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Research paper

Fed intestinal solubility limits and distributions applied to the Developability classification system

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ABSTRACT

For solid oral dosage forms drug solubility in intestinal fluid is an important parameter influencing product performance and bioavailability. Solubility along with permeability are the two parameters applied in the Biopharmaceutics and Developability Classification Systems (DCS) to assess a drug's potential for oral administration. Intestinal solubility varies with the intestinal contents and the differences between the fasted and fed states are recognised to influence solubility and bioavailability. In this study a novel fed state simulated media system comprising of nine media has been utilised to measure the solubility of seven drugs (ibuprofen, mefenamic acid, furosemide, dipyridamole, griseofulvin, paracetamol and acyclovir) previously studied in the fasted state DCS. The results demonstrate that the fed nine media system provides a range of solubility values for each drug and solubility behaviour is consistent with published design of experiment studies conducted in either the fed or fasted state. Three drugs (griseofulvin, paracetamol and acyclovir) exhibit very narrow solubility distributions, a result that matches published behaviour in the fasted state, indicating that this property is not influenced by the concentration of simulated media components. The nine solubility values for each drug can be utilised to calculate a dose/solubility volume ratio to visualise the drug's position on the DCS grid. Due to the derivation of the nine media compositions the range and catergorisation could be considered as bioequivalent and can be combined with the data from the original fed intestinal fluid analysis to provide a population based solubility distribution. This provides further information on the drugs solubility behaviour and could be applied to quality by design formulation approaches. Comparison of the fed results in this study with similar published fasted results highlight that some differences detected match in vivo behaviour in food effect studies. This indicates that a combination of the fed and fasted systems may be a useful in vitro biopharmaceutical performance tool. However, it should be noted that the fed media recipes in this study are based on a liquid meal (Ensure Plus) and this may not be representative of alternative fed states achieved through ingestion of a solid meal. Nevertheless, this novel approach provides greater in vitro detail with respect to possible in vivo biopharmaceutical performance, an improved ability to apply risk-based approaches and the potential to investigate solubility based food effects. The system is therefore worthy of further investigation but studies will be required to expand the number of drugs measured and link the in vitro measurements to in vivo results.

1. Introduction

1.1. Oral drug administration

The pharmaceutical industry favours oral administration as the most common route for drug delivery. The ease of ingestion and familiarity with this route are convenient and known to increase patient compliance and treatment effectiveness when compared with other delivery routes [1]. Notwithstanding these positive characteristics, there are challenges associated with the gastrointestinal tract that might be under estimated when it comes to the choice of this route. The gastrointestinal tract's anatomy and physiology, as well as the drug and medicinal product's physicochemical characteristics are factors that impact performance after oral administration [2]. To be absorbed from the gastrointestinal tract solid drug must first dissolve within the intestinal fluid and then permeate through the tract membranes to gain access to the portal and then systemic blood circulation. Therefore, intestinal solubility [3] along with permeability are two key factors controlling gastrointestinal

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drug absorption. Solubility and permeability are connected in the Biopharmaceutics Classification System (BCS) [4] and the Developability Classification System (DCS) [5], which link in vitro solubility and permeability to provide categorisations that predict a drug's in vivo performance. Intestinal solubility is therefore a key parameter controlling oral absorption behaviour.

Drug solubility in simple aqueous buffers is not necessarily equivalent to intestinal solubility due to the influence of intestinal fluid components such as endogenous bile salt or free fatty acids from digested food [6]. The ultimate measure of intestinal solubility is using sampled human intestinal fluid (HIF). However, it is known that the co-administration of drugs with or after food can significantly influence the rate and extent of drug absorption [7]. Intestinal fluid composition varies between fasted and fed states [8] and the two systems are usually investigated separately [9].

1.2. Fasted and fed states

The gastrointestinal tract's normal physiological function is the digestion and absorption of food. A food effect occurs when a drug's bioavailability significantly varies in the fed state when compared with the fasted state [10]. The fasted state is achieved by overnight fasting to ensure that the stomach and small intestine are devoid of food based materials. The sampled fasted intestinal fluid therefore represents a base level composition of gastrointestinal physiology in the absence of exogenous food based materials [9]. The fed state is a more complex system arising after the ingestion of food resulting in a distinctive gastrointestinal fluid composition and volume, pH, surface tension, osmolality and variability associated with the nature of the food consumed [11]. Drug absorption in the fed state can therefore be influenced by the type of meal (solid or liquid), its calorie content, fluid ingestion, nutrient composition (high-fat meals, high-protein, or high-carbohydrate), volume and the temperature of the meal [11–14]. Fed state conditions are also associated with post prandial changes of the GI physiological variables for example bile flow, pH, different gastric emptying times and small intestinal transit times, changes in luminal metabolism along with direct food-drug interactions. All these factors can result in an increase (positive food effect) or decrease (negative food effect) in the overall extent of bioavailability [10,14–16].

1.3. Human and Simulated intestinal fluid

A recent modification of the DCS [17] specified the preferred usage of HIF in order to provide improved standardised and biorelevant conditions for solubility determination. However, there are multiple practical issues that hamper HIF application in routine studies. The process of collecting HIF aspirates is complicated as it requires human volunteers and an invasive and variable technique [9]. Due to these limitations HIF from either fasted (FaHIF) or fed (FeHIF) states is expensive to obtain and inconsistent as it varies depending on different sampling protocols, storage conditions [6,18], along with variability between different locations of the gastrointestinal tract and inter and intra subject variability [8,19,20].

To mitigate HIF collection and variability issues, simulated intestinal fluids (SIF) were developed and multiple recipes are available in the literature [21] covering both fasted (FaSIF) and fed (FeSIF) states. Drug solubility varies with SIF recipes [19,22], which complicates the decision on which recipe is optimal [21]. The variability and complexity of fasted and fed SIF media systems was revealed in recent design of experiment studies (DoE) [23–26] that aimed to investigate the impact of SIF media components on drug solubility. These studies highlighted that intestinal solubility was a range and multiple media factors influenced solubility. To refine SIF recipes a subsequent publication [27] studied fasted and fed HIF sample compositions obtained from twenty volunteers [8] using a five dimensional (a dimension was either pH, bile salt, phospholipid, free fatty acid or cholesterol concentration) mathematical analysis. This identified for both the fasted and fed states eight media compositions that statistically characterised over 95 % of the HIF samples' component variation and calculated a centre point through a Euclidean approach. The nine fed SIF recipes have been utilised to determine the equilibrium solubility of a range of drugs previously studied in the fed DoE systems [28]. This study reported statistical equivalence to the previous small scale fed DoE studies [23,29], along with the larger scale study [26] once solubility values from non-biorelevant media compositions were removed. In a similar manner to the fasted nine media system [30,31] the fed version is more likely to represent the fed intestinal solubility range than the previous fed DoE studies [23,25,26,29], with due recognition of the original HIF collection study's limitations [8],

