material which in this case is 20. By knowing the L_f value and the liquid medication (W), it is then possible to calculate the amount of carrier "Q" and coating "q" using Equations (2.4) and (2.5). Ultimately, the final admixtures were compacted manually or automatically depending on its flow properties on a 10mm flat-faced punch and die set using a single punch tableting machine (type 3, Manesty Machines Ltd., Liverpool, UK). For high unit dose weight, each sample unit was divided into 2 or 4 tablets so that they contain 40mg of metformin dose. This is to ensure that tablet is within a practical hardness and size. Different formulations had different compression force (to obtain tablets with suitable hardness of 47N) depending on various factors in the formulation such as the tablet weight and the preparations and the use of

the non-volatile vehicle. It is important to mention that non granulated glibenclamide and metformin-HCl were compressed manually due to poor powder flow. Also, sustained release metformin-HCl using HPC-H, 30% metformin-HCl using PEG®400 and Synperonic®PE/L44 were automatically compressed without the aid of granulation. Therefore, only 30% and 60% Cremophor®ELP and 60% PEG and Synperonic®PE/L44 were granulated using PVP (see section 2.4.1).

Example calculation for glibencalmide unit dose per tablet with Synperonic®PE/L44 as a liquid vehicle:

From Figure 2.5 and Equation 2.3, $Lf = 0.26 + 0.12\,(1/20) = 0.266$

From Equation 2.4, W for 10% drug in liquid vehicle (the dose of the drug is 4mg) = 4mg drug+36mg Synperonic®PE/L44 = 40, hence 0.266 = 40/Q; Q = 150.4mg

Thus from Equation 2.5, q can be determined; q = 7.5mg

2.2.3.2. Determination of the angle of slide for Eudragit®RLPO and Eudragit®RL30D as a liquid vehicle (used in metformin-HCl)

For the purpose of choosing the best liquid vehicle, three different grades of dispersion of Eudragit®RS30D, NS30D and RL30D were screened. According to the optimum flowability test Eudragit®RL30D was chosen as a liquid vehicle. Then angle of slide was performed using Eudragit®RLPO and Eudragit®RLPO: Avicel®PH102 (3:7) as carrier systems. The best flowability results were obtained using Eudragit®RLPO with Avicel®PH102 (3:7) and Eudragit®RL30D as a non-volatile liquid vehicle.

2.3. Preparation of Liquisolid powders and compacts

2.3.1 Glibenclamide and Meformin-HCl (immediate release)

Flow chart Figure 2.4 shows the steps for liquisolid powder preparation. Glibenclamide granulated liquisolid formulations denoted as G1 to G6 (Table 2.1) including conventional liquisolid and metformin-HCl ungranulated liquisolid denoted as M1 to M6 (Table 2.2) including conventional liquisolid powder and F1 to F6 (Table 2.2) granulated liquisolid metformin-HCl were prepared using PEG®400, Synperonic®PE/L44 and Cremophor®ELP as liquid vehicles, with 10% and 30% drug concentration (gibenclamide) and 30% and 60% (metformin-HCl). From Table 2.1 and Table 2.2, the exact amount of drug and excipients were measured using a digital weighing balance (PJ Precisa Junior, Swiss quality, Switzerland). Batches of 100 tablets were prepared. The drugs were dispersed in the liquid vehicle (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) with continuous mixing using pestle and mortar. The mixing process was performed for around 5 min until no drug particles is seen undispersed. This was then followed by the gradual addition of the appropriate amount of carrier which was Avicel®PH102. Again, the time of mixing was between 5 to 10 min until proper paste has been formed. Following that, the Silica which acts as a coating material was added to convert the wet mixture into dry powder under continuous mixing (refer to Figure 1.4). The Avicel®PH102 and Cab-O-Sil®M-5 were used at a fixed powder ratio (R) 20. Finally, a 5% w/w of potato starch which acts as a disintegrant was added into the admixture and mixed for 10 min. Then the magnesium stearate 0.75% was added at the end. The above steps were repeated with each liquid vehicle and drug concentration.

Table 2-1: Formulation of Liquisolid systems of Glibenclamide granules

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor *(Lf)*	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
G1	PEG® 400	10	20	0.17	36.00	4.00	238.10	11.90	15.40	2.31	308
G2	PEG® 400	30	20	0.17	9.33	4.00	79.30	3.97	5.10	0.77	102
G3	Cremophor® ELP	10	20	0.52	36.00	4.00	76.90	3.80	6.40	0.96	128
G4	Cremophor® ELP	30	20	0.52	9.33	4.00	25.60	1.28	2.10	0.30	42 to 250mg
G5	Synperonic® PE/L44	10	20	0.27	36.00	4.00	150.40	7.50	10.50	1.60	210
G6	Synperonic® PE/L44	30	20	0.27	9.33	4.00	50.10	2.50	3.50	0.52	70 to 250 mg
Conventional	_	_	_	_	_	4.00	238.10	11.90	13.50	2.00	270

Table 2-2: Formulation of Liquisolid systems of immediate release metformin-HCl (M=no granulation; F= with granulation)

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
M1	PEG® 400	30	20	0.17	93.30	40.00	794.00	40.00	51.00	8.00	1026.30
M2	PEG® 400	60	20	0.17	26.70	40.00	397.00	20.00	26.00	4.00	513.70
M3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501.00	25.00	35.00	5.20	699.50
M4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251.00	13.00	18.00	2.60	351.30
M5	Cremophor® ELP	30	20	0.52	93.30	40.00	256.00	12.80	21.00	3.20	426.30
M6	Cremophor® ELP	60	20	0.52	26.70	40.00	128.00	6.40	11.00	1.60	213.70
Conventional	–	–	–	–	–	40.00	397.00	20.00	24.20	3.60	485.00
F1	PEG 400®	30	20	0.17	93.30	40.00	794.00	40.00	51.00	8.00	1026.30
F2	PEG 400®	60	20	0.17	26.70	40.00	397.00	20.00	26.00	4.00	513.70
F3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501.00	25.00	35.00	5.20	699.50
F4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251.00	13.00	18.00	2.60	351.30
F5	Cremophor® ELP	30	20	0.52	93.30	40.00	256.00	12.80	21.00	3.20	426.30
F6	Cremophor® ELP	60	20	0.52	26.70	40.00	128.00	6.40	11.00	1.60	213.70

2.3.2. Liquisolid metformin-HCl using hydroxypropylcellulose (HPC) as a sustained release carrier and Eudragit®RL30D as a non-volatile liquid vehicle

Similarly, the above steps (Section 2.2.3.1) were used to prepare sustained release metformin-HCl liquisolid using HPC (the sustaining agent) as a carrier instead of Avicel®PH102 at two drug concentrations 30% and 60% (HPC1-HPC6) (Table 2.3). The powders were then automatically compressed using the single punch tableting machine.

Additionally, Eudragit®RLPO (carrier powder) and Eudragit®RL30D as non-volatile liquid vehicle were used to prepare liquisolid metformin-HCl in 30% and 60% drug concentrations and are denoted as LSS1 to LSS4 (Table 2.4). Metformin-HCl was weighed, followed by the addition of the liquid vehicle Eudragit®RL30D. All four liquisolid preparations (LSS1-LSS4) have the same liquid vehicle Eudragit®RL30D, however different carriers were used in LSS1 and LSS2, the carrier was Avicel®PH102. In LSS3 and LSS4, 3:7 of Eudragit®RLPO:Avicel®PH102 was used as a carrier system, after mixing for obtaining a homogenous carrier mix, in a turbular mixer (WAB Turbula, system Schatz, Willy A. Bacheofen machine, AG Maschinenfabrik, Glen Creston Ltd., Switzerland) and then were added to the drug and liquid vehicle admixture. Followed by the addition of coating (Cab-O-Sil®M-5), disintegrant (potato starch) and lubricant (magnesium stearate). Using wet granulation process (See section 2.4) the liquisolid powders were turned into granules using Eudragit®RL30D as a liquid binder and were compacted automatically into desired hardness tablets (refer to flowchart Figure 2.4 for details).

Table 2-3: Liquisolid formulations of sustained release metformin-HCl using hydroxypropylcellulose high grade (HPC-H)

Liquisolid system	Non-volatile liquid vehicle	Drug concentration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg) HPC-H	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
HPC1	PEG® 400	30	20	0.17	93.30	40.00	794.00	40.00	51.00	8.00	1026.30
HPC2	PEG® 400	60	20	0.17	26.70	40.00	397.00	20.00	26.00	4.00	513.70
HPC3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501.00	25.00	35.00	5.20	699.50
HPC4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251.00	13.00	18.00	2.60	351.30
HPC5	Cremophor® ELP	30	20	0.52	93.30	40.00	256.00	12.80	21.00	3.20	426.30
HPC6	Cremophor® ELP	60	20	0.52	26.70	40.00	128.00	6.40	11.00	1.60	213.70
Conventional	_	_	_	_	_	40.00	397.00	20.00	24.20	3.60	485.00

Table 2-4: Liquisolid formulation of metformin-HCl using Eudragit®RLPO as a carrier and Eudragit®RL30D as a non-volatile liquid vehicle.

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
LSS1	Eudragit RL 30D	30	20	0.80	93.30	40.00	166.60	8.33	16.40	2.50	327.00
LSS2	Eudragit RL 30D	60	20	0.80	26.70	40.00	83.30	4.17	8.18	1.20	164.00
LSS3	Eudragit RL 30D	30	20	0.20	93.30	40.00	666.50	33.30	44.20	6.60	884.00
LSS4	Eudragit RL 30D	60	20	0.20	26.70	40.00	333.35	16.67	22.10	3.30	442.00

Note: * Eudragit®RLPO:Avicel®PH102 (3:7) = carrier

2.4. Wet granulated liquisolid formulations

2.4.1. Wet granulation using Polyvinylpyrrolidone (PVP)

The liquisolid powder (with glibenclamide and metformin-HCl) were formed into granules using 10% Polyvinylpyrrolidone (PVP). The PVP was prepared by weighing 10g of PVP and 90g of distilled water, and then was placed in water bath at 60 °C to dissolve the PVP in water. Furthermore, the required amount of PVP was added to the prepared liquisolid powder in intervals and mixed for approximately 2-3 minutes until a so wet mass reached by feeling the wet mass. The correct moisture content was reached when the powder felt like an agglomerated mass. The next step was to perform the “agglomerate mass experiment” (Leuenberger, 2011), which is to take a hand full of wet mass and form an agglomerate mass and drop it into a tray from half a meter above the surface, when the agglomerate breaks into large pieces or only cracks, means the correct amount of moisture was added to the wet mass. Finally, the wet mass was then sieved using two mesh sizes 500µm-710µm. The formed granules were placed to dry at 45 °C for 120min. It is important to mention that the granulation step was performed after the formulation of the liquisolid powders and just before the compaction step, therefore, it was done as a final step before tablet formation (as shown in flowchart Figure 2.4).

2.4.2. Wet granulation of liquisolid powder admixtures using Eudragit®RL30 D as a liquid binder

Similarly, the liquisolid metformin-HCl powders (flowchart Figure 2.4) using the liquid vehicle (Eudragit®RL30D), at two different drug concentrations (30 and 60%) undergone wet granulation process using Eudragit®RL30D as a non-volatile liquid vehicle as well as a liquid binder. The experiment was also used to achieve the end point of the granulation step, along with same temperature and time constants as above-mentioned granulation using PVP (Section 2.4.1).

Furthermore, due to poor compressibility/compactibility of HPC liquisolid formulations, wet granulation step with 10% PVP has been applied as well for formulations containing Cremophor®ELP, Synperonic®PE/L44 (liquid vehicles) where good quality tablets were produced.

2.5. Pre-compression study characteristics

2.5.1. Determination of flow and packing properties of the prepared liquisolid powders

The powder flow and packing properties of each prepared liquisolid compact was determined using tap volumeter (J. Engelsmann AG, Ludwigschafen, Germany). The liquisolid powder was weighed then poured into a 100ml-measuring cylinder. The volume of the poured powder was recorded as V_b and the tapped volume (after sufficient taps), which recorded to give a constant volume on a tap density apparatus, was noted as V_t.

Hence, from the recorded volumes, the density of the liquisolid formulation was calculated using the below eq. to give the bulk density (p_b) and tapped density (p_t) in g/mL. p_b = Weight of powder/ V_b p_t = Weight of powder/V_t

From there, the compressibility index (CI %) of the formulations was calculated using Equation 2.6 to investigate the flowability of the powders:

$$\boldsymbol{CI}\ \% \ = \ 100\ x\ (p_{tapped} - p_{bulk}) / \ p_{tapped}$$ (Albertini, et al., 2008) **Equation 2.6**

According to the British Pharmacopoeia (2017), the smaller the value of CI% the better the flow properties of the powder. Thus, CI% below 25 represents passable flow properties and anything-above 40% are indicative of poor powder flow. The powders were then compacted manually (non-granulated liquisolid powder) or automatically (liquisolid granules) depending on their flow properties on a 10mm flat-faced punch and die set using a single punch tableting machine (type 3, Manesty Machines Ltd., Liverpool, UK). Different formulations had different compression force depending on various factors in the formulation such as the tablet weight. For G4 and G6 (refer to Table 2.1) due to the small unit dose, extra Avicel® PH102 was added as a filler to increase the tablet weight to 250mg unit dose and to get a stronger and bigger tablets.

2.5.2. Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra were performed on all relative excipients, materials and formulations (refer to Tables 2.1-2.4). Using Spectrum BX FTIR Spectrophotometer (Perkin–Elmer,

Cambridge, UK). The frequency ranged from 4000 cm^{-1} to 550 cm^{-1} at 1.0 cm^{-1} resolution. Small amount of individual sample was loaded into the system just to cover the diamond and the peaks were obtained for reading and analysis.

2.5.3. Differential scanning calorimetry (DSC)

Using DSC Refrigerated Cooling System (Model Q1000, TA Instruments, UK), the DSC thermograms of glibenclamide, metformin-HCl and all relative excipients and formulations were analysed. DSC aluminium pans were used to weigh samples of about 3-6 mg and then were hermatically sealed and transferred into the equipment for analysis. Prior to running the samples, the instrument was calibrated with sapphire and indium. Thus, the thermal behavior of each sample was studied at a scanning rate 10°C/min, from 0° C to 300°C.

2.5.4. Scanning electron microscopy (SEM)

Electron microscope (model: Hitachi S3000N, Hitachi High-Technologies UK-Electron Microscopes, Wokingham Berkshire, UK) was used to perform SEM on glibenclamide, metformin-HCl and all relative excipients and formulations used.

The samples were prepared by adding a small amount of each sample to 15mm diameter aluminium specimen stubs that has double sided carbon adhesive tabs (Mikrostik adhesive, Agar Scientific). Prior to examination, a thin layer of gold/palladium mixture coated the samples for electric conductivity. The samples were then exposed to argon atmosphere at 10 Pa and were then coated at current of 18-20mA for 3x15s.

2.6. Characterisation of liquisolid tablets (quality control tests)

The liquisolid compacts were evaluated via quality control tests in accordance to the British Pharmacopoeia, 2017 specifications.

2.6.1 In-vitro dissolution studies for immediate release formulations

In vitro dissolution studies were performed for each batch of liquisolid formulation (refer to Tables 2.1 and 2.2 for details), pure drug and conventional tablets for glibenclamide and metformin-HCl immediate release and for liquisolid formulations using Eudragit®RL30D (LSS1-LSS4, refer to Table 2.4) using USP dissolution apparatus II (Caleva Ltd., Dorset, UK); distilled water (glibenclamide and metformin-HCl) and phosphate buffer, pH 7.6 (glibenclamide), were used as dissolution media. According to British Pharmacopoeia 2017, the liquisolid tablets were placed in dissolution medium of 900mL at a temperature of 37± 0.1^0C and stirred at a paddle speed of 100rpm. Ten millilitres samples were collected at intervals of 5, 10, 15, 20, 25, 30, 45, 60 and 90 min and were replaced by 10 ml of the dissolution medium to maintain a constant volume. The collected samples of glibenclamide and metformin-HCl were then analysed using UV/Vis spectrophotometer single beam at 229nm (glibenclamide) and 233 nm (metformin-HCl) for determination of drug content using the calibration curve (Figures 2.1 and 2.2). The dissolution experiment in triplicate was achieved to compare the percentage drug release of liquisolid tablets, pure drug and conventional tablets. Dissolution for

placebo tablets was performed (to be used as blanks) to eliminate any absorbance interference from excipients.

2.6.2. In-vitro dissolution studies for sustained-release metformin-HCl tablets (HPC-H) using flow through cell method

A USP apparatus 4 (CE 7 Smart with CP7 piston pump, Sotax AG, Switzerland) with 22.6 mm flow through cells was used during the dissolution study, hence the sink condition was maintained. Each cell was prepared by placing a 5-mm ruby bead in the apex of the cone to protect the inlet tube, and roughly 1 spoonful of 1 mm glass beads were added to the cone area to form a glass bead bed. The temperature of the flow cell unit was 37.0±0.5°C. The flow rate of the liquid (distilled water) was set to 10 ml/min. Because this is an open loop flow though cell, the washed solution was collected as waste in a separate reservoir. The open-loop configuration is shown in Figure 2.6

Furthermore, the sample analysis was performed by following the solution absorbance (Agilent 8453 spectrophotometer, Santa Clara, California 95051, United States). Metformin-HCl samples were analysed at 233 nm with a 0.5 cm cell. All samples for analysis were filtered through glass microfiber filters (Whatman, GF/ D). The dissolution run was set to 12 hours, taking samples at, 10, 15, 20, 25, 30, 45, 60, 90, 120, 240, 360, 480, 600 and 720 min. Before analysis, calibration curve using serial dilutions was prepared and entered into the system to get E value, which was then used, for percentage drug release calculation. The UV reader was blanked with distilled water before running the process. The “flow-through” method allows constant optimal sink conditions. This is

due to the continuous flow of fresh solvent and continuous sampling. It uses large volume of solvent and that poor sink can avoid non-sink condition. Therefore, it is an ideal apparatus to show an improved in-vitro/in-vivo correlation tests particularly for poor soluble and modified release drugs forms.

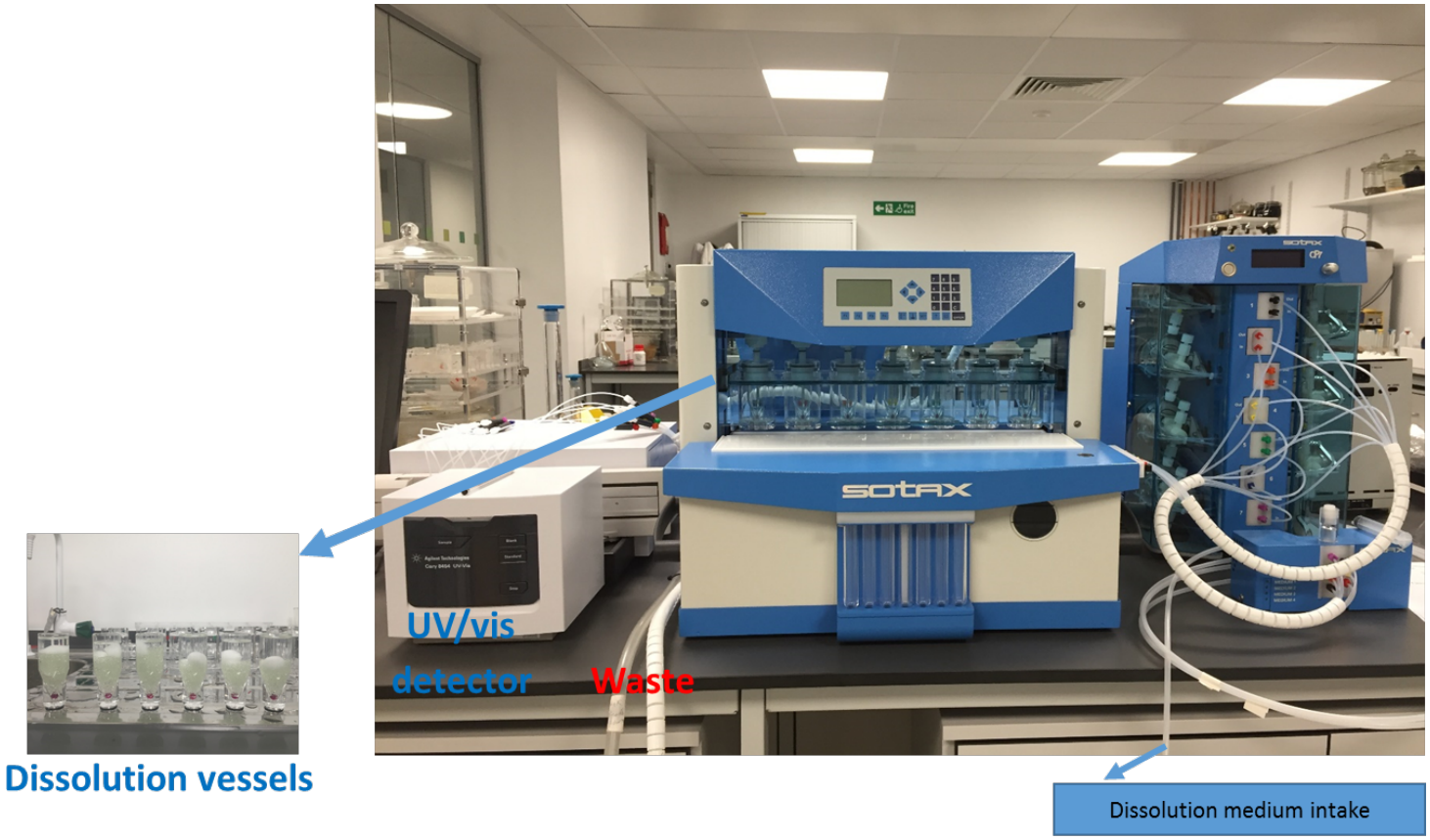


Figure 2.6: Flow through cell (open loop) dissolution station

2.6.3. Uniformity of tablet weight

Twenty tablets were selectively chosen from each formulation and weighed individually. The average mass of the twenty tablets was calculated and the percentage deviation of each tablet was determined.

2.6.4. Tablet hardness

Six tablets were selected from each formulation of the two drugs and the force in Newton needed to crush them was examined using the hardness tester (Model 2E/205, Schleuniger and Co., Switzerland). In addition to that, before the test was performed.

2.6.5. Friability

The friability tester (FRV 1000, Copley Scientific, UK) was used to measure the friability of 10 selective tablets from different formulation. The weight of the tablets was recorded before and after using the tester. The drum was rotating at 25rpm for 4 min. The weight difference was recorded and the percentages of friability were calculated using Equation (2.7).

$$\%\, Friability = (loss\ of\ mass/initial\ mass)\ x\ 100 \quad \textbf{Equation 2.7}$$

2.6.6. Disintegration

The disintegration test was performed on glibenclamide, immediate release metformin-HCl and metformin-HCl using Eudragit®RL30D. Six tablets from different formulations at 37± 1 °C in distilled water using the disintegration unit (Manesty Machines Ltd., Liverpool,

UK) were used. The time of disintegration of the tablet was recorded when there was no residue (soft mass with no palpably firm) in the cylindrical transparent tube, which indicated that the tablet has fully disintegrated.

2.6.7. Drug Content uniformity

2.6.7.1. Glibenclamide content uniformity

Tablets containing glibenclamide were weighed and crushed by mortar and pestle in order to determine the drug content in classical liquisolid, wet granulated liquisolid and conventional tablets. Then, the powder was dissolved in 50 ml 0.2M NaOH to give a concentration of 4mg/50ml (80mg/l). The solution was then filtered and diluted as appropriate. Finally, the samples were analysed for determining the drug concentration using UV spectrophotometer at 229 nm. The percentage of drug content with respect the theoretical amount was determined. The experiment was repeated three times to ensure accurate results.

2.6.7.2 Metformin-HCl Content uniformity

Metformin-HCl tablets, were crushed and placed in 100ml volumetric flask using distilled water to give a concentration of 400mg/L. The solution was filtered and diluted with distilled water. The samples were prepared for each formulation and the experiment was repeated in triplicates and samples were analysed at 233nm using single beam UV-spectroscopy.

2.7 Kinetics model analysis of drug release

In order to inspect the mechanism of metformin-HCl release from the immediate release and sustained release liquisolid three kinetic release models were applied on data obtained from dissolution tests. These models are: Zero order, First order and Higuchi model of kinetics. With the aid of excel, the graph was conducted to obtain the equation of the line and hence the highest square of coefficient of determination (R^2) for the indication of the most appropriate model to represent the release of metformin-HCl from immediate and sustained release liquisolid formulations. Zero order model, is a system that all drug particles transfer mechanism to dissolution medium is disclosed to the surface area of the system. Hence, the cumulative percentage data of the drug release is then plotted against the time. Additionally, first order kinetics drug release is related to the drug concentration and can be plotted logarithm of the cumulative percentage release of the remaining drug versus the time. Moreover, in Higuchi model, linear plotting of the cumulative percentage of the drug release against the square root of time is expected (Dash, et al., 2010).

2.8 Stability study of metformin-HCl (immediate release) liquisolids

The stability of the prepared tablets containing immediate release metformin-HCl and its conventional tablet was performed in a stability chamber with 21°C and 75 % humidity for 12 month period time.

In vitro dissolution tests, DSC and FTIR analyses were applied after the storage periods and the result were compared and evaluated to the fresh sample data.

2.9 Statistical analysis

In the tests of glibenclamide and metformin-HCl tablets evaluation, statistical analysis was performed. Paired t-test was used to compare between the permeability test of liquisolid formulation contained immediate release metformin-HCl using PEG®400, Synperonic®PE/L44 and Cremophor®ELP. Additionally, one-way ANOVA using SPSS was used to statistically analyse the dissolution drug release of metformin-HCl and glibenclamide liquisolid formulations (Appendix for Chapter 3, 4 and 5). Consequently, homogeneity of variance was not significant meaning that data were normally distributed and hence ANOVA and T-test were applied in this research.

2.10. Metformin-HCl permeability test using Franz cell diffuser

Franz cell (Figure 2.7) diffuser was used to perform metformin-HCl permeability test. The membrane used was male Wistar rat gut, cut into thin slice, washed and cleaned using Tyrode buffer (prepared as in section 2.1). Individual tablets of each formulation (refer to Table 2.2, F1-F6) were crushed and dissolved into 20ml distilled water. The sample was then filtered and 1ml was withdrawn using micropipette and injected into the donor chamber; the receiver chamber was filled with 14 ml HCl, 0.2M (pH 1.4), and was kept at a constant temperature (37 ± 3 °C) (Figures 2.7 and 2.8). Furthermore, using small needle and syringe, 1ml sample was withdrawn from receiver chamber every 5 min, 10min, 15min, 20min, 25min, 30min, 45min, 60 min, 90min, 120min and 2 hours. Then, samples were analysed using single beam UV-vis spectrophotometer at 233nm. The withdrawn sample was then returned into the receiver chamber to keep constant volume. Additionally, the experiment was repeated five times.

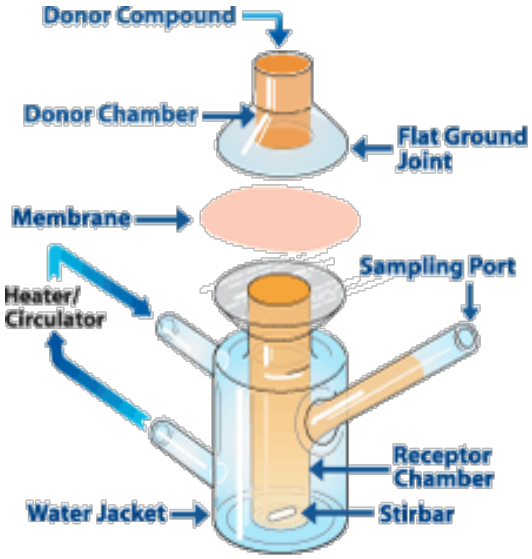


Figure 2.7: Franz cell diffusion equipment description (Permegear, 2015)

Figure 2.8: Franz cell diffusion apparatus setting

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Chapter 3 - Glibenclamide liquisolid preparation with the application of wet granulation technique

(Results and Discussion)

3.1. Introduction:

Via several screening programmes, about 40% of the newly drug synthesis was identified to be hydrophobic in nature. Thus pharmaceutical companies in manufacturing similar drugs for oral delivery will face challenges with solubility and bioavailability difficulties. One of these drugs is glibenclamide, which has been chosen in this research project to be the model drug. Additionally, for the preparation of liquisolid compacts, a series of non-volatile liquid vehicles have been selected such as, “PEG®400, Synperonic® PE/L44 and Cremophor®ELP. Several researchers have mentioned the use of the above liquid vehicles in the manufacturing of liquisolid immediate release tablets (Tiong & Elkordy, 2009). However, those authors have not used glibenclamide. PEG®400 (polyethylene glycol) is a stable hydrophilic surfactant in solution and has a hydrophilic-lipophilic balance (HLB) value of 12. It can be used to form liquid medication with a hydrophobic drug that allows miscibility in water. According to Tiong and Elkordy 2009, naproxen liquisolid tablets containing PEG®400 and Cremophor®ELP display a higher dissolution rate than Synperonic®PE/L44. Therefore, PEG®400 is a good non-volatile solvent to be used in preparation of immediate release liquisolid tablets. The second non-volatile liquid vehicle used in this section is Synperonic®PE/L44 (Poloxamer 124), which is an ethylene and propylene oxide block copolymer. Synperonic®PE/L44 is a water-soluble surfactant with an effective wetting properties. Therefore, its application in liquisolid system using hydrophobic drugs is ideal. Additionally, it has an HLB value of 12 and is classified as a non-toxic substance. Polyoxyl 35 Hydrogenated Castor Oil, Cremophor®ELP, similarly, is a non-volatile liquid vehicle. Its HLB value is 13 and is viscous in nature. Javaheri et al 2014 (the author of this thesis), demonstrated the use of Cremophor®ELP using

glibenclamide and showed the highest dissolution rate than PEG®400 and Synperonic® PE/L44. Additionally, some non-ionic surfactants such as Cremophor®ELP can be considered as effective pharmaceutical excipients for inhibiting p-glycoprotein function and hence promoting the intestinal absorption of different drugs (Shono, et al., 2004). Therefore, the uses of the water-soluble surfactants along with their desirable physicochemical properties make them suitable for liquisolid technique.

Figure 3.1: Flowchart summary of the liquisolid work using glibenclamide as model hydrophilic drug

Table 3-1: Formulation of liquisolid systems of glibenclamide granules

Liquisolid system	Non-volatile liquid vehicle	Drug Concertation in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor *(Lf)*	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
G1	PEG 400	10	20	0.17	36.00	4.00	238.10	11.90	15.40	2.31	308.0
G2	PEG 400	30	20	0.17	9.33	4.00	79.30	3.97	5.10	0.77	102.0
G3	Cremophor ELP	10	20	0.52	36.00	4.00	76.90	3.80	6.40	0.96	128
G4	Cremophor ELP	30	20	0.52	9.33	4.00	25.60	1.28	2.10	0.30	42 to 250 mg with filler
G5	Synperonic PE/L44	10	20	0.27	36.00	4.00	150.40	7.50	10.50	1.60	210
G6	Synperonic PE/L44	30	20	0.27	9.33	4.00	50.10	2.50	3.50	0.52	70 to 250 mg with filler
Conventional	_	_	_	_	_	4.00	238.10	11.90	13.50	2.00	270

3.1. Determination of the angle of slide for Avicel® PH 102 (MCC) and Cab-o-sil® M-5 (fumed silica):

In order to calculate the required ingredient quantities, the flowable liquid retention potentials (Φ values) of powder excipients were utilised which are required for the calculation of L_f value. The angles of slides for Avicel® PH102 and fumed silica were measured with the following liquid vehicles: PEG®400, Synperonic®PE/L44 and Cremophor®ELP. The Φ_{CA} value and Φ_{CO} values decide the amount of carrier and coating material required to produce a free flowing, non-adherent, readily compactible and dry-looking liquisolid formulations. High Φ value is advantageous as it results in smaller sizes of the formulated tablets (Spireas , et al., 1992). It is vital to measure the angle of slide because it is the preferred method to determine the flowability of powders with particle sizes of less than 150μm (Spireas , et al., 1992). In this study, as stated by the manufacturer, the particle sizes of Cab-o-sil® M-5P and Avicel®PH102 were 133.8μm and 0.2-0.3 μm respectively. In addition, Spireas et al (1992) and (Elkordy, et al., 2013) clarified that when the drug particle dissolved in the liquid vehicle, the liquid initially absorbed into the interior of the particles gets captured by its internal structure, thus adsorption of the liquid starts taking place onto the internal and external surfaces of the porous carrier particles after saturation state of this process has occurred (Fahmy & Kassem, 2008). Furthermore, the coating material (Cab-O-Sil®M-5), which has high adsorptive property and large surface area, gives the liquisolid system the desirable flow characterisation. Thus determination of the flow properties of the powder excipient and liquid/powder admixture is significant to ensure a success in liquisolid formulations.

Additionally, the viscosity of the liquid vehicle plays an important role in the formulation of the liquisolid compacts. The viscosity of PEG®400 and Cremophor®ELP are 105-130 and 650-800 mPa s, respectively (Rowe, et al., 2003). (Tiong & Elkordy, 2009), reported that the viscosity of the liquid vehicle is inversely proportional to the amount of carrier and coating material required in a liquisolid formulation and accordingly the L_f increases with increased viscosity of the liquid vehicle. High viscosity liquid vehicle such as Synperonic®PE/L44 and Cremophor®ELP leads to poor powder flowability and compactibility of liquisolid admixture even with application of compressibility index as described by Spireas ((Spireas & Bolton, 1999) (Suliman, et al., 2013) studied the compressibility index of norfloxacin liquisolid powder using PEG®200 and Synperonic®PE/L61 that required the application of an equation described by (Spireas & Bolton, 1999). However, this method is very time consuming especially that the equation needs to be understood thoroughly before application, which is not convenient for manufacturing purposes. The application of compressibility index requires several samples as a result loses excessive amount of powder before the liquisolid powders are compressed, which is again a great disadvantage to the pharmaceutical industry. Nevertheless, although the flowability of liquisolid powder using Synperonic®PE/L61 was enhanced using the compressibility index as stated by (Suliman, et al., 2013) it is yet not a promising method for enhanced flowability of liquisolid admixtures using high viscosity liquid vehicles such as Synperonic®PE/L44 or Synperonic®PE/L61 and Cremophor®ELP. Accordingly, wet granulation was applied in this research just before compaction (Flow chart Figure 3.1) to overcome these problems, with hope that this process will be beneficial for large-scale production in pharmaceutical industry.

3.2. Determination of flow and packing properties of the prepared liquisolid powders-discussion

The determination of the powder flowability is important in handling, packing, mixing and compression. One major obstacle pharmaceutical industries face is the poor flow properties of powders. This affects the uniformity of flow out of the hopper and further problems in compaction (Tiong & Elkordy, 2009); (Spireas , et al., 1992) (Jain, et al., 2014), because uniform and reliable flow from the hoppers into the die of the compressing machine ensures uniform tablet weight and drug content. There are several factors that can affect the flow properties of the liquisolid powder including physical, environmental as well as mechanical. Therefore, the Carr's compressibility index was calculated for each liquisolid powder to determine its flow properties. This means the flow properties are affected by particle shape, particle size, porosity, density, moisture content and surface roughness. Additionally, Carr's compressibility index is a good parameter in reflecting interparticle friction. The calculated Carr's compressibility index of glibenclamide liquisolid granules is presented in Table 3.2 and Figure 3.2. It is a good parameter to evaluate the flow of the prepared powder. The lower the Carr's compressibility index is, the better the flow of the powder would be. In this research project, as shown in Table 3.2. G1 and G5 (for composition refer to Table 3.1), conventional liquisolid powders were 12.6%, 8%, and 18%, respectively, all have CI below 25%. On the other hand, G2 and G3 results showed poor flowability with CI% being above 25% (30% and 28% respectively). This can be related to the amount, type and property of the liquid vehicle used (PEG®400, Synperonic®PE/L44 and Cremophor®ELP). Nevertheless, the flowability results obtained were only for granulated liquisolid powder. This is due to the poor flowability

properties of the ungranulated liquisolid powder using PEG®400, Synperonic®PE/L44 and Cremophor®ELP (viscous liquid vehicles). Thus, this shows that the properties of the liquid vehicle can interfere with the flow property of the formulation making it better or poor flowing. Moreover, in addition to the coating properties of Cab-o-Sil®M-5 (fumed silica), it can also act as a glidant (concentration of 0.1-0.5 %) to improve powder flowability. Furthermore, these granulated liquisolid powders were compacted automatically (electronically) into tablets.

Table 3-2: Carr's compressibility index of glibenclamide liquisolid compacts.

Carr's index (Cl%) of prepared liquisolid powders of glibenclamide		
Liquisolid system*	**Carr's index (Cl%)**	**Type of Flow**
G1	12.60	Excellent
G2	30.00	Poor
G3	28.00	Poor
G4	25.00	Poor
G5	8.00	Excellent
G6	16.00	very Good
Conventional	18.00	Fair

* For the composition of each formula refer to Table 3.1.

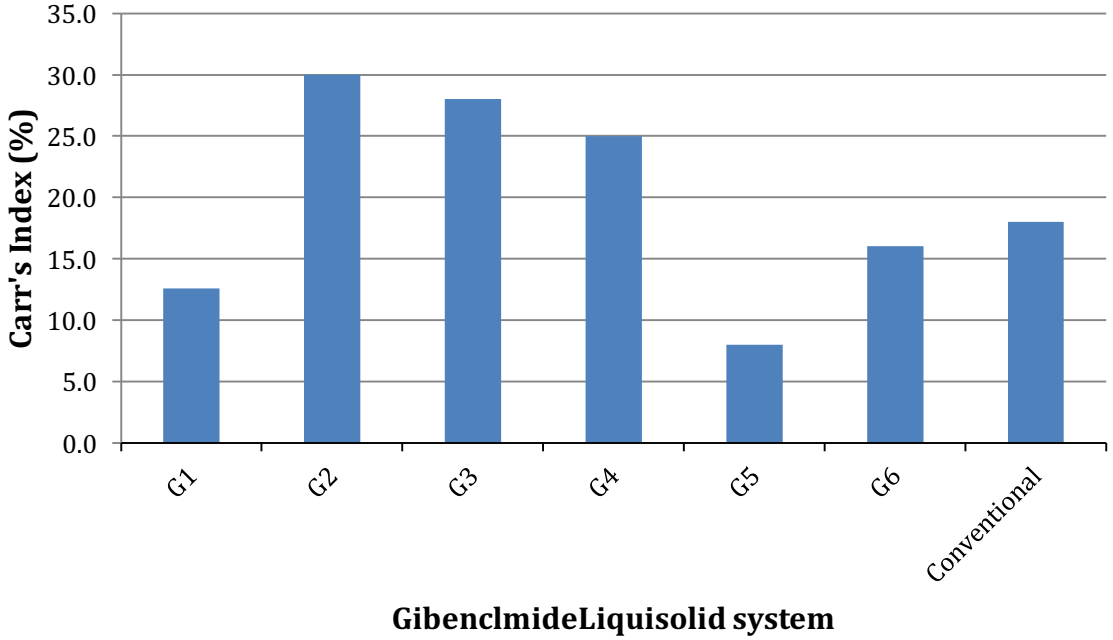


Figure 3.2: Carr's compressibility index glibenclamide liquisolid granules and conventional powder. For formulations' composition refer to Table 3.1

It can be seen from Table 3.2, that the liquisolid granule formulations using Cremophor®ELP (G5) showed best flowability outcome (due to the addition of excess Avicel®PH102 (carrier) that was added to increase the weight of the tablet to 250 mg unit dose). Therefore, it is clearly visible that granulation with the aid of excess Avicel®PH102 addition has enhanced the flowability properties of the liquisolid powder using one of the viscous liquid vehicle i.e. Cremophor®ELP. On the other hand, liquisolid powders using all three vehicles used in this study were not compacted automatically unless formed into granules. This is due to poor flowability and compressibility properties of the liquisolid powder and due to the elasticity properties of the powders with liquid vehicle used. Elkordy et al 2013 experienced two liquisolid formulations using Synperonic® PE/L61 with Solutol HS 15 (1:1) at both drug concentrations 10% and 30% which were incompactible,

and hence no tablets were formed. The given explanation was linked to the liquid vehicle used that lead to elastic behaviour of the powder that caused this incompactibility. The powder deformed upon compression and returned to its original shape after removal of the compaction force. This is clearly seen in this current research in the case of glibenclamide liquisolid with Cremophor®ELP (see Figure 3.3). Compression of ungranulated powder admixture was unsuccessful due to the elastic behavior of the liquisolid formulation. As it is exhibited in Figure 3.4, the powder not only was not compressed into tablets, but also formed a compressed unwanted shape, which is not desirable for the industry (Figure 3.4). Therefore, granulation was used to overcome this problem. After using granulation, the powder was compressed automatically into tablets (Figures 3.5 and 3.6) with no capping or cracking which complied with quality control test of the British Pharmacopeia 2017.

Before granulation

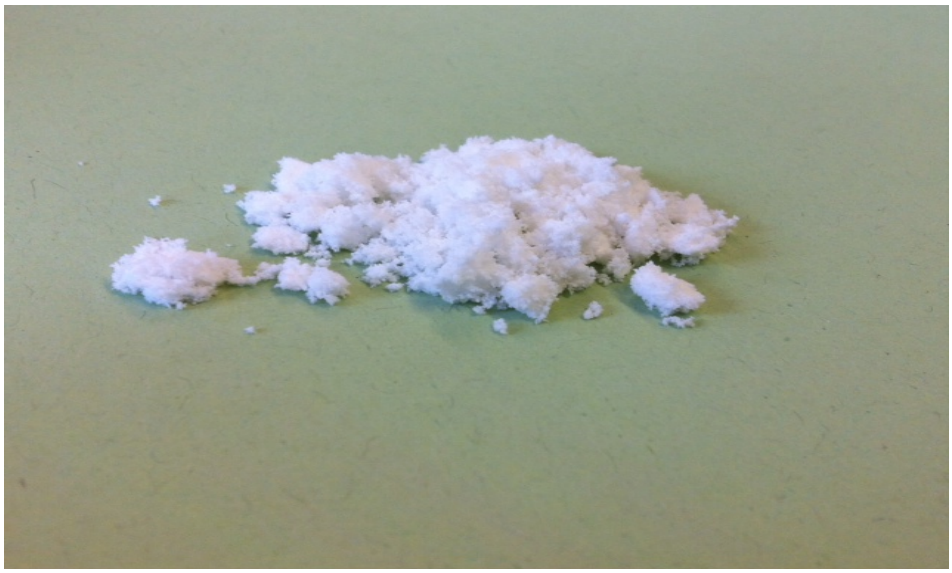


Figure 3.3: Glibenclamide and10% Cremophor®ELP powder without granulation

Figure 3.4: Glibenclamide and10% Cremophor®ELP without granulation in the tablet press.

After granulation

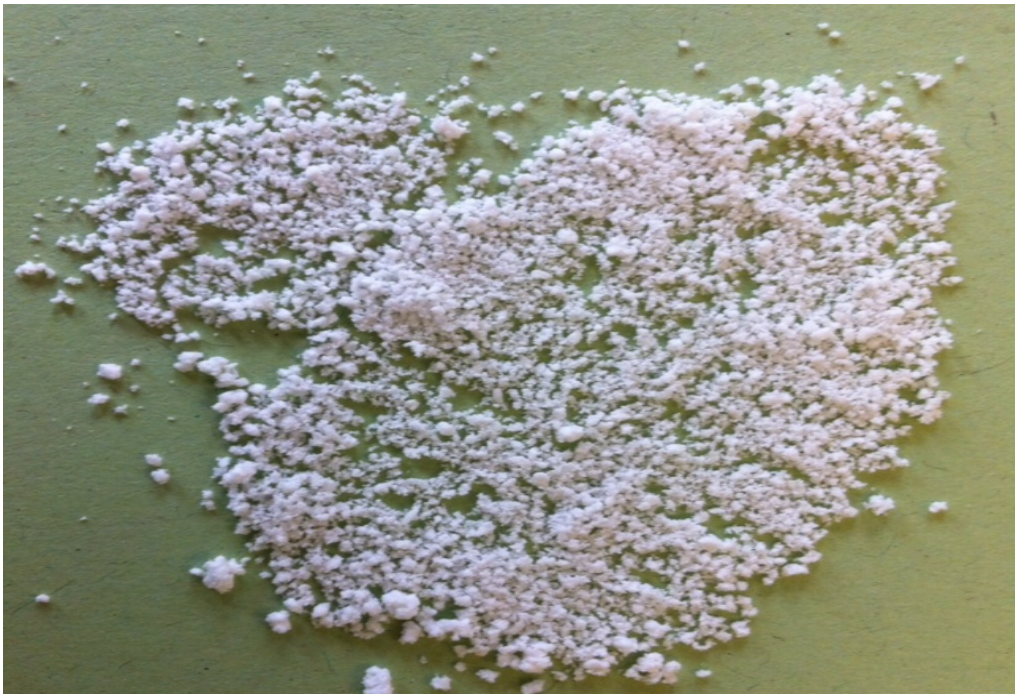


Figure 3.5: Glibenclamide and 10% Cremophor®ELP granules after granulation

Figure 3.6: Glibenclamide and 10% Cremophor®ELP tablets after granulation

In a concentrated suspension (liquid vehicle and drug), weak Van der Waal forces hold flocculated particles together. This often results in plastic flow ((Mahato & Narang, 2012). If the shear stress is low, such materials might possess elastic behavior. However, when the maximum yield stress is exceeded where the Van der Waal forces are broken, plastic flow is exhibited. There must be a balance for plasticity. Although plastic deformation helps the solid links to form between particles within the powder mix, excessive plasticity leads to the formation of tablets with high extent of hardness. The powder mix produced with Avicel®PH101 and the liquid vehicle takes advantage of the elastic properties of the liquid vehicle and the plastic properties of Avicel®PH101. Thus, in the current research, only few ungranulated liquisolid tablets were manually compressed for the purpose of dissolution comparison test study. As shown in table3.1, liquisolid granules using Cremophor®ELP (G5) showed best flowability outcome compared to PEG®400 (G1). According to (Tiong & Elkordy, 2009), liquisolid naproxen showed best flowability with PEG®400 and not with Cremophor®EL (poor flowability). This is due to the high viscosity behaviour of the non-volatile liquid and due to load factor being above 0.25. The higher the liquid load factor the poorer the flowability characteristics of the liquisolid powder (see Table 3.1 for details). This is because there will be fewer amounts of carrier and coating available in the formulation and excess amount of liquid vehicle being available leading to agglomerates ((Altememy & Altememy, 2014) (Darwish & El-Kamel, 2001), (Shangraw, 1989). Also, in this current study, Synperonic®PE/L44 (G3) showed poor flowability compared to the conventional powder (fair flow) but both granules were automatically compactible. This is clearly linked to the amount of liquid vehicle present that delays the flow of the powder. According to (Javadzadeh, et al., 2007) using HPMC or PVP as

microsystems can improve flowability. Hence, Avicel®PH 102 was added as an additional filler to produce 250mg tablets to improve tablets characteristics.

