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Small scale in vitro method to determine a potential bioequivalent equilibrium solubility range for fed human intestinal fluid

Maria Inês Silva [a], Ibrahim Khadra [a], Kate Pyper [b], Gavin W. Halbert [a,*]

[a] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom
[b] Department of Mathematics and Statistics, University of Strathclyde, Livingstone Tower, 26 Richmond Street, Glasgow G1 1XH, United Kingdom

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ABSTRACT

Intestinal drug solubility is a key parameter controlling oral absorption but varies both intra and inter individuals and between the fasted and fed states, with food intake known to alter the bioavailability of many compounds. Intestinal solubility can be measured in vitro either using sampled fed human intestinal fluid (FeHIF) or simulated fed intestinal fluid (SIF) but neither approach is optimal. FeHIF is difficult to obtain and variable, whilst for fed SIF multiple recipes are available with no consensus on the ideal version. A recent study characterised FeHIF aspirates using a multidimensional approach and calculated nine simulated media recipes that covered over ninety percent of FeHIF compositional variability. In this study the equilibrium solubility of thirteen drugs have been measured using the nine simulated media recipes and compared to multiple previous design of experiment (DoE) studies, which have examined the impact of fed SIF media components on solubility. The measured nine media solubility data set is only statistically equivalent to the large scale 92 media DoE in 4 out of 13 drug comparisons, but has improved equivalence against small scale DoEs (9 or 10 media) with 6 out of 9 or 10 out of 12 (9 and 10 media respectively) equivalent. Selective removal of non-biorelevant compositions from the 92 media DoE improves statistical equivalence to 9 out of 13 comparisons. The results indicate that solubility equivalence is linked to media component concentrations and compositions, the nine media system is measuring a similar solubility space to previous systems, with a narrower solubility range than the 92 point DoE but equivalent to smaller DoE systems. Phenytoin and tadalafil display a narrow solubility range, a behaviour consistent with previous studies in fed and fasted states and only revealed through the multiple media approach. Custom DoE analysis of the nine media results to determine the most statistically significant component influencing solubility does not detect significant components. Indicating that the approach has a low statistical resolution and is not appropriate if determination of media component significance is required. This study demonstrates that it is possible to assess the fed intestinal equilibrium solubility envelope using the nine media recipes obtained from a multi-dimensional analysis of fed HIF. The derivation of the nine media compositions coupled with the results in this study indicate that the solubility results are more likely to reflect the fed intestinal solubility envelope than previous DoE studies and highlight that the system is worthy of further investigation.

1. Introduction

The introduction of high throughput screening systems allowed the development of thousands of new molecules through in vitro assays, but resulted in an increase of compounds presenting low aqueous solubility [1,2]. The pharmaceutical industry continues to prioritise oral drug administration because of its improved patient compliance and cost effectiveness [3]. However, a drug's solubility and therefore dissolution rate in gastrointestinal luminal fluids is a limiting step for this route, since a drug must be in solution before absorption [4,5]. Poorly soluble drugs can lead to low oral bioavailability and compromise the druǵs therapeutic effect [6].

The Biopharmaceutics Classification System (BCS) divides drugs into four classes by linking the drug's in vitro dose/solubility ratio with its in vivo bioavailability, highlighting that the rate and extent of drug absorption is controlled by the drug's solubility and gastrointestinal

Abbreviations: FeHIF, fed human intestinal fluid; DoE, design of experiment; SIF, simulated intestinal fluid; BCS, biopharmaceutics classification system; HIF, human intestinal fluid; FeSSIF, fed simulated intestinal fluid; FaSSIF, fasted simulated intestinal fluid.

* Corresponding author.
E-mail address: g.w.halbert@strath.ac.uk (G.W. Halbert).

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permeability [7]. Poorly soluble drugs (class II and IV) are a challenge for the industry, requiring the development of new in vitro methods that allow gastrointestinal solubility assessment for these compounds.

When compared to simple aqueous solubility, gastrointestinal solubility is influenced by the presence of bile salts, phospholipids, food digestion products (e.g. monoglycerides and free fatty acids) and pH, all of which have the potential to enhance, depending upon physicochemical properties, drug solubility and dissolution [8]. Bile salts within the gastrointestinal tract form endogenous micelles, and digested fats present in food form mixed micelles (with phospholipids and bile salts), vesicles and colloids resulting in an increase in the solubilizing capacity for poorly soluble drugs, especially after food intake [9–11]. Therefore, the higher concentrations of these intestinal components in the fed state when compared to the fasted state, can significantly impact the bioavailability of many compounds [12].

To measure gastrointestinal solubility, the most relevant fluid is human intestinal fluid (HIF). However, the collection of HIF is a difficult and expensive process that requires human volunteers and varies depending on the protocols and storage conditions applied [4,6,13]. Multiple HIF studies from both fed and fasted states, highlight the problems with its collection, variability in different parts of the gastrointestinal tract and between individuals [14–16]. Thus, HIF is not a viable option for routine drug solubility studies.

The alternative to HIF is simulated intestinal fluid (SIF) that replicates in vitro the luminal gastrointestinal conditions for both fasted (FaSSIF) and fed states (FeSSIF) [4,17,18]. SIF utilises the HIF components with known roles in drug solubility for example pH, bile salt, phospholipids and food digestion products such as free fatty acids and monoglycerides. FeSSIF media aims to mimic FeHIF and multiple recipes have been published [19] however, there is no consensus on the optimum recipe due to media solubility differences [20].

In this research group, a design of experiment (DoE) study was conducted as part of the EU IMI Oral Biopharmaceutical Tools research program [21], to statistically examine the influence of media components on equilibrium solubility in simulated fed media [20]. This DoE studied eight media components (bile salt, phospholipid, buffer, salt, pH, enzyme, fatty acid and monoglyceride) using a D-optimal design that required 92 experiments (see Table 4). The study identified the significant media components affecting solubility for the acidic (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast), basic (aprepitant, carvedilol, tadalafil, bromocriptine) and neutral (fenofibrate, felodipine, probucol, itraconazole) BCS II drugs investigated. Highlighting that for acidic compounds pH was the most significant media component, whilst for basic and neutral drugs the combination of pH and amphiphile (bile salt, phospholipid, free fatty acid and monoglyceride) concentration was significant. The study also identified various interactions between media components and unusual drug specific solubility behaviour, emphasising that solubilisation in fed simulated media is a complex interplay of factors [22,23]. However, this approach requires a large number of experiments and was not appropriate for routine application and early development studies. Therefore, reduced DoEs that combined both fasted and fed states in either 32 experiments [24] or a dual level design with 20 experiments (10 experiments each in fasted and fed states) [25] were investigated (see Table 4). A further attempt to reduce the experimental load with 9 experiments for the fed state [26] was also studied. All the studies [20,24–26] successfully quantified the drug's equilibrium solubility and were in general agreement with previous literature solubility values. However, the studies all utilised a DoE approach, which measures conditions that are statistically hypothesized to reflect the component variation within the experimental system or simulated fluid and not necessarily reflective of the natural composition. Thus, DoE approaches whilst capable of determining the impact of a media component on drug solubility and its interactions with other components, they do not present a direct association to individual HIF sample compositions.

