Identification and study of the physico-chemical changes during dissolution in simulated intestinal fluids using novel techniques.

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Declaration of Authenticity and Author's Rights

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Date:

Acknowledgments

To complete this work, two things were needed every day, one is something I look to and another is something to look forward to. Now first of, I want to thank my Allah the most Gracious because this is something that I look up to, He graces my life with opportunity that I know it is out of my hands and that I could never have done it without the faith I have in Him.

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Abstract

The oral route is the preferred option for drug administration but contains the inherent issue of drug absorption from the gastro-intestinal tract (GIT) in order to elicit systemic activity. A pre-requisite for absorption is drug dissolution, which is dependent upon drug solubility and the variable milieu of GIT fluid, with poorly soluble drugs presenting a formulation and biopharmaceutical challenge. Multiple factors within GIT fluid influence solubility ranging from pH to the concentration and ratio of amphiphilic substances such as phospholipid, bile salt, monoglyceride and cholesterol. To aid in vitro investigation, simulated intestinal fluids (SIF) covering the fasted and fed state have been developed. SIF media is complex and statistical design of experiment (DoE) investigations have revealed the range of solubility values possible within each state due to physiological variability along with the media factors and factor interactions which influence solubility. In this research a dual level, reduced experimental number (20) DoE providing three arms covering the fasted and fed states along with a combined analysis has been investigated. The results indicate that this small scale investigation is feasible and provides solubility ranges that encompass published data in human, simulated fasted and fed fluids and published DoE results. The study also correctly identifies the major single factor or factor interactions which influence solubility but it is evident that lower significance factors are not picked up due to the lower sample number.

Moreover, several approaches were made to modify drug formulation in an aim to enhance drug solubility and dissolution including sloid dispersion and the usage of excipients. In this work, examining the effect of six representative types of excipients on solubility were examined using the design of experiment. The results indicated specific drug- excipient behavior and that each excipient will have different effect on drug solubility. Smaller molecular weight or lower concentration of excipients as mannitol for example, had no impact on solubility and or dissolution. On the other hand, higher molecular weight and higher concentration of the excipient like chitosan for example, significantly reduced drug solubility. The results also indicated that the effect of the excipient on solubility will be dependent on the type of excipient under investigation, the concentration of the used excipient and on the media state (fasted or

fed) under examination. This outcome improved the feasibility of the design to be used as a prognostic tool to examine the effect of excipients on solubility

Finally, dissolution testing of carvedilol in presence of excipients were carried out. The results indicated that carvedilol dissolution rate were not affected in presence of lower molecular weight excipients as mannitol for example but it significantly reduced in the presence of higher molecular weight excipients as chitosan for example. Moreover, this influence of the excipient on drug dissolution were found to be due to the interaction between the excipient with the different media components and or with the drug. The results also showed that dissolution testing correlate well with the equilibrium solubility testing where, excipients that found to affect solubility showed to affect dissolution also.

These studies illustrate that these approaches therefore represent a useful initial screening tool that can guide further in depth analysis of a drug's behavior in gastrointestinal fluids and in the presence of excipients. The outputs can potentially be applied in drug formulation testing for improved bioavailability.

Table of contents

Acknowledgments 3

Abstract 4

Table of contents 6

List of Figures 12

List of Tables 15

List of Abbreviations 16

1 Oral drug absorption 18

1.1 Solubility 18

1.2 Dissolution 20

1.2.1 Parameters affecting dissolution rate 21

1.2.2 Summary 23

1.3 Permeability 24

1.4 GI media composition, drug physicochemical characteristics and their impact on drug absorption 26

1.4.1 Composition of simulated GI fluids media 26

1.4.1.1 pH 27

1.4.1.2 Buffer capacity 27

1.4.1.3 Amphiphiles and digestion products 29

1.4.1.4 Osmolality 33

1.4.1.5 Surface tension 34

1.4.2 Drug physicochemical characteristics 37

1.5 Biopharmaceutics Classification System (BCS) 38

1.5.1 Early biopharmaceutical classification system 38

1.5.2 Devolapability Classification System (DCS) 39

1.5.3 BCS for determination of in vivo performance 40

1.5.4 Application of BCS to guide dissolution testing 41
1.6 Approaches to improve drug solubility and dissolution 43
1.7 Excipients 44
1.7.1 Sources and functions of excipients 44
1.7.2 Effects of excipients on drug absorption 46
1.8 Oral Biopharmaceutical tools (OrBiTo) 48
1.9 Design of experiment 48
1.9.1 Two Level Full Factorial design of experiment 49
1.9.2 Studies applied statistical investigation of simulated intestinal media. 49
1.10 Aims and objectives 51
2 Dual level statistical investigation of equilibrium solubility in simulated fasted and fed intestinal fluid* 53
2.1 Introduction 53
2.1.1 Gastrointestinal Solubility Factors 53
2.1.2 Gastrointestinal Media 54
2.1.3 Statistical Investigation of Simulated Intestinal Media 54
2.1.4 Dual Range Design of Experiment Study 55
2.2 Materials and Methods 56
2.2.1 Materials 56
2.2.2 Dual Level Design of Experiment and Data Analysis 58
2.2.3 Equilibrium Solubility Measurement 59
2.2.4 Preparation of Stock Systems 59
2.2.5 Preparation of Measurements Solutions 60
2.2.6 Validation of HPLC method 62
2.2.6.1 HPLC equipment 62
2.2.6.2 Chromatographic conditions 62

2.2.6.3 Analysis....62
2.3 Results....64
2.3.1 Equilibrium solubility measurements....64
2.3.2 Statistical comparison....65
2.3.3 Solubility influence of individual DoE factors in fasted and fed study arms 67
2.3.4 Solubility influence of individual DoE factors and factor interactions in the combined study arm....71
2.3.5 Statistically significant solubility factor and factor interactions....74
2.4 Discussion....76
2.4.1 Equilibrium solubility measurements....76
2.4.2 Statistical comparisons of solubility....76
2.4.3 Standardised Effect Values....79
2.5 Conclusions....80
3 Dual level design of experiment investigating effect of fresh and frozen fasted/fed simulated intestinal fluids....81
3.1 Material and method....81
3.1.1 Material....81
3.1.2 Dual level design of experiment and data analysis....81
3.1.3 Equilibrium solubility measurements and Preparation of Stock systems 82
3.1.4 Preparation of experimental measurements solutions....83
3.1.5 Preparation of the frozen stock solutions....83
3.1.6 HPLC method....84
3.2 Results....84
3.2.1 Equilibrium solubility measurements....84

3.2.2 Statistical comparison 85
3.2.3 Influence of DoE factors on solubility in fasted and fed study arms ... 90
3.2.3.1 Influence of individual DoE factors on solubility 90
3.2.3.2 Influence of factor interactions on solubility 95
3.2.4 Influence of DoE factors on solubility in combined study arm 100
3.2.4.1 Influence of individual DoE factors on solubility 100
3.2.4.2 Influence of factor interactions on solubility 104
3.2.5 Statistically significant solubility factors and factor interactions 109
3.3 Discussion 114
3.3.1 Equilibrium solubility measurements 114
3.3.2 Statistical comparisons of solubility 114
3.3.3 Standardised effect values 116
3.4 Conclusions 117
4 Dual level statistical investigation of the effect of excipients on equilibrium solubility of fenofibrate and carvedilol in simulated fasted and fed intestinal fluid 118
4.1 Material and method 118
4.1.1 Materials 118
4.1.2 Dual level design of experiment and data analysis 119
4.1.3 Equilibrium solubility measurement 120
Preparation of lipid suspension 121
Preparation of sodium oleate solution 121
Preparation of buffer solutions 121
4.1.4 Preparation of measurement solutions 121
4.2 Results 121
4.2.1 Equilibrium solubility measurements 121
4.2.2 Statistical comparison 124

4.2.3 Influence of individual DoE factors and type of excipient on solubility values in the fasted and fed arms 127

4.2.4 Influence of factor interactions and type of excipient on equilibrium solubility in fasted and fed arms 133

4.2.5 Influence of individual DoE factors and factor interaction and the excipient effect on equilibrium solubility in combined arm 138

4.2.6 Comparing equilibrium solubility value of the standard in each DoE recipe with the corresponding solubility value in excipient containing media . 145

4.3 Discussion 160

4.3.1 Equilibrium solubility measurements 160

4.3.2 Statistical comparisons of the overall distribution of solubility 160

4.3.3 Standardised effect values and the individual media tubes solubility values 162

4.4 Conclusion 166

5 Dissolution testing using DoE simulated media 167

5.1 Material and method 167

5.1.1 Examining the effect of excipients on carvedilol dissolution when both excipients and drug are within the disc 167

5.1.1.1 Preparation of the dissolution sample disc in Sirius T3 167

5.1.1.2 Preparation of the dissolution sample disc in Sirius SDI 168

5.1.1.3 Preparation of the fasted and fed simulated dissolution media ... 168

5.1.2 Examining the effect of excipients on carvedilol dissolution when excipients are in media 169

5.1.2.1 Preparation of the fasted and fed simulated dissolution media ... 169

5.1.3 The dissolution experiment 169

5.1.4 Molar extinction coefficient 169

5.1.5 Dissolution data comparison 169

5.1.6 Calculation of the *f*2 similarity factor 170

5.2 Results 170
5.2.1 Dissolution testing of carvedilol using Sirius T3 170
5.2.1.1 Dissolution testing of carvedilol control 170
5.2.1.2 Examining the effect of excipient on dissolution of carvedilol when excipient and carvedilol are both in disc. 172
5.2.1.3 Examining the effect of excipient on dissolution of carvedilol when excipient is in simulated media 177
5.2.2 Dissolution testing of carvedilol using Sirius SDI 180
5.2.2.1 Dissolution testing of carvedilol control 180
5.2.2.2 Examining the effect of excipient on dissolution of carvedilol when excipient and carvedilol are both in disc. 183
5.2.2.3 Examining the effect of excipient on dissolution of carvedilol when excipient is in simulated media 189
5.2.3 Comparing equilibrium solubility and concentration of released drug in dissolution testing 189
5.3 Discussion 197
5.3.1 Dissolution testing of carvedilol 197
5.3.2 Dissolution testing of carvedilol with excipients 198
5.3.3 Correlation between solubility and dissolution 201
5.4 Conclusion 201
6 Conclusion and future work 203
7 References 208

List of Figures

Figure 1.1: Absorption stages in GIT after administration of an oral dosage form. ..20

Figure 1.2: Passive and active transport (taken from Watson, 2015). 25

Figure 1.3: Chemical structures of different bile salts 29

Figure 1.4: A schematic representation of mixed micelle. A structure composed of monolayer of different amphiphilic molecule (taken from Pavlović et al., 2018). 30

Figure 1.5: Biopharmaceutics classification system (BCS) 39

Figure 1.6: Devolopability classification system (DCS) 40

Figure 2.1: Chromatographic Analysis of 13 selected drugs using HPLC. 63

Figure 2.2: Equilibrium solubility measurements for each drug in DoE media compositions detailed in Table2.1. 64

Figure 2.3: Statistical Comparison of Design of Experiment Equilibrium Solubility Measurements. 66

Figure 2.4: Standardised Effect Values for DoE Factors on Equilibrium Solubility in Fasted and Fed Study Arms. DoE standardised effect values for factors (as listed in Figure y-axis) on equilibrium solubility. 69

Figure 2.5: Standardised Effect Values for DoE Factors and Factor Interactions on Equilibrium Solubility in Combined Study Arm. 73

Figure 2.6: Average Absolute Standardised Effect Values for DoE Factors on Equilibrium Solubility in Fasted, Fed and Combined Arms. 75

Figure 3.1: Schematic diagram of the design of experiments three arms 82

Figure 3.2: Schematic diagram of freeze stock solutions preparations 84

Figure 3.3: Design of experiment equilibrium solubility measurements. Equilibrium solubility measurements for each drug in the three different stock media solution. ..85

Figure 3.4: Statistical comparison of design of experiment equilibrium solubility measurements. 89

Figure 3.5: Standardised effect value for all DoE factors on equilibrium solubility in fasted and fed study arms 94

Figure 3.6: Standardised effect value for all DoE factor interactions on equilibrium solubility in fasted and fed study arms.....99

Figure 3.7: Standardised effect value for all DoE factors on equilibrium solubility in combined study arm. 102

Figure 3.8: Standardised effect value for all DoE factor interactions on equilibrium solubility in combined study arm. 108

Figure 3.9: Significant standardised effect value for all DoE factors and factor interactions on equilibrium solubility in all three study arms (fasted, fed and combined). 113

Figure 4.1: Design of experiment equilibrium solubility measurements for (a) fenofibrate and (b) carvedilol in presence of different types of excipients in media. 123

Figure 4.2: Statistical comparison of design of experiment equilibrium solubility measurements for both fenofibrate and carvedilol 126

Figure 4.3: Standardised effect values for individual DoE factors on equilibrium solubility in fasted and fed study arms of both fenofibrate and carvedilol. 133

Figure 4.4: Standardised effect values for individual DoE factor interaction on equilibrium solubility in fasted and fed study arms of both fenofibrate and carvedilol 138

Figure 4.5: Standardised effect values for individual DoE factors on equilibrium solubility in combined arm of both fenofibrate and carvedilol:. 143

Figure 4.6: Equilibrium solubility data of fenofibrate and carvedilol control along with the equilibrium solubility data of drugs when different excipients added to the media 159

Figure 5.1: Sirius T3 dissolution results of carvedilol in fasted and fed DoE media. (a) and (b), dissolution testing of carvedilol. 171

Figure 5.2: Sirius T3 dissolution results of excipients +control disc in fasted and fed state media.. 176

Figure 5.3: Sirius T3 dissolution results of carvedilol when excipients were in fasted and fed simulated media. 179

Figure 5.4: SDI dissolution results of carvedilol control in fasted and fed media. (a) and (b), dissolution testing of carvedilol. 182

Figure 5.5: SDI dissolution results of carvedilol when excipient pressed in disc with the control in fasted and fed simulated media. 188

Figure 5.6: Images of the SDI dissolution process of carvedilol when chitosan and HPMC E50 are in media. 189

Figure 5.7: Equilibrium solubility values from DoE of carvedilol control and carvedilol +different excipient in media together with the concentration released of carvedilol in same media of carvedilol +the different excipients in disc with the control. 194

Figure 5.8: 1. Solubility values of carvedilol control in fasted and fed DoE media along with media containing the excipient. 2. Concentration of the released carvedilol control along with the concentration of carvedilol with the excipient in both fasted and fed media. 3. Concentration of the released carvedilol with HPMCE50 after 5 hours duration of the experiment. 196

List of Tables

Table 1.1: Characteristics of the human intestinal fluids 35

Table 1.2: Composition of the commonly used fasted and fed bio-relevant media ...36

Table 1.3: Traffic light used in the 'Oral Physchem Score' (Taken from Lobell et al., 2006) 38

Table 2.1: Fasted and Fed media components and concentration levels. 56

Table 2.2: Physicochemical properties of the studied drugs 57

Table 2.3: Stock Mixture Concentrations (15x lower, mid and upper limits) 59

Table 2.4: Fatty Acids volumes (5x upper limit).....59

Table 2.5: HPLC Analysis Conditions.....61

Table 2.6: Comparison of the Statistical Significance of DoE Factors across Studies. 70

Table 3.1: Comparison of the statistical significance of DOE factors across experiments. 103

Table 4. 1: The composition level of the added excipient 120

Table 4. 2: Excipient volume to be added to the media in fasted and fed state at (0.5% w/v) and (5% w/v) 120

Table 4. 3: Excipient volume to be added to the media in fasted and fed state at 0.5%and 5% concentration of HPMC (E3 and E50) 120

Table 4.4: Comparison of the statistical significance of DoE factors across the experiments 144

Table 5.1: Values of *f*2 similarity factor of the different tests (yellow lines are the significant values). 174

List of Abbreviations

API, Active Pharmaceutical Ingredients

A1, Fasted DoE media where all media components in lower level

BA, Bioavailability

BCS, Biopharmaceutics Classification System

BS, Bile Salt

BS: PL, Bile Salt to Phospholipid ratio

CA, Cholic Acid

CDCA, Chenodeoxycholic Acid

CMC, Critical Micelle Concentration

DCS, Developability Classification System

DoE, Design of Experiment

FaSSIF, Fasted State Simulated Intestinal Fluid from Glaia et al., 1998

FDA, Food and Drug Administration

FeSSIF, Fed State Simulated Intestinal Fluid from Glaia et al., 1998

F2, Fed DoE media where all media components in lower level

GI, Gastrointestinal

GIT, Gastrointestinal Tract

HIF, Human Simulated Fluid

HPLC, High Performance Liquid Chromatography

HPMC E3, Hydroxyl Propyl Methyl Cellulose lower viscosity grade

HPMC E50, Hydroxyl Propyl Methyl Cellulose higher viscosity grade

IMI, Innovative Medicine Initiatives

IPEC, International Pharmaceutical Excipient Council

IVIVC, In-Vivo In Vitro Correlation

MG, Monoglyceride

MW, Molecular Weight

OA, Oleic Acid

OrBiTo, Oral Biopharmaceutical Tools

P (app), Apparent Permeability

P (eff), Effective Permeability

PBPK, Physiologically Based Pharmacokinetics

PEG, polyethylene Glycol

PL, Phospholipid

PVP HG, Polyvinylpyrrolidone Higher Grade

PVP LG, Polyvinylpyrrolidone Lower Grade

SDI, Surface Dissolution Imaging

SGF, Simulated Gastric fluids

SIF, Simulated Intestinal fluids

TPSA, Topological Polar Surface Area

1 Oral drug absorption

Oral drug intake is considered as the preferred route of drug administration considering the ease of administration and patient compliance (Sugano et al., 2007). Though, when drugs passes to the gastrointestinal tract (GIT) they face several physical and chemical barriers to their systemic bioavailability ranging from the acidic environment of the stomach and enzymatic degradation to the variable pH levels and fluid compositions in the intestine (Dressman &Reppas, 2000). In addition, the oral bioavailability of drugs depends on several factors including solubility, dissolution and permeability across the biological membrane. According to the Biopharmaceutics classification system (BCS) (Amidon et al., 1995), some of these drugs are insufficiently soluble in aqueous media and or have poor permeability leading to a poor bioavailability after oral administration (Bergström et al., 2007). Therefore, studying drug solubility and dissolution plus providing in vitro methodologies to measure these properties are of great importance in a manner to predict and enhance the bioavailability of poorly absorbed drugs (Clarysse et al., 2011; Sugano et al., 2007).

1.1 **Solubility**

The equilibrium solubility or thermodynamic solubility is the concentration of a solute in a saturated solution, when an extra amount of solid is present and the solvent and solute are at equilibrium. If a solute has the potential to ionize, the intrinsic solubility is the equilibrium solubility of the unionized form. The term aqueous solubility is applied when measuring solubility in distilled water or in aqueous buffer solution (Szab et al., 2013). Aqueous solubility is a vital molecular property that effects the absorption of an orally administered drugs from the GIT. For an orally administered medicinal product, to attain a desirable systemic exposure the drug has to dissolve in the gastrointestinal (GI) fluids and then pass through the gut membrane or wall to reach systemic circulation (Figure 1). A process driven mainly by the breakdown of the solute –solute bond and the interaction between the solute and solvent in the solution (Yang et al., 2002).

The general solubility equation (Equation 1) was developed to give realistic predictions of the aqueous solubility for a wide variety of compounds by relating the molar aqueous solubility (S_w) to the melting point (mp °C) and the octanol-water partition coefficient (K_{ow}) (Yang et al., 2002). The melting point provides a sensible clue of the intermolecular strength of solute-solute interactions and thus solubility is lower for those compounds with higher melting point due to stronger intermolecular forces. The log *P* value of the compound gives another reasonable clue for solubility as the more affinity of a compound to a polar solvent such as water the more it will be portioned to the solvent ending up with a good solvation (Pouton et al., 2013). The melting point and log *P* value can be attained experimentally or predicted from in silico tools.

$$\text{Log } S\text{w} = 0.5 - 0.01(25 - \text{mp}) - \log K\text{ow} \quad \text{(Equation 1)}$$

Although the primary driver for solubility in the GI fluids is the aqueous solubility of the drug, other factors in the GI tract may facilitate drug solubility such as pH of the media and the naturally occurring surfactants and food components. The different pH ranges of the stomach and the small intestine are of great importance for drugs that are ionised at this range. For a monobasic and a monoacidic compound, solubility can be calculated from the Henderson-Hasselbalch equation (Equation 2 and Equation 3). Where C_s is the total solubility of the non-ionised acid or base (intrinsic solubility), $C_s.0$ is the total solubility (the sum of the ionised and the non-ionised solubility) (Dressman et al., 2007).

$$C\text{s} = C\text{s}.0\ (1 + 10^{\text{pH} - \text{p}K\text{a}}) \quad \text{(Equation 2)}$$

$$C\text{s} = C\text{s}.0\ (1 + 10^{\text{p}K\text{a} - \text{pH}}) \quad \text{(Equation 3)}$$

The benchmark protocol for solubility measurement is the shake flask method which determines solubility at equilibrium by the addition of an excess amount of the solid drug to the medium of interest for a predetermined time and temperature. When equilibrium is reached, samples are removed, the solid separated by filtration or centrifugation and the drug's concentration in the liquid phase will be quantified

(Bergström et al., 2014). Potentiometric acid-base titration is another method for solubility determination where solubility is measured across the whole pH range based on a specific solubility shift leading to precipitation during titration curve (Glomme et al., 2005). In addition, chasing equilibrium solubility (CheqSol) has been developed for measuring intrinsic solubility of ionisable drugs. An appropriate amount of the drug was first dissolved by adjusting the pH so the drug will exist in the ionised form, then the pH were changed so the drug will be in the neutral form and precipitate. The process continues by adding strong acidic or basic titrant to adjust the pH to discover its equilibrium condition, then the intrinsic solubility of the drug in the neutral form could be determined (Stuart & Box, 2005; Box et al., 2009; Schmidt et al., 2011).

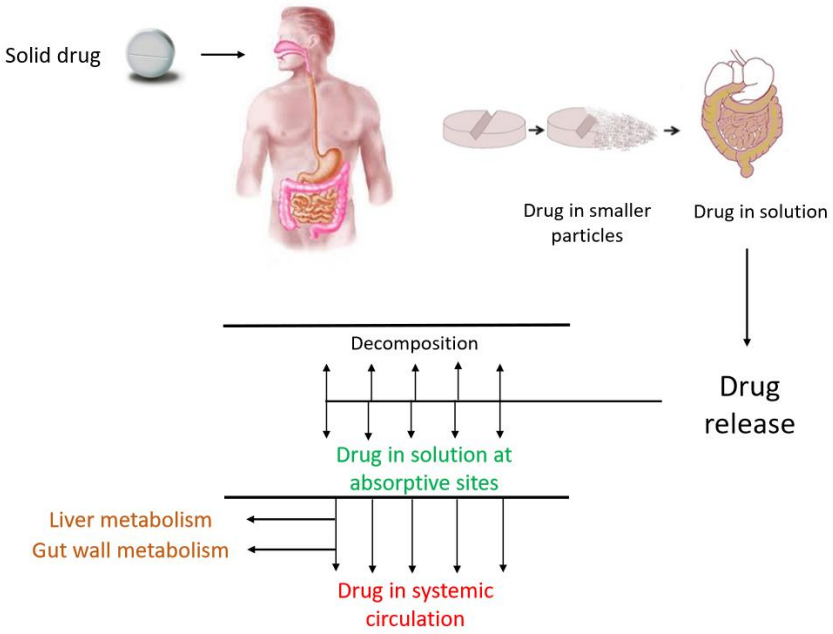


Figure 1.1: Absorption stages in GIT after administration of an oral dosage form.

1.2 **Dissolution**

Dissolution is the process of the drug's solubilization in the solvent to produce a solution which is an important feature for the in vivo performance of the solid oral dosage product. The complete pharmacological effect of an orally taken drugs cannot be attained unless these formulations undergo dissolution before a molecule can pass through the biological membrane of the GIT to finally reach the systemic circulation

and produce their response (Nurhikmah et al., 2016). Hence, dissolution testing has become important to guide the pharmaceutical scientist to maximise drug absorption by improving the dissolution of the compound for better effect. To provide the best media for dissolution testing, the factors that are known to have an effect on drug dissolution must be identified. The modified Noyes-Whitney equation (Equation 4) displays these factors (Dressman & Reppas, 2016).

$$DR = \frac{A.D}{h} . (C_s - \frac{Xd}{V}) \quad \text{(Equation 4)}$$

Where DR is the dissolution rate, A is the surface area of the drug, D is the diffusion coefficient of drug in solution, h is the thickness of the diffusion layer, C_s is the concentration of the saturated drug in the GI conditions, V is the volume of the dissolution medium and Xd is the amount of the drug that is already dissolved.

1.2.1 Parameters affecting dissolution rate

1.2.1.1 Particle size/Surface area and wetting

For the active ingredient to be dissolved into a solution it should first be wetted by the solvent, which occurs by the spread of the solvent molecules over the surface of the solute followed by the immersion of the solid solute into the solvent. Increasing the surface area will increase the area for dissolution. The smaller the particle size of the drug the higher is the surface area and dissolution rate. The presence of native surfactant of the GIT such as bile salts will also influence the dissolution rate of the drug through the decrease of the surface tension and thus improving the wetting process (Dressman & Reppas, 2016). Surface tension is lower in fed than in fasted state due a higher level of surfactant and presence of food components. Additionally pH can affect the wetting process through the ionization of the different amphiphiles present in the media (Pepin et al., 2001), which illustrates the importance of taking pH and presence of surfactant into account when conducting in vitro dissolution tests.

1.2.1.2 Diffusion coefficient/Viscosity of the luminal contents

Viscosity exerts an important influence on a compound's diffusivity and the disintegration of the formulation. Stokes- Einstein's equation (Equation 5) describes the most important factors that affect the diffusion of a drug (Kleberg et al., 2010).

$$D = \frac{k\,T}{6\pi\rho r} \quad \text{(Equation 5)}$$

Where D is the diffusion coefficient, k is the Boltzman constant, T is the temperature (°C), ρ is the fluid viscosity and r is the radius of the drug molecule. From the equation, viscosity is inversely proportional to the diffusion coefficient of the drug. Viscosity is highly dependent on the feeding state and type of meal, intestinal fluid viscosity tends to be increased with dietary fibers which may decrease the dissolution and absorption of a drug. Therefore, viscosity is of greatest important parameter to be considered when setting dissolution testing taking into account the different luminal contents that might affect this parameter.

1.2.1.3 Boundary layer thickness/Motility and flow rate

The hydrodynamics in the gastrointestinal tract vary with the motility pattern, how good the luminal contents are mixed and the flow rate in the gastrointestinal tract. This has a significant role in dissolution of drugs through their impact on the boundary layer thickness.

1.2.1.4 Lipophilicity of the drug/Bile and food components

In addition to the food and fluids ingested with the dosage form, various fluids are secreted from the GIT such as bile salts. Bile salts can highly affect the solubility and dissolution of lipophilic compounds through the incorporation of such drugs in to the formed mixed micelles and consequently enhancing the diffusion and dissolution of the drug

1.2.1.5 Volume of GI content

The volume into which the drug will dissolve is a function of the co-administered fluids together with the fluids in the GIT secreted from the para gastrointestinal organs (liver, salivary glands, pancreas). The amount of fluids ingested orally could be about 2 liters per day though it varies from person to person depending on body weight, physical activity and personal habit (Dressman et al., 1998). The luminal conditions may vary widely between subjects according to type of meal taken, co- administration of certain type of dugs as well as disease state. Dose: solubility ratio is an important parameter to determine whether GI fluid's volume is enough to completely dissolve the taken dose of the drug or not. A dose: solubility ratio greater than 250 ml indicates less than optimal condition for dissolution (Dressman & Reppas, 2000). In dissolution testing, it is important to adjust the volume of the medium according to the site in GIT that needs to be simulated since the volume of these fluids will be different in fasted than in fed state and in stomach than in small intestine. The volume of gastric fluids in fasted state may be as little as 20-30 mL and reaches to 1500 mL with gastric secretions (Dressman et al., 1998) while in intestine, fluids may reaches to a total of 6 liters per day owing to the endogenous secretions (saliva, pancreatic juice, bile output) as well as the intestinal secretion of water as a component of mucus (Dressman et al., 1998).

1.2.2 Summary

By screening the main factors that affect dissolution from the Noyes-Whitney equation, one can realize that along with the physical parameters of the drug, there are many other physiological parameters that can control the dissolution rate of a medicinal product. Therefore, to improve quality of dissolution testing for predicting in vivo performance of an API, an in vitro-in vivo correlation (IVIVC) studies were established in 1997 (Guidance for industry, 1997 b). The development of IVIVC should lead to a reduction in work needed for formulation development and reduction in the number and size of clinical studies required (Dressman et al., 1997). Because of the importance of dissolution, regulations for development of dissolution testing was established by employing a dissolution test using paddle or basket apparatus with

a dissolution medium of 900-1000 ml at pH 6.8 phosphate buffer (British Pharmacopeia, 2009; United State Pharmacopeia, 2011).

However recent research focus is on the use of more bio relevant media for dissolution testing which simulate the gastric and small intestinal fluids. The research group of Dressman et al in 1998 developed a more bio relevant media such as simulated gastric fluid (SGF), fasted state simulated intestinal fluids (FaSSIF) and fed state simulated intestinal fluids (FeSSIF) which provides a better determination of the in vivo performance of a drug (Galia et al., 1998).

Physiological dissolution profiling has been done using a large volume of dissolution medium (500-900) which becomes so expensive due to the large amount of the bile salt and lecithin required plus the high amount of the drug required to saturate the medium (Bergström et al., 2014). Currently several automated miniaturized methods such as the µDISS Profiler™, T3 platform dissolution apparatus and Sirius SDI (surface dissolution imaging) which can measure dissolution rate from discs (insert size/diameter in mm) or powder in small volumes of bio-relevant medium. These systems also allow control of the pH or temperature (Fagerberg et al., 2010; Bergström et al., 2014;.Avdeef & Tsinman, 2008; Tsinman et al., 2009)

1.3 **Permeability**

Physiological bilayer membranes consist of a hydrophobic core and a hydrophilic surfaces and therefore they are described as an amphiphilic system. Oral drugs have to permeate the cell membrane to reach systemic circulation. Drug permeation can occur through several processes such as paracellular diffusion, transcellular diffusion and active transport (Figure 1.2). Permeation of most drugs occur by passive diffusion either across the lipid membrane (transcellular) or between the epithelial cells through water filled pores in the tight junction complex (paracellular). For each drug, several parameters such as molecular size, hydrogen bonding, charge and lipophilicity are important for the permeation through these biological membranes (Bergström et al., 2014). The permeability of a drug is evaluated by measuring the passages of drug across the membrane between the donor and the acceptor compartments and thus the

permeability coefficient (*P in cm/sec*) can be calculated according to the following equation (Dressman & Reppas, 2016):

$$P=\frac{dQ}{dt}\ x\ \frac{1}{A\ X\ Cdonor} \quad \text{(Equation 6)}$$

Where (dQ/dt) is the amount of drug transported per unit time, A is the surface area of the barrier and C_{donor} is the drug concentration at the donor compartment.

Several systems can be used for a permeability assessment such as artificial membranes and Caco-2 cell system which is the industry standard for screening of a drug candidate and can be used to assess the apparent permeability (P_{app}) (Dressman & Reppas, 2000). The in situ intestinal perfusion that best simulates the in vivo situation can be used to assess the effective permeability (P_{eff}) and the results showed a good correlation with the in vivo human permeability for passively absorbed drugs (Fagerholm et al., 1996). According to the biopharmaceutics classification system (BCS), a drug considered to be highly permeable when the fraction absorbed is at least 90% (Amidon et al., 1995).

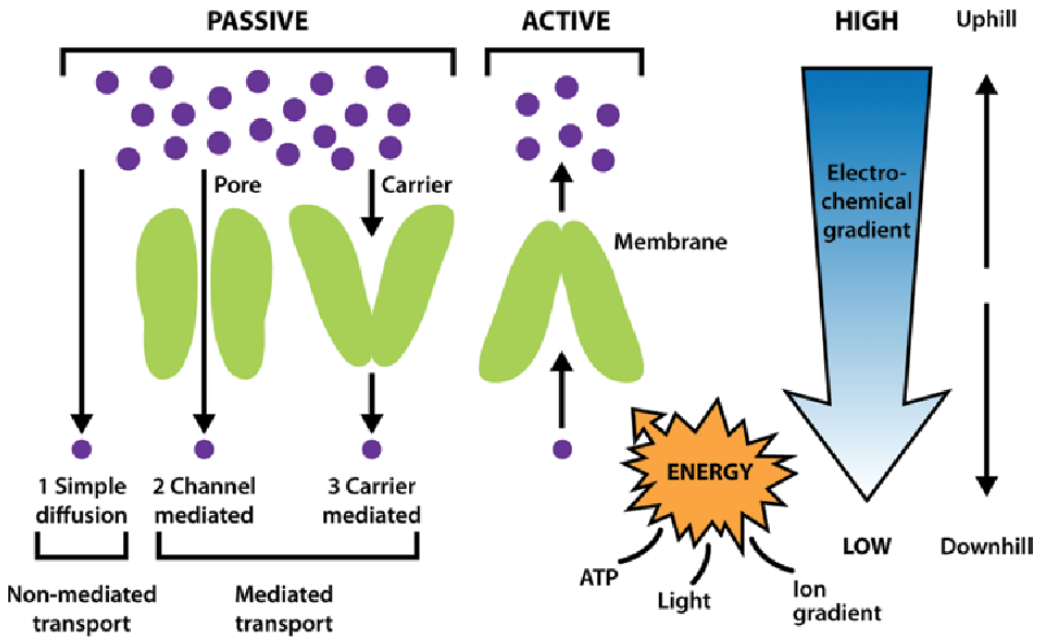


Figure 1.2: Passive and active transport (taken from Watson, 2015).

1.4 GI media composition, drug physicochemical characteristics and their impact on drug absorption

The small and large intestine in the abdominal cavity are responsible for a variety of functions mainly the digestion of food and absorption of nutrients. The large surface area of the small intestine allows this part of the gastrointestinal tract to be almost solely responsible for the absorption of the nutrients (Kiela & Ghishan, 2016). However, the GI media has it is own complexity and variability (Dressman et al., 2007) with several factors that can affect drug solubility and absorption such as bile salt, buffer capacity and food effects. The intake of a meal makes several changes in the GI tract such as increased volume due to GI secretions and changes in gastric and intestinal pH (Dressman & Reppas, 2016). In addition to the GI media effect, several drug specific factors, for example, pKa, log *P*, chemical structure, and properties (i.e., acidic, basic, or neutral), are known to affect aqueous solubility generally and also in intestinal media. This section summarizes these aspects that can impact the drug absorption and bioavailability.

1.4.1 Composition of simulated GI fluids media

Multiple studies have been published, directed at achieving an improved understanding of drug solubility in the GIT and its impact on oral bioavailability (Augustijns et al., 2014; Klein et al., 2010). Research has been performed to provide in vitro derived media which simulates and resembles human intestinal fluids by containing all of the components that are known to play a role in drug solubility, such as pH, bile salt, buffer, lecithin, and lipid degradation products (Galia et al., 1998) in both fasted and fed state. Table1.1 reviews the different parameter levels in human gastric, jejunum and duodenum fluids in both fasted and fed state. Table1.2 reviews some commonly used bio-relevant media in literature studies.

1.4.1.1 pH

Values of gastric pH reported to range between 1.7 and 3.3 in the fasted state (Dressman et al., 1990; Pedersen et al., 2000; Kalantzi et al., 2006; Lindahl et al., 1997). Therefore, simulated gastric media in fasted state will have pH values between these values. Higher gastric pH values will be found in patients undergoing gastric antacid treatment or with patients above 65 years old (Dressman et al., 1998). After food intake, gastric pH will be buffered by the food components leading to an increase in gastric pH value to a range between 4.5 and 5.8 depending on the food components. Over time, between one and four hours and upon secretions of gastric fluids, the pH level will return back to fasted state values (Dressman & Reppas, 2016). Accordingly, only drugs that are taken with or directly after meal will be affected by the increased pH level of the stomach under normal physiological conditions (Dressman et al., 1998).

The intestinal pH values are significantly higher than the gastric pH values due to pancreatic secretions of bicarbonate ion which neutralizes the incoming acid with a tendency for increasing pH values when moving from duodenum to jejunum (Dressman et al., 1997). The fasted pH values were found to range between 5.6 and 7 in the duodenum and 6.5 and 7.8 in the jejunum (Clarysse et al., 2009; Bergström et al., 2014; Brouwers et al., 2006; Psachoulias et al., 2011). In the fed state, the pH value of the duodenum is decreased to a value of 5.7 to 6.5 (Dressman et al., 1990; Vertzoni et al., 2012; Mansbach et al., 1975) as it is affected by the chyme arriving from the stomach while the distal parts of the intestine is more stable with a pH value of 7 to 7.5 (Dressman et al., 1997). Simulated intestinal fluid media employ a pH range of 5-6.7 in all different bio-relevant media formulations (Table1.2).

1.4.1.2 Buffer capacity

The pH value in the diffusion boundary layer of an API is an important element for the dissolution of an ionizable drugs. Different factors can control the pH value in the diffusion boundary layer including the ionization constant, the drug's intrinsic

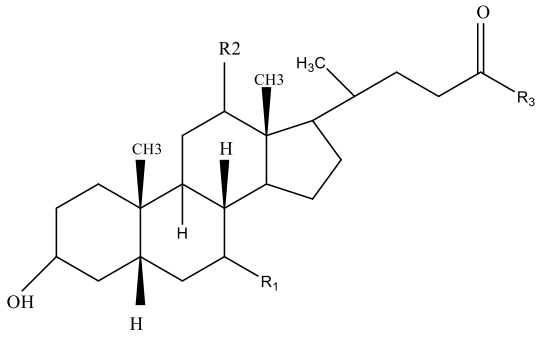
solubility, media pH and the media's buffer capacity (Dressman et al., 1997). The buffer capacity in the fasted state is between 13.3- 19.0 mM /Δ pH in gastric fluids, 5.6-8.5 mM / Δ pH in duodenum (Kalantzi et al., 2006a; Pedersen et al., 2013) and a median of 4 mM /Δ pH in jejunum with a tendency of buffer capacity to decrease going from stomach to jejunum (Bergström et al., 2014). In the fed state, buffer capacity increases for gastric (19.5 mM /Δ pH), duodenal (24-30 mM /Δ pH) and jejunal (13.9 mM /Δ pH) fluids and the increase will also depend on the ingested food components (Bergström et al., 2014).

The intestinal biological buffer is bicarbonate which forms through a dynamic equilibrium of dissolved carbon dioxide, carbonic acid and bicarbonate ion. The in vivo multistep equilibrium and the pH value instability makes it difficult to simulate in vitro and thus, it was avoided in simulated media (Dressman et al., 1998). Dressman and coworkers first presented the fasted state simulated intestinal fluids (FaSSIF) (Dressman et al., 1998; Galia et al., 1998) where they used phosphate as the buffer. This version was then improved to a more bio relevant one and called the fasted state simulated intestinal fluid version 2 (FaSSIF-V2) (Jantratid et al., 2008), where the phosphate buffer was changed to maleic acid. Maleic acid was found to retard the rancidity of fat and oil (Dressman et al., 1998). In addition, it complies more with the physiological conditions as it reached buffer capacity within the pH range (5.4-6.5) of both the fasted and the fed state without exceeding the relevant physiological osmolarity (Jantratid et al., 2008). However, maleate was found to increase the oxidation of troglitazone drug (Vertzoni et al., 2004b). In addition, maleate has chromophoric property which may interfere with drug analysis during the HPLC assay (Mauger, 2017). It is important to note that buffer capacity of the media will have a great effect on drug dissolution and solubility therefore, in order to attain bio relevant pH and buffer capacity, buffers should be chosen to meet the desired combination of pH, buffer capacity and osmolarity (Jantratid et al., 2008).

1.4.1.3 Amphiphiles and digestion products

Bile salt

Bile salts (BS) are physiological surfactants that are produced from cholesterol in the liver. Two main human bile salts are found, cholic acid (CA) and chenodeoxycholic (CDCA) acid and they are conjugated to either taurine or glycine (Bergström et al., 2014). Bile salts have a specific chemical structure characterized by the rigid and planner hydrophobic steroid nucleus with hydroxyl group that vary in number, position and orientation (Pavlović et al., 2018; Poša et al., 2015) along with a flexible acidic side chain (Figure 1.3). This amphiphilic property allows bile salts in combination with other amphiphilic substances to form a supramolecular aggregates or micelles (Figure 1.4) which plays a crucial rule in the solubilization of the lipophilic components in food (Dressman &Reppas, 2016; Pavlović et al., 2018; Wilson et al., 2016).



Name	R1	R2	R3
Cholic acid	OH	OH	OH
Chenodeoxycholic acid	OH	H	OH
Deoxycholic acid	H	OH	OH
Ursodeoxycholic acid	OH	H	OH
Lithocholic acid	H	H	OH
Glycocholic acid	OH	OH	NHCH2COO-
Taurocholic acid	OH	OH	NHCH2CH2SO3-

Figure 1.3: Chemical structures of different bile salts

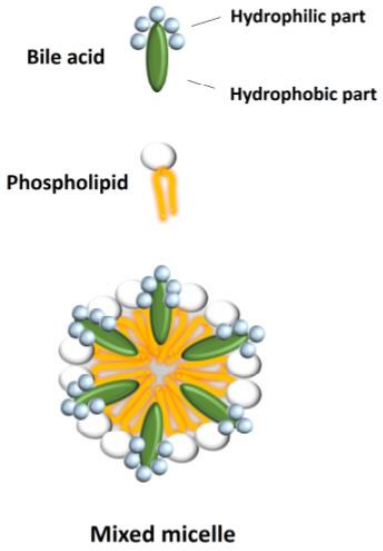


Figure 1.4: A schematic representation of mixed micelle. A structure composed of monolayer of different amphiphilic molecule (taken from Pavlović et al., 2018).

Several studies have indicated the solubility enhancing effect of amphiphilic substances to different poorly soluble BCS class II compounds (Zughaid et al., 2012; Kleberg et al., 2010). In addition to the micelle forming capacity of bile salts, bile salts have been found to increase drug solubility and dissolution through their wetting effect and decreasing the interfacial tension between the drug and the media in which it's dissolved (Pavlović et al., 2018).

Taurocholate and glycocholate are the most abundant bile salts in human. They have different pKa values, 1.5 for taurocholate and 3.7 for glycocholate and at the pH range of the small intestine only taurocholate bile salt will be fully ionized and that's why it is most often used in bio-relevant media (Clarysse et al., 2011).

Fasted bile salts level vary between 0.0-0.8 mM in gastric fluids (Pedersen et al., 2000) due to reflux of the bile salt from duodenum. However, it has been debated that duodenal reflux of bile salt was induced by the cannula used for fluids sampling in that study and therefore presence of bile salt in stomach is not physiologically relevant (Bergström et al., 2014). Bile salt level was reported to be about 3-5 mM in intestinal fluids (Peeters et al., 1980; Dressman et al., 1998). In the fed state, bile salts level

were found to be lower than fasted state in gastric fluids (Schindlbeck et al., 1987) due to the dilution of the bile content by food components. In the duodenum, bile salt level vary between 0.03 and 36.18 mM with a mean of 4.61 mM in the fasted state. After food intake, the level of bile salt that was reported in literature was wide range varying between 3.6 mM and 24 mM with a median of 11.8 mM (Riethorst et al., 2015; Bergström et al., 2014; Armand et al., 1996; Hernell et al., 1990).

Phospholipid

Phospholipids (PL) are amphiphilic compounds composed of a choline and glycerophosphoric acid head with two fatty acid tails. Phosphatidylcholine (PC) is the most common phospholipid in bile secretion and has been used in bio relevant media as a model (Galia et al., 1998). Commonly, eggs or soy bean are used as a source of PL, both contain phosphatidylcholine but egg phospholipid contain more saturated fatty acid (Wang & Wang, 2008). In the lumen of the small intestine, PC is converted to lyso PC which is able to form a micelle by itself while the former can form vesicles. However PC will interact with bile salt to form a micelle (Kleberg et al., 2010b). The interaction of bile salt and lecithin will form large micelles which have been found to alter the solubility of various drugs (Zughaid et al., 2012).

In the fasted state, gastric phospholipid concentrations are approximately 0.029 mM (Dewar et al., 1982; Bergström et al., 2014) with a level of 0.2 mM found in the duodenum (Clarysse et al., 2009; Riethorst et al., 2015). In the fed state, PL concentration are reported to be 0.022 mM in stomach (Dewar et al., 1982; Bergström et al., 2014) and have a range of 1.2 to 6.0 mM in duodenal fluids (Armand et al., 1996; Vertzoni et al., 2012; Riethorst et al., 2015). However, the concentration of PL might be higher after a meal depending on the type of the administered meal for example, egg will increase the level of PL (Bergström et al., 2014). In simulated intestinal media, the amount of PL was changed from 0.75 mM in FaSSIF to 0.2 mM in FaSSIF-V2 (Table1.2) to match the in vivo osmolarity and to maintain the stability of the media over 7 days (Jantratid et al., 2008).

Lipid digestion products: monoglyceride, free fatty acids and cholesterol

Monoglycerides and free fatty acids are surfactants that are formed during the digestion of triglyceride from food by the lipase enzyme (Bergström et al., 2014). Cholesterol is also present in the small intestine either derived from the ingested food or secreted with the bile secretion (Cohn et al., 2010). Thus the modification of FaSSIF and FeSSIF by the addition of lipid digestion products to the fasted simulated media of FaSSIFV2 plus and FaSSIF-V3 and to the fed simulated media of FeSSIF-V2 and Copenhagen recipe were found to achieve improved IVIVC (Table1.2) in comparison to the previous recipes. These surface active agents can then interact with bile salt and phospholipid to form mixed micelles. The presence of these colloidal aggregates in the small intestine was found to have the ability to solubilize lipophilic drugs in particular by the formation of vesicular structures (Pavlović et al., 2018; Kleberg et al., 2010).

Very few studies reported the levels of monoglyceride and free fatty acids especially in stomach and fasted state as they are a lipid digestion product (Bergström et al., 2014; Kleberg et al., 2010). A level of 0.6 mM free fatty acids and 0.1 mM for both monoglyceride and cholesterol were reported in human intestinal fluids (HIF) during fasted state (Riethorst et al., 2015). In the fed state, a level of 5.95 mM of monoglyceride and 39.4 mM for free fatty acids were reported in duodenum (Fatouros et al., 2009). Riethorst et al reported a level of 9 mM of monoglyceride, 1mM of cholesterol and 6.5 mM of free fatty acid (Riethorst et al., 2015).

BS: PL ratio

The ability of bile salts and other amphiphilic substances to form micelle depends on the presence of these substances at a concentration above a certain concentration termed the critical micellar concentration (CMC) (Pavlović et al., 2018). A total concentration of bile salt and lecithin between 2 and 3mM was required to start micelle formation.

The molar ratio between bile salt and PL is reported to be 11.5 in the fasted duodenal fluids (Bergström et al., 2014). In the fed state, a ratio of 2.3 was reported in gastric fluids (Dewar et al., 1982) and a range of 2 to 16 in duodenal fluids with the majority of ratios between 2 and 4 (Mansbach et al., 1975; Hernell et al., 1990; Armand et al., 1996; G.A. et al., 2007). The BS: PL ratio is significantly lower in fed state than in fasted state which can be due to the increased PL content after food ingestion (Dressman &Reppas, 2000; Bergström et al., 2014).

The original intestinal bio relevant media introduced by Dressman and collogues in the FaSSIF version used a level of 3mM of bile salt and 0.75 mM of PL resulting in a BS: PL ratio of 4 in both fasted and fed state (Galia et al., 1998; Dressman et al., 1998). In FaSSIF-V2 and FaSSIF-V2 plus , the amount of PL in fasted state was reduced to be 0.2 mM to comply more with the osmolality in-vivo, resulting in an increase in the BS: PL ratio from 4 to 15, while in fed state the ratio was 5 with increasing levels of both bile salt and PL (Jantratid et al., 2008). In 2010, Kleberg et al shifted the ratio back to 4 (Table1.2) in both fasted and fed Copenhagen simulated media (Kleberg et al., 2010).

1.4.1.4 Osmolality

Osmosis is the diffusion of water across a semipermeable membrane under the control of the osmotic pressure which is caused by an imbalance of molecules on either side of the membrane. Osmolality measures the balance between water and fluid solute. By far, sodium (Na^+) and chloride (Cl^-) are the most predominant cation and anion in extracellular fluids (Sugano et al., 2007). The regulation of osmolality is achieved by balancing the intake and excretion of sodium and other solutes with that of water keeping the cells isotonic, this will equalize the osmotic pressure on either side of the membrane. The isotonic osmolality of plasma fluids ranges between 270-310 mOsm/kg. Since sodium is the main electrolyte, a typical bio-relevant media is recommended to be composed of sodium buffer. Sodium hydroxide and potassium dihydrogen phosphate are recommended in British Pharmacopoeia for dissolution testing (British pharmacopeia, 2009).

In the fasted state, stomach fluid osmolality was found to range between 119-221 mOsm/kg (Lindahl et al., 1997; Pederson et al., 2000; Kalantzi et al., 2006a; Pederson et al., 2013). Osmolality was found to range between 137 and 224 mOsm/kg in duodenal fluids (Kalantzi et al., 2006a; Clarysse et al., 2009; Dressman et al., 2006). In the fed state, osmolality found to be higher than in the fasted state as a result of the chyme and intestinal secretions although the increase will depend on the type of food, sampling time and the inter-individual physiological variability (Bergström et al., 2014; Dressman &Reppas, 2000). The fed fluid osmolality found to be 388 mOsm/kg in gastric fluids (Kalantzi et al., 2006a) and ranged between 267 and 416 mOsm/kg in duodenal fluids (Kalantzi et al., 2006a ; Clarysse et al., 2009).

1.4.1.5 Surface tension

Surface tension is a parameter that measures the wetting property of the media thereafter potentially affecting drug dissolution. The presence of amphiphilic substances such as bile salt, lecithin, free fatty acid and cholesterol will increase the wetting property of the media by decreasing the surface tension and thus improving drug dissolution. In the fasted state, gastric surface tension was found to be between 31and 45 mN/m (Pedersen et al., 2013; Kalantzi et al., 2006a) which is lower than the water surface tension (72 mN/m). This is due to the presence of pepsin enzyme and the refluxed bile salt from duodenum (Bergström et al., 2014). The duodenal surface tension is similar to the gastric fluids with a tendency to decrease in surface tension going from stomach to jejunum owing to the increased pancreatic secretions and in particular, the secretion of bile salts (Bergström et al., 2014). In the fed state, surface tension found to be 30.5 mN/m in gastric fluids (Kalantzi et al., 2006a) and 27.8-35.4 in duodenal fluids (Kalantzi et al., 2006a; Clarysse et al., 2009).

Table 1.1: Characteristics of the human intestinal fluids

Factors	Fasted stomach	Fasted duodenum	Fasted jejunum	Fed stomach	Fed duodenum	Fed jejunum
BS (mM)	0.0-0.8 (0.28)	2.5-5.9 (3.25)	1.4-5.5 (2.52)	0.051-0.31	3.6-24 (11.8)	4.5-8.0
PL (mM)	0.029	0.26	0.19	0.022	1.2-6.0 (2.15)	2.0-3.0
BS:PL		11.5	15.5	2.3	2-16 (3.4)	2.3-2.7
MO (mM)		0.5 *			25*	
Cholestrol (mM)		0.1*			1*	
pH	1.7-3.3 (2.5)	5.5-7 (6.3)	6.5-7.8 (6.9)	4.5-6.7	5.4-6.5 (6.0)	6.1
Osmolality (mOsm/Kg)	119-221 (202)	137-224 (202)	200-300 (280)	388	276-416	
Salt (mM)	Na^+19-122 (68)		Na^+111-165 (142)			
Surface tension (mN/m)	31-45 (36.8)	31-45 (36.8)	30-35	30.5	27.8-35.4	30
Buffer capacity (mmol/L.pH)	13.3-19 (14.3)	5.6-8.5	4	19.5	24-30	13.9

All data from Bergström et al., 2014, value in brackets is (median value of several studies), * data taken from Riethorst et al., 2015.

Table 1.2: Composition of the commonly used fasted and fed bio-relevant media

	FaSSIF	FaSSIF-V2	FaSSIF-V2 plus	FaSSIF-V3	FeSSIF	FeSSIF-V2	Copen. Fasted	Copen. fed
BS (mM)	3	3	3	TC/GC 1.4/1.4	15	10	2.5	5.0-20
PL (mM)	0.75	0.2	0.2	PC lyso/PC 0.035/0.315	3.75	2	PC lyso/PC 2.5/0.625	1.25- 5
BS:PL	4	15	15	9	4	5	4	4
MO (mM)	-	-	-	-	-	5	-	0-10
OA (mM)	-	-	0.5	0.315	-	0.8	-	0-45
OA:MO			0.16					2-7.5
Cholesterol (mM)	-	-	0.2	0.2	-	-	-	-
Buffer	phosphate	maleate	maleate	maleate	acetate	maleate	Trizma maleate	Trizma maleate
pH	6.5	6.5	6.5	6.7	5	5.8	6.5	6.5
Osmolality (mOsm/Kg)	270	180	180	220	635	390	270	varying
Salt (mM)	KCl 103	NaCl 96	NaCl 96	NaCl 93.3	KCl 204	NaCl 125	NaCl	NaCl
Buffer capacity (mM/pH)	10	10			75	25		
Surface tension (mN/m)	45.5	-	-	-	46.3	40.45	-	-
Reference	1	2	3	4	1	2	5	5

BS, bile salt; PL, phospholipid; lyso-PC, lysophosphatidylcholine; MO, monooelate; OA, sodium oleate; copen., copenhagen Refrence: 1, Galia et al., 1998; 2, Jantratid et al., 2008; 3, Psachoulias et al., 2012; 4, Fuchs et al., 2015; 5, Kleberg et al., 2010.*FaSSIF-V3 has multiple recipes but FaSSIF-V3 TC/GC Chol is the leading prototype.

1.4.2 Drug physicochemical characteristics

Lipophilicity, solubility and solid state form are several drug physiochemical properties that play an important role in drug absorption. These parameters will significantly affect drug bioavailability in GIT since they control drug dissolution, permeability and drug interaction in the stomach and the small intestine (Bergström et al., 2014). Several scoring systems have been developed to identify the drugs with an acceptable biopharmaceutical profile including BCS (Amidon et al., 1995b), Lipinski's rule of five (Lipinski e al., 2001) and the 'Oral physChem Score' (Lobell et al., 2006).

Lipinski and co-workers highlighted that good drug absorption required a molecular weight lower than 500 Da, an Octanol/water partition coefficient (log *P*) lower than 5, hydrogen bond acceptors lower than 10 and hydrogen bond donors lower than 5 (Lipinski et al., 1997).

Lobell and co-workers presented a traffic light method (Table1.3) were the drug values of molecular weight, calculated log *P*, calculated solubility, number of rotatable bonds and the topological polar surface area (TPSA) are all summed and the lower the score is, the better is the biopharmaceutical properties of the drug.

Ritchie and co-workers also published a system to identify drug developability depending on the number of the aromatic ring (Ritchie et al., 2011a). An aromatic ring number greater than 3 tends to decrease developability and solubility with the increase in the lipophilicity and serum albumin binding (Ritchie & Macdonald, 2009; Ritchie et al., 2011b). However, these systems provide only rough estimation of the physicochemical properties (Bergström et al., 2014).

Table 1.3: Traffic light used in the 'Oral Physchem Score' (Taken from Lobell et al., 2006)

Traffic light color (green, 0; yellow, 1; red, 2)

value	solubility (µg/mL)	Log *P*	Mw	PSA ($A^{\circ 2}$)	Rotatable Bonds
0	≥50	≤3	≤400	≤120	≤7
1	10-50	3-5	400-500	120-140	8-10
2	<10	>5	>500	>140	>11

1.5 **Biopharmaceutics Classification System (BCS)**

1.5.1 Early biopharmaceutical classification system

In 1995, a collaboration between academic scientists and the FDA led to the introduction of the biopharmaceutics classification system (Amidon et al., 1995) which classifies drugs into four groups based on drug solubility and permeability (Figure1.2). Class I: high solubility- high permeability drugs, class II: low solubility-high permeability drugs, class III: high solubility- low permeability drugs, class IV: low solubility- low permeability drugs. In the BCS classification, a drug considered to be highly soluble when the highest dose can be dissolved in 250 mL of aqueous media or less over a pH range of 1 to 8 at 37°C (Galia et al., 1998).

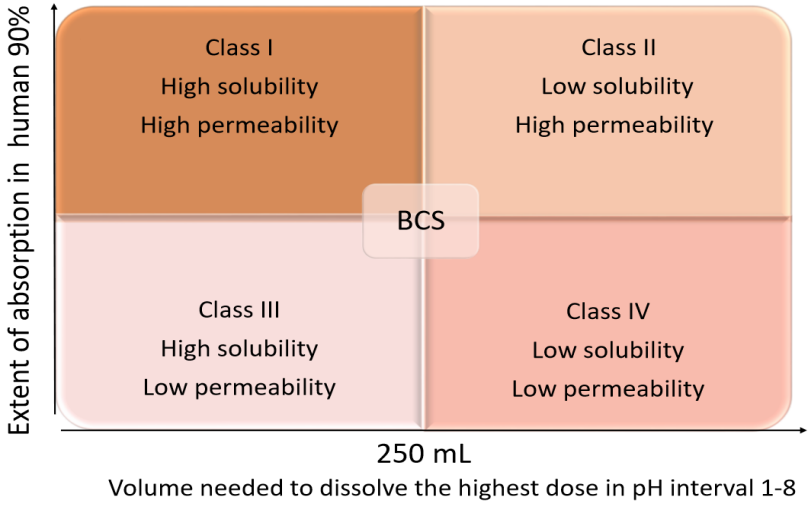


Figure 1.5: Biopharmaceutics classification system (BCS)

1.5.2 Devolapability Classification System (DCS)

Further expansion of the BCS to the developability classification system (DCS) (Figure 1.3) was developed with the aim to accurately classify drugs based on the rate limiting factors of oral absorption (Butler & Dressman, 2010). The BCS had an influence on the development of immediate release dosage forms through prediction of in vivo drug's performance from in vitro measurements of solubility and permeability (Benet, 2013). However, the system is still strict with regards to the classification of BCS class II drugs where absorption is limited by solubility and or dissolution rate. The main difference between the BCS and DCS is that the volume required to dissolve the maximum dose was extended to 500 ml, this was to compensate for other factors that affects drug solubility in the GIT such as pH dependent solubility and the presence of solubilisers in the gut from biliary secretions and food intake (Butler & Dressman, 2010). In addition, the BCS class II was divided into two groups, IIa which is the dissolution rate limited and IIb which is the solubility limited drugs. For class IIa drugs, complete absorption of the solid dosage from can be attained when permeability is high, however other factors controlling the drug release from a formulation such as surface area, particle size and wettability will be critical for complete absorption (Butler & Dressman, 2010). On the other hand class IIb drugs require a solubilised form to reach complete absorption (Butler & Dressman, 2010).