The new technique of wet granulation was used in this research. It has shown enhanced flowabiity of liquisolid powders that contain viscous liquid vehicles such as Cremophor®ELP. Not only did it enhance the flowability, but it also allowed for automatic compression of the liquisolid granules, which was impractical, to achieve without granulation. Also, the excipients used play a great role in the flowability and compactibility of the liquisolid powder. For example, microcrystalline cellulose (MCC, Avicel®), is a widely used excipient in pharmaceutical tableting, because of its good compactible properties. This can be explained by the chemical properties of the microcrystalline cellulose. The microcrystalline particles are held together with hydrogen bonds, which in return affect the strength and cohesiveness of the tablets. Therefore, MCC (Avicel®PH102) was used as a filler to increase the tablet size to a medium size tablet of 250mg unit dose. By the addition of MCC, the tablets were compacted evenly without any cracking or capping. Nevertheless, glibenclamide and Cremophor®ELP 10% and 30% without granulation were difficult to compact neither manually nor automatically but they became elastic and showed an unexpected outcome, they were poorly flowable and were cohesive as seen in Figures 3.3 and 3.4. Therefore, pre-granulation liquisolid powders did not undergo flowability test. On the other hand, as seen in Figures 3.5 and 3.6; the granulated liquisolid powder of glibenclamide using Cremophor®ELP showed better shape with enhanced flowability and thus compactible tablets.

Therefore, granulation is a successful technique to convert incompactible elastic powder into good flowability and compactability powders with excellent flowability properties according to Carr's compressibility index.

3.4. Characterisation of liquisolid formulations

3.4.1 In-vitro dissolution studies

The dissolution profiles of glibenclamide formulations were performed in both distilled water (non-sink conditions) and phosphate buffer (pH 7.6; for sink conditions) to study the effect of liquisolid technique on aqueous dissolution enhancement of the drug. The dissolution profile of glibenclamide with 30% drug in Cremophor®ELP (Figure 3.7a and b) shows highest drug dissolution rate ($P < 0.05$) (refer to Appendix for Chapter 3) when compared to most formulations including the pure drug (which showed no drug release at all in water). Also, the liquisolid glibenclamide with granulation showed higher drug release than without granulation ($P<0.05$). It is shown in Figure 3.7, where G1 to G6 (refer to Table 3.1 for composition) showed a higher drug release when granulation step was performed and inversely, the drug release was to a minimum when manual compaction was performed in the non-granulated form.

Therefore, it can be concluded that the in-vitro dissolution of liquisolid tablets is greatly influenced by granulation and by other three major factors: the type of vehicle, the drug-vehicle ratio and type of disintegrant in agreement with (Javadzadeh, et al., 2007); (Nokhodchi, et al., 2005). Some carriers could have an effect on the drug dissolution, for example in this case, microcrystalline cellulose (Avicel®PH102) was used and from

previous research it showed that microcrystalline cellulose (MCC) has disintegration property which could facilitate disintegration of tablets and thus dissolution of drug (Javadzadeh, et al., 2007). In addition, a study by (El-Gizawy, 2007), suggested that single disintegrant has no significant effects on drug dissolution. Also, when explaining the dissolution characterisation and the percentage drug release of the formulation better, the Noyes-Whitney equation was used.

$$\frac{dC}{dt} = k.A\,(Cs - Cb)$$

where dC/dt is the dissolution rate of the drug particle; “k” is a constant, which is affected by the viscosity of the dissolution medium and experimental conditions; “A” is the surface area exposed to dissolution; “Cs” is the saturation solubility of the drug in the diffusion layer; “Cb” is the concentration of drug in the bulk solution. Moreover, all dissolution tests were done under same paddle speed of 100rpm and same dissolution medium. Thus, the factors that will affect the dissolution outcomes of the liquisolid compacts are A and (Cs - Cb). Additionally, there are some factors that can affect the solubility of the solid drug in the dissolution medium such as temperature (endothermic or exothermic), dissolution medium (acidic or neutral), crystalline structure, presence of solubilising agents and molecular structure of the solute. In this study, the temperature was maintained constant at 37±0.1^{0}C and the dissolution medium as have been mentioned previously was kept constant for all formulation. Therefore, what could have affected the outcome is the presence of other compounds, which are the non-volatile liquid vehicles. As a result, each formulation had different dissolution profile (Figure 3.7), depending on its liquid vehicle and its wetting property (El-Say, et al., 2010).

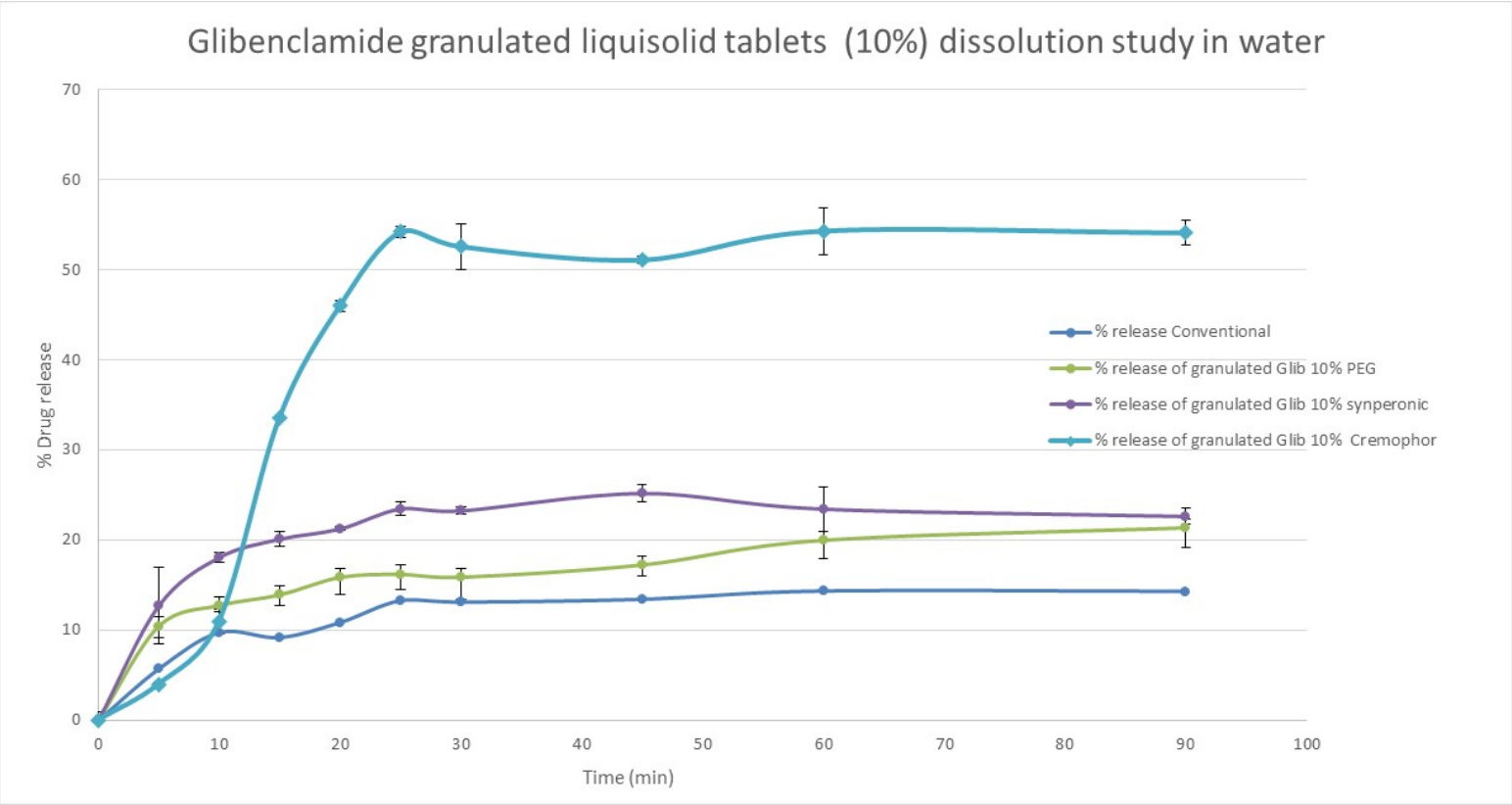


Figure 3.7a: Dissolution results of glibenclamide granulated liquisolid and conventional tablets (10% drug concentrations) in distilled water.

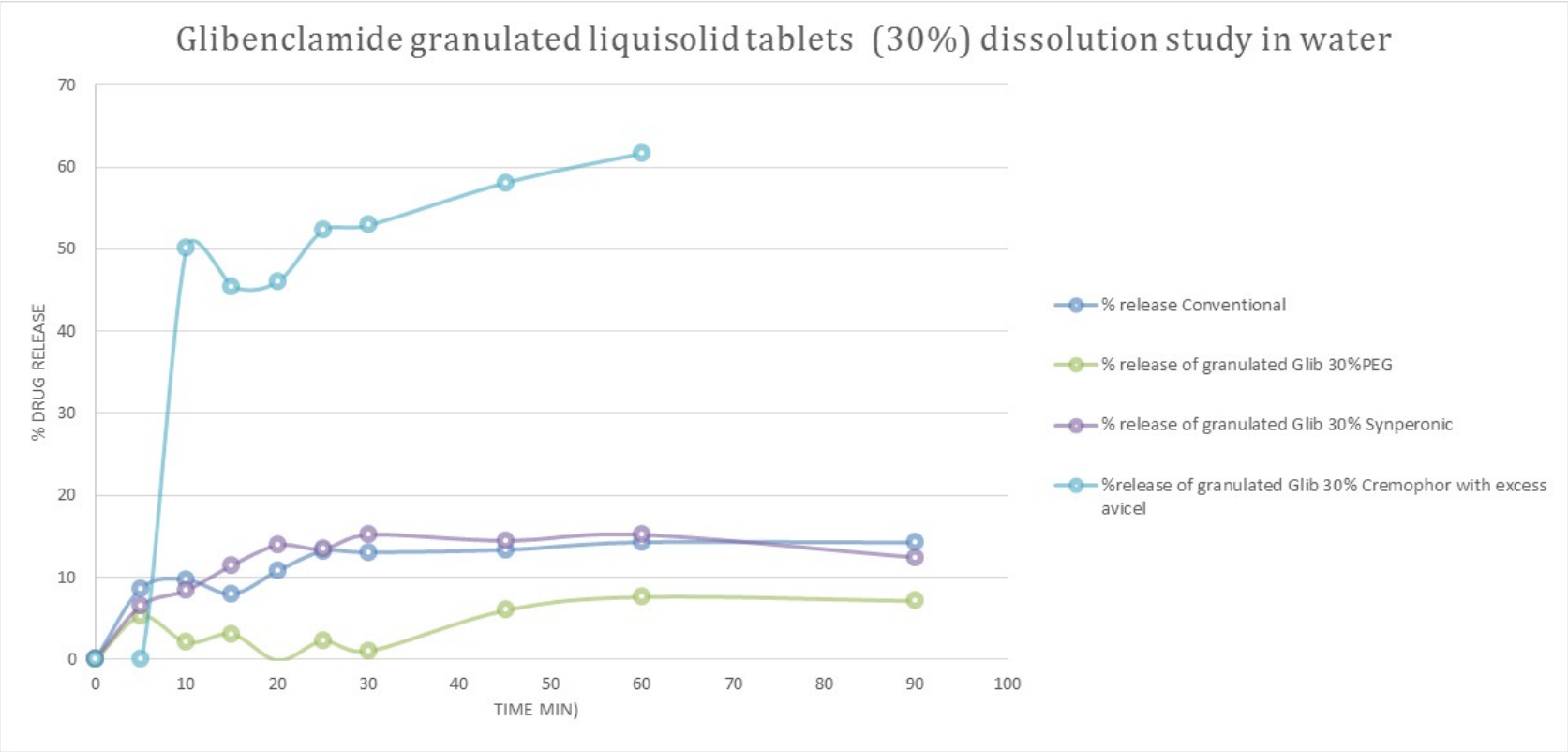


Figure 3-7b: Dissolution results of glibenclamide granulated liquisolid and conventional tablets (30% drug concentrations) in distilled water.

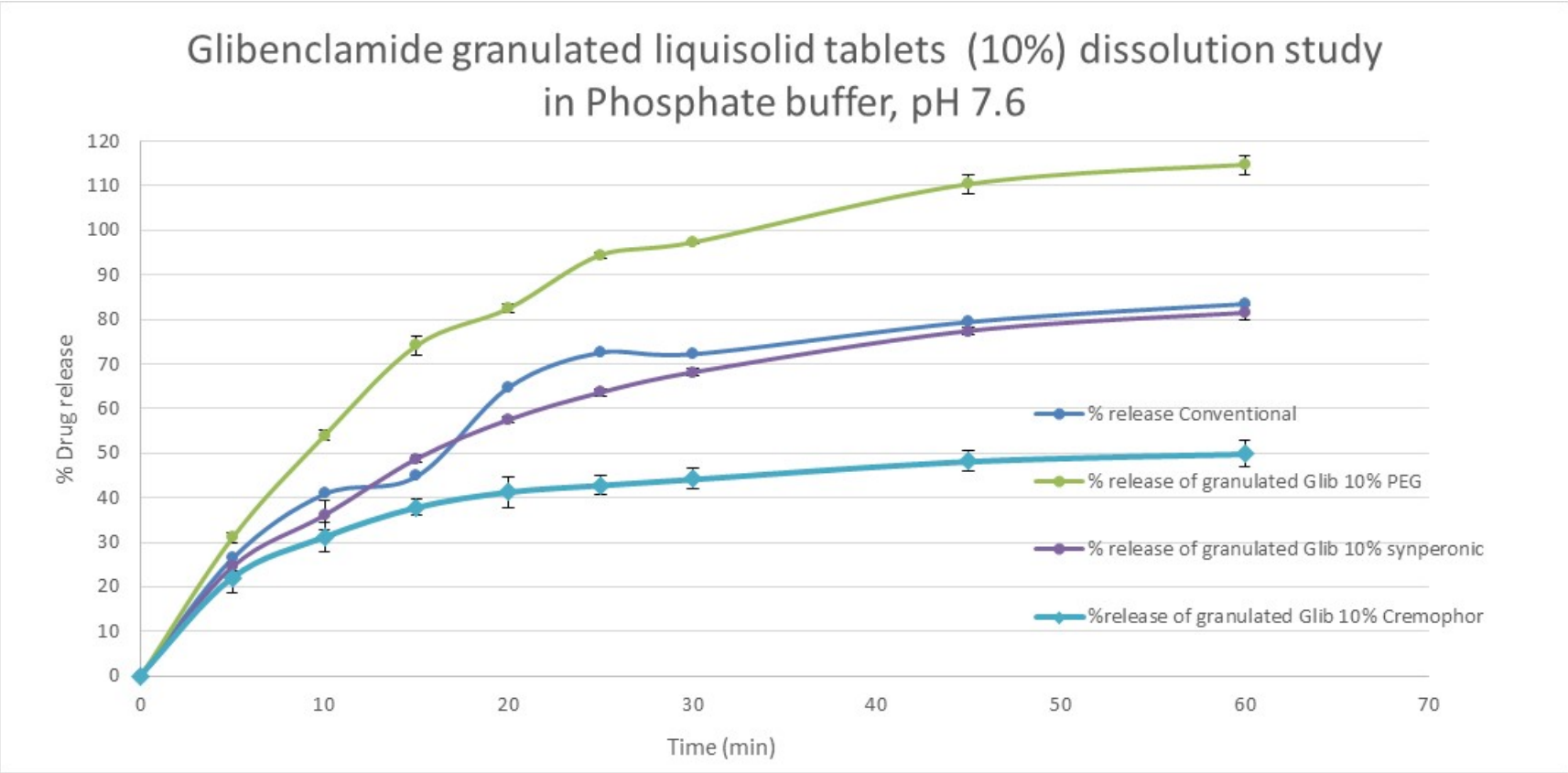


Figure 3.8a: Dissolution results of glibenclamide granulated liquisolid and conventional tablets (10% drug concentrations) in phosphate buffer, pH 7.6.

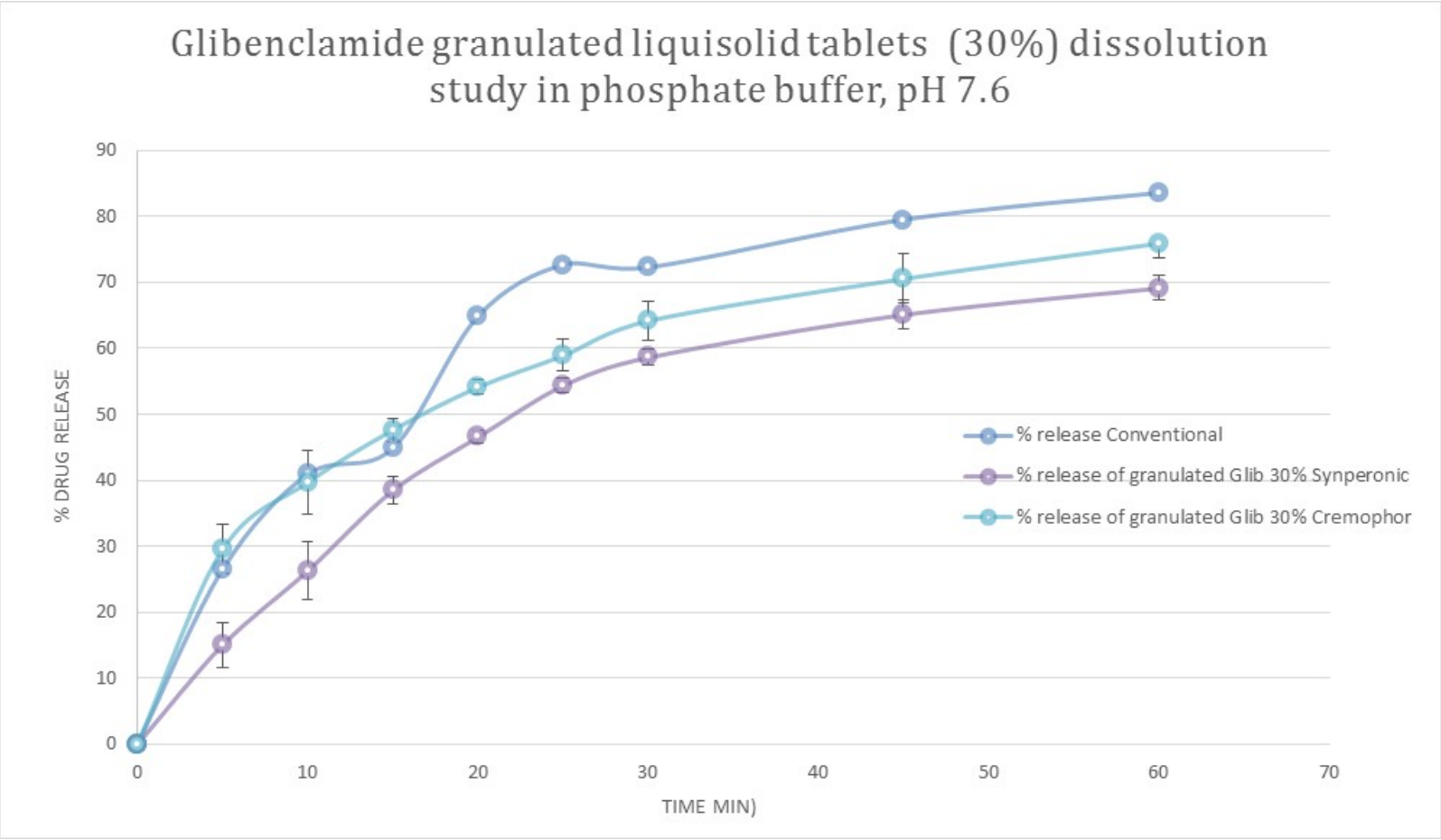


Figure 3-8b: Dissolution results of glibenclamide granulated liquisolid and conventional tablets (30% drug concentrations) in phosphate buffer, pH 7.6.

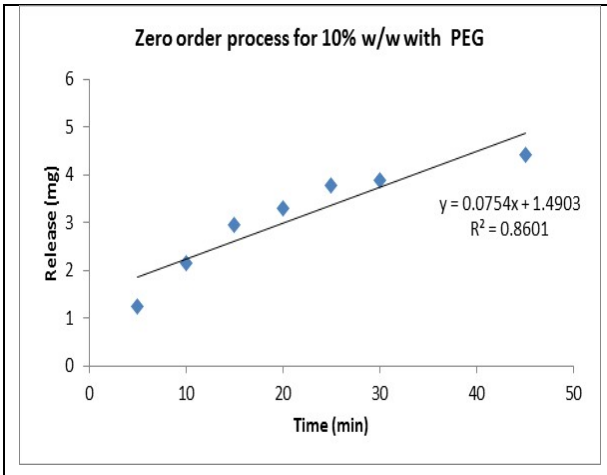
Basically, enhanced dissolution rate revealed in liquisolid formulations is due to the mechanical treatment with the hydrophilic liquid vehicle, which increased the contact between the drug, and the dissolution medium which was distilled water (Yadav & Yadav, 2010). In other words, surfactants increase the wettability of the drug making them more available for water molecules to penetrate and thus increase the drug dissolution and improve the absorption from the GI tract. Hence, to relate the results to the equation, when formulating the liquisolid tablet, the drug particle was dispersed in the hydrophilic liquid vehicle which increases the diffusion coefficient of the dissolved drug particle, which is affected by the viscosity of the dissolution medium. In this study, PEG®400 behaved with glibenclamide differently when compared to other drug in other literature as it showed enhanced dissolution (Javadzadeh, et al., 2007), but with glibenclamide no great effect was observed (see Figure 3.7). Therefore, not all liquisolid vehicles work to the same extent with all hydrophobic drugs. The reasoning behind one formulation having higher dissolution rate than another is due to the solubility effect of the liquid vehicle itself. In other words, if the drug was soluble in that liquid then it can be predicted that formulation will have higher dissolution profile. On the other hand, comparing drug release from conventional tablets, the extent of the drug release was about 15% (Figure 3.7) and only 10% of the drug were released after 10 minutes. For PEG®400 containing liquisolid formulations, the drug release was lower (however not significant $P>0.05$) in the case of ungranulated compacts prepared manually. Presence of Synperonic®PE/L44 and Cremophor®ELP enhanced glibenclamide release better than PEG®400 did. Hence, based on this study, PEG®400 is not a good choice of liquid vehicle to enhance glibenclamide dissolution in water. However, higher values were obtained in phosphate

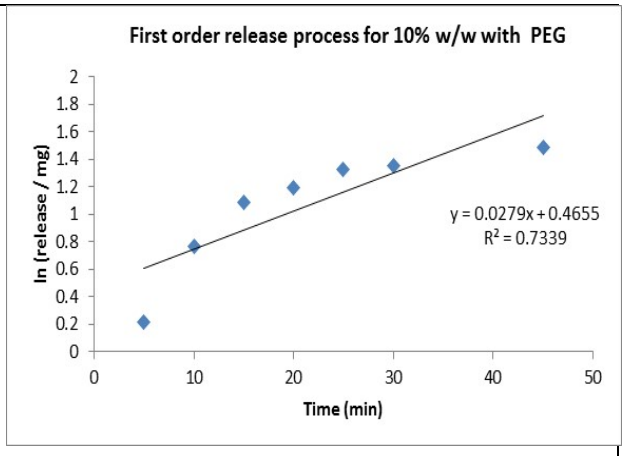
buffer.

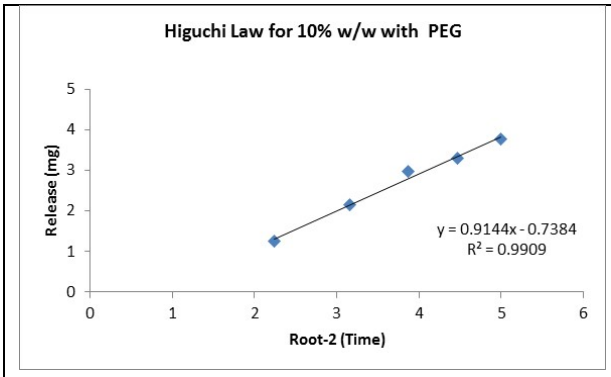
Interestingly, Cremophor®ELP containing liquisolid compacts, which was prepared after granulation resulted in the highest drug release (>50% after 20 min in dissolution media) (Figure 3.7 & Figure 3.8). The same formulation without the wet granulation step could not be compacted into tablets even manually (Figure 3.4). On the other hand, as seen in Figure 3.7, the granulated liquisolid tablets showed better release and thus higher dissolution rate compared to the ungranulated ones. In a similar research paper, (Javadzadeh, et al., 2007) used PVP not as a liquid binder to produce granules. PVP was used to increase the load factor of the liquisolid formulations and therefore witness an increase in dissolution rate with the presence of PVP. Moreover, because of its excellent solubility, PVP normally has no delaying effect on the dissolution rate of the pure drug and higher rate of dissolution is seen than that of the pure drug. According to Javadzadeh et al 2007, the reason behind this enhancement could be due to the “crystal growth inhibition”, which refers to one the characteristics of PVP that inhibits the precipitation of the drug from the saturated solution. Additionally, as a result of the adsorption of the drug on the carrier, this allows the drug to be exposed to the dissolution medium with a larger surface area leading to a higher dissolution rate. Thus, wet granulation step has shown an advantage to the liquisolid formulation in the release of the hydrophobic drug and enhancement of the dissolution rate (Figure 3.7b) which may be leading to improved oral bioavailability.

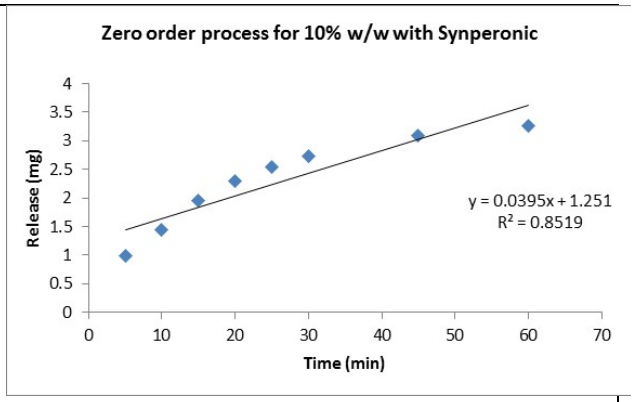
For choosing the most applicable kinetic model of the drug release in phosphate buffer from the granulated glibenclamide liquisolid formulations, goodness of fit test must be performed (Refer to Figure 3.9 for examples). The results for coefficient of determination

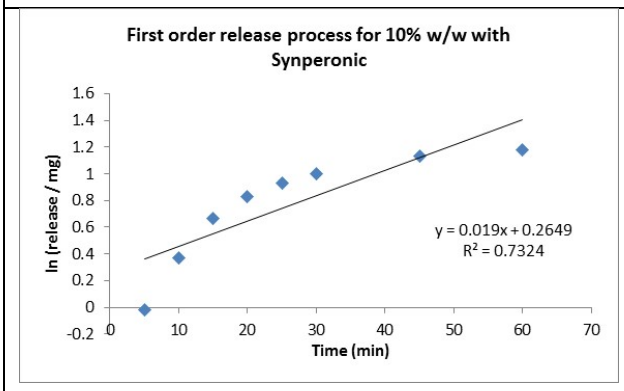
(R^2) for glibenclamide liquisolid granulated tablets were revealed that the release of the drug was governed by the Higuchi model (Figure 3.8). Higuchi model describes the release of the drug from a matrix, in this case the matrix is the carrier mixed with the liquid vehicle, as a square root of time, which is linked to Fick's Law of diffusion (Prabakaran, et al., 2003) This means that the driving force for diffusion of metformin-HCl molecule from the tablet into the dissolution medium is the drug concentration gradient between tablet and dissolution medium (Elkordy, et al., 2013), (Javadzadeh, et al., 2007). Also, this can explain the slow diffusion rate of the drug as the distance of diffusion is increased which is the Higuchi model (Square root of time). The zero-order model can be best described when drug release rate is independent of its concentration. Whilst the first-order model is when the drug release is dependent on its concentration.

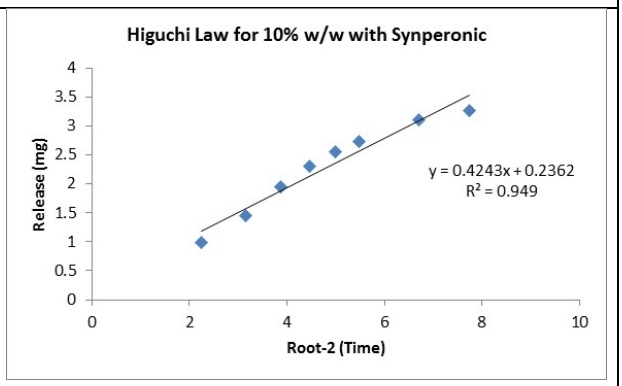


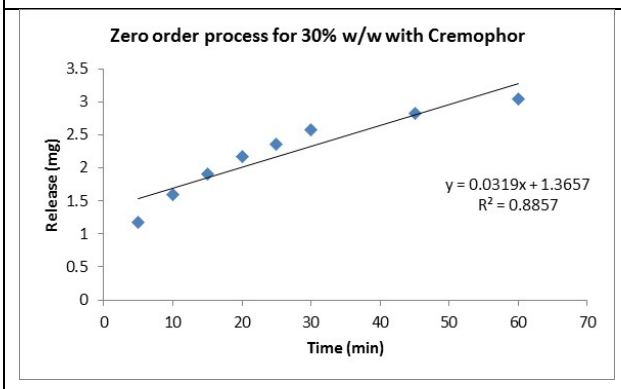


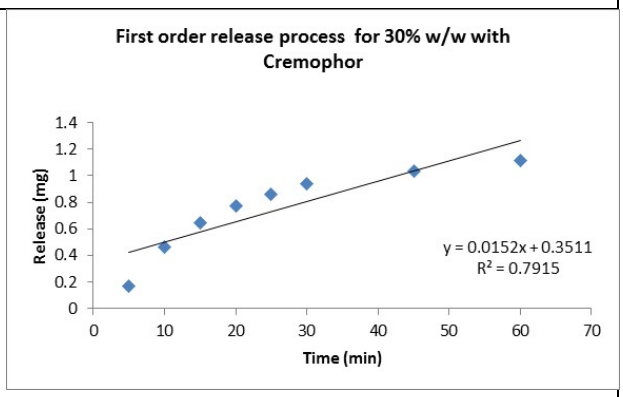

Higuchi Law for 10% w/w with PEG
Release (mg)
Root-2 (Time)
y = 0.9144x - 0.7384
R² = 0.9909


Zero order process for 10% w/w with Synperonic
Release (mg)
Time (min)
y = 0.0395x + 1.251
R² = 0.8519


First order release process for 10% w/w with Synperonic
ln (release / mg)
Time (min)
y = 0.019x + 0.2649
R² = 0.7324


Higuchi Law for 10% w/w with Synperonic
Release (mg)
Root-2 (Time)
y = 0.4243x + 0.2362
R² = 0.949


Zero order process for 30% w/w with Cremophor
Release (mg)
Time (min)
y = 0.0319x + 1.3657
R² = 0.8857


First order release process for 30% w/w with Cremophor
Release (mg)
Time (min)
y = 0.0152x + 0.3511
R² = 0.7915

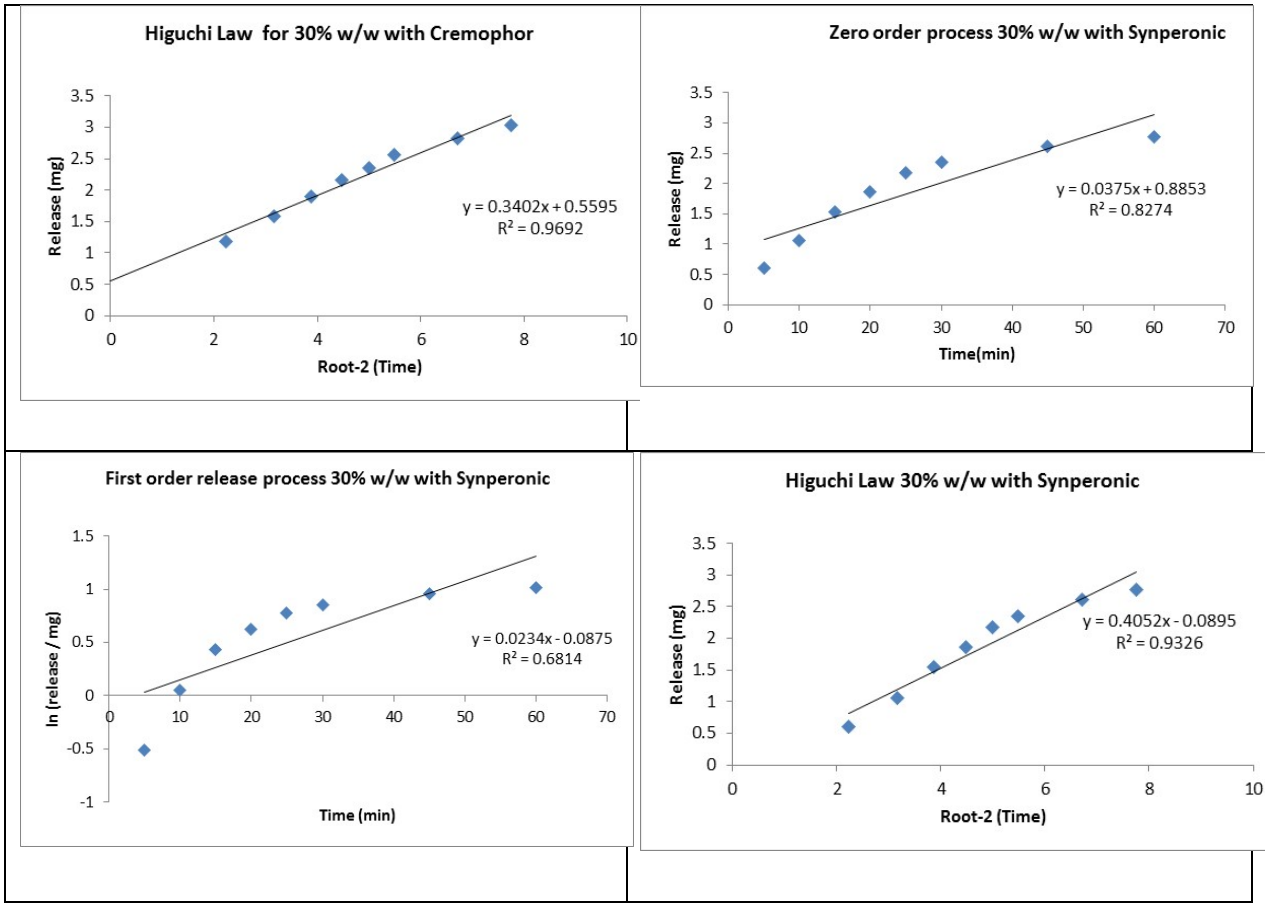


Figure 3-9: Kinetic release models of glibenclamide from some granulated liquisolid tablets (10% and 30% drug concentrations) in phosphate buffer, pH 7.6.

3.4.2. Uniformity of tablet weight

The permitted weight variation in BP is, not more than two tablets differ from the mean by more than 5% if the average weight of the tablet was 250 mg or more, and for less than 250 mg, the percentage deviation is 7.5 %. In this case, all prepared formulations (Table 3.3), including the conventional tablet except Synperonic®PE/L44 and glibenclamide 30%w/w (G4), did comply with British Pharmacopeia (BP) specifications. The above uniformity of weight test was performed only on granulated liquisolid tablets whilst non-granulated tablets were not tested for any quality control measures due to elastic properties of these powders.

3.4.3. Tablet hardness

Generally, ideal tablet hardness should be produced without applying excessive compression force where rapid tablet disintegration and drug dissolution are maintained at the same time. In this study, G1 and G6 (table 3.1) showed best tablet hardness (5.93 Kg/f (58N) and 5.03 kg/f (49 N) respectively) compared to the other formulations. There are several factors tablet hardness depends on such as particle size, interparticle force and most importantly compression force. Usually, as compression force increased, tablet hardness and fracture resistance also increases. Additionally, (El-Say, et al., 2010) found a relationship between liquid load factor and the hardness of the tablets in the formulae having approximately the same powder excipient ratio. When liquid load factor increased, the hardness of the tablet decreased. However, this was not supported in this study; the data obtained showed no relationship to the liquid load factor of the liquid vehicle used. Our hypothesis is that not only the liquid load factor has an effect on the hardness of the tablet, but also granulation process that was performed before compaction (refer to table 3.3). For example, G3 formulation, although hardness was small (1.8kg), but the tablet complied with friability test (<1%).

3.4.4. Friability

All the liquisolid and conventional tablets complied with British pharmacopoeia 2017 (BP) limits (less than 1%) of the percentages of weight loss in friability tests (Table 3.3). The tablets showed no crack, chipped or broken marks during the test. Hence, liquisolid

tablets can withstand any handling and packaging attritions applied to them without losing their physical properties.

Table 3-3: Summary of results expressed by the average of tablet hardness (N), tablet uniformity weight (mg), friability (%) and drug content (%) for all granulated liquisolid (G1-G6) and conventional glibenclamide tablets.

FORMULATION	HARDNESS (N)	CRUSHING LOAD (KG/F)	FRIABILITY (%)	DRUG CONTENT (%)	UNIFORMITY OF WEIGHT (MG)
G1 (GRANULATED)	58.21 ± 11.2	5.93 ± 1.14	0.60	107.00	307 ± 7.70
G2 (GRANULATED)	84.86 ± 12.9	8.65 ± 1.32	0.05	98.00	109 ± 1.90
G3 (GRANULATED)	17.82 ± 5.63	1.82 ± 0.57	0.40	102.00	211 ± 2.80
G4 (GRANULATED)	23.87 ± 3.44	2.43 ± 0.35	0.23	85.00	179 ± 15.3
G5 (GRANULATED)	38.10 ± 0.74	3.88 ± 0.08	0.06	104.00	248 ± 7.40
G6 (GRANULATED)	49.38 ± 1.34	5.03 ± 0.14	0.50	103.00	251 ± 1.30
CONVENTIONAL	52.32 ± 16.5	5.33 ± 1.68	0.60	98.00	256 ± 10.1

3.4.5. Disintegration

The presence of a non-volatile liquid vehicle is expected to delay the disintegration time of the tablet. However, the presence of microcrystalline cellulose (MCC) speeds the process of disintegration giving a low disintegration time (Javadzadeh, et al., 2007). In addition to that, some literatures suggest that high hardness tablets are associated with long disintegration time. Disintegration time can be affected by the type of liquid vehicle used and also by other properties such as tablet porosity and pore structures (Tiong & Elkordy, 2009). Studies suggest that there is a positive relationship between tablet hardness and disintegration time. In other words, when the hardness increases, the disintegration time increases (Kitazawa, et al., 1975). Additionally, in this research, G6 (refer to table3.1 for composition) records the lowest disintegration time of about 9 seconds and the longest time was seen in G2 (15 min). Figure 3.10 presents the average

disintegration time results of the liquisolid and conventional formulations. All formulations including the conventional tablets showed disintegration time below 15 min except for G2. This proves to some extent that the higher the crushing force, the longer the disintegration time. Hence, the relationship between tablet hardness and disintegration time is directly proportional. Thus, the way to overcome poor disintegration time is to reduce the compression force. Nevertheless, all glibenclamide liquisolid tablets including the conventional tablet did comply with BP specification of disintegration time meaning that liquisolid technique was successful to produce enhanced disintegrated (hence enhanced drug release as shown above) glibenclamide tablets.

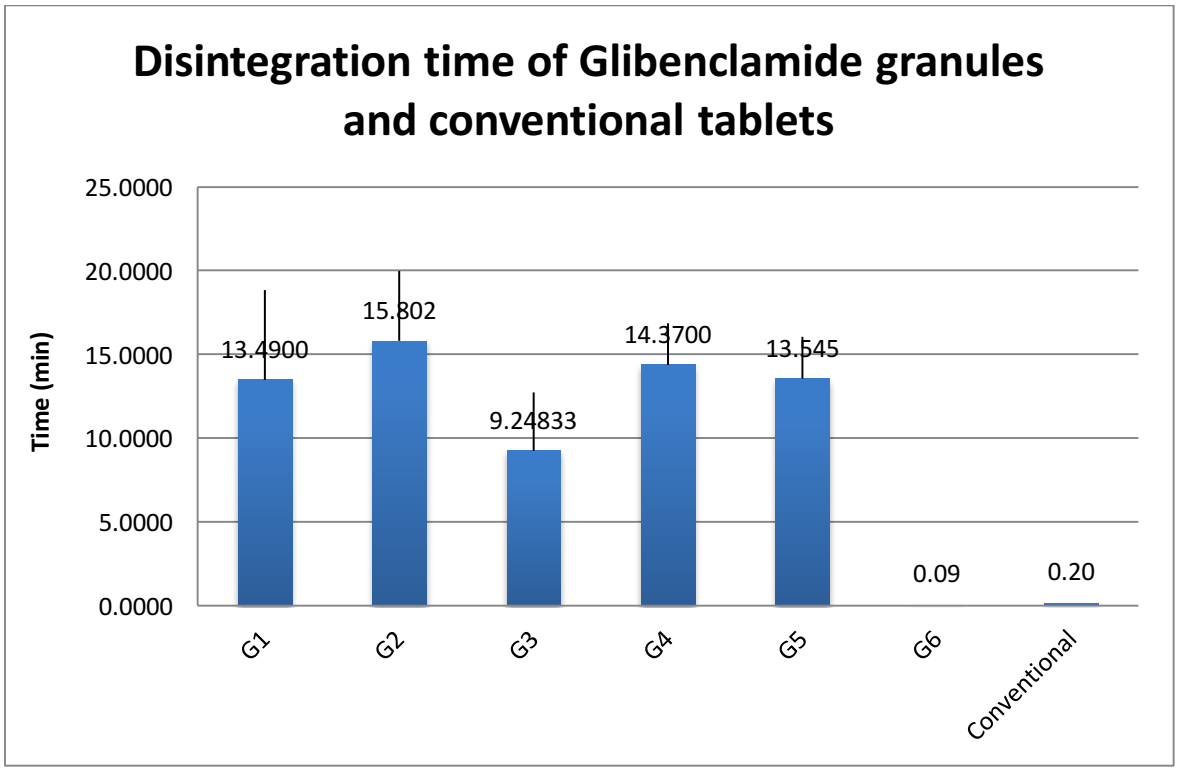


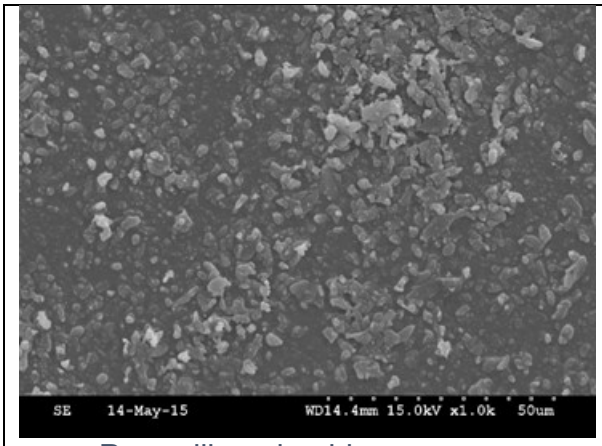
Figure 3-10: Average disintegration time of glibenclamide liquisolid granulated tablets and conventional tablets. (Refer to Table 3.1 for formulations' composition)

3.4.6. Drug content

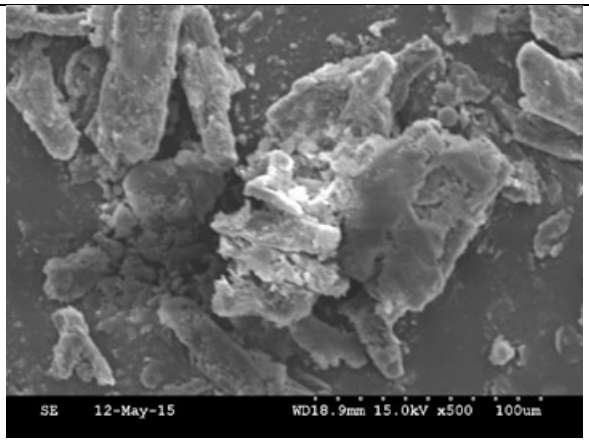
British Pharmacopoeia 2017 drug content uniformity criteria suggests an accepted limit between 85% and 115% of the average drug content. Accordingly, all liquisolid (G1-G6 granulated, and G1-G4 ungranulated) (Table 3.3) preparations except conventional tablets complied with BP limit test. However the drug content for G4 was less than other granulated formulations and this can be explained on the basis of large standard deviations obtained from weight uniformity results of G4. Moreover, there are no significant differences ($p > 0.05$) between either granulated and ungranulated as well as conventional tablets. In other words, the presence of PVP and different quantities of liquid vehicles carrier and coating did not affect the drug uniformity results. Thus, liquisolid granules prepared by wet granulation technique using PVP show similar results to non-granulated liquisolid tablets. Literature suggests that the adsorptive properties of liquisolid system onto the carrier, which increases the surface area, could lead to a homogeneous drug distribution within the batch (Fahmy & Kassem, 2008). It was also suggested by (Elkordy, et al., 2012), that the large volume of the liquid vehicle, carrier and coating being used affects the drug uniformity. Their results showed batches with higher liquid vehicle concentration (10% w/w) complied with BP content uniformity specifications (Cremophor®EL 20%, Synperonic®PE/L61 20% and Capryol™ 90 20%. The reason is that the drug would need larger volume of liquid vehicle to be dispersed in, thus a larger amount of carrier and coating would be needed to produce a free flowable powder which leads to an even drug content.