1.4. Fed Developability classification system and solubility driven food effects

The importance of studying solubility under physiologically relevant conditions was highlighted by Zaki N., et al [32] who demonstrated that some BCS Class II compounds when tested using relevant media (FaSSIF, FeSSIF and phosphate buffer pH 6.5) may perform differently in vivo and change their BCS Class. The authors emphasised that physiologically relevant conditions should be considered in all stages of drug discovery to produce better formulations. The published DCS analyses [5,17] utilises solubility values for the fasted state but does not apply this to fed state. Since it is well known that solubility can vary between the fasted and fed state, the inclusion of fed solubility values would increase the information about a drug's behaviour in both states. This is especially important for poorly soluble drugs due to the potential for greater solubility changes in the fed state.

In this study, drugs originally tested in the fasted DCS [5] (see Table 1, furosemide, ibuprofen, mefenamic acid, paracetamol, acyclovir, griseofulvin and dipyridamole) were utilised to measure their equilibrium solubility in the fed intestinal fluid media compositions [27,28] (Table 2). The solubility range determined using these media recipes are more likely to be bioequivalent, in a similar manner to the fasted state [31], since they originated from sampled FeHIF [8]. It should be noted that there is a limitation since the fed state in the original study was obtained via the administration of the liquid feed Ensure Plus™. The aim of this study was to apply the fed state solubility range to the DCS grid and associated calculations, which to our knowledge is not available in the literature. To assess the solubility behaviour across the population a solubility frequency distribution was also determined. However, intra- and inter-subject variability cannot be analysed using this approach because the frequency distribution arises from the combined measured HIF samples of the twenty volunteers in the original study.

An obvious comparison would be the fed data measured in this paper against the previous fasted state study [31]. However, in order to limit paper size and focus discussion on the fed state DCS this manuscript will be restricted to a basic comparison of fed vs fasted results. A more detailed fasted vs fed comparison with a view to elucidating possible detection and quantification of solubility based food effects will be covered in a subsequent paper.

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

Table 1
Physicochemical properties and molecular structures of drugs.

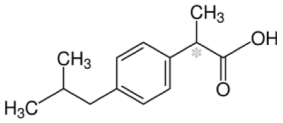
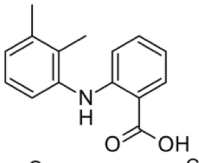
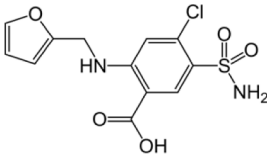
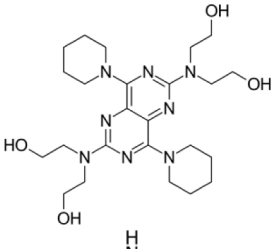
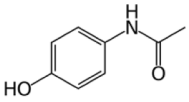
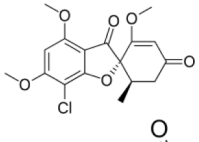
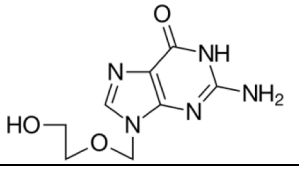
Compound	a/b/n	pKa	LogP	Structure
Ibuprofen	a	5.3	3.97	
Mefenamic Acid	a	4.2	5.12	
Furosemide	a	3.9	2.03	
Dipyridamole	b	6.2	3.77	
Paracetamol	n	–	0.46	
Griseofulvin	n	–	2.18	
Acyclovir	n	2.52/9.35	−1.56	

Table 2
Fed Media Compositions.

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

Scientific. The active pharmaceutical ingredients griseofulvin, furosemide, dipyridamole and acyclovir were purchased from Merck Chemicals ltd. Ibuprofen was purchased from BSAF chemical company, paracetamol was obtained from Mallinckrodt Pharmaceuticals and mefenamic acid from Sigma Aldrich. 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 solubility experiments

Lipid stock solutions 2.5 times greater than the required concentrations for each of the 9 recipes (Table 2) were prepared. Bile salts (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 and an aliquot of Solution B (100 µl) transferred to Solution A, stirred and the chloroform evaporated 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 (5 ml) and made to volume with water. Sodium oleate stock solution was prepared by weighing 913.32 mg 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. [28].

2.2.2. Fed simulated intestinal fluid (FeSSIFv2)

The media was prepared according to the instructions provided by the manufacturer (Biorelevant).

2.2.3. Equilibrium solubility measurement

The method applied was based in the previous papers in the fed [28] and fasted states [30,31] along with DoE studies [23–26,29]. An excess of the study drug was weighed into a centrifuge tube (15 ml Corning® tubes), and the fed biorelevant media stock, buffer stock, salt stock, FFA stock and water were added as shown in Table 3 to a final volume of 4 ml. The pH of each tube was adjusted as required (Table 1, pH ± 0.02) using KOH or HCl. The tubes were then shaken for 1 h at room temperature and the pH readjusted. FeSSIF-v2 media (4 ml) was added to separate tube with an identical excess of drug and pH 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 visually confirmed and 1 ml of each solution transferred to 1.5 ml Eppendorf tubes and centrifuged at 10,000 rpm for 15 min (Hettich Zentrifugen Mikro 20). The supernatant was analysed by HPLC for drug content. Three replicate measurements of each media system were performed.

2.2.4. HPLC analysis

HPLC analysis was performed using a Shimadzu High Performance Liquid Chromatography Prominence-I LC-2030C system in the conditions specified in Table 4. The HPLC method has been previously applied to quantify the concentration of the drug of interest [28,30]. For each drug, calibration curves were constructed, and the linés equation was used to interpolate drug concentration.

2.2.5. Data analysis

Data analysis and comparison was conducted using Graphpad Prism

Table 3
Fed Media Stock Solution 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

Table 4
HPLC Method Details.

Drug	Mobile Phase	Column	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
Acyclovir	Mobile Phase A: 10 mM Ammonium Formate pH 3 in H20	b	0.5	10	254	2.21
Dipyridamole	Mobile Phase B: 10 mM Ammonium Formate in ACN:H20 (9:1 V/	b	1	10	291	1.60
Furosemide	V)	b	1	10	254	1.07
Griseofulvin**		a	1	10	291	1.69
Ibuprofen		a	1	10	254	2.06
Mefenamic Acid		b	1	10	291	1.71
Paracetamol		b	1	10	254	1.08

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; **Analysis performed using an Agilent Technologies 1260 Series Liquid Chromatography system with Clarity Chromatography software.