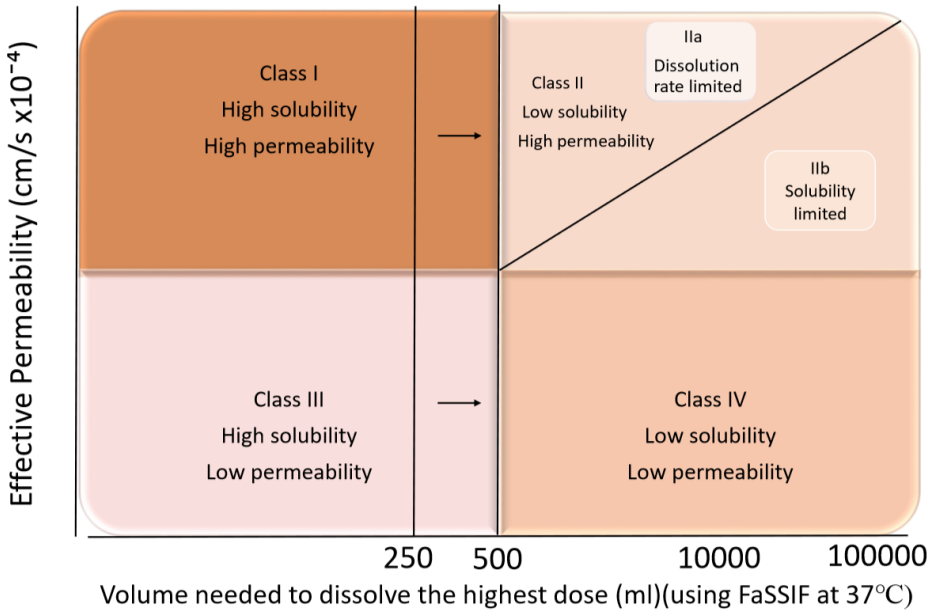


Figure 1.6: Devolopability classification system (DCS)

1.5.3 BCS for determination of in vivo performance

An essential feature of the development of a pharmaceutical product is to find the in vitro formulation properties that predict their in vivo performance and thus it is important for these formulations to undergo dissolution tests. Dissolution testing is most often associated with the assessment of in vivo performance in order to choose the dosage form with the most suitable release profile (Dressman et al., 1998).

In 1998, Dressman et al specified the four possible sources of incomplete drug absorption following oral administration:

i. The drug is not delivered to the site of absorption in the GI tract due to incomplete release from the dosage form at an appropriate time.

ii. The drug forms a non-absorbable complex or decomposed in the gastrointestinal tract.

iii. Insufficient transportation of the drug across the gut wall.

iv. Metabolism or elimination of the drug prior to entry into the systemic circulation.

Therefore, the BCS points cover two (i and iii) of the four limitations of oral drug absorption. Drug solubility which is physicochemical limitations of the drug resulting in insufficient release from the dosage form and permeability limitations due to insufficient drug transportation across the gut wall (Dressman et al., 1998).

1.5.4 Application of BCS to guide dissolution testing

According to the BSC, drugs had been classified to a low and high permeability-solubility classes and the in vitro in vivo correlation could be specified by choosing the most suitable tests for each drug class.

Class I drugs are able to dissolve directly in aqueous media and are well absorbed, consequently the dissolution is not the rate limiting step for oral absorption of this class. For immediate release dosage form, gastric emptying rate is the rate limiting step for oral absorption and no correlation with in vitro dissolution is expected (Amidon et al., 1995). As an alternative, one point dissolution testing of an immediate release dosage form with about 85% dissolved in 0.1N HCL within 15 minutes can be used as an indirect measure for bioequivalence (Shah et al., 1997).

Class II drugs are the class of drugs where dissolution is the rate limiting step of absorption (Amidon et al., 1995). This class of drugs has high permeability but poor solubility in the aqueous media of the gastrointestinal tract under usual conditions (Dressman &Reppas, 2000). Since dissolution depends on several factors such as surfactants (e.g bile salt), buffer capacity, pH and dissolution volume, the chosen media for dissolution testing should be closely similar to the GI conditions in order to achieve a good in vitro in vivo correlation (Galia et al., 1998) for this class of drugs. In an effort for better prediction of the in vivo performance of this class of drugs, media that simulates the fasted and fed state in the GI tract were developed (Galia et al., 1996). Studies showed that the rate of dissolution was higher after food intake (Persson et al., 2005) which referred to the increased GI volume by food and the induction of the gastric and intestinal secretions such as bile salt and phospholipids. Also the food components and their enzymatic degradation products were found to

play an important role in the solubility of many class II compounds (Sunesen et al., 2005). In vitro studies carried out (Bates et al., 1966) highlight the importance of bile salt for enhanced griseofulvin solubility which proves the importance of surfactants in improving the bioavailability of lipophilic compounds.

Food- induced pH changes may also affect the dissolution and absorption of poorly soluble weak acidic or basic compounds as gastric pH increases after food intake, which will be beneficial for the solubility of weak acids with pKa lower than 5 such as ketoprofen (Dressman &Reppas, 2016). In addition, Gu et al., found that bioavailability of ibuprofen was increased after food intake (Gu et al., 2007). This is not the case if the administered drug is a weak basic compound with a pKa near to the fed gastric pH (4.5- 5.8), as solubility can be decreased after food intake as is the case with albendazole (Dressman &Reppas, 2016). However, the absorption of ketoconazole (weak base) was found to be improved after food intake (Daneshmend et al., 1984) as the unfavorable lowering solubility effect induced by the increase in gastric pH was balanced with the higher level of bile salt and the larger volume of GI fluids (Galia et al., 1998).

In conclusion there are several postprandial changes that can affect the absorption of class II compounds and thus the choice of the dissolution tests will depend on several parameters including the ionization state of the drug, pH range of the GIT fluids and the concentration of the solubilizing components.

Class III drugs have high solubility and low permeability with the gut wall representing a permeability barrier and the rate limiting step during absorption and thus dissolution is not critical (Amidon et al., 1995). As class I, the dissolution description is valid for immediate release dosage forms where the gastric emptying rate is the controller of the drug input in to the intestinal wall.

Class IV drugs present major problems for actual oral delivery as they are characterized by both low solubility and low permeability (Dressman & Reppas,

2000). Due to the limited permeability and aqueous solubility of this class, drugs are suspected to have very poor absorption and significant inter and intra individual variability with limited or no IVIV correlation (Amidon et al., 1995). Development of an effective formulation is a massive challenge for this class of drugs.

1.6 **Approaches to improve drug solubility and dissolution**

Several factors were found to affect drug solubility and dissolution in the GIT including the physicochemical properties of the drug (pH, *log P*….etc*),* food effect and the large changes in physiology and the different GI media components (Dressman &Reppas, 2016). Many drugs show low solubility and slow dissolution in aqueous media which will consequently leads to poor bioavailability of these poorly soluble drugs (Paus et al., 2015). Therefore, several approaches were developed to modify drug formulation with the aim to improve drug solubility and dissolution rate (Savjani.et al., 2012; Ràfols et al., 2018; Bevernage et al., 2010; Khadka et al., 2014). This includes chemical modifications using surfactants, solid dispersion, soluble prodrug and lipid based delivery system (Vemula et al., 2010; Pinnamaneni et al., 2002). Physical modification could also be applied through pH adjustment, salt formation, particle size reduction and modification of the crystal habit (Vemula et al., 2010; Horter et al., 2001; Lingam et al., 2009). Attaining fine spreading and diffusion of poorly soluble drugs at the absorption level through solid dispersion was one of the most important formulations approaches taken to maximise dissolution and solubility (Choi et al., 2016; Balasaheb et al., 2014; Vasconcelos et al., 2007; Ghule et al., 2018). This can be performed by incorporating the poorly soluble drugs with the different types of carriers (water soluble, amphiphilic, lipid soluble) (Rask et al., 2106; Knopp et al., 2015). Therefore, exploring the solubility and dissolution behavior of poorly soluble drugs in the presence of excipients was essential in the prediction of drug solubility and dissolution during formulation.

1.7 Excipients

Excipient was defined by the international pharmaceutical excipient council (IPEC) as substances other than the API present in the pharmaceutical formulations and have been appropriately tested for safety (Apte et al., 2003). Excipients were added to help in the processing, manufacturing, protection or to improve stability and bioavailability or patient acceptability or to advance any features of the safety of the drug delivery system (Ahjel & Lupuliasa, 2008; Nadavadekar et al., 2014;.Furrer, 2013).

1.7.1 Sources and functions of excipients

Excipients can arise from different origins including plant origins such as cellulose and sugars, animal origins such as gelatin and lactose, mineral such as calcium phosphate and silica or synthesized as povidone and polysorbates (Pifferi & Restani, 2003). Today there are over one thousand different excipients are used in pharmaceutical industry with a broad range of functionalities such as, bulking agents, disintegrants, lubricants, coloring agents, flavors, and solubility enhancer. There are multiple types of excipients each with different characteristic and function. In this study only 4 representative excipients (mannitol, higher and lower grade Polyvinylpyrolidone (PVP), hydroxyl propyl methyl cellulose (HPMC) E3 and E50, and chitosan) were chosen to study their effect on solubility and or dissolution of drugs and they will be detailed in the following section. Mannitol and chitosan were chosen as they were examined by Oral Biopharmaceutical tools (OrBiTo) and mannitol showed to act as an inert excipient while chitosan showed an effect. Moreover, PVP and HPMC were chosen as they are commonly used excipients in drug manufacturing.

Mannitol: is a naturally occurring excipient from plant origin (marine algae, fresh mushrooms) that is widely used in the pharmaceutical industries as a sweetener (taste masking) and diluents (Chaudhary et al., 2010, Basalious et al., 2014). It is about half as sweet as sucrose and considered desirable in masking bitter tastes. Mannitol is a polyol sugar alcohol that is present in white crystalline powder and readily soluble in water (Shawkat et al., 2012).

PVP: is a synthetic linear chain homo-polymer consisting of 1-vinyl-2-pyrrolidone group and also known as povidone. PVP is among the most frequently used excipients as carriers for solid dispersion due to its high molecular weight and solubility in both hydrophilic and hydrophobic solvents (Rask et al., 2016). This is because of the formation of the hydrogen bond between the (C=O) group of PVP and the solvent (Li et al., 2017). It is obtained in varying viscosity grades for example higher and lower grade going from low to high molecular weight (2500-3,000,000 g/mol) and coded by the *K* value in pharmacopeias (Rask et al., 2016). PVP used as an excipient for stabilizing , binding properties, and solubility enhancement (Vadlamudi & Dhanaraj, 2017; Gupta et al., 2004; Loftsson et al., 1996; Madsen et al., 2106). Furthermore, PVP showed to increase dissolution rate of poorly soluble drugs when the drug dispersed in PVP due to the surface tension lowering effect of the excipient (Yadav et al., 2013).

HPMC: is a water soluble hydrophilic, non-ionic cellulose ether. The structure has hydroxyl propyl substitution (-OCH2CH (OH) CH3) that has a secondary hydroxyl group on carbon number 2, this substitution produced by processing pulp cellulose with caustic soda followed by reaction with methyl chloride and propylene oxide (Chan et al., 2003). The hypromelloses are identified by codes E, F or K that relates to the degree of substitution. In this study, HPMC E was used which relate to the grade that has the methoxy and hydroxypropyl substitution (Li et al., 2005). HPMC is used in drug formulation as disintegrant, drug loading, lubricant, binder, viscosity increasing agent and glidant (Lee et al., 1999; Shihora et al., 2011; Tompkins et al., 2010; Majumder et al., 2016). Low viscosity grade of HPMC act as a surfactant which will enhance the wetting properties of the drug and consequently enhance the solubility (Vadlamudi & Dhanaraj, 2017). However, HPMC swells and forms a gel when in solution, the swelling was faster when excipient with higher viscosity grade was used. This will lead to the formation of a turbid gel that resists dilution resulting in a slower drug diffusion and drug release (Wan et al., 1991). Charged drugs for example interact with the gel leading to the increase in the diffusion time of these drugs through the gel structure (Fyfe & Welsh, 2000;.Li et al., 2005). Nevertheless, medium and high

viscosity grades of the excipient are used in sustained release matrix formulation (Guo et al., 1998; Phadtare et al., 2014).

Chitosan: chitin is a naturally occurring polysaccharide that exists in the exoskeleton of crab shells, insects and fungal cell walls. Chitin is a derivative of cellulose with the replacement of the hydroxyl group with an amine group which makes the structure polycationic (Ahsan et al., 2017). Chitosan is the major derivative of chitin where the alkaline deacetylation of chitin was obtained. Chitosan is sparingly soluble in aqueous solutions under basic or neutral conditions and its solubility increased in acidic solution due to the protonation of the amino group (Ahsan et al., 2017). Chitosan displays a variety of features that facilitate the use of this excipient in drug delivery. These features include a mucoadhesive property and the ability to open the epithelial tight junction (Ahsan et al., 2017), the availability of functional groups that facilitate chemical modification (Du et a., 2015; Zargar et al., 2015; Jayakumar et al., 2010; Zhang et al., 2010), as well as the disintegrant property (Ritthidej et al., 1994; Rasool et al., 2012). In addition, chitosan has a swelling property that permits the control of the drug release rate in oral sustained drug delivery system (Sun et al., 2013). This allows the use of chitosan as a drug carrier and coating molecule (Park et al., 2010) . Nevertheless, the low solubility of chitosan in biological fluids (pH=7.4) limits its use in drug delivery (Park et al., 2003).

1.7.2 Effects of excipients on drug absorption

The pharmaceutical excipient market has experienced a significant growth related to the excipient's aiding of the API to achieve better functionality and to overcome solubility and dissolution problems (Vadlamudi & Dhanaraj, 2017). Several researches have studied the effect of different types of excipients on solubility and or dissolution of poorly soluble drugs (Leuner & Dressman, 2000; Widanapathirana et al, 2015) in simulated gastrointestinal fluids (Javeer et al., 2013; Taupitz et al., 2013) and in human intestinal fluids (Kubbinga et al., 2015). Studies revealed that several types of excipients found to enhance the gastrointestinal absorption of poorly soluble drugs. For example, polysaccharide such as cyclodextrin found to improve the

dissolution rate of eflucimibe (Rodier et al., 2005). Sugars such as mannitol are often used in formulations (Liao et al., 2005) and their effect on solubility and dissolution was examined and revealed no significant effect (Paus et al., 2015). Moreover, synthetic excipients such as poly ethylene glycol (PEG) and PVP have also been examined. Studies showed that both excipients found to increase indomethacin and naproxen solubility (Paus et al., 2015). In addition, PVP found to inhibit crystallization of a variety of drugs such as ketoprofen (Di Martino et al., 2004) and piroxicam (Tantishaiyakul et al., 1999) which consequently improves solubility, dissolution rates and bioavailability. This enhancement effect was attributed to the high affinity of PVP to form hydrogen bond with the drug (Paus et al., 2015). However, the solubility enhancement of PVP found to be drug specific and dependent on the intermolecular interaction between the excipient and the drug under investigation (Widanapathirana et al., 2015). For example, a study found that HPMC is a better stabilizer for itraconazole compared to solutions with PVP (Widanapathirana et al., 2015; Miller et al., 2008). In addition, low viscsity grade HPMC found to enhance solubility and dissolution of felodipine (Won et al., 2005) and simvastatin (Pandya et al., 2008). Chitosan found to increase insulin absorption through the bioadhesion and transient widening of the tight junctions in the membrane (Thanou et al., 2001). In addition, chitosan found to enhance the dissolution rate of naproxen solid dispersion (Zerrouk et al., 2004). Nevertheless, examination of excepients found also to delay the dissolution rate or decrease solubility of some poorly soluble dugs. For example, ethyl cellulose found to delay the dissolution rate of indomethacin (Ohara et al., 2005). In additoin, Stappaerts et al found that cyclodextrin significantly lowered the solubility of itraconazole in the presence of bile salt and phosphatidylcholine (Stappaerts et al 2016). Moreover, chitosan found to significantly decrease the absorption of acyclovir which was attributed to the interaction of chitosan with the drug, bile salt or the epithelial membrane (Kubbinga et al., 2015). This indicates that the type of excipient and type of the drug plus the media where the drug dissolved in are an important parameters to be taken into account when examining the effect of excipients on solubility and or dissolution.

1.8 Oral Biopharmaceutical tools (OrBiTo)

OrBiTo is a European project funded by the Innovative Medicines Initiative (IMI) program that aims to enhance the understanding of oral drug absorption, and to provide new laboratory experiments (in vitro) and computer models (in silico) that will improve the prediction of a drug's performance in patients (Lennernäs et al., 2014). The project focuses on several areas including the investigation of the effect of bio relevant simulated GI media on solubility and dissolution of API and formulated drugs (Bergström et al., 2014). In addition, the project focuses on the improvement of in silico and simulated physiologically based pharmacokinetic PBPK models which become a successful area for IVIVC (Harwood et al., 2013). PBPK modelling software such as GastroPlus™ (Kuentz et al., 2006) and Simcyp® simulator (Shaffer et al., 2012) have been developed to deliver complementary information for preclinical in vivo studies. The project works by integrating data from many existing studies with the initiation of new studies that will produce improved data, which consequently will reduce the need for animal experiments and human clinical studies in the future.

1.9 Design of experiment

Design of experiment (DoE) is a powerful statistical data collection and analysis tool that designed to determine the connection among factors affecting the outcome (response) of an experiment. It permits for multiple input factors to be employed determining the effect of these factors on the output. Employing multiple factors at the same time allows to determine the important interactions which may be missed when examining only one factor at a time. The key ideas behind the design of experiment include randomization, blocking and replication. Randomization means that the experiment was running in a randomized sequence and that each run is independent and not affected by the previous run. Blocking is the arrangements of the similar experimental units in groups. Replication allows the estimation of the experimental error. There are variable types of DoE and the most common are One Factor Design and the Factorial Designs (for example: General Full Factorial Design, two level Full Factorial, two level Fractional Factorial, and Taguchi). In this study,

only the two level factorial design and specifically the two level fractional factorial design will be used.

1.9.1 Two Level Full Factorial design of experiment

A factorial design of experiment is a design where multiple number of factors are investigated and factors can be quantitative (e.g. pH) and or qualitative (acid or base). In the two level full factorial design, the factors have two levels, high and low and they are expressed as (+1) for high and (-1) for low. The number of the experimental runs can be calculated from the following formula 2^n where "n" is the number of factors needing to be examined. The two level full factorial designs gives all possible combinations of all factor levels with the possibility for the addition of replication (Montgomery, 2018). This will allow the main effect of the individual factor and the factor interactions to be determined and not confounded. Nevertheless, the number of experiments can be very high and resource intensive especially when there are numerous factors that needed to be examined. As an example, the usage of the statistical design of experiment to explore the effect of simulated media factors on solubility of drugs which required large numbers of experiments (Khadra et al., 2015). Accordingly, a fractional factorial design could be a more practical option (Gunst et al., 2006). A fractional factorial design select a fraction of the full design which may end up with reduced statistical resolution and confounded effects. However, by careful selection of the critical factors and the fraction of DoE, valuable information can be gained with an acceptable number of experiments.

1.9.2 Studies applied statistical investigation of simulated intestinal media.

Three recent studies have applied a structured statistical design of experiment (DoE) approach to examine the significance of media components individually and in combination using simulated intestinal media on the solubility of a range of acidic, basic and neutral BCS class II drugs. The first one was covering the fasted range (Khadra et al., 2015), the second one the fed range (Zhou et al. 2017), and the third the full fasted and fed range (Perrier et al., 2018).

In the fasted DoE publication, a quarter of the full factorial design with seven factors (either a component concentration or a system parameter such as pH) and two levels was constructed and analysed using Minitab which generated 66 different experiments (32 experiments by upper/lower limits and one centre point) using various combination of the upper and lower levels of these seven factors (bile salt, lecithin, salt, buffer, pH, pancreatin and sodium oleate). The fed DoE paper applied a D-Optimal design with eight factors (fasted factors plus monoglyceride as an additional fed media component) and two levels was constructed and analysed using MODDE which generated 92 different experiments (44 experiments by upper/lower limits each measured in duplicates and four centre points) using various combination of the upper and lower factor levels. In the full range DoE, a quarter of the full factorial design with seven factors and two levels (upper and lower limits) was constructed and analysed using Minitab requiring 32 measurements that conducted in duplicate which generated a total of 64 measurements. The lower level values are derived from the fasted DoE and the upper level values are derived from the fed DoE.

The results indicated that an individual drug's solubility could vary over three orders of magnitude in either the fasted or fed state, solubility in fasted media was lower than fed and published literature solubility values in either human intestinal fluids (HIF) or simulated media were in agreement (Augustijns et al., 2014). For acidic drug in fasted or fed simulated media pH was the most important individual solubility driver with only a minor contribution from sodium oleate, bile salt and lecithin, significant combination of factors were limited to pH either with oleate or bile salt. For basic drugs, pH was an important individual solubility driver in both fasted and fed media systems but the magnitude of the effect was equivalent to sodium oleate, bile salt and lecithin. Interactions between media factors were slightly greater in number and again involved the factors which were individually significant. For neutral drugs in both media systems pH, sodium oleate, lecithin and bile salt were roughly equivalent as single factors with a lower significance for monoglyceride. Since these drugs are noninoizable the impact of pH must be mediated through ionization of media components and this is evident in an increased number of significant pH based factor interaction influencing solubility.

Both fasted and fed DoE studies illustrated the applicability of this statistical method for determining the media factors affecting drug solubility and the possible range of solubility values that might arise. However, both fasted and fed DoE required large numbers of experiments (66 and 92 respectively) which is a high experimental load that separately or in combination is resource intensive and not suited to early development studies where drug availability may be limited. Consequently, the full range study was conducted to combine both fasted and fed state in one reduced experiment. This was found to be feasible and gave comparable solubility results to the larger published fasted and fed studies but with a lower statistical ability to identify the significant factors and factor interactions. Therefore, further statistical and experimental improvement is required to tease out the differences between fasted and fed states.

1.10 **Aims and objectives**

Although extensive research has been published on the composition of GI fluids (Riethorst et al., 2015; Bergström et al., 2010), the variability in GI fluids and media components makes it difficult to understand the exact impact of all of the factors on performance of drug solubility and dissolution. Simulated bio-relevant media helps to figure out IVIV correlations but experiments still lack a systematic design that permit comparison and determination of the effect magnitude of factors on bioavailability. The aim of this work was to gain better knowledge of the solubility and dissolution of the poorly water soluble dugs using simulated intestinal media and or in the presence of specific types of excipients.

The objectives of this work was to build a statistical design of experiment that covers both fasted and fed state in one smaller single experiment to investigate the solubility of a list of acidic, basic and neutral drugs using the shake flask method and comparing the results to the published fasted and fed DoE's. Moreover, the feasibility of using frozen stock solution rather than preparing fresh one for each study was investigated through examining the effect of these factors on solubility of drugs using frozen stock

solution with different storage duration and compare the results of solubility to the fresh prepared media using Minitab statistical analysis. In addition, the effect of the addition of 6 different types of excipients (Mannitol, Chitosan, HPMC E50, HPMC E3, PVP HG and PVP LG) to the simulated intestinal media on equilibrium solubility of fenofibrate and carvedilol was examined using the design of experiment. Finally, based on Noyes-Whitney equation, dissolution tests were performed to assess the relationship between solubility and dissolution using selected fasted and fed media from the design of experiment in the presence of the excipients that were used to explore equilibrium solubility.

2 Dual level statistical investigation of equilibrium solubility in simulated fasted and fed intestinal fluid*.

2.1 Introduction

The worldwide demand for new drug therapies is growing rapidly, driven by ageing of populations and an increasing number of diseases (Chalmers & Chapman, 2001), which has led to the growth of drug discovery research. The oral dosage form is optimal (Sugano et al., 2007) as it is the most convenient, cost effective and route of administration with the highest patient compliance. For oral dosage forms to attain the required systemic exposure, the drug needs to dissolve in the gastro-intestinal fluid, which can be influenced by its variable composition (Dressman et al., 2007) and for poorly water soluble drugs, low solubility coupled with low dissolution rate can result in limited and variable absorption. Studying drug solubility is therefore of critical significance in order to understand the behavior of low solubility drugs in the gastrointestinal tract (GIT) and thus improve drug absorption and bioavailability (Sugano et al., 2007; Clarysse et al., 2011).

2.1.1 Gastrointestinal Solubility Factors

Several drug specific factors for example, pKa, log *P*, chemical structure and properties (i.e. acidic, basic or neutral) are known to affect aqueous solubility generally and also in intestinal media. In addition, multiple factors constitutively present in the GIT fluid such as bile salts, buffer capacity and food composition (Amidon et al., 1995) can further influence drug solubility. In the fasted state, bile salt and lecithin concentrations are lower than in the fed state, where their concentrations are increased due to the ingestion of food and the presence of associated lipid digestion products (Dressman& Reppas, 2000). The formation in GIT fluid of mixed micelles consisting of "bile salts, lecithin and lipolytic products" tends to have a solubilizing ability for poorly soluble drugs (Clarysse et al., 2011).

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2.1.2 Gastrointestinal Media

Multiple studies have been published, directed at achieving an improved understanding of drug solubility in the GIT and its impact on oral bioavailability (Augustijns et al., 2014). The obvious media to employ is human intestinal fluid (HIF) samples, aspirated either from the fasted or fed state (Dressman et al., 2007) however, HIF is difficult to obtain (requiring human volunteers or patients), variable and therefore not ideal for routine solubility studies (Reppas and Vertzoni et al., 2012; Kleberg et al.,2010). To avoid the issues associated with human sampling research has been performed to provide in vitro derived media which simulates and resembles HIF by containing all of the components that are known to play a role in drug solubility such as bile salt, buffer, lecithin and lipid degradation products (Galia et al., 1998). Thus fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) have been developed. Further research has extended these initial media with the addition of food based constituents for example cholesterol (Khoshakhlagh et al., 2015) and multiple media recipes are now available for both fasted (Khadra et al., 2015) and fed (Zhou e al., 2017) states.

2.1.3 Statistical Investigation of Simulated Intestinal Media

Two recent studies have applied a structured statistical design of experiment (DoE) approach to examine the significance of media components individually and in combination in fasted (Khadra et al., 2015) and fed (Zhou e al., 2017) simulated media on the solubility of a range of acidic, basic and neutral BCS class II drugs. The results indicated that an individual drugs solubility could vary over three orders of magnitude dependent in either the fasted or fed state, solubility in fasted media was lower than fed and published literature solubility values in either HIF or simulated media were in agreement (Augustijns et al., 2014). For acidic drugs in fasted or fed simulated media pH was the most important individual solubility driver with only minor contributions from sodium oleate, bile salt and lecithin, significant combinations of factors were limited to pH either with oleate or bile salt. For basic drugs pH was an important individual solubility driver in both fasted and fed media systems but the magnitude of the effect was equivalent to sodium oleate, bile salt and lecithin. Interactions between media factors were slightly greater in number and again involved the factors which

were individually significant. For neutral drugs in both media systems pH, sodium oleate, bile salt and lecithin were roughly equivalent as single factors with a lower significance for monoglyceride. Since these drugs are non-ionisable the impact of pH must be mediated through ionization of media components and this is evident in an increased number of significant factor interactions influencing solubility. Both DoE studies illustrated the applicability of this statistical method for determining the media factors affecting drug solubility and the possible range of solubility values that might arise. However, the fasted DoE required sixty six individual media experiments and the fed ninety four, an experimental load that separately or in combination is resource intensive and not suited to early development studies where drug availability may be limited.

2.1.4 Dual Range Design of Experiment Study

In this paper a dual range DoE covering fasted and fed states in a smaller single experiment with bio relevant factor levels (see Table2.1) was applied to determine the equilibrium solubility of BCS class II compounds. This was achieved through removing salt and buffer as media factors since they were not statistically significant (Khadra et al., 2015; Zhou et al., 2017), adding cholesterol and monoglycerdie as new factors (Fuchs et al., 2015; Riethorst et al., 2016) in both fasted and fed states, resulting in a media consisting of seven factors (bile salt, lecithin, sodium oleate, monoglyceride, cholesterol, pH and BS: PL ratio). A 1/16 of the full factorial DoE design with two levels (upper and lower) was constructed separately for the fasted and fed states (8 experiments with upper and lower levels and 2 center point in each state) then the two experimental tables were employed as an input for a factorial custom DoE which combined the fasted and fed data into a single DoE. The DoE therefore has three arms, two small arms of 10 experiments each covering fasted and fed, with a third arm based on the combination of fasted and fed. This has the advantage of examining both fasted and fed states within the same experiment coupled with the ability to combine the data to provide an overall solubility assessment for both states. The equilibrium solubility of nine BCS class II drugs was investigated: two acids

(Phenytoin and Indomethacin), four bases (Aprepitant[1], Tadalafil, Zafirlukast, Carvedilol) and three neutral drugs (Felodipine, Fenofibrate, Probucol) and compared to the published fasted and fed DoE studies.

Table 2.1: Fasted and Fed media components and concentration levels.

Component	MW (g/mol)	Substance	Fasted State		Fed State	
			lower	upper	lower	upper
Bile salt	515.70	Sodium Taurocholate	1.5mM	5.9mM	3.6mM	15mM
Lecithin	750.00	Phosphatidylcholine	0.2mM	0.75mM	0.5mM	3.75mM
Fatty acid	304.44	Sodium oleate	0.5mM	15mM	0.8mM	25mM
Mono-glyceride	358.57	Glyceryl mono-oleate	0.1mM	2.8mM	1mM	9mM
Cholesterol	386.65	Cholesterol	0.1mM	0.26mM	0.13mM	1mM
pH	Sodium hydroxide/hydrochloric acid		5	7	5	7
BS:PL ratio			7.5	7.9	7.2	4

2.2 Materials and Methods

2.2.1 Materials

The physicochemical properties of the representative compounds are shown in Table 2.2. Sodium taurocholate, monosodium dihydrogen phosphate, ammonium formate, formic acid, sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), cholesterol, chloroform, fenofibrate, indomethacin and phenytoin were purchased from Sigma-Aldrich, Poole, Dorest UK. Lecithin S PC

[1] Aprepitant has been classified as a basic drug in order to simplify comparison with published DoE results, references (Khadra et al., 2015; Zhou et al., 2017).

(phosphatidylcholine from soybean 98%) was supplied from Lipoid, Germany. Sodium oleate was from BDH chemical Ltd. Poole England. Monoglyceride (Glyceryl mono-oleate) was kindly supplied from CRODA. The BCS class II compounds felodipine, aprepitant, tadalafil, carvedilol and zafirlukast were provided through OrBiTo by Dr. R Holm, Head of Preformulation, Lundbeck, Denmark. All water used was ultrapure Milli-Q water. Methanol and acetonitrile were purchased from VWR Prolabo Chemicals, UK.

Table 2.2: Physicochemical properties of the studied drugs

Compound	MW	Category	pKa	Log p
Indomethacin	357.7	acid	4.5	4.2
Phenytoin	252.2	acid	8.3	2.4
Carvedilol	406.4	base	7.8	4.2
Tadalafil	389.4	base	10	1.7
Zafirlukast	575.6	base	4.3	5.4
Aprepitant	534.4	weak base	9.7	4.5
Felodipine	384.2	neutral	-	3.8
Fenofibrate	360.8	neutral	-	5.2
Probucol	516.8	neutral	-	10

Molecular weight in g/mol (MW), lipophilicity octanol/water (log p), Dissociation constant (pKa).

2.2.2 Dual Level Design of Experiment and Data Analysis

For each media parameter (bile salt, lecithin, sodium oleate, monoglyceride, cholesterol, pH and BS: PL ratio) lower and upper limit concentration values for fasted and fed states were defined, (Table 2.1). Using Minitab® 17.2.1 and a custom experimental design, a 1/16 of the full factorial DoE with the seven factors and two levels (lower and upper limits) was constructed (8 experiments around the upper and lower levels plus two centre points) separately for the fasted and the fed states. These two tables were then applied as an input for a factorial custom design of experiment which combined the fasted and fed using all twenty data points to provide an overall analysis. The study therefore consists of three arms, two smaller (10 data point) fasted and fed arms, which are then merged into a larger (20 data point) combined arm.

When designing and analysing the DoE, only a factor's main effects and two way interactions have been considered and three way interactions or more were not included. For each DoE the magnitude for each factor's effect on equilibrium solubility was determined by the standardized effect value for all of the individual factor and the significant two way interactions. This value was used to articulate whether these factors are increasing or decreasing drug solubility. Due to the design and the low number of experiments, the standardized effect values calculated for the smaller fasted and fed state arms indicate a significant increase in drug solubility when it is greater than +4 and a decrease when it is less than -4. For the combined fasted and fed state arm the value of the standardized effect is considered to indicate a significant increase in drug solubility when it is greater than +2 and a decrease when it is less than -2. Finally, two way interactions could only be determined for the combined DoE arm with the larger number of data points.

The Kolmogorov normality test was used in Minitab® to assess the normality distribution of each data set. A Mann-Whitney test was used to evaluate the median between two data sets (not normally distributed) and the two-sample t-test was used to evaluate the mean of two data sets (normally distributed).

2.2.3 Equilibrium Solubility Measurement

The concentration of each stock solution has been designed to be 15 times greater than the upper limit concentration value required for the DoE with the exception of oleate where only a 5 times concentration was possible (Table2.3&2.4).

Table 2.3: Stock Mixture Concentrations (15x lower, mid and upper limits)

Component	Fasted State			Fed State		
	Lower	Middle	Upper	Lower	Middle	Upper
Bile salt	22.5mM	55.5mM	88.5mM	54mM	139.5mM	225mM
Lecithin	3mM	7.125mM	11.25mM	7.5mM	31.8mM	56.25mM
Monoglyceride	1.5mM	21.75mM	42mM	15mM	75mM	135mM
Cholesterol	1.5mM	2.7mM	3.9mM	1.95mM	8.475mM	15mM

Table 2.4: Fatty Acids volumes (5x upper limit)

Component	Fasted State			Fed State		
	Lower	Middle	Upper	Lower	Middle	Upper
Sodium Oleate	16µL	248µL	480µL	25.6µL	412.8µL	800µL

2.2.4 Preparation of Stock Systems

Preparation of Lipid Suspension

Sodium taurocholate, monoglyceride, lecithin and cholesterol were weighed and transferred into a flask then 2 ml of chloroform was added to dissolve all the solid material. A stream of nitrogen gas was applied in order to remove the chloroform and to ensure the formation of a dried film. Water was added to reconstitute the dried film

and mixed to obtain a homogenous suspension, transferred to a 5 ml volumetric flask and made up to volume with water.

Preparation of Sodium Oleate Solution

Sodium oleate (1.90 g) was weighed into a 50 ml volumetric flask, dissolved in water, with the assistance of gentle heating to aid dissolution and then made up to volume with water and kept under heat to aid solubilisation.

Preparation Buffer Solution

A concentration of 0.3 M monosodium dihydrogen phosphate buffer was prepared by adding 20.4 g into a 500 ml volumetric flask and making up to volume with water. This is split into two and the pH adjusted to 5 and 7 using 0.5 M HCL or 0.5 M KOH.

2.2.5 Preparation of Measurements Solutions

Preparation of Individual Design of Experiment Solutions

The solution was prepared by the addition of an excess amount (10 mg, above the estimated solubility) of solid for each compound investigated to a centrifuge tube (15 ml Corning®) followed by the addition of each component of the simulated intestinal fluid media according to the run order generated by the DoE. After all of the media components were added, pH was adjusted to 5, 6 or 7 according to the run order using 0.1 M HCl or 0.1 M KOH and tubes were capped and placed in a tube rotator (OS 5 basic Yellowline, IKA, Germany) for 1 h at 37°C after which the pH was readjusted if required. The 20 different tubes were then shaken in the tube rotator for 24 h at 40 rpm at 37 °C to simulate intestinal fluid conditions. After 24 hours, a 1 mL amount was taken from each of the 20 tubes and transferred to a 1.5 mL Eppendorf® tube then centrifuged at 15,000 rpm for 5 minutes. Following centrifugation 0.5 mL of the supernatant solution was transferred to an HPLC vial to analyse drug solubility using HPLC (Table2.5).

Table 2.5: HPLC Analysis Conditions.

Drug	Mobile phase	Flow Rate (mL/min)	Injection Volume (µL)	Detection (nm)	Retention time (min)	R2**	LOQ (µM)
Phenytoin	Mobile phase A: Ammonium formate 10 mM pH 3.0 in H_2O Mobile phase B: Ammonium formate 10 mM pH 3.0 in ACN/H_2O (9:1 v/v)	1	10	260	2.3	0.9998	26
Indomethacin		1	10	254	2.5	0.9999	12.4
Aprepitant		1	100	254	2.7	0.9992	83
Tadalafil		1	10	291	1.7	0.9996	24.5
Zafirlukast		1	10	260	3.1	0.9996	2.6
Cravedilol		1	10	254	1.2	0.9989	78.3
Felodipine		1	10	260	3.1	1.0000	7.8
Fenofibrate		1	10	291	3.6	0.9999	19
Probucol		1	10	254	4.3	0.9995	15

Apparatus Agilent Technologies 1260 Series Liquid Chromatography system with clarity Chromatography software: Gradient method: Time 0, 70%A: 30%B, 3 min 0%A: 100%B, 4min 0%A: 100%B, 4.5 min 70% A: 30%B total run 8 mins. Column X Bridge C18 column /186003108 / 50mm x 2.1 mm id. 5 µ. **R2 Linear regression coefficient curve, n=6 or more. ACN: acetonitrile. LOQ: Limit of Quantification.

2.2.6 Validation of HPLC method

2.2.6.1 HPLC equipment

Thirteen BCS class II drugs have been selected as model compounds and they were representative of the three drug categories: acid (naproxen, indomethacin, phenytoin, and piroxicam), basic (aprepitant, carvedilol, zafirlukast, tadalafil, albendazole) and neutral (fenofibrate, griseofulvin, felodipine and probucol). HPLC analysis were performed using Apparatus Agilent Technologies 1260 Series Liquid Chromatography system with clarity Chromatography software. The column used for the analysis of the drugs was ACE 3 C18 reverse phase HPLC column (50 mm x 3.0 mm ,3 µm) and guarded by Phenomenex C18 (4 mm × 3.0 mm, Macclesfield, UK).

2.2.6.2 Chromatographic conditions

Similar chromatographic conditions (mobile phase, flow rate and injection volume) used above in Table2.5 (section 2.2.5) were applied for the analysis of the 13 model drugs for method validation.

2.2.6.3 Analysis

The analysis presents an optimized generic method using a narrow-bore column packed with 1.5-microm nonporous particles and a completely automated HPLC workstation configured for the best efficiency, throughput, and robustness with this column. A test mixture of 13 compounds with a wide polarity range were separated within 3.5 minute with a cycle time of 8 minutes (Figure 2.1).

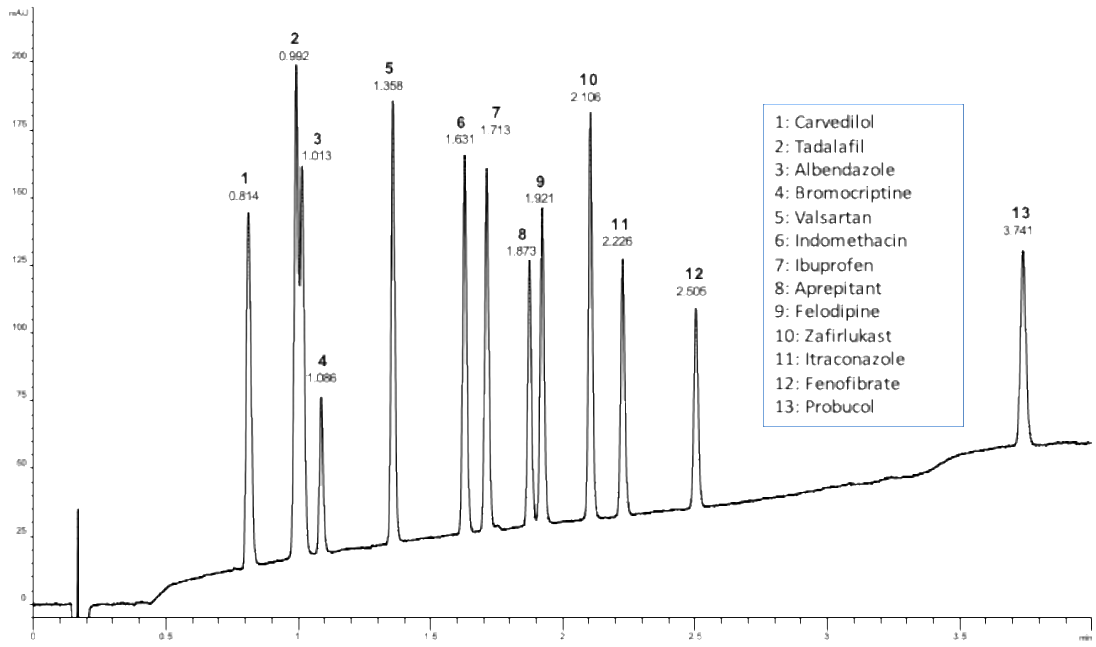


Figure 2.1: Chromatographic Analysis of 13 selected drugs using HPLC.

2.3 Results

2.3.1 Equilibrium solubility measurements

The results of all the equilibrium solubility measurements are presented in Figure 2.2, and illustrate that a broad range of solubility values are observed depending on the drug and the media state (fasted or fed) investigated. As a comparison literature solubility values where available for the drugs in simulated intestinal fluid (SIF) and/or human intestinal fluid (HIF) in both fasted and fed states (Augustijns et al., 2014) are plotted in Figure 2.2. The results also indicate that drug specific factors are influencing solubility, tadalafil has a smaller solubility variation than fenofibrate for example, a feature that has been previously reported (Khadra et al., 2015; Zhou et al. 2017) for these types of studies.

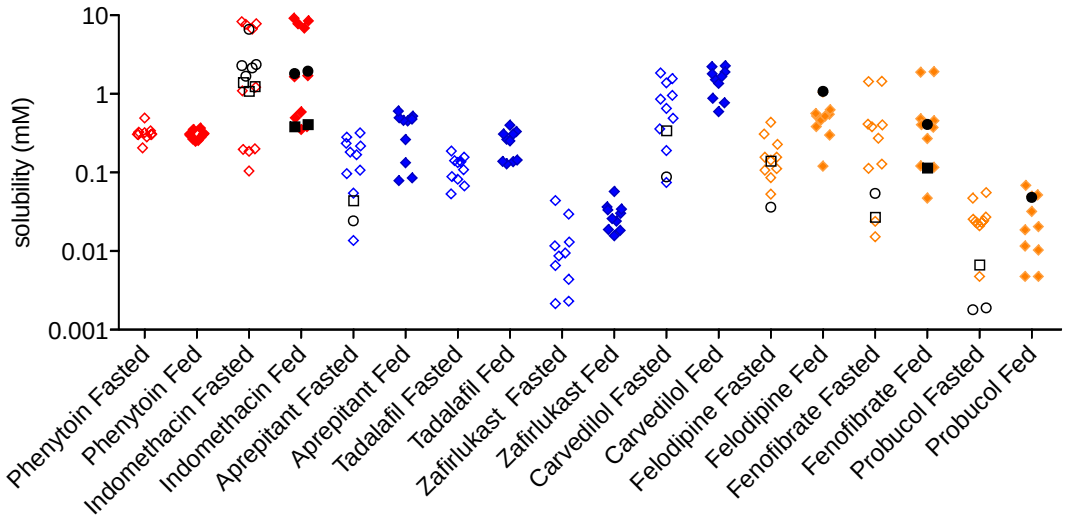


Figure 2.2: Equilibrium solubility measurements for each drug in DoE media compositions detailed in Table2.1. Red data points for acidic drugs, blue basic drugs and yellow for neutral drugs – open symbols for fasted media conditions, closed symbols for fed media conditions. O reported solubility values for individual drugs in fasted (open symbol) simulated intestinal fluid and fed (closed symbol) simulated intestinal fluid media respectively, □ reported solubility values for individual drugs in fasted (open symbol) human intestinal fluid and fed (closed symbol) human intestinal fluid respectively, all values from (Augustijns et al., 2014).

2.3.2 Statistical comparison

In Figures 2.3 a-c the dual level equilibrium solubility results for the fasted and fed states are presented alongside a box and whisker plot of published fasted (Khadra et al., 2015) and fed (Zhou et al. 2017) measurements along with a statistical comparison of the distributions. It is important to note that slightly different levels of factors are used in this dual design when compared to the published fasted and fed data. The results indicate that fasted solubility is in the majority of cases lower than fed solubility and that the solubility values from the dual level study are comparable, with some exceptions (tadalafil fasted for example) to the published data. For the current dual level DoE statistical examination indicated that nine out of a possible eighteen data sets had a normal distribution. This is in marked comparison to the published data where all eighteen data sets had non-normal distributions. A statistical comparison between the published fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) data indicates that for all nine drugs there is a significant difference with fasted solubility lower than fed. Statistical comparison of the current dual level fasted against fed data indicates that there is a significant difference in only four (tadalafil, zafirlukast, carvedilol and felodipine) out of the nine drugs tested and in these cases the fasted solubility is lower than the fed. Finally, comparison of the dual level with the published data indicates that for fasted six (phenytoin, aprepitant, tadalafil, felodipine, fenofibrate, and probucol) out of the nine results are significantly different and for the fed the value is four significantly different (phenytoin, aprepitant, carvedilol and fenofibrate) out of nine.

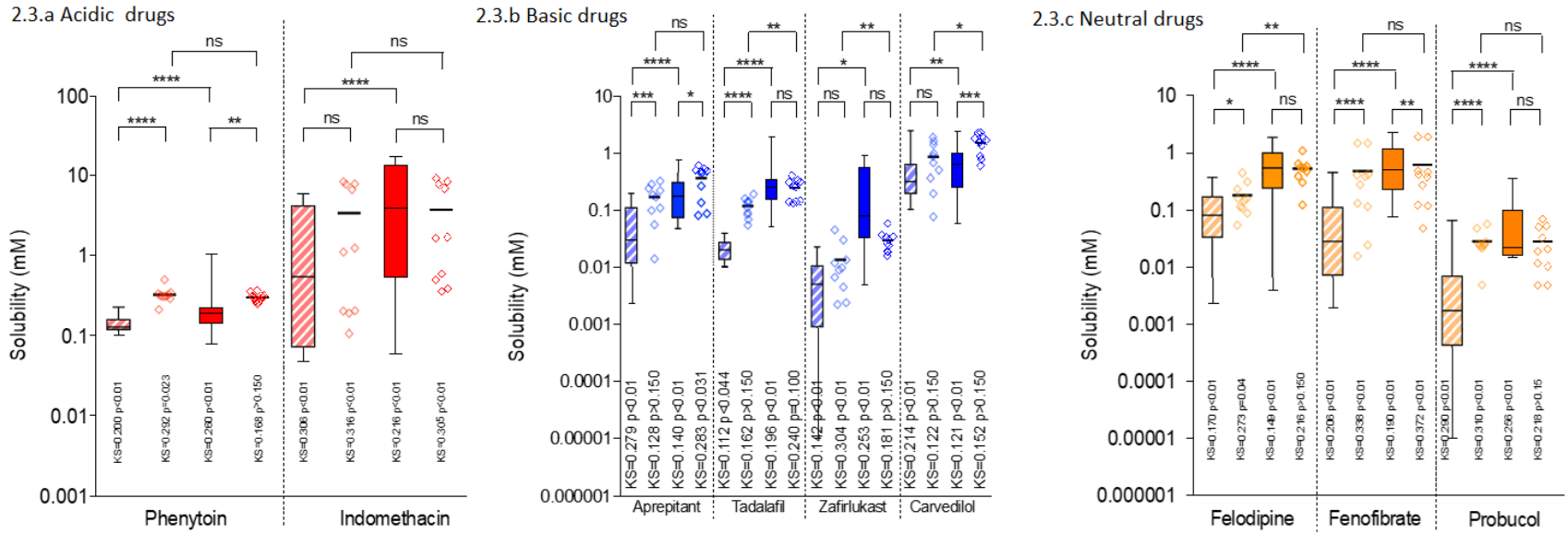


Figure 2.3: Statistical Comparison of Design of Experiment Equilibrium Solubility Measurements. Box and Whisker plots, published fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) design of experiment solubility data. Scatter plots separate fasted and fed design of experiment equilibrium solubility data current study, bar indicates arithmetic mean. KS Kolomogrov normality test on the data set, $p < 0.05$ indicates the distribution is not normal. Comparison bars Mann-Whitney test, not significant (ns) if $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ and **** $p \leq 0.0001$. Published fasted (lined box) and fed (closed box) DoE equilibrium solubility data, Current study fasted (light diamonds) and fed (dark diamonds) equilibrium solubility data. (a) red for acids , (b) blue for bases and (c) orange for neutrals.

2.3.3 Solubility influence of individual DoE factors in fasted and fed study arms

The standardised effect value for each factor in the fasted and fed study arms are presented in Figure 2.4, due to the small experimental data set a value of greater than ±4 is significant and two way factor interactions cannot be determined. Out of the possible 126 values only 29 (around 23%) are significant and drug dependent behaviour is evident since some drugs (tadalafil and carvedilol) have no significant factors, whilst felodipine has eight out of a possible thirteen (around 62%). A comparison with published significant effect values in larger fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) studies is presented in Table2.6, in these studies (where comparable) out of a possible 81 values 64 (around 80%) are significant, indicating that this study is finding a lower incidence of significant factors. Agreement between this study and the published data arises in 32 out of the 64 (50%) possible comparisons (Table2.6) with the level varying between the factors for example, pH seven out of eighteen agree lecithin eleven out of eighteen but for bile salt only two out of eighteen agree. Further comparison indicates that where the factor is significant in the published studies the current study only agrees in around 28% of cases, but if the published data indicates that the factor is not significant the agreement is around 61%.

For the acidic compounds (Figures 2.4a and b) pH is the most significant factor in both fasted and fed state which is identical to the two previously reported DoE studies. Indomethacin matched the previous studies with respect to pH but phenytoin was contrasting as pH showed a negative effect on solubility. The effect of pH on indomethacin is attributable to drug ionization (pKa = 4.5) in the experimental pH range. The negative pH effect on phenytoin (pKa = 8.1), which will be predominantly un-ionised in the experimental pH range must be related to changes in the media composition, most notably the incorporation of cholesterol, between experiments impacting on media behavior and solubility. For example, the significant negative solubility effect of cholesterol for phenytoin has not been previously reported. Sodium oleate, cholesterol and the BS:PL ratio showed significant effects in fasted

phenytoin, but not with the fed state and all other factors showed no significant influence on solubility.

For all the basic compounds (Figures 2.4c to f), there were no significant factors influencing solubility in the fed state and for tadalafil and carvedilol there were no significant factors influencing solubility in both states. Only aprepitant and zafirlukast showed an influence by the media factors in fasted state with sodium oleate, lecithin and monolyceride for aprepitant and pH, cholesterol and monoglyceride for zafirlukast significant. This low incidence of significant factors is in marked contrast to the published studies (see Table2.6), however pH, sodium oleate and lecithin have previously been shown to be significant. The positive effect of cholesterol on zafirlukast solubility has not been previously reported in the literature nor the negative effect of monoglyceride, however both of these factors have not been previously studied in fasted DoE systems.

For the neutral compounds (Figures 2.4 g to i), sodium oleate was significant for all drugs in both fasted and fed state which is in compliance with the published fasted (Khadra et al., 2015) and fed (Zhou et al.,2017) studies. Lecithin was significant in both fasted and fed states in case of felodipine and fenofibrate which is in compliance with the published studies but did not agree for probucol. pH was significant in both fasted and fed states for fenofibrate and fasted for felodipine but not for probucol, which was significant in the published studies. The effect of pH on the solubility of the neutral compounds must be through an indirect effect on ionization of the different media components. Bile salt had no significant impact on solubility in the fasted state, which is at variance with the literature for felodipine and fenofibrate but not probucol. Cholesterol which has not been previously studied did not show a significant impact on solubility. Monoglyceride showed positive effect on felodpine solubility in the fasted and fed state, which is not in agreement with published data.

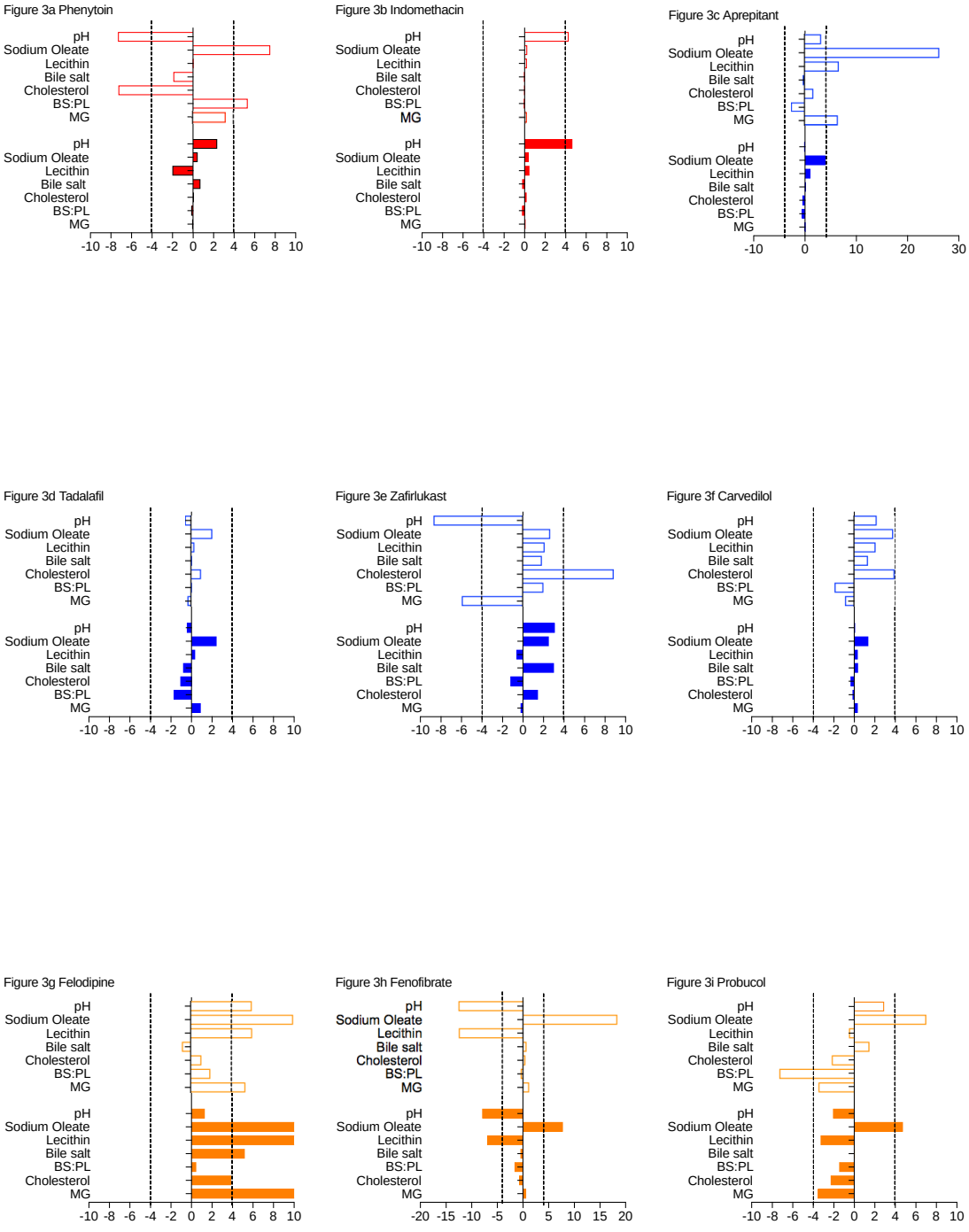


Figure 2.4: Standardised Effect Values for DoE Factors on Equilibrium Solubility in Fasted and Fed Study Arms. DoE standardised effect values for factors (as listed in Figure y-axis) on equilibrium solubility. Separated Fasted result empty histogram bar, Separated Fed result closed histogram bar. Vertical black lines indicate statistical significance ($P < 0.05$ NB Significance value = ±4 due to small sample number in separate fasted and fed study), horizontal bar direction indicates direction of effect, to the right of 0 on axis is positive effect on solubility, bar length indicates the magnitude of the effect.

Table 2.6: Comparison of the Statistical Significance of DoE Factors across Studies.