3.5. Scanning electron microscopy (SEM)

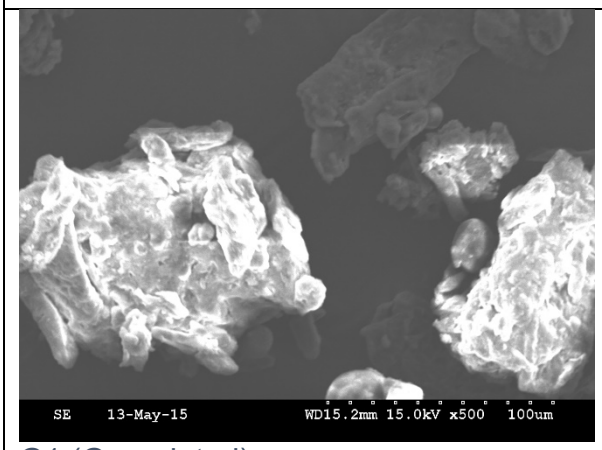
SEM photomicrographs that show the surface morphology of the liquisolid formulations (G1-G6 granulated, G1–G6 ungranulated) including conventional and pure drug are displayed in Figure 3.11. Pure glibenclamide powder observed to be crystalline in nature with fine particles which consists of flat shape. Also, conventional preparation showed crystals on the surface of the carrier material. The scanning electron microscopy of the liquisolid particles reveals irregular shapes with some long or rod-like structures which usually represents the shape of Avicel®PH102 (Elkordy, et al., 2013). The change in shape provides additional surface for deposition of the drug particle into the liquid vehicle. Thus, the disappearance of glibenclamide crystals to large extent suggests solubilisation of the drug into the liquisolid admixture, as will be confirmed by the DSC data. This may be contributed to the enhanced dissolution properties of the drug (Altememy & Altememy, 2014). Furthermore, as seen in Figure 3.11, the SEM photomicrographs show different particle shape between granulated and ungranulated formulations. The granulated glibenclamide liquisolid formulations present with joint agglomerates, whereas the ungranulated particles appear to be less bonded with rod-like structure shapes. This can be indicative of the enhanced flowability using PVP in wet granulation. Liquisolid drug particles after granulation become combined into a general sphere like shape that not only means the drug has been dispersed well with the carrier and coating materials, but also demonstrates the unique flow characteristics associated with this technique.



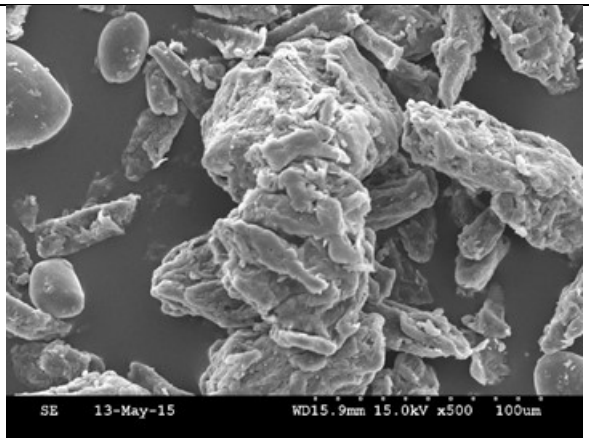
Pure glibenclamide



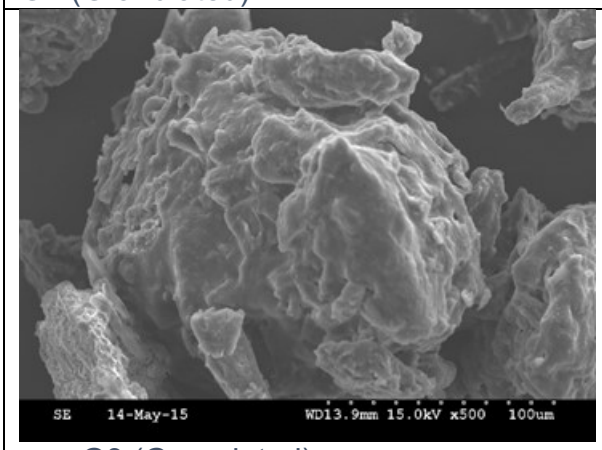
Conventional glibenclamide



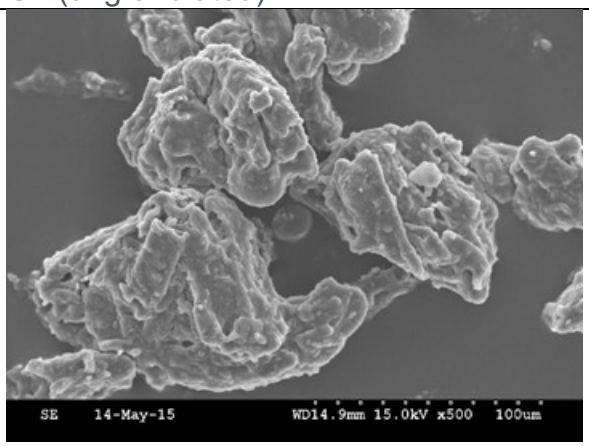
G1 (Granulated)



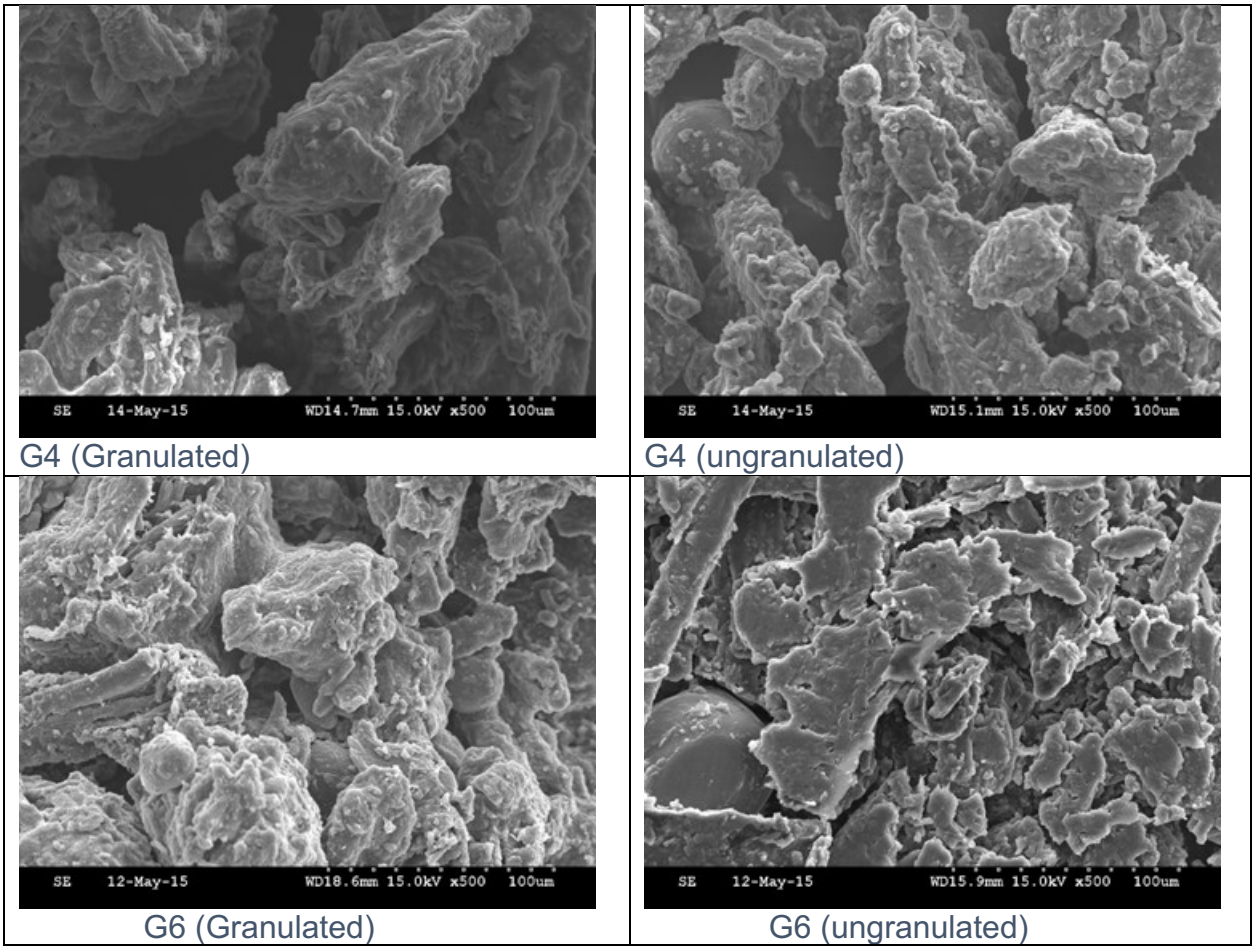
G1 (ungranulated)



G3 (Granulated)



G3 (ungranulated)



G4 (Granulated) G4 (ungranulated)

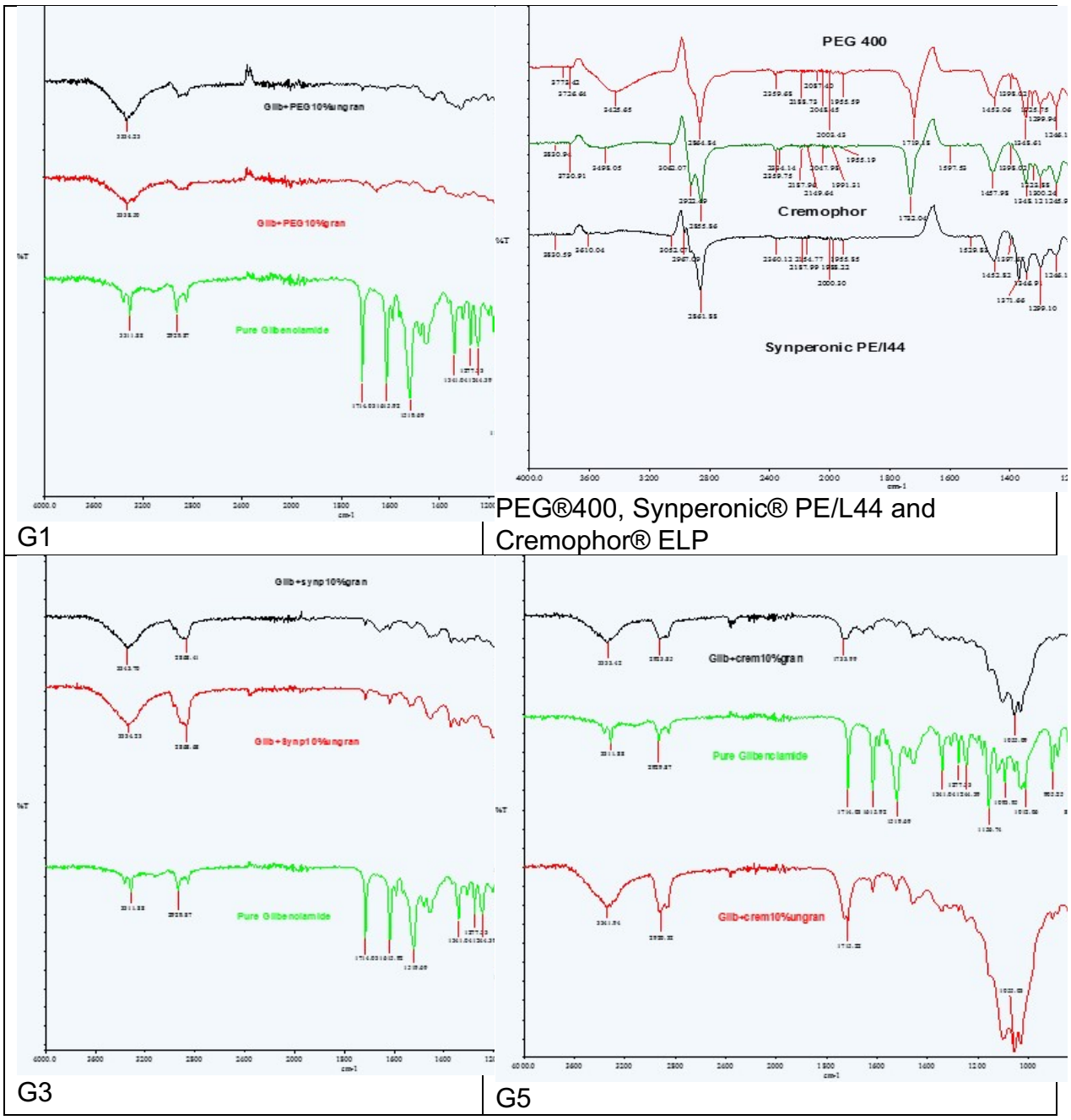
G6 (Granulated) G6 (ungranulated)

Figure 3-11: SEM photomicrographs for pure (x1000) and conventional glibenclamide, and glibenclamide liquisolid powders and granules (x500). Refer to Table 3.1 for formulations' composition.

3.6. Fourier transform infrared spectroscopy (FTIR)

Figures 3.12 illustrate the Fourier transform infrared spectral information of pure glibenclamide powder, excipients (Avice®PH102, Cab-O-sil®M-5, potato starch, Mg stearate and PVP) and non-volatile liquid vehicles (PEG®400, Synperonic®PE/L44, Cremophor®ELP). Additionally, liquisolid formulations (G1-G6) (refer to Table 3.1 for composition) in both granulated and ungranulated forms. The characteristics fingerprint FTIR peaks between 1500 and 1800 cm^{-1} for glibenclamide, the C=O stretching region

can be seen in Figure 3.12. It shows noticeable change in the IR spectra associated with liquisolid preparations. The peaks were either shifted or disappeared with even reduced intensity, suggesting interaction between the drug (glibenclamide) and the excipients used (Suliman, et al., 2013). Moreover, bands between 1000-1200 cm^{-1}, show reduction in percentage transmittance which is the C-O stretching region, implies to an interaction between drug and non-volatile liquid vehicles leading to hydrogen bonding between glibenclamide and the liquid vehicle. One of the contributing factors that can affect the drug's solubility is the formation of hydrogen bonding between the drug and liquid vehicles and thus accelerates dissolution rate (Elkordy et al 2013). Also, the comparison between ungranulated and granulated liquisolid glibenclamide do not show any change in their IR spectra, both correspond to the same peak number. Thus, the granulation process using PVP does not lead to any interaction with glibenclamide. Consequently, FTIR spectra analysis complies with DSC results where the crystallinity of glibenclamide was decreased leading to the solubilisation of glibenclamide crystals in the liquid vehicles.



G1 | PEG®400, Synperonic® PE/L44 and Cremophor® ELP

G3 | G5

Figure 3-12: FT-IR spectra of pure glibenclamide powder, liquisolid formulations (G1, G3 and G5) in powder and granules form and excipients used (PEG®400, Synperonic®PE/L44 and Cremophor®ELP. Refer to Table 3.1 for formulations' composition.

3.7. Differential scanning calorimetry (DSC)

The DSC thermograms of pure glibenclamide, granulated and ungranulated liquisolid glibenclamide including conventional powder should be in the range of the melting temperature of glibenclamide in order to define the effect of liquisolid and wet granulation on the drug. The presence of any change or shift in the melting temperature indicates the outcome of PVP in wet granulation as well as the effect of liquid vehicles and other excipients. Formulations G1-G6 (refer to Table 3.1 for composition), the excipients, pure glibenclamide and conventional DSC thermograms are shown in (Appendix for Chapter 3) and Table 3.4 exhibits the DSC transition temperatures. The thermogram of pure glibenclamide clearly shows a melting temperature of 175.9 ^{0}C and an enthalpy value of 91.5 J/g. At the 175.9 ^{0}C, the drug was in melting state with a sharp peak, which corresponds to the crystalline form of the drug (Baskaran, et al., 2016). Furthermore, in the liquisolid formulations (PEG®400, Synperonic®PE/L44 and Cremophor®ELP), the sharp endothermic peak at the drug melting point was either disappeared from granulated liquisolid containing PEG®400 (G1 and G2), and Cremophor®ELP (G3 and G4) liquid vehicles (which corresponds to the solubilisation of the drug in the liquid vehicle or causing change to the amorphous state of glibenclamide which was reflected in enhanced drug release as shown above in Figure 3.7a and b) or broadened with reduced enthalpy with granulated liquisolid containing Synperonic®PE/L44 (G5 and G6). In addition to that, no interaction between drug and excipients were shown in the DSC thermograms but compatibility was shown. In more detail, PVP granulated liquisolid formulations thermograms display small changes at a temperature range of 110-130 ^{0}C in their endothermic peaks compared to the ungranulated formulations. The probable reason

could be that the interaction between the non-volatile liquid vehicles and drug particles are much larger than that between PVP, excipients and glibenclamide. Nevertheless, a slight shift in the endothermic peaks in all granulation methods is evident compared to the ungranulated formulations. Also, (Albertini, et al., 2004), debated whether the appearance is due to drug interaction with PVP or with other excipients. It can be concluded that the enhanced dissolution rate of glibenclamide is not only due to the interaction between the drug and the excipients or reducing the crystallinity of the drug, but also due to the wettability of the drug particles in the liquisolid systems by the aid of the liquid vehicle, this is in agreement with the study by (Javadzadeh, et al., 2007).

Table 3-4: DSC transition temperatures of pure glibenclamide conventional powder, drug liquisolid granulated and ungranulated formulations (G1 – G6).

Composition*	Transition temperature (^{0}C)
Pure glibenclamide	175.88
Conventional	109.38, small peak at about 176
G1 (granulated)	110.19
G1 (ungranulated)	130.47
G2 (granulated)	118.56
G2 (ungranulated)	117.87
G3 (granulated)	131.79
G3 (ungranulated)	133.94
G4 (granulated)	127.27
G4 (ungranulated)	136.17
G5 (granulated)	111.69, 193.26
G5 (ungranulated)	106.70, 141.08, 189.85
G6 (granulated)	127.64, small peak at 174
G6 (ungranulated)	139.27

* Refer to Table 3.1 for formulations' composition.

3.8 Conclusion

Liquisolid technique changes the properties of glibenclamide by inclusion of the drug particles (10%w/wand 30%w/w) in non-volatile liquid vehicles (PEG® 400, Synperonic®PE/L44 and Cremophor ®ELP). Wet granulation of prepared liquisolid powders successfully produced automatically compactible tablets using the above-mentioned liquid vehicles. The best dissolution enhancement was seen with combination of Cremophor® ELP with glibenclamide (10%w/w). However, the viscous behavior of Cremophor ®ELP makes the flowability and automatic compaction of liquisolid powders containing Cremophor® ELP very challenging. Using the new technique of wet granulation developed in this study, those problems were resolved and well compressed tablets were developed that were in compliance with B.P standards. Therefore, it can be concluded, that the problem with liquisolid compacts, which is flowability and compressibility, has been resolved by the introduction of wet granulation system to liquisolid powder admixture before compression. This new technique enhances not only the dissolution of the hydrophobic drug, but also the formation of wet tablets into strong well-compacted tablets with no lamination or capping. The liquisolid system can use any type of viscous liquid vehicles in its composition and by application of wet granulation technique liquisolid powders can be compacted into tablets.

Chapter 4 - Application of liquisolid technology using wet granulation on metformin-HCl: Immediate release and permeability studies

Results and discussion

Figure 4.1: Flowchart summary of immediate release metformin-HCl liquisolid preparation

4.1. Introduction

The main goal in diabetic patients is to achieve controlled blood glucose levels with the aid of insulin or antidiabetic drugs such as metformin-HCl. However, successful use of metformin-HCl therapy is challenged due to the high incidence of associated gastrointestinal symptoms such as diarrhoea and abdominal discomfort (BNF 73, 2017). Additionally, metformin-HCl has a multiple dosage regimen of 500mg two to three times daily or 850 mg once or twice per day. Therefore, such complications are not desirable and should be avoided to maintain patient compliance. Moreover, metformin-HCl presents other formulation challenges such as poor compressibility and poor permeability (Nanjwade, et al., 2011). According to the biopharmaceutical classification system (BCS), metformin-HCl belongs to class III with high water solubility and low permeability properties and due to metformin-HCl's poor compressibility properties, direct compression is difficult on the commercial scale. This leads to poor content uniformity as well as poor weight content uniformity, hardness and friability (Nanjwade, et al., 2011).

The aim of the work presented in Chapter 4 is to utilise liquisolid-based formulations of metformin-HCl as immediate release preparations whereas in the following chapter the formulation of metformin-HCl as a sustained release drug will be discussed. By use of liquisolid technique, a reduction in drug dosage was successfully achieved for both release types, meaning less costs for pharmaceutical industry and fewer side effects for patients. For both metformin-HCl liquisolid immediate and sustained release formulations, PEG®400, Synperonic®PE/L44 and Cremophor®ELP were used as non-volatile liquid vehicles. Additionally, wet granulation using PVP was applied to both systems in order to

achieve acceptable powder flowability and compressibility. Also tested and discussed in this chapter is the immediate release liquisolid metformin-HCl tablets used to perform permeability test using FRANZ Diffusion Cell to study the permeability release of immediate release metformin-HCl through Wistar rat stomach membrane.

4.2. Determination of the angle of slide (θ) for determination of flowable liquid retention potential (Φ values) for Avicel®PH102 (MCC) and Cab-O-sil®M-5 (fumed silica)

Flowchart Figure 4.1 shows the newly designed procedure. (Spireas & Sadu, 1998) claimed that the liquisolid technology theory indicates that drug, upon dissolving in the liquid vehicle, becomes merged into the carrier material. The carrier (Avicel®PH102), has entangled fibres as cellulose in its interiors and also has a porous surface where adsorption and absorption occurs. In other words, the non-volatile liquid gets absorbed in the interior of the particles where then the saturation of this procedure leads to the adsorption of the liquid onto the internal and external surface of Avicel®PH102 (carrier). Thus, the adsorptive coating material and its large surface area offers the desirable flow properties to the liquisolid system. Additionally, the angle of slide is used to determine the flowable liquid-retention potential in order to calculate the Lf (as explained in sections 1.4 and 3.1). According to Spireas et al 1992, angle of slide is the preferred technique to obtain the flowability of small particle size powders (less than 150μm.) The chosen non-volatile liquid vehicles were PEG®400, Synperonic®PE/L44 and Cremophor®ELP. The Φ values for Avicel®PH102 (MCC) and Cab-O-sil®M-5 using the three liquid vehicles were obtained. Drugs in liquisolid formulations, with lower concentration require more liquid

vehicles, carriers and coating materials to produce larger tablets. Additionally, literature values show that the liquid load factor (Lf) is inversely proportional to the amount of carrier and coating used, and it increases with high viscosity liquid vehicles (refer to section 3.1 for more details). Table 4.1 reveals results for Lf value.

Table 4-1: Formulation of liquisolid systems of immediate release metformin-HCl (m=no granulation; F= with granulation)

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
M1	PEG® 400	30	20	0.17	93.30	40.00	794	40.00	51.00	8.00	1026.30
M2	PEG® 400	60	20	0.17	26.70	40.00	397	20.00	26.00	4.00	513.70
M3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501	25.00	35.00	5.20	699.50
M4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251	13.00	18.00	2.60	351.30
M5	Cremophor®ELP	30	20	0.52	93.30	40.00	256	12.80	21.00	3.20	426.30
M6	Cremophor® ELP	60	20	0.52	26.70	40.00	128	6.40	11.00	1.60	213.70
Conventional	–	–	–	–	–	40.00	397	20.00	24.20	3.60	485.00
F1	PEG® 400	30	20	0.17	93.30	40.00	794	40.00	51.00	8.00	1026.30
F2	PEG® 400	60	20	0.17	26.70	40.00	397	20.00	26.00	4.00	513.70
F3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501	25.00	35.00	5.20	699.50
F4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251	13.00	18.00	2.60	351.30
F5	Cremophor® ELP	30	20	0.52	93.30	40.00	256	12.80	21.00	3.20	426.30
F6	Cremophor® ELP	60	20	0.52	26.70	40.00	128	6.40	11.00	1.60	213.70

4.3. Flowability and packing properties of the prepared liquisolid powders

Powder flowability plays a crucial role in handling, packing, mixing and producing of pharmaceutical dosage forms where a set of tablets with even drug distribution is required to be compressed. Uniform powder fed through the hopper is required to maintain uniformity of tablet weight and drug content (Ekordy et al 2012, British Pharmacopoeia 2017, (Mahato & Narang, 2012). Additionally, several factors such as physical, environmental as well as mechanical aspects can affect the liquisolid powder flowability properties. Therefore, to determine the flowability of powder, Carr's compressibility index (CI%) is used. It is a suitable parameter to compare between bulk and tapped densities of powder mass, which provides a measure to the reflecting interparticulate friction, and thus is used to measure the flowability of all liquisolid powders. The calculated Carr's compressibility index of metformin-HCl liquisolids before and after granulation is presented in Figure 4.2. Generally, in accordance to British Pharmacopoeia 2017, any formulation with CI% below 15% indicates good flowability and CI% above 25 % suggests formulation with poor powder flowability.

Therefore, the powder flow properties of the prepared granulated and ungranulated liquisolid metformin-HCl were evaluated using CI%. However, liquisolid formulation using Cremophor®ELP powder flow was very poor due to its high viscosity properties, hence no CI% was obtained before wet granulation stage. The calculated CI% results show that granulated liquisolid formulation have better flow properties than ungranulated ones (F1: 24.5%, F2: 18.6%, F3: 15.7%, F4: 14.6%, F5: 24.2%, F6: 24.5%, refer to Table 4.1 for composition) as well as conventional metformin-HCl showed better flow properties in the granulated form (18%, Figure 4.3).

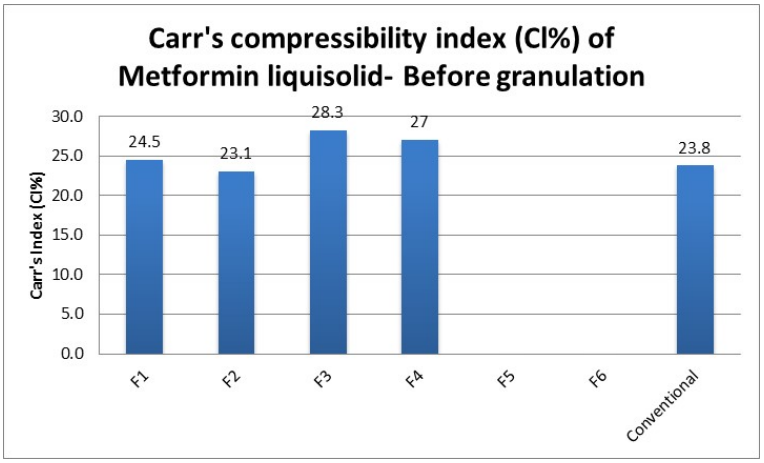


Figure 4.2: Carr's compressibility index of metformin-HCl Liquisolid before granulation. F5 and F6 powders were sticky and hence they were not flowable at all.

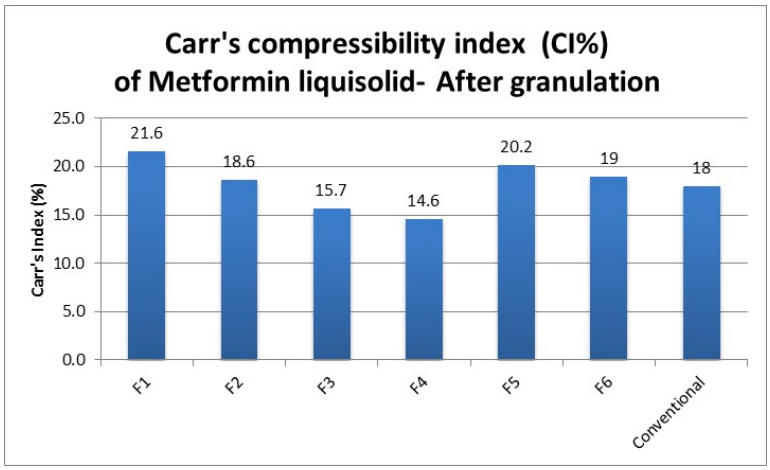


Figure 4.3: Carr's compressibility index of metformin-HCl liquisolid after granulation. For formulations' composition refer to Table 4.1.

This can be related to the amount, type and property of the liquid vehicle used (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) and the effect of wet granulation on the enhancement of powder flow using PVP as a liquid binder. The ungranulated liquisolid metformin-HCl showed fair flowability for F1 and F2 (24.5% and 23.1% respectively) and poor flowability was seen in Synperonic®PE/L44 liquisolid powders with CI% of 28.3% and 27% for F3 and F4 respectively. Also, CI% for F5 and F6 was not calculated due to

its unflowable powder characteristics (Figure 4.1). Thus, this shows that the properties of the liquid vehicle can interfere with the flow property of the formulation making it better or poor flowing.

Literature (Spireas , et al., 1992), (Alderborn, 2013), (Javadzadeh, et al., 2007), (Javazadeh , et al., 2005), (Nokhodchi, et al., 2005) mentioned that the amount of carrier and coating materials in the liquisolid system can play a great role in determination of powder flow property. The liquid vehicle gets adsorbed to the surface of the carrier allowing the coating material (Cab-O-Sil®M-5) to absorb the excess liquid to form a freely flowable powder. Furthermore, only granulated liquisolid metformin-HCl were automatically compacted into tablets. This is due to poor flowability and compressibility properties of the liquisolid ungranulated powder and due to the elasticity properties of the liquid vehicle used, thus powders were manually compressed for the purpose of dissolution and drug content uniformity evaluation. According to (Javaheri, et al., 2014), ungranulated powder admixtures were uncompactible due to the elastic behavior of the liquisolid formulations. The powder not only was not compressed into tablet, but also formed a compactible unwanted shape. Similarly, such powder behavior was experienced by Elkordy et al 2013, where two liquisolid formulations using Synperonic®PE/L61 with Solutol HS 15 (1:1) at both drug concentrations 10% and 30% were incompactible, and hence no tablets were formed. The two non-volatile liquid vehicles have a plastic flow nature when combined, thus tablet formation was unachievable. In a concentrated suspension, weak Van der Waal forces hold flocculated particles together. This often results in plastic flow (Mahato & Narang, 2012). If the shear stress is low, such materials might possess elastic behavior.

However, when the maximum yield stress is exceeded where the Van der Waal forces are broken, plastic flow is exhibited. There must be a balance for plasticity. Although plastic deformation helps the solid links to form between particles within the powder mix, excessive plasticity leads to the formation of tablets with high extent of hardness. The powder mix produced with Avicel®PH102 and the liquid vehicle takes advantage of the elastic properties of the liquid vehicle and the plastic properties of Avicel, leading to the formation of a powder mix that is mainly elastic (Elkordy et al.2013). (Javadzadeh, et al., 2007) suggested using HPMC or PVP as microsystems can improve flowability regardless of the liquid load factor being high. Although the flowability measured using Carr's index in this study was fairly poor after granulation, as per their study, HPMC was only granulated and not the whole formulations. In the current research project, ungranulated F5 and F6 (refer to Table 4.1 for composition) showed the least favourable CI% results of all formulations. The suggested explanation is dependent on the high viscosity property of the liquid vehicle (Cremophor®ELP with viscosity of 650-800 (Rowe, et al., 2003) leading to a non-flowable liquisolid powder admixture. Similarly, (Tiong & Elkordy, 2009) claimed the powder flow of liquisolid naproxen with Cremohpor®EL to be poor due to the high viscosity of the liquid vehicle and high liquid load factor. In agreement with (Javazadeh , et al., 2005), load factor above 0.25 along with high viscosity liquid vehicle can induce poor powder flowability. For the reason that, there will be fewer amounts of carrier and coating materials available in the formulation and excess amount of liquid vehicle being available leading to the formation of agglomerates. As shown in Figure 4.2 and Figure 4.3, liquisolid powders show better Carr's compressibility index when formed into granules.

According to (Javaheri, et al., 2014), liquisolid powders before granulation showed poor flowability and therefore Carr's compressibility index was not performed. However, after using wet granulation using PVP 10%, enhanced flowabiity of liquisolid powder granules was shown even with viscous liquid vehicles such as Cremophor®ELP. Not only did it enhance the flowability, but it also allowed for automatic compression of the liquisolid granules, which was previously unachievable without granulation. Granulated tablets, appeared hard with no capping or cracking and complied with quality control test of the British Pharmacopeia 2017. For this reason, wet granulation system, with a suitable liquid binder, is a successful technique to convert incompactible elastic powder into compactible powders with advanced tablet properties.

4.4. In-vitro dissolution studies of immediate release metformin-HCl

The in-vitro drug release (dissolution profiles) from immediate release metformin-HCl liquisolid tablets (M1-M4 and F1-F6, refer to Table 4.1 for composition), including conventional and pure metformin-HCl were performed in distilled water and are graphically presented in Figure 4.4. It was found that, all the prepared granulated liquisolid tablets released more than 80% of their metformin-HCl content after 60 min. After 5 min in the dissolution apparatus, the percentage released from granulated metformin-HCl liquisolid tablets was found to be 37.6, 41.0, 15.8, 16.0, 20.7 and 20.9 % from F1, F2, F3, F4, F5 and F6 (refer to Table 4.1 for composition) respectively versus 73.8% and 101% from the pure and conventional metformin-HCl respectively. The release for ungranulated liquisolid metformin-HCl showed inconsistent drug release (Figure 4.4). This indicates

that the addition of wet granulation using PVP as a liquid binder to the liquisolid compacts produces a steady with stable release of the drug over the time. Moreover, the extent of drug release for F1-F6 liquisolid formulations after 90 min was 102,103, 91, 100, 105 and 103% for F1, F2 F3, F4, F5 and F6 respectively. Wet granulation process helps in having good flowable liquisolid powders with good compaction and also good drug uniformity content (Table 4.2). The uneven distribution of the drug in the tablets for M3 and M2 causes the drug release to be more than 100% (for M3) and less than 90% (for M2 due to high friability of M2 tablets). This confirms that the liquisolid metformin-HCl when formed into granules using PVP produced a strong binding effect that released the drug gradually. Moreover, the main reason for the stable release of the drug in the presence of PVP might be due to crystal growth inhibition, where PVP may serve to inhibit precipitation of the drug from the supersaturated solution (Usui, et al., 1997) (Javadzadeh et al.2007). According to (Javaheri, et al., 2014), similar outcome was not observed, although 10% PVP was also used for wet granulation process. The ungranulated glibenclamide tablets showed almost no release (however it is a hydrophobic drug but metformin-HCl is a water soluble drug) when compared to the granulated formulations. This can explain, using similar excipients does not necessarily give common results, as the drug's structure and characteristics can play a great role in the release profiles (El-Say, et al., 2010). Additionally, higher dissolution rate was observed in pure metformin-HCl (Figure 4.4) and not for pure gibenclamide; this is basically due to metformin-HCl's solubility properties in water which is not present in the water insoluble drug, glibenclamide.

For the tested liquid vehicles, PEG®400 within granulated liquisolid tablets (30 and 60%

drug concentration) showed fast drug release within the first 10 minutes, while Cremophor®ELP and Synperonic®PE/L44 delayed the drug release over the time (Figure 4.4). This can be explained on the basis of viscosities of the two later vehicles which have effects on the thickness of the diffusion layer around the drug particles (increased thickness) and on the diffusion coefficient (reduce diffusion). Based on the Noyes Whitney equation, these will properties cause the drug release to be reduced. This may be desirable to enhance drug bioavailability by enhancing the drug permeability for the released drug molecules. (Elkordy, et al., 2012).

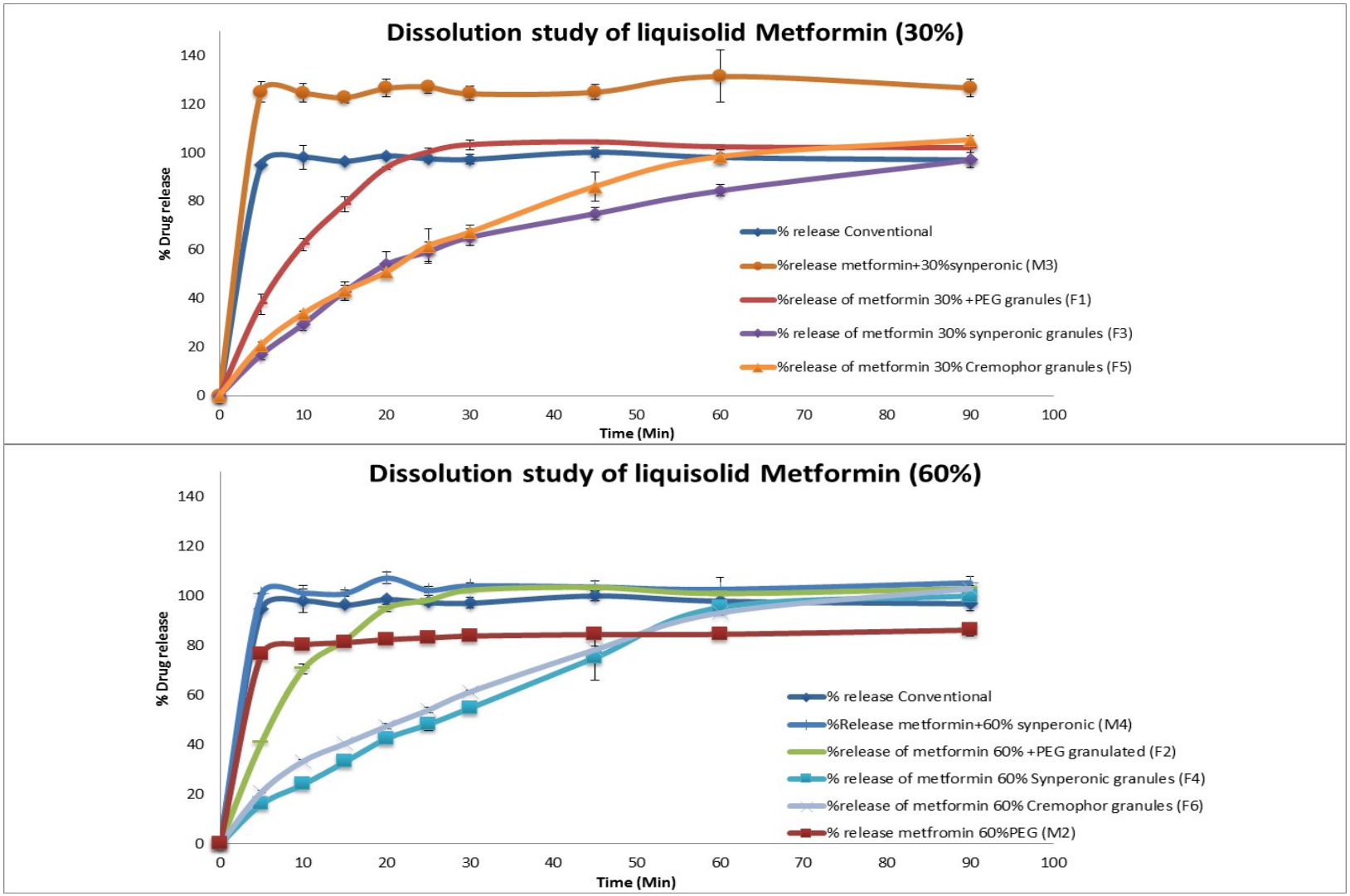


Figure 4.4: Dissolution study of immediate release metformin-HCl liquisolid in distilled water (30% and 60% drug concentrations). Refer to Table 4.1 for formulations' composition.

Enhanced in-vitro dissolution of the drug from liquisolid compacts can be denoted to improved wettability via liquid vehicles Cremophor®ELP, a non-volatile hydrophilic liquid vehicle (surfactant) with HLB of 12-14. It is responsible for wetting of drug particles in the liquisolid system by decreasing the interfacial tension between particle's surface and the dissolution medium causing an increase in the surface area of drug particles. The choice of the carrier can also affect the dissolution of the drug. After exposure to the dissolution medium, the drug being present on the surface of the carrier material dissolves, leading to an initial burst release of the drug that acts as a loading dose. (Butreddy & Dudhipala , 2015). The liquisolid preparations with a higher R value contains a low liquid load factor, a higher amount of microcrystalline cellulose (Avicel®) and a lower amount of the coating material. This was confirmed by the study performed on griseofulvin liquisolid formulations, where the use of microcrystalline cellulose with R-value of 20 resulted in a significant higher percentage of drug release compared to a prepared formulation with low R value (R=10) (Elkordy, et al., 2012)

Several liquisolid studies performed on hydrophobic drugs correlate the enhanced dissolution rate to the increased wettability and surface availability of the lipophilic drug to the liquid medium as a result of drug dispersion within the hydrophilic non-volatile liquid such as PEG®400 ((Butreddy & Dudhipala , 2015). Comparable outcomes were not obtained in the current project due to the high water solubility of metformin-HCl, where the non-volatile liquid vehicle (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) attached their hydrophilic head to the hydrophilic part of metformin-HCl (react differently compared to hydrophobic drugs) allowing for a constant release of the drug. However, the positive effect of liquisolid technique on metformin-HCl was the possibility of dose

reduction (40mg active ingredient) and steady release of the drug by performing wet granulation in the final stage while maintaining the specified therapeutic effect of the drug (permeability enhancement as will be shown later). Nevertheless, liquisolid metformin-HCl release has been prolonged by using HPC-high grade which is another great advantage of applying liquisolid on hydrophilic instead of lipophilic drugs to produce a sustained release formulation (refer to Chapter 5).

Additionally, there are some factors that can affect the solubility of the solid drug in the dissolution medium such as temperature (endothermic or exothermic), dissolution medium (acidic or neutral), crystalline drug structure, presence of solubilising agents and molecular structure of the solute. Moreover, not all liquisolid vehicles work to the same extent with all hydrophobic drugs, as they can work differently depending on the solubility of the drug. Therefore, the presence of other non-volatile liquid vehicles may have affected the outcome. As a result, each formulation had a different dissolution profile depending on its liquid vehicle and its wetting property (El-Say et al. 2010) as seen in this current study (Figure 4.4). Principally, enhanced dissolution rate seen in liquisolid formulations is due to the mechanical treatment with the hydrophilic liquid vehicle, which is believed to raise the contact between the drug, and the dissolution medium (Yadav & Yadav, 2010). In other words, surfactants increase the wettability of the drug making them more available for water molecules to penetrate and thus increase the drug dissolution and improve the absorption from the GI tract. However, the opposite can be seen in some liquid vehicles. According to (Suliman, et al., 2013), conventional tablets gave a higher drug release than the liquisolid formulation using norfloxacin (a hydrophobic drug), this

was explained due to the zwitterionic behaviour of the drug in water. This is observed with hydrophobic nature drugs. Metformin-HCl behaves differently as it is freely soluble in water and results in rapid dissolution from immediate release tablets but this drug is poorly absorbed by the stomach (Hu , et al., 2006). The binding properties of the drug to the liquid vehicle and other excipients too can play a role as exhibited in Figure 4.4. The higher the concentration of the drug (60%) the higher the drug release compared to the 30% containing drug formulations. Ultimately, the hydrophilic liquid vehicle contains two parts, a hydrophilic head and a hydrophobic tail, the tail attracts to the hydrophobic part of the drug allowing for water solubility enhancement. However, in this case since metformin-HCl is highly soluble in water, the opposite is happening, the hydrophilic head binds the hydrophilic part of the drug allowing for gradual release of metformin-HCl (Figure 4.4).

Therefore, it can be concluded that the in vitro dissolution of liquisolid tablets is greatly influenced by wet granulation that causes strong binding, and by other factor such as the type of liquid vehicle used and whether automatic or manual compaction was performed on the powder admixture.

For choosing the most applicable kinetic model of the drug release from the granulated metformin-HCl liquisolid formulations (F1-F6), goodness of fit test must be performed. Table 4.2 shows the results after calculation being performed to get the coefficient of determination (R^2) for metformin-HCl liquisolid granules (F1-F6). The zero-order model can be best described when drug release rate is independent of its concentration. On the other hand, First-order model is when the drug release is dependent on its concentration. The highest coefficient of determination is usually the most desirable model to be used in

which in this case it is the Higuchi model. It describes the release of the drug from a matrix, which is usually insoluble as a square room of time, which is linked to Fick's Law of diffusion (Prabakaran, et al., 2003) This means that the driving force for diffusion of metformin-HCl molecule from the tablet into the dissolution medium is the drug concentration gradient between tablet and dissolution medium. Also, this can explain the slow diffusion rate of the drug as the distance of diffusion is increased which is the Higuchi model (Square root of time).