9 for MacOSX.

3. Results and discussion

3.1. Equilibrium solubility measurements

The measured equilibrium solubility for the nine fed state media recipes and FeSSIF V2 are presented in Fig. 1 along with where available, literature solubility FeSSIF or FeHIF data. The drugs analysed in this study have not been measured in previous fed DoE approaches [23,25,26] and thus a comparison with these data sets is not possible. The majority of the published FeHIF and FeSSIF solubility values (69 %, 18 of 26 values) lie within the solubility range measured in this study, indicating that the solubility range is consistent with literature fed state solubility values. The level of agreement is comparable to the fasted state study where 8 out of 11 (73 %) literature points were inside the fasted solubility range [31]. The variability observed between the literature points and this study could be due to different measurement protocols, different media compositions (for example pH) and that the fed state can be achieved using different meal types [9].

Although the study drugs have not been assessed in previous fed DoE studies the solubility behaviour is, based on the individual drug's physicochemical properties (Table 1), consistent with published DoE results [25,26,29] and the developing fasted state literature [30,31,33]. The acidic drugs exhibit a different solubility behaviour in this study in comparison to the fasted state, which can be connected to the different pH ranges between the two systems (fasted pH 6.64 – 8.04, fed pH 5.97 – 6.59) in relation to drug pKa values, see later sections. Three out of the seven drugs (acyclovir, griseofulvin and paracetamol) provide a narrow solubility range, which is also reported in the fasted state [30,31,33]. This further reinforces that for these drugs variation of media composition is not a major solubility influence and extends this finding into the fed state. Although this might have been expected since the media components utilised in this study are identical to the fasted study [31]. This low solubility range property is not restricted to a specific BCS/DCS class (paracetamol – class I; griseofulvin – class II; acyclovir – class III) in

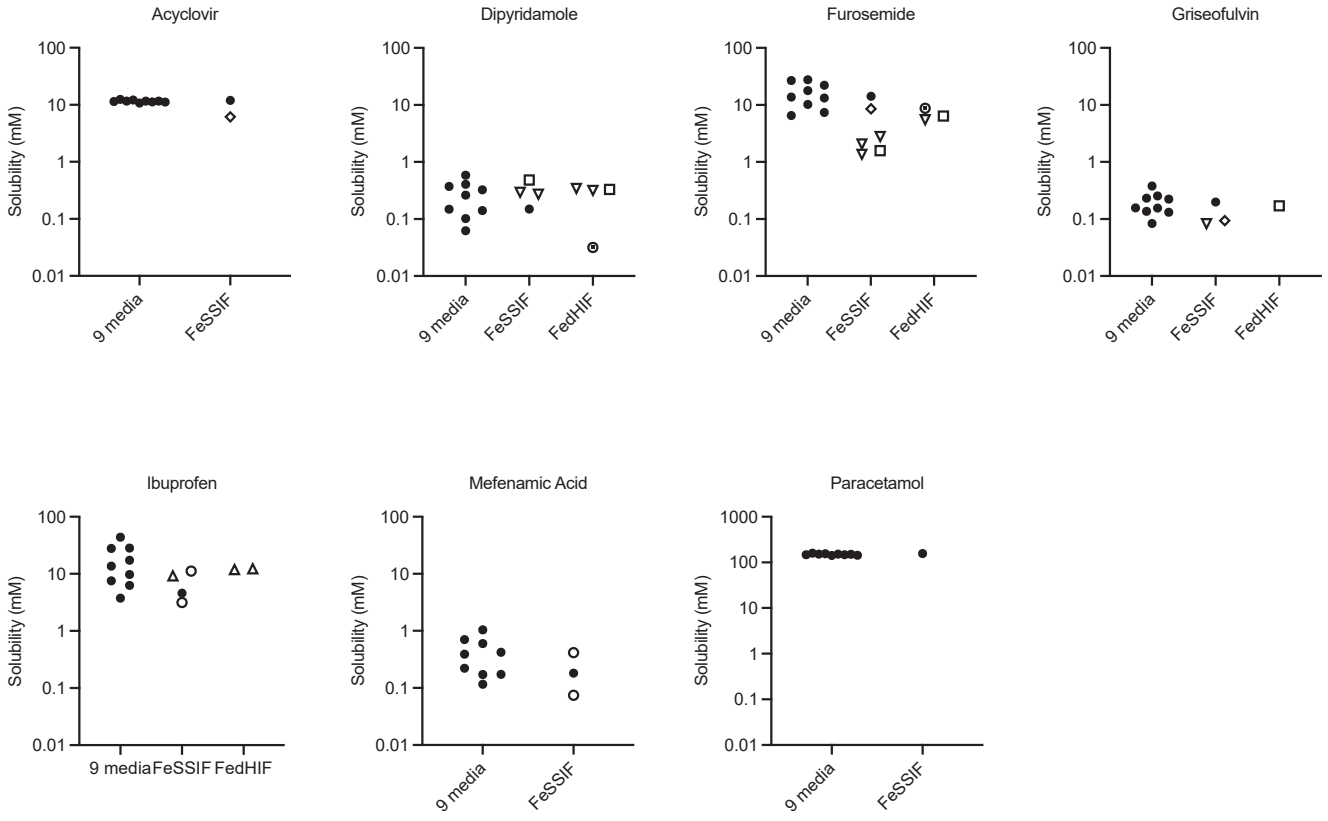


Fig. 1. Measured Fed State Equilibrium Solubility Distributions. Legend: Fig. 1: ● 9 media this study (mean, n = 3); ● FeSSIFv2 (Fed Simulated Intestinal Fluid v2) this study (mean n = 3); FeSSIF (Fed Simulated Intestinal Fluid v1) and HIF (Fed Human Intestinal Fluid) literature values as follows △ from [18]; ◇ from [47]; □ from [19]; ⊙ from [48]; ○ from [49]; ▽ from [50]. NB Paracetamol y-axis different scale.

the fed state and is probably due to a combination of the drug's molecular structure and physicochemical properties. These compounds, when compared to the others in this study, are relatively simple planar molecules with a low log P value (Table 1, albeit griseofulvin log P = 2). In order to completely define this behaviour an increased number of examples would be required. These results highlight for the fed state that an in vitro multi point solubility analysis allows for the detection and study of properties and behaviours that would not be possible using a single point measurement [30,31,33].