	Factor																											
Drug	pH				Sodium Oleate				Lecithin				Bile Salt				Cholesterol				BS:PL				Monoglyceride			
	Fasted		Fed		Fasted		Fed		Fasted		Fed		Fasted		Fed		Fasted		Fed		Fasted		Fed		Fasted		Fed	
	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published	Current	Published
Phenytoin	S	S	NS	S	S	S	NS	S	NS	S	NS	NS	NS	S	NS	S	S	-	NS	-	S	-	NS	-	NS	-	NS	S
Indomethacin	S	S	S	S	NS	S	NS	S	NS	NS	NS	NS	NS	S	NS	S	NS	-	NS	-	NS	-	NS	-	NS	-	NS	NS
Aprepitant	NS	S	NS	S	S	S	NS	S	S	NS	NS	S	NS	S	NS	S	NS	-	NS	-	NS	-	NS	-	S	-	NS	NS
Tadalafil	NS	S	NS	NS	NS	S	NS	S	NS	S	NS	NS	NS	S	NS	S	NS	-	NS	-	NS	-	NS	-	NS	-	NS	NS
Zafirlukast	S	S	NS	S	NS	S	NS	NS	NS	S	NS	NS	NS	S	NS	S	S	-	NS	-	NS	-	NS	-	S	-	NS	NS
Carvedilol	NS	S	NS	S	NS	S	NS	S	NS	S	NS	NS	NS	S	NS	S	NS	-	NS	-	NS	-	NS	-	NS	-	NS	S
Felodipine	S	S	NS	S	S	S	S	S	S	S	S	S	NS	S	S	S	NS	-	NS	-	NS	-	NS	-	S	-	S	NS
Fenofibrate	S	S	S	NS	S	S	S	S	S	S	S	S	NS	S	NS	S	NS	-	NS	-	NS	-	NS	-	NS	-	NS	S
Probucol	NS	S	NS	S	S	S	S	NS	NS	NS	NS	S	NS	NS	NS	S	NS	-	NS	-	S	-	NS	-	NS	-	NS	S
Total Significant	5	9	2	7	5	9	3	8	3	6	2	4	0	8	1	9	2	-	0	-	2	-	0	-	3	-	1	4

Current = Current study results – Figure 2.4, Fasted Published = Data from {Khadra, 2015} Figure 2.3, Fed Published = Data from {Zhou, 2017} Figure 2.3,S = Factor Statistically Significant in Design of Experiment Study, NS = Factor Not Statistically Significant in Design of Experiment Study, - = Comparison not possible, Shaded box = no consistent result between studies

2.3.4 Solubility influence of individual DoE factors and factor interactions in the combined study arm

The standardised effect value for each factor and factor interactions in the combined arm (fasted + fed data) are presented in Figure 2.5, due to the larger experimental data set a value of greater than ±2 is significant and eight two way factor interactions can be determined. Out of the possible 63 values only 16 (around 25%) are significant and drug dependent behaviour is evident since some drugs (phenytoin and zafirlukast) have no significant factors, whilst fenofibrate has three out of a possible seven (43%). No similar DoE studies covering fasted and fed states in this manner have been published, the overall significance level appears low when compared to the previous published larger fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) studies where around 80% are significant, indicating that this study is finding a lower number of significant factors.

For the acidic drugs (Figures 2.5a and b), pH was the only significant factor with an effect on indomethacin solubility which can be attributed to the ionization of the compound over the pH of the DoE, see above (section 2.3.3).

For basic drugs (Figures 2.5c to f), sodium oleate was significant for three (aprepitant, tadalafil and carvedilol) out of the four drugs with in these cases a positive solubility impact which agrees with the previous published fasted and fed data. No significant effect was determined for pH, which was unexpected as was the low significance of lecithin (significant for aprepitant only) and bile salt.

The neutral drugs (Figures 2.5g to i) exhibited a more complicated pattern since for each drug at least three or four factors were significant encompassing all seven factors in the DoE pH, sodium oleate, lecithin, bile salt, cholesterol, BS:PL ratio and monoglyceride. Sodium oleate was the factor with the highest magnitude of effect in all 3 drugs and always positive, followed by lecithin, monoglyceride and then pH, with bile salt and cholesterol only significant for felodipine. This multi-factorial result is

in agreement with the published fasted (Khadra et al., 2015) and fed (Zhou et al., 2107) studies where for neutral drugs multiple factors contributed to solubility.

The increased number of data points available by combining the fasted and fed arms permits the determination of two way interactions and these are also presented in Figure 2.5. Only three out of the nine drugs (phenytoin, zafirlukast and probucol) exhibited significant interactions with an overall rate of around 32% of significant interactions out of the total possible. This overall rate is similar to the previous fasted (Khadra et al., 2015) and fed (Zhou t al., 2107) studies which for the two way interactions matched with this study had significance rates of 33% and 28% respectively. However, the significant interactions were not restricted to the three above noted drugs, for example bile salt*oleate significantly increased the solubility of felodipine and fenofibrate in the fasted study and felodipine and probucol in the fed study, a result not matched in the current study.

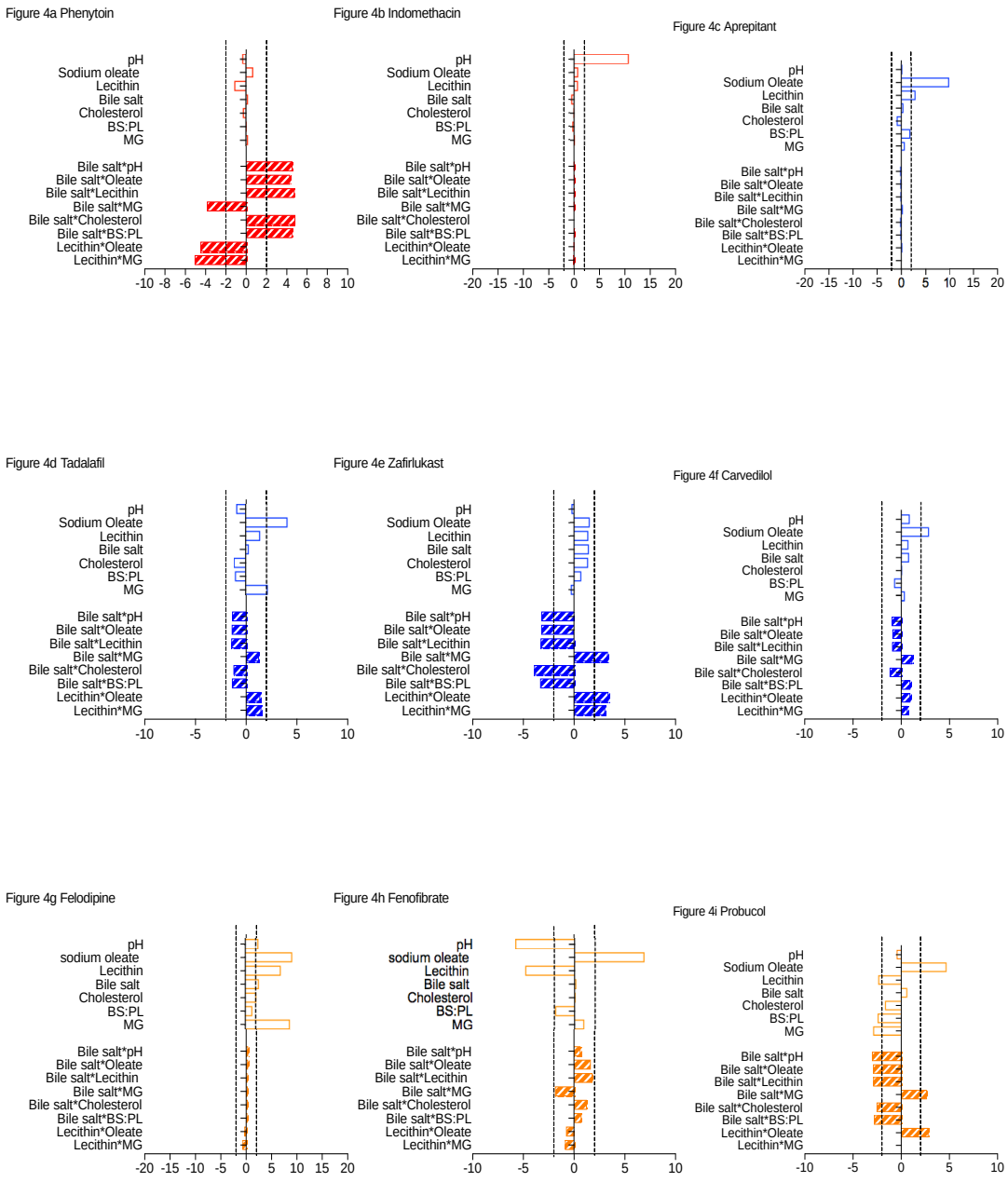


Figure 2.5: Standardised Effect Values for DoE Factors and Factor Interactions on Equilibrium Solubility in Combined Study Arm. DoE standardised effect values for factors and factor interactions (as listed in Figure y-axis) on equilibrium solubility. Combined Fasted and Fed result empty histogram bar, Combined Factor interactions lined histogram bar. Vertical black lines indicate statistical significance ($P < 0.05$ NB Significance value = ±2 due to larger sample number when compared to separate fasted and fed study, Figure 2.4), horizontal bar direction indicates direction of effect, to the right of 0 on axis is positive effect on solubility, bar length indicates the magnitude of the effect.

2.3.5 Statistically significant solubility factor and factor interactions

The mean of the absolute value of all standardised effect values in the three arms of the study arranged by drug group is presented in Figure 2.6 in order to summarise the experimental results. Note that this removes the factor's direction of effect information.

For acidic drugs (Figures 2.6a and b) the only significant single factor is pH in the fasted and combined arms a result that is not surprising based on the published data for acidic drugs in fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) media. In the published DoEs sodium oleate, lecithin and bile salt were also significant, although that result is not reflected in this study. All the two way interactions investigated were significant a result that is due to the impact of phenytoin, since indomethacin had no significant interactions.

For basic drugs (Figures 2.6c and d) sodium oleate is the only significant single factor in the fasted, fed and combined arms. This was also the most significant factor for basic drugs in the fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) studies, however in these studies other factors for example pH, bile salt and lecithin were also significant although with a marginally lower magnitude. No two way interactions were significant in this study, which is at variance with the published studies since bile salt*oleate was significant in the fasted state and lecithin*oleate in the fed.

For neutral drugs (Figures 2.6e and f) in the fasted arm pH, sodium oleate and lecithin are significant, with sodium oleate, lecithin and monoglyceride in the fed and pH, sodium oleate and lecithin in the combined. This is in close agreement with the published fasted (Khadra et al., 2015) where pH, sodium oleate, bile salt and lecithin were approximately equally significant and the fed (Zhou et al., 2017) where the four aforementioned factors were significant with sodium oleate dominant. No two way interactions were significant in this study, which is at variance with the published studies since bile salt*pH, bile salt*oleate and bile salt*lecithin was significant in the fasted state and bile salt*oleate, bile salt*MG, lecithin*oleate and bile salt*lecithin in the fed.

Figure 5a Acidic Drugs - Fasted/Fed

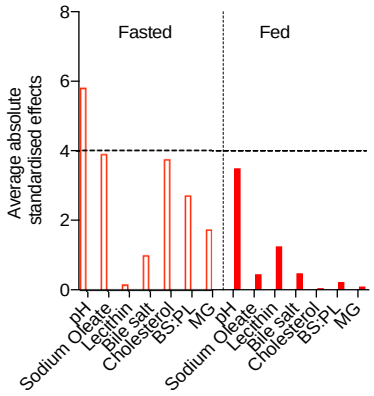


Figure 5b Acidic Drugs - Combined/Interactions

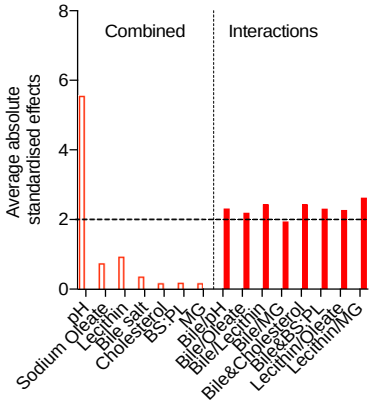


Figure 5c Basic Drugs - Fasted/Fed

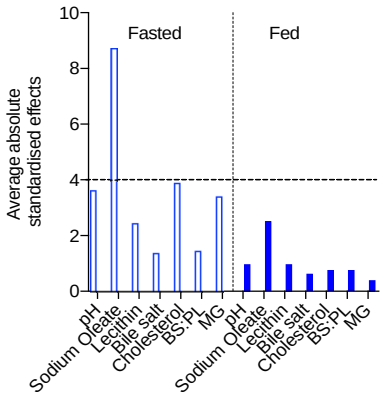


Figure 5d Basic Drugs - Combined/Interactions

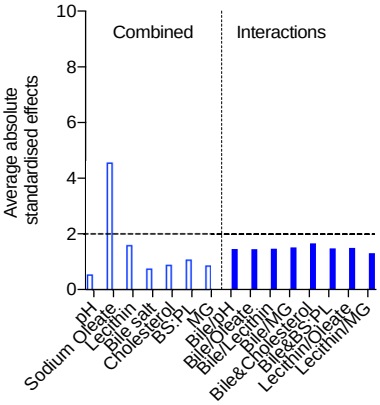


Figure 5e Neutral Drugs - Fasted/Fed

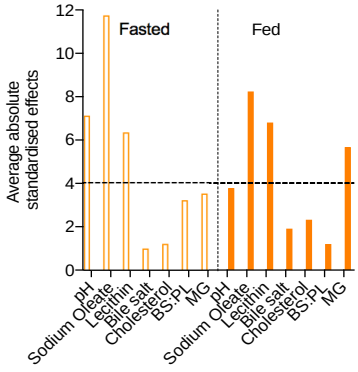


Figure 5f Neutral Drugs - Combined/Interactions

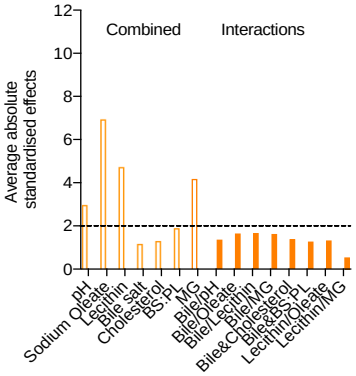


Figure 2.6: Average Absolute Standardised Effect Values for DoE Factors on Equilibrium Solubility in Fasted, Fed and Combined Arms. Average absolute (NB this removes direction of effect information) standardised effect values for individual factors on equilibrium solubility grouped by drug category. Horizontal black line indicates statistical significance ($P < 0.05$).

2.4 Discussion

2.4.1 Equilibrium solubility measurements

The equilibrium solubility results in either arm (fasted or fed) of this study are presented in Figures 2.2 and 2.3 indicate that the measurements are in broad agreement with available published equilibrium solubility data in fasted and fed HIF, simulated intestinal fluids (Augustijns et al., 2014; Liu et al., 2015) and published DoE studies in fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) simulated intestinal media systems. In addition, the results demonstrate individualistic drug behavior, with some drugs providing a low solubility variability for example phenytoin and others large variability for example probucol. A feature that was evident in previous fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) DoE. This indicates that the current study is investigating a similar solubility space to previous simulated studies and comparable to sampled HIF.

2.4.2 Statistical comparisons of solubility

The generation of a solubility data set for each drug permits a statistical comparison with published data and this is presented in Figure 2.3. Examination of the published fasted (Khadra et al., 2015) and fed (Zhou et al., 2017) data indicates that for all of the systems the solubility distribution is non-normal, an unexpected result based on the number of data points in each system (fasted DoE= 66, fed DoE= 92). This analysis is evident but not replicated by the results in this current study where around 50% of the measured distributions in the fasted and fed arms are non-normal. This result may arise through the non-normal sample pattern induced by the DoE structure, the fact that drug solubility is not normally distributed in the sample space or that the statistical sample is not sufficiently large. The former explanation is visually evident in the indomethacin fasted and fed data in this study (Figure 2.3a) where the impact of the three pH levels (Table 2.1, mid-point pH 6 not shown) on solubility creates a non-normal distribution. This stratified variability is likely to be induced by all factors and therefore a non-normal distribution is sensible, although further sampling studies would be required to investigate this phenomenon.

The comparison of the published fasted and fed DoE results indicate that in all cases the fed solubility is statistically significantly higher than the fasted which is in agreement with the literature data (Clarysse et al., 2011; Augustijns et al., 2014; Krupa et al., 2017) and indicates that these published DoEs (Khadra et al., 2015; Zhou et al., 2017) have investigated different solubility spaces. A comparison of the current study fasted and fed arms indicates that in four (tadalfil, zafirlukast, carvedilol and felodipine) out of the nine cases the fasted is statistically significantly different from the fed which has a higher solubility and therefore in agreement with the cited literature. However, in five (phenytoin, indomethacin, aprepitant, fenofibrate and probucol) of the cases in this study there is no statistically significant difference between the fasted and fed arms. For the acidic drugs (Figure 2.3a) indicates that in the case of phenytoin this is related to the narrow solubility distribution, which when coupled with the small sample number is not sufficient to discriminate between the arms. Whilst for indomethacin, since pH is the major factor influencing solubility (see Figure 2.4b) in both fasted and fed states and is identical in the fasted and fed states the lack of a statistically significant difference is understandable. For the basic drug aprepitant (Figure 2.3b) whilst the mean fasted solubility is lower the range of solubility overlaps with the fed and coupled with the small sample number is not sufficient to discriminate between the arms. For the neutral compounds both fenofibrate and probucol (Figure 2.3c) have no significant difference between the fasted and fed arms, which appears to be due to the inability of the fasted arm to measure the lower solubility values evident in the published fasted results, see next paragraph.

Comparison of the fasted arm with published fasted results indicates that in six (phenytoin, aprepitant, tadalfil, felodipine, fenofibrate and probucol) out of the nine cases there is a statistically significant difference between the solubility data sets with the current fasted arm having a higher solubility. Visual examination of Figure 2.3 indicates that this appears to be due to the inability of the fasted arm to measure the lower solubility values evident in the published fasted results. There is a subtle difference in the media compositions, since in this study cholesterol and monolglyceride were included at low levels (Table2.1) based on current literature

(Riethorst et al., 2106) and recent proposed changes to the composition of fasted state simulated media (Fuchs et al., 2015). Both of these factors were not employed in the fasted DoE (Khadra et al., 2015) or the original fasted simulated intestinal fluid recipes (Galia et al., 1998). This is re-enforced by the literature data included in Figure 2.2, where for example the fasted value for probucol, determined in fasted media without cholesterol or monolyceride (Clarysse et al., 2009), is below the values determined in this study. In addition, in fed media it has been demonstrated that increasing the total "surfactant" concentration, which included monoglyceride, increases the solubilisation of fenofibrate (Kleberg et al., 2010). Figure 2.4 indicates that cholesterol does not positively impact the solubility of any of the drugs and negatively impacts phenytoin, whilst monoglyceride positively impacts the solubility of aprepitant and felodipine. The solubility difference therefore is probably due to the presence of cholesterol and monoglyceride in the current fasted media system which increases the amphiphilic phase components by 0.2mM (around 8% of total content) at the lower and 3.06mM (around 12%) at the higher level increasing overall solubilisation capacity.

Comparison of the fed arm with published fed results (Zhou et al., 2017) indicates that in four (phenytoin, aprepitant, carvedilol, fenofibrate) out of the nine cases there is a statistically significant difference between the solubility data sets with the current fed arm generally higher (with the exception of fenofibrate). A similar explanation to that presented above for phenytoin is applicable and for aprepitant and carvedilol the differences seem to be due to a higher solubility than the published range, whilst for fenofibrate it appears to be due to a marginally increased solubility range. Although the overall number of significant differences is smaller a similar explanation to that presented above for the fasted media appears to be applicable. The current study factor ranges are different to the published data set (sodium oleate (current 0.8-25mM vs published 0.8-52mM), bile salt (3.6-15mM vs 3.6-24mM), lecithin (0.5-3.75mM vs 0.5-4.8mM), monoglyceride (1-9mM vs 1-6.5mM)) and cholesterol (0.13-1mM) is included as an additional component in the current media.

2.4.3 Standardised Effect Values

The determined standardized effect values presented in Figures 2.4 and 2.5 and summarized in Table 2.6 for the fasted and fed states indicates that in this setting very few factors have a statistically significant impact on solubility. In the current fasted, fed and combined arms factors were significant in only 46 (around 24%) out of 189 possible cases, which is around one quarter of the incidence determined from the previous fasted (Khadra et al., 2015) and fed (Zhou et al.,2017) DoE studies. Interestingly the fasted study employed a quarter and the current study employs a sixteenth of the full factorial DoE (the fed is not comparable since it employed a D-optimal design) indicating that reducing the number of data points in the study limits the ability to determine significant factors. However, based on the comparison in Table 2.6 and Figure 2.6 the current study has correctly identified the factors with the highest magnitude of effect (for example pH for acidic drugs, sodium oleate for basic drugs and pH, sodium oleate and lecithin for neutral drugs) on solubility. Interestingly though the current study suggests that bile salt has no significant impact on solubility a result that is not in agreement with the literature (Clarysse et al., 2009; Dressman et al., 1998) but a reflection of the statistical power of the current study. Indicating that small scale studies will have inherent statistical limitations.

The use of small numbers in DoE reduces the ability to determine higher level interactions between the factors and in this study only eight could be determined. The level of significant interactions at around 32% of the total possible is similar to the previous studies (Khadra et al., 2015; Zhou et al., 2017) but is restricted to only three (phenytoin, zafirlukast and probucol) out of the nine drugs, which is a lower incidence. In the previously published studies factor interactions generally had a lower standardized effect value to their single factor counterparts and in the current study none of the interactions are on average significant for the basic or neutral drugs. Indicating that the argument presented above with respect to reduced statistical discrimination is also active for factor interactions.

2.5 **Conclusions**

The results indicate that a reduced experimental number design of experiment covering both fasted and fed simulated media states in a single study is feasible and provides equilibrium solubility data and drug related behaviours that are similar to previous studies. The study will provide for a drug, equilibrium solubility values that are comparable to published individual solubility measurements in either fasted or fed sampled human intestinal or simulated media systems. However, the results indicate that changes in the media composition will impact on the solubility ranges determined and when coupled with the reduced number of data points will determine a smaller solubility range than larger scale studies. The system will be able to establish the factors with the largest influence on equilibrium solubility but due to the reduced experimental number and therefore statistical power, factors with a lower influence will not be revealed. In the current study for example bile salt paradoxically has no significant effect on equilibrium solubility. In conclusion it is feasible to apply a small scale DoE to determine the equilibrium solubility range for a drug in either fasted or fed simulated intestinal fluids, this will also indicate the major factors influencing solubility but the statistical limitations of the approach must also be considered.

3 Dual level design of experiment investigating effect of fresh and frozen fasted/fed simulated intestinal fluids

As discussed previously (section 1.9.2), published fasted and fed DoE's necessitate large numbers of experiments which together with the requirement of preparing multiple fresh simulated media, is time consuming and produces a high experimental load. In addition, the 20 dual DoE, showed statistical limitations, as discussed in section 2.5. In order to compensate for the reduced statistical power of the 20 dual DoE, this study was conducted using larger number of experiments (68) with a larger number of stock solutions (34) and the stock solutions were frozen. The results were then examined for the feasibility of using frozen simulated stock solutions rather than preparing fresh solution. Three different drugs (Fenofibrate, Indomethacin and Apprepitant) representing neutral, acidic and basic categories were chosen as model compounds to carry out the experiment. Comparisons of the solubility values from fresh experiment with the frozen experiments were performed to determine the viability of using frozen stock solutions rather than preparing fresh.

3.1 Material and method

3.1.1 Material

The same materials were used as in section 2.2.

3.1.2 Dual level design of experiment and data analysis

For each media parameter (sodium TC, lecithin, sodium oleate, cholesterol, BS: PL ratio and monoglyceride) lower and upper limit concentration values for fasted and fed sate were defined (as in Table2.1, section 2.1.4). Using Minitab 17.2.1 design of experiment and a custom experimental design, a quarter of the full factorial DoE with seven factors and two levels (lower and upper limits) was constructed (32 experiments around the upper and lower levels plus two centre points) separately for the fasted and the fed state. These two tables were then used as an input for a factorial custom design of experiment which combined fasted and fed states using all 68 data points to provide an overall analysis. The study therefore consists of three arms, two smaller (32 data

point) fasted and fed arms which are then merged into a larger (68 data point) combined arm (Figure 3.1).

According to the design, the standardized effect value calculated for the smaller (separated) fasted and fed state arms and the larger (combined) fasted and fed state arm indicates a significant increase in drug solubility when it is greater than +2 and a decrease in drug solubility when it is less than -2.

The Kolmogorov normality test was used in Minitab to assess the normality distribution of each data set. A Mann-Whitney test was used to evaluate the median between two data sets (not normally distributed) and the two-sample *t*-test was used to evaluate the mean of two data sets (normally distributed).

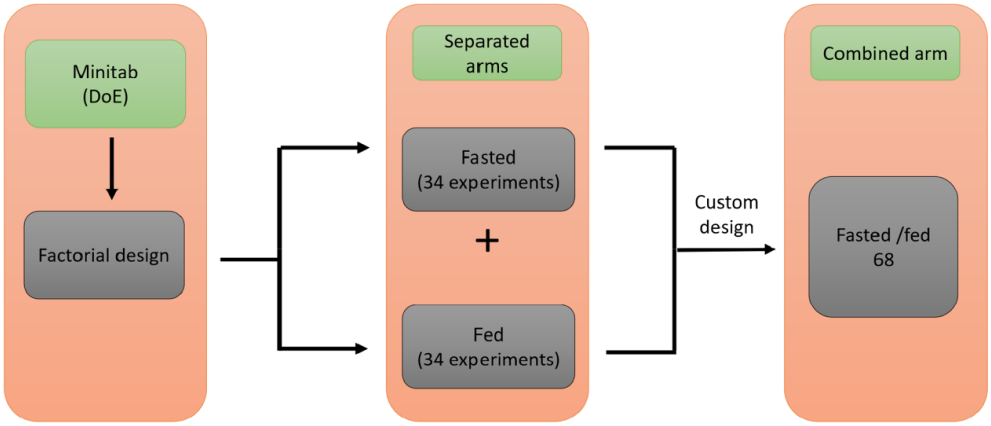


Figure 3.1: Schematic diagram of the design of experiments three arms

3.1.3 Equilibrium solubility measurements and Preparation of Stock systems

The same preparations and measurements performed in section 2.2.3 and 2.2.4 were used in this section.

3.1.4 Preparation of experimental measurements solutions

The same preparations and measurements performed in section 2.2.5 were used in this section.

3.1.5 Preparation of the frozen stock solutions

The experiment designed in section 3.1.2 required 68 solubility experiments generated via 34 stock solutions consisting of bile salt, lecithin, MG and cholesterol which had to be prepared for each compound under examination. The preparation of the 34 stock solutions was time and cost consuming. To avoid these difficulties, an amount of 1 ml was taken from each fresh stock solution and transferred to an Eppendorf tube to be frozen at -80 °C so it could be used later (Figure 3.2). To ensure the feasibility of using frozen stock solution, each compound experiment was repeated three times using the same stock solution but with different storage durations. The first experiment was conducted using fresh stock solution, the second experiment was conducted using frozen1 stock solution (1-week duration of freezing) and the last experiment was conducted using frozen 2 stock solution (2-weeks duration of freezing). The same method was used for all of the three groups and then a statistical comparison between them was performed to examine the consistency.

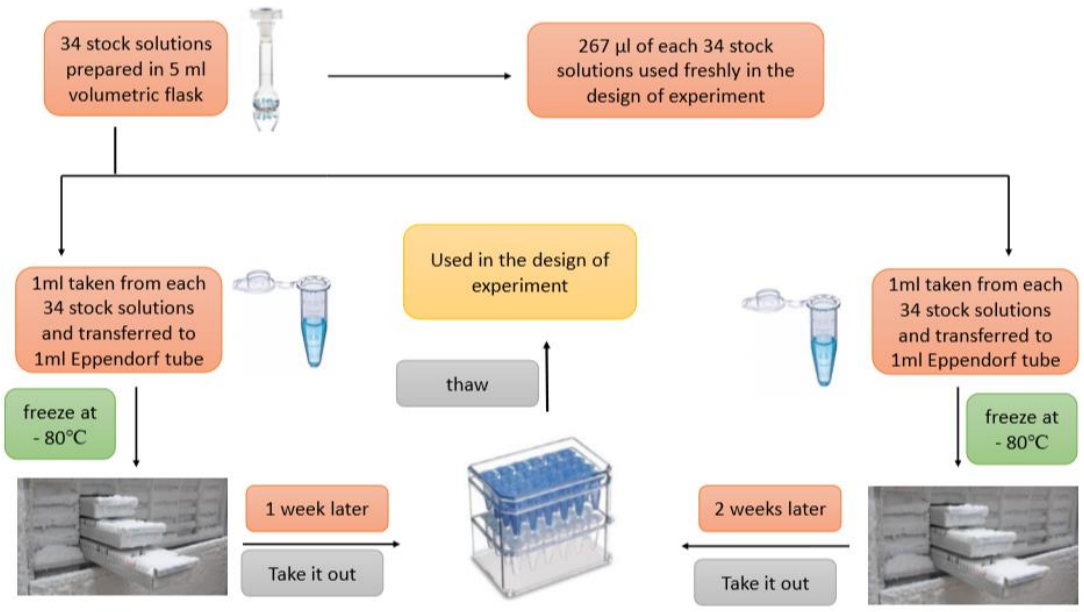


Figure 3.2: Schematic diagram of freeze stock solutions preparations

3.1.6 HPLC method

The same HPLC conditions used in Table2.5 were used for this study.

3.2 Results

The results will present a comparison of the solubility data of drugs using fresh stock solution with the solubility data of drugs using frozen stock solutions.

3.2.1 Equilibrium solubility measurements

The equilibrium solubility measurements for all three drugs in all three replicates (fresh, frozen 1 and frozen 2) are presented in Figure 3.3 (fresh was presented using box and whisker plot) and illustrate that broadly similar solubility ranges have been observed depending on the drug under investigation, the used stock solution (fresh or frozen) and the media state (fasted or fed). The results also indicates that drug specific factors influences solubility, for example fenofibrate showed consistent solubility values in all three stock solutions compared to aprepitant that showed different solubility values between fresh and frozen stock solutions.

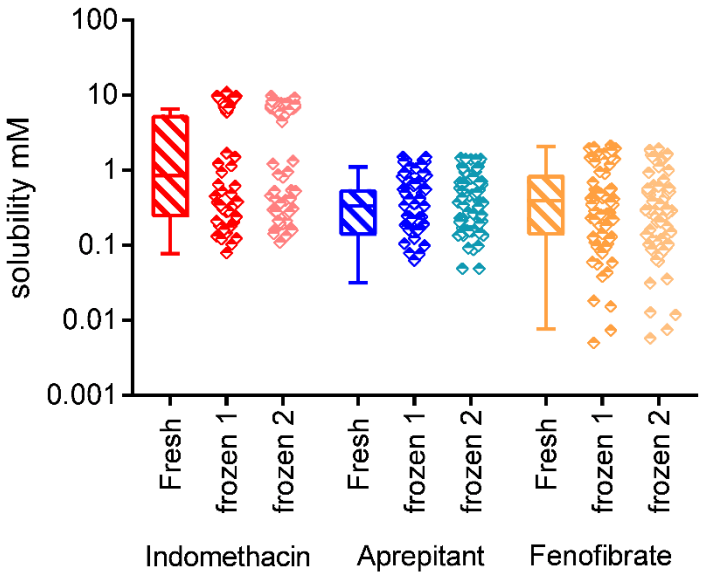


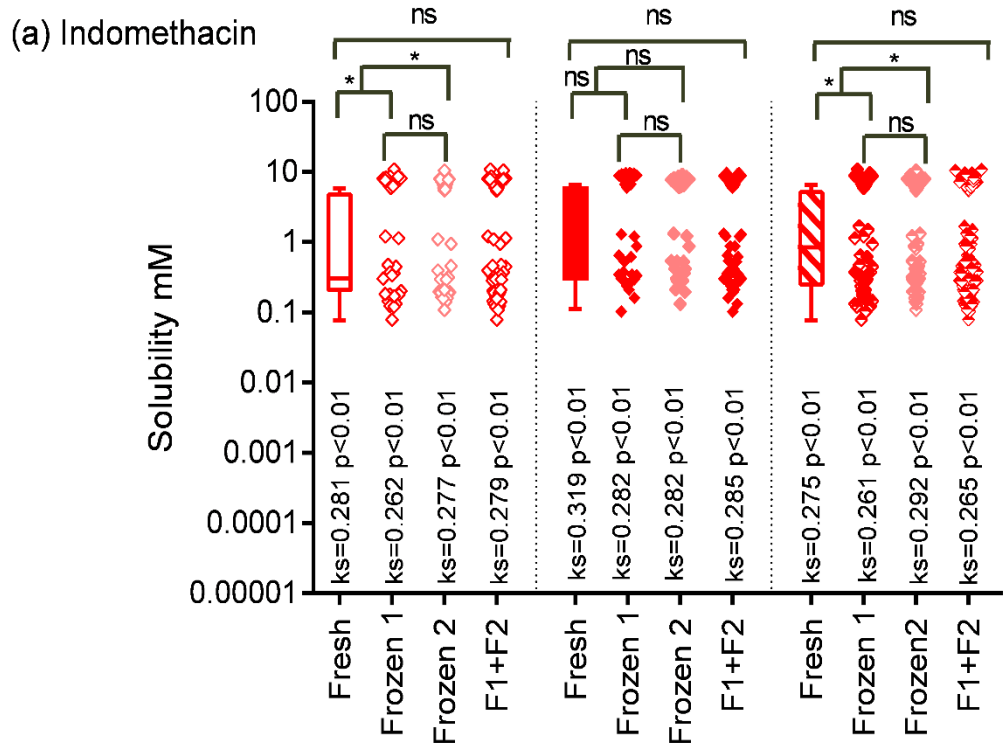
Figure 3.3: Design of experiment equilibrium solubility measurements. Equilibrium solubility measurements for each drug in the three different stock media solution, lined box and whisker plot reported solubility values for the fresh stock solution- Red and white data point for the acidic drug indomethacin, Blue and white data point for the basic drug aprepitant and yellow and white for the neutral drug fenofibrate. Diamonds (◊) scatter plots reported frozen stock solution solubility data- Dark red and white data points for indomethacin in frozen 1 experiment and light red and white for indomethacin in frozen 2 experiment, dark blue and white data points for aprepitant in frozen 1 experiment and light blue and white for aprepitant in frozen 2 experiment, dark yellow and white data points for fenofibrate in frozen 1 experiment and light yellow and white for fenofibrate in frozen 2 experiment.

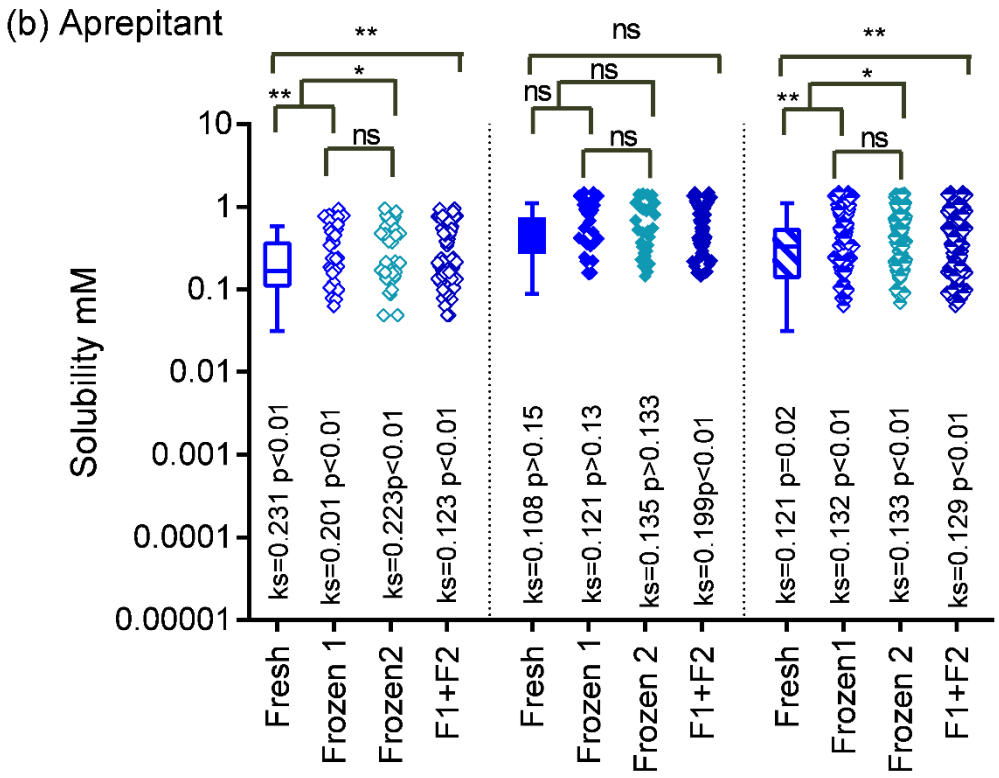
3.2.2 Statistical comparison

The equilibrium solubility results for the fasted, fed and combined states in the three different media stock solutions (fresh, frozen 1 and frozen 2) are presented in Figure 3.4 (the fresh stock solution solubility data were presented using box and whisker plot) along with a statistical comparison of the distributions. It's important to note that media with different storage durations was used in each replicate. First replicate (fresh) was performed using media prepared freshly, second replicate (frozen1) was performed using media after one week of freezing, third replicate (frozen 2) was performed using media after two weeks of freezing).

A statistical examination indicated that 33 out of a possible 36 data sets had non-normal distribution. This is in close comparison to the published fasted and fed (Khadra et al., 2015; Zhou et al., 2017) where all data sets had non-normal distribution. Three out of the possible 36 data sets were normally distributed and all referred to the basic drug aprepitant in the fed arm. The non-normal and the normal distribution of the data sets were discussed in the previous chapter section 2.4.2.

A statistical comparison between the solubility data of drugs using fresh experiment and solubility data using frozen 1 and frozen 2 experiments, showed different responses of drugs to the media changes, and that variability in solubility was specific for each drug and each experiment. For example, fenofibrate showed no effect with the alterations in both frozen 1 and frozen 2 media and in each state (fasted, fed or combined) while aprepitant and indomethacin showed a significant difference between fresh and frozen media in fasted state. Ten out of the 36 data sets had a significant difference with all of these significantly different data sets showing higher solubility values in the frozen experiments than the fresh experiment. In the fasted arm, a statistical examination indicates that five (fresh indomethacin versus frozen 1 and frozen 2 experiments and fresh aprepitant versus frozen 1, frozen 2, and frozen 1+frozen 2) out of nine data sets were significantly different. In the fed state, none of the data showed a significant difference between the fresh and frozen experiments. Combining the fasted and fed arms showed that five (fresh indomethacin versus frozen 1 and frozen 2 experiments and fresh aprepitant versus frozen 1, frozen 2, and frozen 1+frozen 2) out of nine possible values were significantly different


(a) Indomethacin
Solubility mM
100
10
1
0.1
0.01
0.001
0.0001
0.00001
ns
*
ns
ks=0.281 p<0.01
ks=0.262 p<0.01
ks=0.277 p<0.01
ks=0.279 p<0.01
ks=0.319 p<0.01
ks=0.282 p<0.01
ks=0.282 p<0.01
ks=0.285 p<0.01
ks=0.275 p<0.01
ks=0.261 p<0.01
ks=0.292 p<0.01
ks=0.265 p<0.01
Fresh
Frozen 1
Frozen 2
F1+F2
Frozen2


(b) Aprepitant
Solubility mM
10
1
0.1
0.01
0.001
0.0001
0.00001
**
*
ns
ns
ns
ns
ns
**
*
ns
ks=0.231 p<0.01
ks=0.201 p<0.01
ks=0.223p<0.01
ks=0.123 p<0.01
ks=0.108 p>0.15
ks=0.121 p>0.13
ks=0.135 p>0.133
ks=0.199p<0.01
ks=0.121 p=0.02
ks=0.132 p<0.01
ks=0.133 p<0.01
ks=0.129 p<0.01
Fresh
Frozen 1
Frozen2
F1+F2
Fresh
Frozen 1
Frozen 2
F1+F2
Fresh
Frozen1
Frozen 2
F1+F2

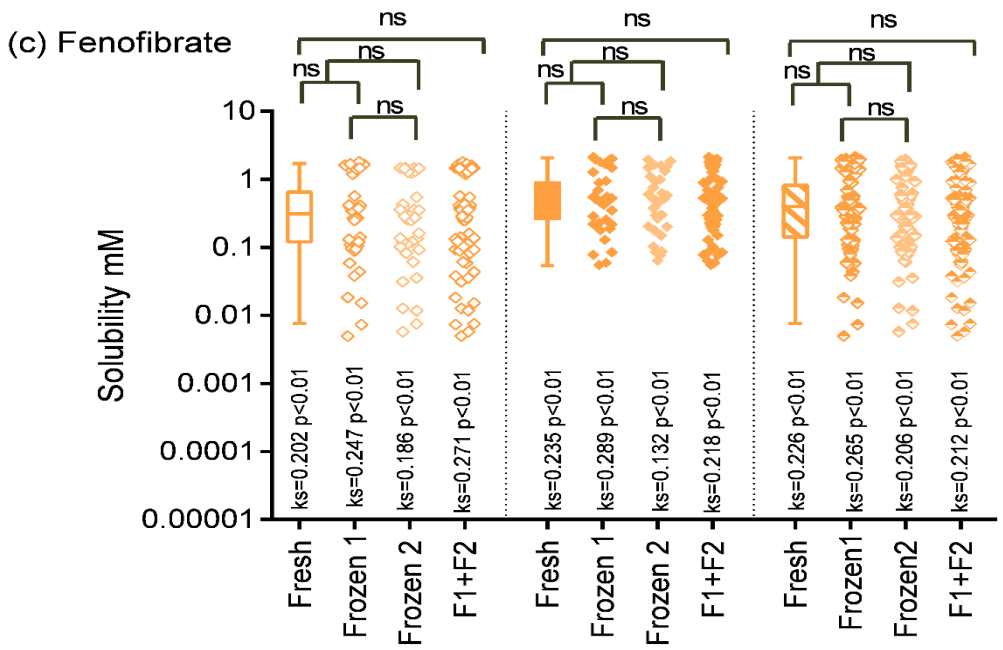


Figure 3.4: Statistical comparison of design of experiment equilibrium solubility measurements. Box and whisker plots, solubility values in fresh experiment. Diamonds scatter plots represents frozen stock solution. Ks Kolomogrov normality test on the data set, $p<0.05$ indicates the distribution is not normal. Comparison bars Mann-Whitney test and two sample t-test; not significant if $p>0.05$,*$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; and **** $p \leq 0.0001$. (a) Comparison of indomethacin. Fasted solubility values in fresh (open red box and whisker plot), frozen 1 (dark red open diamond symbol), frozen 2 (light red open symbol) experiments. Fed solubility values in fresh (closed red box and whisker plot), frozen 1 (dark red closed diamond symbol), and frozen 2 (light red closed diamond symbol) experiments. Combined fasted/ fed values in fresh (red and white box and whisker plots), frozen 1 (dark red and white diamond symbol), and frozen 2 (light red and white diamond scatter plots) experiments. (b) Comparison of Aprepitant. Combined fasted/fed values in fresh (blue and white box and whisker plots), frozen 1(dark blue and white symbol), and frozen 2 (light blue and white symbol) experiments. Fasted solubility values in fresh (open blue box and whisker plot), frozen 1 (dark blue open symbol), and frozen 2 (light blue open symbol) experiments. Fed solubility values in fresh (closed blue box and whisker plot), frozen 1 (dark blue closed symbol), and frozen 2 (light blue closed symbol) experiments. (c) Comparison of Fenofibrate. Combined fasted/ fed values in fresh (yellow and white box and whisker plots), frozen 1 (dark yellow and white symbol), and frozen 2 (light yellow and white symbols) experiments. Fasted solubility values in fresh (open yellow box and whisker plot), frozen 1 (dark yellow open symbol), and frozen 2 (light yellow open symbol) experiments. Fed solubility values in fresh (closed yellow box and whisker plot), frozen 1 (dark yellow closed symbol), and frozen 2 (light yellow closed symbol) experiments. F1+F2 represents solubility data of frozen 1+ frozen 2 in fasted, fed and combined of each drug.

3.2.3 Influence of DoE factors on solubility in fasted and fed study arms

3.2.3.1 Influence of individual DoE factors on solubility

The standardised effect value for each factor in each media replicate in the fasted and fed study arms are presented in Figure 3.5. A standardised effect value of greater than ± 2 is significant. Table 3.1 reports the statistical significance factor of each experiment.

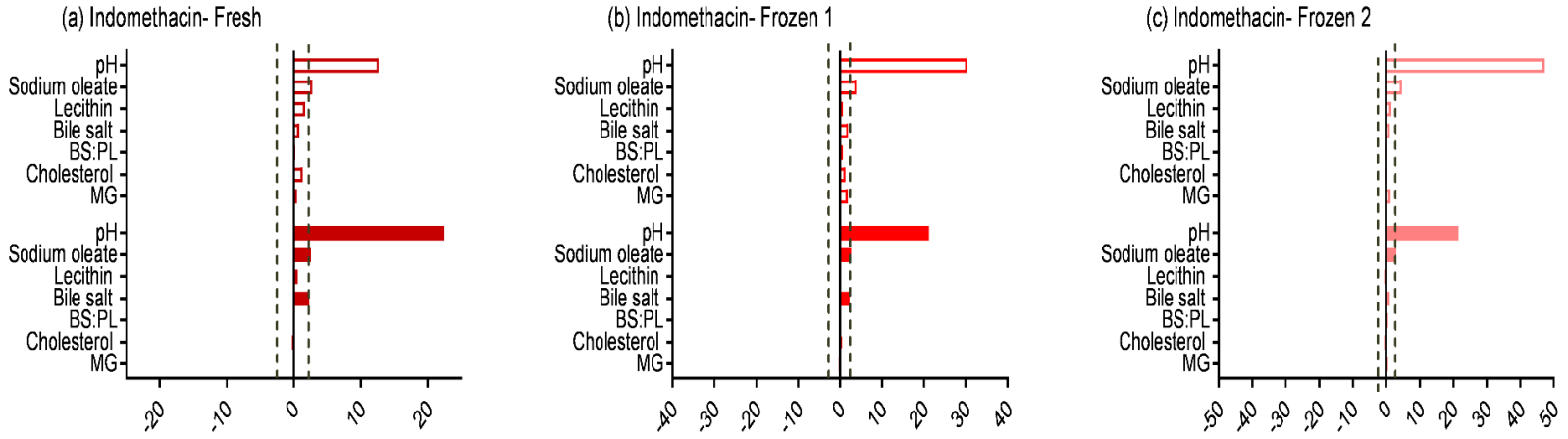
The presence of three stock solutions (fresh, frozen 1, and frozen 2) in the experiment each with seven factors to be examined together with two study arms (fasted and fed) creates 126 possible values to be compared. Starting with the fresh experiment, 12 values (around 29%) out of a possible 42 were significant in both fasted and fed state arms where 6 values in fasted state and 6 values in fed state were significant. Secondly in the frozen1 experiment, in both the fasted and fed study arms, 15 out of 42 possible values were significant with 7 of these significant values were in fasted state and 8 of them were in fed state. Finally, in the frozen 2 experiment, in both the fasted and fed study arms, 14 out of 42 possible values were significant, with 7 in the fasted state and 7 in fed state. This indicates that all 3 experiments had showed comparable incidence of significant factors.

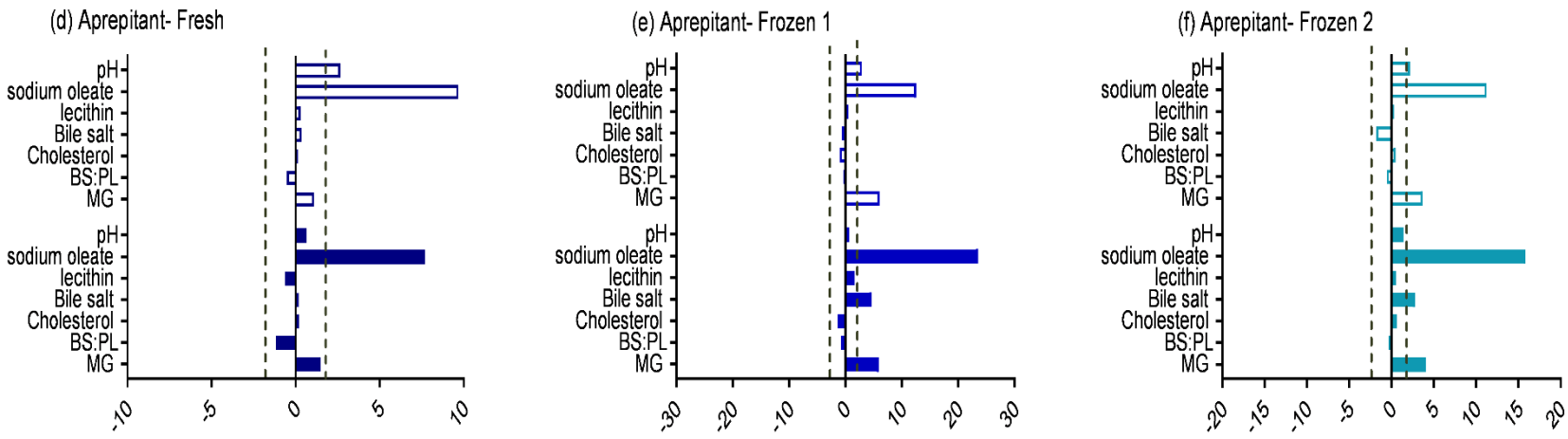
For the acidic drug indomethacin (Figure 3.5(a, b and c), in the fasted study arm, pH and sodium oleate were significant in all three experiments (fresh, frozen 1 and frozen 2). However, the frozen experiments showed higher magnitude of effect with regard to pH factor compared to the fresh experiment. This indicates that physical changes in media composition was induced by freezing the stock solutions. In the fed state, pH, bile salt and sodium oletae were significant in all three experiments, with the exception of frozen 2 experiment, where bile salt was not significant. In contrast to the fasted state, the compliance between the 3 experiments in fed state was in the significant factor effect and magnitude of factor effect.

For the basic drug aprepitant (Figure 3.5 (d, e and f), in the fasted state sodium oleate and pH were significant in all three experiments. However, frozen experiments

showed monoglyceride as a significant factor with the addition to the higher magnitude of oleate effect compared to the fresh experiment. In fed state, sodium oleate was significant in all experiments. However, monoglyceride and bile salt were significant with frozen experiments and there was also a higher magnitude of oleate effect compared to fresh experiment.

For the neutral drug fenofibrate ((Figure 3.5 (g, h and i), the three experiments were compatible with regard to the significant and magnitude of factor effect in both fasted and fed state, where pH and sodium oleate were the significant factors.


(a) Indomethacin- Fresh
(b) Indomethacin- Frozen 1
(c) Indomethacin- Frozen 2
pH
Sodium oleate
Lecithin
Bile salt
BS:PL
Cholesterol
MG
-20
-10
0
10
20
-40
-30
-20
-10
0
10
20
30
40
-50
-40
-30
-20
-10
0
10
20
30
40
50


(d) Aprepitant- Fresh
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
-10
-5
0
5
10
(e) Aprepitant- Frozen 1
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
-30
-20
-10
0
10
20
30
(f) Aprepitant- Frozen 2
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
pH
sodium oleate
lecithin
Bile salt
Cholesterol
BS:PL
MG
-20
-15
-10
-5
0
5
10
15
20

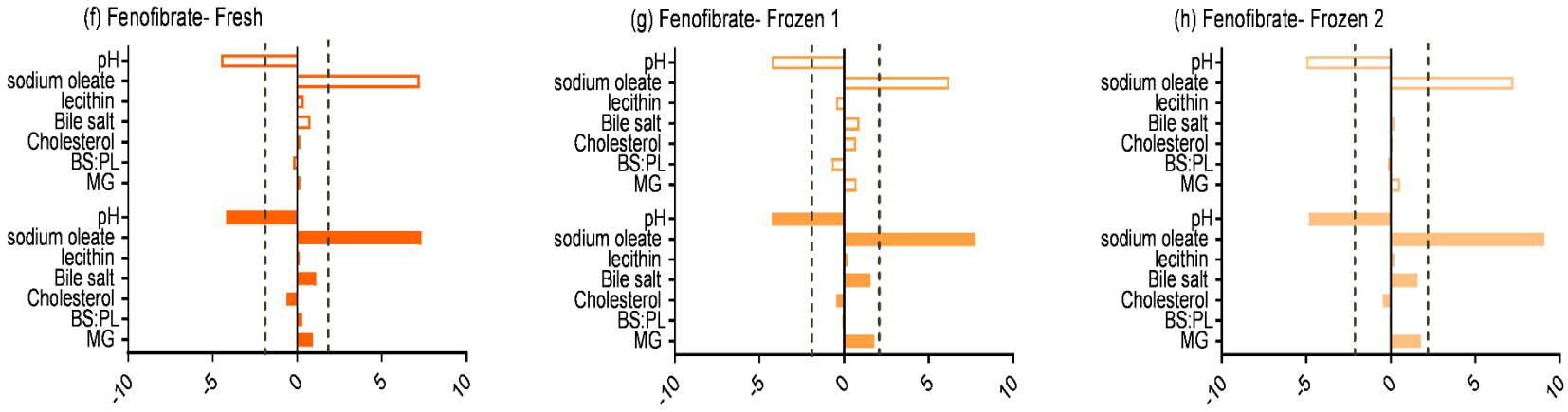


Figure 3.5: Standardised effect value for all DoE factors on equilibrium solubility in fasted and fed study arms. DoE standardised effects values for factors (as listed on y- axis) on equilibrium solubility. Bar colour indicates drug category: red= acid (dark red= fresh, red= frozen 1, light red= frozen 2). Blue= base (dark Blue = fresh, Blue = frozen 1, light Blue = frozen 2). Orange= neutral (dark Orange = fresh, Orange = frozen 1, light Orange = frozen 2).

3.2.3.2 Influence of factor interactions on solubility

The experiment consisted of seven factors which allows a possible of eighteen interactions in each media state (fasted or fed) and in each experiment (fresh, frozen 1 and frozen 2). The standardised effect value for each factor interactions in all three experiments in fasted and fed study arms have been presented in Figure 3.6.

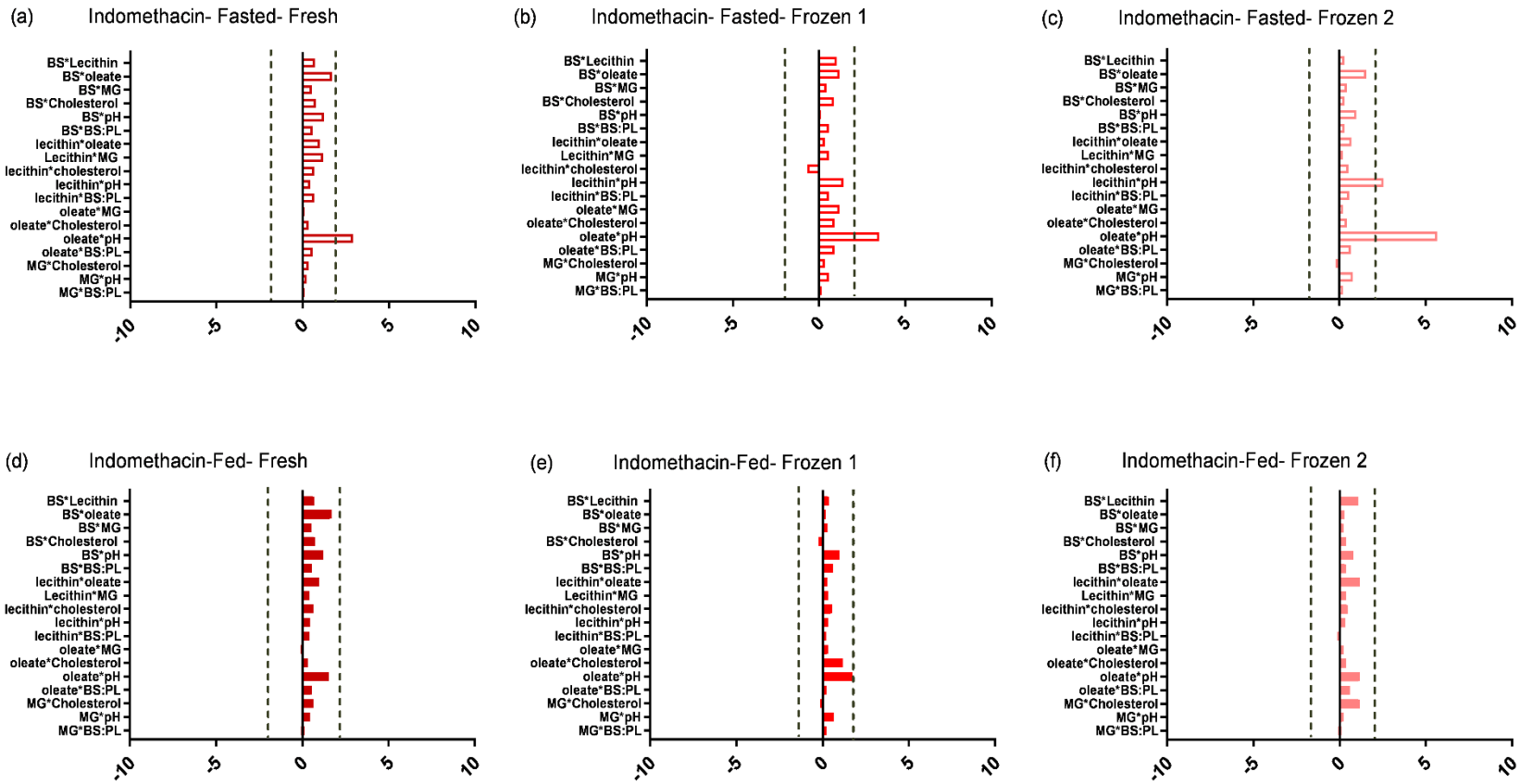
In fasted and fed study arms, eighteen interactions in each experiment and each state gave a total of 324 possible cases but only 41 (around 13%) were significant. The results indicates that drug dependent behaviour in each state was evident since some drugs (aprepitant fasted and indomethacin fed) have no significant factor interactions while fed apreitant and fenofibrate each have 17 (around 32%) out of 54 possible values of the three experiments. The results also indicates that frozen 2 experiment showed the highest incidence of significant factor interactions (19 out of 108 possible values) in both fasted (3 significant values) and fed (16 significant values) state arms, compared to the fresh or frozen 1 experiment where only 11 out of 108 (2 in fasted and 9 in fed) possible values were significant in each experiment.