Table 4-2: Kinetic release mechanism of metformin-HCl

Metformin Liquisolid granules	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)
PEG®400 30% (F1)	0.959	0.869	0.991
PEG®400 60% (F2)	0.872	0.788	0.987
Synperonic®PE/L44 30% (F3)	0.931	0.797	0.988
Synperonic®PE/L44 60% (F4)	0.921	0.790	0.973
Cremophor®ELP 30% (F5)	0.889	0.743	0.972
Cremophor®ELP 60% (F6)	0.931	0.797	0.996

4.5. Tablet hardness, Friability, Disintegration (BP) and Uniformity of drug content and Uniformity of tablet weight

A summary of expressed average and the standard deviations results of tablet hardness, friability, disintegration and drug content for all metformin-HCl liquisolid (F1-F6) and conventional tablets are shown in the Table 4.3. The uniformity of weight test was performed on liquisolid tablets with granulation (F1-F6, refer to Table 4.1 for composition) whilst non-granulated tablets were not tested for any quality control measures due to the

manual compression of the liquisolid powders. Regarding the tablet hardness test, there were variations in tablet thickness and hardness due to the compression force applied. Generally, ideal tablet hardness should be produced without applying excessive compression force where rapid tablet disintegration and drug dissolution are maintained at the same time ((Tiong & Elkordy, 2009), (Fahmy & Kassem, 2008) (Elkordy, et al., 2013). Tablet hardness depends on particle size, interparticle force, and most importantly, compression force. Usually, as compression force increases, tablet hardness and fracture resistance increases as well (Bi, et al., 1999). Additionally, El-Say et al. (2010) found a relationship between liquid load factor and the hardness of the tablets in the formulae having approximately the same powder excipient ratio: when liquid load factor increased, the hardness of the tablet decreased. In this study, M1 has the lowest liquid load factor (refer to Table 4.1) and as a result showed highest hardness (hardness: 11.8 kg/f, 115.7N) and inversely for F1 (8.2kg/f 80.4N), although M1 and F1 have same excipients' ratio but M1 is ungranulated and F1 was prepared from granulated powder mix. The M1 was manually compacted. That would be another reason why the hardness is high in this formulation as a high force was applied when compacting manually. Therefore, our suggestion is that not only the liquid load factor has an effect on the hardness of the tablet, but also the granulation process that was performed before compaction can be a factor and also the binding substance, PVP, as well. In addition, hydrogen bonding between hydrogen groups on Avicel®PH102 molecules can play a role in the strength of the liquisolid tablets (Fahmy & Kassem, 2008). This is because microcrystalline cellulose, MCC (a crystalline structure) undergoes plastic deformation when compression is applied allowing for increased hydrogen bonding through closer contact between the surfaces of

the MCC molecules.

Table 4-3: Crushing load, hardness, friability, drug content uniformity and uniformity of weight for metformin-HCl liquisolid tablets (for formulation compositions, refer to Table 4.1)

FORMULATION	CRUSHING LOAD (KG/F)	HARDNESS (N)	FRIABILITY WEIGHT (%)	DRUG CONTENT (%)	UNIFORMITY OF WEIGHT (MG)
M1	11.80 ± 1.81	115.70	0.26%	103%	270 ± 6.50
M2	7.10 ± 0.52	69.60	10.00%	101%	255 ± 4.50
F1	8.15 ± 0.26	79.90	1.00%	99%	276 ± 1.70
F2	6.83 ± 0.89	67.00	0.87%	99%	263 ± 4.10
F3	6.18 ± 0.26	60.60	0.01%	94%	372 ± 7.40
F4	11.97 ± 0.42	117.40	0.01%	94%	377 ± 14.4
F5	5.98 ± 0.45	58.60	0.10%	96%	231 ± 4.00
F6	6.37 ± 0.24	62.50	0.02%	99%	213 ± 5.90
CONVENTIONAL	4.98 ± 0.97	48.80	0.80%	98%	371 ± 30.0

All liquisolid granulated metformin-HCl tablets complied with BP limits of friability test (<1%). There was no cracking or breaking of tablets after performing the test. However, M2 (refer to Table 4.1 for composition), the ungranulated liquisolid metformin-HCl formulation did not comply with BP and showed a very high friability percentage (10%; this affects its drug release) (Figure 4.4). Therefore, the granulated liquisolid tablets are expected to stay strong and withstand handling, packaging through, transportation.

Rapid disintegration is important during manufacturing of tablets to ensure fast breakup of tablet into small particles, yielding a high surface area for enhanced dissolution (Elkordy et al 2012, (Gubbi & Jarag, 2009), (Chebli & Cartilier, 1998)). Disintegration time can be affected by several factors such as the type of liquid vehicle used, tablet porosity and pore structures (Tiong & Elkordy, 2009). The presence of a non-volatile liquid vehicle

is expected to delay the disintegration time of the tablet. However, the presence of MCC speeds the process of disintegration giving a low disintegration time (Javadzadeh et al. 2007). This is because water uptake into pores is enhanced causing pressure in the tablet pores eventually breaking down of the tablet into smaller particles. In addition, literature suggests a liner relationship between tablet hardness and disintegration time. In other words, as hardness increases, the disintegration time also increases (Kitazawa, et al., 1975). The higher the crushing force, the longer the disintegration time will be. However, this finding was not achieved in this study, as seen in Figure 4.4 and Table 4.3, the slowest disintegration formula was F3 (refer to Table 4.1 for composition) which took 43 minutes to disintegrate followed by F4 (32.5 minutes). The strong binding of PVP and Synperonic®PE/L44 may cause the delay in the disintegration time. Although, microcrystalline cellulose (Avicel®PH102) has disintegration property that can facilitate disintegration and dissolution of drug, However, the presence of non-volatile liquid vehicle (Synperonic®PE/L44) binds to the liquisolid powder and cause a delaying disintegration time (Javadzadeh et al.2007). Therefore, this delay of the disintegration time in F3 in addition to the extensive binding properties of PVP, lower dissolution rate (Figure 4.4). Nevertheless, F1 and F2 showed a faster disintegration time (13.5 min and 13.6 min respectively) compared to F3-F6 (Figure 4.5) and this could be linked to the nature of the non-volatile liquid vehicle used (PEG®400 in F1 and F2). Thereby, F1 and F2 disintegrated within 13.5 min and 13.7 min, respectively and complied with BP disintegration test. Thus, the type of non-volatile liquid vehicle, the quantity of the disintegrant and the quantity and the type of the carrier are the major time controlling factors of the liquisolid tablet disintegration. Taking into consideration, there are no big

difference in hardness of F1, F2, F3, F5 and F6 (Table 4.3 and Figure 4.5).

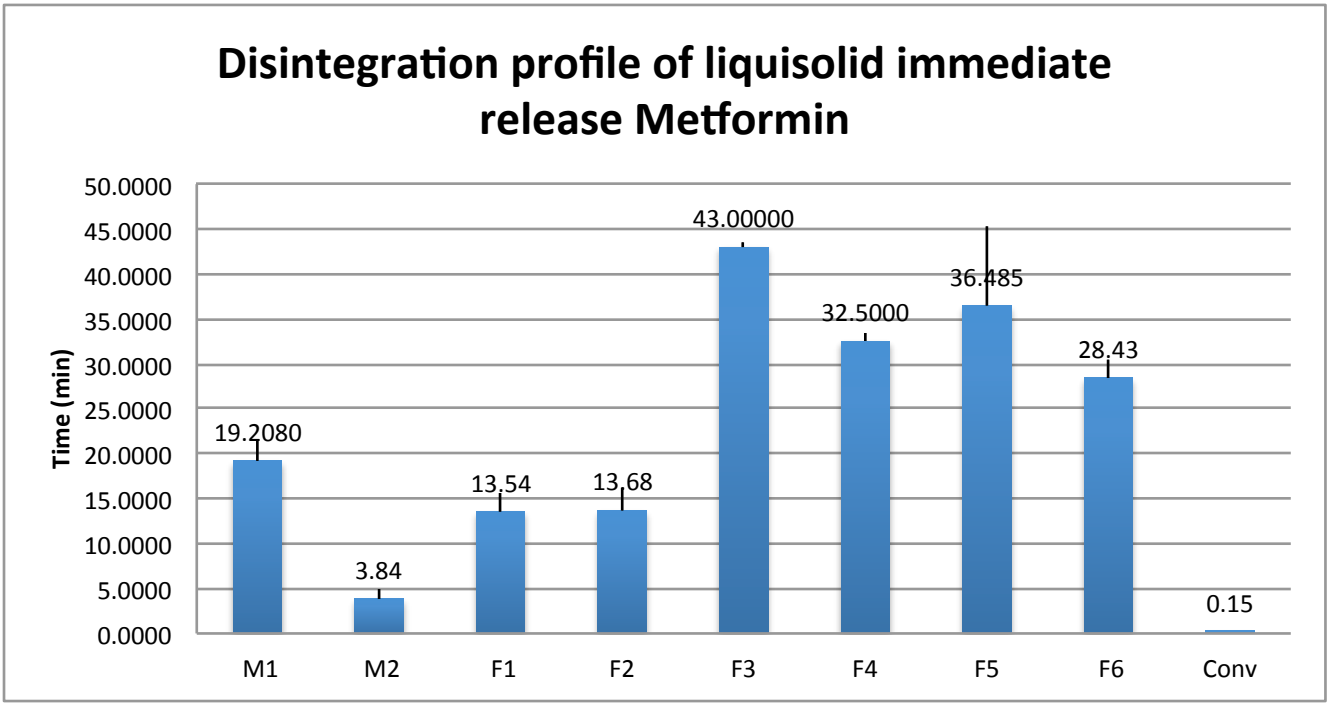


Figure 4.5: Average disintegration time of liquisolid immediate release metformin-HCl. Refer to Table 4.1 for composition.

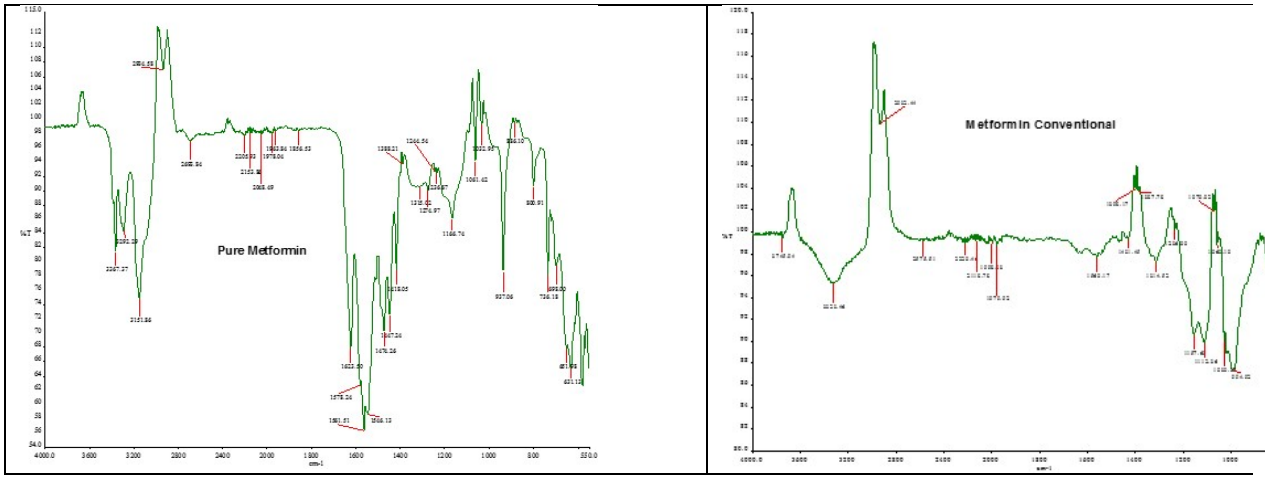
British Pharmacopoeia (2017) specifies the accepted limits of the percentage of the active drug in tablets between 85% and 115% (BP 2017). The drug uniformity content was performed only on granulated tablets (F1-F6, refer to Table 4.1 for composition) and tablets that were manually compactable (M1 and M2). This is due to the lack of good flowability and compactibility properties of M3-M6. Accordingly, all liquisolid formulations including conventional tablet complied with British Pharmacopoeia (BP), 2017 drug content uniformity. From the drug content result, it can be said that the granulated formulation showed uniform drug content. This is a very important finding that drug binding to PVP during granulation allows for better uniformity and distribution in tablets when compacted. According to (Fahmy & Kassem, 2008), the higher the amount of liquid medication and carrier used, the more uniform drug distribution is achieved. This is due

to the adsorption process of the carrier to the liquid vehicle, which yields a higher surface area with a homogeneous distribution of the drug. Moreover, the effect of liquid medication and content of carrier did not play a great role and there was no major difference in drug content, between 30% and 60% drug content of each batch used. In other words, there was no significant differences between liquisolid formulations of different liquid vehicles (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) as well as conventional tablets. The possible reason can be referred to the amount of the carrier excipient in the unit dose of each formulation, which enhances the absorption of the liquid medication into the internal part of the carrier, resulting in a homogeneous distribution of the active ingredient (Spireas , et al., 1992), (Akinlade, et al., 2010) (El-Say, et al., 2010) (Javaheri, et al., 2014).

4.6. Fourier transform infrared (FT-IR) spectroscopy

Within a molecule, the IR-radiation causes the excitation of the vibrations of covalent bonds, which can also be the bending and stretching means. Figure 4.6 below illustrates the infrared spectra of pure metformin-HCl, pure excipients, conventional and some of the liquisolid powders both granulated and ungranulated (M1-M6 and F1-F6). The fingerprint region is usually between 500 – 1800 cm^{-1} and the functional group region is between 2000-4000 cm^{-1}. Metformin-HCl IR studies, revealed two typical bands at 3367 and 3292 cm^{-1} due to N-H primary stretching and a band at 3156 cm^{-1} due to N-H secondary stretching. Additionally, characteristic bands at 1623 and 1561 cm^{-1} were observed to be denoted for N-H asymmetric deformation. The FT-IR can be indicative of shift due to hydrogen bonding interaction between the hydroxyl group of liquid vehicle

and metformin-HCl which was observed in liquisolid formulations (F1-F6, refer to Table 4.1 for composition). Hydrogen bonding is an important contributing factor that determines solubility or dissolution enhancement of the hydrophobic drug. Although metformin-HCl is a hydrophilic drug, the conventional metformin-HCl spectra showed a complete change in peaks when compared to pure metformin-HCl (Figure 4.6). The DSC in Figure 4.7 confirms the rationale behind the FT-IR data of the liquisolids granulated and ungranulated. Additionally, there was no band shifting in granulated an ungranulated formulations, therefore, this could indicate that PVP as a liquid binder has no negative effects on drug release in the neutral dissolution medium and in this situation, PVP will not lead to crystallization. The FT-IR results were also consistent with DSC results, which confirms the solubilisation of metformin-HCl within the liquid vehicles. (Silverstein, et al., 1991), (Sheela, et al., 2010).



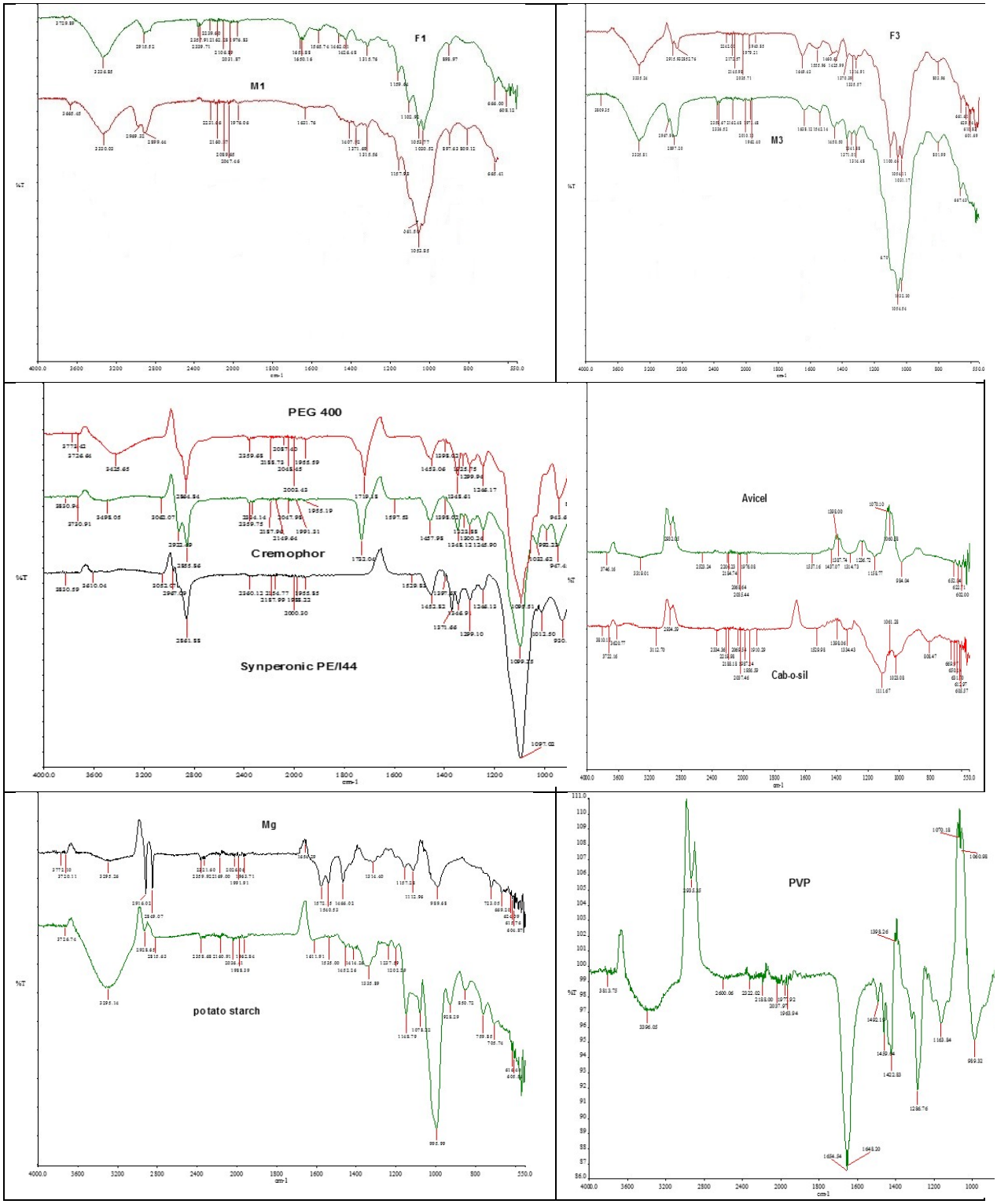
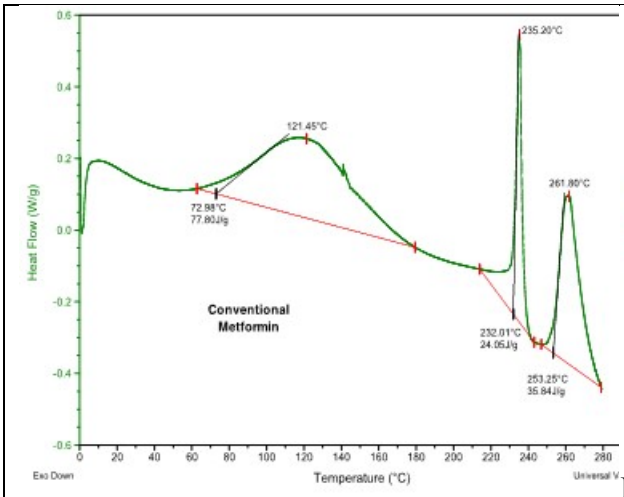
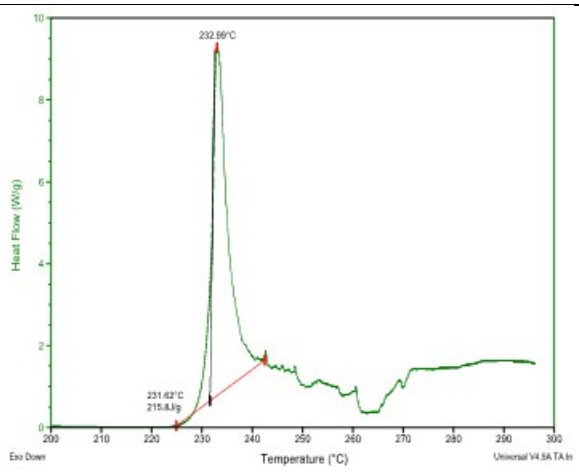


Figure 4.6: FT-IR spectra of pure metformin-HCl, pure excipients, conventional and some of the liquisolid powders both granulated and ungranulated (F1, M1, M3, F3). For formulations' compositions refer to Table 4.1.

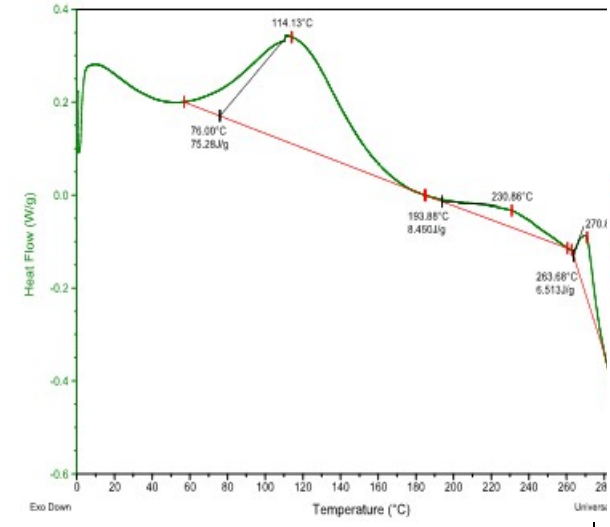
4.7. Differential scanning calorimetry (DSC)

DSC is the most common parameter to determine the physicochemical properties and interaction between the drug and the excipients. Figure 4.7 shows the DSC of pure metformin-HCl, pure excipients, conventional and some metformin-HCl liquisolid formulations. Pure metformin-HCl thermogram showed a sharp endothermic peak at 232.99°C with a melting enthalpy of 215.8J/g. This sharp peak indicates that the drug is in its crystalline anhydrous state (Murra , et al., 2005) (Fahmy & Kassem, 2008). Very broad peaks were displayed in Figure 4.7 for both thermograms of potato starch and Avicel®PH102 with mid points of 139.18^{0}C and 145°C, respectively. In addition, Cab-o-sil®M-5 thermogram showed a small peak at 117.77^{0}c which indicates the amorphous state of the coating material. On the other hand, the DSC thermograms (Figure 4.7) of all liquisolid formulations (except M5 and M6; F5 and F6) showed broadened peaks with reduced intensities instead of the sharp melting peak of metformin-HCl; meaning changing of the drug crystallinity. This signifies to the large solubilisation of metformin-HCl in the liquid vehicle as well as the possibility of an interaction between the drug and the excipients (Tiong and Elkordy, 2009) or a modification to a secondary amorphous forms which were reflected in enhanced dissolution compared to Cremophor® ELP containing formulations M5 and M6 (ungranulated powders) and F5 and F6 (granulated powders). The thermograms of conventional and pure metformin-HCl showed sharp peaks at ~229.29^{0}C and conventional metformin-HCl powder revealed a broad peak at 121.45^{0}C corresponding to the Avicel®PH102 broad peak and a sharp one at 235.20^{0}C which resembles the pure drug melting point, indicating no major changes in crystallinity of metformin-HCl in the conventional system.

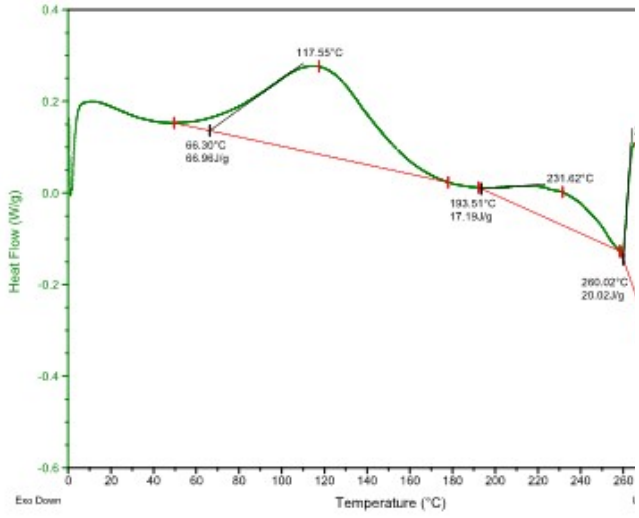




Pure Metformin

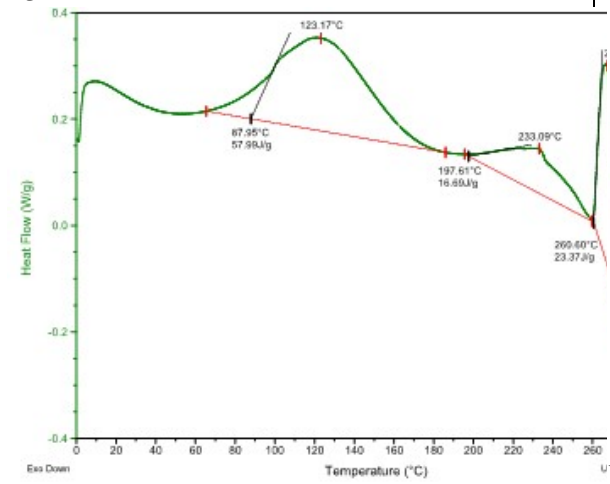


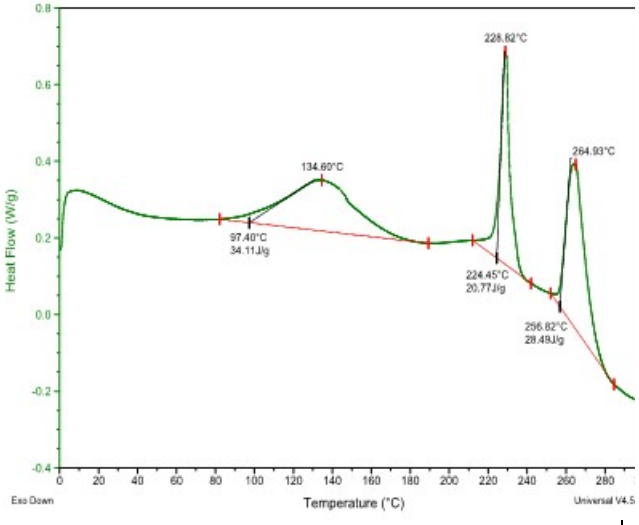
F1



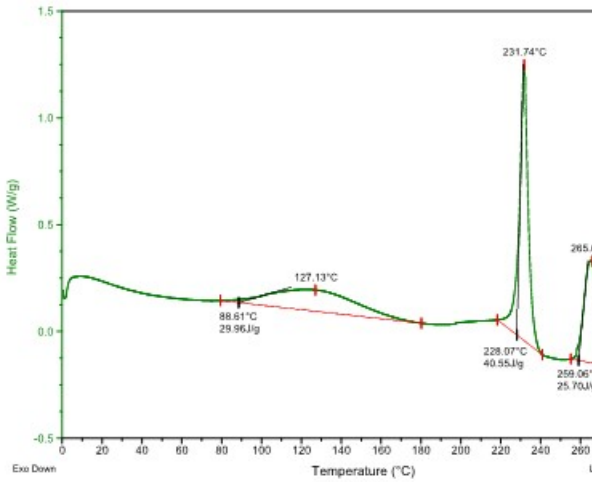
F2

F3





M5



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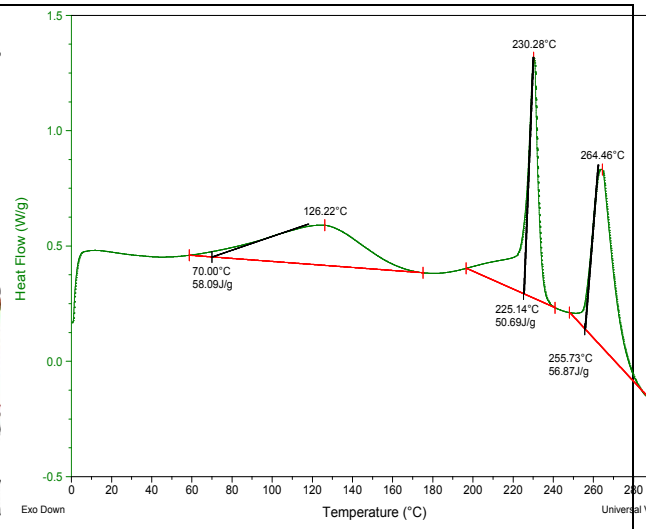
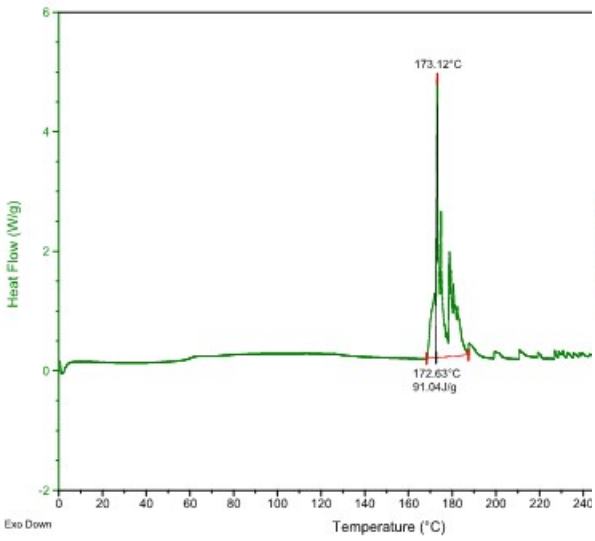
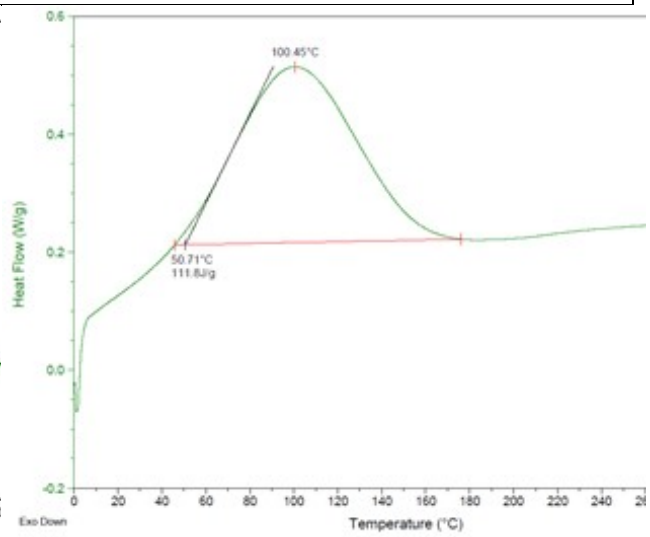


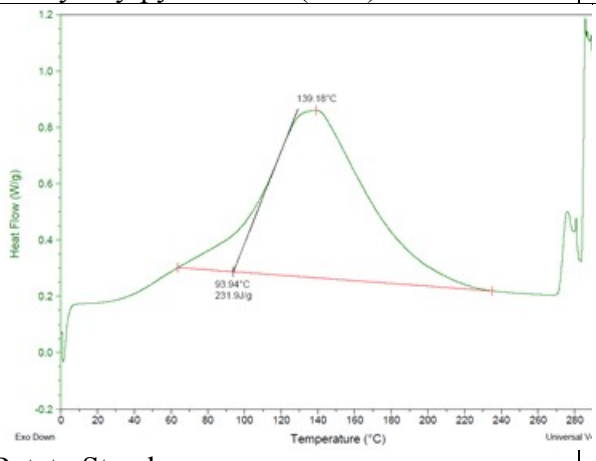
M6



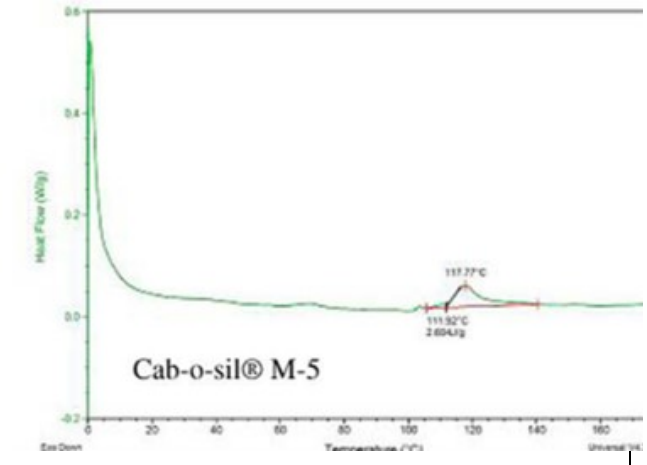
Polyvinylpyrrolidone (PVP)

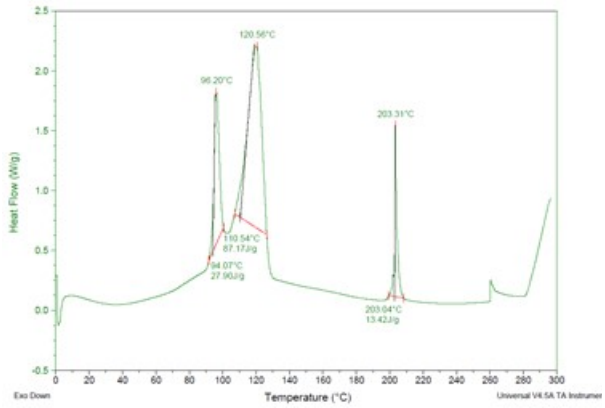


Avicel pH102



Potato Starch





Magnesium stearate

Figure 4.7: DSC thermograms of pure metformin-HCl, some liquisolid formulations, conventional, and pure excipients used. Refer to Table 4.1 for formulations' composition.

4.8. Scanning electron microscopy (SEM)

Figure 4.8 displays the SEM microphotographs of pure metformin-HCl, conventional and some liquisolid powders, granulated and ungranulated (M5, M6, F5, and F6). It can be noted that the pure metformin-HCl powder shows the drug in crystalline form with particles having well defined edges. This can be confirmed by the above results obtained from DSC thermograms. On the other hand, the liquisolid powders present with a rod like shape structure in which no crystals were seen. According to (Elkordy, et al., 2013) the disappearance of the drug crystals indicates that there has been a full inclusion of the drug molecule within the carrier, coating and liquid vehicle used. Moreover, conventional metformin-HCl shows a more defined, smooth edged shape even though liquid medication is not added. Therefore (Elkordy, et al., 2013) findings are confirmed by this study. It can be observed from the images below that the granulated liquisolid metformin-HCl shows a more defined yet smooth edged shape particles compared to the non-granulated powders. It can be concluded that the SEM results below can be a promising

finding for permeability test (Section 4.14) as it indicated that the drug particles are integrated in the liquid vehicles and the other excipients used.

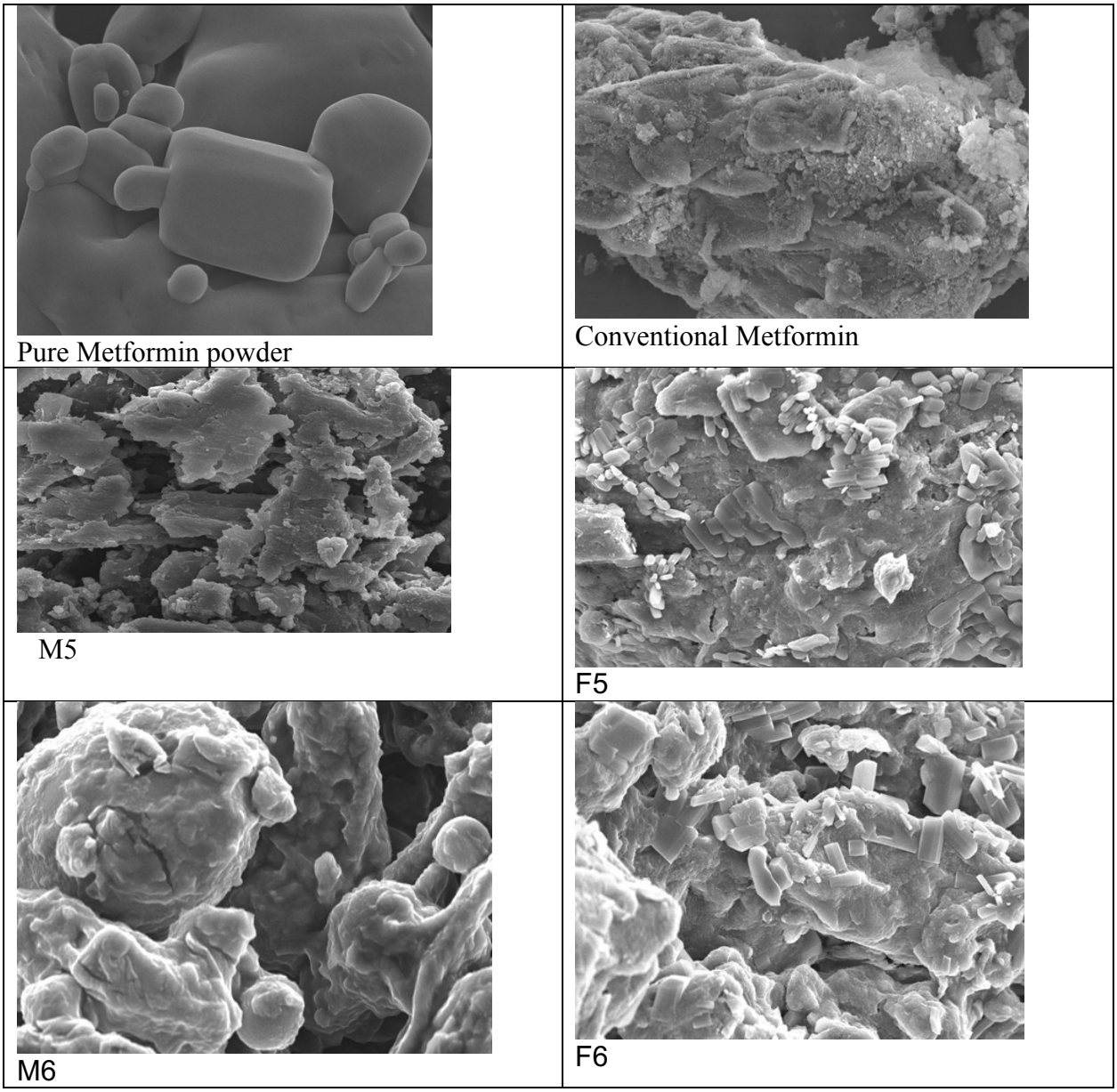


Figure 4.8: SEM microphotographs (x1000) of pure metformin-HCl and liquisolid powders being ungranulated and granulated. Refer to Table 4.1 for composition of formulations.

4.9. Stability study

4.9.1. In-vitro dissolution study for stored metformin-HCl liquisolid tablets

Dissolution stability results of metformin-HCl granulated (F1-F6) liquisolid tablets in figure 4.9 revealed that there was no significant difference ($P > 0.05$) between fresh and store liquisolid formulations after storage at 25^0C with 76% relative humidity. This suggests that metformin-HCl liquisolid formulations are stable under any stressful condition such as high humidity, without affecting the drug properties (as detected from DSC and FT-IR data– see below) or cause any changes in dissolution profile, making the production of metformin-HCl liquisolid viable for pharmaceutical industrial manufacturing.

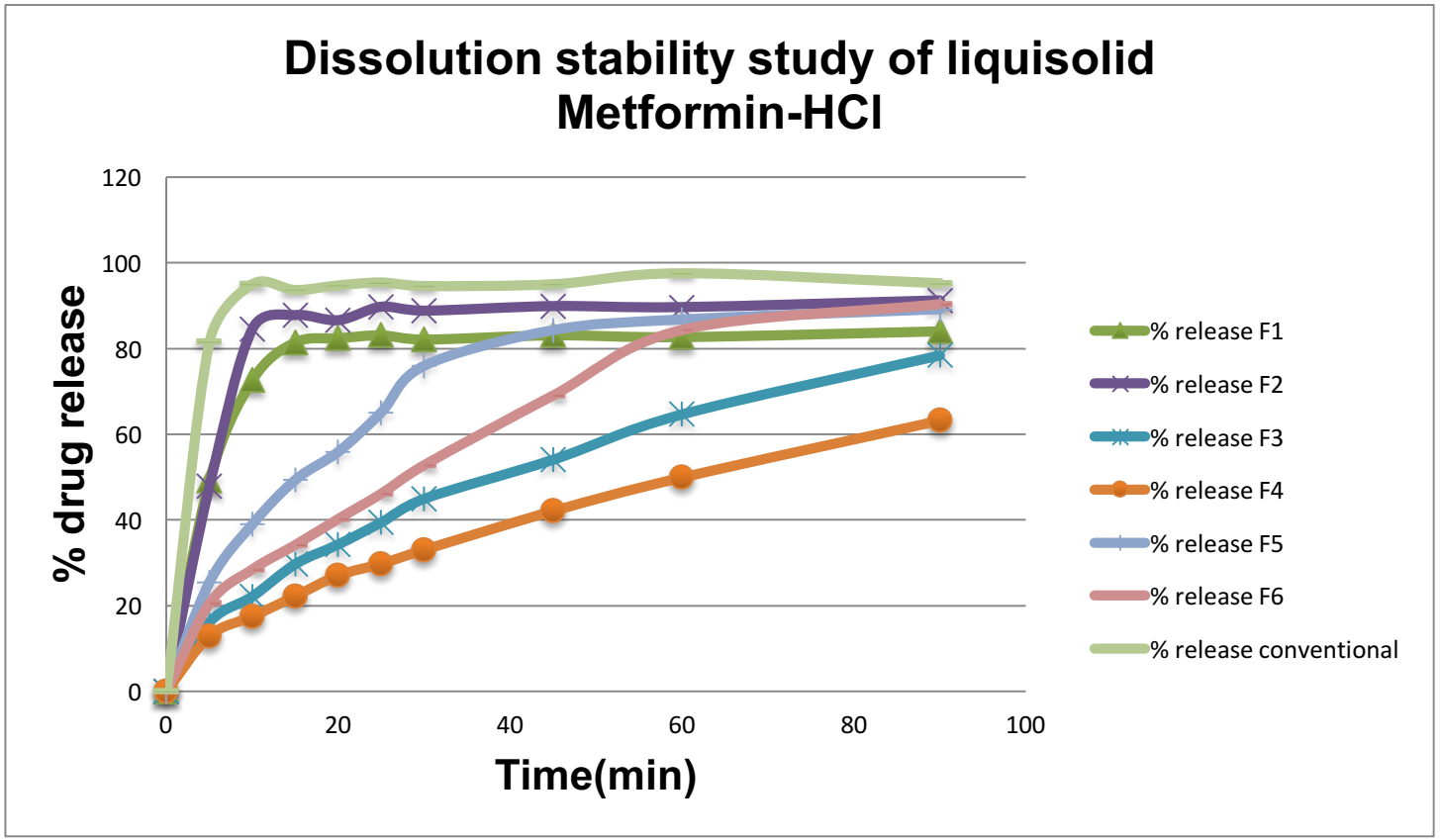


Figure 4.9: Dissolution profiles from stored liquisolid metformin-HCl (F1-F6). For formulations' composition refer to Table 4.1

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4.9.2. Fourier transform infrared (FT-IR) spectroscopy stability study of granulated liquisolid metformin-HCl

The FT-IR results of stored liquisolid metformin-HCl tablets are shown in Figure 4.10. It can be observed that there is no significant difference between the fresh and the stored liquisolid metformin-HCl tablets. This further confirms the obtained dissolution results of stored liquisolid metformin-HCl and that the liquisolid compacts are successful formulations that maintain stability under stressful conditions.