To compare the fed nine media system with the FeSSIFv2 solubility values, a statistical comparison of the nine media centre point solubility value and the mean FeSSIFv2 solubility was performed using a Wilcoxon matched pairs signed rank test. This analysis indicates that there is no statistically significant difference between the two data sets (Fig. 2) which suggests that the existing FeSSIF v2 could be compared with the centre point solubility measured with the fed media system. A non-parametric statistical comparison (Mann Whitney test) of FeSSIFv2 and centre point measurements (n = 3 per drug for both systems) performed for each individual drug did not detect a significant difference. This statistical analysis is however, hampered by the small number of drugs tested and the limitations of a non-parametric test. A larger number of drugs or multiple measurements for individual drugs is required to fully confirm the results of this comparison.

3.2. Solubility range

Collected solubility data is presented in Table 5 along with the dose and Peff values from the original DCS publication [5]. The solubility multiplier was calculated using the maximum and minimum solubility values and ranges from 1.16 for acyclovir to 11.7 for ibuprofen. A skew value was also determined to assess distribution symmetry around the centre point media. A value of 1 indicates a broadly symmetrical distribution, values > 1 indicate a skew to higher solubility values and conversely < 1 to low solubility values. The calculated values range from 0.694 for dipyridamole to 2.04 for mefenamic acid. The solubility multiplier values are smaller than the original fed DoE [26]. A previous examination of the nine media fed system concluded that this was due to the elimination of non-biorelevant outlier media systems that resulted from the DoE statistical design [28]. Along with greater media concentration variation due to the upper and lower DoE limits. A comparison with the fasted nine media study (Table 5) [31] indicates some differences in multiplier values especially for the acidic drugs. Both mefenamic acid and furosemide exhibit a decreased solubility multiplier in the fed study in contrast with ibuprofen that shows an increase. This

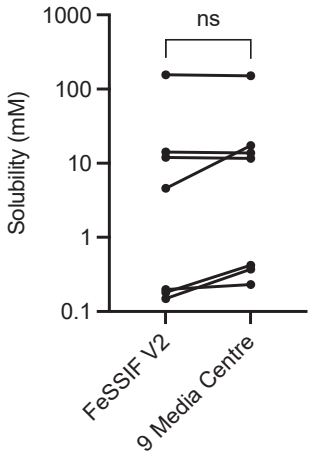


Fig. 2. FeSSIFv2 vs Centre Point Solubility Comparison. Legend: FeSSIFv2 (Fed State Simulated Intestinal Fluid) and Centre this study. ns no significant difference (P > 0.05), each point mean n = 3.

variation can be explained by the differences in media pH (fed pH 5.97 – 6.59 Δ = 0.62 / fasted pH 6.64 – 8.04 Δ = 1.4) in combination with the drugs' pKa values (see Table 1). This result is consistent with the previous finding that pH is the main factor controlling acidic drug solubility [24,26]. Based on the solubility multiplier furosemide in the fed state has a narrow solubility range (similar to griseofulvin). In general, drugs with the lowest solubility multiplier also have the lowest skew value a result in agreement with the fasted study. However, dipyridamole is an exception in this fed study and presents the lowest skew value with a larger solubility multiplier, which maybe an example of a complex drug behaviour in the fed state. Individualistic drug behaviours in these media systems has been previously reported [34,35], however a larger number of data points is required to fully explore this behaviour.

3.3. Developability classification system analysis

The Developability Classification System [5,17] was developed to cover the fasted state and this is the first investigation of fed solubility data using this approach. The fed nine media solubility data was combined with the drug's normal oral dosage in the original DCS paper [5], to calculate a dose/solubility ratio for each media measurement and plotted at the respective permeability value. The results are presented in Fig. 3, where it is possible to visualise the drug's fed DCS dose/solubility range. The fed nine media compositions were designed to cover greater than ninety-five percent of the intestinal fluid variation within a data set of fed HIF samples [27] (collected from twenty healthy volunteers [8]). Therefore it is reasonable to assume that the measured dose/solubility ratio ranges represent a drug's solubility behaviour in fed intestinal space. As detailed previously there is a caveat to this assumption, the fed state in the original study [8] was obtained using 400 ml of Ensure Plus as a liquid meal representative of a standard meal. This may not be equivalent to alternative fed states induced by solid meals [9]. The lowest solubility or largest dose/solubility ratio could be interpreted as the worst scenario in a drug's solubility profile during the fed state with more than ninety percent of the dose/solubility distribution lower than this value. Thus, formulation selection and compound screening based on the lowest solubility values could be applied to early drug development as a worst case scenario instead of centre point or FeSSIF values. This could facilitate quality by design development approaches and reduce the risk of unexpected solubility induced behaviour. The knowledge of lowest solubility values for the fed state could also be especially useful since it might be able to highlight the impact of food effects on solubility, when compared to the fasted state.

For the acidic drugs, mefenamic acid, furosemide and ibuprofen, the solubility behaviour is highlighted with respect of media pH in Fig. 4. The main conclusion is that solubility increases (thus dose/solubility volume decreases) with increasing pH, with some variations occurring due to media amphiphilic factors. As above this is consistent with pH as the major solubility driver for acidic drugs as identified in the original fed DoE study [26] and subsequent studies [23,29]. Also comparable with the fasted system [31], indicating that acidic drug solubility behaviour remains consistent with the fed media.

An interesting biopharmaceutical result is a drug's position within the DCS. Fig. 3 indicates that three drugs (paracetamol – Class I, furosemide and acyclovir – Class III) are within class boundaries, with four drugs (ibuprofen Class I – II, mefenamic acid and griseofulvin Class IIa – IIb, dipyridamole Class I - IIa – IIb) spanning across boundaries. This is markedly different to the fasted nine media result where only mefenamic acid crossed a classification boundary [31]. In the fed state mefenamic acid crosses a DCS boundary, from IIb (solubility limited) to IIa (dissolution limited) with the center point and FeSSIFv2 values both located in IIa and only a single lowest solubility value located in IIb. In the fasted state mefenamic acid also crossed between IIa and IIb, but the centre point was located on the class boundary. Similarly, for ibuprofen a single low solubility value crosses from class I into IIa. Griseofulvin is similar crossing from IIa to IIb, but in this instance only the single

Table 5
Collected Solubility Data and Analysis.