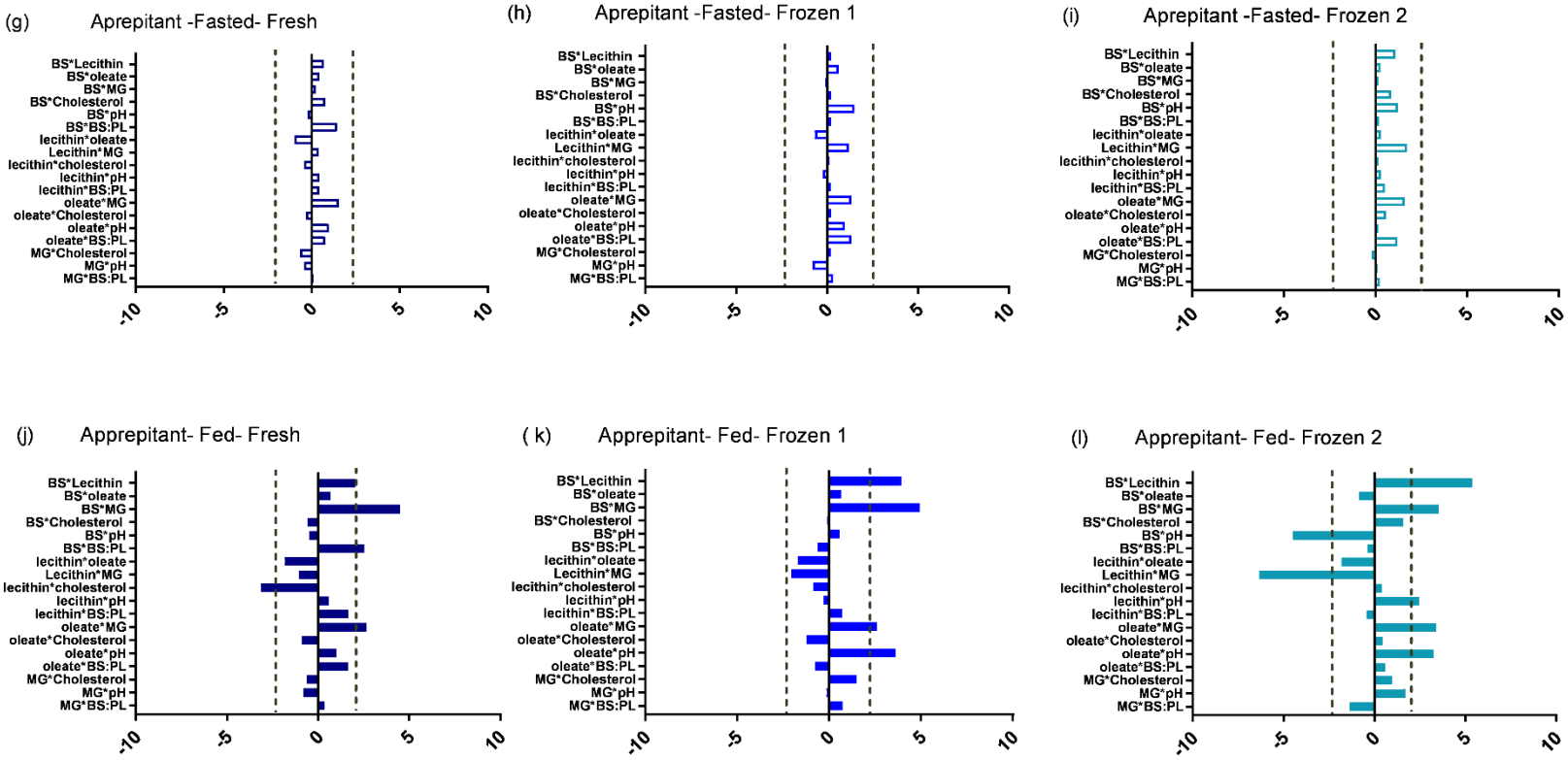
For the acidic drug indomethacin (Figure 3.6 (a-f)), in fasted state, the interaction between oleate and pH was significant in all three experiments. However, frozen 2 experiment showed an additional significant interaction which is the interaction between lecithin and pH. In the fed state, none of the possible interactions were significant in all three experiments.

For the basic drug aprepitant (Figure 3.6 (g-l)), in the fasted state, no significant interactions were indicated in all three experiments. In the fed state, similar significant interactions between frozen experiments and fresh experiment were found in bile salt with either lecithin or monoglyceride and sodium oletae with monoglyceride. However, additional significant interactions that is different in each experiment were indicated (Figure 3.6 (g-l)), with frozen 2 experiment in total showed higher number

of significant interactions (7 out of 18) compared to the frozen 1 or fresh (5 out of 18) experiment.

For the neutral drug fenofibrate (Figure 3.6 (m-r)), in fasted state comparable results were found between fresh and frozen media solutions since the interaction between oleate and pH was significant in all three experiments. In the fed state, comparable results between all three media solutions were found in bile salt with either lecithin or monoglyceride, oleate with monoglyceride and oleate with pH as all these factor interactions were significant. However, a variation in results between the fresh experiment and frozen experiments were found with the frozen 2 solution since more significant interactions (bile salt with either oleate or pH; lecithin with oleate, MG or pH) were found.


(a) Indomethacin- Fasted- Fresh
(b) Indomethacin- Fasted- Frozen 1
(c) Indomethacin- Fasted- Frozen 2
(d) Indomethacin-Fed- Fresh
(e) Indomethacin-Fed- Frozen 1
(f) Indomethacin-Fed- Frozen 2
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*Cholesterol
oleate*pH
oleate*BS:PL
MG*Cholesterol
MG*pH
MG*BS:PL
-10
-5
0
5
10


(g) Aprepitant -Fasted- Fresh
(h) Aprepitant -Fasted- Frozen 1
(i) Aprepitant -Fasted- Frozen 2
(j) Apprepitant- Fed- Fresh
(k) Apprepitant- Fed- Frozen 1
(l) Apprepitant- Fed- Frozen 2
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*Cholesterol
oleate*pH
oleate*BS:PL
MG*Cholesterol
MG*pH
MG*BS:PL
-10
-5
0
5
10

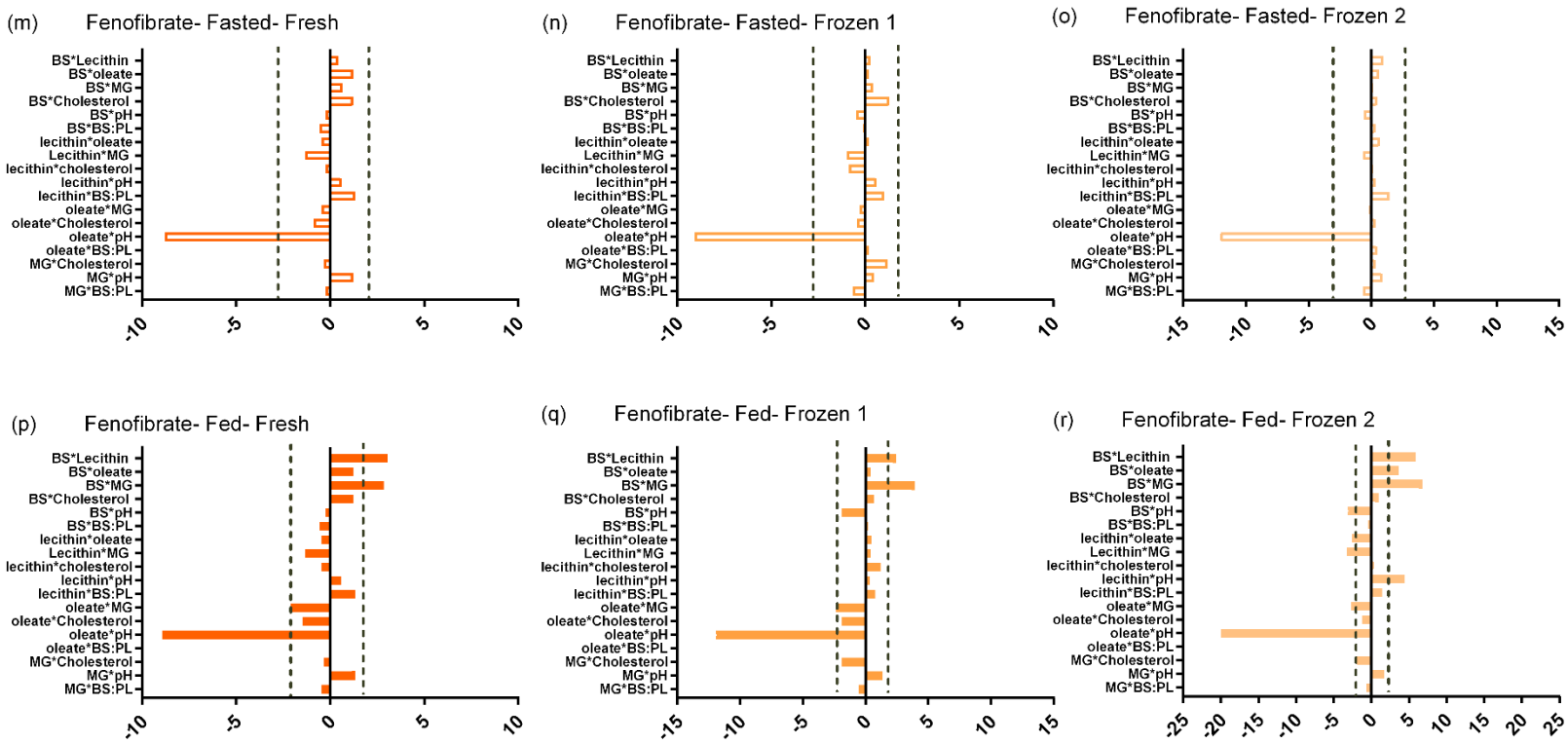


Figure 3.6: Standardised effect value for all DoE factor interactions on equilibrium solubility in fasted and fed study arms. DoE standardised effects values for factor interactions (as listed on y- axis) on equilibrium solubility. Bar colour indicates drug category: red= acid (dark red= fresh, red= frozen 1, light red= frozen 2). Blue= base (dark Blue = fresh, Blue = frozen 1, light Blue = frozen 2). Orange= neutral (dark Orange = fresh, Orange = frozen 1, light Orange = frozen 2).

3.2.4 Influence of DoE factors on solubility in combined study arm

3.2.4.1 Influence of individual DoE factors on solubility

The standardised effect value for each factor in each experiment in the combined (fasted +fed) study arm have been presented in Figure 3.7. Table 3.1 reported the statistical significant factor of each experiment.

The presence of three experiments each with seven factors to be examined in the combined state (fasted +fed) allows for 63 possible values to be compared. Out of the possible 63 values, 26 (around 41 %) were significant. Starting with the factors affecting drug solubility in the fresh experiment, out of the possible 21 values, 8 were significant. Secondly, in frozen 1 experiment, 9 out of the possible 21 values were significant. Finally, in frozen 2 experiment, 9 out of the possible 21 values were significant. This indicates that all three experiments had showed comparable incidence of significant factors.

For the acidic drug indomethacin (Figure 3.7 (a,b,c)), all three experiments showed similar significant factors, where pH, oleate and bile salt were significant. However, the compatible effect was in the significant effect but not in the magnitude, as both frozen1 and frozen 2 experiments showed a higher magnitude of effect and this is with regard to pH effect.

For the basic drug aprepitant (Figure 3.7 (d,e,f)), frozen experiments showed several differences compared to the fresh experiment since bile salt (in both frozen1 and frozen 2), cholesterol (in frozen 1), and pH (in frozen2) were significant which is in contrast with the fresh experiment since none of these factors were significant. In addition, the ratio between bile salt and lecithin was significant with the fresh experiment but not with either of the frozen experiments. However, sodium oleate and monoglyceride had a significant effect in the frozen experiments, which matched with the significant effect of the same factors in the fresh experiment.

For the neutral drug fenofibrate (Figure 3.7 (g,h,i)), all three experiments showed similar results where oleate and pH were significant. The compatibility between the three experiments were in regard to the significant effect and the magnitude of factor effect.

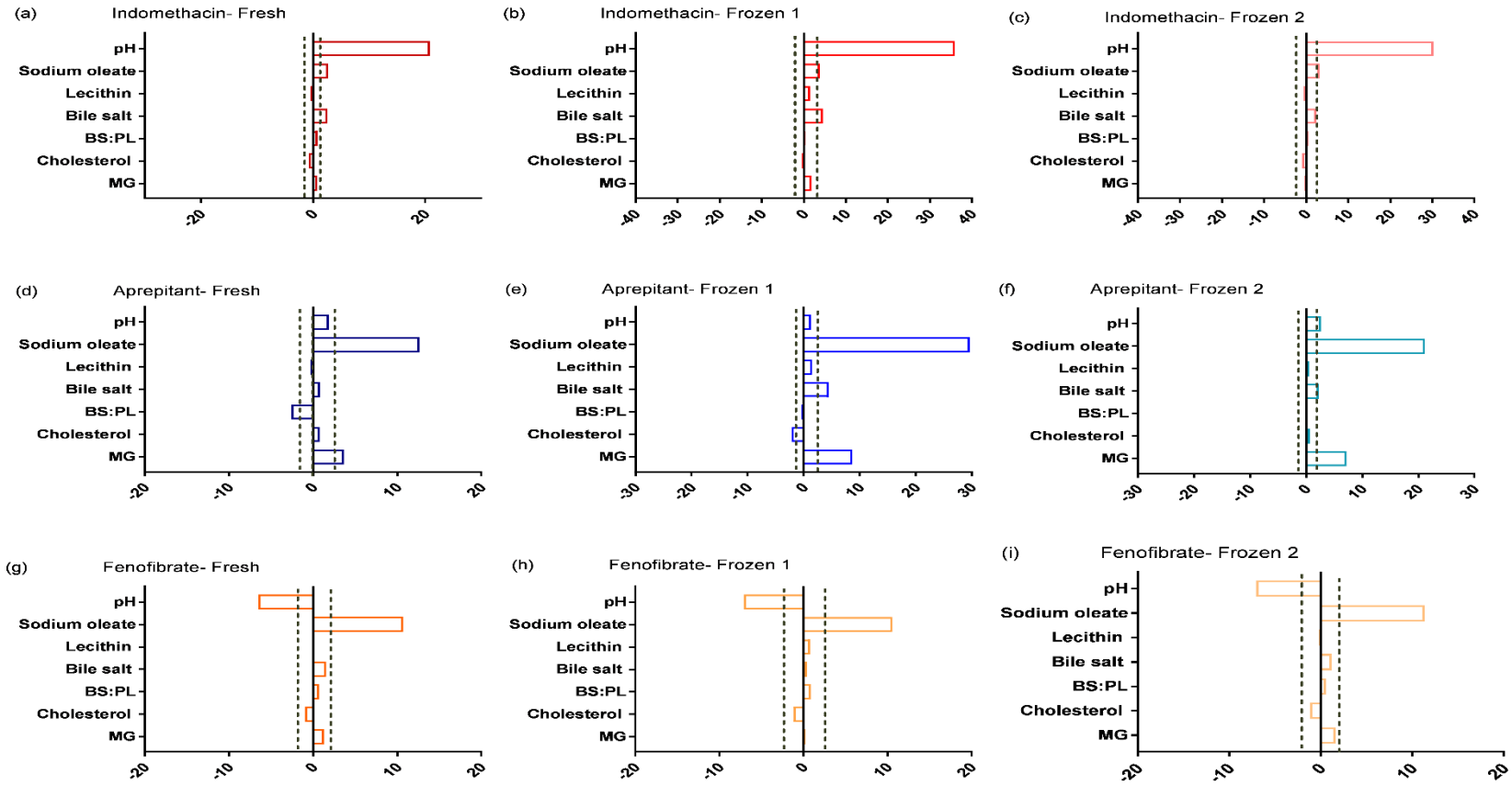


Figure 3.7: Standardised effect value for all DoE factors on equilibrium solubility in combined study arm. DoE standardised effects values for factors (as listed on y- axis) on equilibrium solubility. Bar colour indicates drug category: red= acid (dark red= fresh, red= frozen 1, light red= frozen 2). Blue= base (dark Blue = fresh, Blue = frozen 1, light Blue = frozen 2). Orange= neutral (dark Orange = fresh, Orange = frozen 1, light Orange = frozen 2).

Table 3.1: Comparison of the statistical significance of DOE factors across experiments.

	factors																				
	pH			oleate			lecithin			bile salt			cholesterol			BS:PL			monoglyceride		
Drugs	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed
Indomethacin fresh.	S	S	S	S	S	S	NS	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Frozen 1	S	S	S	S	S	S	NS	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Frozen 2	S	S	S	S	S	S	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Aprepitant fresh	S	NS	NS	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	S
Frozen 1	S	NS	NS	S	S	S	NS	NS	NS	NS	S	S	NS	NS	S	NS	NS	NS	S	S	S
Frozen 2	S	NS	S	S	S	S	NS	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	S	S	S
Fenofibrate fresh	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Frozen 1	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Frozen 2	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

The red row for acidic drug, blue row for basic drug and yellow row for the neutral. S means the factor had a significant effect, NS means the factor had a non-significant effect. The significant results are highlighted with grey.

3.2.4.2 Influence of factor interactions on solubility

In the combined state (fasted +fed), an experiment consisting of seven factors allows a possible of twenty-one interactions in each experiment (fresh, frozen 1 and frozen 2). The standardised effect value for each factor interaction for each experiment in the combined arm have been presented in Figure 3.8.

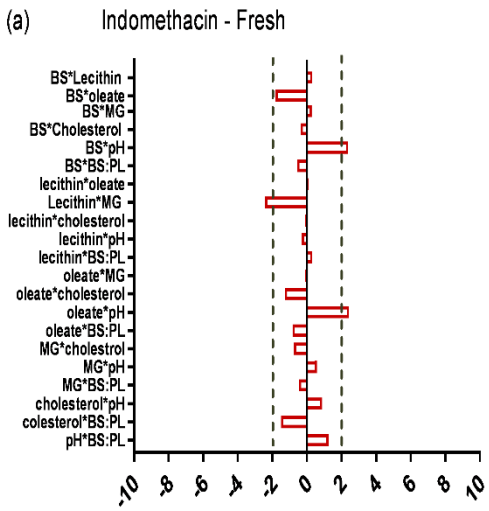
In the combined arm (fasted +fed), twenty-one interactions in each experiment gave a total of 189 possible values, and out of the 189 possible values 53 (around 28%) were significant. In each experiment, out of the 63 possible values 17, 18 and 20 significant values were reported in fresh, frozen 1and frozen 2 experiments respectively. This indicates that all three experiments showed comparable incidence of significant factors.

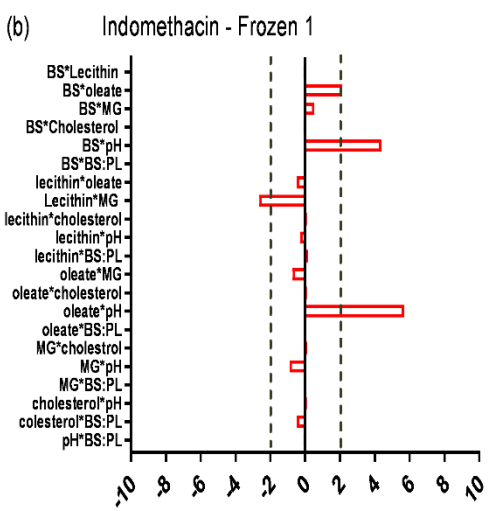
For the acidic drug indomethacin (Figure 3.8 (a, b ,c)), the frozen experiments showed compatible results with the fresh experiment in all significant factor interactions seen with the fresh one(Figure 3.8) but with varying magnitude of factor interaction effect (frozen experiments showed higher values). The varied results were seen in the significant effect of factor interaction between bile salt and oleate since it was significant with both frozen experiments but not in the fresh experiment.

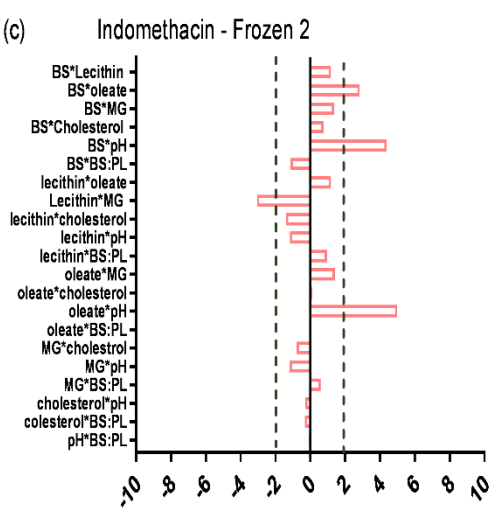
For the basic drug aprepitant (Figure 3.8 (d, e, f)), comparable results between both frozen experiments and fresh experiment were seen only in five (bile salt with lecithin or MG; MG with lecithin or oleate and oleate with pH) out of the ten significant values, obtained with fresh experiment (Figure 3.8 (d)) which is around 50%. The frozen experiments showed significant interaction between lecithin and pH which is in contrast to the fresh experiment since this interaction was not significant.

For the neutral drug fenofibrate (Figure 3.8 (g, h, i)), all significant interactions seen in fresh experiment (Figure 3.8 (g)) were seen with the frozen experiments (Figure 3.8 (h and i)). However, frozen experiments showed different results compared to fresh experiment where more factors indicated to be significant such as oleate with MG in

both frozen experiments, MG with pH in frozen 1 experiment and pH with the ratio between BS: PL in frozen 2 experiment (Figure 3.8 (h and i)).


(a)
Indomethacin - Fresh
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
MG*pH
MG*BS:PL
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10


(b)
Indomethacin - Frozen 1
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
MG*pH
MG*BS:PL
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10


(c)
Indomethacin - Frozen 2
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
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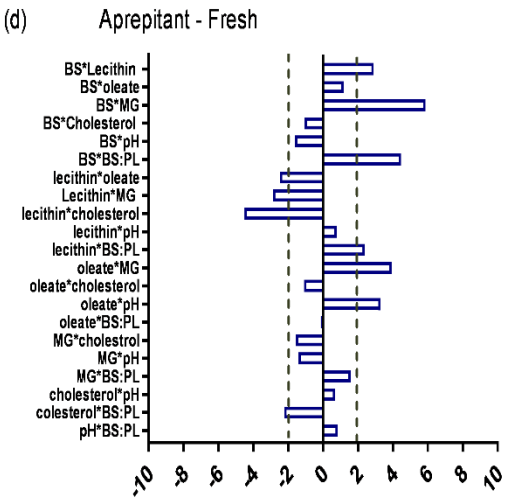
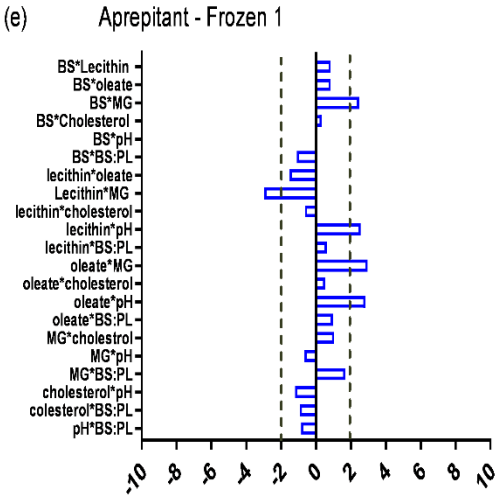
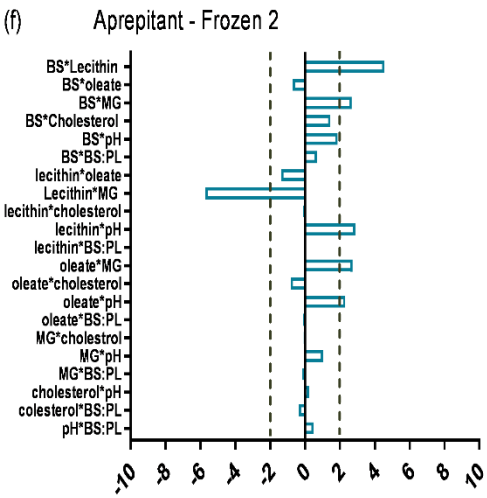
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10


(d)
Aprepitant - Fresh
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
MG*pH
MG*BS:PL
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10


(e)
Aprepitant - Frozen 1
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
MG*pH
MG*BS:PL
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10


(f)
Aprepitant - Frozen 2
BS*Lecithin
BS*oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
lecithin*oleate
Lecithin*MG
lecithin*cholesterol
lecithin*pH
lecithin*BS:PL
oleate*MG
oleate*cholesterol
oleate*pH
oleate*BS:PL
MG*cholestrol
MG*pH
MG*BS:PL
cholesterol*pH
colesterol*BS:PL
pH*BS:PL
-10 -8 -6 -4 -2 0 2 4 6 8 10

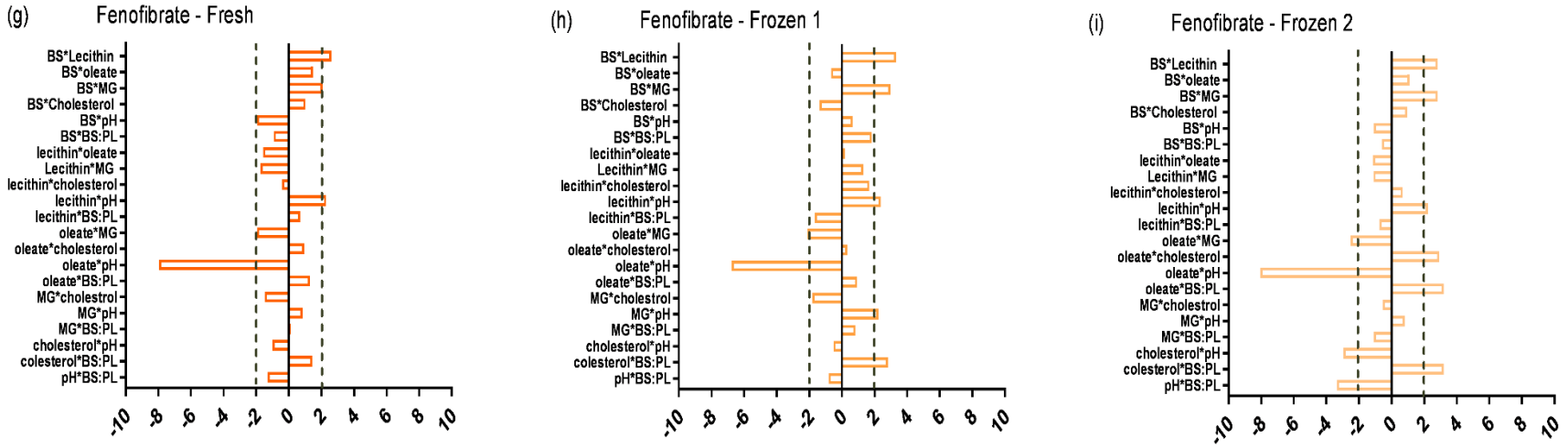


Figure 3.8: Standardised effect value for all DoE factor interactions on equilibrium solubility in combined study arm. DoE standardised effects values for factor interactons (as listed on y- axis) on equilibrium solubility. Bar colour indicates drug category: red= acid (dark red= fresh, red= frozen 1, light red= frozen 2). Blue= base (dark Blue = fresh, Blue = frozen 1, light Blue = frozen 2). Orange= neutral (dark Orange = fresh, Orange = frozen 1, light Orange = frozen 2).

3.2.5 Statistically significant solubility factors and factor interactions

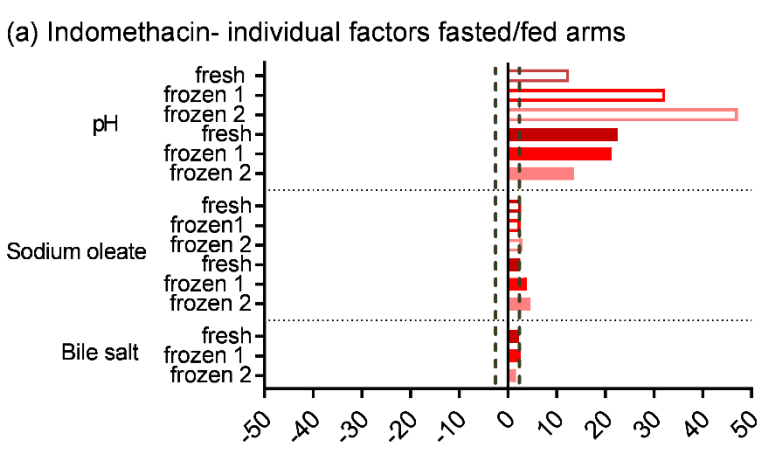
The significant standardised effect value for each factor and factor interactions in the fresh fasted, fed and combined (fasted +fed) study arms have been presented in Figure 3.9, along with the significant standardised effect value for same factor and factor interactions in the fasted, fed and combined study arms of frozen experiments (frozen 1 and frozen 2) in order to summarize the experimental results.

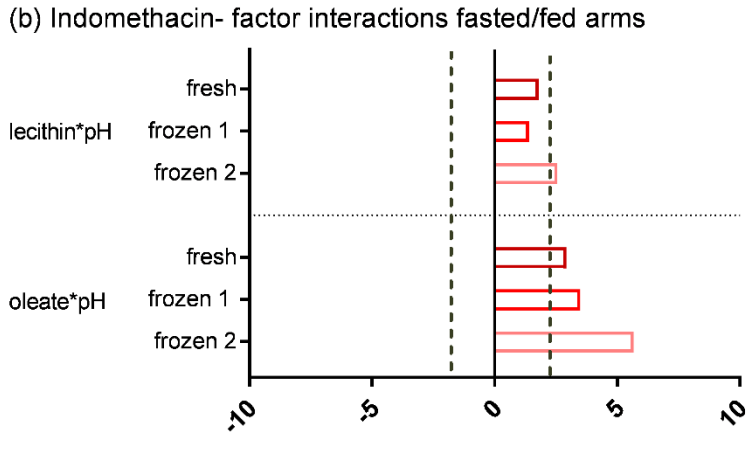
For the acidic drug indomethacin (Figure 3.9 (a-d)), comparing fresh experiment with the frozen experiments in all three study arms, compliance was found in 12 out of 12 (100%) in frozen 1 and 11 out of 12 (around 92%) in frozen 2 of the determined significant values of individual factors and factor interactions but with a varying degree in magnitude of effect. Agreement between frozen 1 and frozen 2 experiments were found in almost all significant factors and factor interactions. The frozen experiments were able to indicate the most significant factors affecting drug solubility which is in compliance with published literature (Khadra et al., 2015; Zhou et al., 2017) and with the fresh experiment, but with the frozen experiments experiencing higher amount of factor effect (pH in fasted state arm). Two factor interactions (bile salt with oleate and lecithin with pH) were found to be significant with the frozen experiments but not in the fresh. These differences indicate that the changes occurred in the media composition might be due to changes in the pH of the media, leading to the increased ionization of these media components (oleate, bile salt and lecithin).

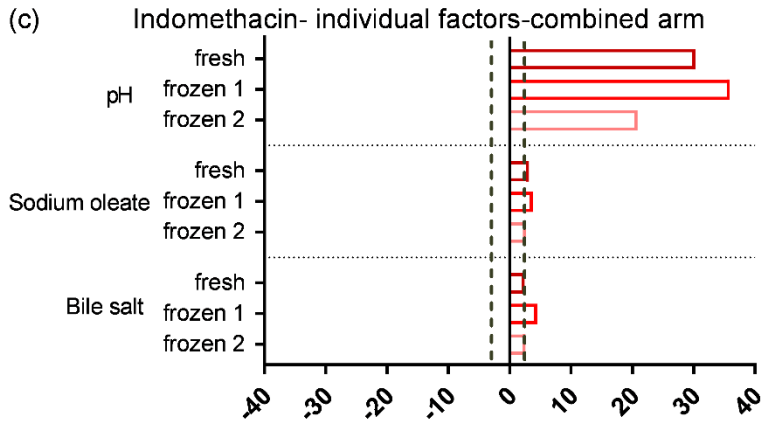
For the basic drug aprepitant (Figure 3.9 (e-h)), in all three study arms, the frozen experiments were able to indicate the most significant factors affecting drug solubility with oleate and pH significant in the fasted state arm (Khadra et al., 2017) and oleate in the fed and combined arms (Zhou et al., 2017; Perrier et al., 2018). Comparing fresh experiment with the frozen experiments indicates that frozen 2 experiment detected a higher incidence of significant factors and factor interaction in all three study arms with, 21 significant values in fresh, 21 in frozen 1 and 23 in frozen 2. However, although same number of significant factors were found in both fresh and frozen 1 but the latter experiment showed different significant factors and a higher

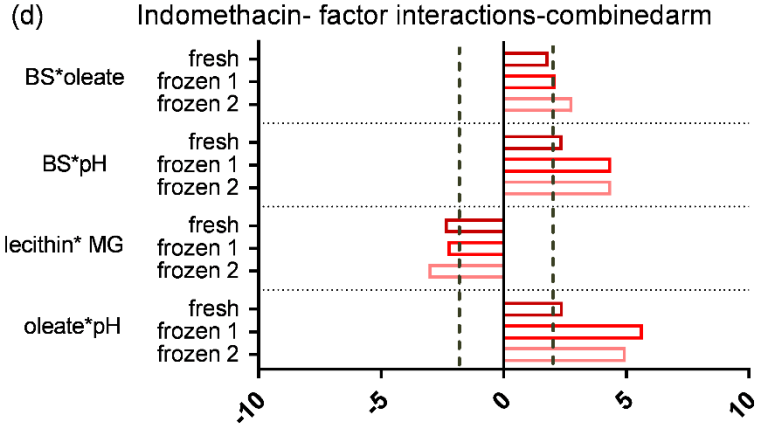
magnitude of factor effect (sodium oleate, Figure 3.9 (e and g)). Differences between the experiments appeared either due to a different magnitude of factor effect, or due to the presence of other media factors that are significant with the frozen experiments but not in fresh experiment, such as bile salt in both the combined and fed state arms and monoglyceride in the fed state arm. These different additional significant factors together with the higher magnitude of oleate effect in the frozen experiments make the explanation regarding media changes presented above for indomethacin applicable for aprepitant too.

For the neutral drug fenofibrate (Figure 3.9 (i-l)), in all three study arms compliance between the fresh experiment and frozen experiments was found for all individual factors in both the significant effect and the magnitude of effect. Differences between the three experiments were found in factor interactions where 9 significant values were determined with the fresh experiment, 12 with frozen 1 and 20 with frozen 2 in all three study arms. This indicates that with fenofibrate, differences between the three experiments occurred only in the factor interactions and not in the individual factor effect nor the magnitude of factor effect. This indicates that these changes might be due to experimental or analytical differences and not to the media changes induced by freezing the stock solution.

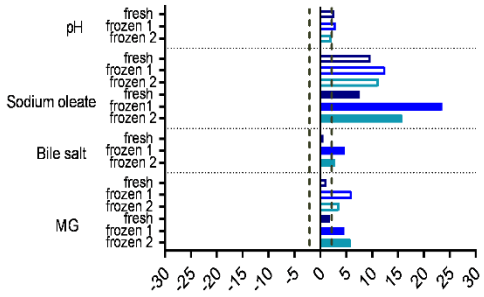

(a) Indomethacin- individual factors fasted/fed arms
pH
Sodium oleate
Bile salt
fresh
frozen 1
frozen 2
frozen1
-50
-40
-30
-20
-10
0
10
20
30
40
50


(b) Indomethacin- factor interactions fasted/fed arms
lecithin*pH
oleate*pH
fresh
frozen 1
frozen 2
-10
-5
0
5
10

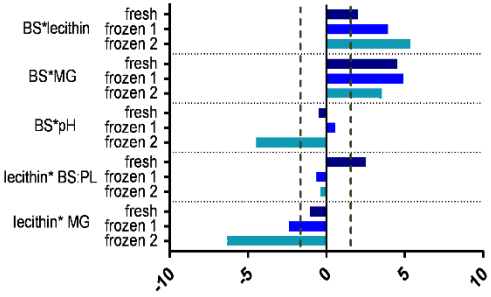

(c) Indomethacin- individual factors-combined arm
pH
Sodium oleate
Bile salt
fresh
frozen 1
frozen 2
-40
-30
-20
-10
0
10
20
30
40


(d) Indomethacin- factor interactions-combinedarm
BS*oleate
BS*pH
lecithin* MG
oleate*pH
fresh
frozen 1
frozen 2
-10
-5
0
5
10

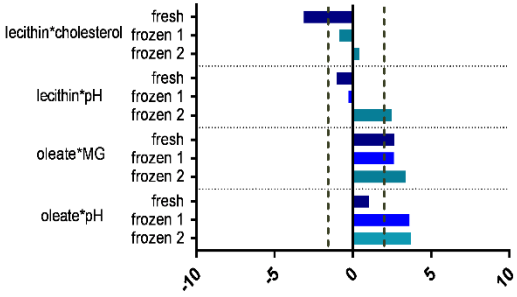
(e) Aprepitant- individual factors fasted/fed arms



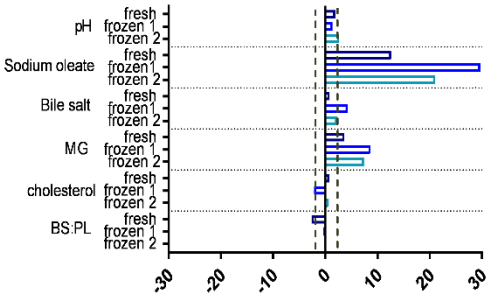
(f) Aprepitant- factor interactions fasted/fed arms



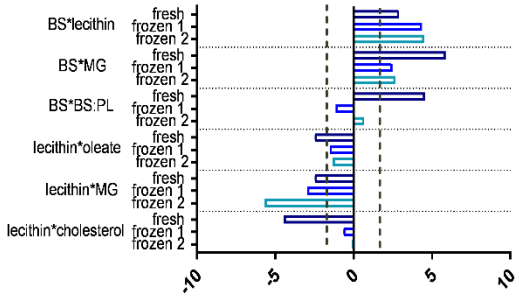
(f) Aprepitant- factor interactions fasted/fed arms -cont..



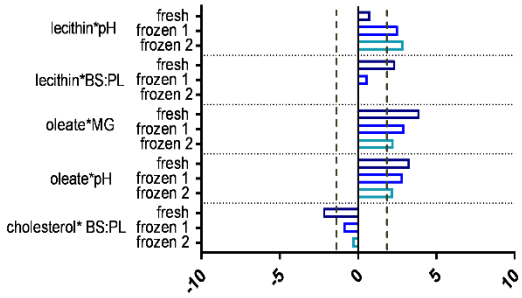
(g) Aprepitant- individual factors combined arm



(h) Aprepitant -factor interactions combined arm



(h) Aprepitant -factor interactions combined arm- cont..



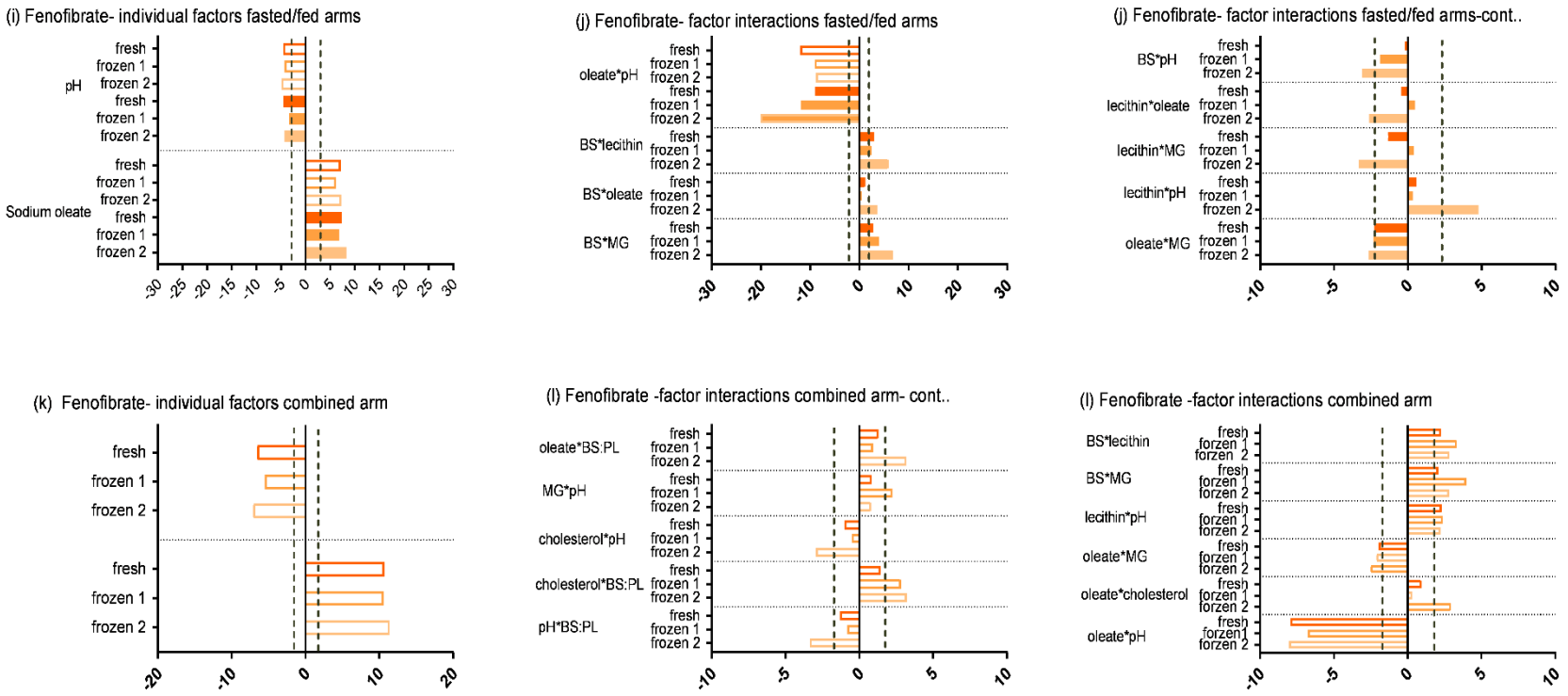


Figure 3.9: Significant standardised effect value for all DoE factors and factor interactions on equilibrium solubility in all three study arms (fasted, fed and combined). DoE standardised effects values for factor or factor interactions (as listed on y- axis) on equilibrium solubility. Bar colour indicates drug category: red= acid (dark red= fresh, red= frozen 1, light red= frozen 2). Blue= base (dark Blue = fresh, Blue = frozen 1, light Blue = frozen 2). Orange= neutral (dark Orange = fresh, Orange = frozen 1, light Orange = frozen 2).

3.3 Discussion

3.3.1 Equilibrium solubility measurements

The equilibrium solubility results in all three arms of the study and three experiments (fresh, frozen 1, frozen 2) are presented in Figure 3.3 and 3.4 and indicates that the measurements of drug solubility of the frozen experiments are in a broad agreement with drug solubility using freshly prepared stock solution. In addition, the results showed individualistic drug behaviour, with aprepitant and indomethacin exhibiting minor variabilities between fresh experiment and frozen experiments in fasted arm and combined arm while fenofibrate showed no variabilities in all three study arms (fasted, fed and combined).

3.3.2 Statistical comparisons of solubility

The generation of solubility data sets for each drug in each replicate permits a statistical comparison between fresh and frozen experiments and this is presented in Figure 3.4. The examination of the fresh and frozen experiments indicates that for all of the results (except aprepitant in the fed state) the solubility distribution is non-normal, a feature that is in compliance with the published fasted, fed and full range study (Khadra et al., 2015; Zhou et al., 2017; Perrier et al., 2018). The non- normal distribution of all data sets together with the normal distribution of aprepitant in the fed state were discussed in the previous chapter section 2.4.2.

Comparing the equilibrium solubility data set of the fresh experiment with the frozen experiments indicates that variabilities were detected in 10 out of the possible 36 solubility data sets in all study arms, with in all significant differences, solubility values in the frozen experiments were higher than the fresh solubility values. Figure 3.9 indicates that these differences arise due to the higher number of significant factors affecting solubility when examined in the frozen experiments compared to the fresh one. A feature that when coupled with the higher factor magnitude in these experiments (frozen 1 and frozen 2) leads to a higher solubility values.

For the acidic drug indomethacin, in the fasted study arm the comparison between the solubility data in fresh experiment and frozen experiments indicates that the frozen experiments showed higher solubility values than the fresh one. This might be related to the fact that freezing of biologics can cause complex physical and chemical changes in media conditions (Kolhe et al., 2010; Singh et al., 2009; Kolhe et al., 2012) which will consequently affect the different media micellar components. Indomethacin is an ionisable drug and pH was reported in literature as the main solubility driver of the drug (Khadra et al., 2015; Zhou et al., 2017) which is in compliance with the results in this study. Interestingly, the frozen experiments indicated that pH was also the main solubility driver but with a higher magnitude of effect comparing the three replicates (fresh= 12.6, frozen 1= 32.33, frozen 2 = 47.26). This indicates that freezing might cause media modifications which might influence other factors in media and consequently increasing the impact of the main drug solubility driver. In the fed state, none of the frozen media showed a significant differences compared to the fresh media. This might be related to the lower influence of pH (main solubility driver) in the fed media state compared to the fasted by comparing the magnitude of factor effect in each state as per graph (3.4), where it is obvious that fasted state solubility values are more affected by the three pH levels of the experiment.

For the basic drug aprepitant, significant differences between fresh experiment and frozen experiments arose in the fasted state. Aprepitant is an ionisable drug whose solubility is known to be affected by pH (Khadra et al., 2015; zhou et al., 2017). In addition it has a log *p* value of 4.5 and consequently lipophilic media components such as oleate will have an influence on solubility (Khadra et al., 2015; Zhou et al., 2017). In this study sodium oleate was the main solubility driver with the highest number of magnitude. Remarkably, frozen experiments indicated similar factors but with a higher magnitude of oleate effect comparing the three experiments (fresh= 9.68, frozen 1= 19.55, frozen 2= 15.28). This make the analysis above for indomethacin relevant for aprepitant too, where freezing the media leads to several changes that will consequently increase the impact of the main solubility driver (oleate) of the drug. However, more studies and replication of the experiments are required to indicate if these differences were due to the freezing of the media or it's related to the

experimental or analytical differences. Interestingly, the fed state showed no significant differences between the three experiments which indicates the lower sensitivity of that state to changes due to media freezing compared to the fasted state. A result that was similar to the finding with the indomethacin drug where solubility of the fasted state was more affected than the fed state.

With the neutral drug fenofibrate, none of the frozen experiments in each media state (fasted or fed) showed a significant difference with the fresh experiment. This indicates that the non-ionisable fenofibrate drug was not affected by freezing the media in the fasted state nor in fed state. The results showed that pH was significant with the different experiments of fenofibrate and this effect was reported in published fasted and fed DoE (Khadra et al., 2015; Zhou et al., 2017) as an effect of pH on the different media components and not on the drug itself.

3.3.3 Standardised effect values

The determined significant standardised effect values presented in Figure 3.9 for all three arms (fasted, fed and combined) indicates that frozen experiments determined more significant factor and factor interactions that affect drug solubility. In the fasted, fed and combined study arms of fresh experiment, out of the 159 possible values 48 were significant while, in frozen experiments, 52 in frozen 1 and 62 in frozen 2 were significant. Interestingly, differences in main factors affecting drug solubility were seen only with the ionisable drugs indomethacin and aprepitant but not with the neutral drug fenofibrate, indicating that freezing the stock solution will causes some media changes that will affect ionisable drugs' solubility. Based on Figure 3.9, the frozen experiments were able to determine the most significant factors known to affect drug solubility but with a higher number of magnitude, which make the differences in solubility between the replicates significantly different in comparison with the fresh experiment. Differences between both frozen media solutions were seen mainly in the factor interactions even when there is no significant differences in solubility between solutions. These differences were obvious also with fenofibrate since the frozen 2 experiment showed higher incidence of significant factors compared to the

fresh experiment, although there were no significant differences in solubility between the three replicates. In addition to the experimental or analytical differences, this might also be an effect of freezing. As ice crystal grow they remove water from solutions and exclude the solute leading to a zone were solute is at higher concentrations (Singh et al., 2009; Bhatnagar et al., 2008). As an impact of the post frozen structure of the system, different response of each drug and each stock solution to the available factors will occur.

3.4 **Conclusions**

The results indicate that using frozen stock solution rather than freshly prepared solution will provide comparable equilibrium solubility values in both fasted and fed study arms. The results also indicate that changes in media composition due to the freezing of the stock solution will affect solubility ranges of ionisable drugs leading to an overestimated solubility values but not for the non-ionisable drug. The results also indicates that freezing simulated media will affect solubility of ionisable drugs in fasted state and that fed state solubility will not be affected. The outcomes also indicate that frozen experiments will be able to determine the main factors affecting drug solubility but with a greater magnitude and with the addition to the appearance of other significant factors. However, in order to have the full image, more drugs in each category should be examined to indicate if the effect of freezing will be affecting ionisable drugs only or it will be drug specific effect. Moreover, replication of each frozen experiments should be carried out to trace the media changes occurring due to freezing of the solution. In conclusion, frozen experiments are valuable investigations that would save effort though more work required.

4 Dual level statistical investigation of the effect of excipients on equilibrium solubility of fenofibrate and carvedilol in simulated fasted and fed intestinal fluid

This study shows the modification of the 20 dual range DoE study (Ainousah et al., 2017) by the addition of different types of excipient to the media to investigate their effects on the solubility of fenofibrate and carvedilol. This involved expanding the 20-experiment dual range design to 36 experiments in order to avoid the statistical limitation of the reduced experimental number while preserving an acceptable experimental load. A 36-experiment dual range DoE covering both fasted and fed states in a single experiment with bio relevant factor levels (Table2:1, see above section 2.1.4) was conducted to determine the equilibrium solubility of the model drugs fenofibrate and carvedilol in the presence of chitosan, mannitol, HPMC E3, HPMC E50, PVP lower grade and PVP higher grade at concentrations of (0.5% w/v) and (5% w/v). These concentrations were chosen to examine the effect of excipients on solubility of the representative drugs when the excipient is at lower concentration (0.5 % w/v) acting as a bulking agent for example and at higher concentration (5% w/v) when the excipient act as disintegrants for example. This was achieved by using seven gastrointestinal media parameters (bile salt, lecithin, sodium oleate, monoglyceride, cholesterol, pH and BS:PL) as factors to determine whether each affects drug solubility positively or negatively, while the different excipients were added to the media to identify any significant effect on total solubility.

4.1 Material and method

4.1.1 Materials

The same materials used as above in section 3.2.1 with the addition of Polyvinylpyrrolidone (pharmaceutical grade, Plasdone ^{TM} K-12 (batch number 0001885427) and Plasdone ^{TM} K-29/32 (batch number 0001954666) from Ashland. Methocel E50 (batch number DT 238358) and Methocel E3 (batch number DT426742) (pharmaceutical grade premium LV hydroxypropyl methylcellulose) were obtained from Colorcon Ltd., Kent, UK. Mannitol (pharmaceutical grade) was obtained from

Blackburn Distributions, Nelson, UK. Chitosan (from crab shells, practical grade) were purchased from Sigma-Aldrich, Poole, Dorset, UK. NB: From the nine BCS class II compounds (Phenytoin, Indomethacin, Aprepitant, Tadalafil, Zafirlukast, Carvedilol, Felodipine, Fenofibrate, Probucol), Only fenofibrate and carvedilol were used in this experiment.

4.1.2 Dual level design of experiment and data analysis

For each media parameter (bile salt, lecithin, sodium oleate, monoglyceride, cholesterol, pH and BS: PL ratio), lower and upper limit concentration values for fasted and fed states were defined (Table3:1). Using Minitab® 17.2.1 and a custom experimental design, a 1/8 of the full factorial DoE with seven factors and two levels (lower and upper limits) was constructed (16 experiments around the upper and lower levels plus two centre points) separately for the fasted and the fed states. These two tables were then used as input to a factorial custom design which combined the fasted and fed states using all 36 data points to provide an overall analysis. The study therefore consisted of three arms: two smaller (18 data point) fasted and fed arms, which were then merged into a larger (36 data point) combined arm.

Due to the design and the low number of experiments, the standardized effect values calculated for individual factor effects in the fasted and fed state arms and in the combined arm indicate a significant increase in drug solubility when it is greater than +2 and a decrease when it is less than -2. For the two-way interactions in the fasted and fed arms, of which seven were identified, the standardized effect is considered to indicate a significant increase in drug solubility when it is greater than +4 and a decrease when it is less than -4. In the combined arm, 21 possible interactions were found, all negative for all data.

The Kolmogorov normality test was used in Minitab® to assess the normality distribution of each data set, the Mann-Whitney test was used to evaluate the median between two data sets (not normally distributed) and the two sampled t-test used to evaluate the mean of two data sets (normally distributed).

4.1.3 Equilibrium solubility measurement

Lipid suspension and oleate preparation: see above section 2.2.4

Excipient solution: The concentration level of the excipient was calculated to be (5% w/v) or (0.5% w/v) of the total solubility of the drug in the fed state (Table 4:1). The concentration of each excipient was designed to be 15 times greater than the highest solubility value of the drug (fenofibrate or carvedilol) in the fed state (Table4:2) except for the HPMC and chitosan where only a five times concentration was possible (Table4:3).

Table 4. 1: The composition level of the added excipient

Drug	Excipient (mM)	
	0.5% w/v	5%w/v
Fenofibrate	0.0095	0.095
Carvedilol	0.011	0.11

Table 4. 2: Excipient volume to be added to the media in fasted and fed state at (0.5% w/v) and (5% w/v)

Concentration	0.5 % w/v		5% w/v	
Media	Fasted	Fed	Fasted	Fed
Volume (µl)	20.2	26.7	202.2	267

Table 4. 3: Excipient volume to be added to the media in fasted and fed state at 0.5%and 5% concentration of HPMC (E3 and E50)

Concentration	0.5 % w/v		5% w/v	
Media	Fasted	Fed	Fasted	Fed
Volume (µl)	60	80	589.5	800

Preparation of lipid suspension

See above section 2.2.4

Preparation of sodium oleate solution

See above section 2.2.4

Preparation of buffer solutions

See above section 2.2.4

4.1.4 Preparation of measurement solutions

Individual DoE run solutions were prepared as follows. An excess amount (10 mg, above the estimated solubility) of solid for each compound investigated was added to a centrifuge tube (15 ml Corning® Centristar cap, polypropylene RNase/DNase free, nonpyrogenic), then each component of the simulated intestinal fluid media was added according to the run order generated by the DoE. After that the excipient to be examined was added to each tube. Once all of the media components had been added with the excipient the same followed steps in section 2.2.5 were done.

4.2 Results

4.2.1 Equilibrium solubility measurements

The results of the equilibrium solubility measurements in fenofibrate and carvedilol are presented in (Figure 4:1 (a) and (b)). In comparison with the control, some of the data indicate a uniform solubility range, while others show varied solubility values depending on the type and concentration of excipient and the media state (fasted or fed) investigated. The results indicate that each excipient has its own characteristics which, together with the used concentration, produced a specific effect on the solubility of fenofibrate or carvedilol. It is visually evident that Mannitol, for example, had no effect on solubility with both drugs, while chitosan change both drugs solubility at the higher concentration (5% w/v). (Figure 4:2 a-l) depicts the dual level

equilibrium solubility results for the control and each excipient at different concentrations (0.5 % w/v and 5% w/v) in the fasted and fed states, along with a statistical comparison of each excipient and its concentration solubility values with that of the control.