To circumvent the statistical construction of DoE systems a recent publication has studied HIF composition using data collected from fasted and fed HIF samples obtained from volunteers [27]. This study performed a multidimensional mathematical analysis of HIF composition that treated the fluid as a 5 dimensional system covering pH, bile salt, phospholipid, fatty acid and cholesterol concentrations [28]. These media components were based on the DoE results that indicated the importance of these components and their interactions for drug solubility. The 5 dimensional analysis identified 8 bioequivalent media compositions (see Table 1 and 4) that statistically characterised over 90% of the component variation within the HIF sample set in the fed state and calculated a centre point through a Euclidean approach in 5-dimensional space.

In this study we have applied the calculated fed state compositions from the multidimensional analysis to measure the equilibrium solubility of thirteen drugs originally studied in the first fed state DoE [20], see above for drug list. The aim of this study is to compare the two approaches (multidimensional analysis vs DoE) for measuring drug solubility in simulated fed intestinal systems. The equilibrium solubility data was compared to the original fed DoE [20] and to the reduced experiment DoEs [24–26] where appropriate data was available. The aim is to determine similarities between the measured solubilities and the feasibility of utilising fed simulated media recipes derived using the multidimensional approach. This will provide a direct comparison to the approach applied to the fasted media systems [29].

2. Materials and methods

2.1. Materials

Sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl) and formic acid were purchased from Merck Chemicals Ltd. Lecithin S PC (phosphatidylcholine from Soybean "98%") was purchased from Lipoid® Germany. Chloroform was obtained from Rathburn Chemical® and FeSSIF-v2 media from Biorelevant.com Ltd. Sodium phosphate monobasic monohydrate ($NaH_2PO^4{\cdot}H_2O$) was from Fisher Scientific.

The active pharmaceutical ingredients carvedilol, tadalafil, valsartan, fenofibrate, bromocriptine, phenytoin, itraconazole, indomethacin, probucol and ibuprofen were purchased from Merck Chemicals Ltd. Aprepitant and felodipine were provided through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark and zafirlukast was purchased from Stratech Scientific Ltd.

The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

2.2. Methods

2.2.1. Stock media solutions for fed solubility experiments

Biorelevant media stock solutions.

Table 1
Fed 9 Media Compositions [28].

Media	Bile Salt (mM)	Phospholipid (mM)	Free Fatty Acid (mM)	Cholesterol (mM)	pH
1	4.94	2.02	10.5	0.95	5.97
2	19.04	7.94	47.51	0.34	6.59
3	5.65	2.43	18.06	0.1	6.13
4	16.65	6.59	27.63	3.45	6.42
5	15.66	5.1	10.92	0.5	6.24
6	6	3.14	45.68	0.65	6.32
7	7.34	6.17	21.82	0.57	5.97
8	12.81	2.6	22.85	0.58	6.59
9 (Centre)	10.94	4.02	23.38	0.32	6.26

Bile Salt (sodium taurocholate): Phospholipid (Soya bean phosphatidylcholine): Free Fatty Acid (Oleic Acid).

Lipid stock solutions 2.5 times greater than the required concentrations for each of the 9 recipes (Table 1) were prepared using the following method. The required quantity of bile salt (sodium taurocholate) and phospholipid (soybean lecithin) were added to a flask and dissolved in 3 ml of chloroform – Solution A. Cholesterol was weighed (x250) in a separate flask and dissolved with 10 ml of chloroform – Solution B. An aliquot of Solution B (100 µl) was transferred to Solution A, stirred and the chloroform evaporated from using a stream of nitrogen gas until a dry film formed. The lipid dry film was resuspended with water and transferred to a 5 ml volumetric flask and the volume completed with water. A stock solution of sodium oleate 913.32 mg of the powder was added to a 10 ml flask, dissolved in water with the aid of sonication and an elevated temperature, made to volume and kept at 50 °C to aid solubilisation. Stock solutions of buffer (sodium phosphate monobasic monohydrate; 28.4 mM) and salt (sodium chloride; 105.9 mM) were prepared in water.

2.2.2. Equilibrium solubility measurement

Into a centrifuge tube (15 ml Corning® tubes) an excess of drug (that exceeds the limit of its solubility) was weighed, and the fed biorelevant media stock, buffer stock, salt stock, FFA stock and water were added as shown in Table 2. The pH of each tube was adjusted (Table 1, pH ± 0.02) using KOH or HCl as required, then shaken for 1 h at room temperature and the pH readjusted if necessary. FeSSIF-v2 media (4 ml) was added to another tube with an identical excess of drug and pH was adjusted if necessary. The tubes were then placed in an orbital shaker (Labinco L28 Orbital Shaker) for 24 h at 37 °C and 240 rpm. After incubation, the presence of solid drug was confirmed in all tubes and 1 ml of each solution transferred to 1.5 ml Eppendorf tubes and centrifuged at 10,000 rpm for 15 min at room temperature (Hettich Zentrifugen Mikro 20). The supernatant was analysed by HPLC for drug content. Three replicate measurements of each individual media was performed (see Table 3).

2.2.3. HPLC analysis

HPLC analysis was performed using a Shimadzu High Performance Liquid Chromatography Prominence-I LC-2030C system in the conditions specified in table 3. The HPLC method has been previously applied to quantify the concentration of the drug of interest [24,29]. For each drug, calibration curves were constructed, and the linés equation was used to extrapolate the drug concentration.

2.2.4. Data analysis

The data was compared using a non-parametric Kruskal-Wallis test with Dunn's multiple comparison correction using Prism 9 for MacOSX and only the comparisons represented in the figures were analysed. In order to calculate the significant factors influencing solubility, the media concentration values (Table 1) were used as input for a factorial custom design of experiment using Minitab®19.

Table 2
Fed Media Preparation – Stock Media Volumes.