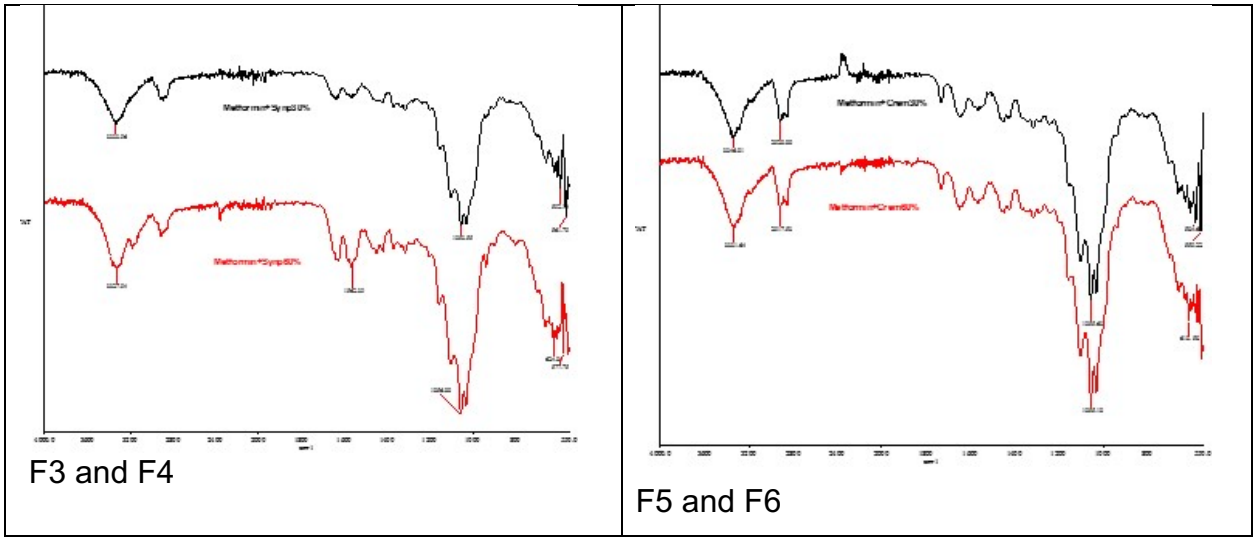
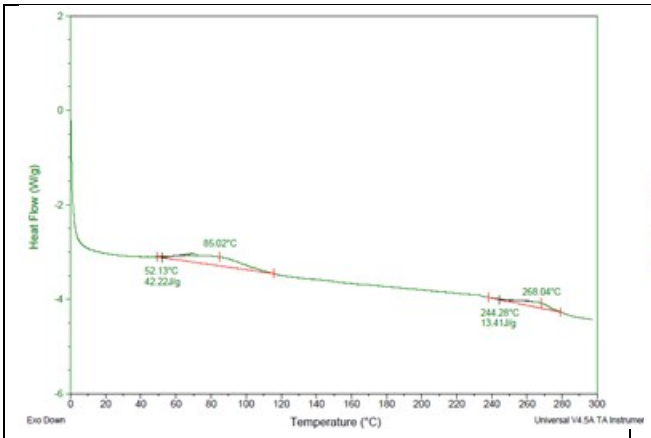


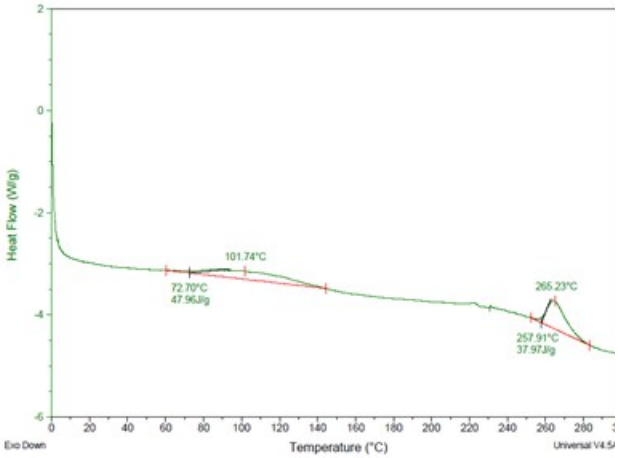
Figure 4.10: Fourier transform infrared (FT-IR) spectroscopy stability study of liquisolid metformin-HCl. For composition refer to Table 4.1.

4.9.3. Differential scanning calorimetry (DSC) of stored liquisolid metformin-HCl

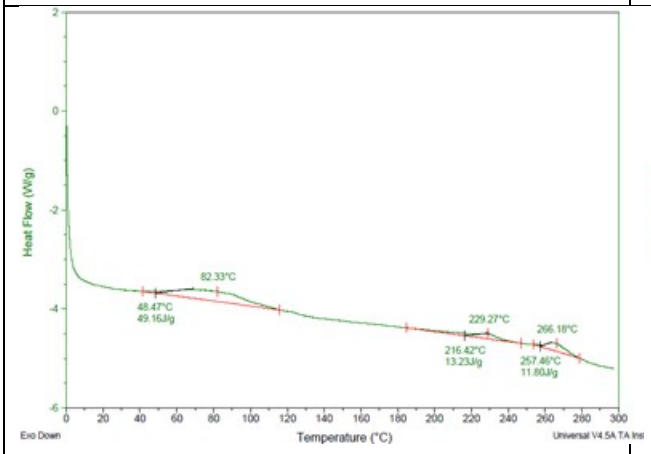
Figure 4.11 shows the DSC thermograms of liquisolid metformin-HCl (F1-F6) after storage (at 76% humidity and 25°C) for 12 months. The thermograms revealed no significant difference between fresh (Figure 4.4) and stored metformin-HCl liquisolid tablets (Figure 4.9).



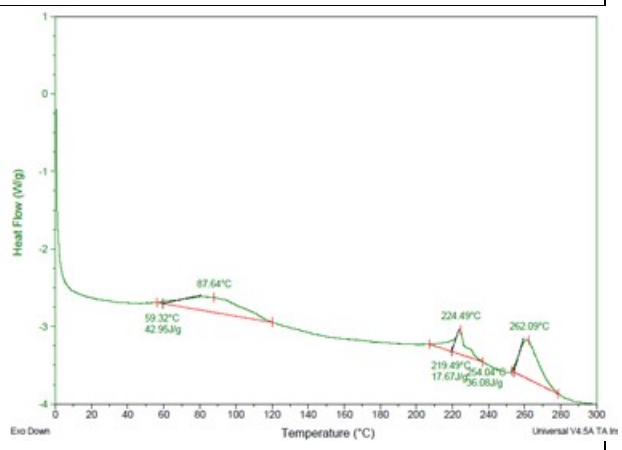
F1



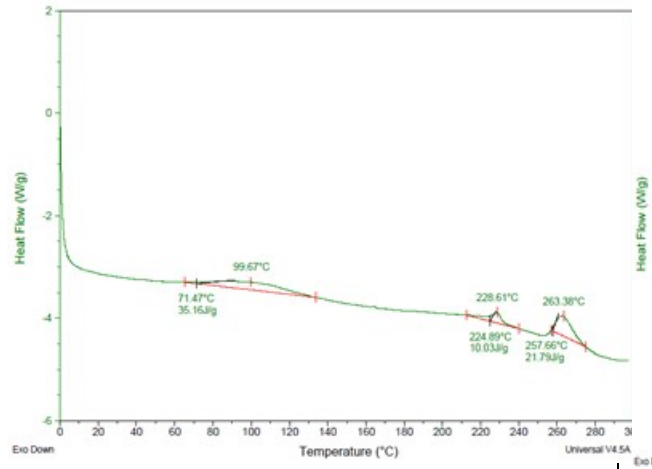
F2



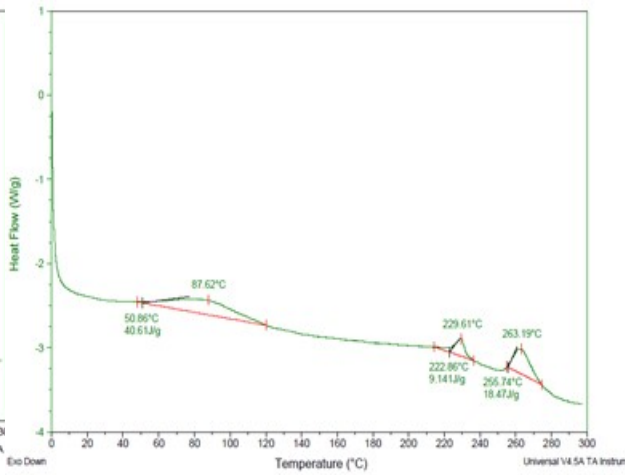
F3



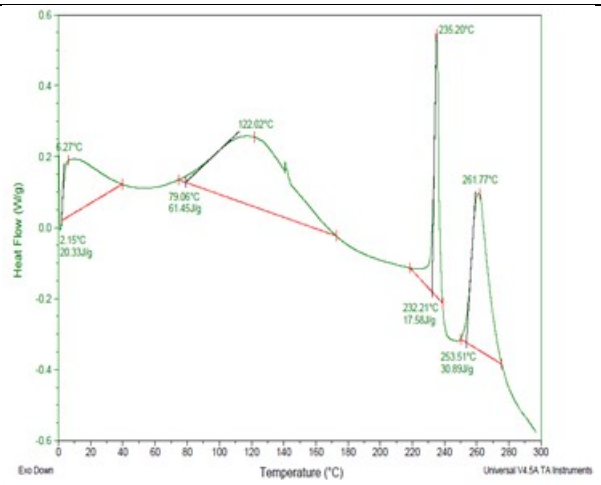
F4



F5



F6



Conventional metformin

Figure 4.11: Differential scanning calorimetry (DSC) of stored liquisolid metformin-HCl formulations, refer to Table 4.1 for composition.

4.10. Metformin-HCl permeability test

The major goal in the design, drug selection and production of drugs intended for noninvasive delivery is the prediction of human drug absorption. In different phases of drug discovery and development, several techniques were employed to evaluate the extent of drug absorption. Screening protocols include a variety of preclinical methodologies to evaluate drug absorption such as in-vitro, in-vivo and ex-vivo. To assess the absorption of drugs in preclinical studies, animal studies are the gold standards despite the fact that this is an expensive and time consuming protocol that may not precisely predict the drug in human behavior (Sarmento, et al., 2012). Additionally, in the selection of compounds for clinical development, the in-vitro models that mimic the mucosa in the human body are most appropriate protocol for absorptions data. The presently used techniques for drug absorption and permeability tests other than using human volunteers for some particular drugs in clinical trials, cell culture models, which offers a highly defined tool, as well as human cell lines are currently considered as

convenient models. In order to thoroughly resemble the in-vivo tissues, the in-vitro cell based model have been optimized which incorporates more than one single type of cell. In an attempt to shorten drug development time, new and improved tissues based in-vitro models are used to estimate the drug absorption. An alternative approach to test the activity of drug across the biological tissue is the consideration of ex-vivo experiments that closely represent human conditions. Based on the in-vitro drug permeation profiles, the in-vitro-in-vivo correlation must provide a drug's in-vivo pharmacokinetics prediction (Pelkonen, et al., 2001). The key factor in the absorption and distribution of drugs is the permeability across the biological membranes leading to poor absorption across the gastrointestinal mucosa and distribution throughout the human body. This can also be due to membrane based efflux mechanisms as well as structural features. Additionally, the permeability classification can be determined directly by measuring the mass rate transfer across the human intestinal or stomach membrane. An alternative method is by indirect estimation of the amount of drug absorption in pharmacokinetics studies in humans. Several other systems can be used for oral drug absorption prediction in humans as well as for classification of drugs according to their permeability, such as intestinal perfusion studies (in-situ methods), in-vitro cell cultures (Caco-2), artificial membranes and ex-vivo studies using intestinal segments from animals or humans (Balimane, et al., 2000). In this section, a review on the different types of drug permeability tests used and their application in different publication is discussed. The goal of this work is to demonstrate the use of prepared liquisolid metformin-HCl tablets in an ex-vivo experiment using Franz Cell Diffusion. The permeability test was performed on Wistar male rats stomach tissue to compare the absorption between pure metformin-HCl and liquisolid

formulations. To the best of our knowledge, no study or publication has been performed on the use of liquisolid formulations in permeability test using Franz cell diffusion and particularly using stomach tissue. Therefore, all the acquired data are purely based on the experiments performed during the laboratory work, and the typical available methods of drug permeability test such as Caco-cell based tests were not used.

4.11. Different permeability studies

4.11.1 Intestinal permeability test using Single-pass Intestinal Perfusion

A study by (Song, et al., 2006) used the Single-pass Intestinal Perfusion (SPIP) technique to characterize the intestinal transport and mechanism of metformin-HCl in rats. They have stated that this procedure is used in anesthetized rats whereby a section of intestine is isolated and perfused with a solution of the drug. In the experimental procedures of single-pass Intestinal Perfusion, modifications can be made to the length of the perfused intestine, concentration of the drug and the flow rate. Additionally, as determined by the difference between the inlet and outlet concentrations, the loss of compound is attributed to absorption. The preliminary studies may consist of stability studies in the homogenates of the intestine and the buffer intestine. As a result, it was found that metformin-HCl could be absorbed from the whole intestine with the main absorption site being the duodenum. Metformin-HCl is transported by passive and active carrier mediated mechanisms, which is also concentration-dependent. Furthermore, the Peff values obtained, the human fraction dose absorbed for metformin-HCl is estimated to be 74%-90% along human intestine. Intestinal perfusion studies are closer to in-vivo conditions. Limitations of this

method are associated with the technical complexity of studies in anesthetized animals making the method very low throughput. The permeability measurement is based on disappearance of the drug from the intestinal lumen as the only indication of absorption and a large number of animals are needed for a statistical significance

(Zakeri-Milania, et al., 2007) used SPIP in rats to predict the human intestinal permeability and fraction absorbed upon oral dosing. They determined the permeability coefficients in anaesthetized rats using fourteen compounds. The appropriate SPIP method used is through the perfusion of the drug solution through a single-pass intestinal perfusion (SPIP) in phosphate buffered saline (PBS) with 0.21ml/min flow rate. At different time points, up to 90min, the samples were taken from outlet tubing. Using HPLC, drug concentrations in samples were determined and permeability coefficients (Peff) were calculated. In conclusion, the SPIP could be utilized with precision to predict the human intestinal permeability. Additionally, this particular technique provides a complete blood supply and functional intestinal barrier that simulates a normal physiological state, which can be used as a reliable procedure for the prediction of the absorbed dose following oral administration in humans.

4.11.2 In-vivo permeability study of liquisolid formulations using animals such as beagle dogs and rabbits

Khaled et al. 2001, carried out an in-vivo drug permeability test to examine the absorption properties Hydrochlorothiazide liquisolid tablets by using six male beagle dogs. The liquisolid hydrochlorothiazide was orally and intravenously administered in dogs. Moreover, 25mg of a single oral liquisolid and commercial tablets in a randomized two-

way crossover design was administered on two occasions. The results of the oral administration showed significant difference ($P < 0.05$) between the liquisolid and the commercial tablets in the peak plasma concentration, and the absolute bioavailability. Additionally, liquisolid tablets showed 15% higher absolute bioavailability than the commercial tablets, indicating greater bioavailability of the liquisolid tablets. Consequently, the absorption of liquisolid hydrochlorothiazide tablets showed significantly higher extent of absorption than the commercial tablets. Thus, upon performing the in-vivo test, production of liquisolid formulation has the potential to be used for clinical trials on humans to provide large scale production of liquisolid hydrochlorothiazide tablets by pharmaceutical industries (Khaled, et al., 2001).

(El-Houssieny, et al., 2010) studied the bioavailability and biological activity of repaglinide liquisolid and its effect on glucose tolerance in rabbits. The rabbits were orally administered with liquisolid repaglinide and blood samples were used to determine the pharmacokinetic parameters of the liquisolid drug and then compared to pharmacokinetic parameters of marketed tablets (Novonorm, 2 mg). For pharmacokinetic studies, high-performance liquid chromatography was used to determine and validate the concentration of repaglinide in the plasma of rabbits. It was found that the relative bioavailability of repaglinide liquisolid tablets was significantly increased in comparison to the marketed tablets. In regards to insulin levels, the liquisolid repaglinide tablets increased insulin level with a significant change of 37.6% while an insignificant change of 3.52% was seen in commercial tablets. Additionally, the glucose tolerance test showed a reduction in blood glucose level (18.1 % change) upon administration of the commercial whereas the blood glucose level percentage change in groups treated with liquisolid formulation decreased

29.98%. Consequently, the bioavailability of liquisolid repaglinide was enhanced upon oral administration of the drug which showed significant decrease in blood glucose levels and more control of blood sugar than in commercial tablets by stimulating the release of insulin from the β-cell of the pancreas (El-Houssieny, et al., 2010).

4.11.3 Effect of Freezing and Type of Mucosa on Ex-Vivo Drug Permeability Parameters

(Caon & Simões, 2011) performed an ex-vivo permeability study of carmabazepine (CBZ) and triamcinolone acetonide (TAC) on the porcine esophageal and buccal mucosa with different permeabilities and physicochemical properties using a Franz diffusion cell system and HPLC as A detection method. The evaluation of freezing effects on drug permeability parameters by comparing fresh and frozen tissues was tested. As a result, it was found that fresh and frozen esophageal mucosae provided higher permeation of triamcinolone acetonide than on buccal mucosae while the permeability parameters obtained for carmabazepine were similar on both mucosae. Additionally, involving carmabazepine, porcine esophageal mucosa could be used as a substitute for buccal mucosa on ex-vivo studies whereas triamcinolone acetonide cannot be used for buccal mucosa study. With great care, substitution of frozen tissues could be used instead of fresh in both cases with special attention to the results of previous tests due to results obtained could differ depending on the tested drug. Thus, in permeability studies involving carmabazepine and triamcinolone acetonide, frozen porcine mucosae could be substituted by fresh porcine mucosae. Also, only in ex-vivo experiments comprising of carmabazepine, esophageal mucosa could be used as a substitute for buccal mucosa.

Consequently, to study the buccal drug permeability, consideration of drug type and its physicochemical characteristics are relevant during the development of an ex-vivo mode. (Caon & Simões, 2011).

4.11.4 Caco-2 Model

Caco-2 cells have been considered the “gold standard” assay for the prediction of intestinal absorption, since they are extracted from human colorectal carcinoma (Hidalgo, 2001). No passive diffusion is constituted in Caco-2. Additionally, intestinal epithelium permeability is a vital property that determines the extent and the rate of human absorption which eventually affects the bioavailability of drug candidates. Additionally, by allowing rapid assessment of membrane permeability, the caco-2 permeability assay rank compounds as high, medium and low permeability. Moreover, majority of the morphological and functional characteristics of intestinal cells that are responsible for absorption are expressed by enterocytes. On the other hand, the permeability of hydrophilic drugs are generally low in the Caco-2 monolayer that are transported by the paracellular pathway. This is due to the tighter junction of the hydrophilic drugs (Sun, et al., 2008)

4.12. Passive diffusion fundamentals of drug substances

The main target upon drug administration by extravascular route is the systemic absorption followed by distribution to tissues and organs. At the side of action, drug receptor interaction usually takes place to exert the pharmacological or toxic effect of the drug. The transport of drug molecules across the cellular barrier involves two types of

movements or diffusion caused by an external force such as hydrodynamic flow or gravity: transcellular (across the cell) and paracellular (inbetween cells). Diffusion in the presence of a concentration gradient causes a net transport of molecules. The movement of drug molecules from a higher to a lower concentration without the need of energy is known as “passive diffusion” and this type of movement or diffusion does not involve a carrier which can mediates either transcellular or paracellular transports (Shargel and YU 1999). Additionally, according to Cao et al. (2006), about 80-95% of drug are absorbed by passive diffusion and is affected by physicochemical properties such as hydrogen bonding, pKa and lipophilicity.

4.13. Franz Cell

The most common in-vitro release testing (IVRT) method that is currently being used is called Franz diffusion cells. There are few studies for orally administered drugs using the Franz diffusion cell. Traditionally, they are used with synthetic membranes such as skin and only few studies have employed Franz diffusion cells to study drug permeability across the stomach or small intestine tissue (Dezani, et al., 2013). The membrane is kept between the two compartments that separates donor from the receptor chamber. The donor compartment contains the test product and the receptor compartment is filled with the collection medium. There are several advantages of using Franz diffusion cells including the need for a small amount of drug substance and the minor amount of tissue handling and the similarities between rat and human tissues (Cao, et al., 2008).

4.14. Permeability study of metformin-HCl using stomach tissue and Franz cell

The permeability study of liquisolid immediate release metformin-HCl was performed on male Wistar rats' stomach tissue (Refer to Appendix for Chapter 4). The tissue was stored in freezer (-80 ^{0} C) and prior to the test, the tissue was defrosted and placed in freshly prepared tyrode solution. Throughout the experiment, fresh solutions of tyrode and 0.2M HCl were prepared. The tyrode's solution resembles lactated Ringer's solution, but contains magnesium, glucose uses bicarbonate and phosphate as a buffer. The isotonic solution was invented by an American pharmacologist Maurice Vejux Tyrodeis and it is used in physiological experiments and tissue culture. Metformin-HCl liquisolid tablet containing roughly 40mg of active ingredient was dissolved in distilled water and filtered using 0.2μm size filter. The stomach tissue, upon cleaning the inner lining and cutting into appropriate thickness, was placed on the Franz diffusion cell opening designated for the membrane. The chamber was filled with 14 ml of HCl, 0.2M solution. The Franz cell was kept at a constant temperature over a magnetic stirrer. Using a micropipette,1 ml of the filtered metformin-HCl solution was injected in the donor chamber and samples were withdrawn from the receiver chamber after specific time intervals. The results of the performed permeability study are shown in Table 4.4.

As an anti-diabetic biguanide, metformin-HCl through a reduction in gluconeogenesis, works by suppressing the unnecessary production of hepatic glucose. It has a half-life of about 5 hours with a renal clearance rate of 510±120 ml/min. Moreover, metformin-HCl is not metabolized and is excreted unchanged only in the urine. The drug is distributed into the tissues of the body, liver, kidney as well as intestines by cationic transporters

(Gong, et al., 2012). The current performed ex-vivo permeability study on the male wistar rat stomach has shown a permeability difference between pure metformin-HCl and liquisolid formulations. According to (Caon & Simões, 2011), the difference between fresh and frozen tissues were insignificant. Thus, although the results shown in Table 4.4 were using frozen tissue, the results obtained were promising and can be performed on different types of liquisolid drugs. The possible explanation to the new findings could be linked to several factors including: the properties of the surfactants (PEG®400, Synperonic®PE/L44 and Cremophor®ELP), the excipients (Avicel®PH102 and Cab-O-Sil® M-5) and the use of polyvenylpyrolidone (PVP). Volker Bühler (2005) presented a work linking the effect of PVP (Povidone) with the bioavailability of different active drugs administered via several routes. The oral bioavailability of a nifedipine coprecipitate in the rat, phenobarbital coprecipitate in rabbits and upon percutaneous administration of hydrocortisone coprecipitate on the human skin was evaluated. Additionally, similar dose of the pure active substance without PVP was applied for reference. Interestingly, after two hours, the plasma level of the nifedipine coprecipitate reached twice the plasma level of pure substance, and in the case of the phenobarbital suppositories, it reached three times the level of the pure active substance (Bühler, 2005). Therefore, a strong correlation between the effect of PVP on the drug permeability and bioavailability is observed. Thus, it is a suggested reason, other than the benefits of liquisolid technique on the enhancement of drug bioavailability but also wet granulation by using PVP can contribute to the permeability improvement.

Table 4-4: Efflux (μg/ml/cm^2) of metformin-HCl liquisolid in HCl (0.2M) solution using Franz diffusion cell for permeability study.

0.2M HCL	Efflux (μg/ml/cm^2)		
Time (min)	**PEG 30%**	**PEG 60%**	**Synperonic 30%**
5	0.87	1.25	0.50
10	0.97	1.12	0.51
15	1.00	1.09	0.61
20	1.02	1.08	0.73
25	1.02	1.11	0.84
30	1.10	1.13	0.94
45	1.18	1.21	1.12
60	1.33	1.34	1.31
90	1.95	1.46	1.65
120	2.64	1.61	1.20
Synperonic 60%	**Cremophor 30%**	**Cremophor 60%**	**Pure Metformin**
0.61	0.62	1.07	0.31
0.68	0.71	0.90	0.30
0.96	0.74	0.75	0.32
0.99	0.78	0.77	0.35
1.01	0.77	0.95	0.36
1.08	0.80	1.43	0.48
1.15	0.86	1.52	0.57
1.46	0.97	1.72	0.67
1.71	1.16	1.79	0.85
2.03	1.41	2.21	0.93

Table 4-5: Percentage of metformin-HCl penetrated from liquisolid formulations using Franz diffusion cell for permeability study.

0.2M HCL	Percentage of the drug permeated (%)		
Time (min)	**PEG 30%**	**PEG 60%**	**Synperonic 30%**
5	0.61	0.87	0.35
10	0.68	0.78	0.36
15	0.70	0.77	0.43
20	0.71	0.75	0.51
25	0.72	0.78	0.59
30	0.77	0.79	0.66
45	0.82	0.85	0.78
60	0.93	0.94	0.91
90	1.37	1.02	1.15
120	1.85	1.13	0.84
Synperonic 60%	**Cremophor 30%**	**Cremophor 60%**	**Pure Metformin**
0.42	0.44	0.75	0.25
0.47	0.50	0.63	0.21
0.65	0.52	0.52	0.23
0.69	0.54	0.54	0.25
0.70	0.54	0.67	0.25
0.76	0.56	1.00	0.34
0.80	0.60	1.06	0.40
1.02	0.68	1.21	0.47
1.20	0.81	1.25	0.59
1.42	0.99	1.55	0.65

Table 4.4 shows the permeability results efflux (µg/ml/cm^2) and Table 4.5 reveals the data as percentage. It can be seen that all liquid vehicles used (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) significantly enhanced the metformin-HCl permeability ($P < 0.05$) (refer to Appendix for Chapter 4), compared to the pure drug, hence they are good penetration enhancers. PEG®400 increased the drug permeability by 3-fold, whilst Synperonic®PE/L44 and Cremophor®ELP increased it by about 2-3 folds (Table 4.5). Those liquisolid preparations are expected to enhance bioavailability if they have been tried in-vivo. A study by (El-Houssieny, et al., 2010) reported the improvement of

bioavailability of liquisolid Repaglinide upon oral administration of the drug which showed significant decrease in blood glucose levels and more control of blood sugar than from commercial tablets by stimulating the insulin release from the β-cell of the pancreas. Additionally, the permeability of hydrophobic drug via the lipid bilayer of the cell membrane could be increased in the presence of surfactants as they can disrupt the lipid bilayer arrangement on the cell membrane causing increased drug permeability; they can enhance the absorption via the passive transcellular route (Swenson & Curatolo, 1992) they (for example Cremophor RH40) can inhibit P-glycoprotein that reduces intestinal absorption of hydrophobic drugs by efflux transportation (Elkordy, et al., 2012), Moreover, some non-ionic surfactants such as Synperonic®PE/L44 and Cremophor®ELP can be considered as effective pharmaceutical excipients for inhibiting p-glycoprotein function and hence promoting the intestinal absorption of different drugs (Shono, et al., 2004). In addition to the role of surfactants in dissolution rate improvements (Kim, et al., 2000). Therefore, the liquid vehicles (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) are a good choice of surfactants to be used in this study to improve metformin-HCl permeability.

4.15. Conclusion

Metformin-HCl is a water soluble drug that gives about 100% drug release for pure and conventional formulation after 10 minutes. By using liquisolid technique with different liquid vehicles used to enhance dissolution, the drug release has not reached 100% after 10 minutes (Figure 4.4) due to: the effect of processing technique; the effect of surfactants (liquid vehicles) and the interaction of the vehicles with the drug. Accordingly, metformin-

HCl was chosen as a water soluble model drug to emphasis on the various impact of liquisolid technique on hydrophobic and hydrophilic drugs using same liquid vehicles. At present, metformin-HCl is commercially available in high dose tablets, 500 mg; the granulated liquisolid formulations may assist in the reduction of this dose. The stability studies showed that the dissolution of liquisolid tablets was not affected by ageing significantly. Franz diffusion cell has been used to test the permeability of metformin liquisolid using Wistar rat stomach tissue. The results have shown promising results in using granulated liquisolid metformin-HCl tablets to enhance the bioavailability of metformin-HCl. Consequently, liquisolid tablets can be further used in alternative animal tests in order to confirm the beneficial use of liquisolid technology on permeability of drugs with poor bioavailability. Figure 5-1 summarises the sustained release liquisolid metformin-HCl preparation.

Chapter 5 - Preparation and characterisation of sustained release liquisolid metformin-HCl formulations using Hydroxypropyl Cellulose (HPC-H)

Results and Discussion

5.1. Introduction

The main goal in diabetic patients is to achieve controlled blood glucose levels with the aid of insulin or antidiabetic drugs such as metformin-HCl; however, successful use of metformin-HCl therapy is challenged due to the high incidence of associated gastrointestinal symptoms such as diarrhoea and abdominal discomfort (BNF 73, 2017). Metformin-HCl has a multiple dosage regimen of 500mg two to three times daily or 850 mg once or twice per day. Therefore, such complications are not desirable and should be avoided to maintain patient compliance. Metformin-HCl presents other formulation challenges such as poor compressibility and poor permeability (Nanjwade, et al., 2011). According to the biopharmaceutical classification system (BCS), metformin-HCl belongs to class III with high water solubility and low permeability properties and due to metformin-HCl's poor compressibility properties. Direct compression is not desirable on the commercial scale as it can lead to poor content uniformity as well as poor weight content uniformity, hardness and friability (Nanjwade, et al., 2011). Consequently, there is a need to provide once daily dosing formulations that would deliver modified release metformin-HCl, which would maintain drug plasma levels for 10 to 12 hours when consumed orally. By applying sustained release on such drugs, minimised systemic adverse effects and greater patient compliance as well as other advantages can be achieved. Thus, researchers have used numerous approaches to modify drug release in the form of tablets and one of the successful methods from the prospect of this research is the use of liquisolid technique. Liquisolid formulations not only have the potential to be optimised for the reduction of drug dissolution rate and thereby production of sustained release

systems for highly water soluble drugs, but also have the potential to enhance flowability, compressibility and permeability of metformin-HCl (Pavani, et al., 2013) (Chandrasekar, et al., 2011). The sustaining agent that has been used in this chapter is HPC –high grade. HPC is a hydrophilic polymer which is solubilised by hydroxypropyl groups. It swells by producing a state of hydrogel in water which then releases the drug slowly over period of time with diffusion.

The aim of the presented work chapter 5, is to utilise liquisolid-based formulations of metformin-HCl as sustained release preparations. By the aid of liquisolid technique, reduction of dose was successfully achieved, meaning less cost for pharmaceutical industry and fewer side effects for patients. For both metformin-HCl liquisolid immediate and sustained release formulations, PEG®400, Synperonic®PE/L44 and Cremophor®ELP were used as non-volatile liquid vehicles. Additionally, wet granulation using PVP was applied to both systems in order to achieve acceptable flowability and compressibility.

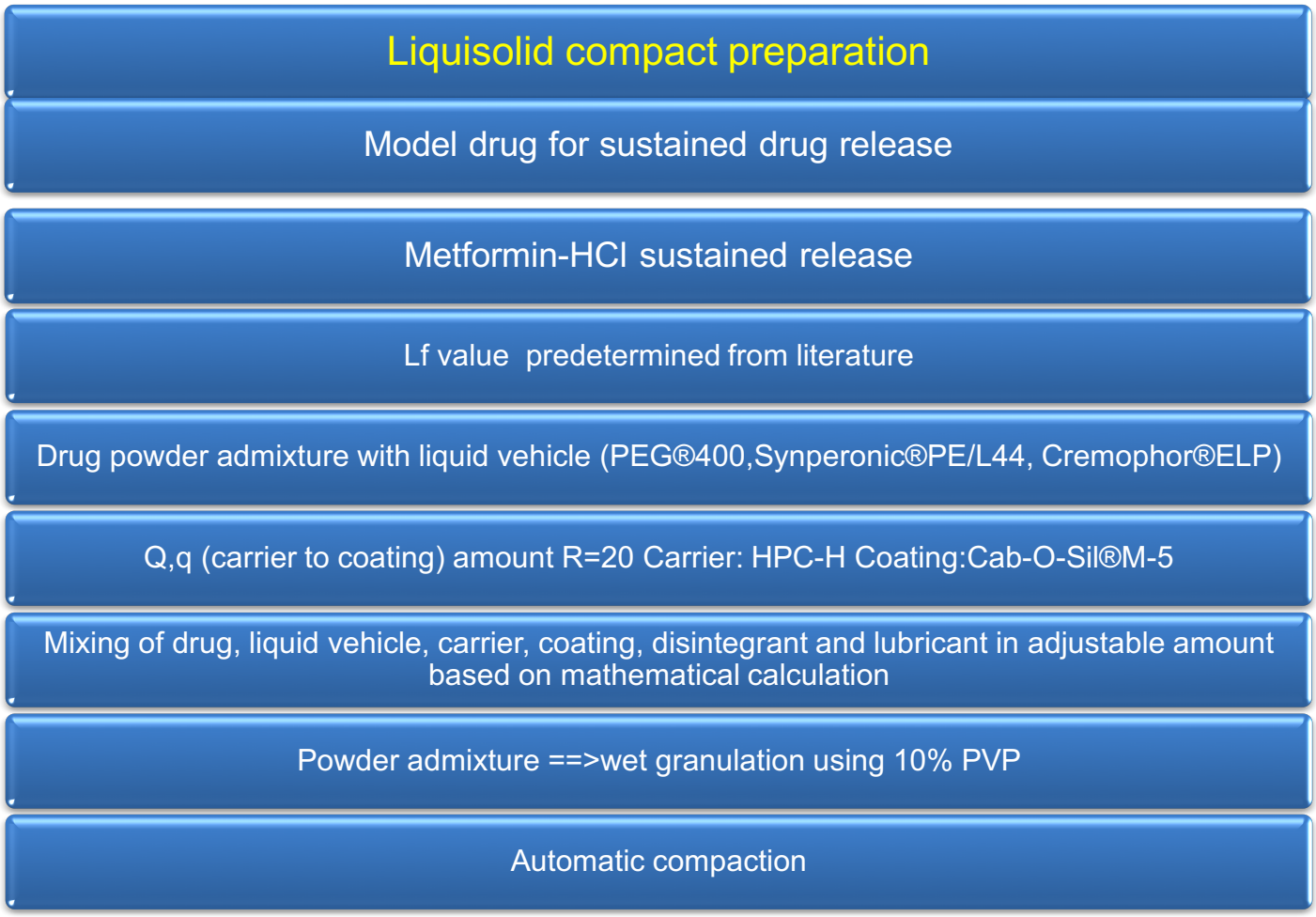


Figure 5.1: Flowchart summary of metformin-HCl sustained release liquisolid preparation using HPC-H

5.2 In-vitro dissolution study of sustained release liquisolid metformin-HCl using HPC-H

Several techniques have been defined to produce sustained release formulations. Among these techniques, a new and promising method, that is the liquisolid formulation, is currently being used in achieving modified release dosage forms. Liquisolid formulations may not only help in reduction in the drug dose but also may help in production of sustained release metformin-HCl formulations. This part of the project explored the role of hydroxypropyl cellulose (HPC-H) as a carrier in liquisolid preparation as well as a sustaining agent in preparation of the modified release metformin-HCl. This work is novel in the field of liquisolid sustaining systems. Other studies tried to introduce new non-volatile liquid vehicle (Kollicoat®SR30D) that comprise of sustaining the drug release while using hydrophilic carrier (Elkordy, et al., 2012). Nevertheless, in this present work, the used non-volatile liquid vehicles are PEG®400, Synperonic®PE/L44 and Cremophor®ELP. The carrier (HPC) will be the main focus of this section. The R-value was also kept constant (20) as an immediate release, meaning the amount of Cab-o-sil® M-5 was kept at 1:20 ratio with the carrier. The dissolution release of metformin-HCl was performed in distilled water to comply with the immediate release conditions. Moreover, in designing the metformin-HCl sustained release formulation, six liquisolid compacts, namely HPC1-HPC6 at 30% and 60% drug concentrations were prepared (Table 5.1). Together with conventional metformin-HCl, HPC1-HPC6 dissolution profiles are shown in (Figure 5.2)

Table 5-1: Liquisolid formulations of sustained release metformin-HCl using HPC-H

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg) HPC-H	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
HPC1	PEG® 400	30	20	0.17	93.30	40.00	794	40.00	51.00	8.00	1026.30
HPC2	PEG® 400	60	20	0.17	26.70	40.00	397	20.00	26.00	4.00	513.70
HPC3	Synperonic® PE/L44	30	20	0.27	93.30	40.00	501	25.00	35.00	5.20	699.50
HPC4	Synperonic® PE/L44	60	20	0.27	26.70	40.00	251	13.00	18.00	2.60	351.30
HPC5	Cremophor® ELP	30	20	0.52	93.30	40.00	256	12.80	21.00	3.20	426.30
HPC6	Cremophor® ELP	60	20	0.52	26.70	40.00	128	6.40	11.00	1.60	213.70
Conventional	_	_	_	_	_	40.00	397	20.00	24.20	3.60	485.00

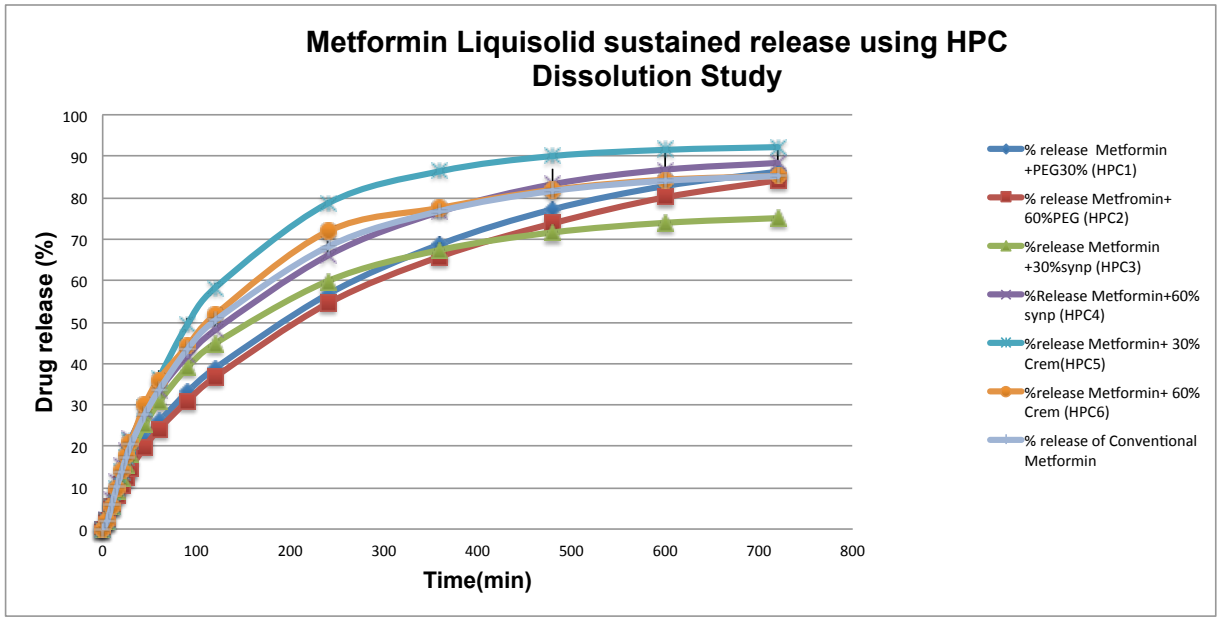


Figure 5.2: The dissolution study of sustained release liquisolid metformin-HCl formulations using HPC-H (refer to Table 5.1 for formulations' composition). Note: Standard deviation is presented but values are very small.

The results of dissolution indicated that HPC1, HPC2, HPC3, HPC4, HPC5, HPC6 (Table 5.1) released 55, 55, 60, 66, 80, and 72%, respectively after 4 hours and released 86, 84, 75, 88, 85 and 85% respectively after 12 hours (Figure 5.2) which showed good release profiles to sustain drug release. In comparison to the immediate release (see Figure 4.4), Cremophor®ELP was the best choice of liquid vehicles to produce immediate release tablets; meaning that this liquid vehicle reduces the effect of HPC as a sustaining release agent. Based on the mathematical calculations the HPC amount (in HPC5, 30% w/w drug concentration in Cremophor®ELP) was less compared to that in HPC1, HPC2 and HPC3 (Table 5.1). On the other hand, PEG®400 (HPC1, HPC2) and Synperonic®PE/L44 (HPC3) enhanced the action of HPC to sustain release of metformin-HCl compared to conventional tablets which contain no liquid vehicle (Figure 5.2); confirming the interaction of the liquid vehicles with the HPC to enhance the drug release sustaining effect of the HPC (i.e. conventional tablets contain 397mg HPC similar to drug with PEG®400 (60%w/w) formula tablets that contains 397mg HPC, hence the liquid vehicle enhanced the effect of the HPC). Also, the enhanced sustaining drug release effect of PEG®400 (in HPC1, HPC2) and Synperonic®PE/L44 (in HPC3) can be explained by that those liquid vehicles equipped the formed HPC pores and hence the dissolution medium can penetrate slowly through the pores and accordingly the release of metformin-HCl was retarded.

Comparing all independent dissolution parameters for liquisolid tablets showed that there were no significant differences between the liquisolid sustained release tablets and conventional metformin-HCl ($p>0.05$) (refer to Appendix for Chapter 5). According to

(Diwedi, et al., 2012), a polymer's ability to retard the drug release rate is related to its viscosity. They used HPMC K4M and K15M and K100M which exhibited viscosity values of 4000, 15000, 100000 mPa respectively. And therefore, HPMC K4M and K15M released the drug faster than HPMC 100M. This theory can be implemented on this study where HPC-H has viscosity of 3040mPa.s (Nisso HPC 2012) which showed a retardation effect on metformin-HCl liquisolid tablets. The high drug release observed with HPC5 (drug in Cremophor®ELP, 30%w/w) could be due to the possible interaction between the liquid vehicle and HPC which retarded the drug release over 12 hours and at the same time maintained a high release of drug without trapping it in the matrix (the drug could not diffuse out of the matrix in a short time). Moreover, the prolonged action of metformin-HCl from the sustained liquisolid compacts was due to the concentration and swellable nature of HPC-H polymer. HPC-H is responsible for the retardation of drug release and the use of HPMC was responsible for the retardation of trandolapril release from liquisolid compacts (Butreddy & Dudhipala , 2015). The study showed significant sustained dissolution profiles of all liquisolid compacts using HPMC K15 M, where the percentage drug release of one of the formulations (containing PEG®400 as a liquid vehicle) was 97.3% after 14 hours. This is in agreement with this study by using Cremophor®ELP with HPC-H

Hydroxypropyl Cellulose (HPC-H) is solubilised by the hydroxypropyl groups that impede the hydroxyl group hydrogen bonding of the cellulose. Since HPC is a hydrophilic polymer, it can be an effective controlled release agent by swelling and becoming a hydro-gel in water, through which the dosage composition is released in dissolution medium (Nisso

HPC 2012). The incorporation of HPC in liquisolid formulations demonstrates synergistic effect on liquisolid metformin-HCl release retardation as shown in Figure 5.2. The presence of HPC in liquisolid formulations decreased the percentage of drug release from almost 98% in immediate metformin-HCl release (F5) (see Figure 4.4) to 36% drug release (HPC5) within the first hour. However, the amount of HPC being added to the liquisolid formulations (Table 5.1) affects the retardation of the drug (as the amount of the HPC was different in HPC1 - HPC6, Table 5.1). The amount of HPC added corresponds to the amount of carrier being loaded in the liquisolid formulas. This means that the carrier, Avicel®PH102, for immediate release preparations (Figure 4.4) was replaced by HPC polymer to make this novel discovery of joint use of HPC as a sustaining agent as well as a carrier to be used in the liquisolid systems. (Nokhodchi, et al., 2010) showed agreement with the present outcome where their results demonstrated the direct effect of HPMC concentration on the drug release retardation. As more HPMC was integrated in the formulation of liquisolid, a further reduction in drug release rate was seen where the HPMC concentration of 10%w/w and 15%w/w gave drug percentage release of 31.3% and 26.9% respectively (Nokhodchi, et al., 2010). (Wadher, et al., 2011) displayed the relationship between the concentration of HPMC and the rate of drug release to be inversely proportional. It is documented that some factors can affect the performance of tablets when used with HPC-H. The degree of water solubility and molecular weight of the drug as well as the particle size of the used HPC are considering crucial factors in drug release performances, where some polymers can work beneficially for certain drugs and not for others.

Researchers have tested the effect of the concentrations of the hydrophilic sustaining agent polymer (HPC) on the percentage drug release e.g. (Nanjwade, et al., 2011). Their results suggested that as the increase in concentration of the polyethylene oxide is inversely proportional to the release rate of the drug. It is further explained that the highly water-soluble drug found in polyethylene oxide matrix leads to faster polymer swelling and thus increase in gel thickness which prevents the instant disintegration of the tablet (as seen also in the current research with HPC-H), thus sustaining the rate of drug release. The current project study focusses on the use of liquisolid technique along with wet granulation to possibly reduce the amount of the active ingredient and sustain its release while maintaining the therapeutic efficacy level in order to reduce possible side effects and promote patient compliance. The results promise regarding sustaining the drug release.

In this study, the effect of liquid vehicle on drug release was not significantly different compared to the conventional metformin-HCl (Figure 5.2). A study by Nokhodchi et al (2010) showed that liquisolid compacts containing polysorbate 80 revealed slower drug release compared to conventional tablets without polysorbate 80. Additionally, they examined the effect of coating (silica) to Eudragit ratio and have observed slowest drug release when ratio of silica:Eudragit was 1:2 (Nokhodchi, et al., 2010). Hydroxyethyl cellulose (HEC) matrices drug release unlike HPC can be a combination of erosion, polymer swelling as well as drug diffusion (Sinha & Rohera, 2002). Moreover, HPC demonstrates a low drug release rate with effect of lower penetration rate resulting in moderate swelling. Javadzadeh et al (2007) also showed that formulation containing polysorbate 80 (50%) with Eudragit ®RS as the carrier and HPMC as additive had a low

dissolution rate. The possible explanation can be due to the formation of gel around the disintegrated particles by HPMC, a barrier is built against diffusion of the dissolved drug into the dissolution medium.

In more details, the process of drug release from hydrogel matrix tablet is divided into four stages: water penetration into the hydrogel matrix tablet upon insertion into the dissolution medium, diffusion of the drug through the sustained release matrix and its dissolution, as well as polymeric excipient erosion (Tack-Oon, et al., 2013). Also, diffusion of water into the polymer matrix occurs as soon as the polymer matrices come in contact with water. Due to this, an increase in the mobility of the polymer chains is caused by water penetration, allowing entangled chains to embrace new configuration, causing the outer surface of the polymer to dissolve and erode when becomes in contact with water. High molecular weight polymers, such as HPC-H, form a more viscous layer causing a decrease in the polymer matrix erosion due to the ability of these polymers to seal pores before allowing more liquid to penetrate, resulting in the polymer to swell faster. (Tack-Oon, et al., 2013) and (Wan, et al., 1991). Moreover, the presence of HPC as a drug carrier retards the release of metformin-HCl to a great extent. Since HPC-H swells in water forming stiff matrix retarding metformin-HCl release. The metformin-HCl release mechanism from the HPC matrix. And this can be compared to the immediate release liquisolid metformin-HCl did not show delayed release when Avicel®PH102 was used as a carrier instead of HPC.