Drug	Dose (mg)*	Estimated Human Peff ($cms^{-1}x10^{-4}$)*	FeSSIF V2 Solubility (mg/ml)	Centre Point Solubility (mg/ml)	Minimum Solubility (mg/ml)	Maximum Solubility (mg/ml)	Solubility Multiplier[1]	Skew[2]
Ibuprofen	400	12	0.946	3.58	0.773	9.06	11.7 (4.41)	1.96 (0.772)
Mefenamic Acid	250	14	0.044	0.102	0.028	0.252	9.02 (35.9)	2.04 (29.2)
Furosemide	80	0.6	4.68	4.56	2.17	9.13	4.21 (40.0)	1.91 (3.16)
Dipyridamole	100	1.5	0.076	0.188	0.031	0.297	9.44 (7.48)	0.694 (7.23)
Paracetamol	500	1.3	23.6	22.8	21.4	24.2	1.13 (1.22)	0.919 (1.10)
Griseofulvin	500	8.7	0.070	0.082	0.030	0.133	4.52 (2.32)	0.998 (3.63)
Acyclovir	800	0.25	2.70	2.61	2.42	2.81	1.16 (1.15)	1.059 (0.929)

* Data from Butler [5].
1: Solubility Multiplier = (Maximum Solubility)/(Minimum Solubility).
2: Skew = ((Maximum Solubility − Centre Point Solubility))/((Centre Point Solubility − Minimum Solubility)).
3: Solubility Multiplier and Skew, bracketed values from fasted study [31].

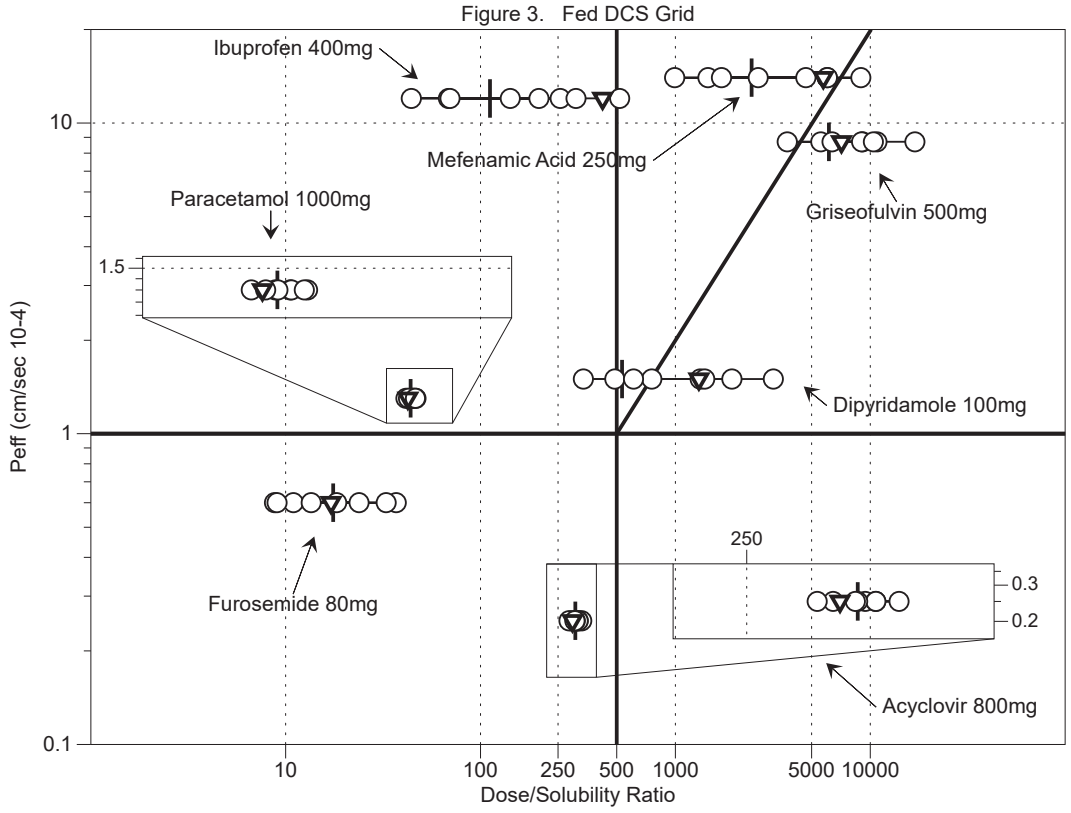


Fig. 3. Fed Nine Media Systems on Developability Classification System Grid. Legend: ▽ FeSSIFv2 (Fed State Simulated Intestinal Fluid); ○ Fed Nine Media data points, I Fed Nine Media centre point. Inset expanded scale for acyclovir and paracetamol. Individual drugs and doses as labelled. Each point mean n = 3.

highest solubility value lies within IIa. This is also different to the fasted state where all points are located within class IIa. This shift to a higher solubility and lower dose/solubility ratio in the fed state correlates with the known effect of food enhancing griseofulvin bioavailability [36], see section 3.5. Dipyridamole, has a Peff value that is very close to the low/high permeability boundary and in the fed nine media system is the only drug to cross two classification boundaries, spanning from two high solubility values in class I, three in class IIa and four (along with FeSSIFv2) in class IIb. This behavior is different to the fasted state where all the measured solubility values were in class IIb. This additional information regarding the variations on drug solubility behaviour in the DCS grid is only available due to the solubility range that results from the multi-point measurements. The same analysis would not be possible with single measurements using FeHIF or FeSIF, and is a further example of the utility of this solubility range approach.

3.4. Fed solubility distributions

Table 2 media compositions were calculated based on the compositional variation of the 172 fed HIF samples in the original analysed data set [27]. Through the application of 5-dimensional Euclidean space it is possible to calculate the proximity of each fed HIF sample to an