Media	Media Stock (ml)	FFA Stock (ml)	Buffer Stock (ml)	Salt Stock (ml)	Water (ml)
1	1.60	0.350	0.267	0.267	1.516
2	1.60	1.584	0.267	0.267	0.282
3	1.60	0.602	0.267	0.267	1.264
4	1.60	0.921	0.267	0.267	0.945
5	1.60	0.364	0.267	0.267	1.502
6	1.60	1.523	0.267	0.267	0.343
7	1.60	0.727	0.267	0.267	1.139
8	1.60	0.762	0.267	0.267	1.104
9 (Centre)	1.60	0.779	0.267	0.267	1.087

3. Results and discussion

3.1. Equilibrium solubility comparisons

The fed 9 media system equilibrium solubility results are in Figs. 1, 2 and 3 for the acidic, basic and neutral drugs along with comparable data (where available) from previous fed DoE studies. A striking feature of all figures is that the initial 92 point DoE [20] has a greater solubility range than the other systems. Statistical comparison of the 9 media equilibrium solubility distribution with the 92 point DoE indicates that four out of thirteen drugs are statistically equivalent, which is in marked contrast to the fasted comparison [29], where ten drugs from twelve were equivalent to the fasted DoE study [30]. Comparison of the 9 media distributions with the 10 media DoE [25] or 9 media DoE [26] provides an improved correlation, with sixteen of twenty one comparisons statistically equivalent. This is similar, to the fasted where fifteen from eighteen were statistically equivalent. The 92 point fed DoE therefore produces a higher level of statistically different equilibrium solubility distributions when compared to the 9 media system than either the 10 media or 9 media DoEs, which is different to the similar comparison of fasted DoEs [29].

The media component concentration values and statistical constructions analysed in these studies are not identical and a synopsis is presented in Table 4. The 92 point fed DoE examined eight components at three concentration values, and three (buffer, salt and enzyme) had no or negligible (when compared to the other components) impact on drug solubility [20]. These components were, therefore not examined in subsequent studies [25,26]. The remaining components' (pH, bile salt, phospholipid, free fatty acid, and monoglyceride) concentration values in the smaller DoE studies [25,26] is consistent between studies (with only minor differences), with the biggest difference the statistical design applied to determine the concentration values for each point. The 92 point DoE [20] utilised a D-optimal design, two concentration levels along with a centre point and required 44 media compositions dictated by the statistical design, measured in duplicate (centre point four replicates). To lessen the experimental load subsequent DoE studies [25,26] reduced the number of media compositions and utilised either smaller fractional designs (10 media DoE) or custom media compositions based on literature data (9 media DoE). The difference between the DoE systems therefore, is not primarily controlled by media component concentration values but the number and variation of media compositions studied. The 92 point DoE with a larger number of statistically guided media compositions contains combinations of media components and concentrations that are not likely to be biorelevant (eg high bile salt concentration combined with low phospholipid and free fatty acid concentration) and or combinations that do not support or impair drug solubilisation [23]. The presence of these compositions is dictated by the statistical design and assists in calculating the impact of each component on drug solubility, but does not link directly to FeHIF composition.

The 9 media system compositions (Table 1 and 4) are based on a multidimensional analysis of FeHIF [28] not a DoE. The component concentrations between the 9 media and DoEs are different (Table 4 and Fig. 4a) especially the low DoE concentrations of bile salt, phospholipid, free fatty acid and pH. This arises from the limited media component concentration information available at the time of the 92 point DoE [13], resulting in component concentrations that are out with the 9 media data cloud [28]. In the fasted media systems comparison these differences are not as pronounced (Fig. 4b) resulting in improved solubility determination equivalence between the DoE and multidimensional media [29]. The 92 point fed DoE therefore includes media component concentrations that are out with the FeHIF data cloud (Fig. 4a), and consequently due to the DoE design a wider variation in media component concentrations and combinations some of which are not likely to be biorelevant (see above), when compared to the 9 media system. This difference can explain the 92 point fed DoE's wider solubility range and lack of statistical equivalence to the 9 media system.

Table 3
HPLC Method Details.

Drug	Mobile Phase	Column	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
Aprepitant		a	1	10	254	2.19
Tadalafil**	Mobile Phase A: 10 mM Ammonium Formate pH 3 in H20	a	1	10	291	1.49
Zafirlukast		a	1	10	254	2.53
Carvedilol	Mobile Phase B: 10 mM Ammonium Formate in ACN:H20 (9:1 V/V)	a	0.7	10	254	1.57
Phenytoin		a	1	10	254	0.97
Indomethacin		a	1	10	254	2.00
Felodipine**		a	1	10	254	2.60
Fenofibrate**		a	1	10	291	3.23
Ibuprofen		a	1	10	254	2.06
Probucol		a	1	10	254	4.36
Valsartan		b	1	10	254	1.27
Itraconazole		b	1	10	254	2.62
Bromocriptine	Isocratic method ACN and 0.1% w/v acetic acid (50:50 v/v)	a	1	10	291	0.58

a- Column: XBridge C18 5 µm 2.1x 50 mm; b- Column: ACE 5 C18 150x3.0 mm
Gradient start 70:30 (A:B), 3 min 0:100, 4 min 0:100, 4.5 min 70:30 total run time 8 min; ACN- Acetonitrile. **HPLC analysis performed using an Apparatus Agilent Technologies 1260 Series Liquid Chromatography system with Clarity Chromatography software.

Table 4
Synopsis of Simulated Fed Media Conditions.

Study	pH	BS (mM)	PL (mM)	FFA (mM)	MG (mM)	Cholesterol (mM)	Buffer (mM)	Salt (mM)	Enzyme (IU/ml)	Number of Media	Statistical Design
Zhou 2017	5/6/7	3.6/ 13.8/24	0.5/2.65/ 4.8	0.8/ 26.4/52	1/3/ 6.5	ns	29/43/ 58	125/164/ 203	50/100/ 150	92[A]	D-optimal design
Ainousah 2017	5/-/7	3.6/9.3/ 15	0.5/2.1/ 3.8	0.8/13/ 25	1/5/9	ns	ns	ns	ns	10	1/16 Full Factorial Custom Design
McPherson 2020	5/-/7	3.6/15/ 24	0.5/2/4.8	6.6/20/ 33	1/5/ 6.5	ns	ns	ns	ns	9	Custom design
9 media This study	6/6.3/ 6.6	5/11/19	2/4/8	10/23/ 48	ns	0.1/0.3/ 3.4	ns	ns	ns	9	FeHIF 5D analysis

BS - Bile Salt (sodium taurocholate): PL – Phospholipid (Soya bean phosphatidylcholine): FFA – Free Fatty Acid (Oleic Acid): MG – Monoglyceride (glyceryl monooleate): Buffer – Zhou 2017 – Maleic acid, Ainousah 2017 and McPherson 2020 – Phosphate salt: Salt – Sodum Chloride: Enzyme – Porcine Pancreatin.
ns: Not studied as a variable in system indicated. A: 92 media consisted of 44 duplicate measurements and centre point measured 4 times.