Additionally, the release of drug from matrix is highly dependent on the amount of polymer used as seen in Figure 5.2. This is in agreement with (Tack-Oon, et al., 2013) who investigated drug release from hydrophilic polymer matrices such as HPMC with different

drug to polymer ratios. It was found that as the concentration of the polymer increased, the viscosity of the gel also increased and diffusional path being formed and thus drug release rate was reduced by diffusion.

In vitro drug testing is crucial process to study the quality assurance as well as determination of stability release properties of drugs over a certain period of time. Thus, basket and paddle methods resemble the most popular approaches which operate under closed sink condition, yet cannot mimic the GI system. In this study, the dissolution test of the liquisolid sustained release metformin-HCl was performed using USP 4 operating in open-loop manner. The flow through cell (open-loop) system is greatly beneficial for low-solubility drugs as it can easily provide sink conditions by maintaining the continuous flow of fresh medium throughout the dissolution period. However, in this project, open loop configuration was used for high water soluble metformin-HCl to study its sustained effect using this system, where results obtained are expressed as the drug instantaneous concentration. Additionally, the open loop configuration (USP4) is strongly proficient in simulating the digestive system environment and has the potential to provide in-vitro and in-vivo relationship that can be easily applied by laboratories (Gao Z., 2009).

5.3 Drug release mechanism and kinetics for metformin-HCl sustained release formulations

To interpret the kinetic release mechanisms of the metformin-HCl from matrix systems (sustained release HPC), different Kinetic equations such as zero-order, first-order and

Higuchi's equations were applied. The best plot with higher squared coefficient of determination (R^2) was found to represent the best kinetic release model formulation (Wadher, et al., 2011).Table 5.2 represents the values of the squared coefficient of determination for metformin-HCl liquisolid sustained release (HPC1-HPC6, refer to Table 5.1 for details), using zero order, first order and Higuchi kinetic release models. Different kinetic equations were implied on release data in order to describe the kinetics of drug release from matrix tablets (Figures are included in the appendix for chapter 5). Kinetics analysis of in-vitro release data could be best expressed for all formulations containing hydroxypropyl cellulose (HPC1-HPC6) by Higuchi's equation, as the plot showed highest linearity coefficient of determination (R^2) of more than 0.97 (Table 5.2). The drug release kinetic using Higuchi model describes the release from an insoluble matrix as a square root of a time-dependent process based on Fickian diffusion (Pavani, et al., 2013).

Constant drug release, from the beginning to the end, in a zero order kinetic model is an ideal matrix system (Pather, et al., 1998). With time, drug release from matrix tablets becomes progressively slower. The zero-order kinetics is when the drug release rate is independent of its concentration. On the other hand, the first order kinetic model describes the release rate is concentration dependent (Wadher, et al., 2011).

Table 5-2: Release kinetics of metformin-HCl liquisolid sustained release formulations (for composition details, refer to Table 5.1)

Metformin Liquisolid sustained (HPC) formulations	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)
PEG®400 30% (HPC1)	0.959	0.686	0.999
PEG® 400 60% (HPC2)	0.965	0.699	0.999
Synperonic®PE/L44 30% (HPC3)	0.954	0.699	0.998
Synperonic® PE/L44 60% (HPC4)	0.941	0.656	0.977
Cremophor® ELP 30% (HPC5)	0.974	0.678	0.998
Cremophor® ELP 60%(HPC6)	0.944	0.661	0.994

Kinetic models which are more suitable for controlled release formulations are zero order and Higuchi kinetic models. (Higuchi, 1963) declared that the drug release from non-swellable matrices is governed primarily by diffusion. Systems that follow Higuchi kinetic model show a decrease as a function of time in the rate of drug release from matrixes containing water-soluble drug and a water insoluble carrier. This is due to the increase in the drug release diffusional path length with time as the solvent moves towards the center of the matrix (Higuchi 1963). Furthermore, drug release from the tablets was primarily controlled by polymer swelling followed by drug diffusion through the swollen polymer and then slow erosion of the tablet matrix (Nanjwade, et al., 2011).

5.4. Tablet hardness, friability and uniformity of weight of sustained release metformin-HCl using HPC

Table 5.3 presents physical information about several quality control tests which include:

tablet thickness, hardness, friability test, and drug weight uniformity test for the liquisolid tablet formulations of metformin-HCl sustained release including conventional tablets using HPC (HPC1-HPC6). The aim of making those tests is to investigate the effect of wet granulation using PVP and the use of HPC on the physicochemical characteristics of the prepared liquisolid tablets. All liquisolid tablets except HPC 4 (2.94%) and conventional tablets (1.54%) passed and complied with pharmacopeia BP required specifications for friability test where the loss of the powder was less than 1%. This indicates that the prepared liquisolid tablets were of sufficient strength to endure the handling and packing process. The wet granulated liquisolid formulations using PVP as liquid binder have a tremendous resistance to any adhesive forces with the wall of the friability tester or cohesive forces among the tablets. Furthermore, using compression force to produce hardness of 30-45N, liquisolid tablets with the hierarchical thickness of 3.35 to 4.47 mm were prepared. The thickness uniformity could be considered as a further control to the increased reproducibility of liquisolid tablets (El-Say, et al., 2010). Also, the acquired results in Table 5.3 show acceptable thickness uniformity for all metformin-HCl liquidsolid tablets (HPC1-HPC6).

Considering the integration of liquid vehicle in the formation of liquisolid metformin-HCl sustained release formulation, wet granulation was applied on formulations (HPC1-HPC6) (Table 5.1) using PVP in order to enhance the flowability and compressibility of the liquisolid powder. HPC is freely flowable and therefore due to the absence of non-volatile liquid in the conventional metformin-HCl no granulation step was performed; however, the friability result was >1%.

Table 5-3: Thickness, crushing load, hardness, friability and uniformity of weight for sustained release metformin-HCl liquisolid tablets using HPC (for formulations' composition, refer to Table 5.1.)

FORMULATION	THICKNESS (MM)	HARDNESS (KG/F)	HARDNESS (N)	FRIABILITY WEIGHT (%)	UNIFORMITY OF WEIGHT (MG)
HPC1	4.06 ± 0.06	4.67 ± 0.69	45.78±6.76	0.38	288 ± 3.00
HPC2	4.47 ± 0.01	5.93 ± 0.81	58.21±7.99	0.75	293 ± 4.40
HPC3	3.56 ± 0.28	1 ± 0.00	9.81	0.97	279 ± 3.70
HPC4	3.34 ± 0.01	1.80 ± 0.85	12.43±5.50	2.90	246 ± 4.40
HPC5	3.55 ± 0.17	<1 ± 0.00	9.81	0.05	247 ± 4.40
HPC6	3.64 ± 0.09	1.12 ± 0.12	10.95±1.15	0.26	276 ± 2.80
CONVENTIONAL	3.35 ± 0.02	4.52 ± 0.78	44.31±7.68	1.54	283 ± 1.50

As the diameters for all tablets have approximately similar values (using same tablet press), the cause for variations in the tablet thickness can be explained in terms of the dissimilarity in the weights of the tablets which also complied with BP limit specifications. Tablet hardness were shown to be good for HPC1, HPC2 and conventional (4.67, 5.93 and 4.52 kg/F = (45.78N, 58.21 N, 44.31 N) respectively where HPC3 - HPC6 showed poor crushing load profile (1kg/f) even though granulation process was applied. A correlation between tablet hardness and its porosity was observed: increasing the compression force and hardness of tablets leads to the reduction of porosity among the particles in the dosage and reduction in drug release (Figure 5.2 for HPC1 - HPC2). Formation of solid bridges among the particles of the tablets can result, leading to a decrease in the intermolecular distance (Pavani, et al., 2013). Moreover, a relationship exists between tablet hardness and the quantity and type of the non-volatile liquid vehicle as well as the amount and type of the carrier and coating that are being used in the formulation of liquisolid tablets (Spireas & Bolton, 1999). It was found that a relation can be found between tablet hardness and the liquid load factor (Lf), where the relationship

is inversely proportional. That is, when Lf increased, tablet hardness decreased and this finding is illustrated in Table 5.3. This finding is confirmed by the following data where the tablet hardness of HPC1-HPC6 is 4.67, 5.93, 1, 1.80, 1 and 1.12 kg/F respectively with a corresponding Lf values of 0.168 (HPC1 and HPC2), 0.266 (HPC3 and HPC4) and 0.52 (HPC5 and HPC6). The proposed explanation to this inverse relationship is that as the Lf value increases, the amount of liquid used also increases causing a reduction in the quantity of powder excipients used, causing a drop in the tablet hardness. In a previous study by Javaheri et al, 2014, it was found that wet granulation in combination with liquisolid system can show positive impact on the hardness of the tablets. However, this scientific fact was not seen in the liquisolid formulation of sustained release metformin-HCl using HPC as carrier and as sustaining agent. The possible explanation could be due to the high viscosity property of Cremophor®ELP creating an unfeasible environment in having freely flowable powders. Nevertheless, the application of wet granulation has shown promising results with such highly viscous non-volatile liquids (Javaheri, et al., 2014) however, HPC in high grade has a gel type characteristic that makes it unpractical to granulate easily using any applicable liquid binders. Therefore, it can be suggested that for future, encapsulation of HPC 5 and HPC6 into size zero capsules would be strongly recommended. Additionally, in regards to uniformity of weight, all liquisolid metformin-HCl tablets were found to be practically within BP limits. Overall, granulated liquisolid formulations of sustained metformin-HCl using HPC showed acceptable good tablets within limits uniformity of weight, low friability with acceptable thickness and hardness. Different liquid binders were tested in this research, and amongst them, only PVP worked

appropriately with HPC and was a suitable liquid binder that was used to granulate HPC, however, poor friability tablets were obtained.

5.5. Scanning electron microscopy of sustained release metformin-HCl using HPC

The scanning electron microscope microstructure of liquisolid formulation using HPC (HPC1-HPC6) including pure metformin-HCl and pure HPC are shown in Figure 5.3. It can be seen from the images that pure drug is different in shape from the liquisolid formulations indicating its crystalline nature of metformin-HCl. Generally, the SEM photomicrographs of liquisolid powders (HPC1-HPC6) demonstrate the drug particles are entrapped onto the surface of the carrier material (HPC) or within them and causes surface modification of the drug particle (Nnamani, et al., 2016); ((Butreddy & Dudhipala , 2015).

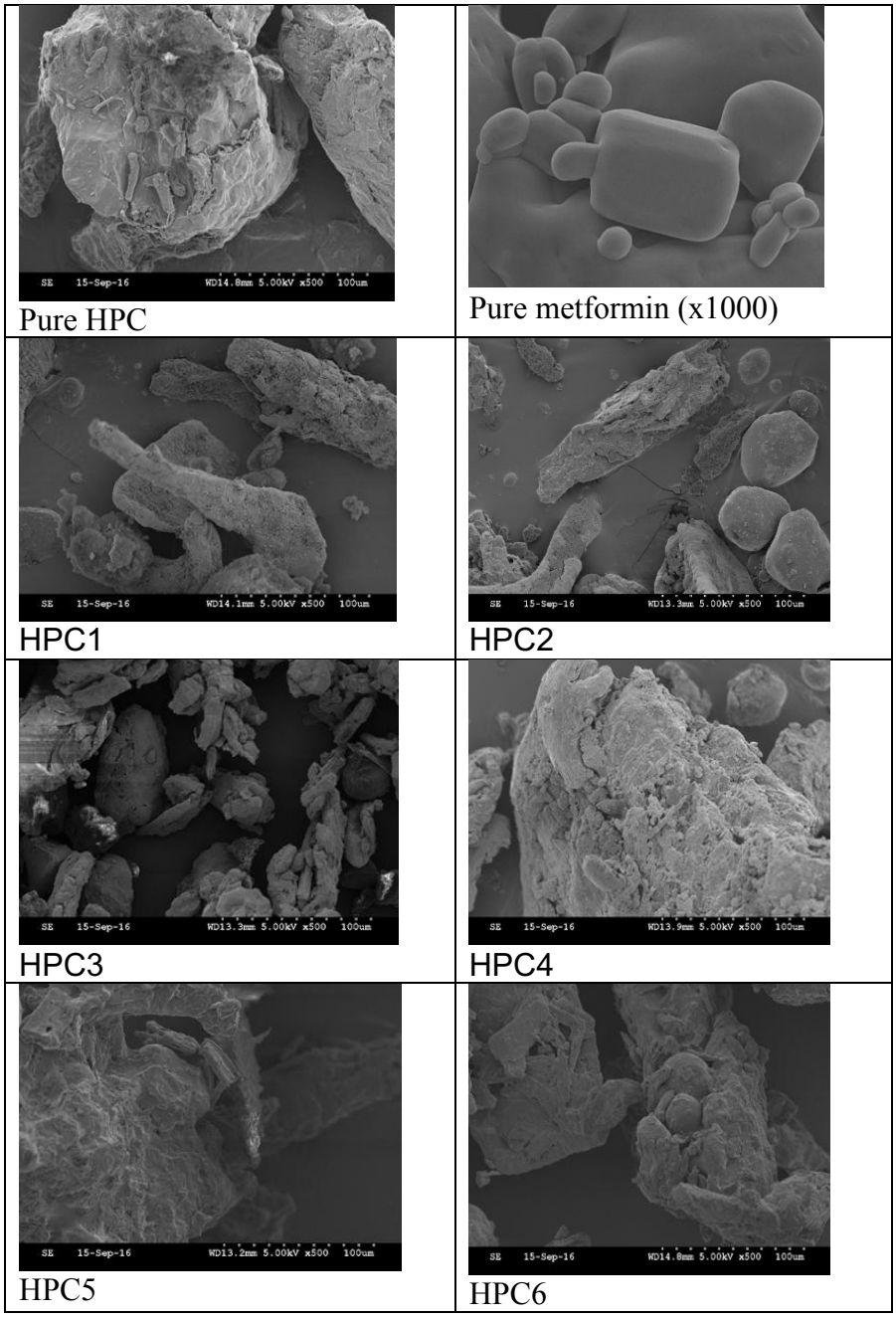
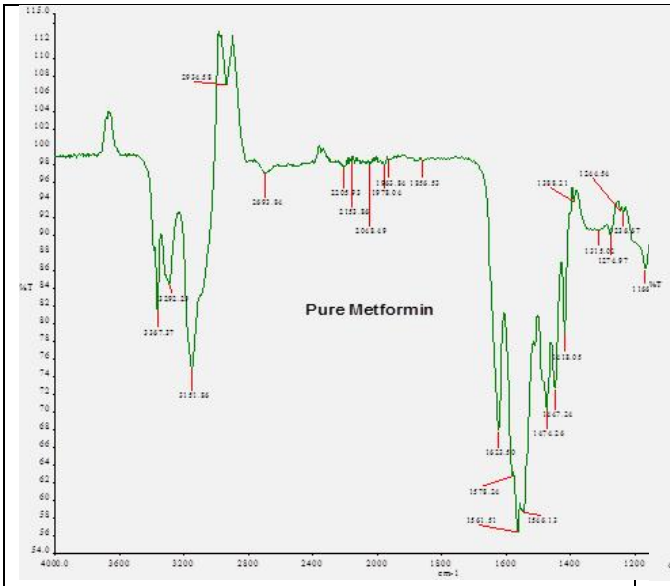


Figure 5.3: SEM microphotographs of pure metformin-HCl and sustained release metformin-HCl liquisolid powders using HPC. Refer to Table 5.1 for formulations' composition

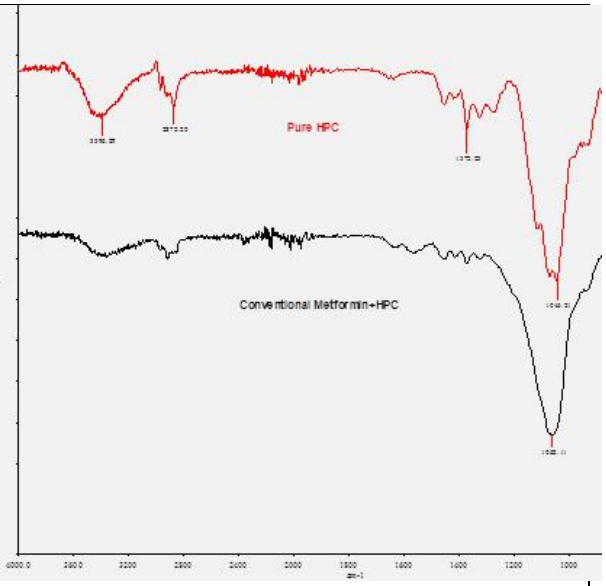
5.6. Fourier transform infrared spectroscopy (FTIR) of HPC formulations

According to (Silverstein, et al., 1991), the interaction between drug and excipients or liquid vehicles cause a shift in the peaks corresponding to the functional groups of the drug to different wavenumbers. Additionally, the absorption bands of the functional groups arise from stretching and deformation vibrations.

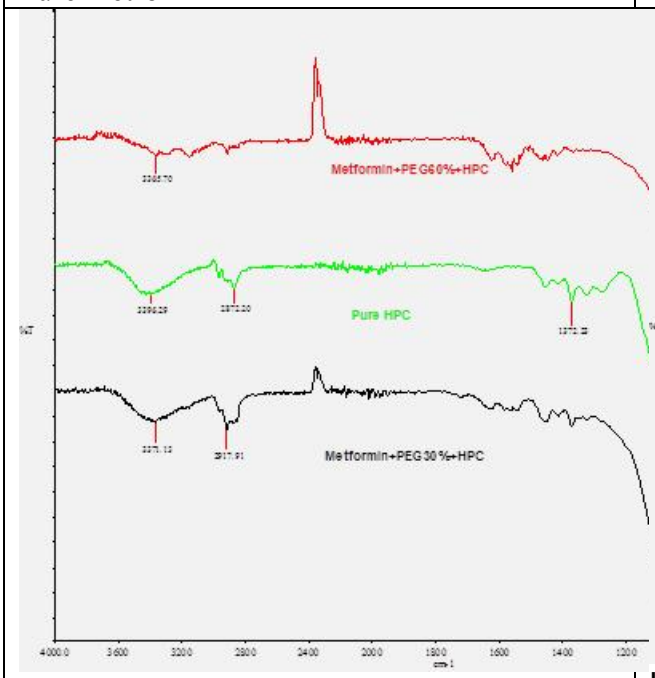
Figure 5.4. metformin-HCl IR studies, revealed two typical bands at 3367 and 3292 cm^{-1} due to N-H primary stretching and a band at 3156 cm^{-1} due to N-H secondary stretching. Additionally, characteristics bands at 1623 and 1561 were observed to be denoted for N-H asymmetric deformation. Furthermore, shifts or reduction in intensity for the FTIR bands of pure metformin-HCl and disappearance of the sharp peak of metformin-HCl was observed in liquisolid formulations (Figure 5.4) which depict clear change in the structure of the drug after the interaction between the liquid vehicle, excipients and metformin-HCl. HPC forms a peak at 1046 cm^{-1} and is clearly seen in all liquisolid formulations including conventional powder and the fingerprint regions of liquisolid metformin-HCl and HPC are superimposed. The study shows drug concentration has no impact on the FTIR spectra and thus no difference appears between 30% and 60% drug concentrations in liquisolid properties.



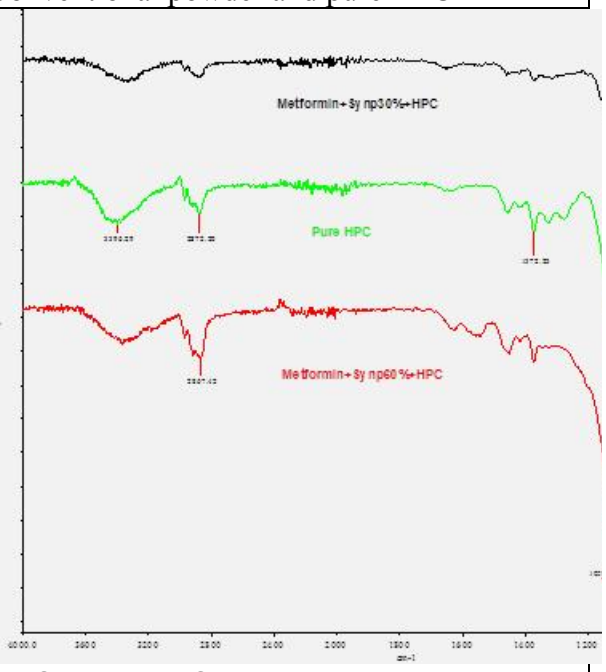
Pure metformin



Conventional powder and pure HPC



HPC1 and HPC2



HPC3 and HPC4

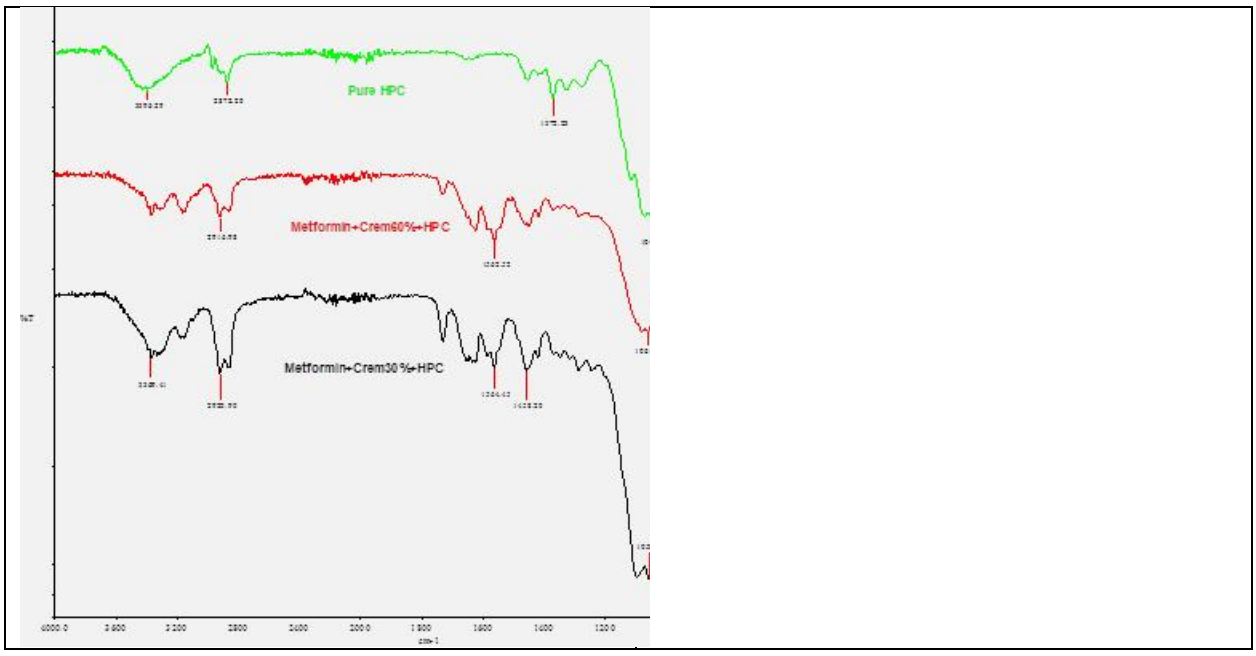


Figure 5.4: FT-IR spectra of sustained release metformin-HCl liquisolid using HPC.

5.7. Differential scanning calorimetry (DSC) of sustained release liquisolid metformin-HCl

DSC thermograms of metformin-HCl HPC1-HPC6 are shown in Table 5.4. The analyses were used in order to investigate the possible solid-state interactions between the components (drug and excipients) and compatibility in all the examined liquisolid formulations. DSC thermogram of pure metformin-HCl in table 5.4 exhibited a sharp endothermic peak at T_m of 232.99 ^{0}C (melting point) and enthalpy of 215.8J/g indicating fusion and crystallinity of metformin-HCl. The thermograms of all liquisolid metformin-HCl (HPC1-HPC6) showed broad endothermic peaks and reduction in melting point (~1-3°C, Table 5.4). In comparison with the pure metformin-HCl thermogram, the results of the liquisolid formulations displayed shifted endothermic peaks towards the lower temperature and greatly reduced the enthalpy, indicative of reduced drug crystallinity in HPC and non-volatile liquid vehicles and solid-solid interaction (Nafady, et al., 2014). This can also be explained on the bases of diluting effects or that most of metformin-HCl was dissolved in the liquid vehicle, thus reducing the number of drug crystals particles in the liquisolid formulations, this is in agreement with (Nokhodchi, et al., 2010). Moreover, the thermogram of conventional metformin-HCl HPC shows peaks of drug melting point 233 ^{0}C, meaning crystallinity of the drug. For HPC1 to HPC6 (refer to Table 5.1) the drug endothermic peak became broader and lost its sharpness, indicating the drug dispersion and solubilisation in the liquid vehicle and the rest of the excipients including HPC within the liquisolid system. However, the DSC thermograms of HPC1-HPC6 including conventional show a second peak with melting point ranging between 99 ^{0}C - 117 ^{0}C that is not seen in pure metformin-HCl thermogram. Thermograms of HPC1-HPC6 have

endothermic peaks of 116.7 ^{0}C, 115.41^{0}C, 110.77^{0}C, 112.5^{0}C, 98.44^{0}C and 99.05 ^{0}C, respectively with enthalpy of 40.38J/g, 46.30J/g, 45.22J/g, 44.13J/g, 43.7J/g and 43.74J/g, respectively. Those peaks are due to water evaporation or the presence of excipients. In conclusion, the delayed dissolution rate of metformin-HCl liquisolid compacts can be linked to the changes in crystallinity of the drug and formation of complex between the drug and the excipients.

Table 5-4: DSC thermograms results of pure metformin-HCl and sustained release metformin-HCl liquisolid formulations using HPC, for formulations' composition refer to Table 5.1.

Formulation	Temperature (^{0}C)	Enthalpy (J/g)
Pure metformin	232.99	215.80
HPC1	116.74, 230.73	40.38, 09.93
HPC2	115.41, 231.92	46.30, 20.24
HPC3	110.77, 230.66	45.22, 08.08
HPC4	112.51, 232.32	44.13, 22.16
HPC5	98.44, 229.60	43.71, 14.78
HPC6	99.05, 229.69	43.74, 36.28
Conventional	98.02, 233.12	51.20, 23.21

5.8. Conclusion

Sustained release metformin-HCl was prepared using HPC by the application of liquisolid technique. Three different liquid vehicles (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) were used with 30% and 60% drug concentrations. Among the above mentioned liquid vehicles, Cremophor®ELP using 30% drug concentration showed the highest (92%) and the most sustained drug release over 12 hours. HPC shows moderate swelling coupled with slower rate of erosion. Drug release profiles on model fitting followed zero order and Higuchi models. Additionally, in this research chapter, the flow-through method with open loop configuration has been used to perform the dissolution

study of the sustained release metformin-HCl. Therefore, USP flow-through cell with open loop configuration was a successful dissolution apparatus to study the release of metformin-HCl over 12 hours.

Chapter 6 – Orodispersible liquisolid preparation of metformin-HCl immediate release using Eudragit®RL-30D as a non-volatile liquid vehicle: A Novel discovery

Results and Discussion

Figure 6.1: Flowchart metformin-HCl liquisolid formulation using Eudragit®L30D as non-volatile liquid vehicle summary work.

6.1. Introduction:

Eudragit®RL30D and Eudragit®RLPO are biocompatible copolymers synthesized from acrylic and methacrylic acid esters. The structures of Eudragit®RL differ from the rest of the Eudragit polymers only in the extent of the quaternary ammonium substitutions, more substitution is seen with Eudragit®RL. Additionally, their water permeability is unaffected by pH and water can also permeate more freely into Eudragit®RL due to the relative hydrophilicity of the RL polymer (Patra , et al., 2017). This can be linked to the availability of ammonium groups as salt, which makes Eudragit®RL more permeable. Eudragit® RL has an improved protective and sustained-release performance along with an outstanding gastrointestinal tract targeting (Evonik 2015), (Singh & Rana, 2013).

Liquisolid technique is initially designed to enhance the dissolution rate of poorly water-soluble drugs and in recent studies has been used to sustain the release of water soluble drugs such as metformin-HCl (as shown in chapter 5). However, in this section, liquisolid is applied on metformin-HCl, a water soluble drug, using a novel non-volatile liquid vehicle (Eudragit®RL30D), to produce tablets with enhanced properties. Eudragit®RL is a polymer which is widely used in pharmaceutical industry to sustain drug release instead of hydrophilic carrier or retarding agents (such as HPMC) (Azarmi, et al., 2002) in the liquisolid formulations. In this current study, it did not have the potential to sustain the release of metformin-HCl. Instead, it was capable of producing high yet steady release of the drug with high standard quality control properties for the prepared tablets, and an excellent fast disintegrating profile that makes them a successful discovery and addition to the liquisolid system scheme as an orodispersible liquisolid tablets. This may be due

to the interaction between the drug and Eudragit®RL.

Inability to tablet taking is a common difficulty in most patient groups and predominantly for geriatric, pediatric and psychiatric patients that fail in compliance with taking tablets due to complications such as hand tremors, dysphasia and immature nervous and muscular systems. The physical difficulty of swallowing is a common occurrence which leads to poor efficiency of treatment. The emergence of fast dissolving tablets as an alternative has resulted in overcoming the aforementioned disadvantages. Fast dissolving tablets also known as orodispersible tablets swiftly break and disintegrate in the mouth within 60 seconds and liquefy in saliva without the need for chewing or water. These uncoated tablets are convenient and provide instant action that make them suitable dosing with pronounced patient compliance due to ease of swallowing (Revathi, et al., 2015), (Salunkhe, et al., 2013).

Flowchart 6.1 summarises the work performed in this chapter.

6.2. Determination of the angle of slide for Avicel®PH102 (MCC), Eudragit®RLPO, Eudragit®RLPO: Avicel®PH102 (3:7) (as the carrier) and Cab-o-sil®M-5 (fumed silica) using Eudragit®RL30D as a non-volatile liquid vehicle

In the formulation of liquisolid tablets, determination of angle of slide is one of the primary steps of the system preparation. The flowable liquid retention potentials (Φ values) of powder excipients were used for the calculation of L_f value. The L_f is used to calculate the amount of liquid vehicle (Eudragit®RL30D) to be used in the liquisolid formulation. As explained previously, the Φ_{CA} value and Φ_{CO} value determine the amount of carrier and

coating materials required to produce a free flowing, non-adherent, readily compactible and dry- looking liquisolid formulations (Tiong & Elkordy, 2009). The preferred method over other approaches to establish the flowability of powders is the determination of angle of slide (θ) of fine powders with particle diameter less than 150μm. Moreover, this method was applied due to the small particle sizes of Cab-o-sil®M-5P and Avicel®PH102 (133.8μm and 0.2-0.3 μm respectively). Therefore, in order to calculate the required ingredient quantities, the flowable liquid retention potential (Φ values) of powder were utilised. The values of the flowable liquid retention potential for Avicel®PH102 and Avicel®PH102:Eudragit®RLPO (7:3) and Cab-o-sil®M-5 with Eudragi®RL30D (the liquid vehicle) were respectively calculated. Furthermore, the loading factor (Lf), the appropriate amount of carrier (Avicel®PH102 and Avicel®PH102: Eudragit®RLPO (7:3) (Q) and the required quantity of coating material (Cab-O-sil®M-5) (q) to convert a given mass of liquid medication (W) into an reasonably flowing and compressible liquisolid admixture were calculated using equations mentioned in chapter 2 (section 2.3.1). A sample calculation is presented below:

1) $Lf = \Phi CA + \Phi\, CO\ (1/R);\ R = 20$

$$Lf = 0.8 + 0\,x\,(0.05) \rightarrow \text{from angle of slide}$$

$$Lf = 0.8$$

2) $Lf = W/Q$ ……. For 30% w/w drug in liquid vehicle

$$Q = W/Lf$$

$$Q = 133.3/0.8$$

$$Q = 166.66$$

$$W = 30mg\ (drug) \rightarrow 70mg\ (liquid\ vehicle)$$

For 40mg drug/unit dose ➔ liquid vehicle amount in mg will equal $40 * 70/30 = 93.3mg$

Hence,

$$W = 40 + 93.3 = 133.3\, mg/tablet$$

$$Q/q = 20, hence\ q = 8.33mg/tablet$$

Table 6.1 shows the calculations' results and the prepared formulations.

Table 6-1: Liquisolid formulation of metformin-HCl using Eudragit®RLPO as a carrier and Eudragit®RL30D as a non-volatile liquid vehicle.

Liquisolid system	Non-volatile liquid vehicle	Drug Concetration in liquid medication (%w/w)	Carrier: Coating (R)	Liquid load factor (Lf)	Liquid vehicle (mg)	Active ingredient (mg)	Carrier (Q) (mg)	Coating (q) (mg)	Disintegrant (maize starch) (mg)	Lubricant (mg)	Unit dose (mg)
LSS1	Eudragit RL 30D	30	20	0.80	93.30	40.00	166.60	8.33	16.40	2.50	327.00
LSS2	Eudragit RL 30D	60	20	0.80	26.70	40.00	83.30	4.17	8.18	1.20	164.00
LSS3	Eudragit RL 30D	30	20	0.20	93.30	40.00	666.50	33.30	44.20	6.60	884.00
LSS4	Eudragit RL 30D	60	20	0.20	26.70	40.00	333.35	16.67	22.10	3.30	442.00

Note: * Eudragit®RLPO:Avicel®PH102 3:7 = carrier

Several attempts have been made in order to achieve the optimum flowable retention using different Avicel®PH102 to Eudragit®RLPO ratios. A ratio of 1:1 was also used and the successful ratio of 7:3 (Avicel®PH102:Eudragit®RLPO) gave optimum flow and good compactable tablets.

6.3. In-vitro dissolution study of immediate release liquisolid metformin-HCl using Eudragit®RL30D and Eudragit®RLPO

In-vitro dissolution tests were performed to study the release behaviours of liquisolid formulations in dissolution media (distilled water) (refer to Figure 6.2).

Dissolution profiles of liquisolid compacts using Eudragit®RL30D as a non-volatile liquid vehicle (LSS1-LSS4) are shown in Figure 6.2. It is clear that the percentage of metformin-HCl release in the distilled water dissolution medium is significantly high in a short time. After 10 minutes, the percentage of the drug release was over 85% in all liquisolid tablets. In this profile, after 2 hours LSS1 to LSS4 reached 100% release. It is clear from Figure 6.2 that the tablets prepared by liquisolid technique (LSS1 and LSS2) which contain Avicel®PH102 as a carrier show faster drug release rate properties (100% after 1 hour) in comparison with LSS4 and LSS5 drug release (93.5% and 87.4%, after 1 hour) matrix tablets. The results showed that the percentage of drug release from liquisolid matrices containing Eudragit®RLPO and Avicel®PH102 in the ration of 3:7 showed lower release rate but ($P>0.05$). This could be due to difference in water permeability of those two polymers as water can permeate more freely into Eudragit®RLPO than into Avicel®PH102 containing tablets due to the relative hydrophilicity of the RL polymer (Azarmi, et al., 2002); (Javadzadeh, et al., 2008). Additionally, the drug concentration in liquid medication

used was 30% and 60% for all liquisolid tablets. Liquisolid formulations with 30% drug concentration (high amount of liquid vehicle) showed insignificant higher percentage drug release than 60% drug concentration. Comparably LSS1 and LSS2 have liquid load factor of 0.8, thus higher percentage drug release was seen in these two formulations and not in LSS3 and LSS4 where their liquid load factors were 0.2. This finding is also confirmed by the comparison between LSS4 and LSS5. Higher drug release was seen in LSS4 (93.4%) with 30% drug concentration than LSS5 (87.4%) which contains 60% concentration of drug. Therefore, the increase in the amount of liquid vehicle leads to an increase in percentage drug release however insignificant ($P > 0.05$).

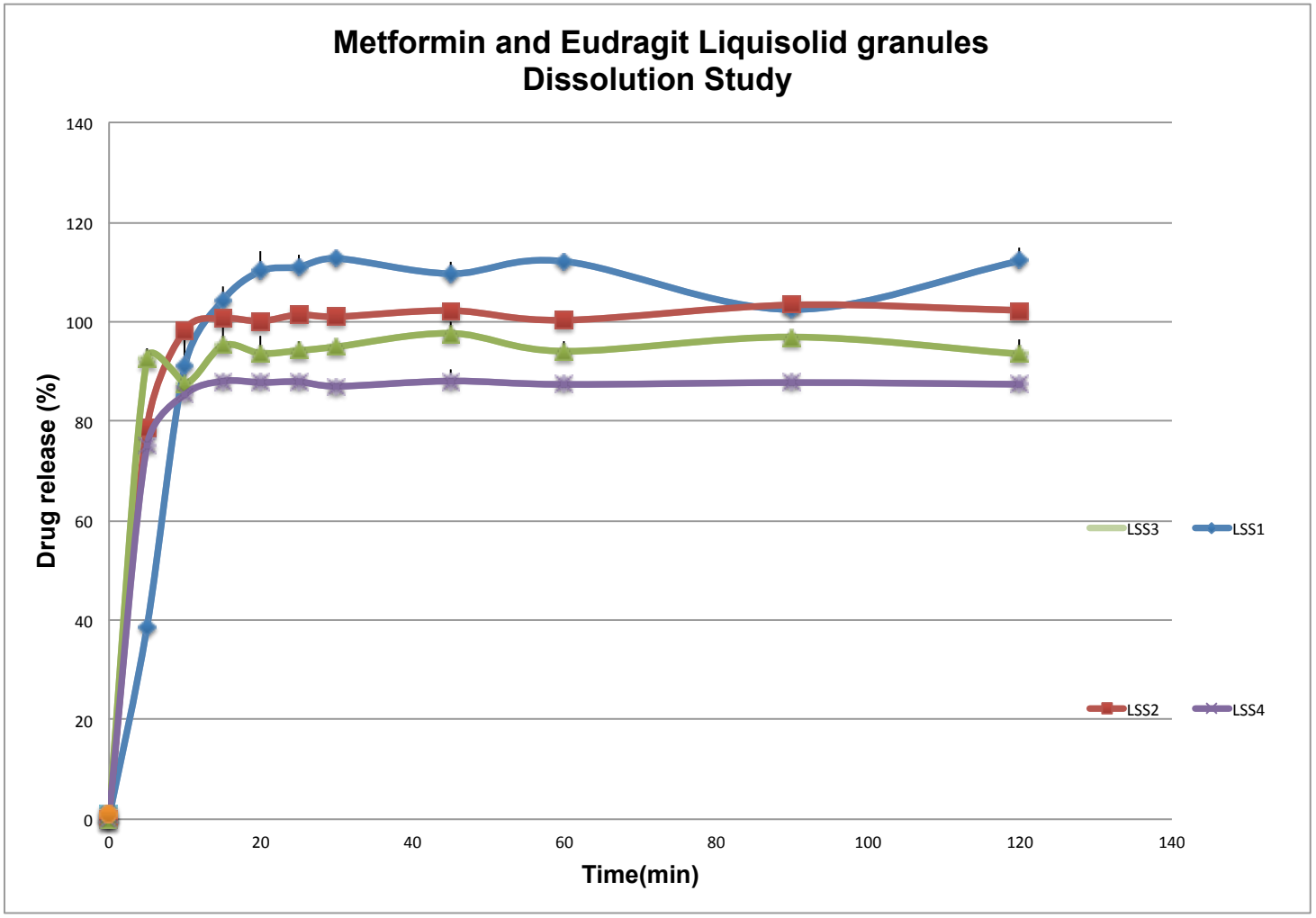


Figure 6.2: Dissolution study of immediate release liquisolid metformin-HCl (LSS1-LSS4) using Eudragit®RL30D as a novel non-volatile liquid vehicle (refer to Table 6.1 for composition).

As can be seen in Figure 6.2, no sustained-release effects were observed with metformin-HCl LSS1-LSS5 liquisolid tablets which were prepared with Eudragit®RL30D as the non-volatile liquid vehicle and Eudragit®RLPO as the carrier (in combination with Avicel®PH102). Fast dissolution rate was observed using Eudragit®RL30D although literature, such as (Boyapally, et al., 2009), suggests sustaining property reported for such polymers. This can be attributed to the hydrophilic nature of Eudragit®RLPO, when exposed to the dissolution medium, water, diffused into the free spaces of the matrix. Upon solvation of the polymer chains due to polymer relaxation, an increase in the dimensions of the polymer molecules can be observed.

Additionally, there was no significant difference among liquisolid tablets in their drug release (p-value is >0.05). This finding can be related to the drug's nature and the way it interacts with the liquid vehicles and other excipients. Similarly, in a study performed by Elkordy et al. (2013), they prepared liquisolid spironolactone tablets using Kollicoat®SR30D as the non-volatile liquid vehicle and observed no sustained-release effects. The proposed explanation was due to the drug's interaction with the excipients, rapid disintegration of tablets due to poor hardness or insufficient concentration of Kollicoat®SR30D to produce sustained release effect. Therefore, this can also be a valid explanation to the current results with metformin-HCl, taking into account that metformin-HCl is highly water soluble and therefore could have reacted differently when being in contact with Eudragit®RL30D and Eudragit®RLPO which was confirmed by FTIR and DSC results.

A study by Javadzadeh et al. (2008) publicised the impact of granulation process on the retardation of propranolol liquisolid release. In the same study, the authors claimed using conventional tablets without the application of granulation process showed higher drug release with Eudragit®RL than RS. However, when wet granulation was applied using HPMC as a liquid binder on the same formulations, the results were the opposite; Eudragit® RL showed slower drug release rate than those prepared using Eudragit®RS. The possible explanation was linked to the use of HPMC in the granulation process and its effect on release pattern as a result of drug particle coating containing Eudragit®RL and not Eudragit® RS. Moreover, in this current research the use of Eudragit®RL30D as the non-volatile liquid vehicle and liquid binder for the process of wet granulation did not have any retardation effect on the release of metfromin liquisolid. On the contrary, it produced steady and immediate release tablets. Unlike the polysorbate 80 which, is also a non-volatile liquid vehicle used by Javadzadeh et al. (2008) along with Eudragit to sustain the release of propranolol. One of the interesting properties of polysorbate 80 by which it can reduce the glass transition temperature of the polymers in which leads to release prolongation of liquisolid tablets due to its plasticity effect.

A study by (Shah, et al., 2012) performed a study on formulation of microsphere based orodispersible tablets of itopride HCL, and in the dissolution profile, where the pH of the medium was 1.2, it was observed that within the first 10 minutes, 90% of the drug was released. Accordingly, in this current research project, the dissolution study performed in distilled water and the result outcome was very interesting. After 10 minutes of dissolution run, LSS1 released 91% of liquisolid metformin-HCl, followed by LSS2, LSS3 and LSS4 where the release was 98%, 87% and 85%, respectively. Therefore, it is expected that as

soon as Eudragit® dissolves in the acidic environment of the stomach, the drug will be quickly released or even before reaching the stomach i.e. in the mouth (as tablets showed fast disintegrating effect). Nevertheless, Javadzadeh et al. (2008) claimed the use of granulation has greater impact on sustained release behaviour of liquisolid tablets. Conversely, in the current study, wet granulation using Eudragit®RL30D as a liquid binder showed no sustaining release behaviour on metformin-HCl Liquisolid tablets(LSS1-LSS4), instead all tablets showed immediate release with fast disintegration profile (Refer to Section 6.4). Moreover, the reason for choosing Eudragit ®RL over Eudragit®RS was the published research papers. Javadzadeh et al. (2008) prepared liquisolid propranolol as sustained release tablets using Eudragit®RL and Eudragit®RS where they compared the retardation effect in the release of propranolol by using both polymers. As a result, Eudragit®RS showed a more sustained release effect due to its hydrophobic properties which reduces the permeability of water into the matrix. However, upon application of wet granulation technique using HPMC, the outcome was reversed. Wet granulation had remarkable impact with greater retardation effect on propranolol liquisolid tablets containing Eudragit®RL (Javadzadeh, et al., 2008). Yet, after application of the above principle, immediate release and fast disintegrating formulations of metformin-HCl liquisolid tablets were obtained which adds a new approach and discovery to the liquisolid list of achievements. For future work, taste masking excipients can be added to make the metformin-HCl orodispersible tablets more customer friendly for patients.

6.4. Uniformity of tablet weight, Tablet hardness, friability, disintegration and uniformity of drug content

Table 6.2 shows a comparison of the content uniformity, friability, hardness and disintegration tests in terms of means and standard deviations between different metformin-HCl liquisolids using Eudragit®RL30D, as a non-volatile liquid vehicle, and Eudragit®RLPO, as a carrier in conjunction with Avicel®PH102 (3:7) that have been granulated using Eudragit®RL30D as a liquid binder. The drug content uniformity results show that the all formulations (LSS1-LSS4) are within the British Pharmacopoeia 2017 specific limits (85%-115%). Moreover, the changing in the quantities or type of carrier shows significant effect on the uniformity of the drug in these formulations. For instance, LSS1 and LSS2 show drug content of 110% and 104% where the used carrier was Avicel®PH102 (microcrystalline cellulose only). On the other hand, when the carrier constitution was made into a mixture of Avicel®PH102 and Eudragit®RLPO with a ratio of 3:7, the drug content uniformity has dropped to 95% and 87% for LSS3 and LSS4 respectively. As a consequence, the wet granulated formulations using Eudragit®RL30D liquid binder and Eudragit®RLPO: Avicel®PH102 as carrier do affect the distribution of the drug inside the Eudragit®RL30D liquisolid bed systems. The liquid binder (Eudragit®RL30D) is compatible with the liquid vehicle (Eudragit®RL30D) and the formation of the granules sustain the distribution of the liquid vehicle inside the solid framework.