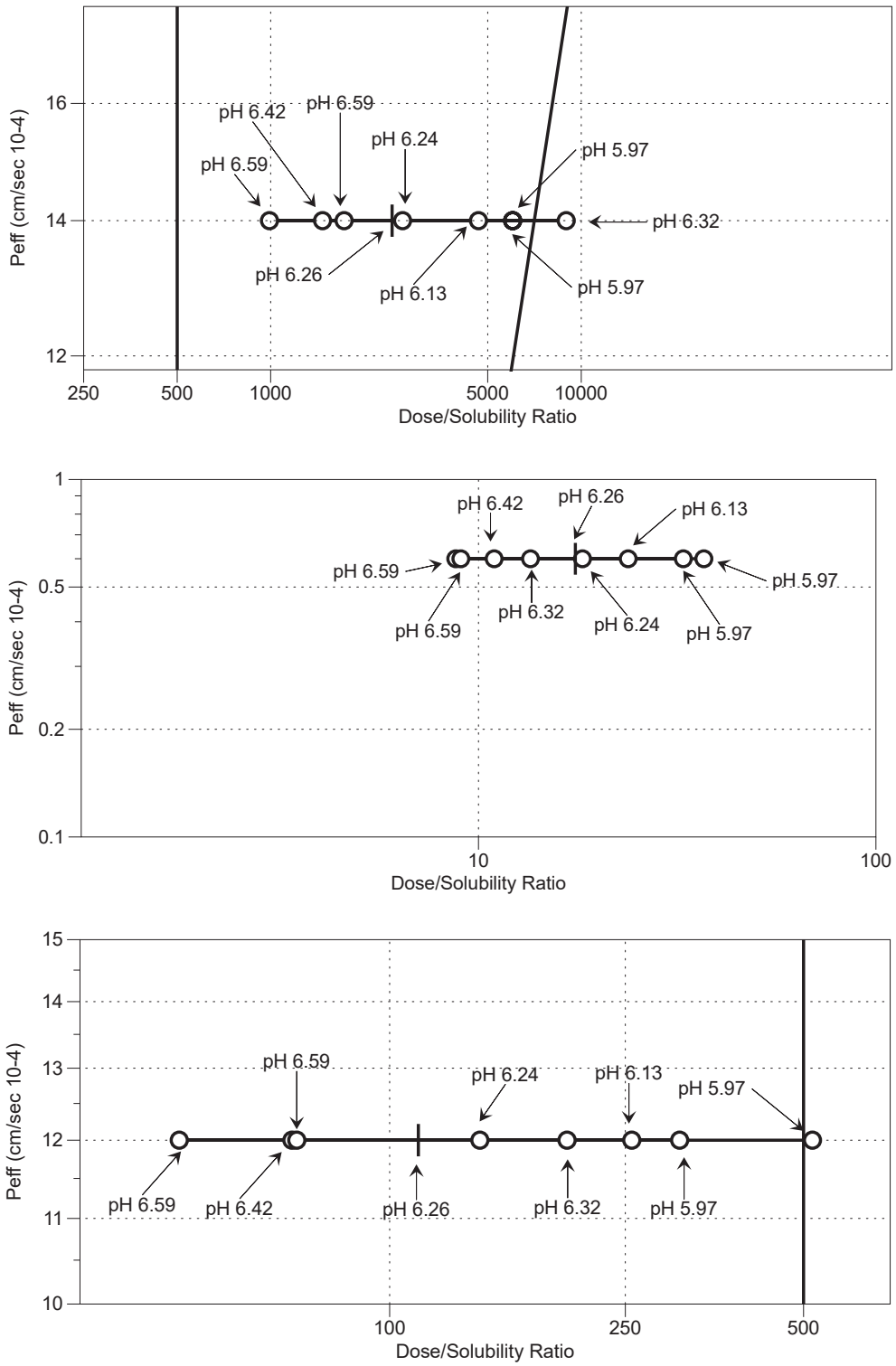


Fig. 4. Acidic Drugs pH driven solubility behaviour. Legend: (a) Mefenamic Acid. (b) Ibuprofen. (c) Furosemide. ○ Fed Nine Media data points, I Fed Nine Media centre point. Media pH values as labelled, see Table 2. Each point mean n = 3.

individual Table 2 media composition to produce a frequency distribution based on the number of HIF samples closest to each media. The equilibrium solubility of each media can then be converted to a dose/solubility volume vs frequency distribution, see Fig. 5a and b. It should be noted that this frequency distribution arises from the sampled fed HIF point compositions [8,27] and cannot be related to individual subject in vivo pharmacokinetic variability [37].

In Fig. 5a the distributions for paracetamol, acyclovir, griseofulvin and dipyridamole are presented. Based on the presentation in Fig. 1 and associated discussion in section 3.1, paracetamol, acyclovir and, griseofulvin all have very narrow frequency distributions with almost vertical cumulative lines, related to the very narrow solubility range for these drugs. Dipyridamole has a broader distribution range but the points are not evenly distributed on the cumulative plot and the centre point is towards the higher end of the plot. In Fig. 5b the distributions for mefenamic acid, ibuprofen and furosemide are presented. Since these