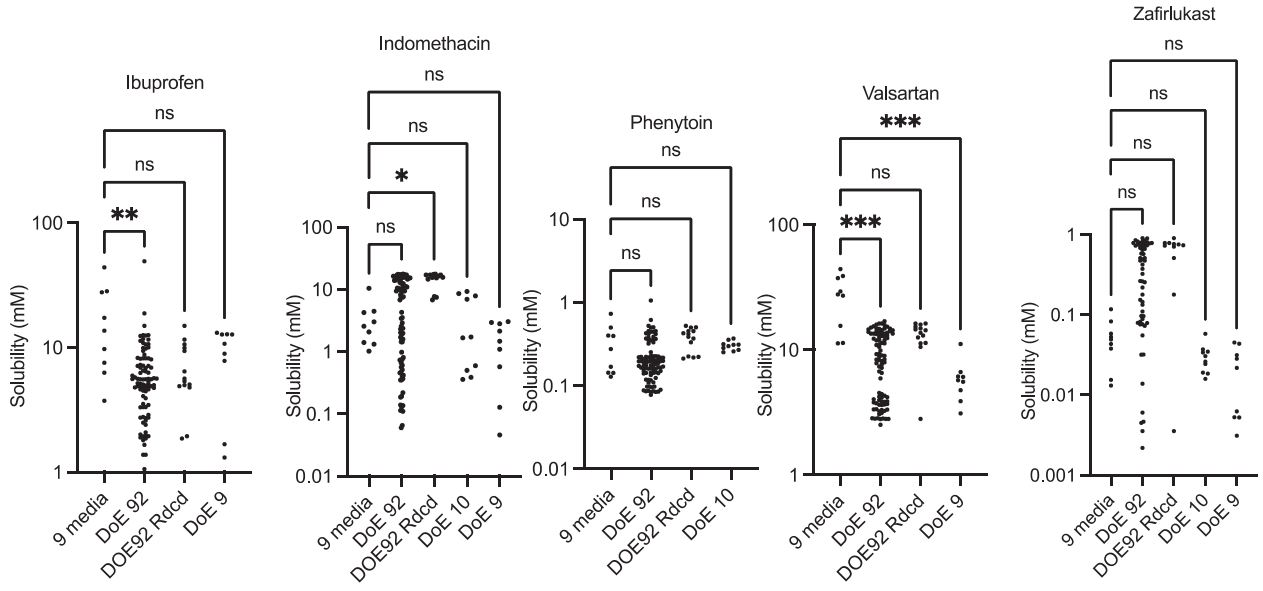

Fig. 1. Measured Equilibrium Solubility Distributions of Acidic Drugs. 9 media – this study; DoE 92 [20]; DoE92 Rdcd see text and Fig. 4a; DoE 10 [25]; DoE 9 [26]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0221; ** p = 0.0051; *** p = 0.0002.

Outlier compositions can be removed from the 92 point DoE (Fig. 4a) to form a reduced distribution consisting of fourteen solubility values, which based on Fig. 4a are more likely to match the solubility behaviour of the 9 media measurements, see Figs. 1, 2 and 3. A statistical comparison of the 9 media system with the reduced 92 point DoE improves the correlation with nine out of thirteen drug solubility distributions statistically equivalent. Highlighting that when the components concentrations lie within similar concentration ranges or limits the two

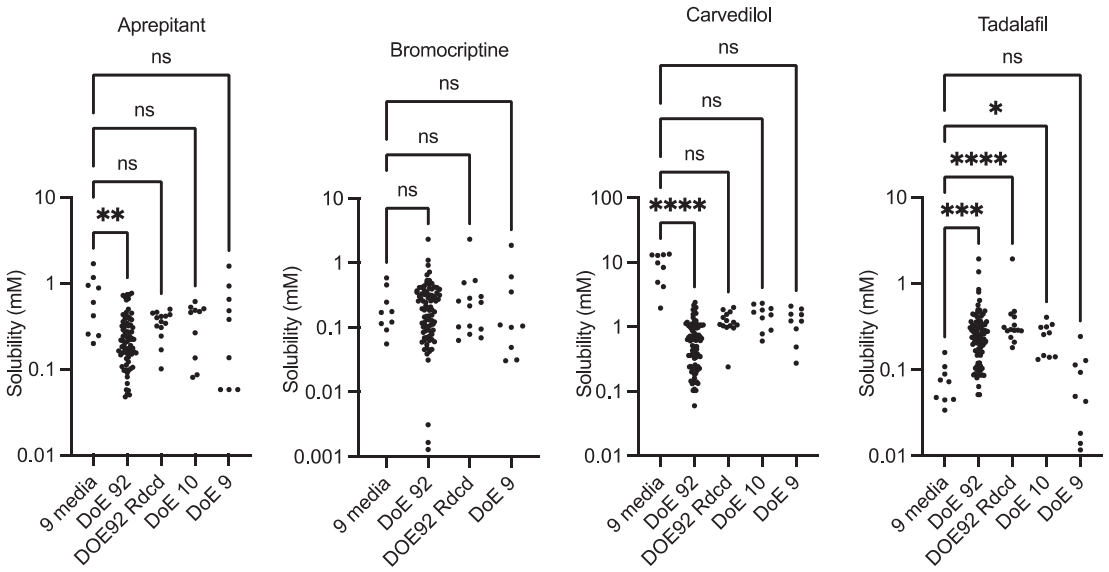

Fig. 2. Measured Equilibrium Solubility Distributions of Basic Drugs. Legend: 9 media – this study; DoE 92 [20]; DoE92 Rdcd see text and Fig. 4a; DoE 10 [25]; DoE 9 [26]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0191; ** p = 0.0029; *** p = 0.0003; **** p < 0.0001.