In regards to the friability tests, none of the liquisolid formulations reached 1% loss. The friability percentage for LSS1-LSS4 are 0.01%, 0.03%, 0.45% and 0.42% respectively.

The results fulfilled the demands of the British Pharmacopoeia 2017 criteria that present an excellent resistance to handling and packaging of the prepared tablets.

In this study, the range of hardness is between 6.95kg/f (68.11N) and 9.25 Kg/f (90.65N). Using Avicel®PH102 as a carrier for LSS1 and LSS2 and Avicel®PH102 with Eudragit®RLPO (7:3) as carriers for LSS3 and LSS4, leads to cover the surface and increase solid bridges among particles. Particles undergo deformation and non-reversible changes of their shapes as a response to compression forces being applied; therefore, it can be said that carriers used in this study are capable of exhibiting plasticity behavior (Fahmy & Kassem, 2008). Plastic deformation is a well-known property of Eudragit® polymers which also have tendency to coat drug particles. (Apu, et al., 2009) noticed highest hardness (216.14 kg/cm$^{2)}$) in matrix tablets containing Eudragit®RLPO and Eudragit®RSPO in ratio of 2:3. They explained that high compression forces can result in hard, low porosity tablets that can markedly affect the drug release rate.

Table 6-2: Crushing load, hardness, friability, drug content uniformity and uniformity of weight for metformin-HCl liquisolid tablets using Eudragit®RL30D as the non-volatile liquid vehicle (for formulation compositions, refer to table 6.1.).

FORMULATION	CRUSHING LOAD (KG/F)	HARDNESS (N)	FRIABILITY WEIGHT (%)	DRUG CONTENT (%)	UNIFORMITY OF WEIGHT (MG)
LSS1	9.25 ± 0.23	90.74 ± 2.30	0.01%	110.00% ± 1.17	174 ± 2.10
LSS2	7.70 ± 0.43	75.54 ± 4.21	0.03%	104.00% ± 2.44	176 ± 1.70
LSS3	6.95 ± 0.21	68.18 ± 2.03	0.45%	95.00% ± 1.63	223 ± 2.10
LSS4	9.03 ± 0.34	88.62 ± 3.38	0.42%	87.00% ± 2.40	239 ± 1.80

For dissolution processes, rapid disintegration time is crucial due to fast division being provided into surface fragments. Furthermore, balancing between disintegration time and tablet hardness is an important factor from the industrial perception. According to the BP specification of disintegration test, in no less than 15 minutes a tablet must disintegrate, in which all liquisolid tablets have complied this specification. The data revealed an average time of about 2.6min for the liquisolid formulations (LSS1-LSS4). The results in Figure 6.3 show that the disintegration time comply with the BP specifications not only for immediate release but also for fast disintegration time (<3min). LSS3 recorded the shortest disintegration time of 15 seconds. Figure 6.3 presents the range of the disintegration time of metformin-HCl liquisolid formulation using Eudragit®RL30D. LSS3 was found to be the fastest formula to disintegrate (16 seconds), followed by LSS4, LSS2 and LSS1 with disintegration time 2.30, 2.70 and 5.2 minutes, respectively. There are fewer variations in the case of the Eudragit®RL30D liquisolid formulations compared with the immediate release metformin-HCl using PEG®400, Synperonic®PE/L44 and Cremophor®ELP formulations (chapter 4, section 4.5).

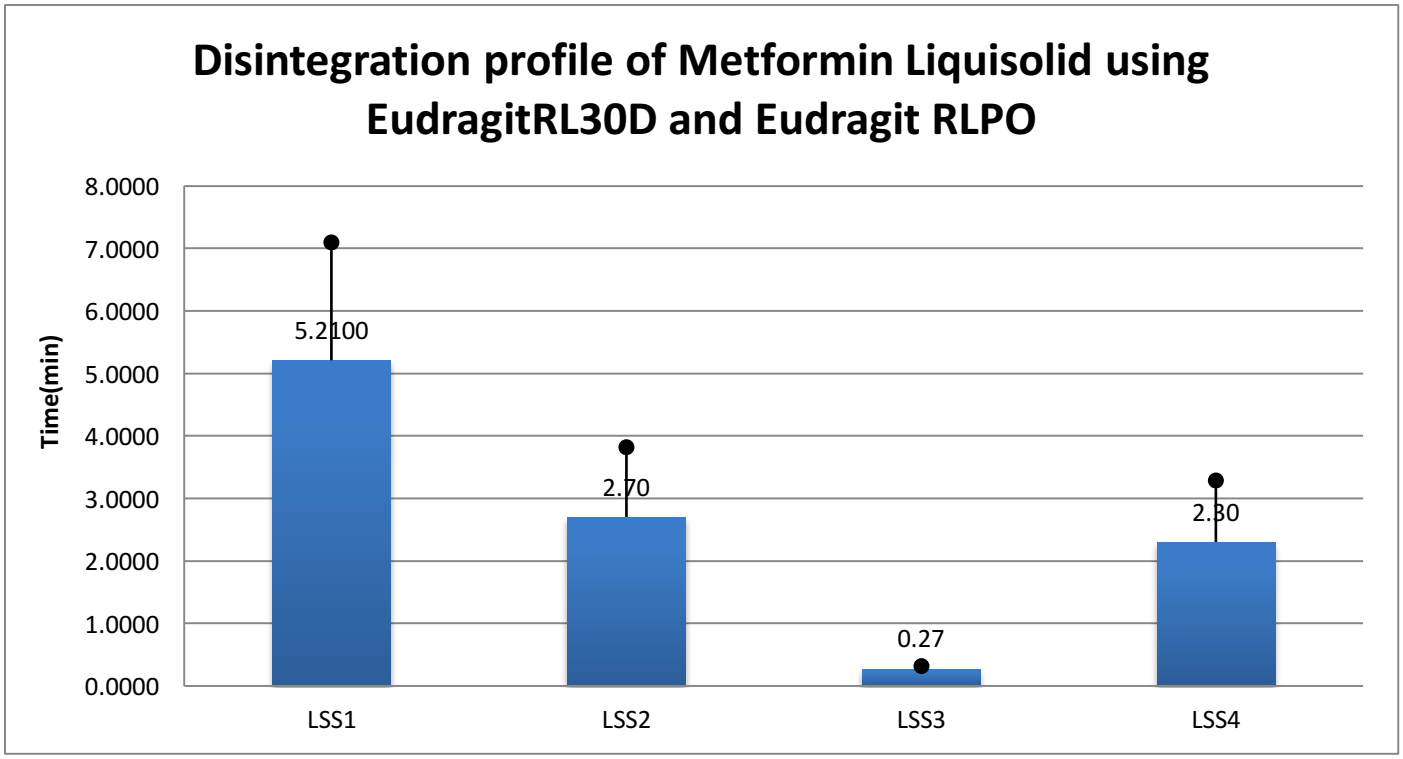


Figure 6.3: Average disintegration profile of liquisolid sustained release metformin-HCl granulated tablets. For formulations' composition refer to Table 6.

Rapid disintegration is important during tablet formation to ensure quick breakage of tablets into smaller segments during dissolution process for a larger surface area. The rapid disintegration of liquisolid tablets might be accredited it to the presence of Avicel®PH102 in conjunction with Eudragit®RLPO powders (7:3). They facilitate the uptake of water into the pores of the tablet initiating an internal pressure which leads to breakage of the tablets into fragments empowering rapid disintegration. Therefore, since all liquisolid tablets disintegrate within 5 minutes (0.27 minutes in LSS3), these liquisolid preparations can be used as fast disintegrating tablets that disintegrate or dissolve promptly into the solution. With these changes, patient compliance can be ensured, particularly for elderly patients who have difficulty in swallowing tablets or any other oral dosage forms that causes a barrier or limitation for their compliance. Due to the effect of Eudragit®RLPO on the disintegration time, with highest amount of carrier (666mg 3:7 Eudragit®RLPO to Avicel®PH102) in LSS3, the LSS3 gave the fastest disintegration time (16 seconds), followed by LSS4 (2.3 minutes). El-Say et al. (2010) found an inversely proportional relationship between liquid load factor (Lf) and disintegration time of the liquisolid tablets. However, similar findings were not observed in this current study. A direct positive correlation was found between the liquid load (L_f) and the disintegration time of the liquisolid tablets. This principle was confirmed from the results of the following formulae, LSS1 and LSS2 having Lf value of 0.8 with a mean disintegration time of 5.2 and 2.7 minutes respectively, and LSS3 and LSS4 having Lf value of 0.2 with mean disintegration time of 0.16 minutes and 2.3 minutes respectively. Similar findings were found in a study by (Egla & Abd Al Hammid, 2017)Lf decrease in two formulations F10 (containing 2.5 mg zolmitriptan, PG as non-volatile liquid vehicle and 9.3mg of CP a

superdisintegrant) and F11 (containing (containing 2.5 mg zolmitriptan, PG as non-volatile liquid vehicle and 9.3mg of CP a superdisintegrant) where loading factors where 0.175 and 0.125 respectively. The wetting time (WT) and disintegration time (DT) of the two specified formulations exhibited a proportional link with loading factor, where decrease in WT and DT was seen as the loading factor was decreased (Egla & Abd Al Hammid, 2017).

The possible explanation was due to the reduction in loading factor, leading to an increase in the amount of Avicel®PH102 (carrier) used. Furthermore, the high porosity of Avicel®PH102 aids in the swelling and disintegration of the orodispersible tablets as a result of water diffusion by capillary action into the hydrophilic matrix generating pressure for rapid disintegration. On the other hand, (El-Say, et al., 2010) proposed a theory where by increasing the liquid load factor the amount of liquid used significantly enhances the wetting properties of the drug, thus increasing the accessibility of the drug to be freely disintegrated causing a reduction in the disintegration time of the liquisolid tablets is incompatible with entire range of liquid vehicles and drugs. In other words, drugs with hydrophobic nature can preserve the above findings and show inverse relationship between disintegration time and liquid load factor (Lf). Thus, when a hydrophilic drug is being used, such as metformin-HCl, this relationship disappears leading to fast disintegrant liquisolid tablets using Eudragit®RL30D as the non-volatile liquid vehicle. In summary, the quantity of the carrier and the quantity of the disintegrants along with the type of the liquid vehicle are the main factors controlling the disintegration time of the liquisolid tablets. Wetting time is an indicator of the simplicity of tablet disintegration in the buccal cavity. The acceptable time range for disintegration of orodispersible tablets must be between 15-30 seconds and 3 minutes according to European Pharmacopoeia (Shah,

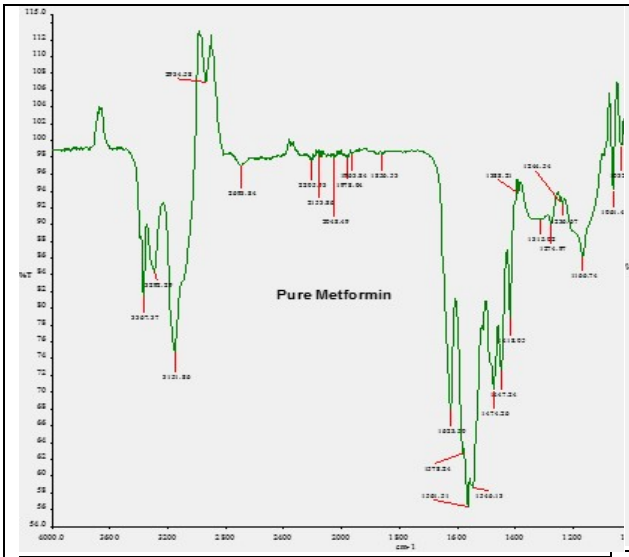
et al., 2012).

In most studies, fast disintegrating tablets were achievable by the use of fast disintegrants such as crospovidone, (Egla & Abd Al Hammid, 2017). In this study they show the importance of a balanced concentration in the orodispersible formulation. An optimal concentration of the super disintegrant leads to an increase in the orodispersibility of the tablet. Such a theory cannot be applied to the current work due to the fact that no superdisintegrant was used, instead only potato starch was added to the liquisolid formulation of metformin-HCl along with Eudragit®. Thus, to the best of our knowledge, the model which played the role of fast disintegrating agent is believed to be Eudragit®RLPO and this has been confirmed by disintegration and dissolution results.

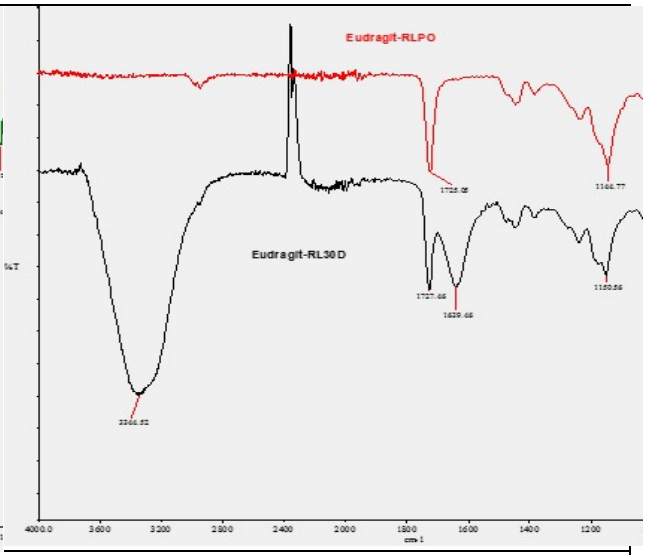
6.5. Fourier transform infrared (FT-IR) spectroscopy

The functional groups absorption bands appear from the stretching, whereas the shape and relative intensity of the IR absorption bands are used for assigning the vibrational mode characteristics with assignments of bands. FT-IR samples of pure metformin-HCl powder, pure Eudragit® and liquisolid metformin-HCl (LSS1-LSS4) granules are shown in Figure 6.4. They were subjected to FT-IR spectroscopic analysis at spectral bands of 550-4000 cm^{-1}. The characteristic absorption bands of pure metformin-HCl are between 3367 and 3292 cm^{-1} due to N-H primary stretching and a band at 3156 cm^{-1} due to N-H secondary stretching (Sheela, et al., 2010)). Additionally, characteristics bands at 1623 and 1561 cm^{-1} were observed to be denoted for N-H asymmetric deformation (Sheela, et

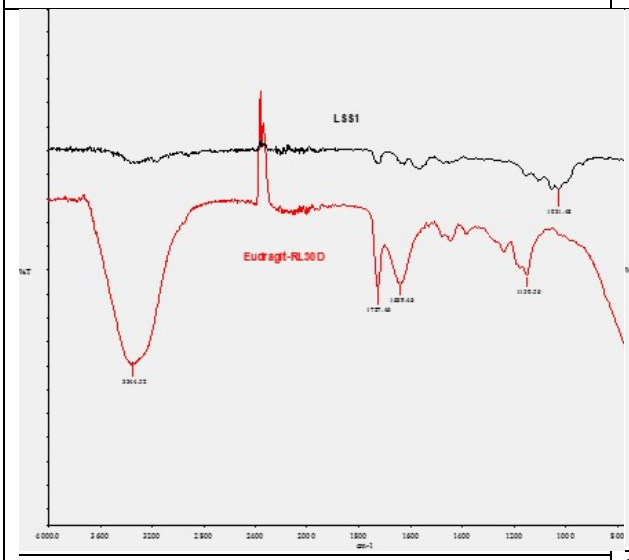
al., 2010). The liquisolid formulations LSS1-LSS4 FT-IR showed complete disappearance of the metformin-HCl pure peak, or reduction in intensity of peaks. This change might be attributed to the interaction between the drug (metformin-HCl) and the excipients used such as Eudragit®RL30D and Eudragit®RLPO.



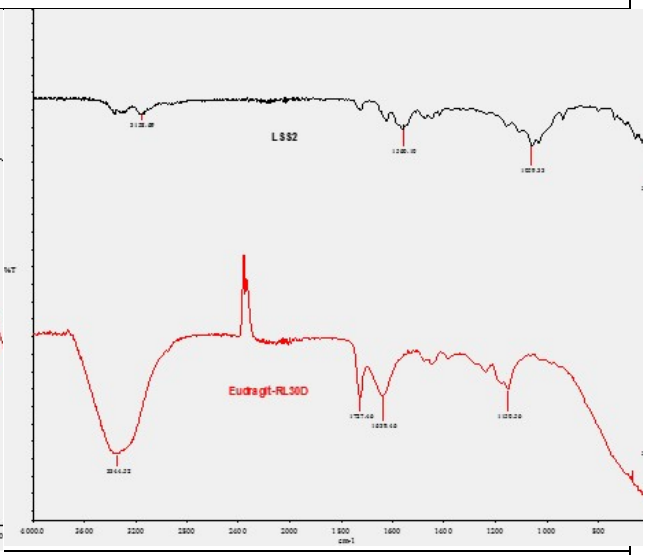
Pure metformin



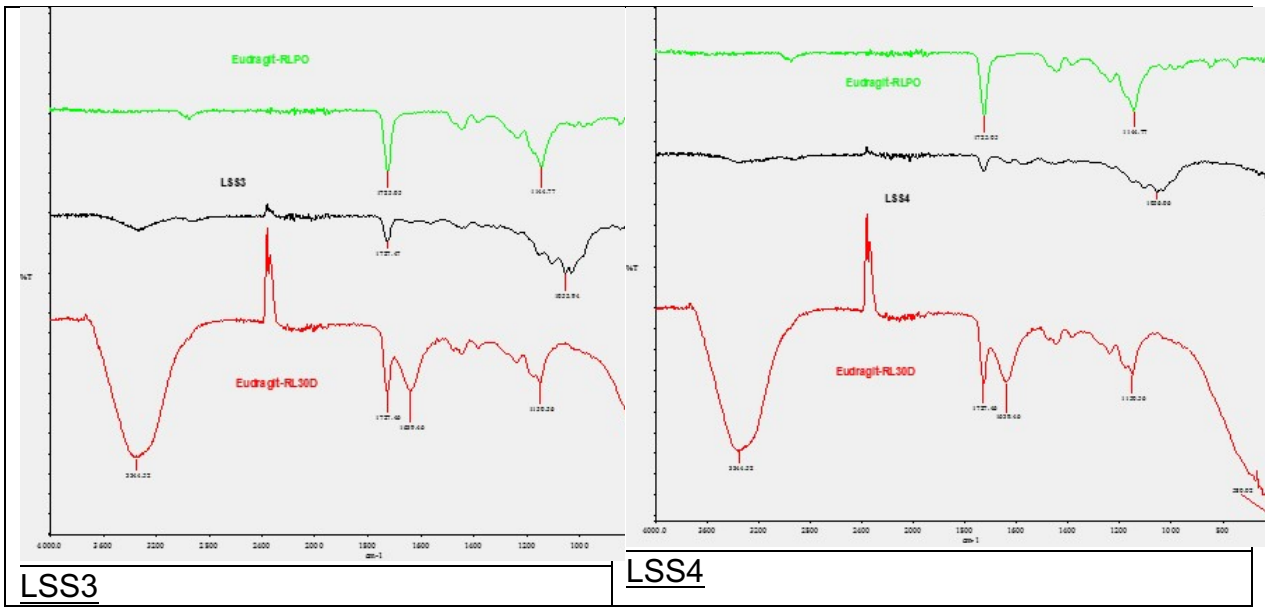
Eudragit®RL30D and Eudragit® RLPO



LSS1



LSS2



LSS3 LSS4

Figure 6.4: FT-IR spectra of metformin-HCl liquisolids using Eudragit® (LSS1-LSS4).For formulations' composition refer to Table 6.1

6.6. Differential scanning calorimetry (DSC)

Differential scanning calorimetry analysis was performed to determine the crystalline nature of the drug and possible incompatibility of the drug with excipients used in the liquisolid formulation development. Figure 6.5 depicts the thermal properties of liquisolid formulations of metformin-HCl using Eudrgit®RL30D. DSC thermograms of pure metformin-HCl showed a sharp endothermic peak at 232.99^{0}C corresponding to its melting point with respective enthalpy of 215.8J/g. This sharp endothermic peak indicates the crystalline nature of drug. The shifts of endothermic peak of metformin-HCl in the DSC thermograms to lower temperatures indicates that the drug is mostly solubilised within liquisolid mixture i.e. drug present mostly in a solution form (Butreddy & Dudhipala , 2015). The small enthalpies of the drug's melting point peak in liquisolid thermograms (Figure 6.4) imply that metformin-HCl in LSS1-LSS4 was mainly dispersed in the metformin-HCl

matrices (fast release observed) or the drug mostly exists in an amorphous form. (Nnamani, et al., 2016).

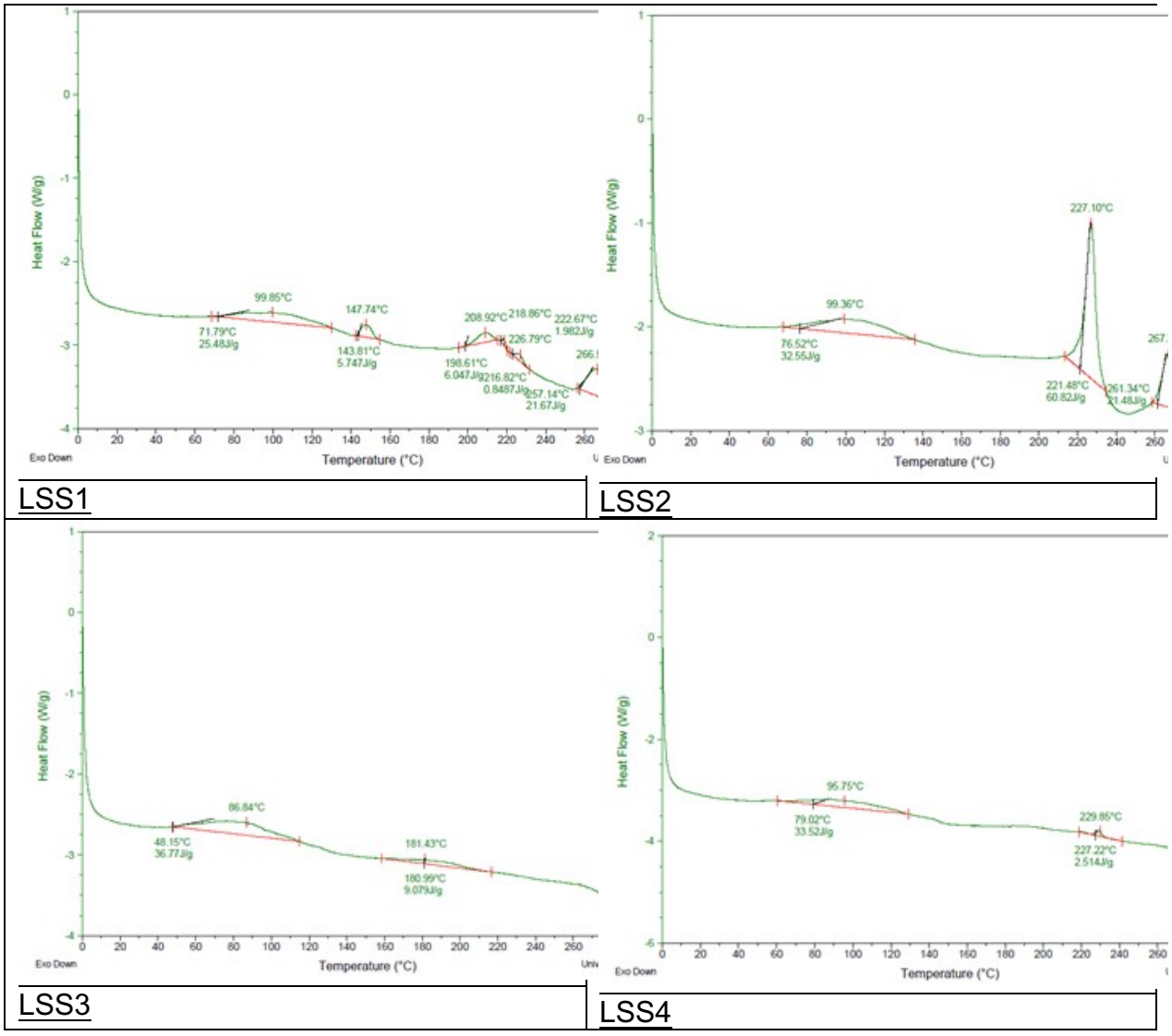
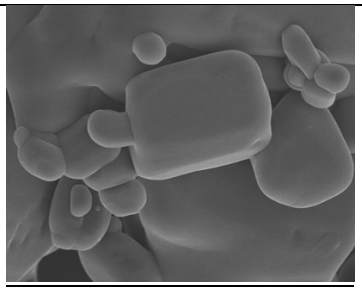


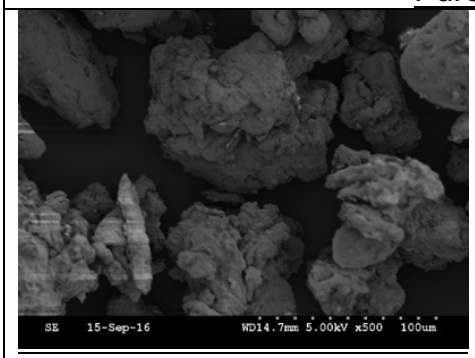
Figure 6.5: DSC thermograms of metformin-HCl liquisolid formulations using Eudragit®RL30D as the non-volatile liquid vehicle (LSS1-LSS4). Refer to Table 6.1 for formulations composition details

6.7. Scanning electron microscopy (SEM)

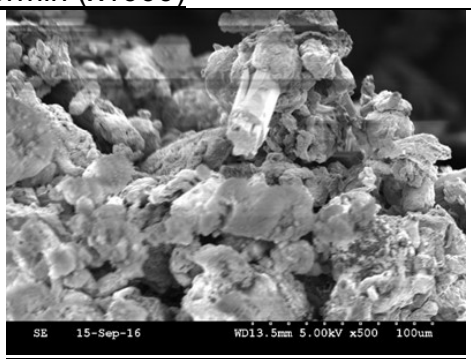
SEM analysis showed pure metformin-HCl of irregular shapes and sizes. The SEM microphotographs of all the liquisolid granules (LSS1-LSS4), shown in Figure 6.6, signify the complete disappearance of metformin-HCl crystals, a fact that indicates that the drug was solubilised in the liquisolid system. The liquisolid formulation hypothesis has been proved by the fact that although the drug is in a solid dosage form, it is held within the powder substrate in solution and it is observed in the dispersed state. This contributes to the idea of free flowing and enhanced drug dissolution properties (Jain, et al., 2014).



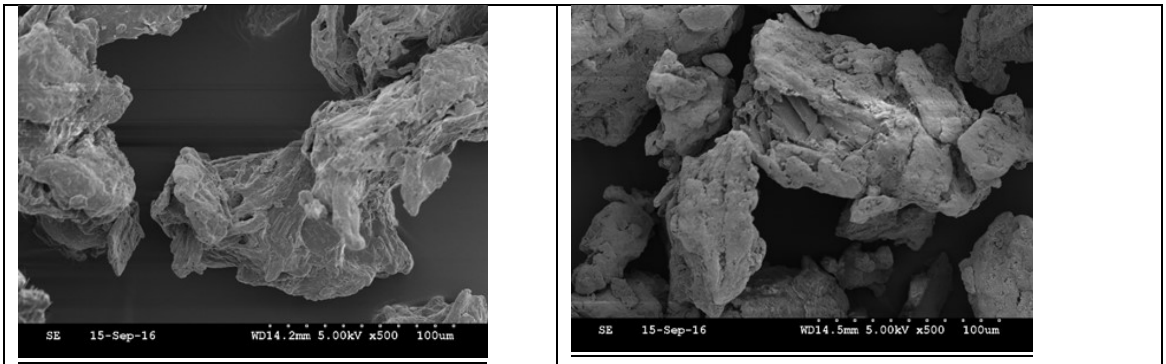
Pure metformin (x1000)



LSS1



LSS2



LSS3 LSS4

Figure 6.6: SEM microphotographs of LSS1, LSS2, LSS3 and LSS4 using Eudragit®RL30D as the non-volatile liquid vehicle. Refer to Table 6.1 for formulations composition details.

6.8. Conclusion

This study demonstrated that the prepared liquisolid metformin-HCl using Eudragit®RL30D as a liquid vehicle and Eudragit®RLPO as a carrier can possess fast oral disintegrating time, thus shorter dissolution release pattern. Therefore, it can be concluded that the rapid disintegration tablets formulated in this manner can easily help in administration of liquisolid metformin-HCl in a greater satisfactory form. Hence, such formulations can ensure higher patient compliance rate with minimum side effects making it a suitable and sought after approach for the pharmaceutical industry. Figure 7-1 shows the summary of liquisolid work performed in this research.

Chapter 7 - Conclusion and Future work

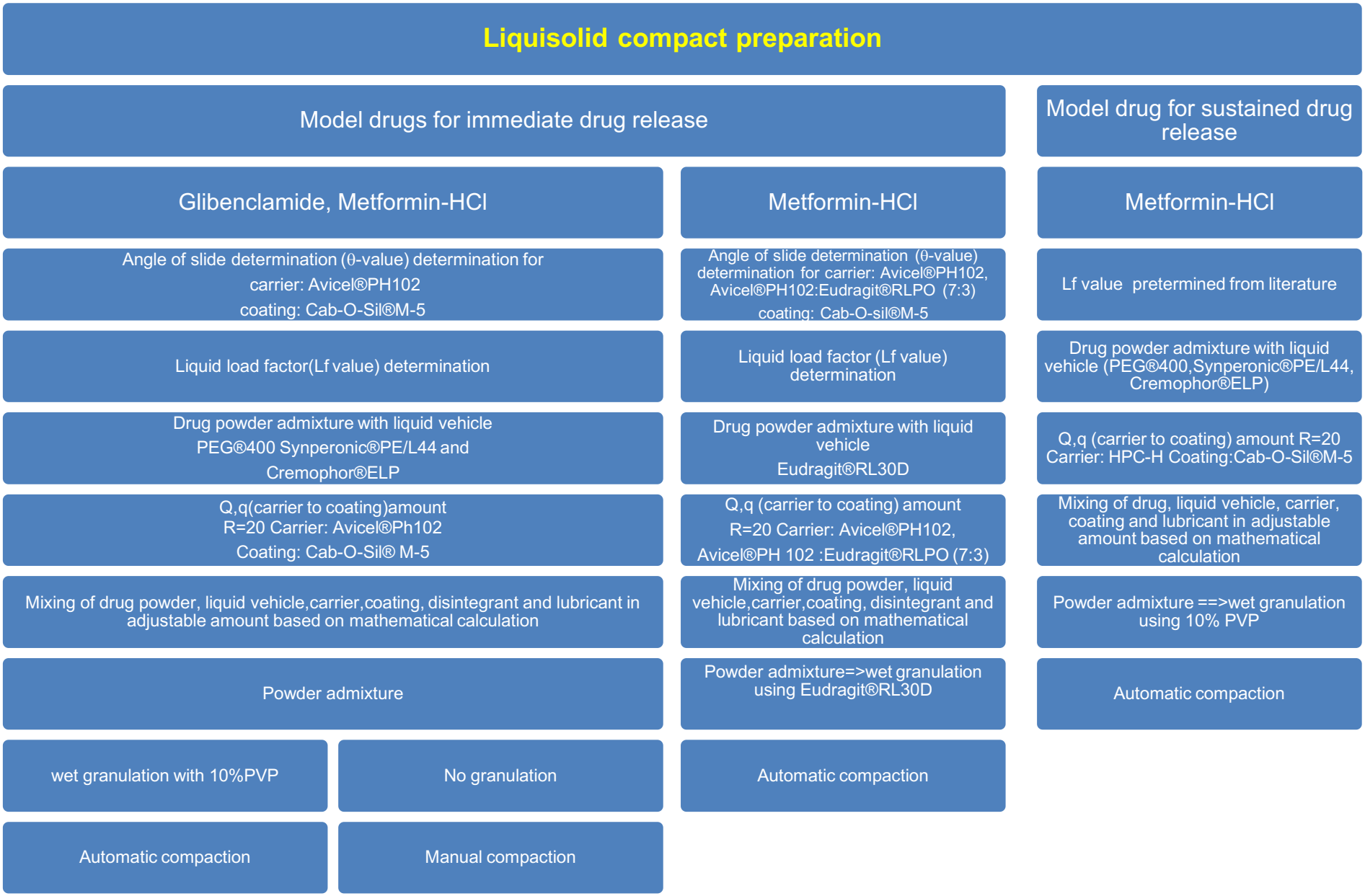


Figure 7.1: Summary of liquisolid work

Conclusion

Solubility and dissolution are the major factors that affect the bioavailability and *in vivo* performance of the drug. Moreover, based on literature, most current drugs have high lipophilicity and poor aqueous solubility resulting in poor bioavailability. In an attempt to improve bioavailability, it is crucial to improve the release behaviors of water insoluble drugs. The aim of this research work was to enhance the solubility and dissolution of hydrophobic drugs by the application of liquisolid technique. Liquisolid technology has the capability to sustain the release water-soluble drugs and allow the development of sustained release formulation with desirable release kinetics based on the work presented in this research. A further aim was to prepare sustained release metformin-HCl using liquisolid technique.

In chapter three, liquisolid technique changed the properties of glibenclamide, a hydrophobic model drug by inclusion of the drug particles (10%w/w and 30%w/w) in non-volatile liquid vehicles (PEG® 400, Synperonic® PE/L44 and Cremophor ®ELP). Wet granulation of prepared liquisolid powders successfully produced automatically compactible tablets using the above-mentioned liquid vehicles. The best dissolution enhancement was seen with combination of Cremophor® ELP with glibenclamide (10%w/w). However, the viscous behavior of Cremophor ®ELP made the flowability and automatic compaction of liquisolid powders containing Cremophor® ELP very challenging. Therefore, the new technique performed in this study (wet granulation by incorporating PVP as a liquid binder) resolved the compactibility issues and produced well-compressed liquisolid glibenclamide tablets which complied with B.P. quality control

tests. Therefore, the combination of the two methods wet granulation and liquisolid not only showed dissolution enhancement of glibenclamide (hydrophobic drug), but also the non-compressible tablets due to wet liquisolid powder mass, were formed into strong well-compacted tablets with no lamination or capping. Simultaneously, incorporation of PVP as a liquid binder did not affect the positive behavior of the liquid vehicles and also, application of heat in the drying process upon wet massing did not lead to any evaporation of the liquid vehicle. Accordingly, liquisolid system can use any type of viscous liquid vehicles in its composition and by application of wet granulation technique liquisolid powders can be compacted into tablets. The above-mentioned liquid vehicles (Synperonic®PE/L44 and Cremophore®ELP) were chosen because there are limited literature on their usage in liquisolid technology due to their viscous behavior and this current research over-come the undesirable viscosity effects on the properties of the liquisolid powders.

In chapter 4 the use of granulated liquisolid technique to generate stable metformin-HCl tablets was tested. Metformin-HCl is a water-soluble drug, which gives about 100% drug release for pure drug and the conventional formulation after 10 minutes. By using liquisolid technique with different liquid vehicles used, the drug release has not reached 100% after 10 minutes due to: the effect of processing technique; the effect of surfactants (liquid vehicles) and the interaction of the vehicles with the drug. Metformin-HCl was chosen as a water-soluble model drug to emphasize on the various impact of liquisolid technique on hydrophobic and hydrophilic drugs using same liquid vehicles. At present, metformin-HCl is commercially available in high dose tablets, 500 mg; the granulated liquisolid formulations may assist in the reduction of this dose. The stability studies

showed that the dissolution of liquisolid tablets was not affected by ageing significantly. Franz diffusion cells have been used to test the permeability of metformin-HCl liquisolid using Wistar rat stomach tissue. The results have shown promising results in using granulated liquisolid metformin-HCl tablets to enhance the drug permeability and hence may enhance drug bioavailability. Consequently, liquisolid tablets can be further used in alternative animal tests in order to confirm the beneficial use of liquisolid technology on permeability of drugs with poor bioavailability.

In Chapter 5, sustained release metformin-HCl was prepared using HPC polymer by the application of liquisolid technique. Three different liquid vehicles (PEG®400, Synperonic®PE/L44 and Cremophor®ELP) were used with 30% and 60% drug concentrations. Among the above mentioned liquid vehicles, Cremophor®ELP using 30% drug concentration showed the highest (92%) and the most sustained drug release over a 12 hour period. HPC shows moderate swelling coupled with slower rate of erosion. Drug release profiles on model fitting followed zero order and Higuchi models. Additionally, the flow-through method with open loop configuration has been used to perform the dissolution study of the sustained release metformin-HCl. Therefore, USP flow-through cell with open loop configuration was a successful dissolution apparatus to study the release of metformin-HCl over 12 hours.

The final chapter, Chapter 6, demonstrated the work on the preparation of liquisolid metformin-HCl using Eudragit®RL30D as a liquid vehicle and Eudragit®RLPO as a carrier, which can possess fast oral disintegrating properties, thus a shorter dissolution release pattern. Therefore, it can be concluded that the rapid disintegration tablets formulated in

this manner can easily help in administration of liquisolid metformin-HCl in a more customer friendly manner. Hence, such formulations can ensure higher patient compliance rate with minimum side effects. This approach can be considered by the pharmaceutical industry. This work discovered a new feature for Eudragit to produce fast disintegrating effect with metformin-HCl and accordingly, this part was extended to formulate liquisolid tablets with Eudragit. However, further future work is required.

In conclusion, although numerous techniques to handle solubility related issues already exist, the liquisolid technology or powder solution technology is a novel and promising technique for modifying the release of active pharmaceutical ingredients and to produce pharmaceutically modified drugs with desirable characteristics. As the liquisolid technology uses similar production processes as followed to develop conventional tablets, this technology aims to improve the release rate of poorly water-soluble drugs via a simple and cost effective method to positively improve patient compliance.

To go over the main points regarding the effect of the three non-volatile liquid vehicles used (PEG®400, Synperonic®PE/L44 and Cremophor®ELP), Cremophor®ELP was the most promising non-ionic surfactant as it enhances the dissolution of glibenclamide significantly and improved the permeability of metformin-HCl. Additionally, it also enhances the effect of Hydroxypropylcellulose(HPC) in sustaining the release of metformin-HCl.

Future work

The current research project commenced with the investigation of applying the liquisolid technique on glibenclamide (hydrophobic model drug) in order to enhance the solubility and dissolution as well as flowability properties of the drug. Additionally, liquisolid technique was also applied on metformin-HCl (hydrophilic model drug) in order to sustain the release of the drug by the use of HPC-H in the aqueous media. The obtained results with liquisolid preparations could be further investigated *in vivo* to check the bioavailability of the drugs and to validate its enhancement to the drug solubility and dissolution.

Furthermore, using HPC-H was successful in sustaining the release of metformin-HCl by the use of liquisolid technique can further be applied on other hydrophilic and can also be filled into capsules, for more convenience. Additionally, stability test can be performed on all liquisolid formulations containing Eudragit®RL30D as liquid vehicle (new orodispersible liquisolid tablets) and HPC-H containing liquisolid metformin-HCl for further quality control testing that is vital for industrial purposes.

The use of Eudragit® RL30D as liquid vehicle was a successful formulation to obtain fast disintegration tablets in which as a result can be beneficial for elderly and vulnerable people who have swallowing difficulties by including some flavor enhancer to mask the taste of Eudragit® and make it more pleasant for patients. Also, similar liquisolid technique can be applied on other available drugs in order to formulate orodispersible tablets using Eudragit®RL30D.

Finally, the current research work showed promising results in enhancing the permeability of metformin-HCl by using liquisolid technique and this test was performed on stomach

membrane of Wistar rats. This research can further be continued by using other membranes such as small intestine in order to investigate the permeability of liquisolid metformin-HCl.

Chapter 8 - References

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Appendix

Chapter 3

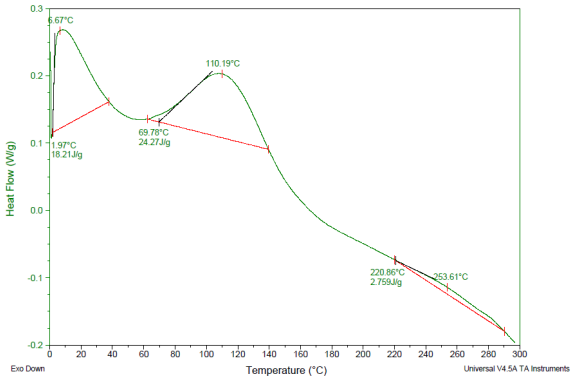
Statistics results for glibenclamide release from granulated liquisolid preparations

Time (min)	% release Conventional	% release of granulated Glib 10% PEG	% release of granulated Glib 30%PEG
0	0	0	0
5	5.698867899	10.44169296	5.291454443
10	9.704406847	12.74716213	2.096145381
15	9.162517902	13.91162574	3.109161793
20	10.81741395	15.85633014	0.111
25	13.23610574	16.18977161	2.291391561
30	13.07318623	15.87783337	1.042256178
45	13.37929179	17.23284525	6.004213042
60	14.30175252	19.96671039	7.625605232
90	14.28095345	21.3437423	7.14204867
T Test	0.004351204	0.001448477	0.001448477
	% release Conventional	% release of granulated Glib 10% PEG	% release of granulated Glib 30%PEG
	vs	vs	vs
	%release of granulated Glib 30% Cremophor with excess avicel	%release of granulated Glib 30% Cremophor with excess avicel	%release of granulated Glib 30% Cremophor with excess avicel

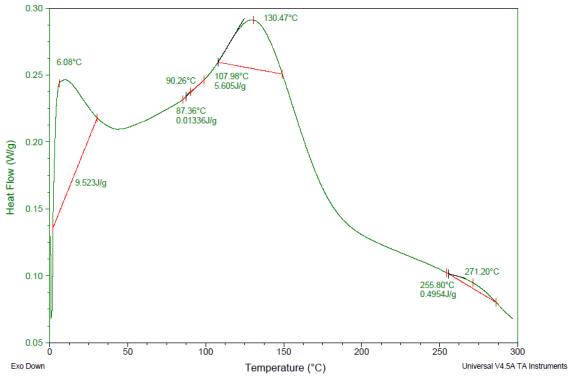
% release of granulated Glib 30% Synperonic	%release of granulated Glib 30% Cremophor with excess avicel
0	0
6.51700323	0
8.391751478	50.16109238
11.4528666	45.44678985
13.88518877	46.07189941
13.42027191	52.33172807
15.17024265	52.93074958
14.44746957	58.08494742
15.1698552	61.67116112
12.41198555	
0.005396878	0.026005073
% release of granulated Glib 30% Synperonic	%release of granulated Glib 30% Cremophor with excess avicel
vs	vs
%release of granulated Glib 30% Cremophor with excess avicel	% release of granulated Glib 10% synperonic

% release of granulated Glib 10% synperonic	%release of granulated Glib 10% Cremophor with excess avicel	
0	0	
12.71988243	3.988841409	
18.0747401	10.94687797	
20.10604626	33.526474	
21.24673841	45.98409448	
23.46577155	54.18281294	
23.2550223	52.57479598	
25.17967281	51.10274215	
23.45457365	54.31200124	
22.63262514	54.10310768	
0.026005073	0.336853453	
% release of granulated Glib 10% synperonic	%release of granulated Glib 10% Cremophor with excess avicel	
vs	vs	
%release of granulated Glib 30% Cremophor with excess avicel	%release of granulated Glib 30% Cremophor with excess avicel	

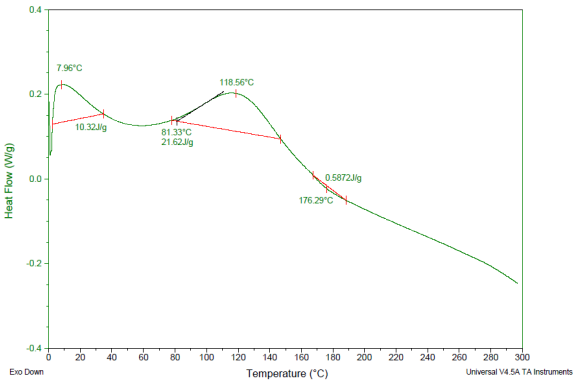
DSC thermograms of G1-G6 as liquisolid powder and liquisolid granules. Pure Glibenclamide, market Glibenclamide, Conventional Glibenclamide as well as nonvolatile liquid vehicles (PEG®400, Synperonic® PE/L44, Cremophor®ELP) and excipients such as Avicel® PH102, Cab-O-Sil® M-5, potato starch, Mg stearate and PVP.