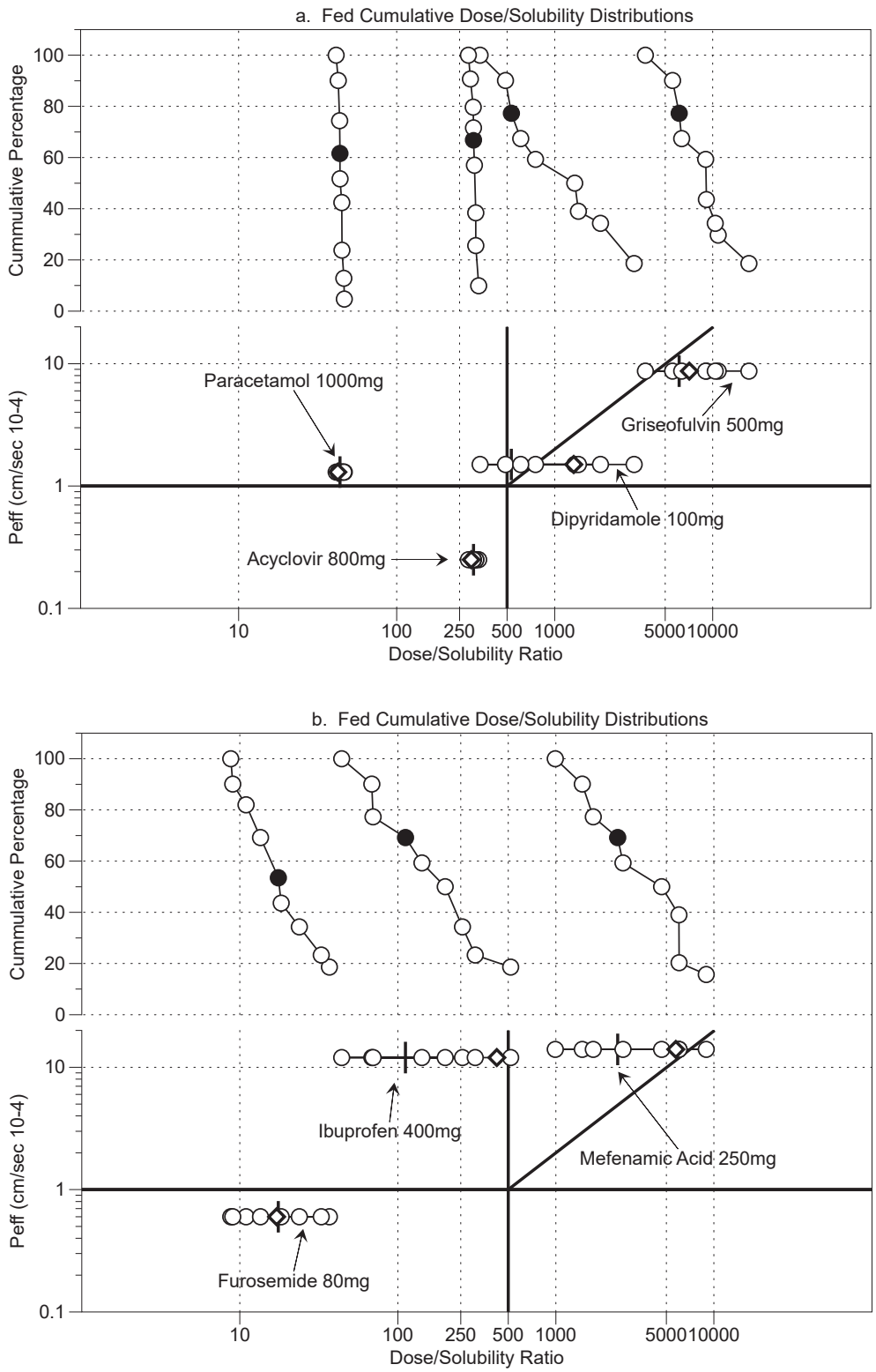


Fig. 5. A and b. cumulative fed dose/solubility ratio distributions. legend: lower graph: developability classification system grid,⋄ FeSSIFv2 (Fed State Simulated Intestinal Fluid); O Fed Nine Media data points, I Fed Nine Media data centre point. Each point mean n = 3. Upper graph: Cumulative percentage incidence of data points, O Fed Nine Media data points, ● Fed Nine Media data centre point.

are all acidic drugs the distributions will be predominantly controlled by pH (see section 3.2 and Fig. 4a, b and c), but also display the same characteristics previously described. Mefenamic acid and ibuprofen also exhibit an increased degree of structure in the cumulative plot with steps in the distribution.

Statistical analysis of the distributions either for normal or log normal behaviour did not produce significant results. Previous statistical analysis of fed SIF DoE solubility distributions [23,25] highlighted that the distributions were not normal, also the fed HIF data points used to calculate the bioequivalent points [27] were not normally distributed.

This result might reflect the well known variability of these fluids [38,39] and the measurement of solubility in them [35,40,41]. This behavior is similar to the fasted system [31] and a comparable analysis highlights that the change from low to high solubility is not a simple vector based on the increasing concentration of a single media component. Therefore, the lack of an organised statistical distribution when traversing the solubility range based on individual discrete points is to be expected. This highlights why a single fed HIF aspirate will not be representative of the entire fed HIF space and single measurements limited by a lack of knowledge of the sample's position in the space, which will be further complicated when drug properties are superimposed.

3.5. Solubility limited Absorbable dose distribution

A solubility limited absorbable dose (SLAD) and target particle size to avoid dissolution rate limiting issues can be determined by applying biopharmaceutical assumptions and calculations [5,17]. The SLAD calculation requires a value for the total volume of intestinal fluid. This has been determined as 1150 ml based on 500 ml for a fasted system [5], plus the volume administered during the fed phase of the intestinal fluid sampling study [8] 400 ml Ensure Plus + 250 ml water. This increase in fed state volume in comparison to the fasted means that the calculated fed SLAD will be 2.3 times higher than the fasted value even if the drug's measured solubility does not change. This calculation has been applied to the centre point and lowest solubility value as a worst case situation (Table 6), using literature Peff values [5] and standard values for other properties.

A comparison of the calculated values for the centre point and lowest solubility measurements not surprisingly exhibit the same relationship described above for solubility. For narrow solubility distribution drugs (paracetamol, acyclovir, griseofulvin and furosemide) there is minimal difference between the values, whilst for the other drugs the difference reflects the discussion above. For paracetamol, acyclovir and griseofulvin this finding matches the fasted state (Table 6, bracketed values). This indicates that a narrow intestinal solubility range might be a useful drug development target, since the drug would be intrinsically resistant to intestinal solubility variability. It could also be surmised that congruent fasted and fed solubility distributions would further enhance resistance to gastro-intestinal food effect solubility issues. The narrow distribution for furosemide is only present in the fed state, this represents a different behavior to the fasted and linked to the lower fed media pH range and drug pKa, see above. This indicates that for furosemide, population plasma concentration variation in the fed state should be lower than the fasted, assuming solubility controlled absorption and no interference from other factors, metabolism for example. In one study the AUC in the fasted state is 2,174 ± 668 ng/ml.h and 1,219 ± 403 ng/ml.h in the fed state [42], whilst a separate study determined that the fasted area was 51.3 ± 7.24 % (with reference to an IV dose) and the fed 43.3 ± 5.94 % [43]. In both cases the fed variability is lower, possibly due to the solubility effect noted, with one study [43] stating, "food seemed to diminish the interindividual differences". Although not conclusive, due to the variations in the studies (Beermann determines that there is a food effect on bioavailability, whilst Hammarlund does not find a food effect), this result indicates the potential utility of comparing the fasted and fed solubility distributions as an indicator of food effects. For four drugs (ibuprofen, mefenamic acid, furosemide, paracetamol), the calculated lowest SLAD is above the administered dose (Table 5 and 6), which indicates that minimal solubility based absorption issues are possible and reflective of their positions on the DCS grid. For three drugs (dipyridamole, acyclovir and griseofulvin), the calculated lowest SLAD is below the administered dose (Table 5 and 6, acyclovir also the centre point value) and therefore the lowest solubility based calculation could be applied as a quality by design parameter for particle size to reduce the risk of absorption issues [17]. By linking a point's SLAD value to the cumulative percentage incidence (see section 3.4), it is possible to determine where solubility limitations no longer apply. This is presented in Fig. 6 for dipyridamole, acyclovir and griseofulvin. For dipyridamole and griseofulvin the plot indicates that solubility limitations will arise in under forty and sixty percent of fed HIF compositions respectively and this information could be applied for a risk assessment based development and formulation. For acyclovir all SLAD points are lower than the dose, however the difference is approximately 100 mg or 12 % of the dose, which may not be critical in vivo.

Table 6
Calculated Biopharmaceutical Data.