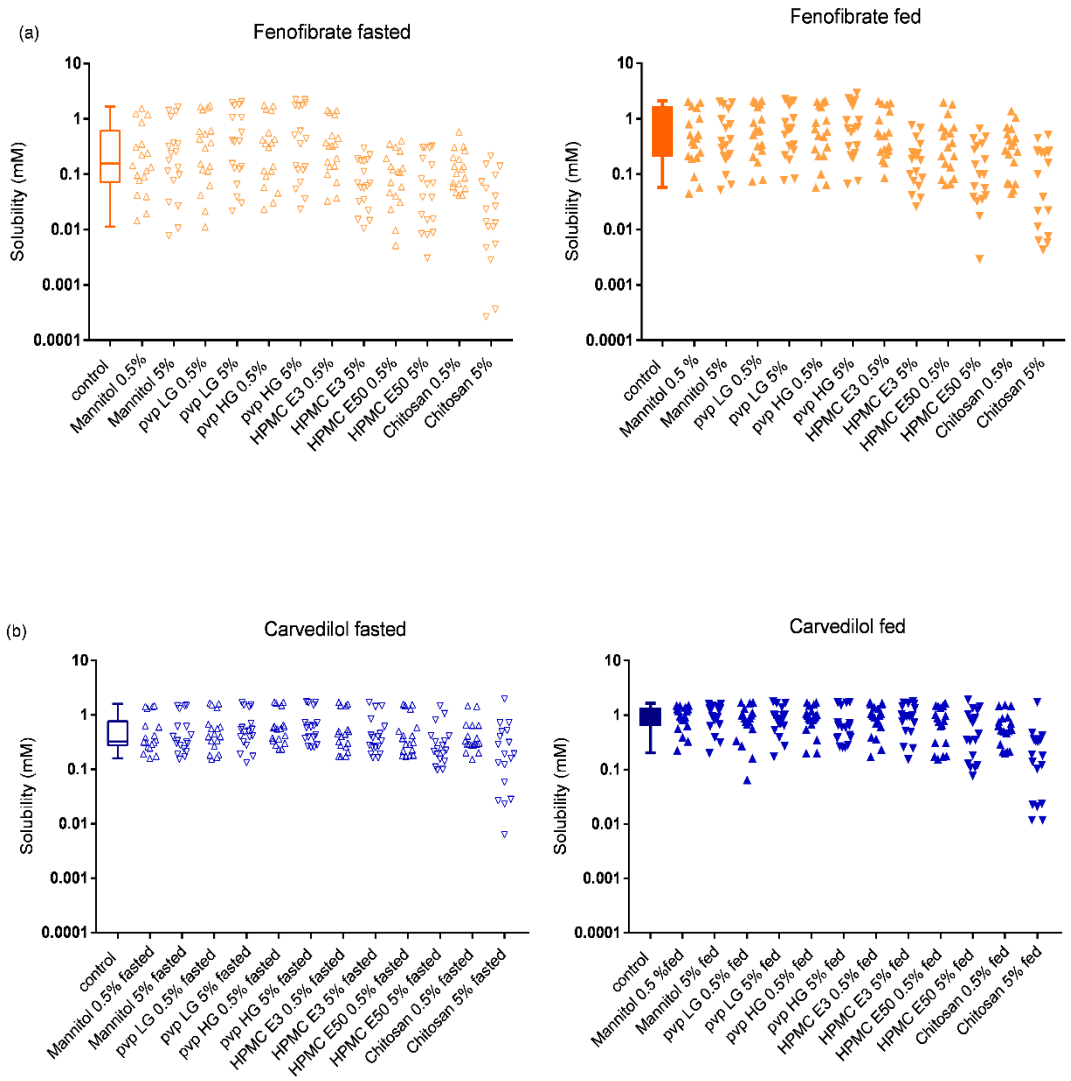
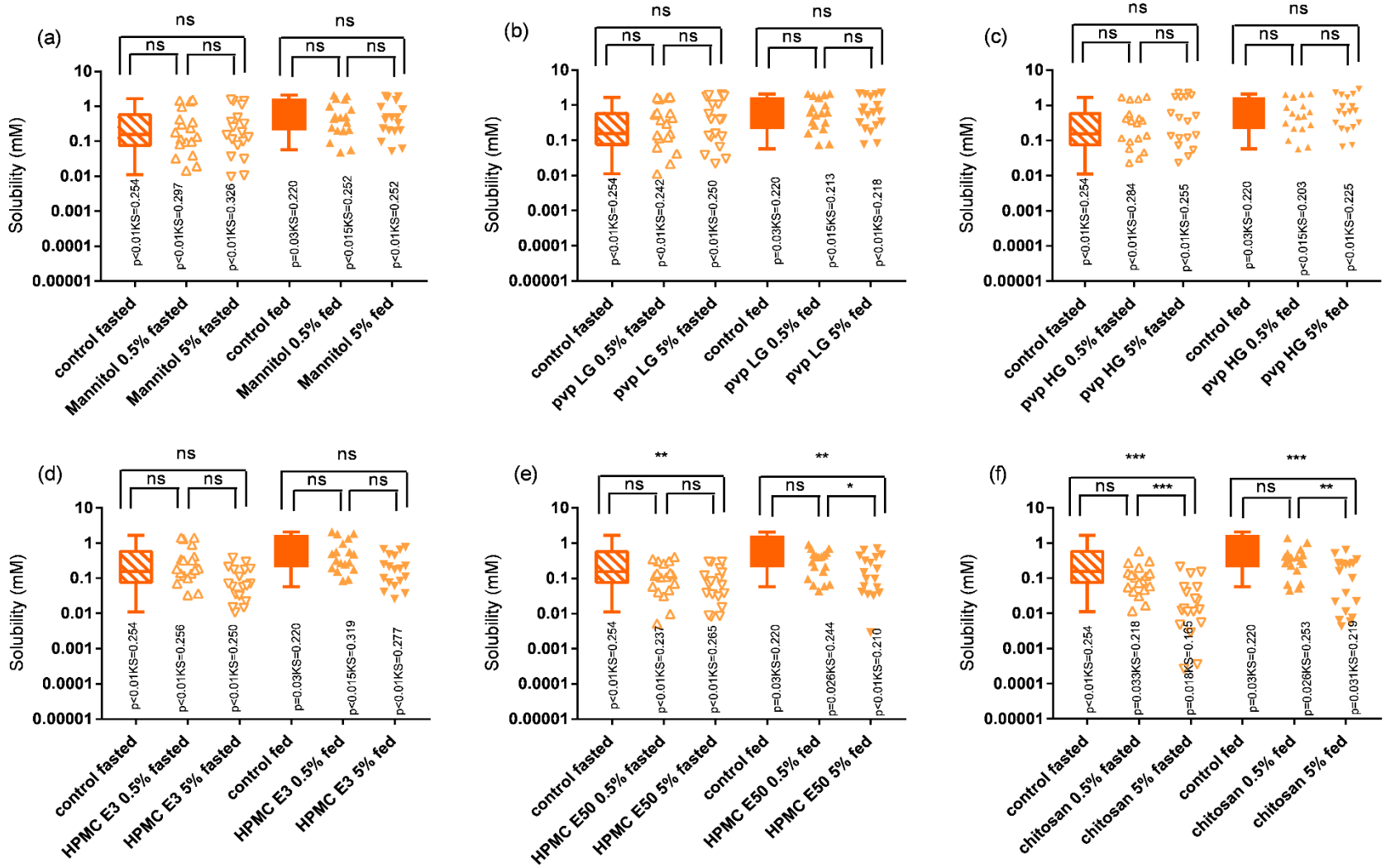


Figure 4.1: Design of experiment equilibrium solubility measurements for (a) fenofibrate and (b) carvedilol in presence of different types of excipients in media: Box and whisker plots for control dark orange for fenofibrate (open box= fasted, closed box= fed) and dark blue for carvedilol (open box= fasted, closed box= fed), from top to bottom the maximum value, 75th percentile, median, 25th percentile and minimum value. Triangle data points for the different excipients in media, orange for fenofibrate and blue for carvedilol. Open symbols for fasted media condition, closed symbols for fed. Δ open symbol for 0.5 % concentration of excipient in fasted state and ▲ closed symbol for 0.5% concentration of excipient in fed state. ▽Open symbol for 5 % concentration of excipient in fasted state and ▼ closed symbol for 5% concentration of excipient in fed state.

4.2.2 Statistical comparison

The generation of a solubility data set for fenofibrate and carvedilol with the different types of excipients permits a statistical comparison between these data which is, presented in (Figure 4:2). A statistical examination indicates that for fenofibrate all of the 26 data sets had non-normal distributions and for carvedilol 12 out of the 26 data sets had normal distribution. The non-normality distribution was discussed in relation to the earlier DoE studies section 2.4.2 (Ainousah et al., 2017) and found to arise either from the non-normal sample pattern induced by the DoE structure, from the fact that drug solubility was not normally distributed throughout the sample space or because the sample was not sufficiently large. The normal distribution of some of the data set in carvedilol was also discussed previously (Ainousah et al., 2017) which was in relation to the narrow range distribution of carvedilol solubility plus the small number of the experiment. A statistical comparison between the control and the different types of excipients indicates that 41 out of the 48 data showed no significant difference in solubility when excipients were added to the control media. Also, 7 out of the 48 studied data showed a significant difference in solubility. A statistical examination between the two different concentrations of the excipients (0.5% w/v and 5% w/v) showed that 20 out of the 24 examined data showed no significant differences in solubility when the concentration of the excipient was increased from (0.5% w/v) to (5% w/v) and that 4 out of the 24 data showed differences in solubility when excipient's concentration increased.

In both fenofibrate and carvedilol, four out of the six examined excipients (mannitol, PVP lower grade and higher grade, and HPMC E3) showed no significant differences in solubility at either concentration (0.5% w/v and 5% w/v) or in both states (fasted or fed), see Figure 4.2 (a-d) and (g-j). For both fenofibrate and carvedilol, the results showed that HPMC E50 showed a significant difference in solubility when the higher excipient concentration (5% w/v) was added to the simulated media in the fed state of each drug (Figure 2.4 (e and k)). For chitosan increasing the concentration to (5% w/v) showed a significant differences in solubility in both fasted and fed state of both fenofibrate and carvedilol (Figure 4.2 (f and l).


(a)
(b)
(c)
(d)
(e)
(f)
Solubility (mM)
10
1
0.1
0.01
0.001
0.0001
0.00001
ns
**
***
*
p<0.01KS=0.254
p<0.01KS=0.297
p<0.01KS=0.326
p=0.03KS=0.220
p<0.015KS=0.252
p<0.01KS=0.252
p<0.01KS=0.242
p<0.01KS=0.250
p<0.015KS=0.213
p<0.01KS=0.218
p<0.01KS=0.284
p<0.01KS=0.255
p<0.015KS=0.203
p<0.01KS=0.225
p<0.01KS=0.256
p<0.01KS=0.250
p<0.015KS=0.319
p<0.01KS=0.277
p<0.01KS=0.237
p<0.01KS=0.265
p=0.026KS=0.244
p<0.01KS=0.210
p=0.033KS=0.218
p=0.018KS=0.165
p=0.026KS=0.253
p=0.031KS=0.219
control fasted
Mannitol 0.5% fasted
Mannitol 5% fasted
control fed
Mannitol 0.5% fed
Mannitol 5% fed
pvp LG 0.5% fasted
pvp LG 5% fasted
pvp LG 0.5% fed
pvp LG 5% fed
pvp HG 0.5% fasted
pvp HG 5% fasted
pvp HG 0.5% fed
pvp HG 5% fed
HPMC E3 0.5% fasted
HPMC E3 5% fasted
HPMC E3 0.5% fed
HPMC E3 5% fed
HPMC E50 0.5% fasted
HPMC E50 5% fasted
HPMC E50 0.5% fed
HPMC E50 5% fed
chitosan 0.5% fasted
chitosan 5% fasted
chitosan 0.5% fed
chitosan 5% fed

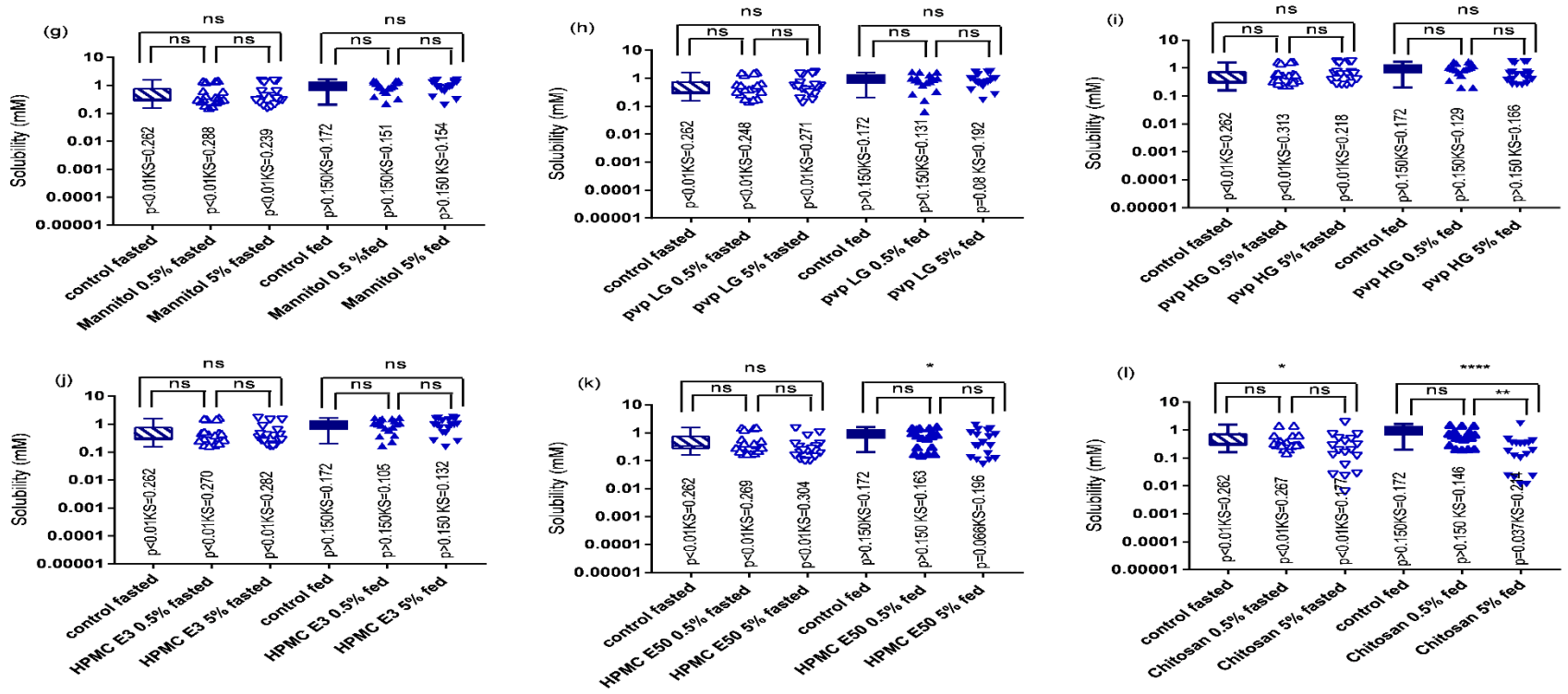


Figure 4.2: Statistical comparison of design of experiment equilibrium solubility measurements for both fenofibrate and carvedilol. Box and whisker plots: separate fasted and fed control equilibrium solubility data. Scatter plots: separate fasted and fed equilibrium solubility data for the different excipients. KS Kolomogrov normality test on the data set, $p < 0.05$, indicates a non-normal distribution. Comparison bars: Mann-Whitney test, not significant (ns) if $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ and **** $p \leq 0.0001$.

4.2.3 Influence of individual DoE factors and type of excipient on solubility values in the fasted and fed arms

The standardized effect values for each factor, in the fasted and fed arms are presented in Figures 4.3, with a value of ±2 significant for the individual factor effect. A comparison between the significant factor in fasted and fed state of the control with those in media containing excipients is presented in Table 4.3.

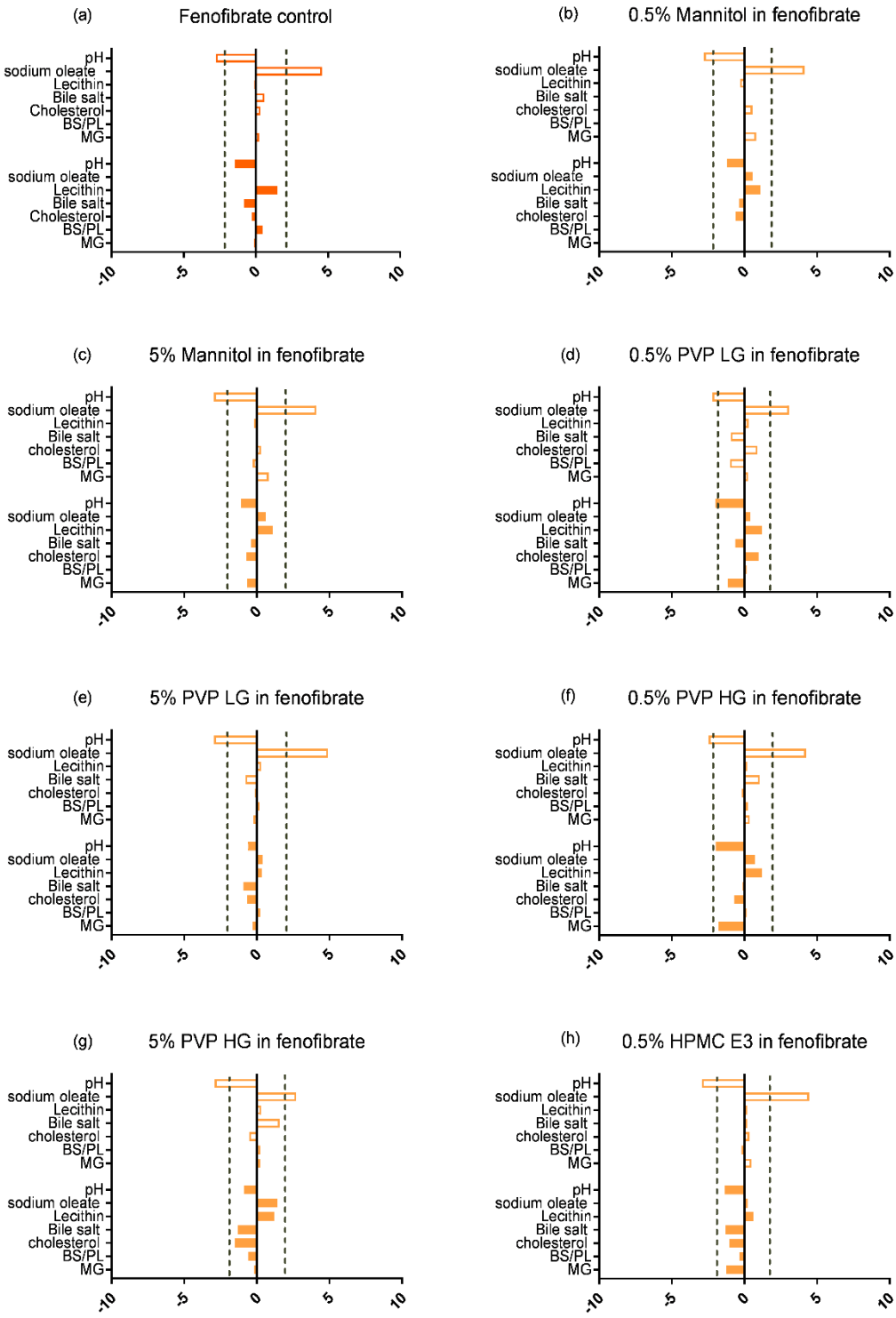
Starting with the control, Figure 4.3 (a) shows that in fenofibrate sodium oleate and pH were the factors with the highest magnitude of effect on solubility in the fasted state and there was no significant factors in the fed state. The effects of sodium oleate and pH on solubility are consistent with the earlier DoE studies in the fasted state (Ainousah et al., 2017; Khadra et al., 2015) but not in the fed state, as both factors were significant (Ainousah et al., 2017; Zhou et al., 2017). This might be due to the difference in experimental number between the studies and the different arrangement of the factors in each design. This is comparable to the results of the 9 DoE study (an OrBiTo work under publication) where the number of the experiments had an influence on the number of significant factors. For carvedilol (Figure 4.3 (n)), oleate and pH were significant in both fasted and fed state and this was in compliance with the published literatures (Khadra et al., 2015; Zhou et al., 2017; Ainousah et al., 2017). It should be noted that there are no published studies examining the effect of the following excipients on solubility in simulated media.

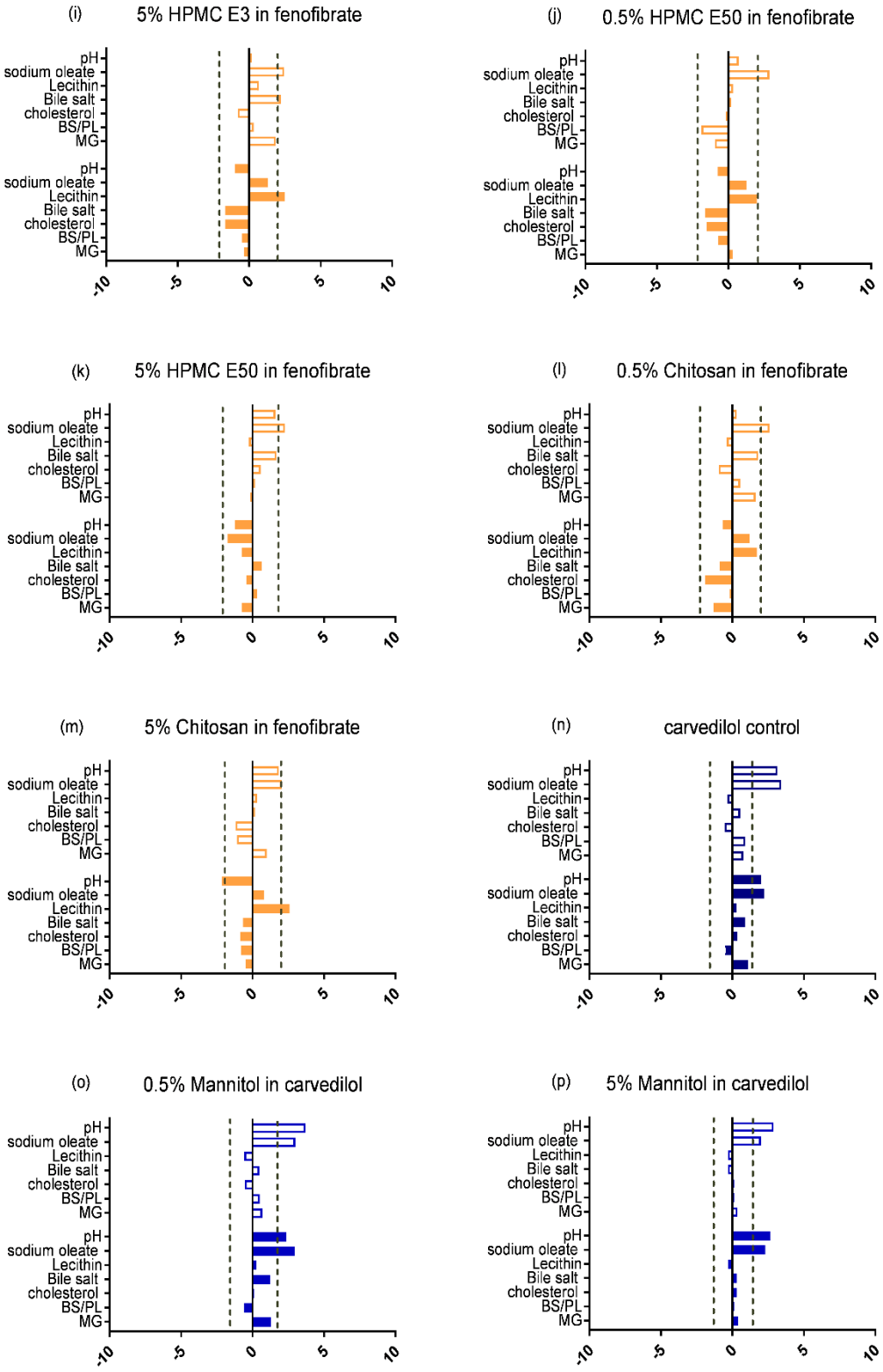
In media where the excipients was added to fenofibrate (Figure 4.3 (b-m), among the 168 possible values of the single factor effect in the fasted and fed arms, compatibility with the control was found in 146 of the different values (around 87%). Furthermore, It was evident that some of the excipients (both mannitol concentrations, both PVP grades concentrations and the lower concentration of HPMC E3) made no changes in the media based on the compatibility of the significant factors and magnitude of standardised effect values. While others (both concentrations of chitosan and HPMC E50 and the higher concentration of the HPMC E3) had an impact on the media leading to changes in the significant factors and magnitude of standardised effect values.

However, increasing the concentration of HPMC E3 to (5% w/v) showed some changes in significant factors where bile salt and pH were significant in the fasted state and lecithin in the fed state. Nevertheless, this was different with HPMC E50 and chitosan where both lower (0.5% w/v) and higher (5% w/v) concentrations showed different significant factors compared to the control. For HPMC E50 both (0.5% w/v and 5% w/v) showed oleate as the only significant factor and pH was not significant in fasted state although fed state showed comparable results with the control as there were no significant factors. For chitosan, the (0.5 % w/v) showed oleate as the only significant factor while increasing the concentration to (5% w/v) showed that only oleate was significant in the fasted state and that lecithin and pH were significant in the fed state where pH was directing the solubility of fenofibrate to the negative way.

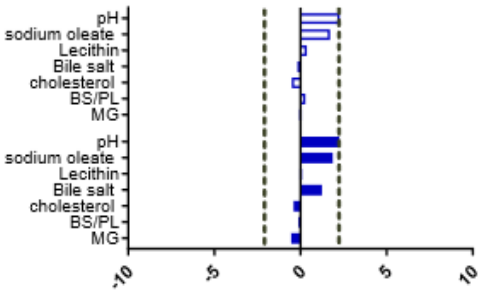
In media where excipient were added to carvedilol (Figure 4.3 (o-z)), among the 168 possible values of the single factor effect in the fasted and fed arms, compatibility with the control was found in 149 of the different values (around 89%) and it was evident that some of the excipients (both mannitol concentrations and the lower concentration of HPMC E3) made no changes in the media based on the compatibility in the significant factors and magnitude of standardised effect values. While others (both concentrations of chitosan, both PVP grades and HPMC E50 and the higher concentration of HPMC E3) impacted on the media leading to alteration in the significant factors and magnitude of standardised effect values. The results demonstrated that both concentrations of mannitol and the (0.5% w/v) concentration of HPMC E3 showed no differences in significant factors compared to the control in both fasted and fed state. However, increasing the concentration of HPMC E3 to (5% w/v) showed an additional significant factor in the fed state which is bile salt. Both PVP grades (LG and HG) and concentrations (0.5% w/v and 5% w/v) showed pH as the only significant factor in fasted and fed state and oleate was not significant in all cases. In the case of HPMC E50 and chitosan, several changes occur to the media where for HPMC E50, the (0.5% w/v) concentration is missing oleate as a significant factor in the fed state and increasing the concentration to (5% w/v) leads to the absence of pH as a significant factor in the fasted state and both pH and oleate in the fed state. For chitosan the (0.5% w/v) concentration showed zero significant factors in both

fasted and fed state while increasing the concentration to (5% w/v) showed that pH in fasted state and oleate in fed state were significant and both were influencing drug solubility to the negative direction.

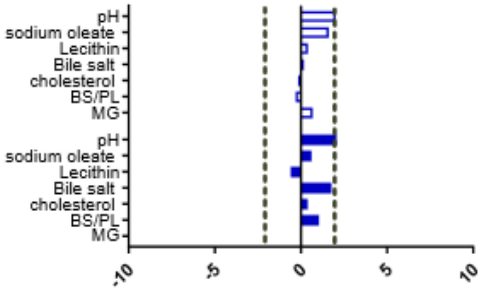

(a)
Fenofibrate control
(b)
0.5% Mannitol in fenofibrate
(c)
5% Mannitol in fenofibrate
(d)
0.5% PVP LG in fenofibrate
(e)
5% PVP LG in fenofibrate
(f)
0.5% PVP HG in fenofibrate
(g)
5% PVP HG in fenofibrate
(h)
0.5% HPMC E3 in fenofibrate
pH
sodium oleate
Lecithin
Bile salt
Cholesterol
cholesterol
BS/PL
MG
-10
-5
0
5
10


(i) 5% HPMC E3 in fenofibrate
(j) 0.5% HPMC E50 in fenofibrate
(k) 5% HPMC E50 in fenofibrate
(l) 0.5% Chitosan in fenofibrate
(m) 5% Chitosan in fenofibrate
(n) carvedilol control
(o) 0.5% Mannitol in carvedilol
(p) 5% Mannitol in carvedilol
pH
sodium oleate
Lecithin
Bile salt
cholesterol
BS/PL
MG
-10
-5
0
5
10

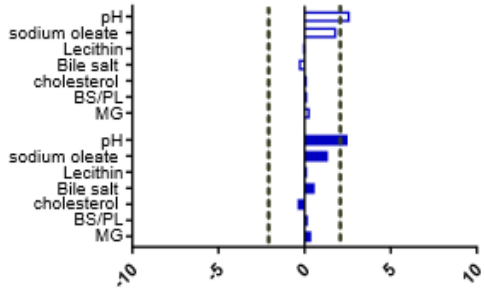
(q) 0.5% PVP LG in carvedilol



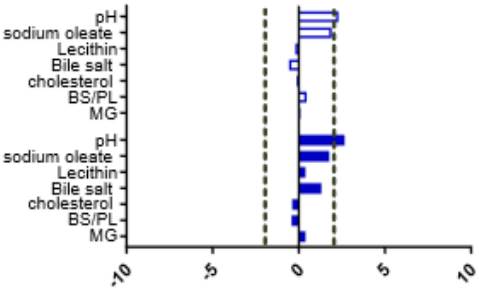
(r) 5% PVP LG in carvedilol



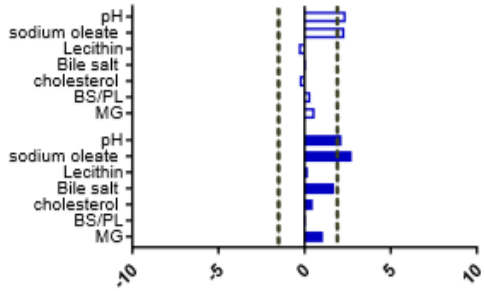
(s) 0.5% PVP HG in carvedilol



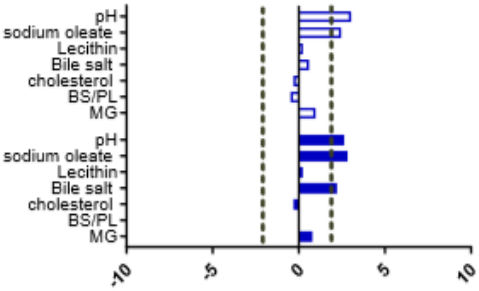
(t) 5% PVP HG in carvedilol



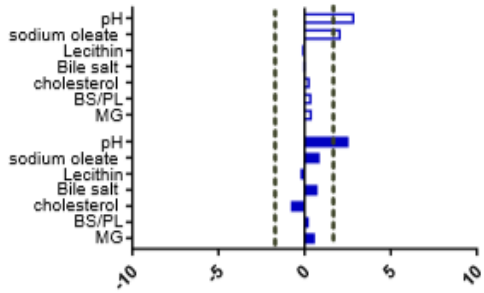
(u) 0.5% HPMC E3 in carvedilol



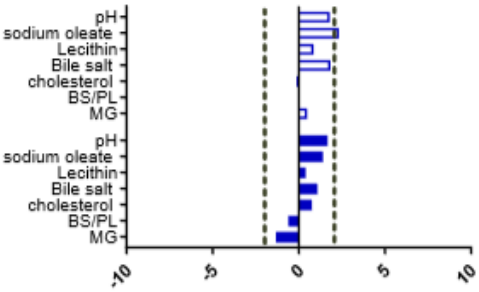
(v) 5% HPMC E3 in carvedilol



(w) 0.5% HPMC E50 in carvedilol



(x) 5% HPMC E50 in carvedilol



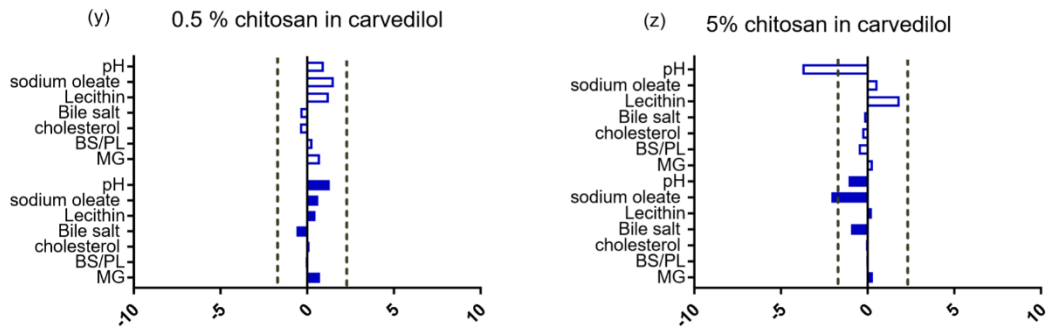


Figure 4.3: Standardised effect values for individual DoE factors on equilibrium solubility in fasted and fed study arms of both fenofibrate and carvedilol: DoE standardized effect values for factors (as listed along y-axis) on equilibrium solubility. Fasted result: empty histogram bar, Fed result: filled histogram bar. Vertical black lines indicate statistical significance ($p < 0.05$, NB significance value = ±2). Horizontal bar direction indicates direction of effect, to the right of 0 on axis means positive effect on solubility, bar length indicates the magnitude of the effect.

4.2.4 Influence of factor interactions and type of excipient on equilibrium solubility in fasted and fed arms

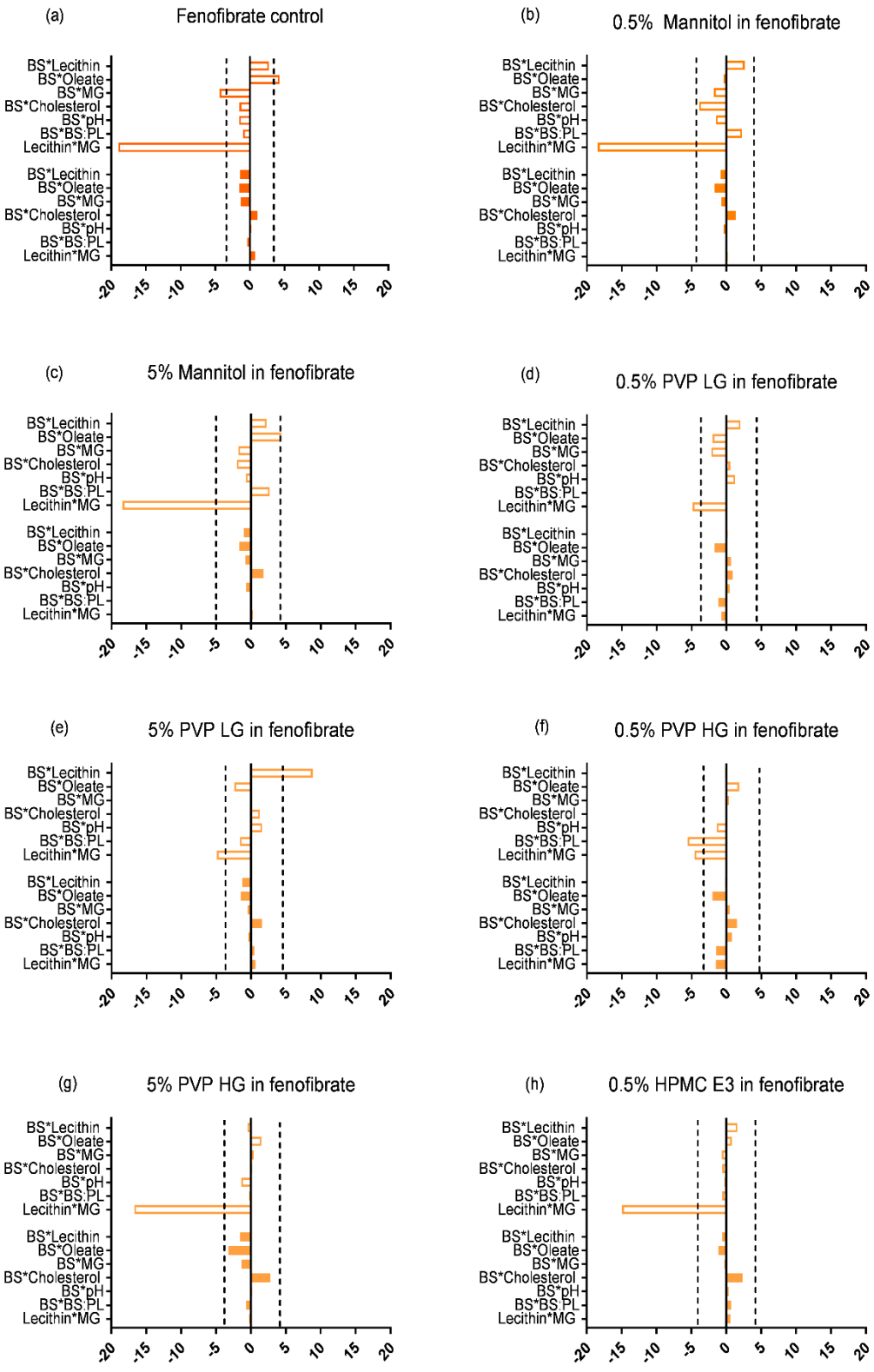
The standardised effect value for each factor interactions in the fasted and the fed state arms are presented in Figure 4.4. Due to the lower number of the experiments in the data set, a value of ±4 is considered to be significant for the factor interactions and only seven interactions could be determined.

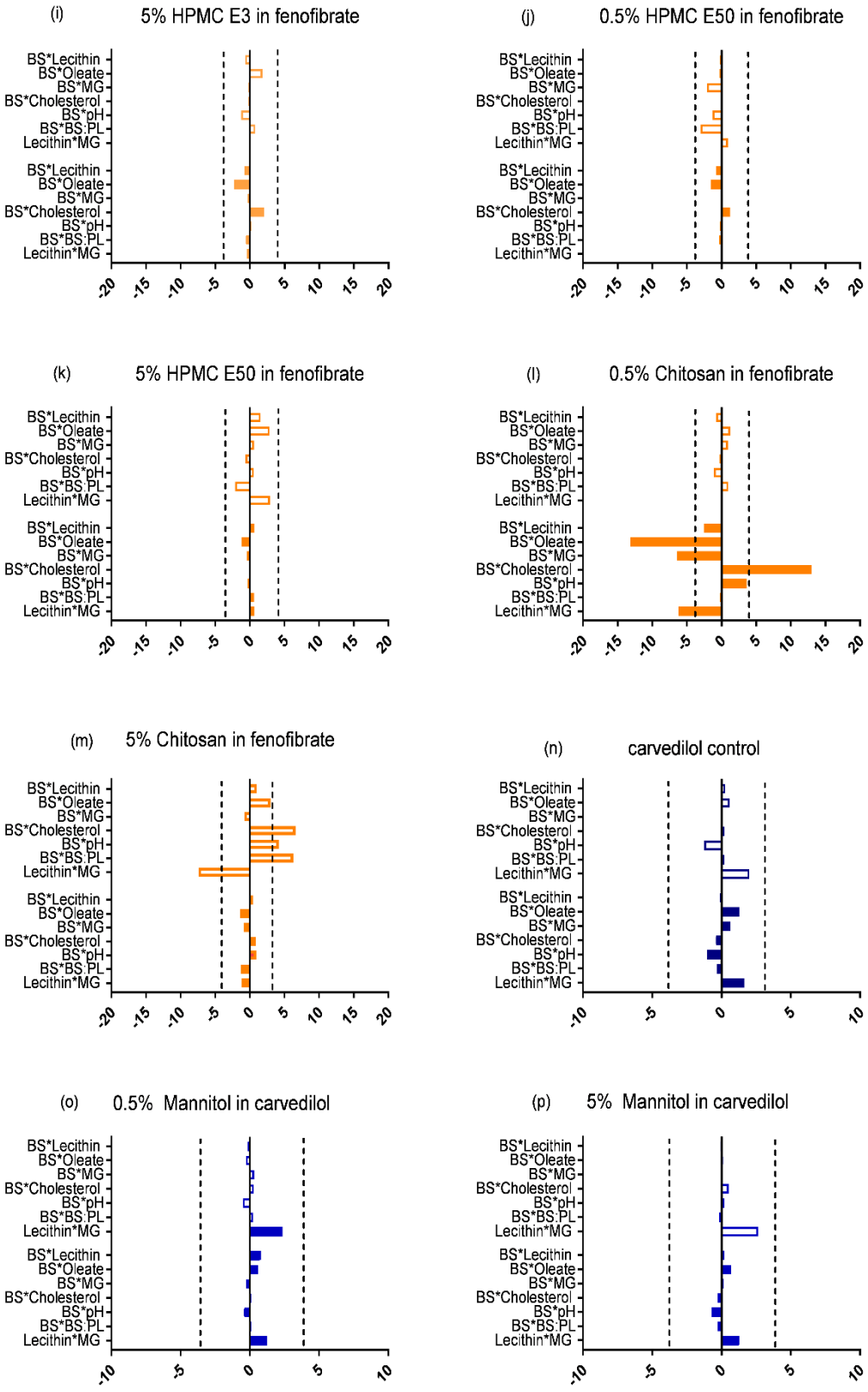
Starting with the control, fenofibrate (Figure 4.4 (a)) shows only three significant interactions (bile salt with oleate or monoglyceride and lecithin with monoglyceride) in the fasted state and no significant interactions were determined in the fed state. For carvedilol control (Figure 4.4 (n)), no interactions were significant in either fasted or fed state.

In media where excipients were added to fenofibrate (Figure 4.4 (b-m), compliance between control and media containing the different types of excipients were found in 131 (around 78%) out of the 168 possible values of the interactions. Differences between the control and the different media containing the excipients arose mainly from either absence of some of the interactions that were significant with the control

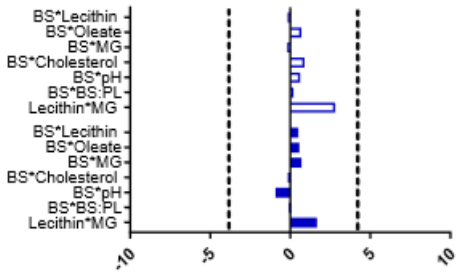
(for example all of the significant fasted state control interactions became not significant with HPMC E50), or they arose from the presence of new significant interactions in media with excipients (for example the significant interaction between bile salt and oleate or monoglyceride in the fed state of media containing chitosan).

In media where excipients were added to carvedilol (Figure 4.4 (o-z)), compliance between control and media containing excipients were found in 162 (around 96%) out of the 168 possible values of the interactions. Compatibility between control and excipients containing media were found in five (mannitol, both PVP grades and both HPMC grades) out of the 6 examined excipients as all showed no significant interactions. Differences arises only in media containing chitosan, where in media with (0.5 % w/v) chitosan ,bile salt with cholesterol and lecithin with monoglyceride were significant and in media with (5 % w/v) chitosan three factor interactions were significant (bile salt with cholesterol or BS:PL and lecithin with monoglyceride).

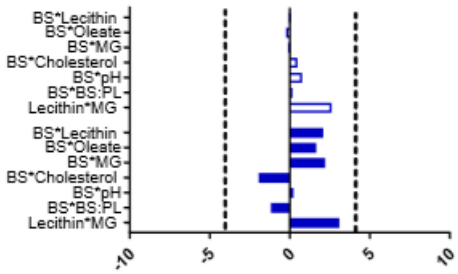

(a)
Fenofibrate control
(b)
0.5% Mannitol in fenofibrate
(c)
5% Mannitol in fenofibrate
(d)
0.5% PVP LG in fenofibrate
(e)
5% PVP LG in fenofibrate
(f)
0.5% PVP HG in fenofibrate
(g)
5% PVP HG in fenofibrate
(h)
0.5% HPMC E3 in fenofibrate
BS*Lecithin
BS*Oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
Lecithin*MG
-20
-15
-10
-5
0
5
10
15
20


(i)
5% HPMC E3 in fenofibrate
(j)
0.5% HPMC E50 in fenofibrate
(k)
5% HPMC E50 in fenofibrate
(l)
0.5% Chitosan in fenofibrate
(m)
5% Chitosan in fenofibrate
(n)
carvedilol control
(o)
0.5% Mannitol in carvedilol
(p)
5% Mannitol in carvedilol
BS*Lecithin
BS*Oleate
BS*MG
BS*Cholesterol
BS*pH
BS*BS:PL
Lecithin*MG

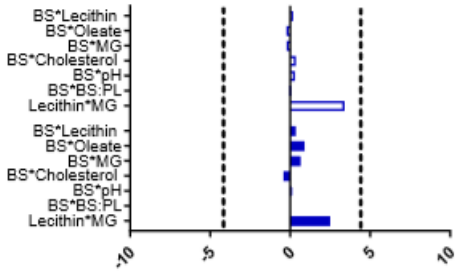
(q) 0.5% PVP LG in carvedilol



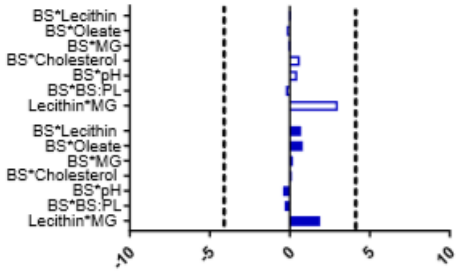
(r) 5% PVP HG in carvedilol



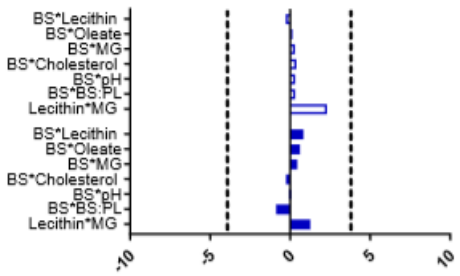
(s) 0.5% PVP HG in carvedilol



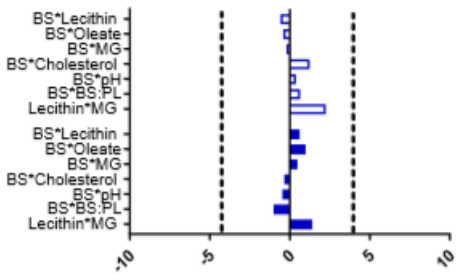
(t) 5% PVP HG in carvedilol



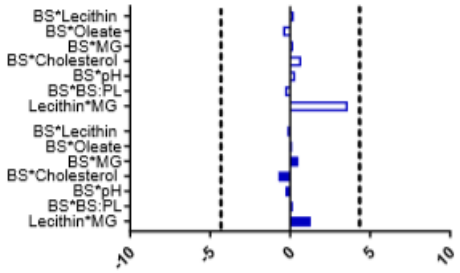
(u) 0.5% HPMC E3 in carvedilol



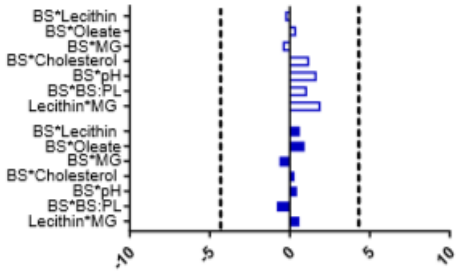
(v) 5% HPMC E3 in carvedilol



(w) 0.5% HPMC E50 in carvedilol



(x) 5% HPMC E50 in carvedilol



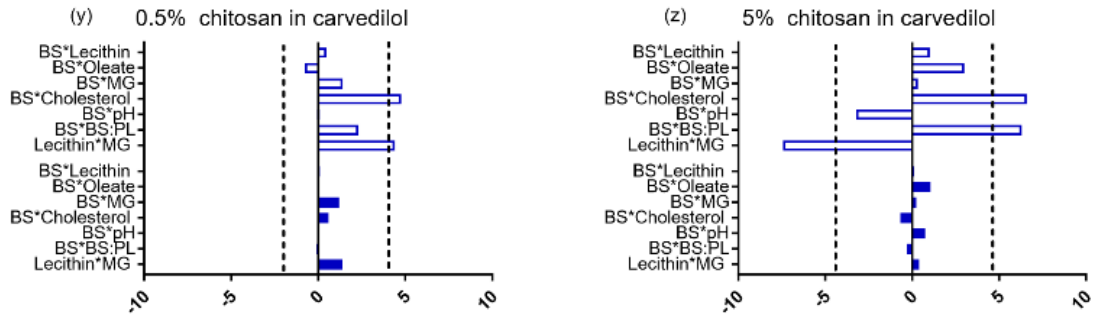


Figure 4.4: Standardised effect values for individual DoE factor interaction on equilibrium solubility in fasted and fed study arms of both fenofibrate and carvedilol: DoE standardized effect values for factors (as listed along y-axis) on equilibrium solubility. Fasted result: empty histogram bar, Fed result: filled histogram bar. Vertical black lines indicate statistical significance ($p < 0.05$, NB significance value = ±4). Horizontal bar direction indicates direction of effect, to the right of 0 on axis means positive effect on solubility, bar length indicates the magnitude of the effect.

4.2.5 Influence of individual DoE factors and factor interaction and the excipient effect on equilibrium solubility in combined arm

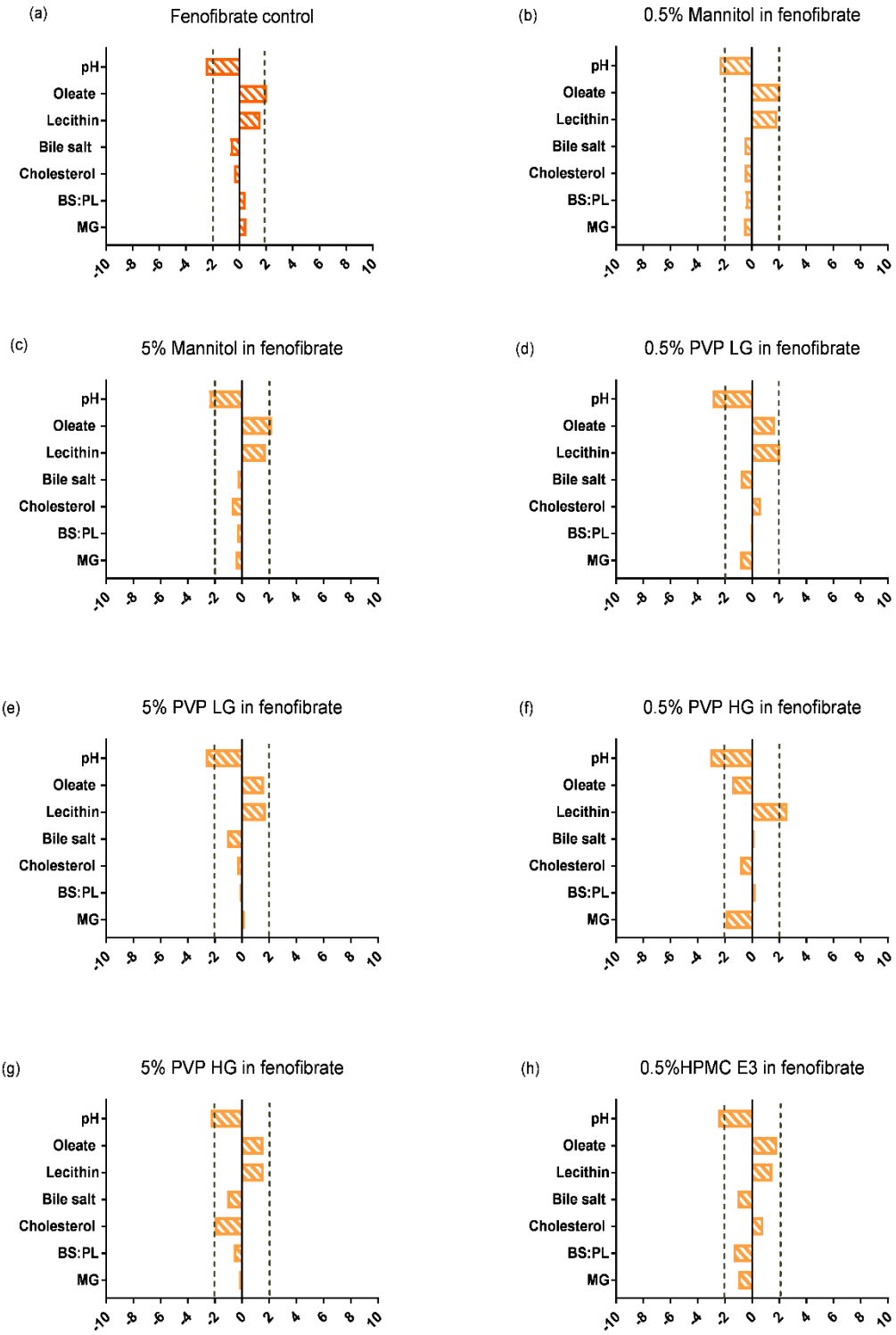
The standardized effect values for each factor in the combined arm, are presented in Figure 4.5. A value of ±2 is significant for the individual factor effect and the factor interaction. No significant interactions were seen with the control or the different excipient's type. A comparison between the significant factor in the fasted and fed states of the control with those in media containing excipients is presented in Table 4.3.

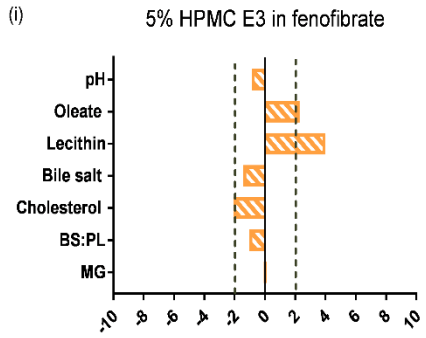
Starting with the control, for both fenofibrate and carvedilol (Figure 4.5 (a & n)) sodium oleate and pH were the factors with the significant effect and no other factors were significant. This was in compliance with the full range study design (Perrier et al., 2018) where sodium oleate was highly affecting solubility of these lipophilic drugs and pH affecting solubility of the ionisable carvedilol drug.

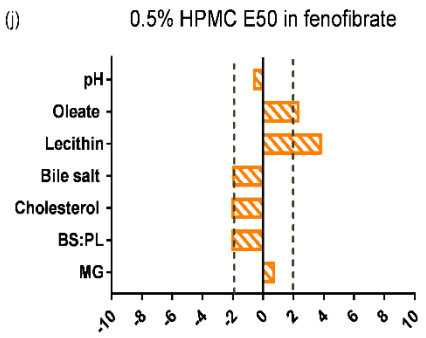
For fenofibrate (Figure 4.5 (b-m), among the 84 possible values compatibility between control and media containing excipient were found in 63 (around 75%) out of 84. Several differences between the control and each excipient were determined with the

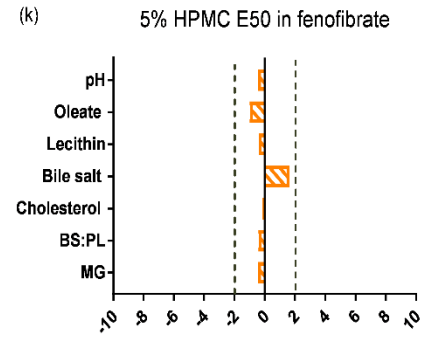
absence of some of the significant factors and the presence of new significant factors. This indicates that the addition of the different types of excipient could make some media changes leading to different drivers of solubility in media compared to the control. Mannitol showed the highest compliance between the different excipients where both pH and oleate were significant with both concentrations. This is followed by both PVP grades, where pH was significant in both lower and higher grades and both concentrations with the absence of the significant effect of oleate and the presence of lecithin as a significant factor. HPMC showed diverse evidence of media changes, where in the lower (0.5% w/v) concentration of HPMC E3 only pH was significant while increasing the concentration to (5% w/v) causes several media changes leading to the presence of lecithin and cholesterol as a significant factors. Though, with HPMC E50 the (0.5% w/v) concentration showed bile salt and cholesterol as significant factors affecting drug solubility while, increasing the concentration to (5% w/v) led to zero significant factors. In media with chitosan, the lower (0.5% w/v) concentration showed only pH as a significant factor, while increasing the concentration (5% w/v) leads lecithin, oleate and cholesterol to be the predominant factors that affects solubility.

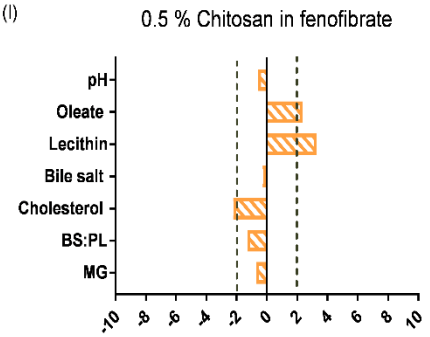
For carvedilol (Figure 4.5 (o-z)), among the 84 possible values, 78 (around 93 %) were compatible with the control. Differences occur in media with HPMC and chitosan where the higher concentration of (5% w/v) E3 showed bile salt as an additional significant factor and the higher concentration (5% w/v) of E50 showed MG as an additional factor that negatively affects solubility. In media with chitosan, lower concentration (0.5% w/v) showed no significant factors, while the higher concentration (5% w/v) showed that pH was affecting solubility, but in a negative way.