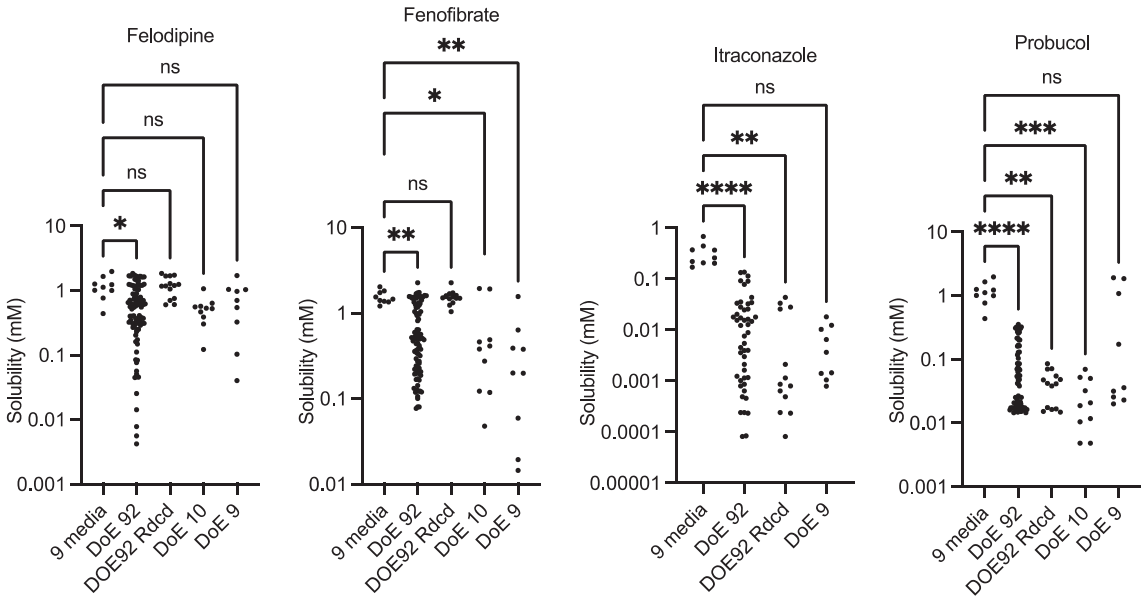

Fig. 3. Measured Equilibrium Solubility Distributions of Neutral Drugs. Legend: 9 media – this study; DoE 92 [20]; DoE92 Rdcd see text and Fig. 4a; DoE 10 [25]; DoE 9 [26]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0382; ** p = 0.0012; *** p = 0.0001; **** p < 0.0001.

systems are measuring the same solubility space.

3.2. Solubility multiple

The initial 92 point DoE highlighted that for some drugs solubility variability was up to three orders of magnitude [20]. For all drugs a solubility multiple was calculated by dividing the highest solubility with the lowest solubility measured in the system. A statistical comparison of the 9 media solubility multiple with the 92 point DoE (Fig. 5a) indicates that there is a significant reduction for all drugs studied (Fig. 5b), whilst a similar comparison with the reduced 92 DoE does not find a statistically significant difference. The results and discussion in the previous section in relation to component concentrations and compositions provides a rationale for this result. For the 9 media system this indicates that the solubility distributions are lower and probably an improved estimate of FeHIF solubility than the 92 point DoE [20], which as discussed contains a large number of non-biorelevant systems.

There are some interesting variations within the solubility multiple values. It is noticeable for two drugs (zafirlukast and itraconazole) that the solubility multiple in the reduced 92 DoE is almost as large as the original indicating that these molecules are extremely sensitive to variations in media composition. Whilst for multiple other drugs (ibuprofen, indomethacin, phenytoin, aprepitant, and felodipine) the reduced 92 DoE has a lower multiple than the 9 media system. In these cases, the restricted media component concentration range (Fig. 4a) of the reduced 92 point DoE is likely to be responsible for this result (see next paragraph with respect to phenytoin), with the discussion in the previous section applicable.

The fasted 9 media system [29] revealed three drugs (phenytoin, tadalafil and griseofulvin (see also [22])) with small solubility multiples and a developability classification system study identified similar behaviour for acyclovir, paracetamol and carbamazepine [31] in the

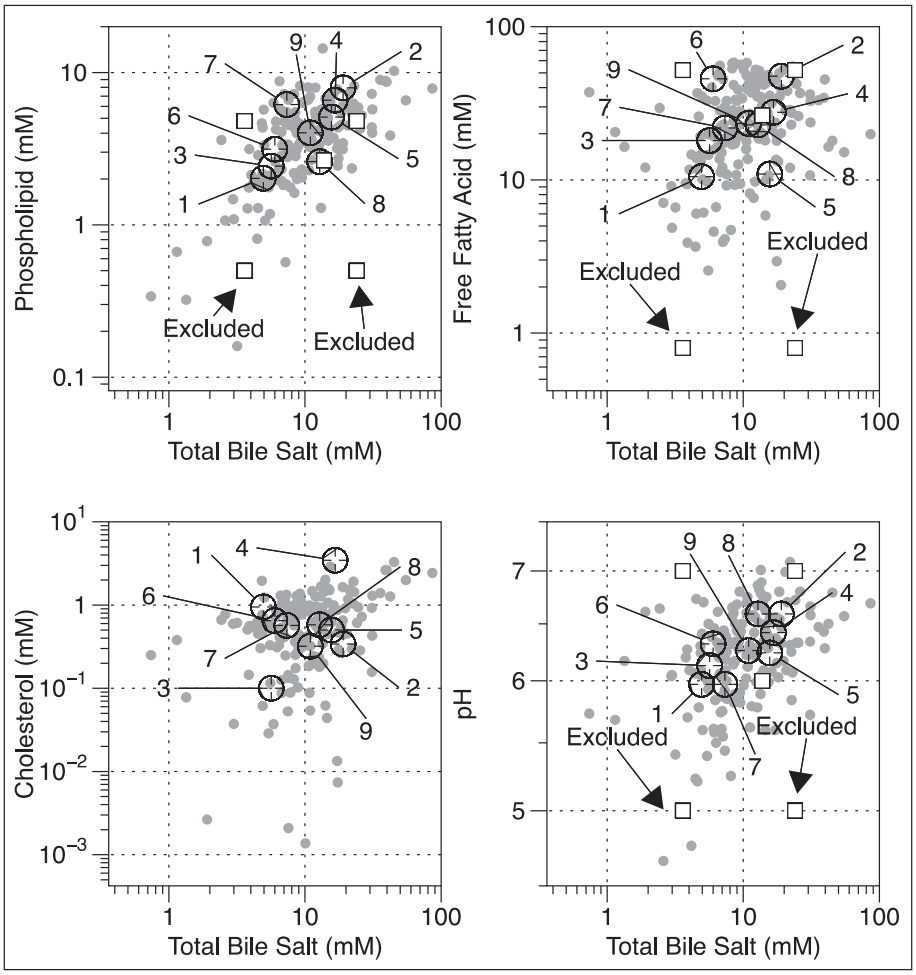

Fig. 4a. Comparison Fed 9 Media Data Points, DoE 92 and Fed Data Cloud. ●Measured FeHIF data points taken from [27,28]; ⊗9 media, this study points numbered as Table 1; □DoE 92 points taken from [20], points excluded for DoE 92 Rdcd as indicated, see text.