Drug	SLAD[1] (mg)		Particle Radius (μm)	
	Centre Point Solubility	Minimum Solubility	Centre Point Solubility	Minimum Solubility
Ibuprofen	47,205 (24,519)	10,199 (8,380)	231	107
Mefenamic Acid	1,539 (193)	431 (90)	39	20
Furosemide	3,009 (1,181)	1430 (114)	261	179
Dipyridamole	310 (10)	52 (6)	53	22
Paracetamol	32,669 (12,357)	30,652 (11,183)	584	566
Griseofulvin	780 (55)	282 (43)	35	21
Acyclovir	718 (3,434)	695 (3,186)	198	194

Solubility Limited Absorbable Dose - SLAD = $S_{INT} \times V \times A_n$ where S_{INT} is the intestinal solubility (mg/ml) measurement as indicated in column header (see Table 3), V is the volume of fed intestinal fluid (1150 ml) and A_n is the absorption number ($A_n = \frac{P_{eff} x T_{si}}{R}$) where P_{eff} is the effective permeability of the intestine to the drug (see Table 4), T_{si} is the small intestinal transit time (3.32 h) and R is the intestinal radius (1.25 cm). Note V value based on 500 ml of fasted system [5], plus volume administered during fed phase of intestinal fluid sampling study [8] 400 ml Ensure Plus + 250 ml water.
Particle radius = $\sqrt{3DxS_{INT}xT_{si}/D_nx\rho}$ where D is the diffusion coefficient (typically at 5×10^{-6} cms^{-1}), S_{INT} and T_{si} are as above, D_n is the dissolution number (set to 1) and ρ is the drug density (typically 1.2 g cm^{-3}).
1: SLAD, bracketed values from fasted study [31].

There are interesting differences between the fasted and fed SLAD analysis for these drugs. For griseofulvin all fasted SLAD values were below the dose, see Fig. 6 [31], whilst in the fed state 60 % of the population is below the dose. For dipyridamole a similar situation exists, in the fed state with a shift to only 40 % of the population below the administered dose. In the fed sate all acyclovir SLAD values are below the administered dose, but this is reversed in the fasted state. Whilst in the fasted state 65 % of mefenamic acid is below the SLAD but no points are in the fed state. As discussed above for griseofulvin this reflects the well known impact of food on bioavailability [36] and food is also known to increase the bioavailability of dipyridamole [44,45]. The literature for acyclovir indicates that it does not exhibit food effects [46]. However, it is a low permeability (Class III), low bioavailability (0.15–0.2) drug and the change in solubility noted in this study might not be sufficient to provide a detectable effect in vivo. Overall the comparison of the fed solubility profile determined in this paper with the previous fasted determination is highlighting differences in vitro between the two states that potentially represents the impact of food in vivo on gastrointestinal solubility.

4. Conclusions

These results indicate that the nine fed media recipes are simple to apply and provide drug equilibrium solubility measurements in agreement with literature fed HIF and SIF values and solubility behaviour in

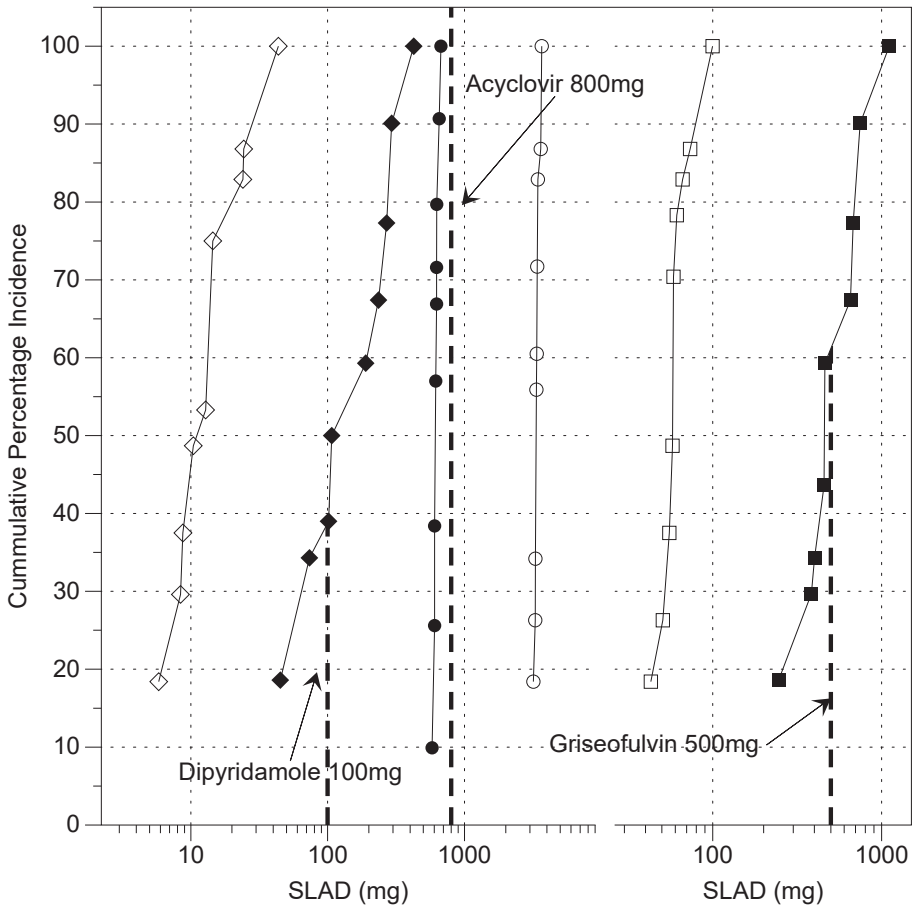


Fig. 6. Cumulative Percentage Incidence of Solubility Limited Absorbable Dose. Legend: Closed symbols Fed Data, Open symbols fasted data from [31]. ⋄ Dipyridamole, ○Acyclovir, □Griseofulvin, dotted vertical line drug dose, value as indicated.

agreement with previous DoE studies. The solubility values can be applied to calculate fed dose/solubility points that can be plotted on the DCS grid, and due to the derivation of the nine fed media recipe compositions are likely to cover > 95 % of the fed intestinal solubility range. Application of standard oral biopharmaceutical parameters also permits the calculation of a SLAD value, which further enhances the available information. The range provides greater information than single point measurements and the lowest solubility value represents a worst case scenario that could be applied to risk assessment or quality by design approaches during drug screening, development and formulation. Solubility values can be linked to the original HIF data set to provide a population frequency distribution that further refines the risk assessment. This approach is comparable to the nine fasted media recipe system [31].

A comparison of the fed values in this study with the fasted values from a previous study [31] reveals some interesting differences in solubility behaviour for griseofulvin, dipyridamole, furosemide and acyclovir. These in vitro fasted vs fed differences can be reconciled with the results from in vivo studies that have examined the impact of food on oral absorption. This indicates that the combination and comparison of the fasted and fed solubility ranges in vitro might be a useful indicator of in vivo behaviour. This will be explored further in a subsequent paper.

Overall the approach is therefore worthy of further development and research to expand the number of drugs analysed, link in vitro solubility to in vivo pharmacokinetics and investigate the fasted fed state comparison.

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.

Data availability

Data will be made available on request.

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