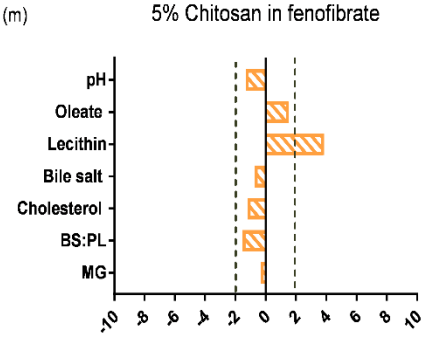

(a)
Fenofibrate control
(b)
0.5% Mannitol in fenofibrate
(c)
5% Mannitol in fenofibrate
(d)
0.5% PVP LG in fenofibrate
(e)
5% PVP LG in fenofibrate
(f)
0.5% PVP HG in fenofibrate
(g)
5% PVP HG in fenofibrate
(h)
0.5%HPMC E3 in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

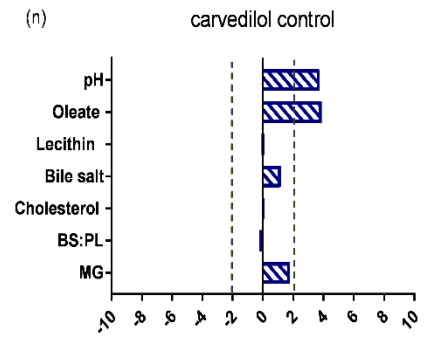

(i)
5% HPMC E3 in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

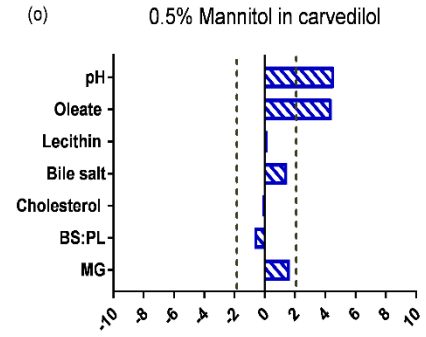

(j)
0.5% HPMC E50 in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

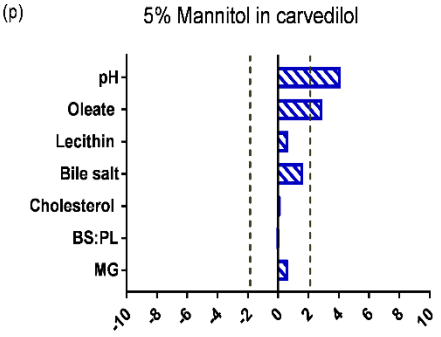

(k)
5% HPMC E50 in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10


(l)
0.5 % Chitosan in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

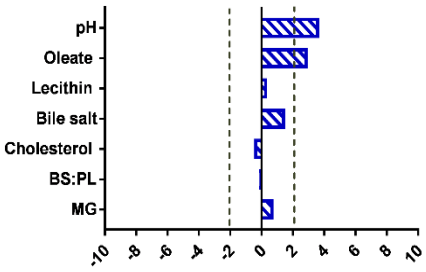

(m)
5% Chitosan in fenofibrate
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10


(n)
carvedilol control
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

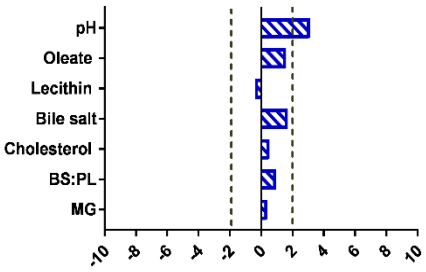

(o)
0.5% Mannitol in carvedilol
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10


(p)
5% Mannitol in carvedilol
pH
Oleate
Lecithin
Bile salt
Cholesterol
BS:PL
MG
-10 -8 -6 -4 -2 0 2 4 6 8 10

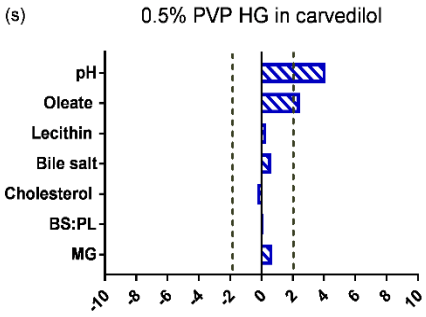
(q) 0.5% PVP LG in carvedilol



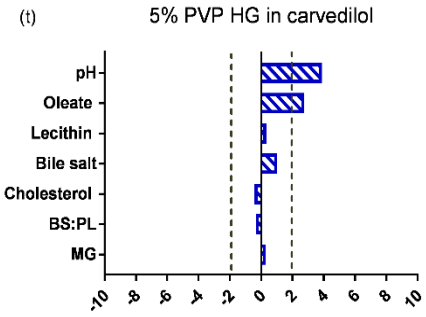
(r) 5% PVP LG in carvedilol



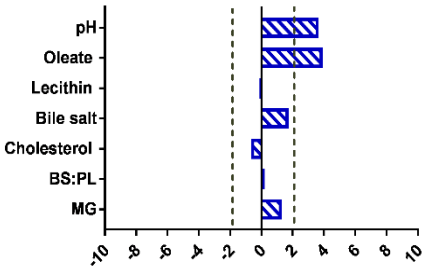
(s) 0.5% PVP HG in carvedilol



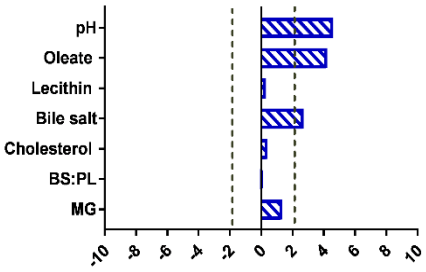
(t) 5% PVP HG in carvedilol



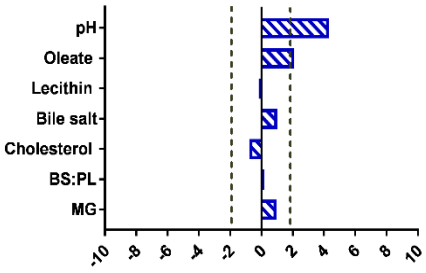
(u) 0.5%HPMC E3 in carvedilol



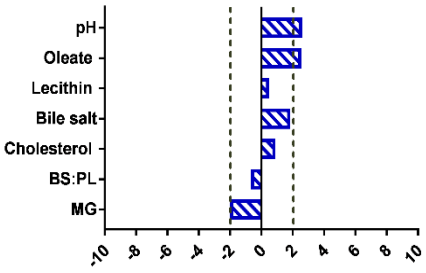
(v) 5%HPMC E3 in carvedilol



(w) 0.5%HPMC E50 in carvedilol



(x) 0.5%HPMC E50 in carvedilol



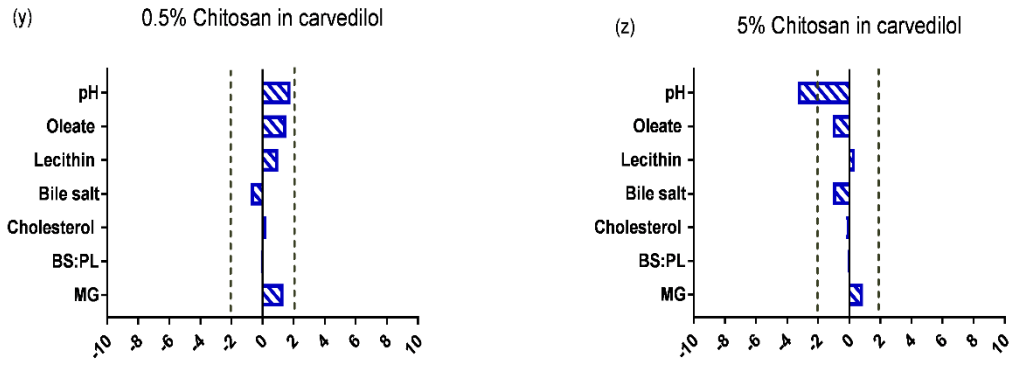


Figure 4.5: Standardised effect values for individual DoE factors on equilibrium solubility in combined arm of both fenofibrate and carvedilol: DoE standardized effect values for factors (as listed along y-axis) on equilibrium solubility. Fasted result: empty histogram bar, Fed result: filled histogram bar. Vertical black lines indicate statistical significance ($p < 0.05$, NB significance value = ±2). Horizontal bar direction indicates direction of effect, to the right of 0 on axis means positive effect on solubility, bar length indicates the magnitude of the effect.

Table 4.4: Comparison of the statistical significance of DoE factors across the experiments

	factors																				
	pH			oleate			lecithin			bile salt			cholestrol			BS:PL			monoglyceride		
Drugs	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed	fa	fed	fa+fed
fenofibrate st.	S	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% mannitol	S	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% mannitol	S	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% PVP LG	S	S	S	S	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5%PVP LG	S	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% PVP HG	S	NS	S	S	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% PVP HG	S	NS	S	S	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5 % E3	S	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5 % E3	NS	NS	NS	S	NS	S	NS	S	S	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
0.5 % E50	NS	NS	NS	S	NS	S	NS	NS	S	NS	NS	S	NS	NS	S	NS	NS	S	NS	NS	NS
5% E50	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% chitosan	NS	NS	NS	S	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
5% chitosan	NS	S	NS	S	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
carvedilol st.	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% mannitol	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% mannitol	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% PVP LG	S	S	S	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5%PVP LG	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5% PVP HG	S	S	S	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% PVP HG	S	S	S	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5 % E3	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5 % E3	S	S	S	S	S	S	NS	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
0.5 % E50	S	S	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% E50	NS	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S
0.5% chitosan	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
5% chitosan	S	NS	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

The blue row for basic drug and yellow row for the neutral. S means the factor had a significant effect, NS means the factor had a non-significant effect. The significant results are highlighted with grey.

4.2.6 Comparing equilibrium solubility value of the standard in each DoE recipe with the corresponding solubility value in excipient containing media

The equilibrium solubility of the control in each fasted and fed state recipe are presented in Figure 4.6 along with the corresponding solubility value in same recipe of media containing the different types of excipients with lower and higher concentration (0.5% w/v and 5% w/v). In excipient containing media, a solubility value 3 times greater or lower than the solubility value of the control considered to be a difference that is induced by the addition of the excipient to the media. A solubility differences lower than 3 times was referred to the analytical or experimental variances.

Starting with fenofibrate, in mannitol containing media the results show (Figure 4.6 (a)) the highest agreement in solubility values with the control in both concentrations (0.5% w/v and 5% w/v) of mannitol and in both fasted and fed states. The solubility values were superimposed over of the control, indicating that mannitol did not causes any media changes that influence drug's solubility. For both lower and higher PVP grades (Figure 4.6 (b &c)), the results indicate that the solubility values of the tubes were near to the solubility values of the control in both fasted and fed states. three times higher solubility values in both PVP grades containing media were seen in tubes number 4, 14 and 18 in fasted state and this is related to the solubility enhancing effect of PVP (Paus et al., 2015). The results also indicate that increasing the concentration from (0.5 % w/v) to (5% w/v) or using higher molecular weight of PVP did not change the effect on solubility.

For HPMC, different behaviours of the lower and the higher grade were evident. In fasted state, the lower concentration (0.5% w/v) of the lower grade HPMC E3 showed higher solubility values in tubes number 3, 4 and 7 which might refer to the surfactant effect of the excipient at the lower molecular weight and lower concentration.(Vadlamudi & Dhanaraj, 2017). However, both (0.5% w/v and 5% w/v) concentrations of E50 and the (5% w/v) of E3 (Figure 4.6 (d &e)) showed lower solubility values of fenofibrate in tubes with high level of oleate and low level of pH (tubes 1, 13, 16 and17). Though, in fed state, media with HPMC E3 solubility values

were comparable to the control when lower concentration (0.5% w/v) was used while increasing the concentration to (5% w/v) showed lower solubility values in a wide range of tubes (Figure 4.6 (d)). Nevertheless, using higher molecular weight of HPMC (E50) showed that in both concentrations, solubility values were lower in almost all of the tubes which indicates that increasing the concentration of the excipient or using a macromolecular weight (E50) will affect solubility due to changes in media components leading to that effect.

For chitosan (Figure 4.6 (f)), fasted state exhibits comparable solubility values with the control fenofibrate when the concentration of the added chitosan was (0.5 % w/v) with the exception of tubes number 13,16 and 17 where oleate, lecithin or cholesterol level were high and pH level was low, where fenofibrate solubility values were three times lower than the control. This indicates that changes in media ionization pattern are the most likely rational for this effect. However, increasing the added chitosan to a concentration of (5% w/v) was accompanied by lower solubility values of fenofibrate in almost all of the tubes in both fasted and fed state. This indicates that higher concentration of the added macromolecular weight of chitosan leads to a lower solubility values of the drug.

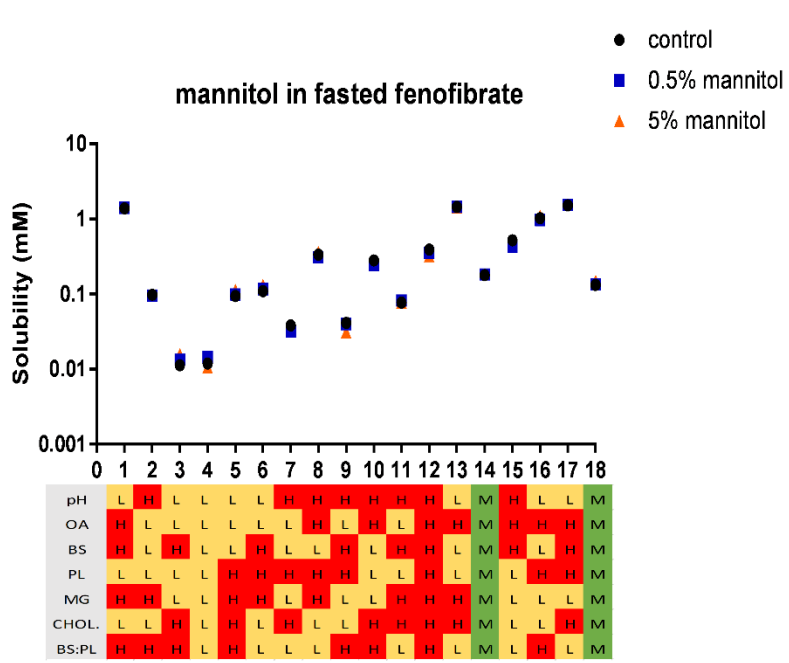
For carvedilol, in media with mannitol and both PVP grades (Figure 4.6 (g-i), solubility values of carvedilol did not change where in mannitol solubility values were superimposed on solubility values of the standard. With PVP solubility values were almost similar to the solubility values of carvedilol using (0.5% w/v or 5% w/v) of the excipient in both fasted and fed state. The solubility enhancing effect of PVP on carvedilol was seen only in tube number 3 in the fasted state.

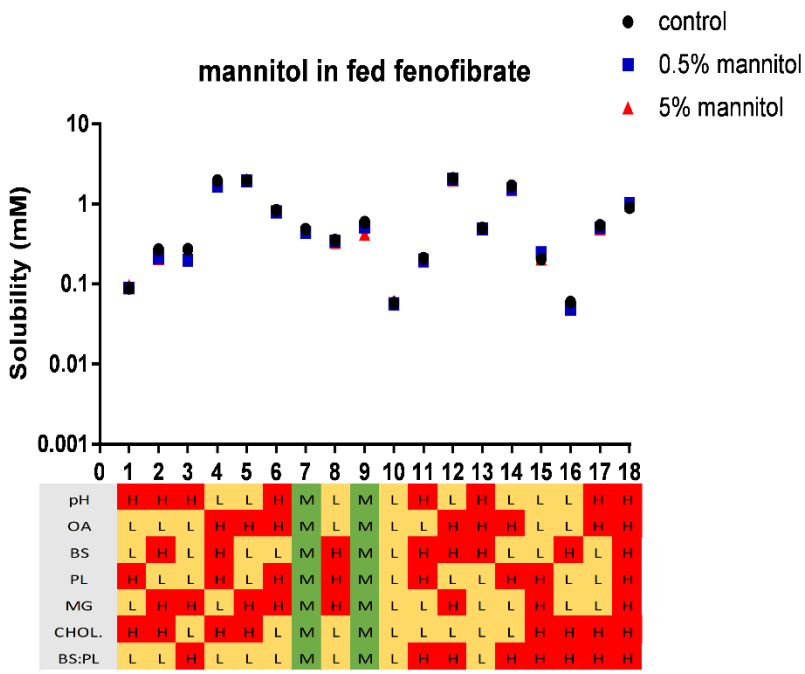
With HPMC (Figure 4.6 j&k)), no differences in solubility was found in both concentrations and in both fasted and fed state of the lower molecular weight HPMC E3. However, increasing the molecular weight to E50 showed almost consistent solubility values in fasted state when using lower and higher concentrations with the exception of tubes number 3 and 18 where solubility values were lower. In the fed

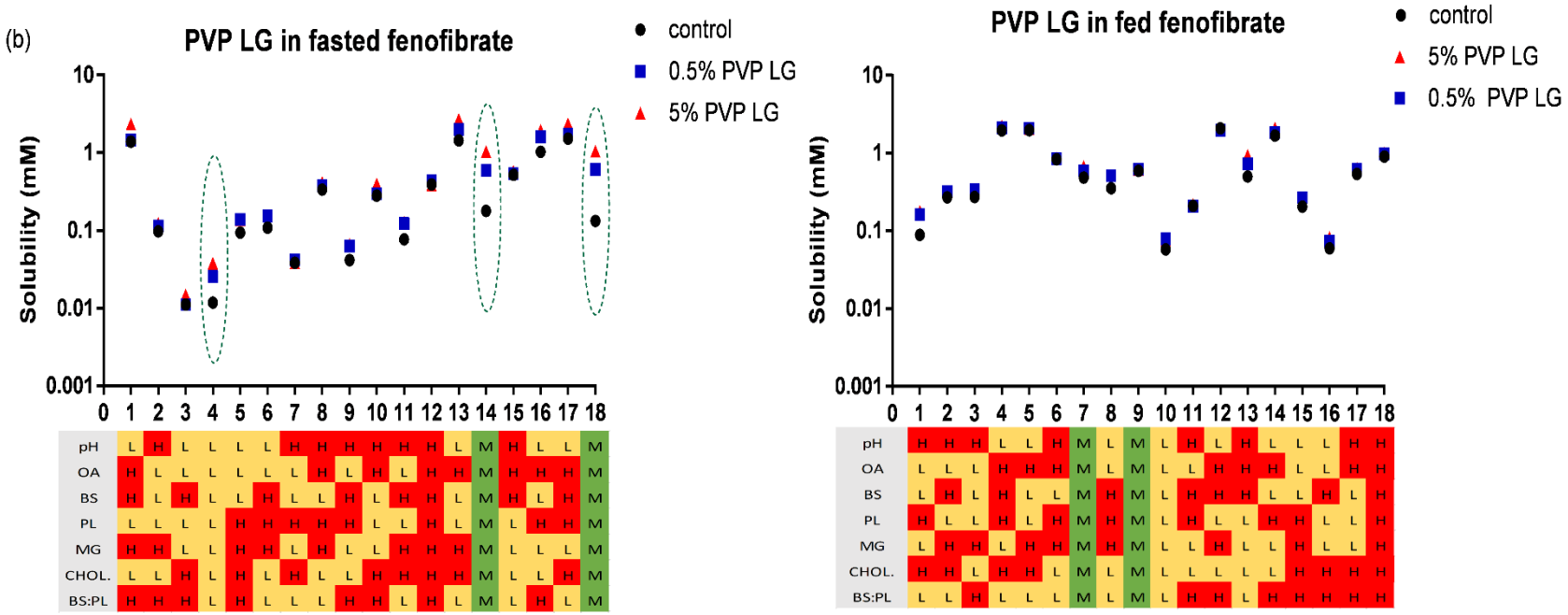
state, increasing the concentration to (5 % w/v) was accompanied by lower solubility values in 8 out of 18 tubes. This indicates that increasing the concentration of the higher molecular weight HPMC E50 could induce some media changes that will influence carvedilol solubility.

With chitosan (Figure 4.6 (l)), fasted state solubility showed that using lower concentration (0.5% w/v) of chitosan showed no differences in carvedilol solubility while, increasing the concentration to (5% w/v) showed diverse effect on solubility. Solubility values were lower in a high number of tubes and remarkably in tubes with high pH and high oleate level. On the other hand, solubility values found to be higher in tubes with low pH and bile salt level and high levels of other surfactants (tubes number 3 and 17). In the fed state, increasing to a concentration of (5% w/v) was accompanied by three time lower solubility values of carvedilol in almost all of the tubes.

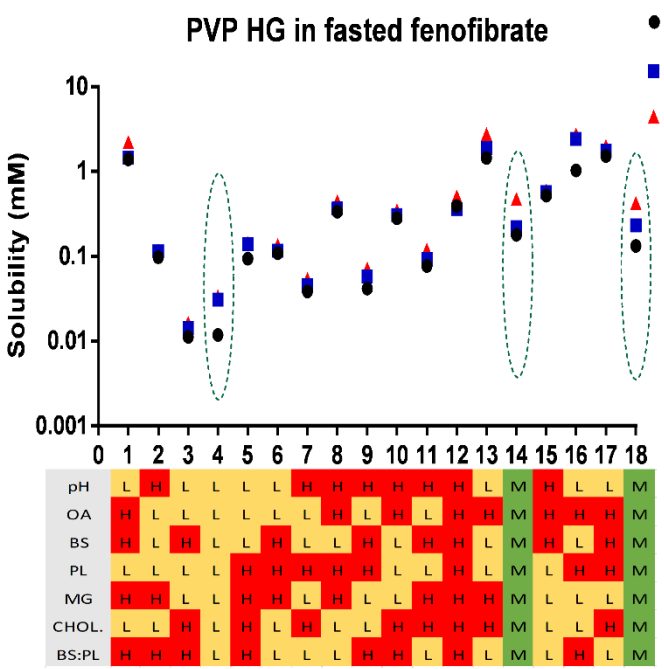
(a)

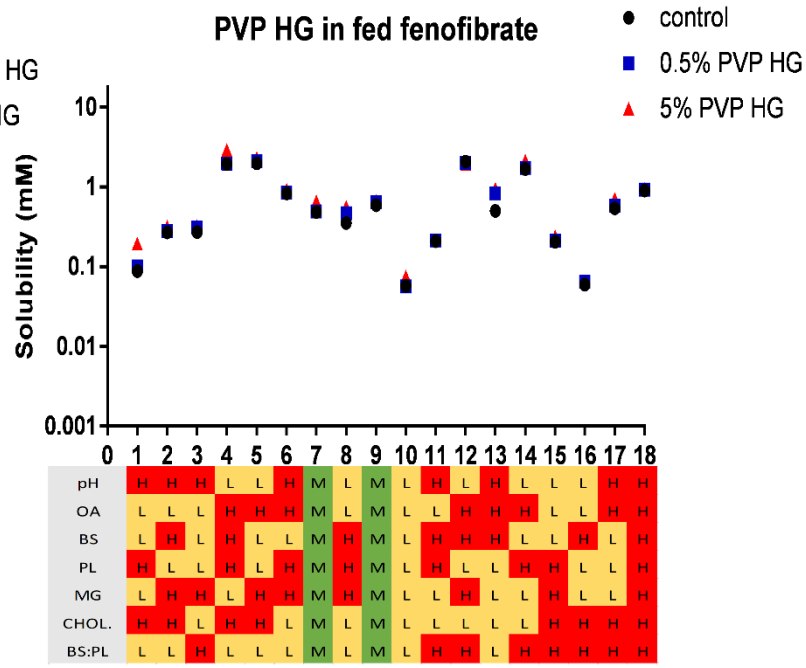

mannitol in fasted fenofibrate
control
0.5% mannitol
5% mannitol
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M


mannitol in fed fenofibrate
control
0.5% mannitol
5% mannitol
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H H

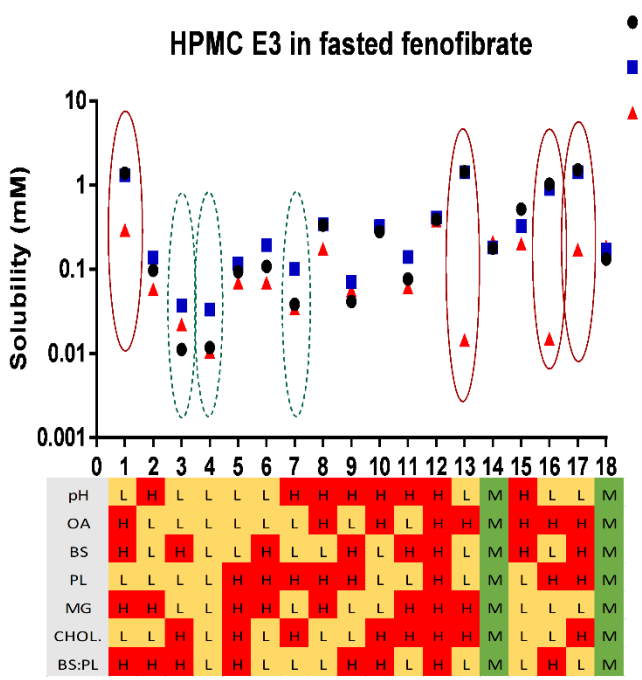

(b)
PVP LG in fasted fenofibrate
control
0.5% PVP LG
5% PVP LG
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M
PVP LG in fed fenofibrate
control
5% PVP LG
0.5% PVP LG
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H H

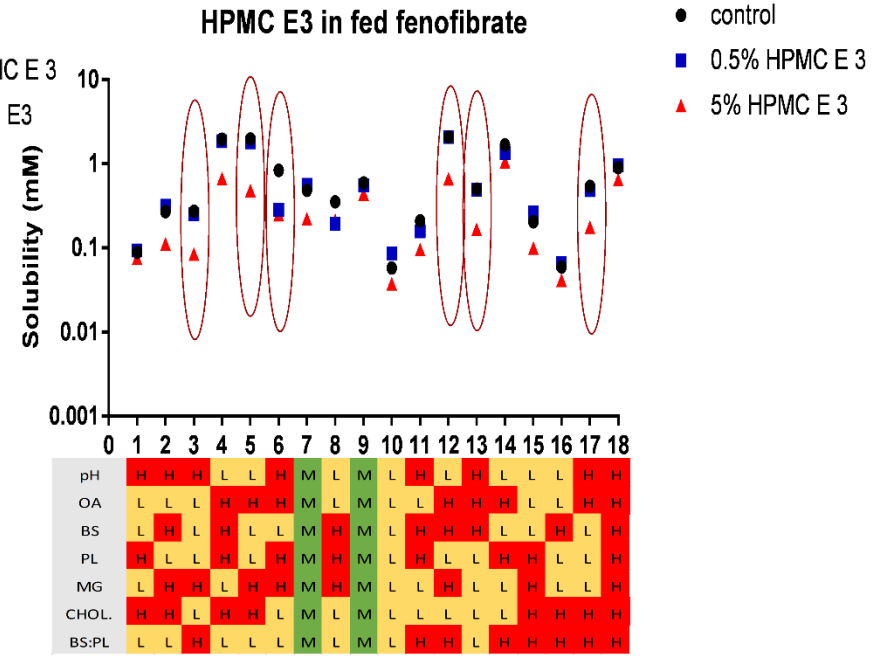
(c)


PVP HG in fasted fenofibrate
control
0.5% PVP HG
5% PVP HG
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M

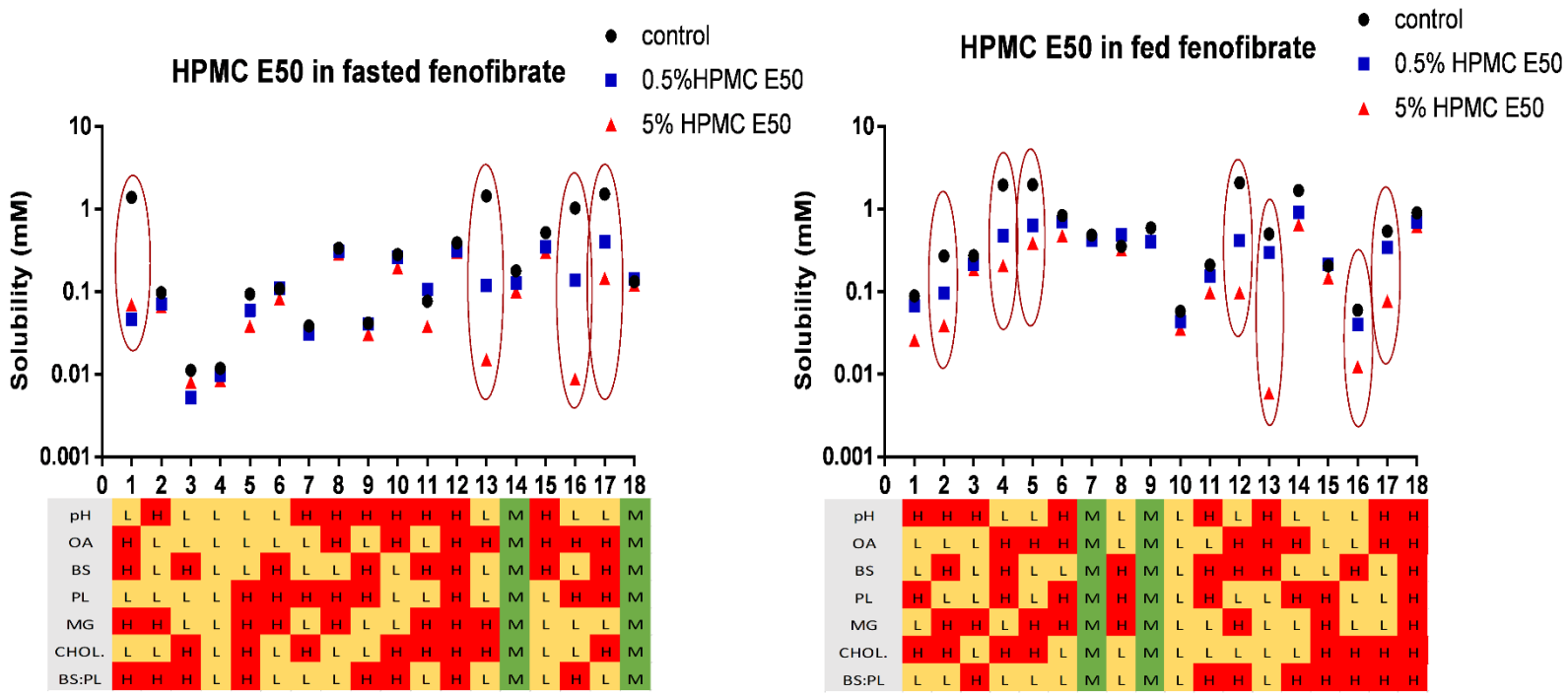

PVP HG in fed fenofibrate
control
0.5% PVP HG
5% PVP HG
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H H

(d)

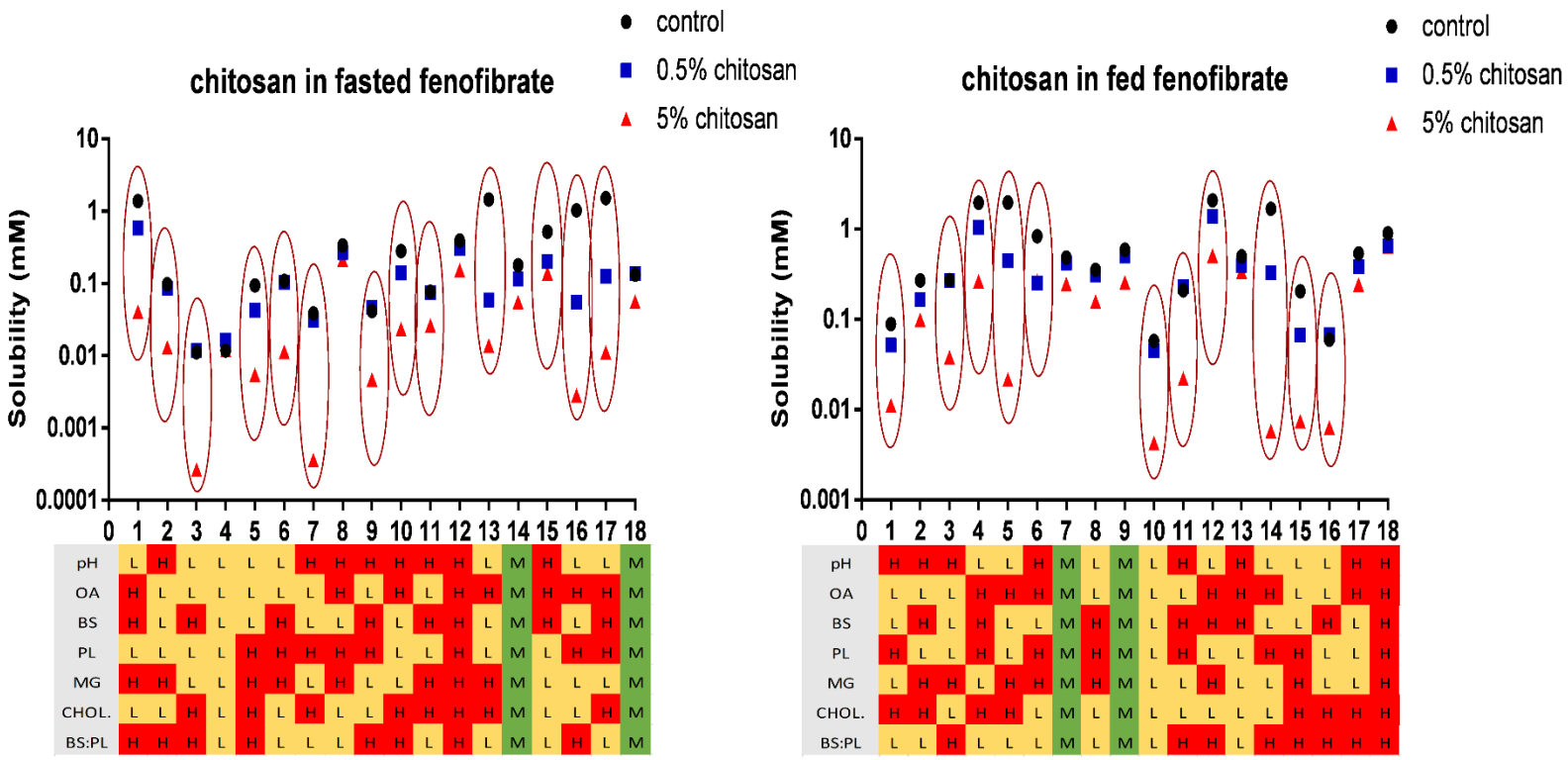

HPMC E3 in fasted fenofibrate
control
0.5% HPMC E 3
5% HPMC E3
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M


HPMC E3 in fed fenofibrate
control
0.5% HPMC E 3
5% HPMC E 3
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H

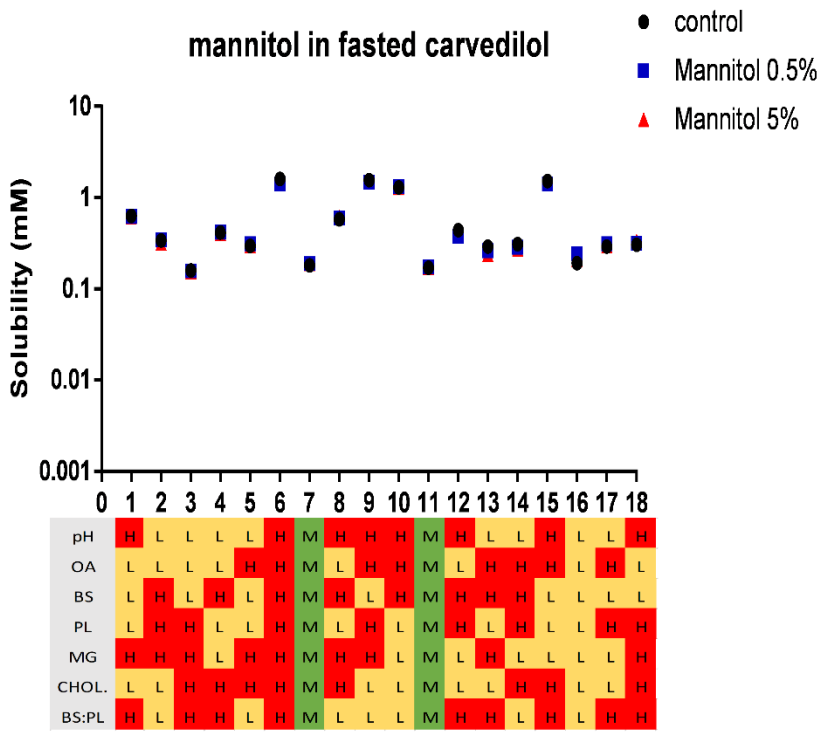
(e)

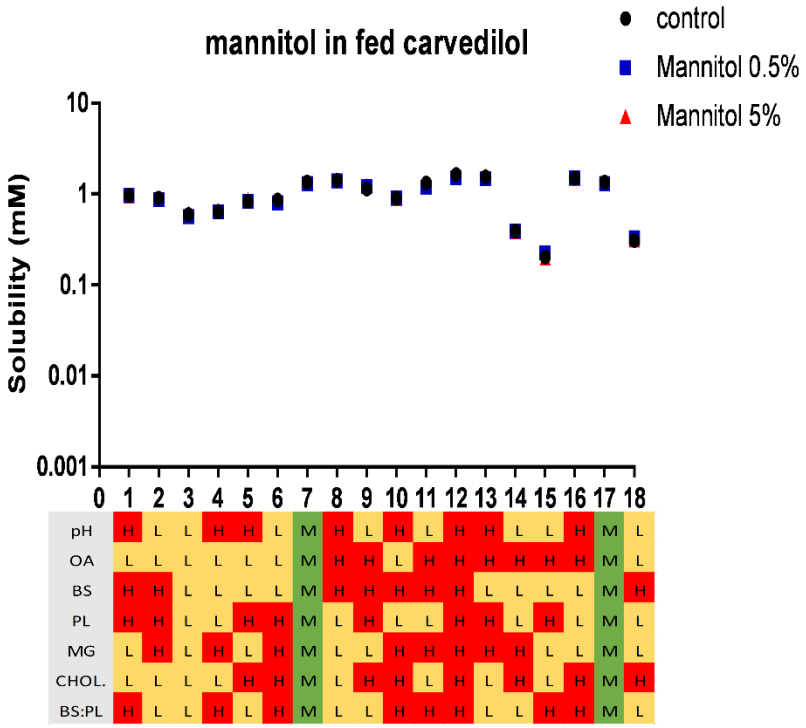

HPMC E50 in fasted fenofibrate
control
0.5%HPMC E50
5% HPMC E50
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M
HPMC E50 in fed fenofibrate
control
0.5% HPMC E50
5% HPMC E50
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H H

(f)

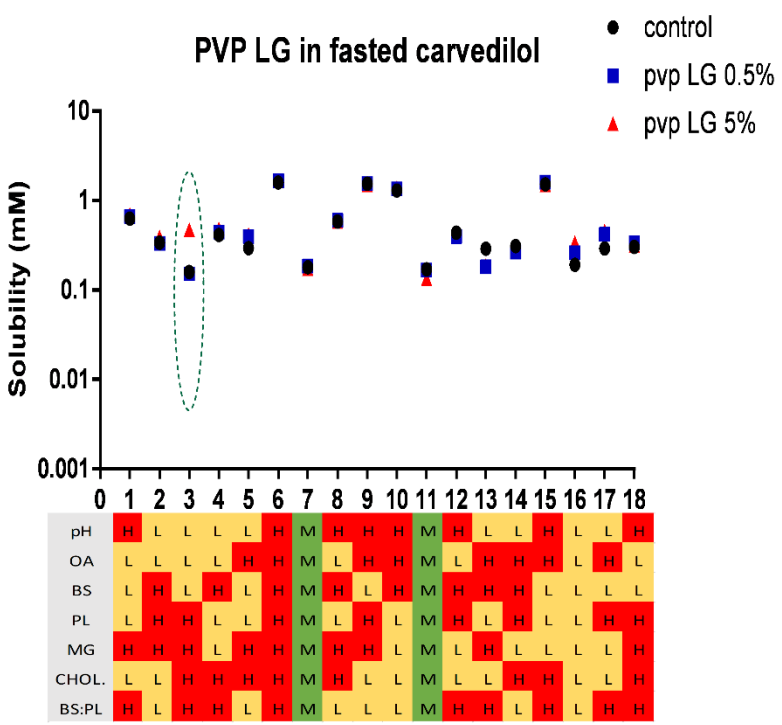

chitosan in fasted fenofibrate
control
0.5% chitosan
5% chitosan
Solubility (mM)
10
1
0.1
0.01
0.001
0.0001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH L H L L L L H H H H H H L M H L L M
OA H L L L L L L H L H L H H M H H H M
BS H L H L L H L L H L H H L M H L H M
PL L L L L H H H H H L L H L M L H H M
MG H H L L H H L H L L H H H M L L L M
CHOL. L L H L H L H L L H H H H M L L H M
BS:PL H H H L H L L L H H L H L M L H L M
chitosan in fed fenofibrate
control
0.5% chitosan
5% chitosan
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H H H L L H M L M L H L H L L L H H
OA L L L H H H M L M L L H H H L L H H
BS L H L H L L M H M L H H H L L H L H
PL H L L H L H M H M L H L L H H L L H
MG L H H L H H M H M L L H L L H L L H
CHOL. H H L H H L M L M L L L L L H H H H
BS:PL L L H L L L M L M L H H L H H H H H

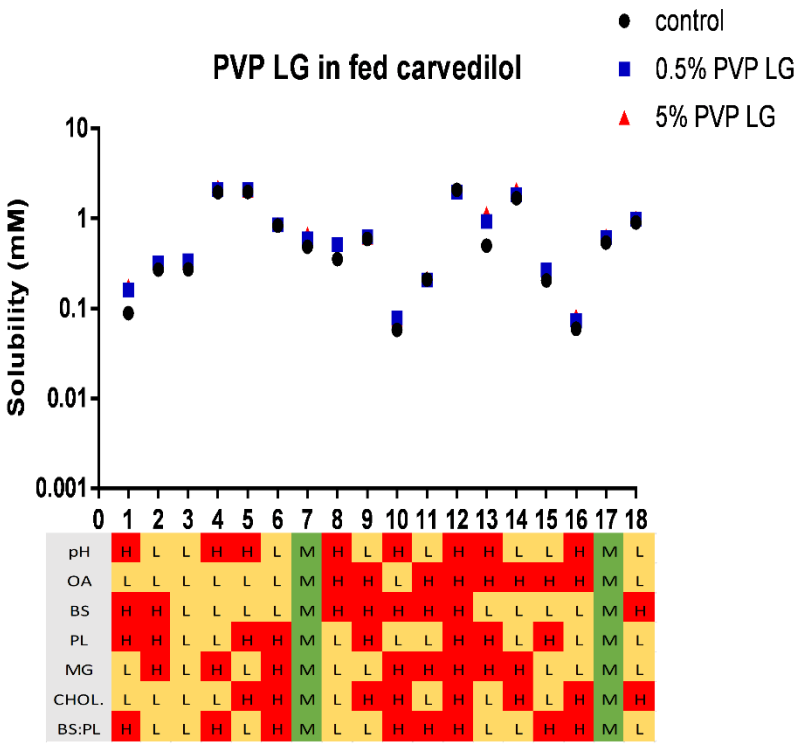
(g)


mannitol in fasted carvedilol
control
Mannitol 0.5%
Mannitol 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L L L H M H H H M H L L H L L H
OA L L L L H H M L H H M L H H H L H L
BS L H L H L H M H L H M H H H L L L L
PL L H H L L H M L H L M H L H L L H H
MG H H H L H H M H H L M L H L L L L H
CHOL. L L H H H H M H L L M L L H H L L H
BS:PL H L H H L H M L L L M H H L H L H H

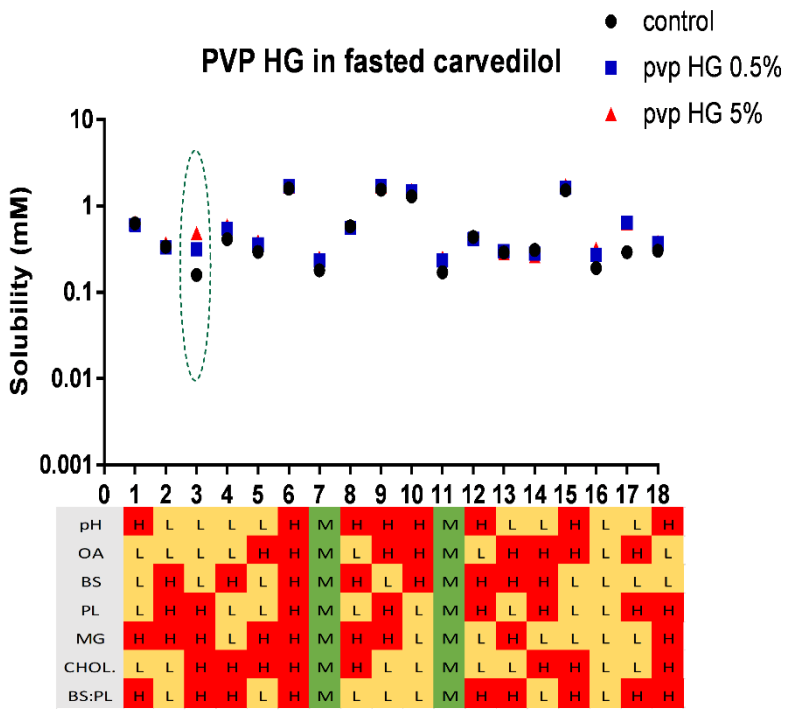

mannitol in fed carvedilol
control
Mannitol 0.5%
Mannitol 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L H H L M H L H L H H L L H M L
OA L L L L L L M H H L H H H H H H M L
BS H H L L L L M H H H H H L L L L M H
PL H H L L H H M L H L L H H L H L M L
MG L H L H L H M L L H H H H H L L M L
CHOL. L L L L H H M L H H L H L H L H M H
BS:PL H L L H L H M L L H H H L L H H M L

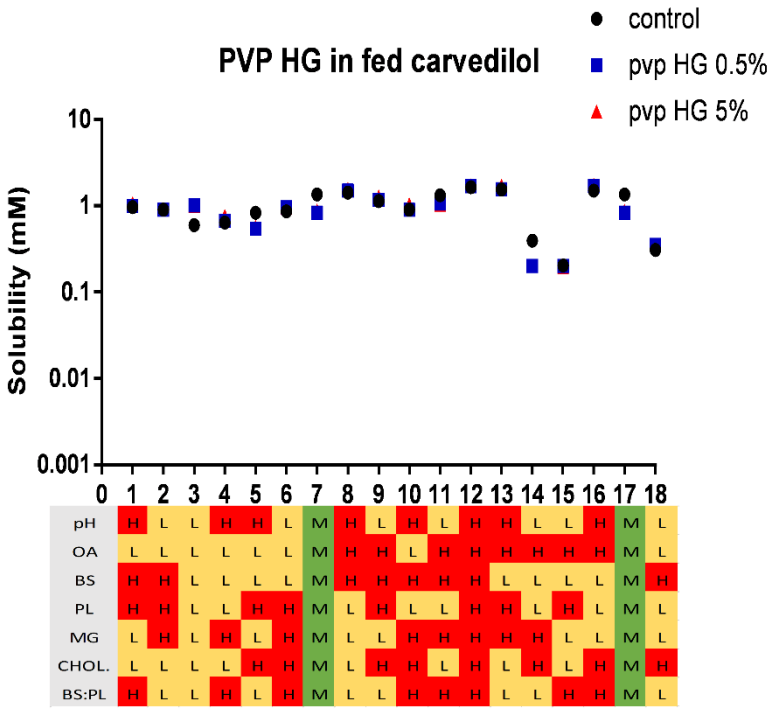
(h)


PVP LG in fasted carvedilol
control
pvp LG 0.5%
pvp LG 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L L L H M H H H M H L L H L L H
OA L L L L H H M L H H M L H H H L H L
BS L H L H L H M H L H M H H H L L L L
PL L H H L L H M L H L M H L H L L H H
MG H H H L H H M H H L M L H L L L L H
CHOL. L L H H H H M H L L M L L H H L L H
BS:PL H L H H L H M L L L M H H L H L H H

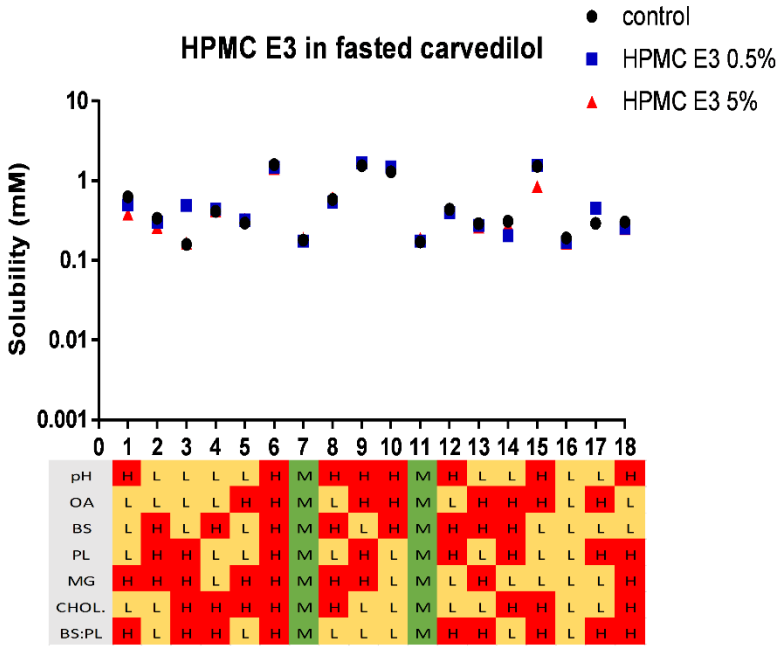

PVP LG in fed carvedilol
control
0.5% PVP LG
5% PVP LG
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L H H L M H L H L H H L L H M L
OA L L L L L L M H H L H H H H H H M L
BS H H L L L L M H H H H H L L L L M H
PL H H L L H H M L H L L H H L H L M L
MG L H L H L H M L L H H H H H L L M L
CHOL. L L L L H H M L H H L H L H L H M H
BS:PL H L L H L H M L L H H H L L H H M L

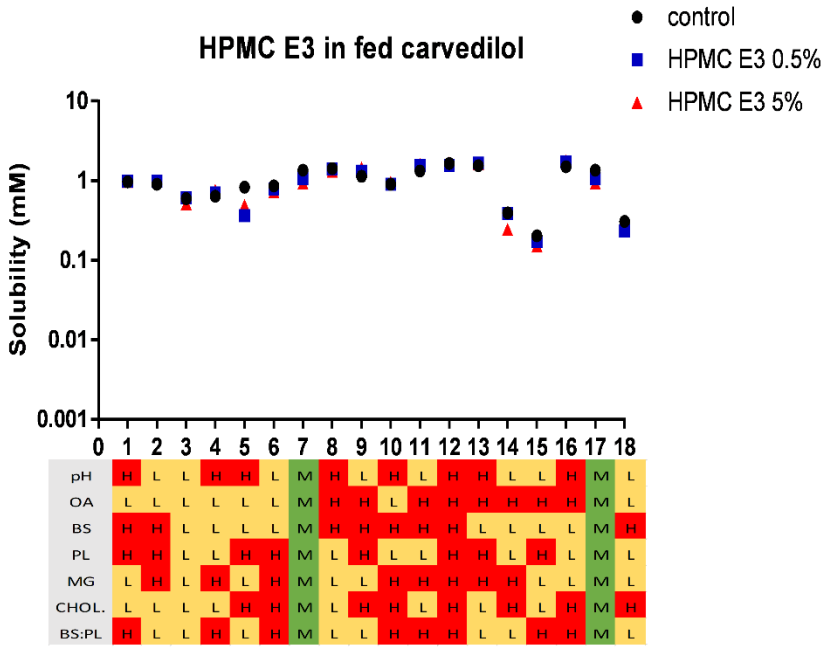
(i)


PVP HG in fasted carvedilol
control
pvp HG 0.5%
pvp HG 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L L L H M H H H M H L L H L L H
OA L L L L H H M L H H M L H H H L H L
BS L H L H L H M H L H M H H H L L L L
PL L H H L L H M L H L M H L H L L H H
MG H H H L H H M H H L M L H L L L L H
CHOL. L L H H H H M H L L M L L H H L L H
BS:PL H L H H L H M L L L M H H L H L H H

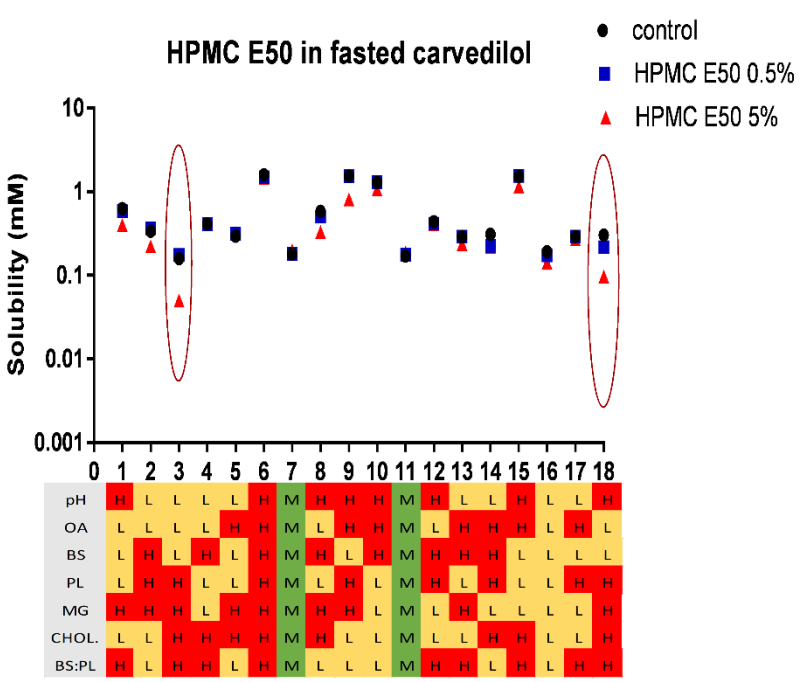

PVP HG in fed carvedilol
control
pvp HG 0.5%
pvp HG 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L H H L M H L H L H H L L H M L
OA L L L L L L M H H L H H H H H M L
BS H H L L L L M H H H H H L L L L M H
PL H H L L H H M L H L L H H L H L M L
MG L H L H L H M L L H H H H H L L M L
CHOL. L L L L H H M L H H L H L H L H M H
BS:PL H L L H L H M L L H H H L L H H M L

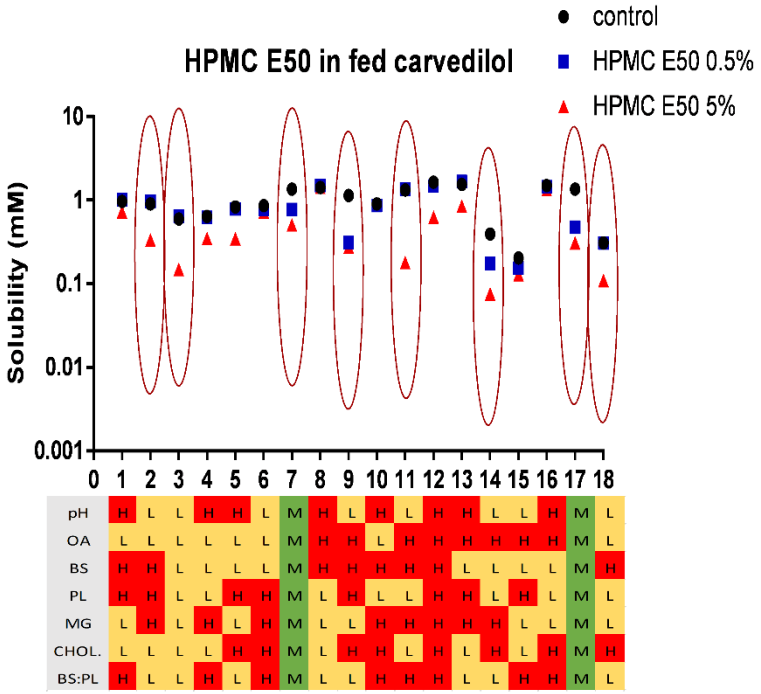
(j)


HPMC E3 in fasted carvedilol
control
HPMC E3 0.5%
HPMC E3 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L L L H M H H H M H L L H L L H
OA L L L L H H M L H H M L H H H L H L
BS L H L H L H M H L H M H H H L L L L
PL L H H L L H M L H L M H L H L L H H
MG H H H L H H M H H L M L H L L L L H
CHOL. L L H H H H M H L L M L L H H L L H
BS:PL H L H H L H M L L L M H H L H L H H


HPMC E3 in fed carvedilol
control
HPMC E3 0.5%
HPMC E3 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L H H L M H L H L H H L L H M L
OA L L L L L L M H H L H H H H H H M L
BS H H L L L L M H H H H H L L L L M H
PL H H L L H H M L H L L H H L H L M L
MG L H L H L H M L L H H H H H L L M L
CHOL. L L L L H H M L H H L H L H L H M H
BS:PL H L L H L H M L L H H H L L H H M L

(k)


HPMC E50 in fasted carvedilol
control
HPMC E50 0.5%
HPMC E50 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L L L H M H H H M H L L H L L H
OA L L L L H H M L H H M L H H H L H L
BS L H L H L H M H L H M H H H L L L L
PL L H H L L H M L H L M H L H L L H H
MG H H H L H H M H H L M L H L L L L H
CHOL. L L H H H H M H L L M L L H H L L H
BS:PL H L H H L H M L L L M H H L H L H H


HPMC E50 in fed carvedilol
control
HPMC E50 0.5%
HPMC E50 5%
Solubility (mM)
10
1
0.1
0.01
0.001
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pH H L L H H L M H L H L H H L L H M L
OA L L L L L L M H H L H H H H H H M L
BS H H L L L L M H H H H H L L L L M H
PL H H L L H H M L H L L H H L H L M L
MG L H L H L H M L L H H H H H L L M L
CHOL. L L L L H H M L H H L H L H L H M H
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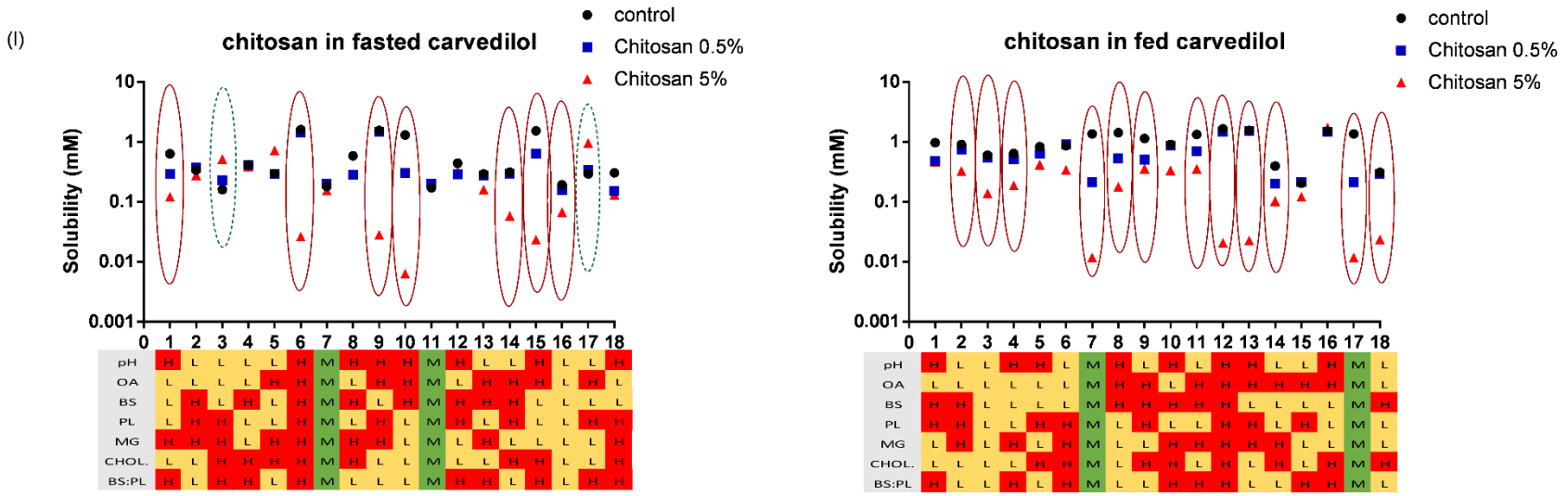


Figure 4.6: Equilibrium solubility data of fenofibrate and carvedilol control along with the equilibrium solubility data of drugs when different excipients added to the media. Black dots represents solubility values of control, blue square represents solubility values when (0.5% w/v) excipient added to the same media and red triangle represents solubility values when (5% w/v) excipient added to the same media. Red oval mark represent media where solubility values were 3 times lower than the control and the dotted green oval mark represent media where solubility values were 3 times higher than the control. X axis represents the number of each tube along with a table that represents the different level of each media components in each tube (listed in the grey column) (Table 2.1, section 2.1.4). H= means factor were in high level, L= means factor were in low level, M= means factor were in intermediate level.