fasted state. A line drawn on Fig. 5b (y = 5.71) at the solubility multiple for phenytoin in the 9 media system, indicates that tadalafil has a lower value along with valsartan and intriguingly all the neutral drugs (felodipine, fenofibrate, itraconazole and probucol). Valsartan was not studied in the fasted system and acyclovir, paracetamol and carbamazepine are not examined in this study. However, the low solubility multiple for tadalafil in combination with phenytoin indicates that this solubility behaviour for these drugs occurs in both fasted and fed states. This is worthy of further examination, as the bioavailability of drugs with this behaviour will not be influenced by intestinal fluid media composition. All the neutral drugs have smaller solubility multiples than phenytoin in the 9 media system, a result that is the reverse of the fasted state (itraconazole was not examined in the fasted study), where the solubility multiple value was larger. For fenofibrate for example the fasted 9 media solubility multiple is 7.65 [29] and in this fed study 1.67. For neutral drugs solubility is controlled by media pH and amphiphilic component concentrations [20] and this finding indicates that in the fed state with higher amphiphile concentrations there is a solubility variability smoothing effect. In a recent study [32] examining the bioavailability of fenofibrate in pigs after an FDA breakfast the $AUC_{0-\infty}$ standard deviation dropped from 24% of the mean value in the fasted state to 9% in the fed state. Multiple other factors for example metabolism or formulation could contribute to this difference, but the solubility finding reported in this study is worthy of investigation for drugs where a food effect is evident and in vitro models required [33]. The low solubility multiple and possible solubility smoothing behaviour are interesting findings and drug dependent properties that are only revealed using a multiple media analysis [29,31].

3.3. *Significant Factor analysis*

Although the 9 media composition is based on a multi-dimensional analysis of FeHIF [28] it is possible to fit the component values into a tailored DoE structure [24]. This permits a standardised effect value to be calculated for the impact of each media component on drug solubility, but does not permit the calculation of two-way or higher interactions. The results are presented in Table 5, along with effect values from the previous equilibrium fed DoE studies [20,25,26]. This reveals that the 9 media system was not able to determine any significant standardised effect values occurring within the system. This is in contrast to the 92 point DoE study [20] where significant media components were identified for almost all drugs. The absence of detection is in agreement with previous results for the fasted 9 media system [29] where the number of significant factors decreases from the large scale DoE to the 9 media system. This also reflects the discussion in section 3.1, relating to the design of the media compositions within each system. The results indicate that if the number of data points is reduced, the data point compositions are not statistically driven, and the solubility variability reduces, the experiment's ability to detect significant media components is severely impaired.

4. Conclusions

The 9 media approach using a small number of media recipes derived

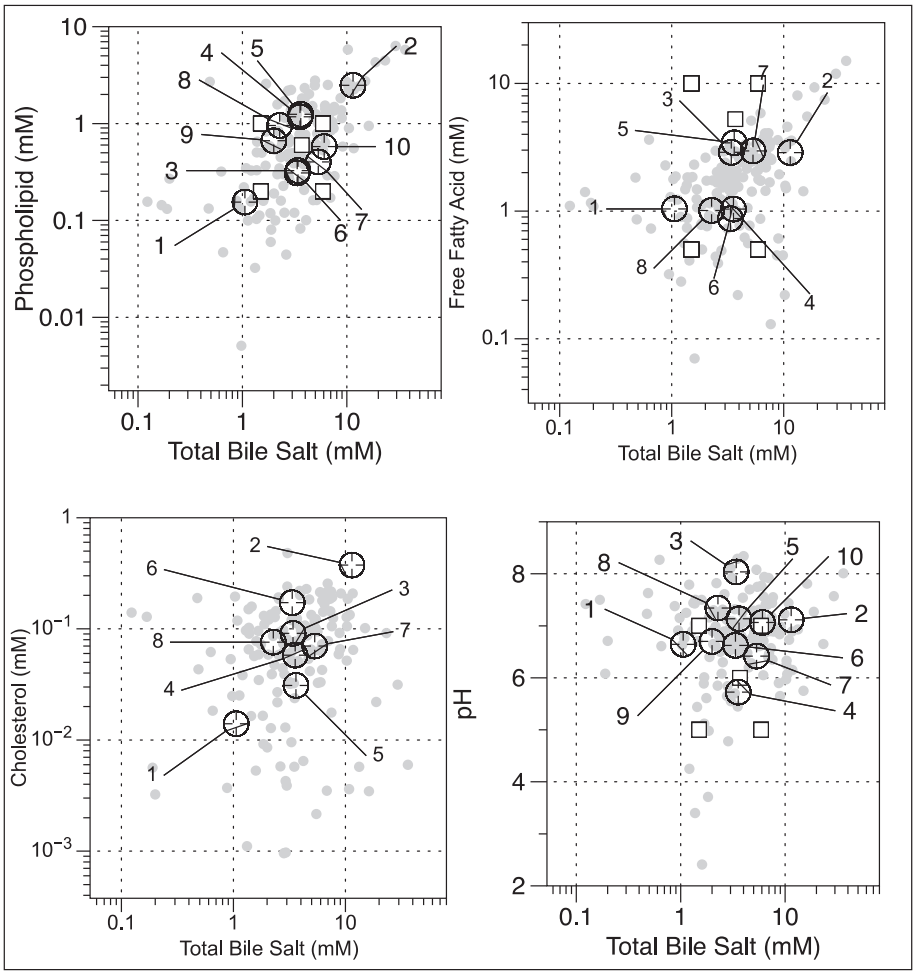

Fig. 4b. Comparison Fasted 9 Media Data Points, DoE 66 and Fasted Data Cloud.● Measured FaHIF data points taken from [27,28]; ⊗9 media, taken from and numbered [29]; □DoE 66 points taken from [30].

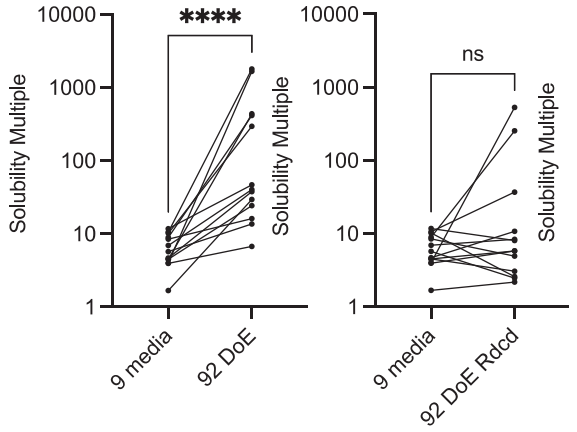

Fig. 5a. Collected Solubility Multiple Comparison.9 media this study, DoE 92 from [20], **** $p < 0.0001$; 92 DoE Rdcd this study (see text); ns = no significant difference.