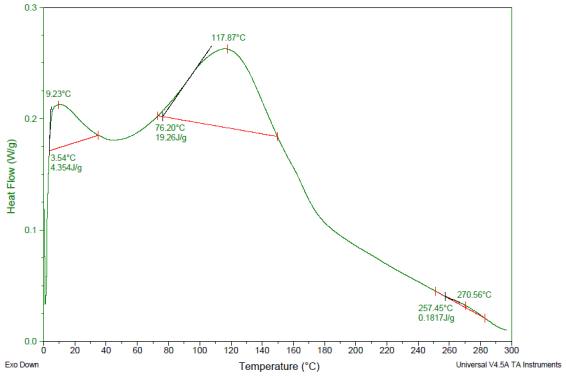
G1



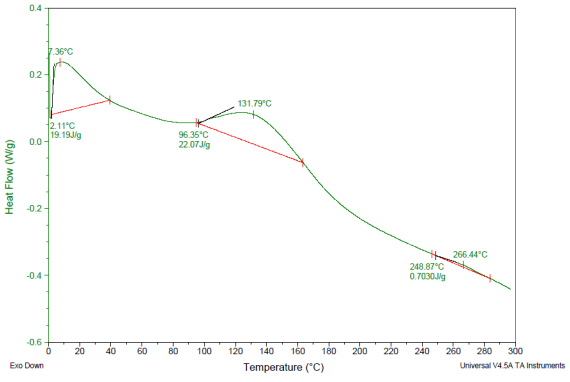
G1 (ungranulated)

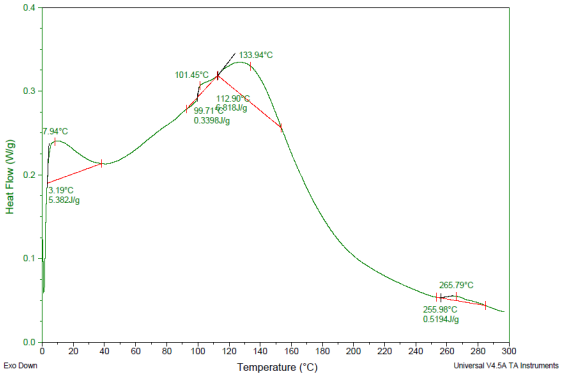


G2

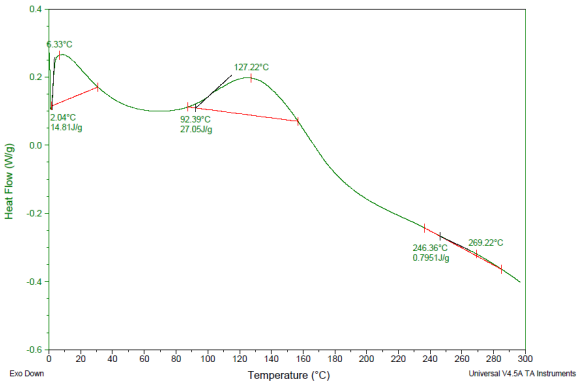


G2 (ungranulated)

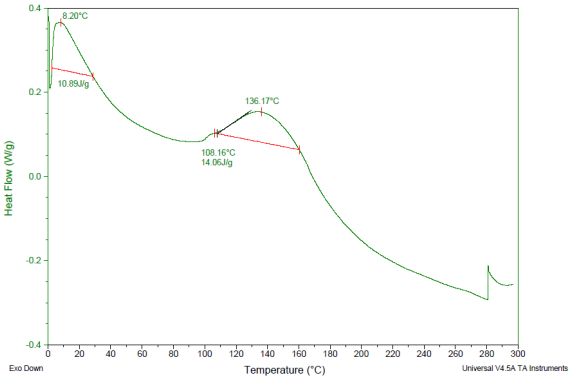




G3



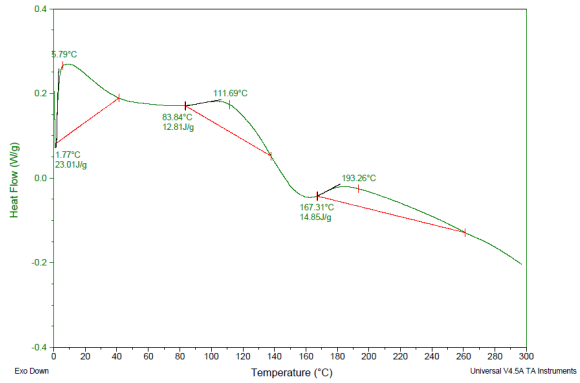
G3 (ungranulated)

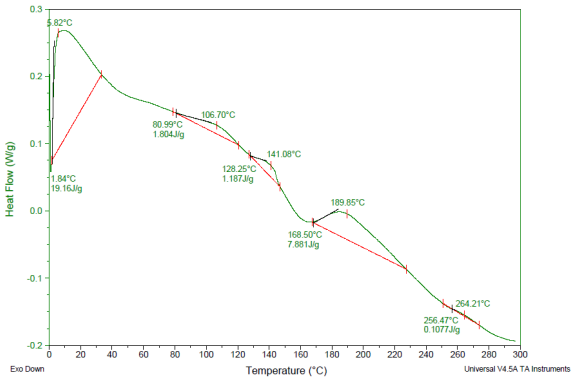


G4

G4 (ungranulated)

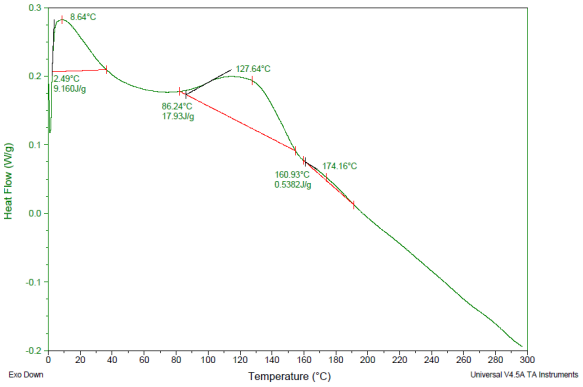
G5

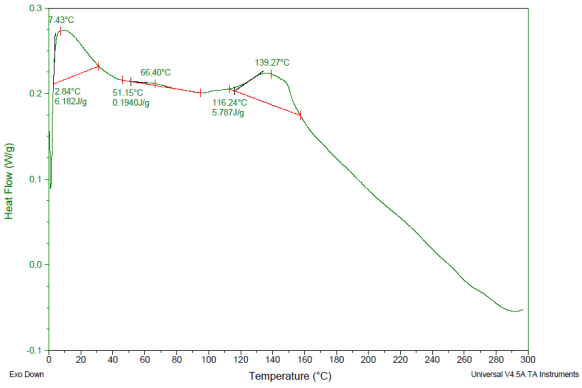




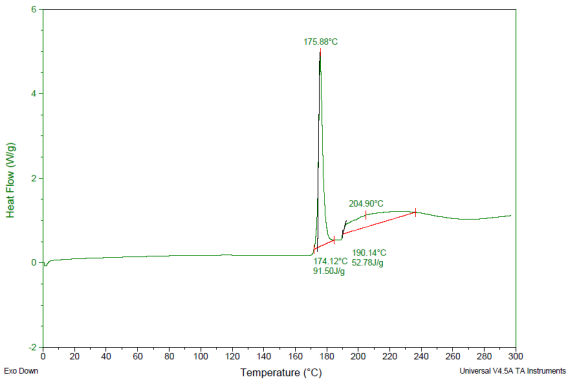
G5 (ungranulated)

G6

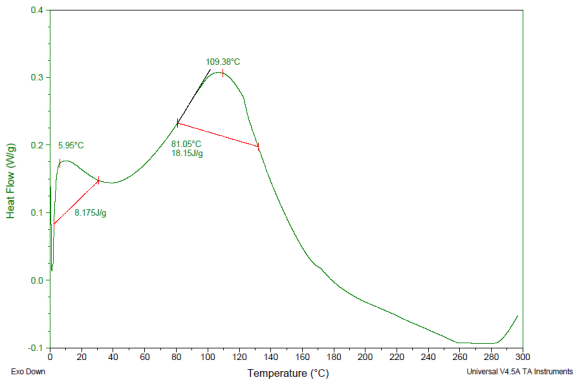




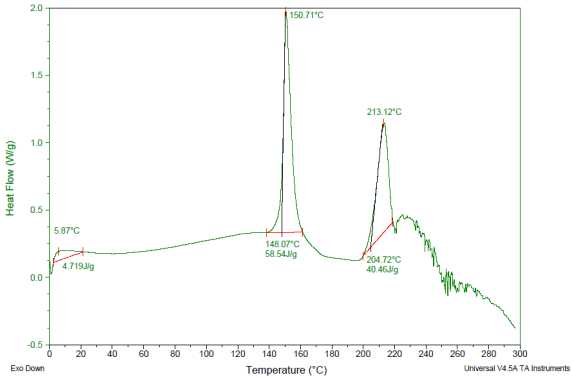
G6 (ungranulated)



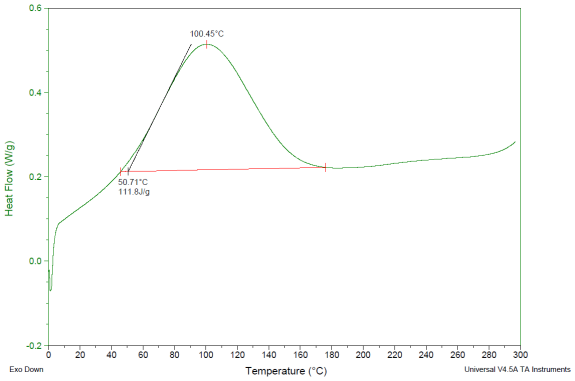
Pure Glibenclamide



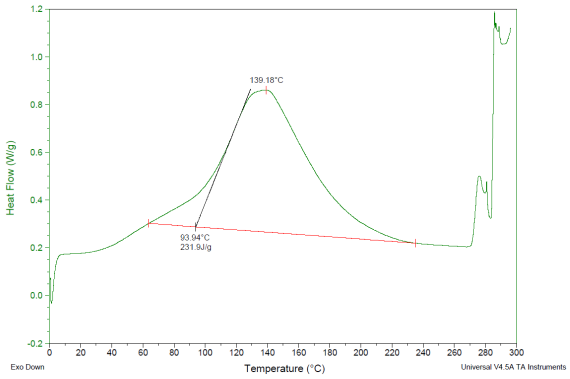
Conventional Glibenclamide



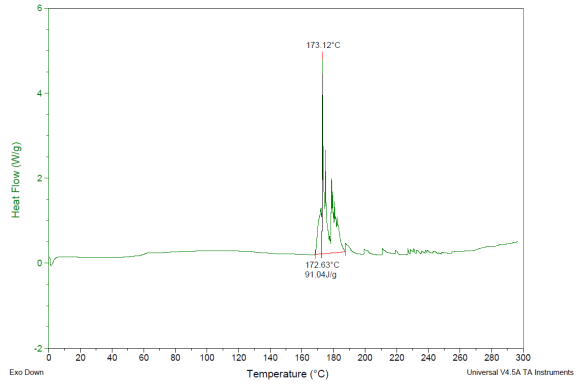
Market Glibenclamide



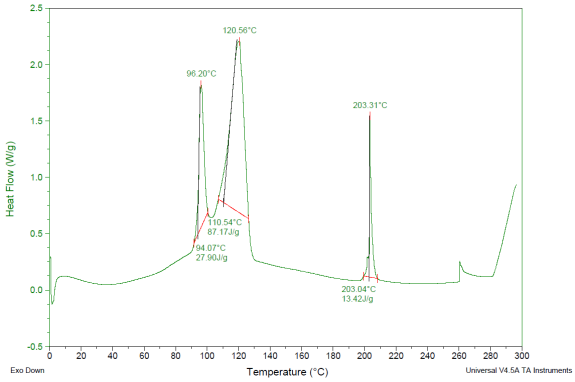
Avicel PH102



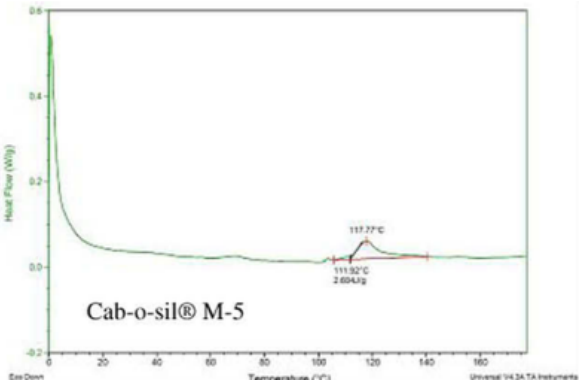
Potato starch



PVP



Magnesium Stearate



Silica

Appendix for Chapter 4

Statistics results for permeability study of metformin using stomach tissue and Franz cell

		PEG 30%	PEG 60%	Synperonic 30%	Synperonic 60%	Cremophor 30%	Cremophor 60%	Pure Metformin
		0.865	1.246	0.497	0.605	0.624	1.069	0.307
		0.973	1.119	0.51	0.675	0.713	0.897	0.294
		0.999	1.094	0.6117	0.935	0.739	0.745	0.324
		1.018	1.075	0.73	0.992	0.777	0.77	0.354
		1.024	1.113	0.84	1.005	0.777	0.953	0.362
		1.1	1.132	0.938	1.081	0.802	1.43	0.481
		1.176	1.214	1.119	1.145	0.859	1.519	0.574
		1.329	1.341	1.305	1.462	0.973	1.722	0.671
		1.951	1.456	1.648	1.709	1.157	1.792	0.849
		2.636	1.614	1.199	2.027	1.411	2.211	0.933
Significance (P value)	versus Pure metformin	5.75211E-18	5.6096E-07	0.008418363	0.001222167	0.002606373	0.000550413	
	amount penetrated in 14 ml							
		12.11	17.444	6.958	8.47	8.736	14.966	4.298
		13.622	15.666	7.14	9.45	9.982	12.558	4.116
		13.986	15.316	8.5638	13.09	10.346	10.43	4.536
		14.252	15.05	10.22	13.888	10.878	10.78	4.956
		14.336	15.582	11.76	14.07	10.878	13.342	5.068
		15.4	15.848	13.132	15.134	11.228	20.02	6.734
		16.464	16.996	15.666	16.03	12.026	21.266	8.036
		18.606	18.774	18.27	20.468	13.622	24.108	9.394
		27.314	20.384	23.072	23.926	16.198	25.088	11.886
		36.904	22.596	16.786	28.378	19.754	30.954	13.062

% penetrated	Time (min)	PEG 30%	PEG 60%	Synperonic 30%	Synperonic 60%	Cremophor 30%	Cremophor 60%	Pure Metformin
	5	0.6055	0.8722	0.3479	0.4235	0.4368	0.7483	0.2149
	10	0.6811	0.7833	0.357	0.4725	0.4991	0.6279	0.2058
	15	0.6993	0.7658	0.42819	0.6545	0.5173	0.5215	0.2268
	20	0.7126	0.7525	0.511	0.6944	0.5439	0.539	0.2478
	25	0.7168	0.7791	0.588	0.7035	0.5439	0.6671	0.2534
	30	0.77	0.7924	0.6566	0.7567	0.5614	1.001	0.3367
	45	0.8232	0.8498	0.7833	0.8015	0.6013	1.0633	0.4018
	60	0.9303	0.9387	0.9135	1.0234	0.6811	1.2054	0.4697
	90	1.3657	1.0192	1.1536	1.1963	0.8099	1.2544	0.5943
	120	1.8452	1.1298	0.8393	1.4189	0.9877	1.5477	0.6531

		PEG 30%	PEG 60%	Synperonic 30%	Synperonic 60%	Cremophor 30%	Cremophor 60%	
Fold	Time (min)							
Increase	5	2.817589577	4.058631922	1.618892508	1.970684039	2.03257329	3.482084691	
	10	3.30952381	3.806122449	1.734693878	2.295918367	2.425170068	3.051020408	
	15	3.083333333	3.37654321	1.887962963	2.885802469	2.280864198	2.299382716	
	20	2.875706215	3.036723164	2.062146893	2.802259887	2.194915254	2.175141243	
	25	2.828729282	3.074585635	2.320441989	2.776243094	2.14640884	2.632596685	
	30	2.286902287	2.353430353	1.95010395	2.247401247	1.667359667	2.972972973	
	45	2.048780488	2.114982578	1.949477352	1.994773519	1.496515679	2.646341463	
	60	1.980625931	1.998509687	1.94485842	2.178837556	1.450074516	2.566318927	
	90	2.297997644	1.714958775	1.941107185	2.012956419	1.362779741	2.110718492	
	120	2.825294748	1.729903537	1.285101822	2.172561629	1.512325831	2.36977492	
		3 fold		2 fold		2-3 fold		
		PEG		Synperonic		Cremophor		

Purchased animal tissue (ethical exemption)

Dear Hoda

Please include the forwarded e mails in the thesis's appendix.

Best wishes
Amal

From: Amal Elkordy
Sent: 20 April 2016 16:48
To: Kimberley Moore
Subject: FW: FW: Hoda Javaheri

Hi Kimberley

I have forwarded Etta' email, we will not need ethical approval.

Many thanks
Amal

From: Etta Evans [etta.evans@sunderland.ac.uk]
Sent: 20 April 2016 16:43
To: Amal Elkordy
Subject: Re: FW: Hoda Javaheri

I think that you would not need ethics approval if you purchase "dead" animal tissue. Kind regards, Etta

On 20/04/2016 16:38, Amal Elkordy wrote:
Many thanks Etta for your reply

Also, if we are going to buy animal tissue from a company, do we need ethical approval?

Many thanks
Amal

From: Etta Evans [etta.evans@sunderland.ac.uk<mailto:etta.evans@sunderland.ac.uk>]
Sent: 20 April 2016 16:32
To: Amal Elkordy
Subject: Re: FW: Hoda Javaheri

Hello Amal,

Michelle asked me a question regarding animal tissue and ethics approval but she did not provide any context .....

Cutting things short, obviously you do not need ethics approval for research on animal tissue purchased at a local butcher's shop.

Sorry for the confusion!

Kind regards,
Etta

--
Dr Etta Evans

Chair of the University Research Ethics Committee
Senior Lecturer in Psychology

University of Sunderland
David Goldman Informatics Centre
St. Peter's Campus
Sunderland, SR6 0DD

Phone: ++44 (0)191 515 2624

Test of Homogeneity of Variances

Drug release

Levene Statistic	df1	df2	Sig.
.393	6	98	.882

ANOVA

Drug release

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1321.609	6	220.268	.237	.964
Within Groups	91242.124	98	931.042		
Total	92563.733	104			

Post Hoc Tests

For metformin HPC formulations' compositions refer to Table 2.3

Multiple Comparisons

Dependent Variable: drug release

Scheffe

(I) formula	(J) formula	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
HPC1	HPC2	1.82376	11.14177	1.000	-38.5874	42.2349
	HPC3	.08757	11.14177	1.000	-40.3236	40.4987
	HPC4	-5.22088	11.14177	1.000	-45.6320	35.1903
	HPC5	-8.72320	11.14177	.996	-49.1343	31.6880
	HPC6	-5.48649	11.14177	1.000	-45.8976	34.9247
	conventional HPC	-4.61883	11.14177	1.000	-45.0300	35.7923
HPC2	HPC1	-1.82376	11.14177	1.000	-42.2349	38.5874
	HPC3	-1.73619	11.14177	1.000	-42.1473	38.6750
	HPC4	-7.04464	11.14177	.999	-47.4558	33.3665
	HPC5	-10.54696	11.14177	.989	-50.9581	29.8642
	HPC6	-7.31025	11.14177	.999	-47.7214	33.1009

	conventional HPC	-6.44260	11.14177	.999	-46.8537	33.9686
HPC3	HPC1	-.08757	11.14177	1.000	-40.4987	40.3236
	HPC2	1.73619	11.14177	1.000	-38.6750	42.1473
	HPC4	-5.30844	11.14177	1.000	-45.7196	35.1027
	HPC5	-8.81077	11.14177	.996	-49.2219	31.6004
	HPC6	-5.57406	11.14177	1.000	-45.9852	34.8371
	conventional HPC	-4.70640	11.14177	1.000	-45.1176	35.7047
HPC4	HPC1	5.22088	11.14177	1.000	-35.1903	45.6320
	HPC2	7.04464	11.14177	.999	-33.3665	47.4558
	HPC3	5.30844	11.14177	1.000	-35.1027	45.7196
	HPC5	-3.50232	11.14177	1.000	-43.9135	36.9088
	HPC6	-.26561	11.14177	1.000	-40.6768	40.1455
	conventional HPC	.60204	11.14177	1.000	-39.8091	41.0132
HPC5	HPC1	8.72320	11.14177	.996	-31.6880	49.1343
	HPC2	10.54696	11.14177	.989	-29.8642	50.9581
	HPC3	8.81077	11.14177	.996	-31.6004	49.2219
	HPC4	3.50232	11.14177	1.000	-36.9088	43.9135
	HPC6	3.23671	11.14177	1.000	-37.1744	43.6479
	conventional HPC	4.10436	11.14177	1.000	-36.3068	44.5155
HPC6	HPC1	5.48649	11.14177	1.000	-34.9247	45.8976
	HPC2	7.31025	11.14177	.999	-33.1009	47.7214

	HPC3	5.57406	11.14177	1.000	-34.8371	45.9852
	HPC4	.26561	11.14177	1.000	-40.1455	40.6768
	HPC5	-3.23671	11.14177	1.000	-43.6479	37.1744
	conventional HPC	.86765	11.14177	1.000	-39.5435	41.2788
conventional HPC	HPC1	4.61883	11.14177	1.000	-35.7923	45.0300
	HPC2	6.44260	11.14177	.999	-33.9686	46.8537
	HPC3	4.70640	11.14177	1.000	-35.7047	45.1176
	HPC4	-.60204	11.14177	1.000	-41.0132	39.8091
	HPC5	-4.10436	11.14177	1.000	-44.5155	36.3068
	HPC6	-.86765	11.14177	1.000	-41.2788	39.5435

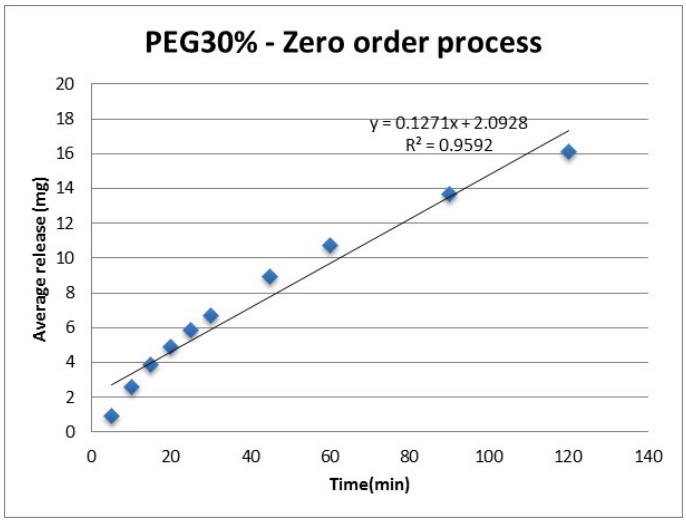
Homogeneous Subsets

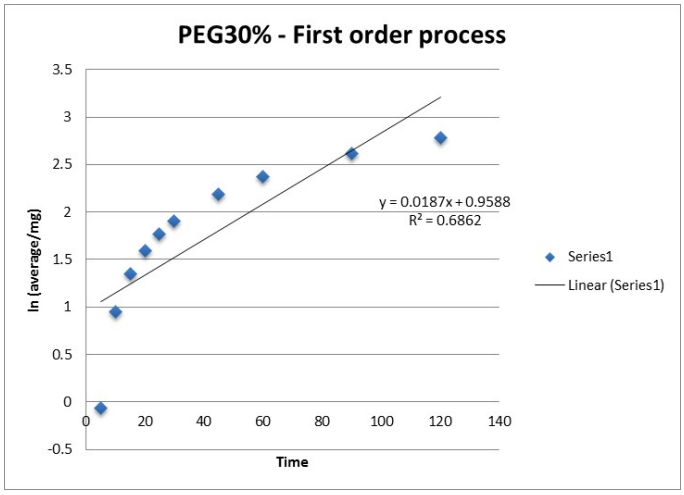
Drug release

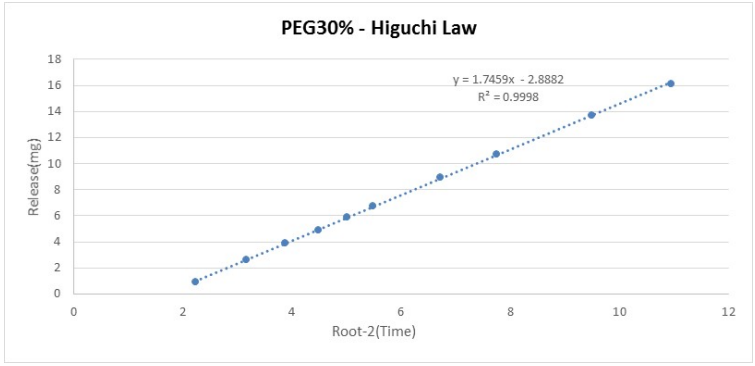
Scheffe[a]

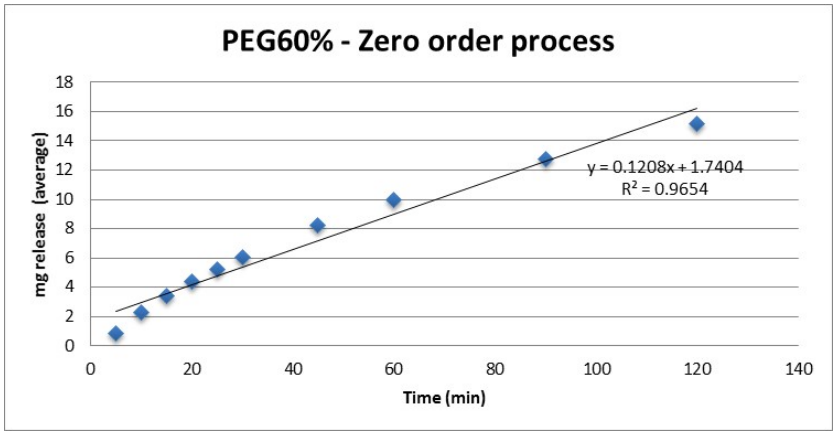
formula	N	Subset for alpha = 0.05 1
HPC2	15	34.9292
HPC3	15	36.6654
HPC1	15	36.7530
conventional HPC	15	41.3718
HPC4	15	41.9739
HPC6	15	42.2395
HPC5	15	45.4762
Sig.		.989

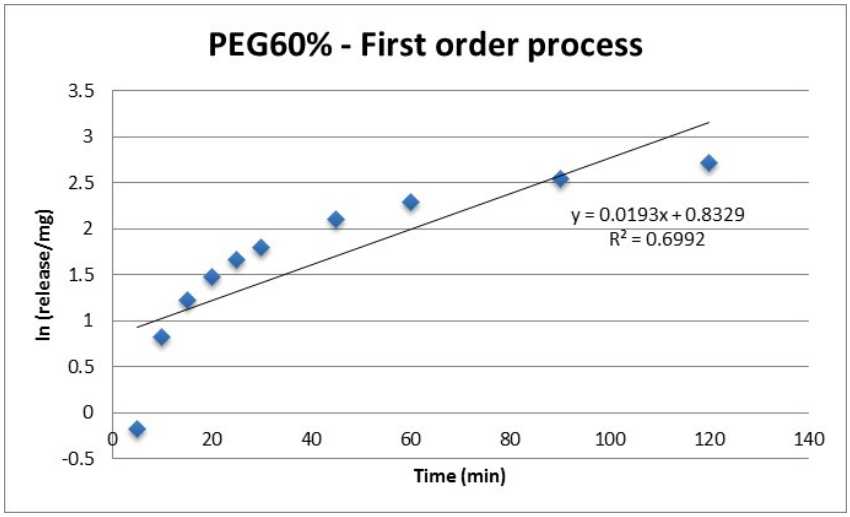
Release Kinetics for HPC metformin liquisolid formulations

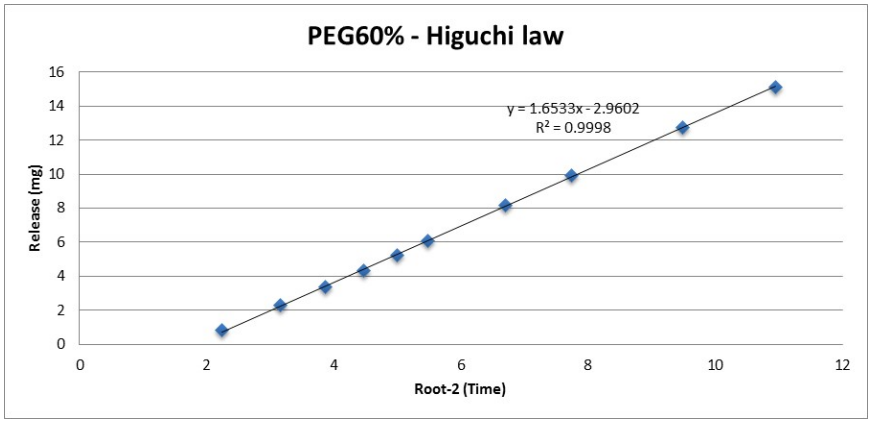


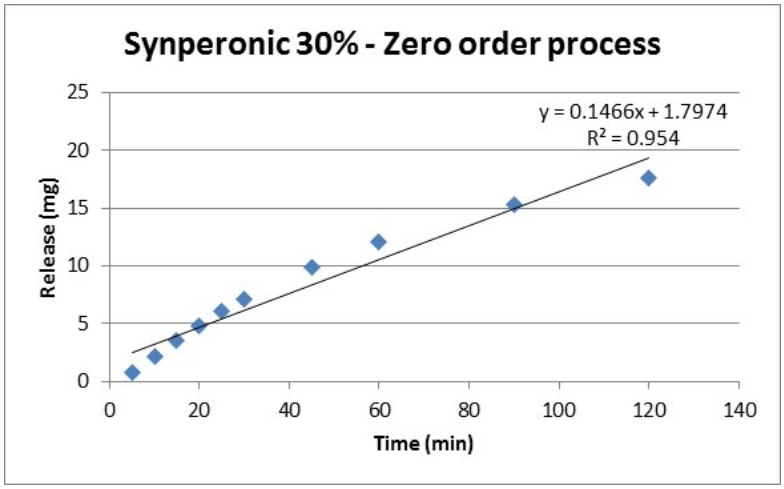


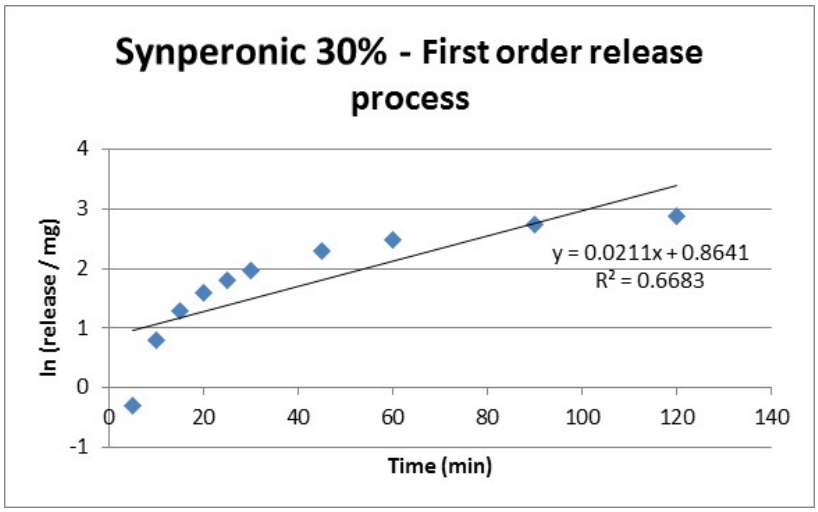


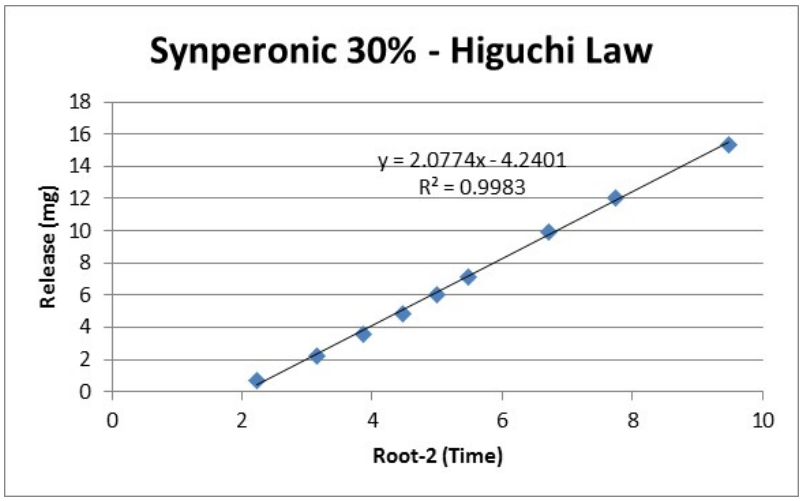

PEG60% - Zero order process
mg release (average)
Time (min)
y = 0.1208x + 1.7404
R² = 0.9654

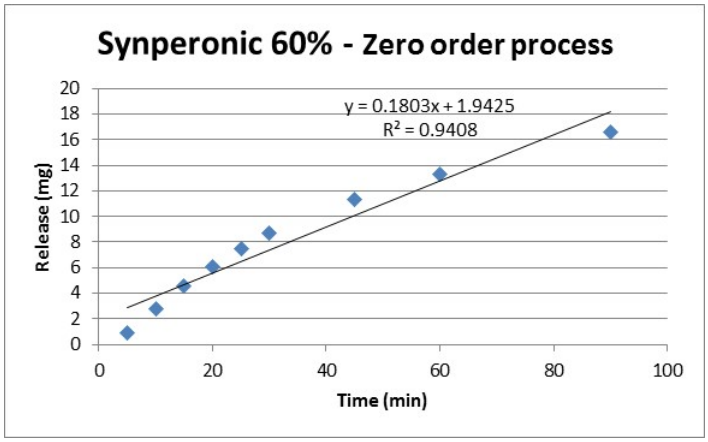

PEG60% - First order process
ln (release/mg)
Time (min)
y = 0.0193x + 0.8329
R² = 0.6992

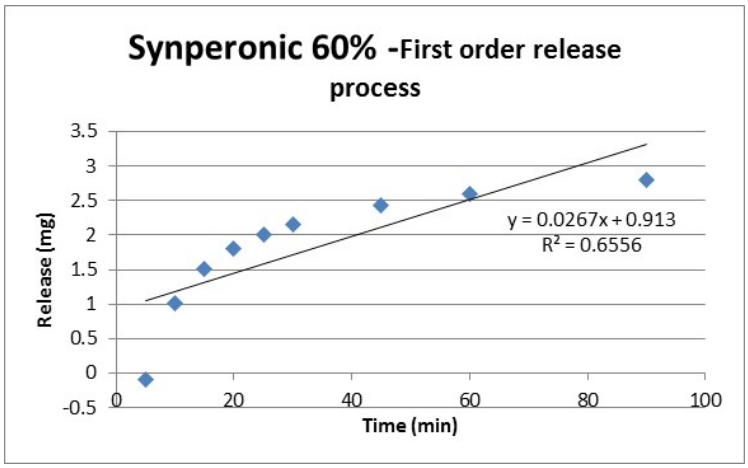

PEG60% - Higuchi law
Release (mg)
Root-2 (Time)
y = 1.6533x - 2.9602
R² = 0.9998

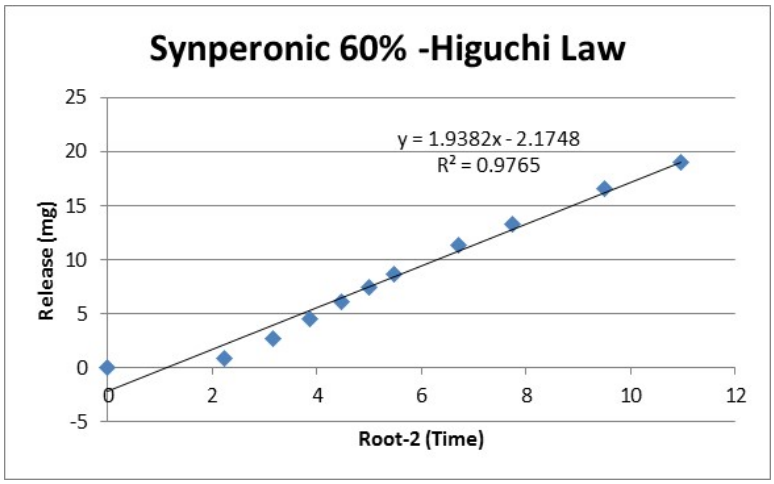

Synperonic 30% - Zero order process
y = 0.1466x + 1.7974
R² = 0.954
Release (mg)
Time (min)
0
5
10
15
20
25
0
20
40
60
80
100
120
140

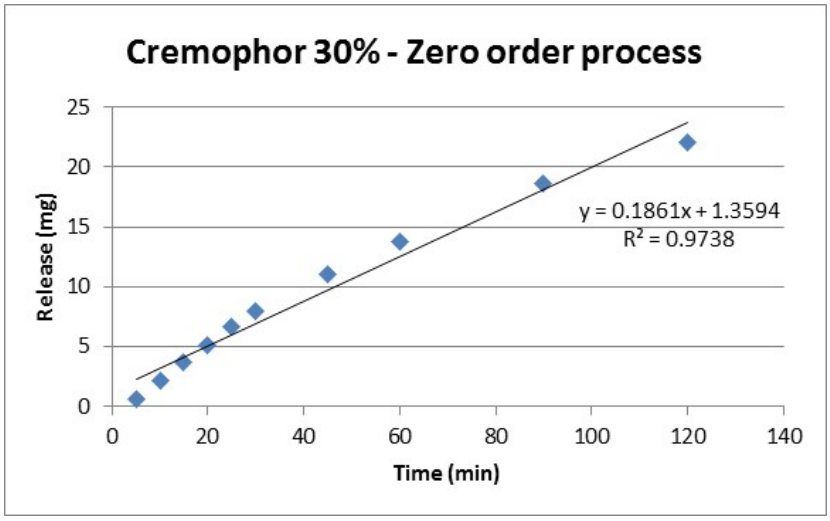

Synperonic 30% - First order release process
y = 0.0211x + 0.8641
R² = 0.6683
ln (release / mg)
Time (min)
-1
0
1
2
3
4
0
20
40
60
80
100
120
140

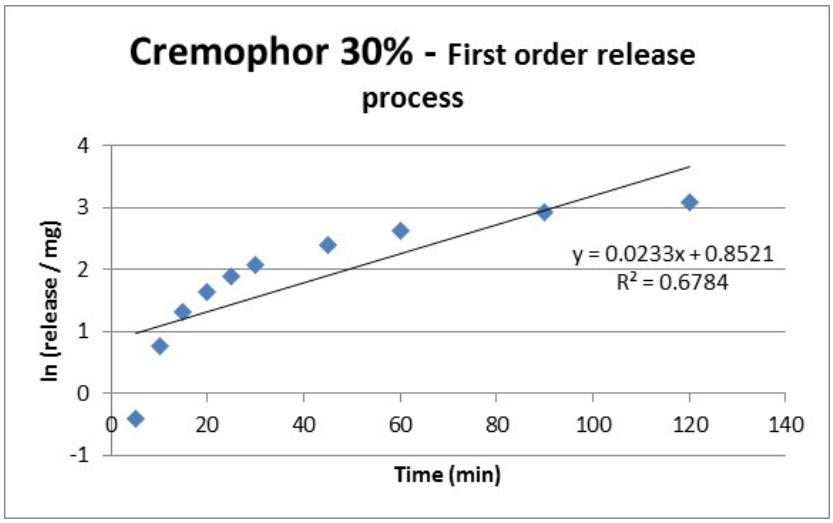

Synperonic 30% - Higuchi Law
y = 2.0774x - 4.2401
R² = 0.9983
Release (mg)
Root-2 (Time)
0
2
4
6
8
10
12
14
16
18
0
2
4
6
8
10

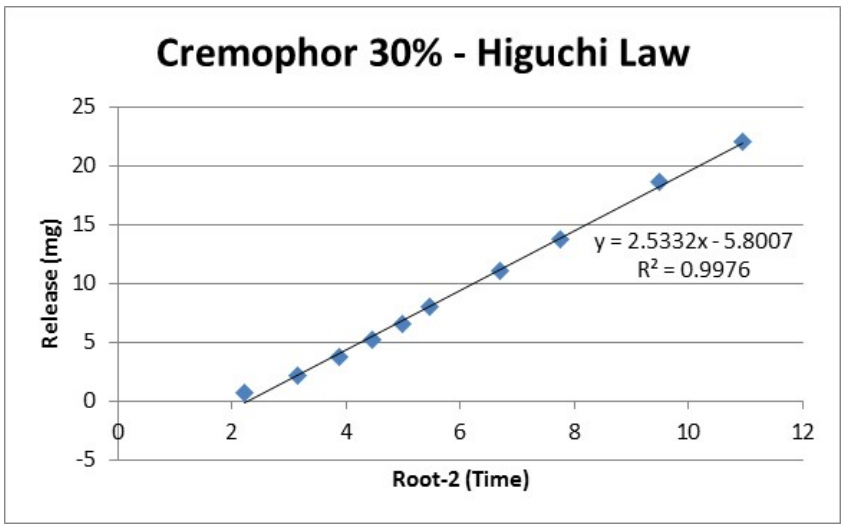

Synperonic 60% - Zero order process
y = 0.1803x + 1.9425
R² = 0.9408
Release (mg)
Time (min)
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100

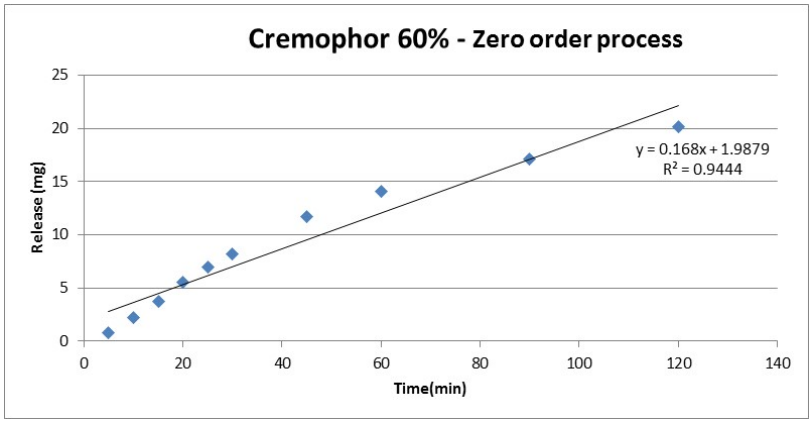

Synperonic 60% -First order release process
y = 0.0267x + 0.913
R² = 0.6556
Release (mg)
Time (min)
-0.5
0
0.5
1
1.5
2
2.5
3
3.5
0
20
40
60
80
100

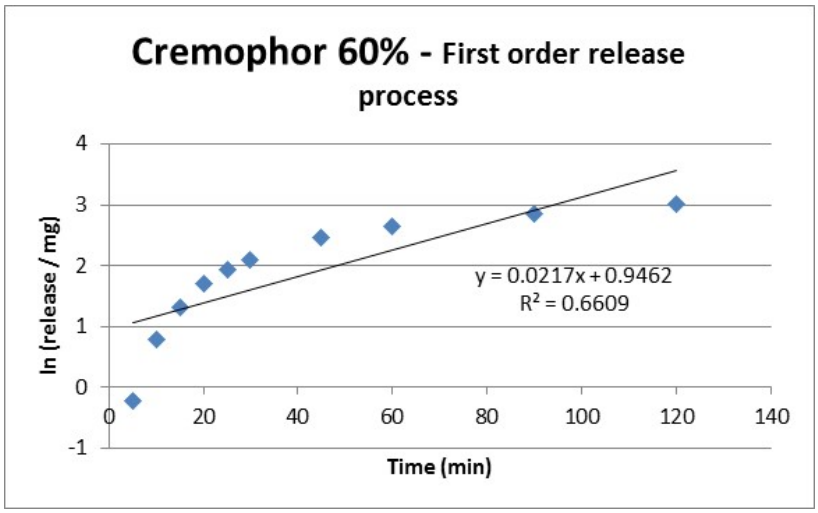

Synperonic 60% -Higuchi Law
y = 1.9382x - 2.1748
R² = 0.9765
Release (mg)
Root-2 (Time)
-5
0
5
10
15
20
25
0
2
4
6
8
10
12

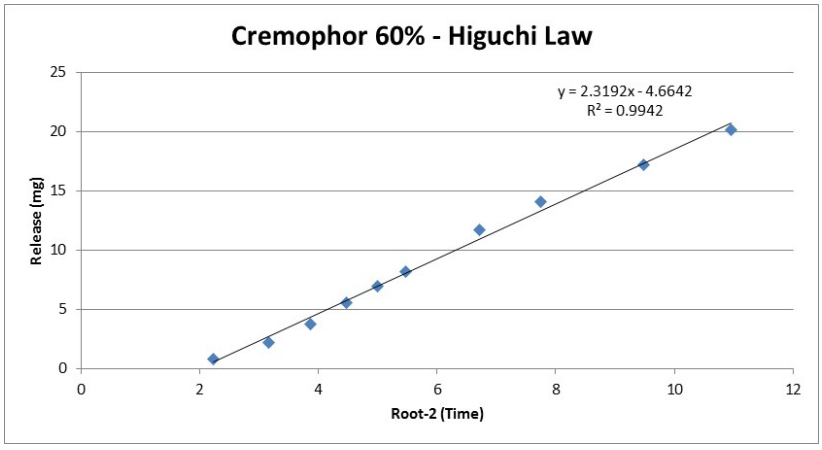

Cremophor 30% - Zero order process
Release (mg)
Time (min)
y = 0.1861x + 1.3594
R² = 0.9738
0
5
10
15
20
25
0
20
40
60
80
100
120
140


Cremophor 30% - First order release process
ln (release / mg)
Time (min)
y = 0.0233x + 0.8521
R² = 0.6784
-1
0
1
2
3
4
0
20
40
60
80
100
120
140


Cremophor 30% - Higuchi Law
Release (mg)
Root-2 (Time)
y = 2.5332x - 5.8007
R² = 0.9976
-5
0
5
10
15
20
25
0
2
4
6
8
10
12


Cremophor 60% - Zero order process
y = 0.168x + 1.9879
R² = 0.9444
Release (mg)
Time(min)
0
5
10
15
20
25
0
20
40
60
80
100
120
140


Cremophor 60% - First order release process
y = 0.0217x + 0.9462
R² = 0.6609
ln (release / mg)
Time (min)
-1
0
1
2
3
4
0
20
40
60
80
100
120
140


Cremophor 60% - Higuchi Law
y = 2.3192x - 4.6642
R² = 0.9942
Release (mg)
Root-2 (Time)
0
5
10
15
20
25
0
2
4
6
8
10
12