4.3 Discussion

4.3.1 Equilibrium solubility measurements

The equilibrium solubility results of the fasted and fed state fenofibrate and carvedilol in the presence of the different types of excipients with different grades and different concentrations are presented in Figure 4.1. This indicates that whether the solubility values of fenofibrate or carvedilol were affected or not was dependent on several parameters including the type of the added excipient, the concentration of the added excipient (0.5% w/v or 5% w/v), the molecular weight of the PVP and the HPMC and on the media state under examination (fasted or fed). For example, visually neither fenofibrate nor carvedilol solubility were affected when mannitol, both PVP grades and HPMC E3 were added to the media which indicates that excipients with low molecular weight or those who did not swell and form a thick gel when in contact with solvent media did not cause any remarkable media changes that influence drug solubility. On the other hand, using excipients with high molecular weight such as chitosan or HPMC E50, showed a remarkable decrease in both drugs solubility which indicates that these two excipient could induce media modification and consequently had an impact on drug solubility

4.3.2 Statistical comparisons of the overall distribution of solubility

The generation of solubility data set for each drug in each excipient permits a statistical comparison between the solubility values of the control fenofibrate or carvedilol and solubility values of these drugs in presence of different types of excipients with different concentrations (0.5% w/v and 5% w/v) and this is presented in Figure 4.2. The results show that mannitol, both PVP grades and the lower grade of HPMC E3 produced no statistical differences in solubility compared to the solubility values of fenofibrate or carvedilol control data.

Mannitol is a highly soluble water excipient (Saffari et al., 2016) that tends to have no effect on drug solubility and has been shown to increase drug solubility only when used in high concentrations (Yadav. et al., 2013). Paus et al., examined the effect of mannitol on solubility of indomethacin and naproxen drugs and the results showed that

mannitol has no obvious influence on solubility with both drugs.(Paus et al., 2015). This is compatible to the results in this study where both fenofirate and carvedilol followed the same trend in the presence of mannitol with no effect on solubility.

Polyvinylpyrrolidone (PVP) is a water soluble polymer that reported in literatures as solubility enhancers for a variety of drugs (Paus et al., 2015;Yadav et al., 2013). However, in this study PVP showed no statistical effect on drug solubility for both fenofibrate and carvedilol at both concentrations (0.5% w/v and 5% w/v).). This might refer to the fact that in this study, different media than literature was used where more complicated media with different micellar components compared to dissolving excess amount of the drug in aqueous solution of the polymer (Yadav et al., 2013). The results also indicates that increasing the molecular weight of the PVP from LG to the HG showed no difference in PVP effect and that solubility was not affected in both fasted and fed states, this is consistent with reports in literature (Knopp et al., 2015; Rask et al., 2016) that increasing the molecular weight of the polymer had no effect on increasing solubility. It should be noted that in this section, the effect of excipients on solubility was compared with the overall solubility of the control in 34 different media and there were no published studies that investigate the effect of excipients on solubility using DoE design with these media.

For HPMC, in this study the lower molecular weight E3 showed to have statistically no significant effect on drug's solubility at both used concentrations (0.5% w/v and 5% w/v) and in both states (fasted or fed). However, using the higher molecular weight HPMC E50 showed no effect on fenofibrate solubility or carvedilol when lower concentration (0.5% w/v) of the excipient was used. Though, increasing the concentration to (5% w/v) showed a statistically significant lowering solubility effect of the excipient in both fasted and fed state of fenofibrate drug and in the fed state of carvedilol drug. This results were found with chitosan also where solubility of the examined drugs were found to be lower in both fasted and fed states when the concentration of the added excipient was raised to (5% w/v). HPMC and chitosan were reported in literature to be used in drug formulation as disintegrants (Nigalaye et al.,1990; Lee et al., 1999) and consequently help the drug to dissolve. However, in

this study the formation of a strong and viscous gel at the higher concentration of the high molecular weight excipients resulted in restricted drug's solubility. The formation of gelatinous layer of these highly molecular weight excipients were reported in literature (Ahsan et al., 2017; Li et al., 2005).

4.3.3 Standardised effect values and the individual media tubes solubility values

The determined standardised effects values presented in Figure (4.3, 4.4, 4.5) indicates that for both fenofibrate and carvedilol control results, sodium oleate and pH were the significant factors in the fasted, fed and combined arm states. The results also indicates that the response of both drugs to the interaction between the drug and the different media components will be highly dependent on the type and the concentration of the added excipient. As for example, mannitol showed consistent significant factors and factor interactions compared with the control fenofibrate or carvedilol. However, in the other systems, chitosan and HPMC E50 showed different response and different significant factors which indicates modification of the media by these excipient leading to different solubility performance.

Figure 4.6 (a, g) presented that mannitol shows consistent solubility values compared to the control fenofibrate and carvedilol. This consistency was evident with the compatible significant factors of both drugs in media containing mannitol with the significant factors in media with the control. However, differences were seen with the factor interactions in media with mannitol compared to the control, for example the significant effect of the interaction between bile salt and lecithin (Figure 4.4 (b)) which might be referred to the analytical or experimental differences.

PVP was slightly different, Figure 4.6 (b, c, h, i) shows that solubility values of fenofibrate were positively influenced in same tubes when lower or higher PVP grade was added and when all media components are in low or in intermediate level. While in carvedilol, solubility values of the different tubes were similar to that of the control. Moreover, Table 4.3 shows that in media with PVP, both drugs responses to the

different media components differently compared to the control media. For example presence of the significant effect of lecithin in case of fenofibrate combined arm or the loss of the oleate as a significant factor in case of carvedilol fasted and fed arms. The higher solubility values of fenofibrate was referred to the solubilizing ability of PVP excipient. This was compatible with literatures where solubility of fenofibrate found to be higher in PVP solid dispersion (Wen et al., 2019; Yang et al., 2019; Chaudhari & Dave, 2016). Several studies showed the enhancement effect of PVP solid dispersion on solubility of carvedilol (Lee et al., 2013; Zoghbi & Wang, 2016). In this study, the effect of PVP on increasing solubility of carvedilol was seen in one tube (tube number 3) in the fasted state. However, different media components were used in these studies (distilled water, 0.1 N H Cl) compared to the complicated media in this study. Moreover, the effect of PVP on solubility found to be drug dependent (Widanapathirana et al., 2015) which interpret the less effect of excipient on carvedilol solubility. Nevertheless, The absence of the significant effect of the leading factor (oleate) and or the presence of other significant factors might be attributed to the hydrogen bond interaction between the C=O group of PVP which act as a hydrogen bond acceptor (Widanapathirana et al., 2015; Chaudhari & Dave, 2016; Rask et al., 2016) and the drug or the different media components that act as hydrogen bond donor. This will lead to media alteration and consequently different factors influencing solubility.

In media with HPMC, excipient effect was highly dependent on the drug under examination, the excipient grade and added concentration, whether fasted or fed state was examined and on the recipe of each tube in the design of the experiment. For example, lower concentration (0.5% w/v) HPMC E3 increased fasted state fenofibrate solubility in a wide range of tubes while in carvedilol it showed no effect. Fasted state fenofibrate showed the oleate surfactant as a significant factor affecting solubility. This significant effect together with the inherent property of HPMC excipient to improve the micelle formation in lower viscosity grade (Javeer et al., 2013;Vadlamudi & Dhanaraj, 2017) leads to a higher solubility values. This effect on fenofibrate was reported in literature where solubility of the drug found to be higher in media with HPMC E5 compared to media with HPMC E15 or HPMC E50 (Chaudhari & Dave,

2016). However, in this study increasing the concentration of HPMC E3 to (5% w/v) or the use of the higher molecular weight E50 decreased fenofibrate solubility in the fasted state remarkably in recipes with high level of surfactants or oleate and low level of pH (Figure 4.6 (d &e). Comparing the significant factors of the fasted arm in experiments with HPMC in media by the significant factors of control showed that pH was no longer significantly affecting drug solubility in all cases (Table4.3). However, in the fed state a higher concentration of the higher grade HPMC E50 decreased both fenofibrate and carvedilol solubility in almost all of the tubes. Comparing the significant factors of media with HPMC in fed state with those in the control showed that with both fenfibrate and carvedilol, HPMC E50 showed no significant factors (Table4.3). Nevertheless, investigating the larger combined fasted and fed arm showed different significant factors compared to the control (Table4.3). The lowering effect of HPMC might be attributed to the increase in the viscosity of the media when higher concentration or higher molecular weight was used (Chaudhari & Dave, 2016). The higher viscosity in media with HPMC together with the formation of a turbid gel will resist drug solubility (Li et al., 2005). Moreover, the differences in the significant factors of media with HPMC and control indicates that an interaction between the numerous hydrogen bond donor in the backbone of HPMC (Widanapathirana et al., 2015 ; Chaudhari & Dave, 2016), and the different media components or the drugs have occurred. This is evident by the negative effect of each of the bile salt, cholesterol (Figure 4.5 (j)) and monoglyceride (Figure 4.5 (x)) on solubility.

In media with chitosan, effect of excipient on solubility showed diverse response depending on the drug under examination, the examined state (fasted or fed) and the media recipe. In the fed state, solubility values were lower in almost all of the tubes with both fenofibrate and carvedilol when higher concentration of excipient was used. This indicates that modification occurred in these media leading to that effect which is evident by the differences in the significant factors when comparing media with excipient and media with control (Table4.3). However, in the fasted state the effect of chitosan was different between fenofibrate and carvedilol where chitosan had a lowering effect in almost all of tubes with fenofibrate. Though, carvedilol response to the addition of chitosan was complex. The excipient decreased solubility in some

tubes and increased solubility in others. Investigating the different factors affecting drugs solubility in the fasted media with chitosan and those with the control showed different drivers of solubility in each media (Table4.3). Interestingly, investigating the significant factors of each media in the larger combined arm showed evidence of media changes between the different experiments where either, the significant effect of the main solubility drivers (oleate and pH) were absent (for example (0.5% w/v) chitosan in carvedilol (Table4.3)) or new factors as lecithin and cholesterol were present (for example ((0.5% w/v and 5% w/v) chitosan in fenofibrate (Table 4.3)). These media changes can probably be attributed to the positively charged functional group of chitosan that will be ionisable over the pH range of the experiment (Huguet et al., 2002; Wang et al., 2006) , and the multiple anionic surfactant present in media. The interaction between the tertiary amino group of chitosan and the anionic group of the fatty acids and bile salts was reported in literature (Kubbinga et al., 2015; Nadai et al., 2006) and found to decrease the bioavailability and absorption of drugs under examination. This finding is in agreement with the results in this study where chitosan has been shown to decrease solubility in high range of recipes. In the case of the neutral non ionisable fenofibrate, the lowering effect might be attributed to the interaction between chitosan and the different media components and consequently weakening the effect of oleate (main solubility driver) on solubility. This is evident by the negative effect of cholesterol and the presence of the significant effect of lecithin (Figure 4.5 (l &m). However, in carvedilol with a pKa value of 7.8-8.25 (Dunn et al., 2019), the drug will be ionised and positively charged over the pH range of the experiment. At low pH level, both carvedilol and chitosan will be positively charged and they will repel each other, though at higher pH range the carvedilol will be in the unionised form and consequently, an interaction between chitosan and the different media components will occur. This will clarify the lower solubility values of carvedilol in tubes with high pH level (pH=7). However, chitosan might interact with the different media components leading to a lower or higher solubility values depending on the level of the amphiphile in each recipe. The complex solubility behaviour of carvedilol was reported in literature (Dunn et al., 2019) where solubility of this drug was dependent on several factors such as pH, total surfactant concentration and the ionization of the surfactant. The media changes occurred due to the addition

of chitosan was evident by the negative effect of pH on solubility (Figure 4.4 (z)) and the presence of several significant interactions as the positive effect of interaction between bile salt and cholesterol and the negative effect of the interaction between lecithin and monoglyceride (Figure 4.5 (z)).

As HPMC E50 and chitosan showed to decrease solubility values of the drugs under investigations, this will increase the dose solubility ratio and consequently might moves the BCS classification. However, as both fenofibrate and carvedilol are not on the border line in class II BCS classification, the lowering effect of the excipients will not shift the class of these drugs.

4.4 **Conclusion**

The results of the present study indicate the feasibility of a reduced experimental number design covering both fasted and fed simulated media states in a single study to provide data on the equilibrium solubility of a drug in the presence of different excipients. It demonstrate specific drug-excipient behaviour and show that each excipient will influence the solubility of the drug differently, some having neutral effects while others significantly reduces solubility. The results also indicates that the effect of the excipient on solubility of drugs will be highly dependent on the concentration of the used excipient, the molecular weight of the excipient, the media state (fasted or fed) under examination and on the pH and the total surfactant level in each recipe. The system will also identify those simulated media factors that have the strongest influence on equilibrium solubility and can be used to investigate differences in the factors predominantly affecting solubility when excipient's type changed.

In conclusion, it is feasible to use this experimental design as a prognostic tool to examine the effect of different excipients on the solubility behaviour of various drugs in simulated intestinal fluids in both fasted and fed states. Moreover, this technique can be used as a screening method to explore the effect of excipients on solubility and dissolution and consequently providing the formulation with the best excipient for optimum drug bioavailability.

5 Dissolution testing using DoE simulated media

The aim of this study is to investigate the dissolution rate of carvedilol in combination with 6 different types of excipients in the fasted and fed DoE media to examine possible correlation with the carvedilol equilibrium solubility in the same fasted and fed DoE media. The dissolution studies were carried out using small scale dissolution assay installed on a Sirius T3 (Sirius Analytical Ltd.) and Sirius SDI (surface dissolution imaging) instruments. Note that fenofibrate dissolution testing was not possible to be carried out referred to the very poor solubility of the drug.

5.1 Material and method

Dissolution testing was carried out using Sirius T3 and Sirius SDI instruments. On each instrument, carvedilol dissolution was tested when each of the 6 types of excipient was pressed with carvedilol in disc. Then, second carvedilol dissolution testing was carried out when each of mannitol, chitosan and HPMC E50 was added to the dissolution media. First test was done by using dissolution media prepared from simulated intestinal media components as in Table 2.2 and the excipient effect on dissolution was examined when the excipient powder was pressed with carvedilol within the disc. Second test was carried out by preparing a disc composed of carvedilol and examining the effect of excipients on dissolution by adding the excipients solution to the dissolution media.

5.1.1 Examining the effect of excipients on carvedilol dissolution when both excipients and drug are within the disc

5.1.1.1 Preparation of the dissolution sample disc in Sirius T3

Dissolution testing was carried out using all the 6 types of excipients that were examined in the simulated intestinal media in the previous section (section 4). To prepare the sample disc, the drug and the excipient were mixed together and the excipient was at a concentration of 15 % (this concentration was chosen in a request to examine the effect of the excipient when it is in an intermediate level, 1% for example is too low and might have no impact while 50 % might have high impact) of

the total amount of the powder. Therefore, 20 mg of carvedilol and 3 mg of the excipient to be examined were weighed and transferred to a mortar for mixing. After mixing, 5 mg of the mixture was compressed into a disc using a 3mm diameter steel die. A compression force of 100 kg was applied and continuously adjusted until constant pressure (+/- 5%) was achieved for 2 minutes (Box et al., 2016). All prepared discs were then examined visually to ensure that their surfaces were smooth and free from any defects. Noting that, an amount of 5 mg of carvedilol was weighed up and the disc was prepared to undergo dissolution testing as a control.

5.1.1.2 Preparation of the dissolution sample disc in Sirius SDI

To prepare the sample disc, the drug and the excipient mixture was prepared as detail above. After mixing, samples were transferred to a 2mm stainless steel cylinder sample cup. A Quickest Minor® torque screwdriver was used to compress the weighted powders at a constant pressure for 10 minutes using the standard Sirius Analytical method (Ward et al., 2017). Noting that, disc containing carvedilol only was prepared and used as control.

5.1.1.3 Preparation of the fasted and fed simulated dissolution media

Dissolution media in each fasted or fed state for carvedilol was chosen from the stock solution combinations where all media components (bile salt, lecithin, MG and cholesterol) including sodium oletae and pH were chosen to be in the lower level. Accordingly, recipe (A1 which is tube number16) was chosen to do dissolution testing in the fasted state and recipe (F2 which is tube number 3) was chosen to do dissolution testing in the fed state. Media were prepared from corresponding powder ingredients and pH adjusted according to the values in Table 2.2.

5.1.2 Examining the effect of excipients on carvedilol dissolution when excipients are in media

5.1.2.1 Preparation of the fasted and fed simulated dissolution media

Dissolution media in each fasted or fed state was prepared according to the previous section (5.1.1.2) then, the excipient under examination was added to the stock solution. The amount of each excipient to be added was calculated to be 5% (this concentration was chosen as it is the concentration where the excipient showed an impact on solubility in section 4) of carvedilol solubility according to the calculations in the previous section (section 4).

5.1.3 The dissolution experiment

An amount of 20 ml of dissolution media was prepared and pH was adjusted according to the recipe in each fasted or fed state and according to the method of examination (whether excipients were in media or in disc) and then added at the beginning of the experiment. The dissolution of the disc was directly monitored by multi-wavelength UV absorption spectroscopy and data recorded over a period of 120 minutes. After the experiment, the UV absorption data were converted to an absolute sample weight using previously determined pH-dependent molar extinction coefficient.

5.1.4 Molar extinction coefficient

A stock solution of 5 mM of carvedilol dissolved in DMSO was prepared and then MEC was determined by a pH titration and UV-spectra was collected.

5.1.5 Dissolution data comparison

Dissolution data of the standard disc and the dissolution data of the drug with the excipient disc were compared and the similarity were assessed by the similarity factor $f2$ (Gohel et al., 2005; Box et al., 2016). The similarity factor was evaluated for the whole duration of the experiment. The dissolution data of carvedilol standard disc were used as a reference when comparing the effects of the different types of excipient

on dissolution of carvedilol. A calculated *f*2 value of 50 or greater (50-100) indicates similarity of the two dissolution profile (M. Gohel et al., 2009).

5.1.6 Calculation of the *f*2 similarity factor

The *f*2 similarity factor is calculated as presented in equation (7) (Diaz et al.,2015) where (Rt) is the mean dissolution value for the reference at time t, (Tt) is the mean dissolution value for the test product at same time point and (n) is the number of time points.

$$f2 = 50 \text{ x } \log 10 \left[\frac{100}{\sqrt{1+\sum_{t=1}^{n}\frac{(Rt-Tt)^2}{n}}} \right] \quad \text{(Equation 7)}$$

5.2 Results

5.2.1 Dissolution testing of carvedilol using Sirius T3

5.2.1.1 Dissolution testing of carvedilol control

The dissolution profile of carvedilol in both fasted and fed state media using Sirius T3 are presented in Figure 5.1. The results shows that an amount of 29.6 µM of carvedilol drug was released in the fasted state and an amount of 48.9 µM in the fed state at the end of the dissolution testing (after 2 hours) which indicates higher dissolution rate of carvedilol in fed state compared to fasted state.

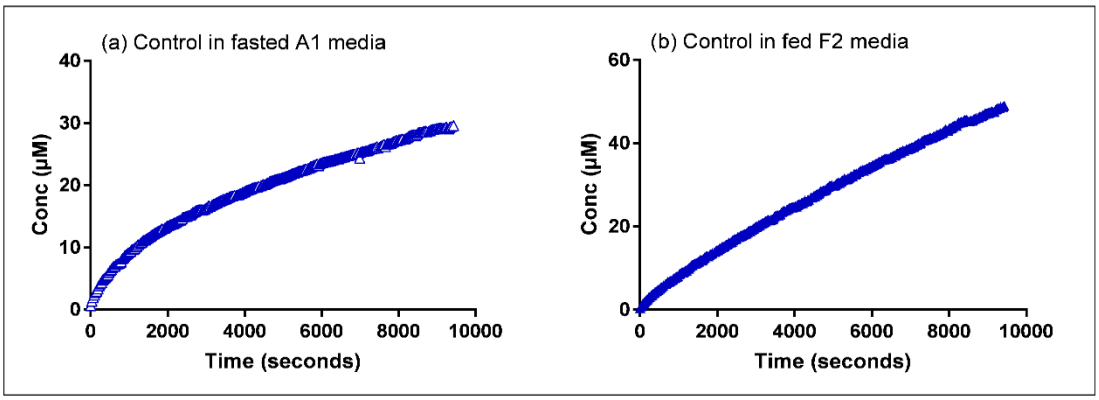


Figure 5.1: Sirius T3 dissolution results of carvedilol in fasted and fed DoE media. (a) and (b), dissolution testing of carvedilol. Open triangle referred to dissolution testing in fasted state and closed triangle referred to dissolution testing in fed state.

5.2.1.2 Examining the effect of excipient on dissolution of carvedilol when excipient and carvedilol are both in disc.

The effect of the different excipients on dissolution of carvedilol are presented in Figure 5.2 where the dissolution data of the control carvedilol disc compared to the dissolution data of carvedilol+ excipient disc in both fasted and fed states. In order to compare between the dissolution profiles of the different experiments, *f*2 similarity factor was calculated (Box et al., 2016) for each dissolution profile in both fasted and fed state (Table5.1).

The results show that the carvedilol dissolution profile from the disc containing excipients revealed some interesting behavior. Carvedilol concentration released from disc containing the following excipients mannitol, PVP HG or LG and HPMC E3 (Figure 5.2 a-h and Table 5.1) showed similar dissolution profile in comparison with the dissolution profile of the control in both fasted and fed state. The corresponding values of *f*2 similarity factor between carvedilol control and the carvedilol+ excipients were 95 and 85 (mannitol+ carvedilol), 70 and 66 (PVP LG+ carvedilol), 60 and 70.15 (PVP HG+ carvedilol) and 55.5 and 57.5 (HPMC E3+ carvedilol) in fasted and fed state respectively. This indicates the similarity in the dissolution profiles of the different experiments. These dissolution results were comparable with the solubility results in DoE (section 4) where solubility of carvedilol did not change with these types of excipients which indicates their neutral effect on carvedilol solubility and dissolution in both fasted and fed simulated intestinal media.

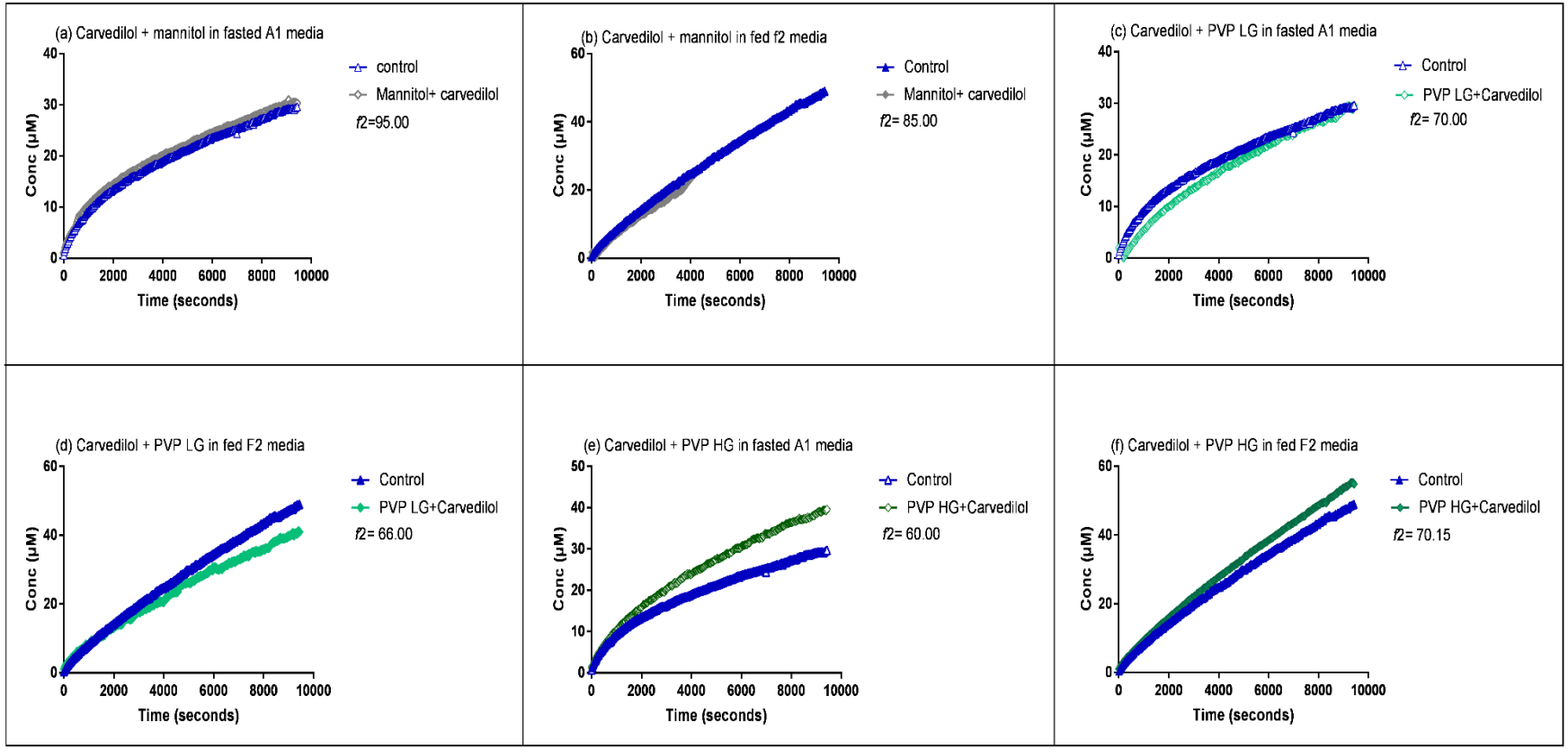
The outcomes were different with chitosan and HPMC E50 where in both fasted and fed state of chitosan and fasted state of HPMC E50 (Figure 5.2 i, k and l), carvedilol release from disc was increasing rapidly from the beginning of the experiment (first minute) reaching the highest concentration within the first hour and then it starts to decrease gradually until it reached the lowest concentration at the end of the experiment. In chitosan, concentration of the released carvedilol reached to 68.1 µM after 45 minutes and 59.2 µM after 1 hour and then decreased to 24.98 µM and 14.03

µM at the end of the dissolution study in both fasted and fed state respectively. The values of the *f*2 similarity factor were 23.9 and 28.95 in fasted and fed states respectively which indicates the differences in carvedilol release when chitosan was added to dissolution testing. In HPMC E50 fasted media (A1), the released carvedilol concentration reached the maximum after 50 minutes (59.4 µM) and then decreased till it reached 3.5 at the end of the experiment. The similarity *f*2 value was 29.15 in fasted state which indicates the differences between the control and the disc with carvedilol and HPMC E50. These results were comparable to the solubility data of carvedilol where solubility values were lower when these types of excipients were in same fasted A1 and fed F2 media.

In F2 fed state of HPMC E50 (Figure 5.2 j), the concentration of released carvedilol was 86.08 µM compared to 44.7 µM of the carvedilol control after 2 hours of the experiment which indicate that HPMC E50 increased the dissolution rate of carvedilol from disc. However, as HPMC E50 showed that it decreases the equilibrium solubility of carvedilol after 24 hours when DoE experiment in F2 media was carried out, a longer dissolution experiment duration to about 5 hours was carried out for HPMC E50 excipient with the carvedilol. The results showed that concentration of the released carvedilol when with the excipient started to decrease after around 3 hours to reach a concentration of 62.1 µM compared to 79.01 µM of carvedilol control at the end of the experiment.

Table 5.1: Values of *f*2 similarity factor of the different tests (yellow lines are the significant values).

Test (disc)	Media	Time (hour)	*f*2 similarity factor
carvedilol+ mannitol	A1 fasted media	2	95
carvedilol+ PVP LG	A1 fasted media	2	70
carvedilol+ PVP HG	A1 fasted media	2	60
carvedilol+ HPMC E3	A1 fasted media	2	55.53
carvedilol+ HPMC E50	A1 fasted media	2	29.15
carvedilol+ chitosan	A1 fasted media	2	23.91
carvedilol	A1 fasted media+ mannitol	2	90
carvedilol	A1 fasted media+ HPMC 50	2	42
carvedilol	A1 fasted media+ chitosan	2	46
carvedilol+ mannitol	F2 fed media	2	85
carvedilol+ PVP LG	F2 fed media	2	66
carvedilol+ PVP HG	F2 fed media	2	70.15
carvedilol+ HPMC E3	F2 fed media	2	57.51
carvedilol+ HPMC E50	F2 fed media	5	31.35
carvedilol+ chitosan	F2 fed media	2	28.95
carvedilol	F2 fed media + mannitol	2	89.52
carvedilol	F2 fed media + HPMC 50	2	32.15
carvedilol	F2 fed media + chitosan	2	25.25


(a) Carvedilol + mannitol in fasted A1 media
control
Mannitol+ carvedilol
f2=95.00
(b) Carvedilol + mannitol in fed f2 media
Control
Mannitol+ carvedilol
f2= 85.00
(c) Carvedilol + PVP LG in fasted A1 media
Control
PVP LG+Carvedilol
f2= 70.00
(d) Carvedilol + PVP LG in fed F2 media
Control
PVP LG+Carvedilol
f2= 66.00
(e) Carvedilol + PVP HG in fasted A1 media
Control
PVP HG+Carvedilol
f2= 60.00
(f) Carvedilol + PVP HG in fed F2 media
Control
PVP HG+Carvedilol
f2= 70.15
Conc (µM)
Time (seconds)

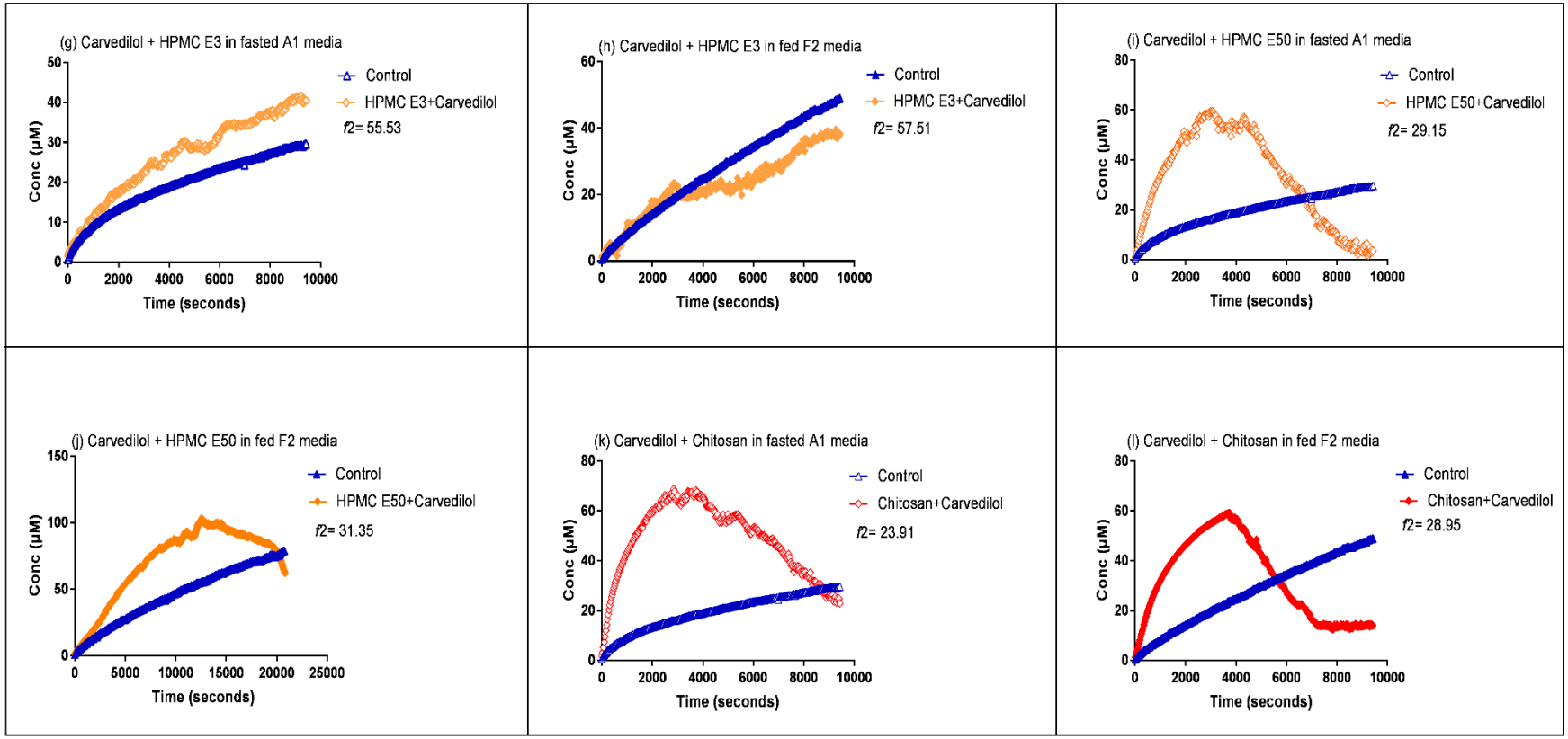


Figure 5.2: Sirius T3 dissolution results of excipients +control disc in fasted and fed state media. Blue open triangle for control in fasted state and closed blue triangle in fed state. All excipients were represented with diamonds (open for fasted and closed for fed), grey for mannitol, light green for PVP LG and dark green for PVP HG, light orange for HPMC E3 and dark orange for HPMC E50, red for chitosan.

5.2.1.3 Examining the effect of excipient on dissolution of carvedilol when excipient is in simulated media

The results in the previous section showed that chitosan and HPMC E50 exhibited unusual behavior when compared to the other excipients during carvedilol dissolution testing. These excipients showed that the released concentration of carvedilol was increased at the beginning of the experiment until it reached to a point where the released concentration starts gradually to decreases until it reached to a concentration lower than that of the standard at the end of the experiment. In a reason to understand the exact behavior and effect of these 2 types of excipients, tests were repeated with the modification that chitosan and HPMC E50 were added to the media rather than to the carvedilol disc and then results compared to the carvedilol control dissolution profile result. In addition, as mannitol showed the most comparable results (superimposed) compared with the carvedilol control dissolution profile in both fasted and fed state, it was repeated also by adding it to the media and used as an additional control for comparison. Figure 5.3 presents the dissolution profile of these excipients when they were added to the media compared to the carvedilol dissolution profile in both fasted and fed state media.

The results showed that dissolution of carvedilol was not changed when mannitol was in disc with carvedilol or when it was in media as the *f*2 similarity values were higher than 50.00 in both conditions (Table 5.1). The concentration of the released carvedilol when mannitol was in media reached to 31.7 µM and 48.5 µM (Figure 5.3 a & b) compared to 30.3 µM and 48.4 µM when mannitol was in disc in fasted and fed state respectively. While for chitosan and HPMC E50 (Figure 5.3 c-f), the results showed that when adding excipient to the media, the steep increase in carvedilol release disappeared and both excipients showed a lower concentration than the control from the beginning until the end of the experiment in both fasted and fed states. The *f*2 similarity values were 46.00 and 25.25 when chitosan and 42.00 and 32.15 when HPMC E50 was added to the media in both fasted and fed stat respectively (Table 5.1). Chitosan and HPMC are excipients that are used as disintegrants in solid oral dosage form (Lee et al., 1999; Illum, 1998) as when they are in disc they will swell and help the disintegration of the disc and consequently the dissolution of the drug.

Nevertheless, the disappearance of the unusual behaviour of chitosan and HPMC E50 on dissolution of carvedilol when they were added to the media probably might be due to the absence of the swelling effect of these types of excipients when they were in discs compared when they were added as solutions to the dissolution media.

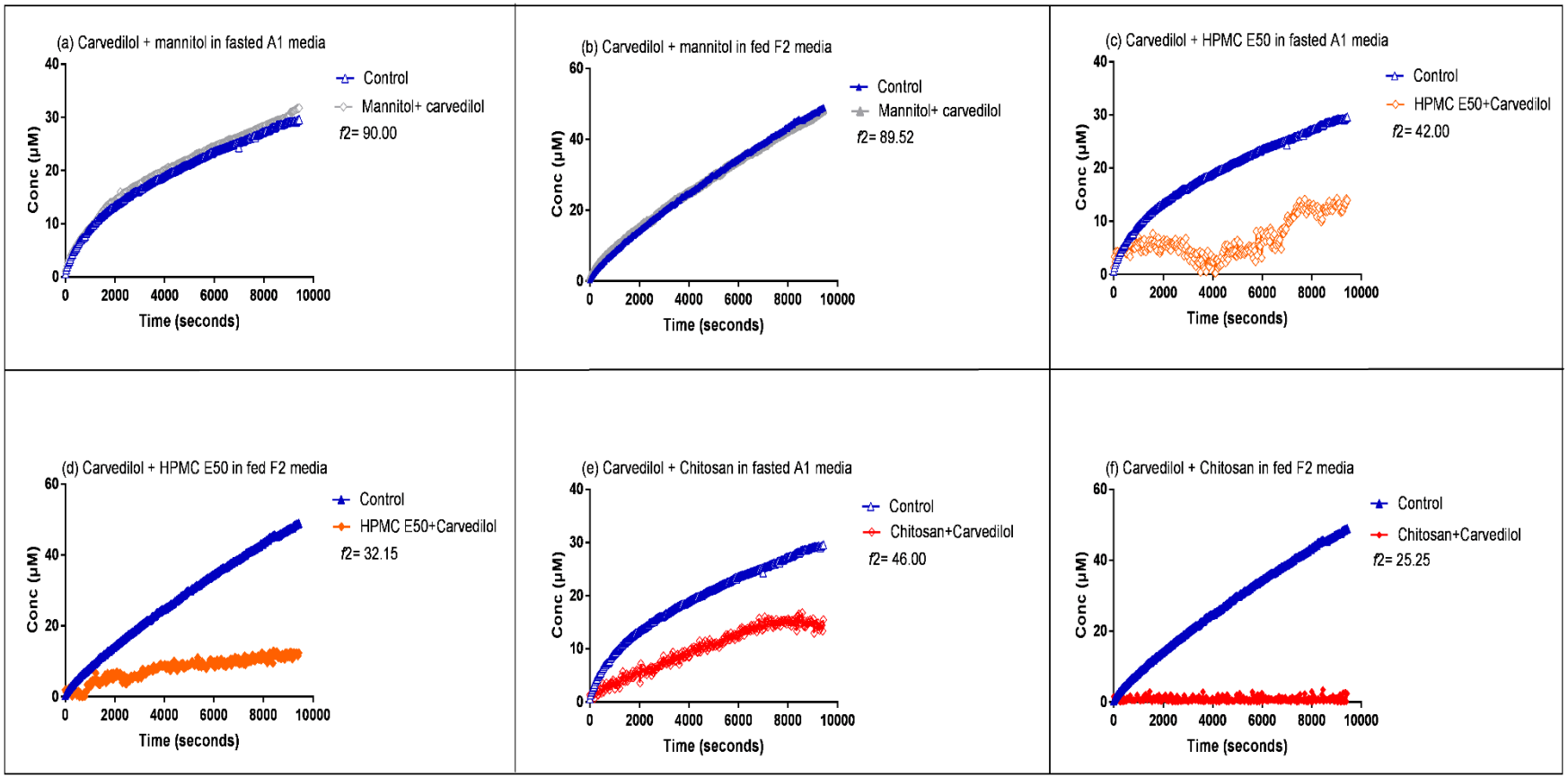


Figure 5.3: Sirius T3 dissolution results of carvedilol when excipients were in fasted and fed simulated media. Blue open triangle for control in fasted state and closed for control in fed state. All excipients were represented with diamonds (open for fasted and closed for fed), grey for mannitol, dark orange for HPMC E50 and red for chitosan.

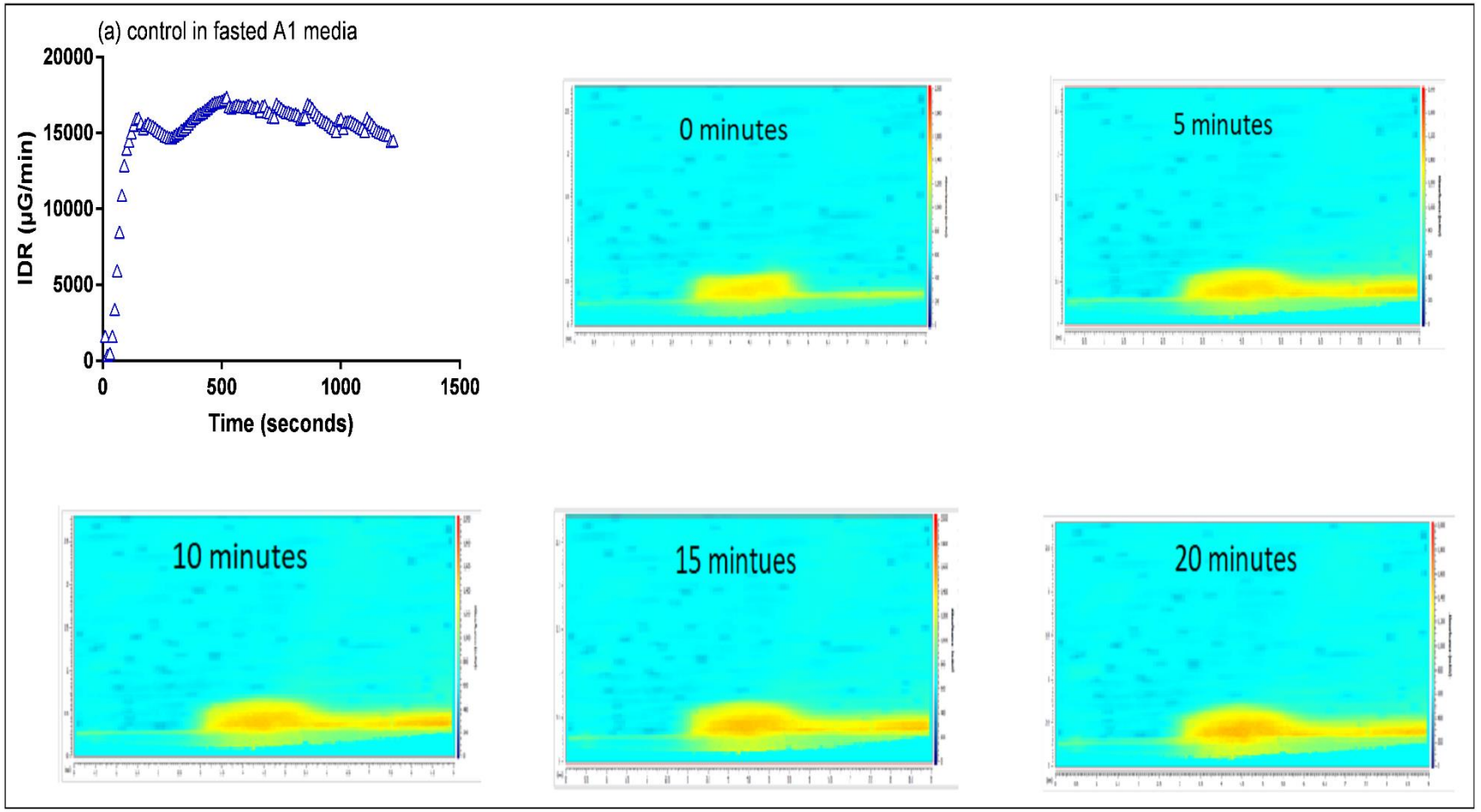
5.2.2 Dissolution testing of carvedilol using Sirius SDI

Sirius SDI is a surface dissolution imaging instrument that provides the ability to look directly at the solid liquid interface as the dissolution process is happening providing the opportunity to understand the dissolution behaviour of the examined samples. Consequently to understand and ensure the swelling effect of chitosan and HPMC E50 in both fasted and fed simulated media, dissolution testing of these two types of excipients were performed using Sirius SDI and compared to the control carvedilol in same simulated media.

5.2.2.1 Dissolution testing of carvedilol control

The dissolution profile of carvedilol control in both fasted A1 and fed F2 media are presented in Figure 5.4 (a & b respectively). In addition, the Figure presented images of the dissolution process of carvedilol in both fasted and fed state media, images were taken every 5 minutes during the experiment.

The results showed that intrinsic dissolution rate of carvedilol reached to about 15000 µg /min in fasted state at the end of the experiment (after 20 minutes) while In fed state, carvedilol showed more complicated dissolution rate where IDR reached to around 9000 µg/min in the first minute of the experiment then starting to fluctuate until it dropped to around 7700 µg/min at the end of the experiment. The images showed the slight swelling of the disc over the experiment when it is in contact with the dissolution simulated media (0 minutes compared with the 20 minutes).


(a) control in fasted A1 media
20000
15000
10000
5000
0
IDR (µG/min)
0
500
1000
1500
Time (seconds)
0 minutes
5 minutes
10 minutes
15 mintues
20 minutes

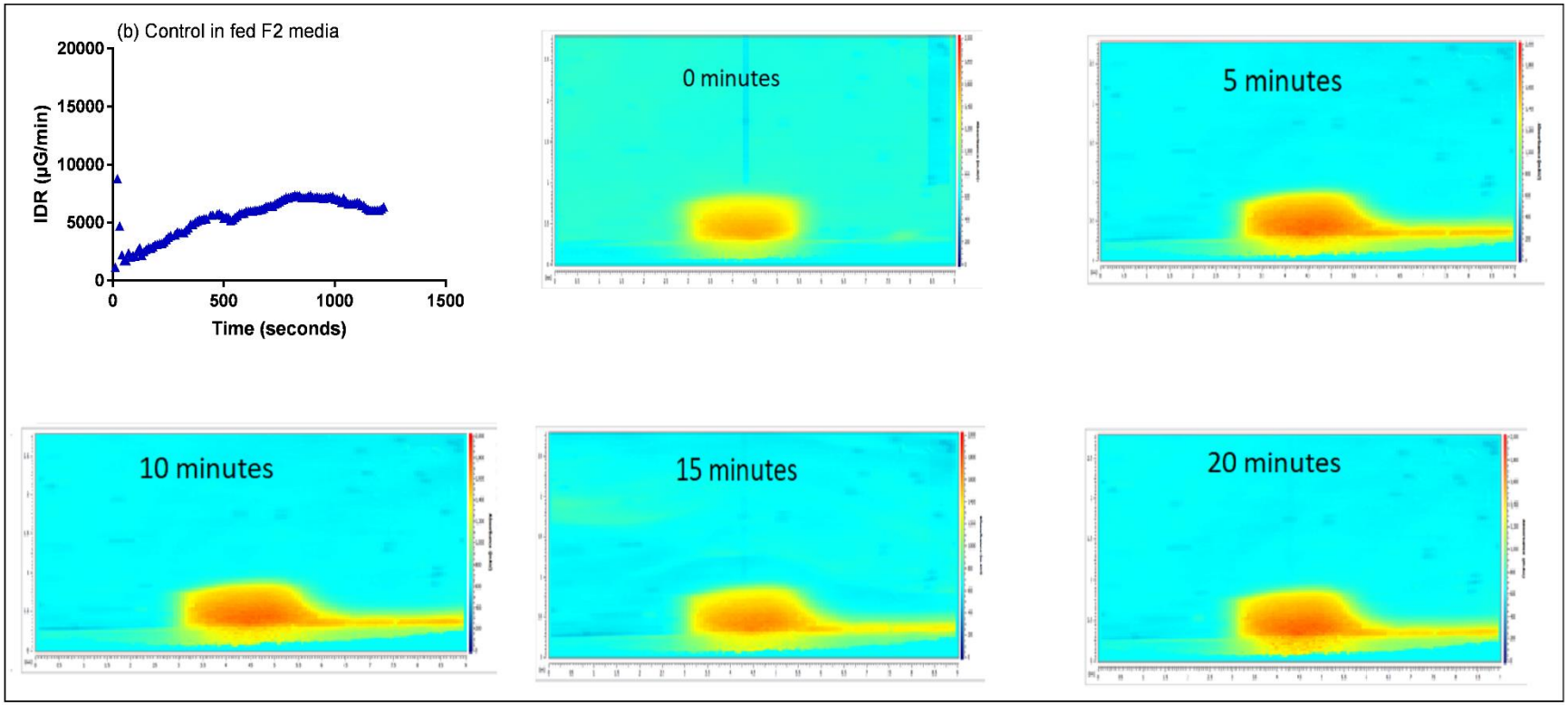


Figure 5.4: SDI dissolution results of carvedilol control in fasted and fed media. (a) and (b), dissolution testing of carvedilol, open triangle referred to dissolution testing in fasted state and closed triangle referred to dissolution testing in fed state. Images showed the disc (2 mm diameter) dissolution process over the experiment where snap shot were taken at the beginning 0 minute, after 5 minutes, after 10 minutes , after 15 minutes and the end of experiment after 20 minutes.

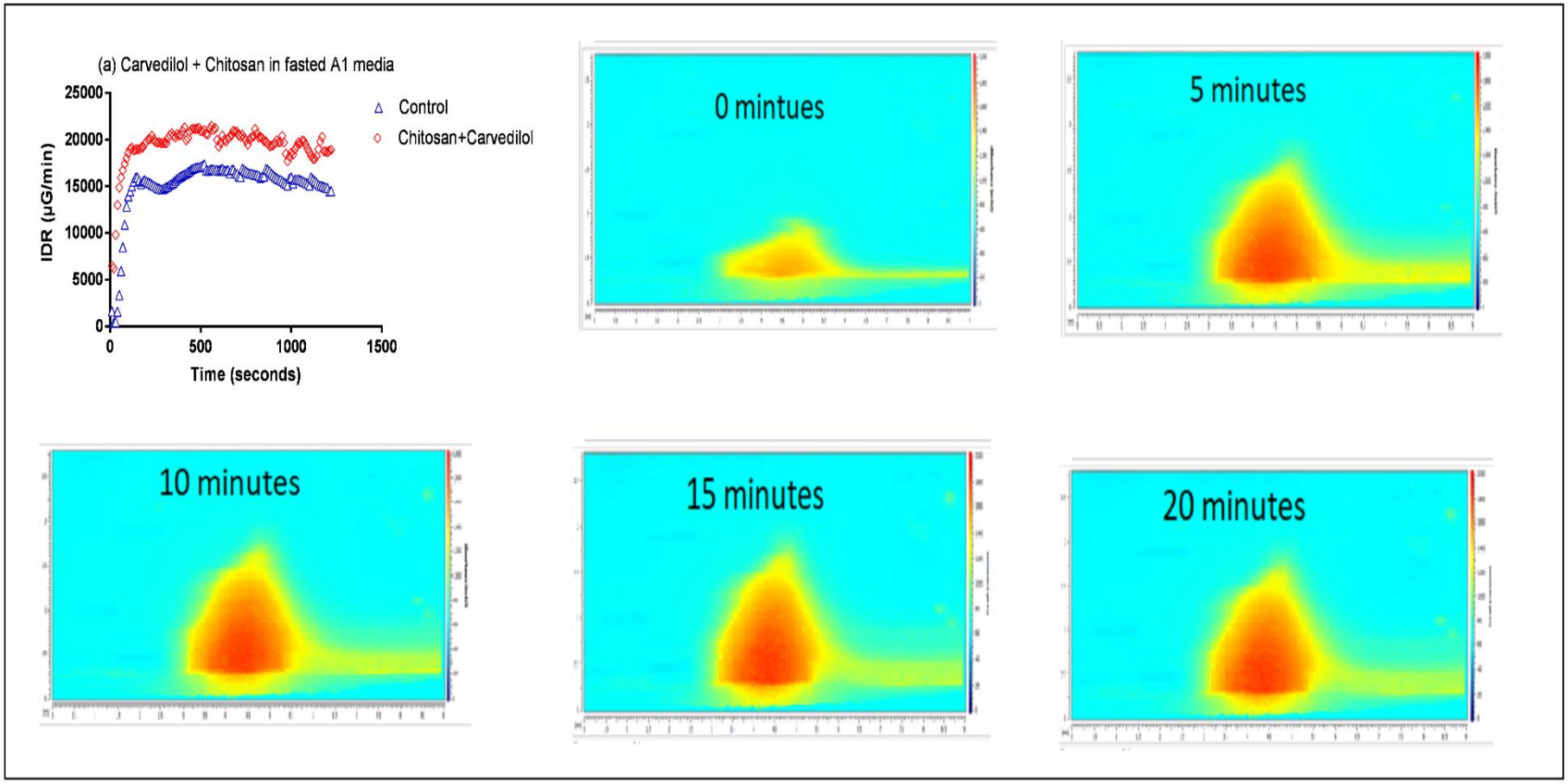
5.2.2.2 Examining the effect of excipient on dissolution of carvedilol when excipient and carvedilol are both in disc.

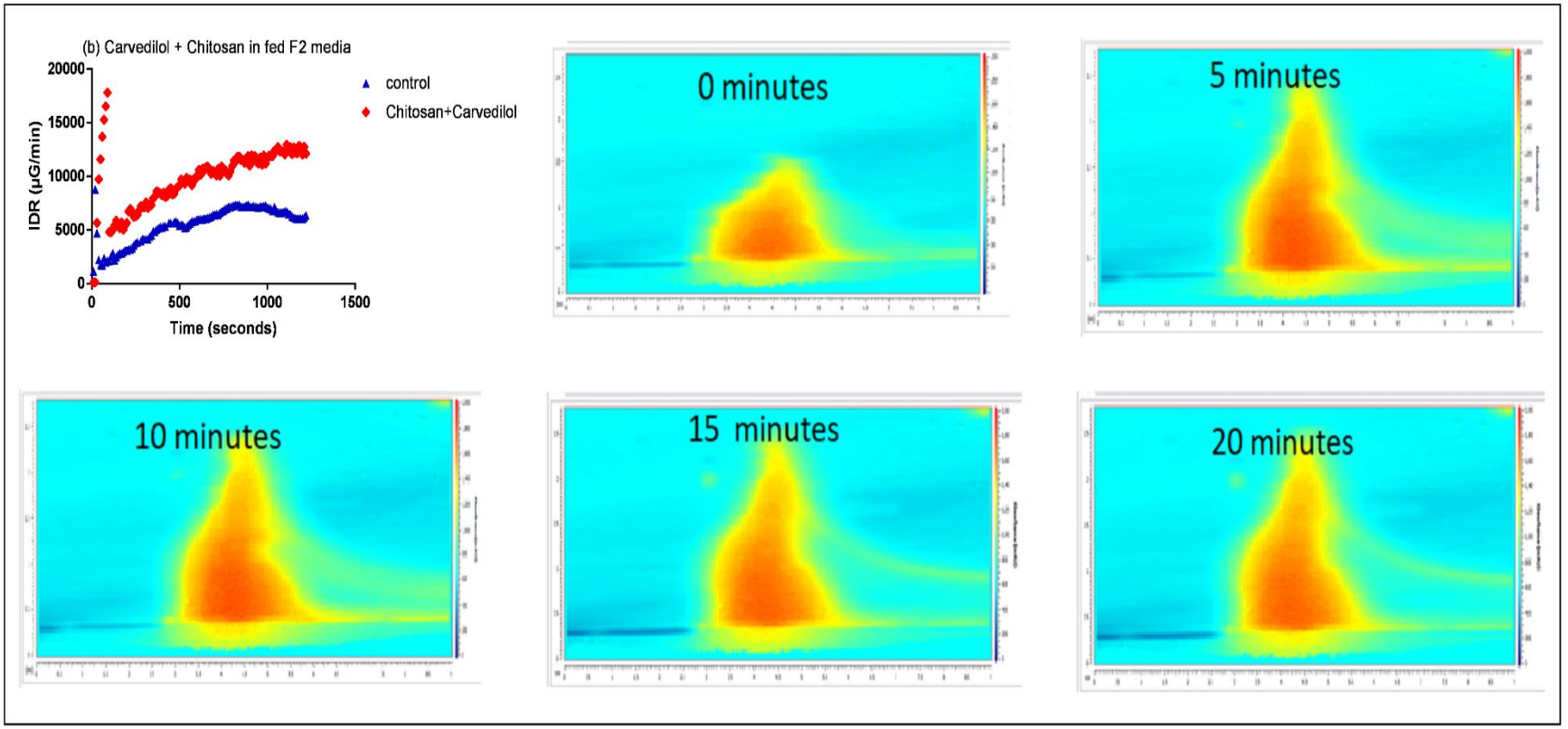
The dissolution profile of carvedilol using Sirius SDI in both fasted and fed state when the chitosan and HPMC E50 are added to the disc are presented in Figure 5.5 along with a snap shot every 5 minutes of the dissolution process of carvedilol with each excipient in each state.