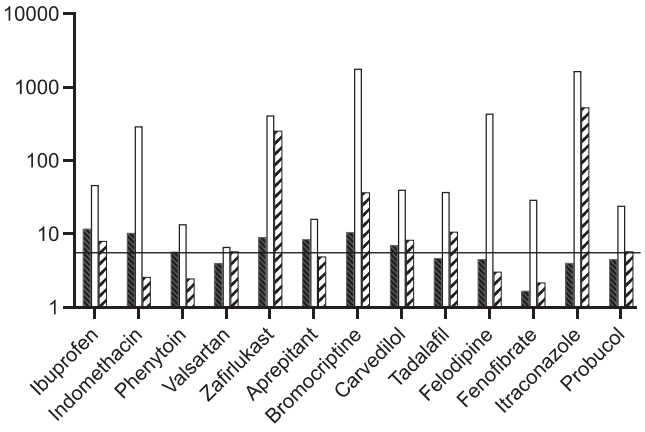

Fig. 5b. Individual Solubility Multiple Results. 9 media, this study; DoE 92 values from [20], DoE 92 Rdcd, see text; Horizontal line y = 5.71 phenytoin 9 media solubility multiple value.

from a multidimensional analysis of sampled FeHIF [28] effectively measured a fed intestinal equilibrium solubility distribution. The equilibrium solubility measured with the 9 media system is only statistically equivalent to the initial fed 92 point DoE [20] in four out of thirteen cases (31%), but equivalent in sixteen out of twenty one cases (76%) to previous smaller DoE studies (DoE 10 [25] and DoE 9 [26]). The result can be related to the differences between the systems in media component concentration ranges, methods applied to determine media compositions and number of data points measured. The initial fed 92 point DoE [20] applies excessive media component concentration ranges compared to the 9 media system and elimination of outlier media compositions improves the statistical agreement to 70%. This highlights that for simulated intestinal fluid systems with similar media component

Table 5
Significant Factor Analysis.

Fed Environment			
Drug	9 media	9 DoE	92 Point DoE
Indomethacin	NS	pH	pH, oleate, bile salt
Ibuprofen	NS	NS	pH
Phenytoin	NS	–	Bile salt, lecithin, pH, oleate
Fenofibrate	NS	Oleate	Oleate, bile salt, lecithin, buffer, monoglyceride
Felodipine	NS	Bile salt	Oleate, bile salt, pH, lecithin
Aprepitant	NS	NS	Oleate, bile salt, pH
Carvedilol	NS	NS	Bile salt, pH, buffer, oleate
Tadalafil	NS	NS	Bile salt, oleate
Zafirlukast	NS	NS	pH, bile salt, oleate
Probucol	NS	NS	Bile salt, monoglyceride, oleate, lecithin, pH
Valsartan	NS	NS	pH, Bile salt
Itraconazole	NS	NS	pH, oleate, bile salt, lecithin
Bromocriptine	NS	NS	NS

NS - No Significant Factors Found

concentration ranges and number of measured data points a statistically equivalent but not necessarily bioequivalent solubility space will exist. Due to the derivation of the 9 media system component concentrations and compositions, this system is more likely to represent the fed intestinal solubility range, present, within the limitations of the initial sampling study [27], than previous DoE approaches [20,25,26]. The comparison with the 92 point DoE indicates, that large scale DoE approaches generate statistically sensible but not biorelevant media compositions.

The solubility multiple (highest solubility / lowest solubility) for each drug observed using the 9 media system was smaller than the value from the initial fed 92 point DoE, a result due to the media differences discussed above and which indicates that the 9 media system probably provides a more realistic estimate of FeHIF solubility. Several drugs display very low solubility multiples, for phenytoin and tadalafil this is similar to their behaviour in the 9 media fasted system [29]. Indicating that in both fasted and fed states the intestinal solubility of these drugs is not sensitive to media composition. The neutral drugs also display very low solubility multiples, a new finding not present in the fasted 9 media system, which potentially impacts biopharmaceutical variability in the fed state in vivo and worthy of further investigation.

The fed 9 media system when analysed as a DoE does not detect any significant media factors influencing solubility. This arises due to the smaller number of media compositions tested, the derivation of the compositions and the lower solubility variability present in the fed state. This result is identical to the fasted systems and highlights that the DoE and multidimensional simulated intestinal media systems are exploring different solubility facets and appropriate choice will provide the required outcome.

The multidimensional fed 9 media system performs in a similar manner to the fasted version but also reveals different solubility behaviours. The system is worthy of further investigation using studies that relate the in vitro behaviour to in vivo performance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, Journal of Pharmacological and Toxicological Methods 44 (2000) 235–249.
[2] K. Sugano, A. Okazaki, S. Sugimoto, S. Tavornvipas, A. Omura, T. Mano, Solubility and dissolution profile assessment in drug discovery, Drug Metabolism and Pharmacokinetics 22 (2007) 225–254.
[3] B. Homayun, X. Lin, H.-J. Choi, Challenges and Recent Progress in Oral Drug Delivery Systems for Biopharmaceuticals, Pharmaceutics 11 (3) (2019) 129.
[4] J.B. Dressman, M. Vertzoni, K. Goumas, C. Reppas, Estimating drug solubility in the gastrointestinal tract, Adv Drug Deliv Rev 59 (2007) 591–602.
[5] B.J. Boyd, C.A.S. Bergstrom, Z. Vinarov, M. Kuentz, J. Brouwers, P. Augustijns, M. Brandl, A. Bernkop-Schnurch, N. Shrestha, V. Preat, A. Mullertz, A. Bauer-Brandl, V. Jannin, Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems, European Journal of Pharmaceutical Sciences 137 (2019), 104967.
[6] D. Dahlgren, M. Venczel, J.P. Ridoux, C. Skjold, A. Mullertz, R. Holm, P. Augustijns, P.M. Hellstrom, H. Lennernas, Fasted and fed state human duodenal fluids: Characterization, drug solubility, and comparison to simulated fluids and with human bioavailability, Eur J Pharm Biopharm 163 (2021) 240–251.
[7] G.L. Amidon, H. Lennernas, V.P. Shah, J.R. Crison, A Theoretical Basis For a Biopharmaceutic Drug Classification - the Correlation Of In-Vitro Drug Product Dissolution and In-Vivo Bioavailability, Pharmaceutical Research 12 (1995) 413–420.
[8] V.H. Sunesen, R. Vedelsdal, H.G. Kristensen, L. Christrup, A. Mullertz, Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug, European Journal of Pharmaceutical Sciences 24 (2005) 297–303.
[9] A.K. Doak, H. Wille, S.B. Prusiner, B.K. Shoichet, Colloid formation by drugs in simulated intestinal fluid, Journal of Medicinal Chemistry 53 (2010) 4259–4265.
[10] K. Kleberg, F. Jacobsen, D.G. Fatouros, A. Mullertz, Biorelevant media simulating fed state intestinal fluids: colloid phase characterization and impact on solubilization capacity, Journal of Pharmaceutical Sciences 99 (2010) 3522–3532.
[11] S. Clarysse, J. Brouwers, J. Tack, P. Annaert, P. Augustijns, Intestinal drug solubility estimation based on simulated intestinal fluids: comparison with solubility in human intestinal fluids, European Journal of Pharmaceutical Sciences 43 (2011) 260–269.
[12] E.D. Gamsiz, M. Ashtikar, J. Crison, W. Woltosz, M.B. Bolger, R.L. Carrier, Predicting the effect of fed-state intestinal contents on drug dissolution, Pharm Res 27 (2010) 2646–2656.
[13] C.A. Bergstrom, R. Holm, S.A. Jorgensen, S.B. Andersson, P. Artursson, S. Beato, A. Borde, K. Box, M. Brewster, J. Dressman, K.I. Feng, G. Halbert, E. Kostewicz, M. McAllister, U. Muenster, J. Thinnes, R. Taylor, A. Mullertz, Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs, European Journal of Pharmaceutical Sciences 57 (2014) 173–199.
[14] P. Augustijns, B. Wuyts, B. Hens, P. Annaert, J. Butler, J. Brouwers, A review of drug solubility in human intestinal fluids: Implications for the prediction of oral absorption, European Journal of Pharmaceutical Sciences 57 (2014) 322–332.
[15] J.H. Fagerberg, E. Karlsson, J. Ulander, G. Hanisch, C.A. Bergstrom, Computational prediction of drug solubility in fasted simulated and aspirated human intestinal fluid, Pharm Res 32 (2015) 578–589.
[16] D. Riethorst, P. Baatsen, C. Remijn, A. Mitra, J. Tack, J. Brouwers, P. Augustijns, An In-Depth View into Human Intestinal Fluid Colloids: Intersubject Variability in Relation to Composition, Mol Pharm 13 (2016) 3484–3493.
[17] E. Galia, E. Nicolaides, D. Horter, R. Lobenberg, C. Reppas, J.B. Dressman, Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, Pharmaceutical Research 15 (1998) 698–705.
[18] M.R.C. Marques, R. Loebenberg, M. Almukainzi, Simulated Biological Fluids with Possible Application in Dissolution Testing, Dissolut. Technol. 18 (2011) 15–28.
[19] N. Bou-Chacra, K.J.C. Melo, I.A.C. Morales, E.S. Stippler, F. Kesisoglou, M. Yazdanian, R. Lobenberg, Evolution of Choice of Solubility and Dissolution Media After Two Decades of Biopharmaceutical Classification System, AAPS J 19 (2017) 989–1001.
[20] Z. Zhou, C. Dunn, I. Khadra, C.G. Wilson, G.W. Halbert, Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs, European Journal of Pharmaceutical Sciences 99 (2017) 95–104.
[21] H. Lennernas, L. Aarons, P. Augustijns, S. Beato, M. Bolger, K. Box, M. Brewster, J. Butler, J. Dressman, R. Holm, K. Julia Frank, R. Kendall, P. Langguth, J. Sydor, A. Lindahl, M. McAllister, U. Muenster, A. Mullertz, K. Ojala, X. Pepin, C. Reppas, A. Rostami-Hodjegan, M. Verwei, W. Weitschies, C. Wilson, C. Karlsson, B. Abrahamsson, Oral biopharmaceutics tools - time for a new initiative - an introduction to the IMI project OrBiTo, European Journal of Pharmaceutical Sciences 57 (2014) 292–299.
[22] Z. Zhou, C. Dunn, I. Khadra, C.G. Wilson, G.W. Halbert, Influence of Physiological Gastrointestinal Surfactant Ratio on the Equilibrium Solubility of BCS Class II Drugs Investigated Using a Four Component Mixture Design, Mol Pharm 14 (2017) 4132–4144.
[23] C. Dunn, J. Perrier, I. Khadra, C.G. Wilson, G.W. Halbert, Topography of Simulated Intestinal Equilibrium Solubility, Molecular pharmaceutics 16 (2019) 1890–1905.
[24] J. Perrier, Z. Zhou, C. Dunn, I. Khadra, C.G. Wilson, G. Halbert, Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs, European Journal of Pharmaceutical Sciences 111 (2018) 247–256.

[25] B.E. Ainousah, J. Perrier, C. Dunn, I. Khadra, C.G. Wilson, G. Halbert, Dual Level Statistical Investigation of Equilibrium Solubility in Simulated Fasted and Fed Intestinal Fluid, Mol Pharm 14 (2017) 4170–4180.
[26] S. McPherson, J. Perrier, C. Dunn, I. Khadra, S. Davidson, B.E. Ainousah, C. G. Wilson, G. Halbert, Small scale design of experiment investigation of equilibrium solubility in simulated fasted and fed intestinal fluid, European Journal Of Pharmaceutics and Biopharmaceutics 150 (2020) 14–23.
[27] D. Riethorst, R. Mols, G. Duchateau, J. Tack, J. Brouwers, P. Augustijns, Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions, Journal of Pharmaceutical Sciences 105 (2016) 673–681.
[28] K. Pyper, J. Brouwers, P. Augustijns, I. Khadra, C. Dunn, C.G. Wilson, G.W. Halbert, Multidimensional analysis of human intestinal fluid composition, Eur J Pharm Biopharm 153 (2020) 226–240.
[29] Q. Abuhassan, I. Khadra, K. Pyper, G.W. Halbert, Small scale in vitro method to determine a bioequivalent equilibrium solubility range for fasted human intestinal fluid, Eur J Pharm Biopharm 168 (2021) 90–96.
[30] I. Khadra, Z. Zhou, C. Dunn, C.G. Wilson, G. Halbert, Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs, European Journal of Pharmaceutical Sciences 67 (2015) 65–75.
[31] Q. Abuhassan, I. Khadra, K. Pyper, P. Augustijns, J. Brouwers, G.W. Halbert, Fasted intestinal solubility limits and distributions applied to the biopharmaceutics and developability classification systems, European Journal of Pharmaceutics and Biopharmaceutics 170 (2022) 160–169.
[32] L.J. Henze, N.J. Koehl, J.P. O'Shea, R. Holm, M. Vertzoni, B.T. Griffin, Toward the establishment of a standardized pre-clinical porcine model to predict food effects - Case studies on fenofibrate and paracetamol, Int J Pharm X 1 (2019), 100017.
[33] C. Pentafragka, M. Symillides, M. McAllister, J. Dressman, M. Vertzoni, C. Reppas, The impact of food intake on the luminal environment and performance of oral drug products with a view to in vitro and in silico simulations: a PEARRL review, Journal of Pharmacy and Pharmacology 71 (2019) 557–580.