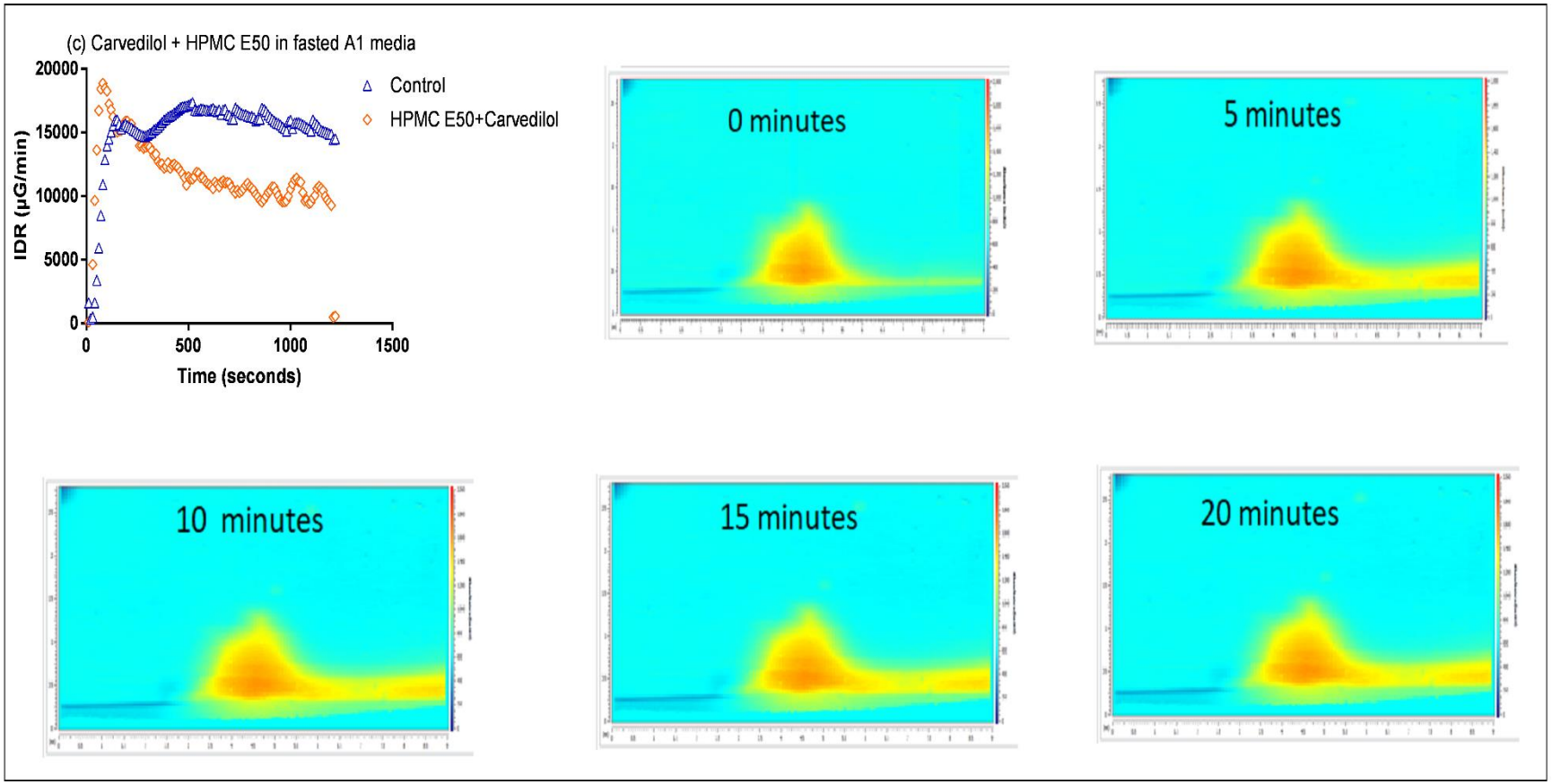
The results showed that with both excipients in both fasted and fed state, the IDR of carvedilol was higher than the carvedilol control at the beginning of the experiment which indicate that these higher dissolution rate might be related to the swelling of the discs containing chitosan or HPMC E50. These results were comparable with the dissolution profile of carvedilol with these excipients using Sirius T3 where the released carvedilol from disc found to rapidly increase at the beginning of the experiment. The snap shot images during the SDI experiment gave an explanation of the higher jump in carvedilol release with chitosan and HPMC E50 once the disc was in contact with the dissolution media. The images showed the huge swelling of the tablet when chitosan or HPMC E50 were added to the carvedilol in disc when compared to carvedilol disc only which causes the drug to be released rapidly form the tablet.

However, using Sirius T3 carvedilol concentration when in disc with chitosan and HPMC E50 was found to gradually decreases after the first hour to reach to a concentration lower than the standard while in Sirius SDI, the IDR found to be constant (with the exception of HPMC E50 in fasted state) till the end of the experiment but it should be noted that the duration in both instruments was different with 2 hours using Sirius T3 compared to 20 minutes using the Sirius SDI. Moreover, the SDI is a flowing rather than a static system where the Sirius T3 is. The simulated media solution including the excipient is constantly removed away in the SDI while in the Sirius T3, the simulated media with the excipient are accumulating and build up. The chitosan was able to swell and release it self while the case was different with HPMC

especially in fasted state with lower media concentrations, the HPMC stick and remain in the system which explain the lowering dissolution profile.


(a) Carvedilol + Chitosan in fasted A1 media
IDR (µG/min)
25000
20000
15000
10000
5000
0
0
500
1000
1500
Time (seconds)
Control
Chitosan+Carvedilol
0 mintues
5 minutes
10 minutes
15 minutes
20 minutes


(b) Carvedilol + Chitosan in fed F2 media
control
Chitosan+Carvedilol
IDR (µG/min)
20000
15000
10000
5000
0
0
500
1000
1500
Time (seconds)
0 minutes
5 minutes
10 minutes
15 minutes
20 minutes


(c) Carvedilol + HPMC E50 in fasted A1 media
IDR (µG/min)
20000
15000
10000
5000
0
0
500
1000
1500
Time (seconds)
Control
HPMC E50+Carvedilol
0 minutes
5 minutes
10 minutes
15 minutes
20 minutes

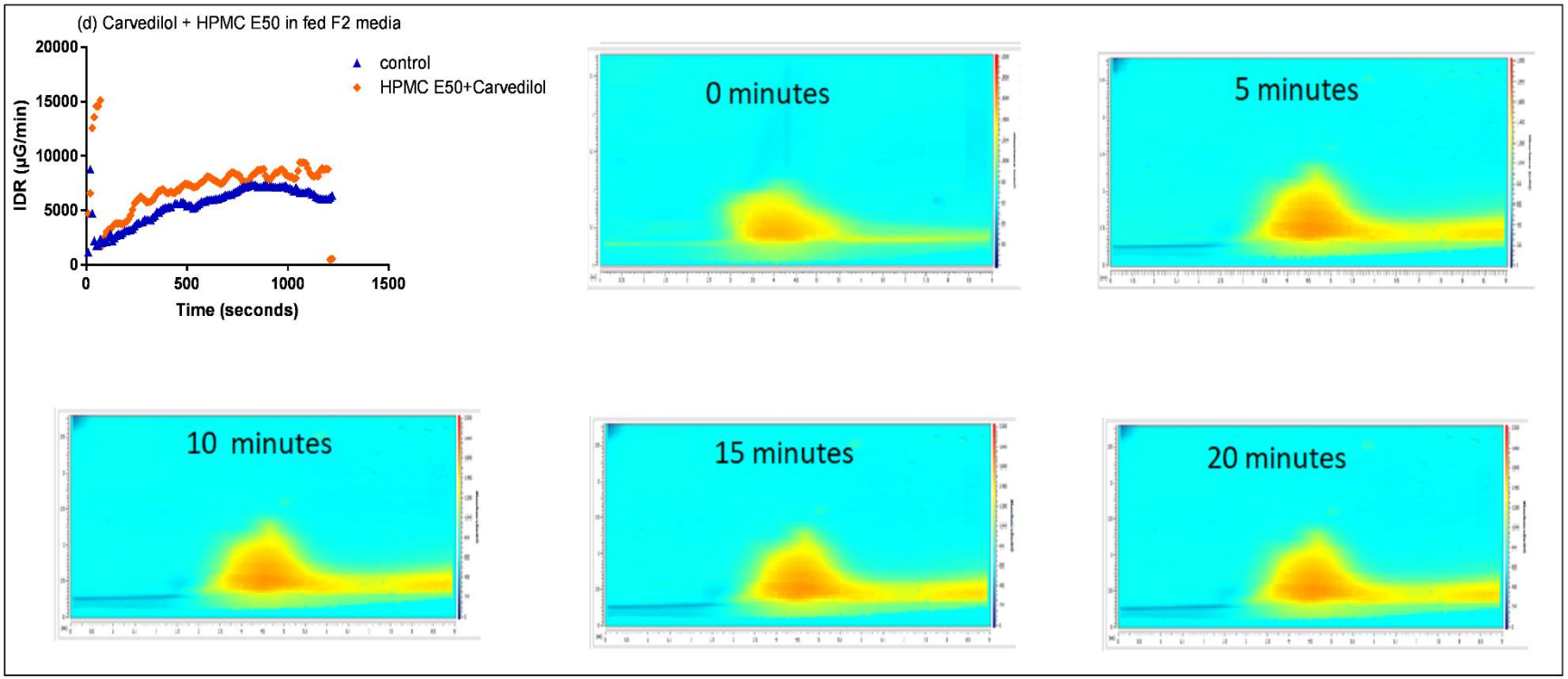


Figure 5.5: SDI dissolution results of carvedilol when excipient pressed in disc with the control in fasted and fed simulated media. Blue data points, dissolution testing for carvedilol control, open triangle referred to dissolution testing in fasted state and closed triangle referred to dissolution testing in fed state. Red data points for chitosan, (a) in fasted A1 media and (b) in fed F2 media. Orange data points for HPMC E50, (c) in fasted A1 media and (d) in fed F2 media. Images showed the disc (2 mm diameter) dissolution process over the experiment where snap shot were taken at the beginning 0 minute, after 5 minutes, after 10 minutes , after 15 minutes and the end of experiment after 20 minutes.

5.2.2.3 Examining the effect of excipient on dissolution of carvedilol when excipient is in simulated media

Examining the dissolution profile of carvedilol when chitosan and HPMC E50 were in media rather than in disc was not able to be detected using Sirius SDI. The dissolution media consisting of simulated biological components such as lecithin and sodium oleate, with different concentrations make the media slightly turbid which became heavily turbid when chitosan or HPMC E50 are added to the fasted or fed simulated media and difficulty arises when there is no light reaching the detector and there is a complete block of the light when the media is so turbid. Figure 5.5 showed images of how turbid the dissolution media became after the addition of chitosan or HPMC E50 compared to the previous images (image 5.4).

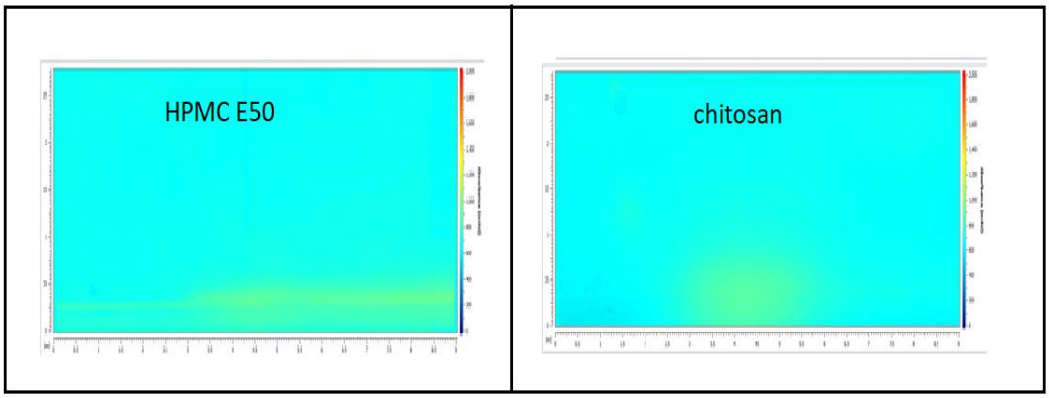


Figure 5.6: Images of the SDI dissolution process of carvedilol when chitosan and HPMC E50 are in media.

5.2.3 Comparing equilibrium solubility and concentration of released drug in dissolution testing

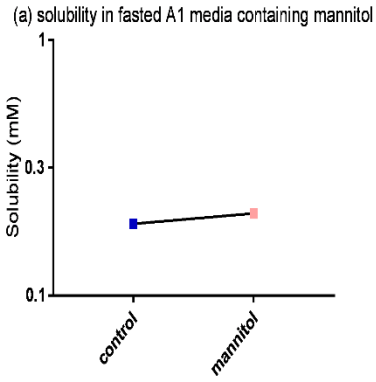
Equilibrium solubility of carvedilol in presence of the 6 different excipients were measured using the design of experiment and discussed in the previous section (section 4). In order to indicate the correlation between solubility and dissolution of carvedilol in presence of these excipients, comparison between the carvedilol control solubility and each excipient and the overall dissolution of the control and each excipient in fasted and fed media were presented in Figure 5.6 (a-x). Figure 5.7 (1 and 2) represent the solubility values along with the released concentration of the control carvedilol and

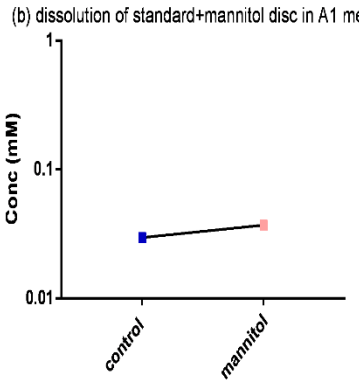
the carvedilol with the excipients. The comparison was done in the same A1 fasted and F2 fed media used for dissolution testing by comparing the equilibrium solubility values of the drug after 24 hours with the concentration of the drug released at the end of the dissolution experiment (2 hours).

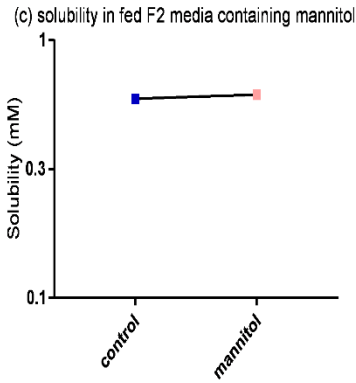
In A1 fasted state, testing carvedilol solubility and dissolution testing containing the following four excipients (mannitol, PVP LG, PVP HG and HPMC E3) showed that solubility values (Figure 5.6 (a, e, i, m)) and (Figure 5.7 (1)) were comparable to the control (see section 4.2.6 regarding the 3 fold differences between control and test). These results were in agreement with the dissolution testing of carvedilol disc containing these four excipients where results (Figure 5.6 (b, f, j, n)) and (Figure 5.7 (2)) showed that for all four excipients, concentrations of released carvedilol were comparable at the end of the experiment (within the range and no more than 3 fold differences). Though, for PVP HG and HPMC E3 the figure showed that concentrations of the released carvedilol were higher compared to the disc containing only carvedilol or carvedilol with mannitol and PVP LG. This might refer to solubilising effect of these excipients (Lee et al., 2013; Zoghbi & Wang et al., 2016; Javeer et al., 2013) on drugs when used in solid oral dosage forms that discussed previously in (section 4 .1.3). The results also showed that with the addition of chitosan and HPMC E50 to the media (Figure (5.6 q, r, u and v)), both solubility and the overall dissolution values of carvedilol (with the exception of dissolution testing of chitosan) were 3 times lower in comparison with the control carvedilol. Chitosan dissolution testing was lower but the differences was 1 fold lower than the control at the end of the dissolution test.

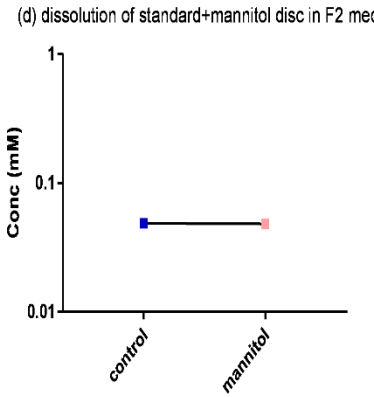
In F2 fed media, solubility and dissolution testing of carvedilol in media containing the following four excipients (mannitol, PVP LG, PVP HG and HPMC E3) showed that solubility and dissolution values of carvedilol in these media (Figure 5.6 (c, g, k, and o) and (Figure 5.7 (1)) were comparable to the control. Yet, (Figure 5.7 (1 and 2)) showed that solubility and dissolution values of the media containing PVP HG was higher compared to other media and this is referred again to the solubilising effect of that excipient (Lee et al., 2013; Zoghbi & Wang et al., 2016). In media containing

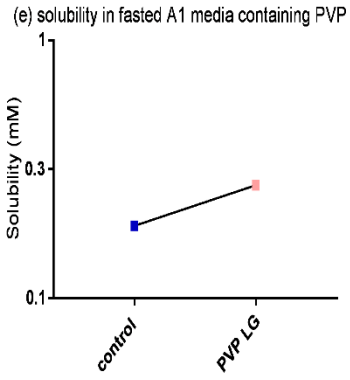
chitosan and HPMC E50, comparable results found in the overall dissolution and solubility testing where statistical investigations showed a significant differences in solubility and dissolution when these excipients were added to the media. Moreover, examination of (Figure 5.6 (s, t, w, x)) and (Figure 5.7 (1 and 2)) showed that both solubility and dissolution values (with the exception of the dissolution testing of HPMC E50) were 3 times lower in F2 media containing both excipients compared to the control. Dissolution testing of HPMC E50 started to decrease after 3 hours and the differences compared to the control was 1 fold lower at the end of the dissolution test.

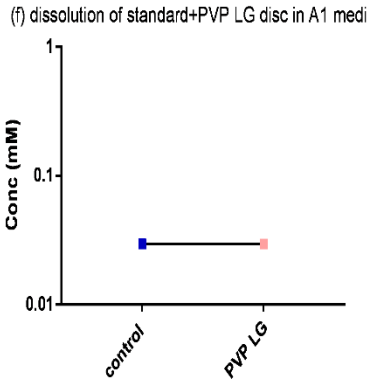

(a) solubility in fasted A1 media containing mannitol
Solubility (mM)
1
0.3
0.1
control
mannitol

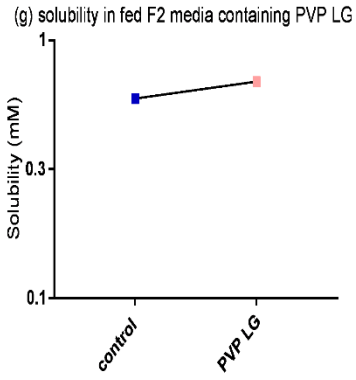

(b) dissolution of standard+mannitol disc in A1 media
Conc (mM)
1
0.1
0.01
control
mannitol

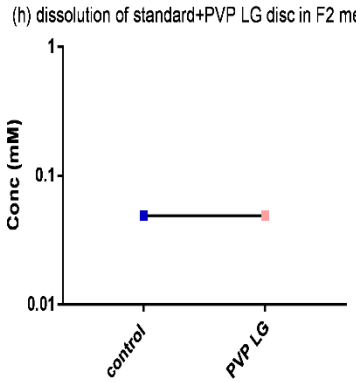

(c) solubility in fed F2 media containing mannitol
Solubility (mM)
1
0.3
0.1
control
mannitol

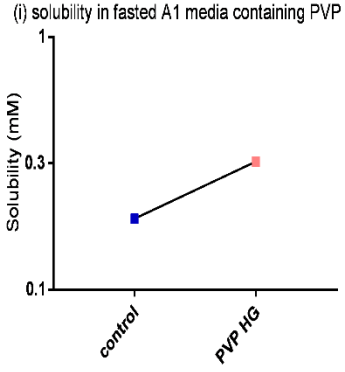

(d) dissolution of standard+mannitol disc in F2 media
Conc (mM)
1
0.1
0.01
control
mannitol

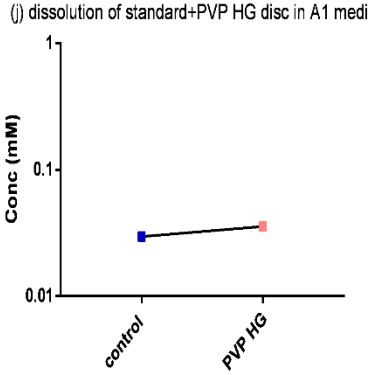

(e) solubility in fasted A1 media containing PVP LG
Solubility (mM)
1
0.3
0.1
control
PVP LG

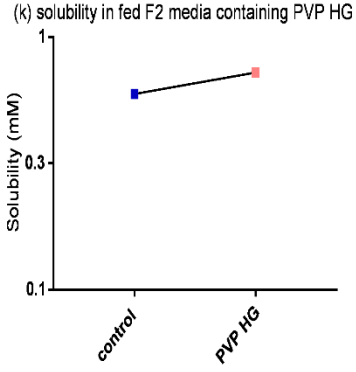

(f) dissolution of standard+PVP LG disc in A1 media
Conc (mM)
1
0.1
0.01
control
PVP LG

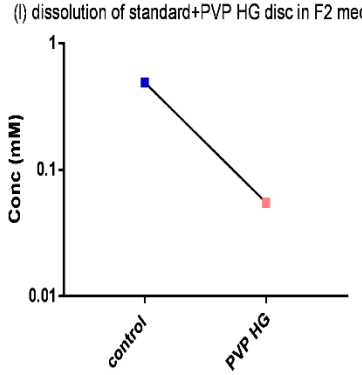

(g) solubility in fed F2 media containing PVP LG
Solubility (mM)
1
0.3
0.1
control
PVP LG

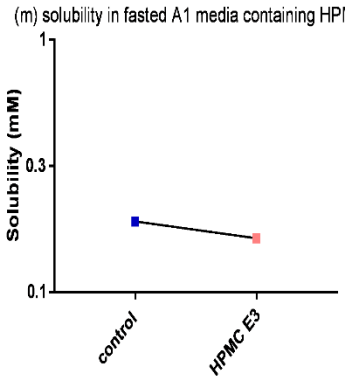

(h) dissolution of standard+PVP LG disc in F2 media
Conc (mM)
1
0.1
0.01
control
PVP LG

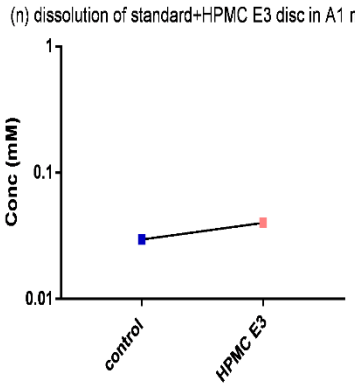

(i) solubility in fasted A1 media containing PVP HG
Solubility (mM)
1
0.3
0.1
control
PVP HG

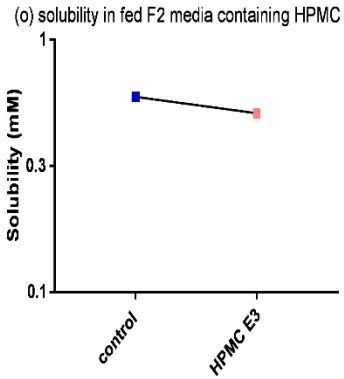

(j) dissolution of standard+PVP HG disc in A1 media
Conc (mM)
1
0.1
0.01
control
PVP HG

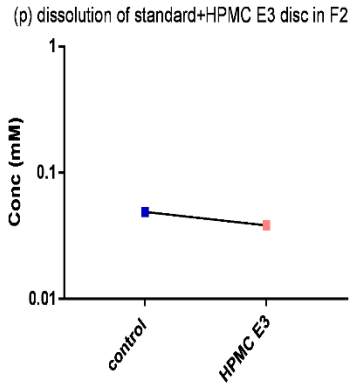

(k) solubility in fed F2 media containing PVP HG
Solubility (mM)
1
0.3
0.1
control
PVP HG


(l) dissolution of standard+PVP HG disc in F2 media
Conc (mM)
1
0.1
0.01
control
PVP HG


(m) solubility in fasted A1 media containing HPMC E3
Solubility (mM)
1
0.3
0.1
control
HPMC E3


(n) dissolution of standard+HPMC E3 disc in A1 media
Conc (mM)
1
0.1
0.01
control
HPMC E3


(o) solubility in fed F2 media containing HPMC E3
Solubility (mM)
1
0.3
0.1
control
HPMC E3


(p) dissolution of standard+HPMC E3 disc in F2 media
Conc (mM)
1
0.1
0.01
control
HPMC E3

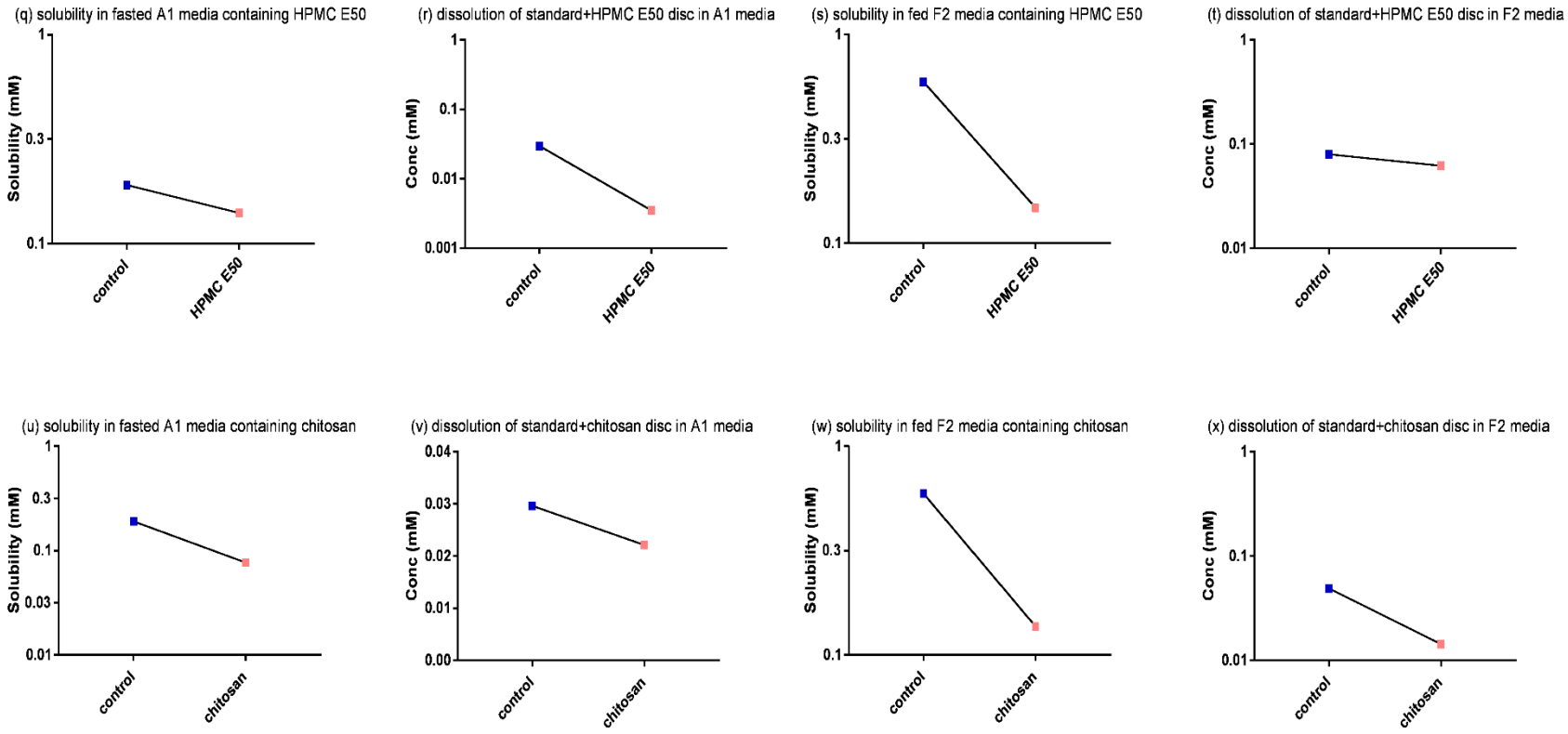
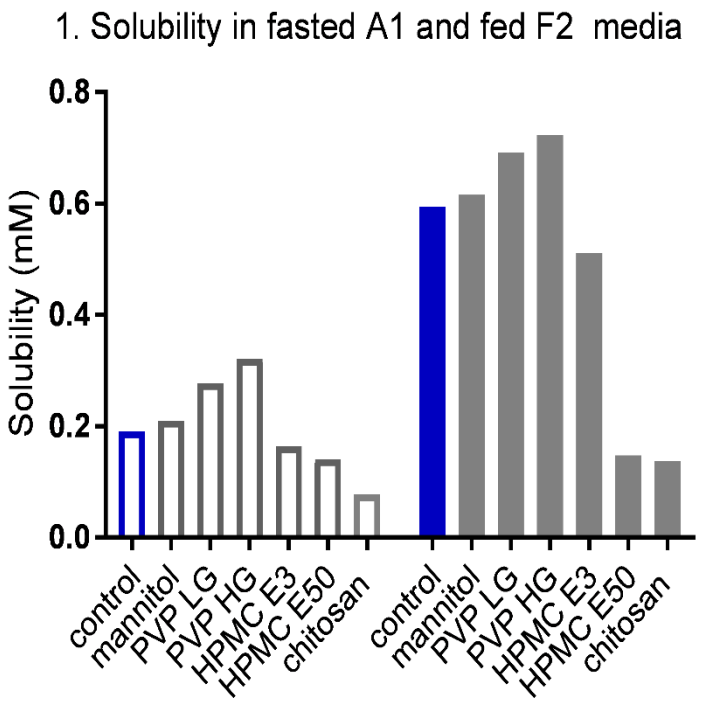
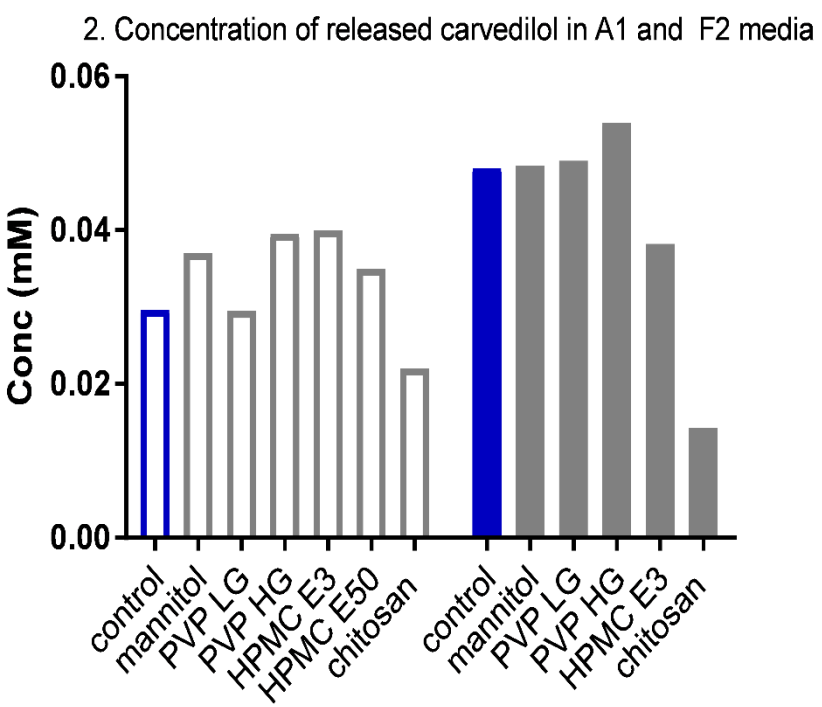


Figure 5.7: Equilibrium solubility values from DoE of carvedilol control and carvedilol +different excipient in media together with the concentration released of carvedilol in same media of carvedilol +the different excipients in disc with the control. Results of solubility was taken from previous chapter (chapter 4). Results of dissolution testing was for Sirius T3 dissolution test.


1. Solubility in fasted A1 and fed F2 media
Solubility (mM)
0.0
0.2
0.4
0.6
0.8
control
mannitol
PVP LG
PVP HG
HPMC E3
HPMC E50
chitosan
control
mannitol
PVP LG
PVP HG
HPMC E3
HPMC E50
chitosan


2. Concentration of released carvedilol in A1 and F2 media
Conc (mM)
0.00
0.02
0.04
0.06
control
mannitol
PVP LG
PVP HG
HPMC E3
HPMC E50
chitosan
control
mannitol
PVP LG
PVP HG
HPMC E3
chitosan

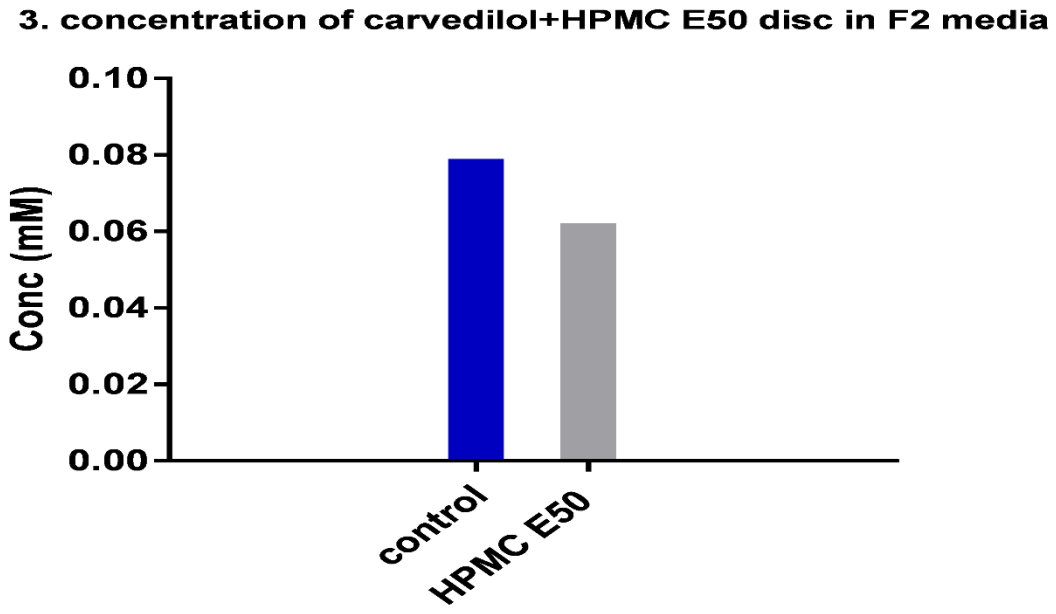


Figure 5.8: 1. Solubility values of carvedilol control in fasted and fed DoE media along with media containing the excipient where open bar for fasted and closed bar for fed. 2. Concentration of the released carvedilol control along with the concentration of carvedilol with the excipient in both fasted and fed media where open bar for fasted and closed bar for fed. 3. Concentration of the released carvedilol with HPMCE50 after 5 hours duration of the experiment. Results of solubility was taken from previous chapter (chapter 4). Results of dissolution testing was for Sirius T3 dissolution test.

5.3 Discussion

5.3.1 Dissolution testing of carvedilol

The dissolution profile of carvedilol using Sirius T3 in intestinal simulated media showed that dissolution of carvedilol was higher in fed state compared to the fasted state where the released carvedilol concentration was 0.0296 mM in fasted A1media compared to 0.0499 mM in fed F2 media at the end of the experiment. Dissolution studies of carvedilol using Sirius T3 was performed by OrBiTo in Strathclyde and showed a concentration of 0.36 mM of carvedilol dissolution in FaSSIF media, though different media was used in this study. The results of dissolution testing in this study was comparable to the solubility studies of carvedilol in the same corresponding media where a value of 0.1908 mM and 0.5944 mM in fasted and fed state respectively was reported in the previous section of carvedilol DoE solubility studies. This indicates that solubility and dissolution of carvedilol in fed media was higher than in fed than in fasted media. The results was also comparable to literature where dissolution and solubility profile of carvedilol was higher in FeSSIF media than FaSSIF media using calibrated USP apparatus type II for the dissolution and solubility testing (Hamed et al., 2016). In Hamed at al study, carvedilol solubility testing was reported to be 0.126 mM in FaSSIF and 2.04 mM in FeSSIF media. This higher dissolution rate of carvedilol in fed than fasted media returned to the higher concentration of media components such as oleate and bile salts in fed state than fasted state which was significantly improving drug solubility and was reported in literature DoE studies (Khadra et al., 2015; Zhou et al., 2017). However, this was not the case when dissolution testing was performed using the Sirius SDI where in fasted state carvedilol dissolution was complete and reached a plateau level after 20 minutes of the experiment. While the fed state, dissolution profile showed a more complicated process where dissolution of carvedilol increased rapidly at the first few minutes then decreased till the end of the experiment. This might be related to the more turbid media of the fed state compared to fasted state due to a higher media components concentration which make it more difficult for the light to reach to the detector.

5.3.2 Dissolution testing of carvedilol with excipients

The effect of 6 different types of excipients on dissolution of carvedilol in both fasted and fed state were studied using Sirius T3 while these excipients were compressed into a disc with carvedilol. The effect of chitosan and HPMC E50 on dissolution of carvedilol were studied using different instruments and methods ranging from Sirius T3 and Sirius SDI and studying the effect on dissolution while excipients are in disc with the drug or in simulated dissolution media.

Starting with the 6 excipients that were studied using Sirius T3, results showed that for mannitol, PVP LG, PVP HG and HPMC E3 there were no significant differences in dissolution profile of carvedilol in both fasted and fed state as *f*2 similarity factor were >50 (Table5.1) in all dissolution profile in both fasted and fed media state. This indicates that these excipients have no influence on dissolution of carvedilol which is comparable to the DoE solubility studies of these excipients discussed in the previous section (section 4). As discussed previously in section 4, these excipients found to increase solubility and dissolution of drugs through their either solubilising effect or the surfactant effect (Zoghbi & Wang, 2016, Javeer et al., 2013;Vadlamudi & Dhanaraj, 2017). Moreover, PVP found to increase solubility and dissolution of carvedilol in solid dispersion dosage form due to the interaction between the drug and the excipient and the enhancement of the wetting property of the drug (Lim et al 2011; Lee et al., 2013; Zoghbi & Wang, 2016; Djuris et al., 2019). This is in contrast with this study where PVP showed no effect on dissolution. However, different simpler media components were applied in these studies (distilled water, 0.1 N HCl and simulated gastric fluid media) compared to the more complex simulated intestinal media consisting of 7 different components in this study. In addition, both literatures used different drug to carrier ratio where significant increase in dissolution rate found when drug to carrier ratio was 1:5 which is different than this experiment where an amount of 3 mg of excipients were added to an amount of 20 mg of carvedilol. In addition, Shim et al., found that dissolution rate of carvedilol was higher than the active pharmaceutical when solid dispersion with HPMC was used (Shim et al., 2012). However, the release behaviour of the solid dispersion was performed using simulated

gastric fluid at pH 1.2. Nevertheless, it should be noted that there are no published literature studies that were investigating carvedilol dissolution in similar media.

In the case of chitosan and HPMC E50 with carvedilol in disc, results showed two distinctive behaviours of the excipients during the dissolution process. At the beginning of the dissolution testing and once the excipient is in contact with the dissolution media, the excipient act as a disintegrants (Lee et al., 1999; Illum, 1998) and consequently swell (Rohindra et al., 2018; Li et al., 2005; Siepmann et al., 2013) and facilitate the release of the drug. The swelling effect of both excipients were proved by the snap shot images taken every 5 minutes from SDI which showed the swelling of the disc containing these excipients over the dissolution process. The disintegrant functional effect of both excipients illustrate the steep rise in the dissolution curve at the start of the experiment as the excipients were acting as a spring. Guzmán et al first describes the concept of a material to facilitate the dissolution of a drug by using the term spring and parachute approach (Guzmán et al., 2007). Spring is the term that used when the drug first dissolves rapidly with the excipient to generate a supersaturated solution and to benefit from this supersaturation state of increasing absorption, the drug has to be maintained at high concentration for a period of time which is the parachute effect (Xua & Dai, 2013;.Brouwers et al., 2009). However, the steep increase in dissolution curve of carvedilol was decreased at the end of the experiment and the statistical similarity factor was lower than 50 in all cases (Table 5.1). This indicates that both excipients failed to maintain the parachute effect for carvedilol dissolution which may indicate interaction between excipients and the different media components.

Examining the effect of these excipients when they are in media showed a different behaviour compared to the disc. In both fasted and fed state and with both chitosan and HPMC E50, the dissolution of carvedilol was significantly lower (f 2 similarity factor was lower than 50 (Table 5.1)) from the beginning to the end of the experiment. This indicates that adding chitosan or HPMC E50 to the carvedilol dissolution media will causes changes in media and lead to lower dissolution rate. The interaction between the excipients and the different media components was also proved by the

failure of the SDI experiment (Figure 5.5) when dissolution testing was not able to be performed owed to the highly turbid media following the addition of chitosan and HPMC E50 as solutions to the media.

Comparing these results with published studies is difficult since literature results showed that both chitosan and HPMC E50 increase the dissolution profile of the poorly water soluble drugs through the enhancement of the wettability of the drug (Shete et al., 2012; Vijaya et al., 2006; Javeer et al., 2013). However, these dissolution studies in literature were performed using distilled water and or simulated gastric fluid media at pH 1.2 which is different to the more complicated and bio relevant media used in this experiment (Table2.2). In addition, the chitosan and the HPMC that were used in literature were either low molecular weight chitosan or low viscosity grade of HPMC compared to higher molecular weight and higher viscosity grade of chitosan and HPMC.

In this study, chitosan and HPMC E50 showed that when the excipient is in disc, it was able to increase the dissolution of the drug at the first hour of the experiment. This indicate that if the drug was able to be absorbed before it precipitate so that the reduced dissolution might have no impact on the absorption of the drug. This will come in compliance with previous studies where dissolution and bioavailability of carvedilol will be higher with these excipients (Sharma et al., 2019; Shete et al., 2012). However, if the drug was not absorbed within one hour where the excipients and media interaction started to affect the dissolution of the drug then the bioavailability might be affected. The dissolution lowering effect of chitosan and HPMC E50 might be related to the higher molecular weight of both excipients. When these excipients are in contact with the dissolution media they will swell and form a gel which make it difficult for the drug to diffuse through the gel into the dissolution media and thus the release of the drug will be retarded and this effect was reported in literature (Shete et al., 2012; Rohindra et al., 2018). Secondly, chitosan and HPMC E50 both contain different functional groups (Wang et al., 2006; Widanapathirana et al., 2015) that make both structures able to create hydrogen bonding with the different ionisable media components as discussed in previous chapter (chapter 4). The ability of chitosan to

decrease solubility due to the interaction with the different media components such as bile salt and fatty acids was reported in solubility studies literature (Kubbinga et al., 2015; Masayuki et al., 2006). Moreover, evidence of media changes was studied and discussed in the previous chapter of solubility of carvedilol in presence of chitosan and HPMC E50. In addition, failure of the SDI experiment due to the highly turbid media when both excipients were added proved the interaction and the media changes that occurred.

5.3.3 Correlation between solubility and dissolution

According to the Noyes-Whitney equation (equation 4), dissolution rate is directly related to the solubility under the same dissolution conditions (media volume, stirring rate, surface area etc...) meaning that drug dissolution will increased when solubility increase in same media and vice versa (Sugano et al., 2007). According to the DoE in section 4, solubility of the drug is highly affected by the media the drug is dissolved in.

According to Figure 5.6, results comes in agreement with the Noyes-Whitney equation and whenever the drug solubility was affected by a certain excipient, the dissolution of the drug was affected too. Solubility of carvedilol in media containing mannitol, PVP LG, PVP HG and HPMC E3 was not significantly affected and so the released carvedilol concentration in both fasted and fed media state. While solubility of carvedilol in A1 and F2 media containing chitosan and HPMC E50 was lower than media containing carvedilol control. Comparing this results with the concentration of the released carvedilol in same fasted and fed media indicates that the overall dissolution was also decreased in media examining dissolution in presence of these excipients compared to media containing carvedilol only in both fasted and fed state.

5.4 Conclusion

The results indicates that dissolution profile of carvedilol in fed simulated media was higher than dissolution profile in fasted simulated media which is in compliance with

literature. Dissolution testing of carvedilol combined with excipients showed different dissolution profile of carvedilol where presence of mannitol, HPMC E3 and both grades of PVP in disc with carvedilol showed no significant Influence on dissolution as the released carvedilol concentration was not changed. The results showed that the presence of chitosan and the higher grade of HPMC (E50) showed a significant influence on dissolution of carvedilol owed to the several changes that occur in media induced by these types of excipients such as the interaction between the functional group of the excipient with the ionisable media components or the carvedilol drug. The results also indicate that dissolution data of carvedilol with the different excipients came in compliance with the results of solubility DoE studies done in previous section of carvedilol in same media where mannitol, PVP and the lower grade HPMC E3 showed no difference in solubility while higher grade HPMC E50 and chitosan showed a significant influence on solubility.

6 Conclusion and future work

A chief parameter for oral drug absorption and good bioavailability is solubility and dissolution (Amidon et al., 1995). In vitro solubility and dissolution testing were performed to predict solubility and dissolution of drugs in the gastro intestinal tract (in vivo). However, the use of simple aqueous solubility media or 6.8 phosphate buffer media for dissolution testing to represent intestinal secretions had its own limitations (Zughaid et al., 2012) owing to the complex mixture of GIT components (Dressman et al., 1998). At that time, in vitro testing were proposed to a bio relevant media that simulate and cover fasted and fed intestinal lumen components by taking into account the most important factors that affect drug absorption (Vertzoni et al., 2003). Statistical design of experiment (DoE) allows for a broad understanding of the solubility profile along with the determination of the components that influence drug solubility. However, DoE requires large numbers of experiments (Khadra et al., 2015; Zhou et al., 2017) which limit the use of large DoE in large scale screening. Consequently, the aim of this work was to achieve better understanding of solubility and dissolution of poorly soluble drugs in the human intestinal fluid, through the use of simulated media that cover both fasted and fed state in one smaller (20 tests) experiment. The design used in this study was effective to cover solubility space in both fasted and fed state with comparable results to the previous published studies in the fasted and fed state and also comparable to the standard FaSSIF and FeSSIF media. In addition the design were able to indicate the critical factors that affect solubility with pH mainly affects drugs that are ionisable at the pH range (5-7) of the media while amphiphiles affects lipophilic drugs. However, the reduced number of experiments was accompanied with the limitation of reduced statistical power with only major factors affecting solubility were indicated while other factors with minor influence were not able to be detected. The experiment proved that the design is able to be used practically to predict solubility in both fasted and fed state in one experiment but statistical boundaries should be well-thought-out.

To overcome the statistical limitation of the 20 dual DoE while preserving acceptable work load, a new larger scale DoE (68 tests) covering both fasted and fed state was

applied and the prepared simulated media components were frozen. This was performed in a vision for using frozen stock solution for further experiments for a reason to minimize the preparation steps of the DoE experiment. The experiments applied suing frozen stock solutions were able to determine solubility ranges that are in wide-ranging agreement with the solubility values using fresh stock solution. In addition, frozen experiments were able to determine the major factors influencing drug solubility similar to the factors indicated with the fresh stock solution. However, although frozen experiments showed to indicate the most significant factors as pH and sodium oleate but the number of factor magnitude was higher compared to the fresh experiment. Moreover, results found that the effect of freezing the media components on solubility was higher in fasted state and specifically for ionisable drugs that known to be affected by the pH level of the media and the different ionisable media components. Freezing the media components for further experimental use is a regarded trial that would save time and effort but further investigation will be required. For example replication of the experimental DoE to track the changes in factor magnitude. In addition, increasing the number of drugs (in each category: acid, base, neutral) under examination will be necessary to indicate if the effect of freezing media components will be specific for ionisable drugs or it will be drug dependent performance.

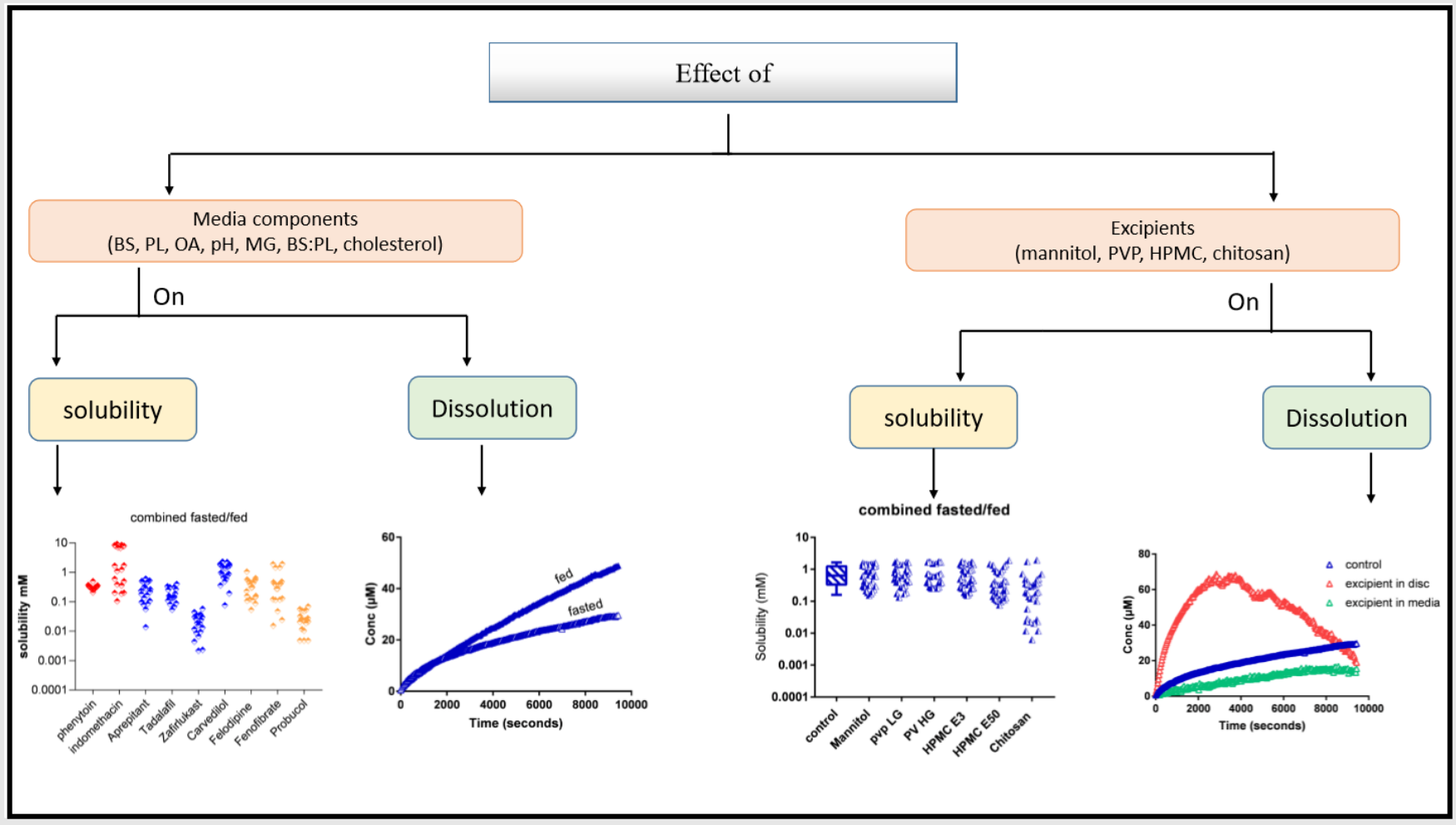
Following the remarkable information on the ability of the design to forecast the effect of the different media components on solubility, the design was used to explore the effect of different types of excipient on solubility. As BCS class II drugs have poor aqueous solubility, this make them susceptible to poor bioavailability after oral administration and consequently strategies to overcome these problems are strongly needed (Amhara et al., 2014). The use of excipients with drug delivery is getting increased devotion in the pharmaceutical industry owed to the ability of excipients to enhance oral bioavailability (Qian et al., 2010). The results indicates that lower concentration (0.5% w/v) of all excipients used showed no influence on drug solubility while increasing the concentration of the excipient to a concentration of (5% w/v) showed varied results. The results showed that excipients with low molecular weight (mannitol, PVP LG.HPMC E50) and or having solubilising or surfactant effect

(HPMC E3 and both PVP grades) showed no significant impact on the overall drug solubility. However, the investigation of drugs solubility in each media tube showed that PVP and the lower concentration of HPMC E3 had an influencing solubility effect in some media where solubility values were three times higher compared to the control. Nevertheless, chitosan and the higher molecular weight of HPMC E50, found to significantly affects the drug overall solubility. Investigation of the solubility of each media tube showed that the effect of chitosan and HPMC E50 on solubility found to be dependent on the drug under investigation, media state (fasted or fed), concentration and molecular weight of the excipient and the pH and total surfactant levels in each media. The experiment performed in this study proved the ability of the design to explore the effect of excipient on solubility. Moreover, the results proves the ability of the design to be used as an excipient screening tool to specify the best concentration, molecular weight and type of excipient to be used in oral drug formulation for improved bioavailability. However, higher number of drugs under examination plus other types, molecular weight and concentration of excipients could be assigned for further investigations.

In vitro dissolution testing is also a key assessment to predict in vivo oral absorption. In this study, dissolution testing were performed to examine the effect of different excipients on dissolution of carvedilol by selecting fasted and fed media from DoE. The results showed good correlation with the DoE solubility results and with the Noyes-Whitney equation where IDR is directly proportional with solubility. The small scale dissolution testing using Sirius T3 allows for predicting the effect of the different excipients on dissolution of drugs. The results showed that mannitol, both PVP grades and HPMC E3 showed no impact on dissolution. The results also showed that chitosan and HPMC E50 showed different effect on dissolution when they were in disc or when they were added to the media. When both excipients were added to the dissolution media, concentration of carvedilol dissolution were lower compared to the control. However, the effect of these excipients on dissolution were different if they are in disc. The excipients act as a spring at the first hour of the experiment but they were not able to maintain the parachute effect and the dissolution starts to go lower after with an overall lower dissolution rate at the end of the experiment.

However, the results showed that if the drug was able to be absorbed before excipients showed their functional effect then the spring effect of the excipients will override the lowering solubility and dissolution effect. The results indicate that this experiment was able to be used in excipient screening to enhance drug solubility, dissolution and bioavailability. However, the results showed that an interaction occurred between higher molecular weight excipients (chitosan and HPMC E50) and the dissolution media components proved by the turning of the media to a turbid solution and the failure of SDI experiments. This will limit the use of the SDI instrument with these media and consequently other dissolution instruments handling turbid media and able to detect the media transformation could give many advantages compared to the current method.

Summary figure for the thesis



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Dual Level Statistical Investigation of Equilibrium Solubility in Simulated Fasted and Fed Intestinal Fluid

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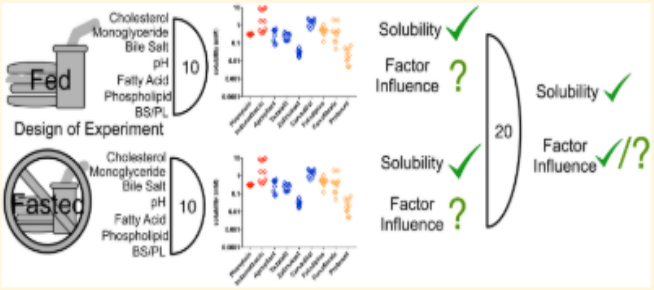

ABSTRACT: The oral route is the preferred option for drug administration but contains the inherent issue of drug absorption from the gastro-intestinal tract (GIT) in order to elicit systemic activity. A prerequisite for absorption is drug dissolution, which is dependent upon drug solubility in the variable milieu of GIT fluid, with poorly soluble drugs presenting a formulation and biopharmaceutical challenge. Multiple factors within GIT fluid influence solubility ranging from pH to the concentration and ratio of amphiphilic substances, such as phospholipid, bile salt, monoglyceride, and cholesterol. To aid in vitro investigation simulated intestinal fluids (SIF) covering the fasted and fed state have been developed. SIF media is complex and statistical design of experiment (DoE) investigations have revealed the range of solubility values possible within each state due to physiological variability along with the media factors and factor interactions which influence solubility. However, these studies require large numbers of experiments (>60) and are not feasible or sensible within a drug development setting. In the current study a smaller dual level, reduced experimental number (20) DoE providing three arms covering the fasted and fed states along with a combined analysis has been investigated. The results indicate that this small scale investigation is feasible and provides solubility ranges that encompass published data in human and simulated fasted and fed fluids. The measured fasted and fed solubility ranges are in agreement with published large scale DoE results in around half of the cases, with the differences due to changes in media composition between studies. Indicating that drug specific behaviors are being determined and that careful media factor and concentration level selection is required in order to determine a physiologically relevant solubility range. The study also correctly identifies the major single factor or factors which influence solubility but it is evident that lower significance factors (for example bile salt) are not picked up due to the lower sample number employed. A similar issue is present with factor interactions with only a limited number available for study and generally not determined to have a significant solubility impact due to the lower statistical power of the study. The study indicates that a reduced experimental number DoE is feasible, will provide solubility range results with identification of major solubility factors however statistical limitations restrict the analysis. The approach therefore represents a useful initial screening tool that can guide further in depth analysis of a drug's behavior in gastrointestinal fluids.

KEYWORDS: *design of experiment, fasted state, fed state, gastrointestinal fluids*

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Dual Level Statistical Investigation of Equilibrium Solubility in Simulated Fasted and Fed Intestinal Fluid

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Introduction: when developing oral drug delivery system for poorly soluble drugs it is important to consider solubility as the rate limiting factor for absorption of a specific drug. Solubility can be investigated in vitro using simulated intestinal fluid containing bile salt, phospholipid and fatty acids. Design of experiment (DoE) investigation was approved to examine the effect of these different factors on solubility. However, these studies require large number of experiment (>60) and are not feasible within drug development. In this study, a smaller dual level (20) DoE covering both fasted and fed states has been investigated and compared to published fasted and fed state papers.

Method: a 1/16 of the full factorial DoE with 7 factors and 2 levels was constructed separately for fasted and fed state using Minitab (8 experiment in each state with 2 centre points) then the 2 experimental Tablewere employed as an input for a factorial custom DoE resulting in an overall 20 experiments. The required fluid composition was mixed from stock solution to provide a final volume of 4 mL in a 15 mL centrifuge tube containing excess amount of the drug and pH was adjusted to 5 or 7. Then tubes were placed in an orbital shaker for 24 h at 37 ℃. Tubes were centrifuged and 500 µl of supernatant removed and concentration determined by HPLC.

Results: the measured equilibrium solubility values indicates that the solubility values are in broad agreement with the available published equilibrium solubility data in fasted and fed HIF , simulated intestinal fluids and published DoE studies in fasted and fed studies fasted and fed DoE data. The design was able to determine the factors with the most significant effect as pH for acidic drugs, oleate for basic drugs and pH, lecithin and oleate for neutral drugs. However, dual design of experiment showed that

bile salt had no effect on solubility which is contrasting to the larger fasted and fed published studies and a reflect of the reduced statistical power of the dual design referred to the lower numbers of experiments compared to the larger published DoE.

Conclusion: the results indicate that a reduced experimental number design of experiment covering both fasted and fed simulated media states in a single study is feasible and provide equilibrium solubility data and drug related behaviours that are similar to previous studies and that the design is able to establish the factors with the largest influence on equilibrium solubility but due to the reduced experimental number and therefore statistical power, factors with a lower influence will not be revealed