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Structured solubility behaviour in bioequivalent fasted simulated intestinal fluids

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A B S T R A C T

Drug solubility in intestinal fluid is a key parameter controlling absorption after the administration of a solid oral dosage form. To measure solubility in vitro simulated intestinal fluids have been developed, but there are multiple recipes and the optimum is unknown. This situation creates difficulties during drug discovery and development research. A recent study characterised sampled fasted intestinal fluids using a multidimensional approach to derive nine bioequivalent fasted intestinal media that covered over 90% of the compositional variability. These media have been applied in this study to examine the equilibrium solubility of twenty one exemplar drugs (naproxen, indomethacin, phenytoin, zafirlukast, piroxicam, ibuprofen, mefenamic acid, furosemide, aprepitant, carvedilol, tadalafil, dipyridamole, posaconazole, atazanavir, fenofibrate, felodipine, griseofulvin, probucol, paracetamol, acyclovir and carbamazepine) to determine if consistent solubility behaviour was present. The bioequivalent media provide in the majority of cases structured solubility behaviour that is consistent with physicochemical properties and previous solubility studies. For the acidic drugs (pKa < 6.3) solubility is controlled by media pH, the profile is identical and consistent and the lowest and highest pH media identify the lowest and highest solubility in over 70% of cases. For weakly acidic (pKa > 8), basic and neutral drugs solubility is controlled by a combination of media pH and total amphiphile concentration (TAC), a consistent solubility behaviour is evident but with variation related to individual drug interactions within the media. The lowest and highest pH × TAC media identify the lowest and highest solubility in over 78% of cases. A subset of the latter category consisting of neutral and drugs non-ionised in the media pH range have been identified with a very narrow solubility range, indicating that the impact of the simulated intestinal media on their solubility is minimal. Two drugs probucol and atazanavir exhibit unusual behaviour. The study indicates that the use of two appropriate bioequivalent fasted intestinal media from the nine will identify in vitro the maximum and minimum solubility boundaries for drugs and due to the media derivation this is probably applicable in vivo. These media could be applied during discovery and development activities to provide a solubility range, which would assist placement of the drug within the BCS/DCS and rationalise drug and formulation decisions.

1. Introduction

1.1. Oral drug administration

The oral route is the most common method of drug administration, permitting self-medication which assists patient compliance and acceptability and allows the pharmaceutical industry to meet this requirement through the provision of adaptable and stable solid oral dosage forms. For a patient oral administration is a simple procedure, however for a pharmaceutical scientist this simplicity masks a complexity that spans through drug discovery, development, physicochemistry, manufacturing, gastro-intestinal physiology (normal and diseased) and patient behaviour, for example food consumption. A constant for solid oral dosage forms is that post administration the solid drug must dissolve and form a solution within the gastro-intestinal tract in order to be absorbed, enter the portal and then systemic circulation and elicit the desired pharmacological response. A fundamental property of oral administration is therefore drug solubility within the gastro-intestinal tract since this controls dissolution [38] and calculated values such as the maximum absorbable dose [13,17,19] and the solubility limited absorbable dose [10,37]. The critical importance of drug solubility for the oral route was formalised in the Biopharmaceutics Classification System [4], that linked solubility and permeability with in vitro and in vivo performance, and refined in the Developability Classification System [10,37]. Solubility is also of increasing importance due to the trend towards development of poorly soluble drugs with respect to dose [28,38]. The ability to measure in vivo intestinal solubility in vitro is therefore a critical stage in a drug development program [16].

Abbreviations: TAC, Total Amphiphile Concentration; FaSSIF, Fasted simulated intestinal fluid; DoE, Design of Experiment.

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Equilibrium solubility can be measured experimentally however physicochemical principles dictate that the value will be controlled by the drug's solid state properties and the "solvent" into which the drug dissolves [22]. It has been recognised that within the gastrointestinal tract different solvent systems are present based on the normal tract physiology and anatomy [8], and patient populations [35,39], and therefore solubility measured in simple aqueous buffers is not applicable [19]. In order to overcome this limitation two approaches have been applied measuring solubility in sampled intestinal fluids [6,27] or simulated intestinal fluids [9,18] that can be utilised in vitro to asses in vivo solubility in various anatomical locations of the intestinal tract and simulating either the fasted or fed states.

1.2. Fasted simulated intestinal fluids

Multiple fasted simulated intestinal fluid (FaSSIF) recipes are now available in the literature [9] ranging from simple systems consisting of a buffer, bile salt and lecithin to more complex systems which include free fatty acid, monoglyceride and enzyme components [25]. Media development has been based around compositional data from sampled fasted human intestinal fluid (FaHIF) along with measured drug solubilities in FaHIF compared to the solubility in the proposed FaSSIF media. This has provided a correlation between drug solubility in FaHIF and FaSSIF [11,37] but despite research it is still not clear which FaSSIF media is optimal [9], new versions are still in development [21] and the measured solubility for drugs varies between media [21]. In addition a single media is unable to simulate the inherent variation that is present in FaHIF [14,34] and therefore biopharmaceutical predictions are limited since only a single solubility value is determined and the value's position in a population is unknown nor is the potential range of solubility behaviours.

Recent design of experiment (DoE) guided studies have examined the impact of FaSSIF media components on drug solubilisation [3,25,29,33], highlighting the potential solubility variability inherent in these systems but also identifying the key media components influencing solubility and the complex interactions between them [40]. Whilst these statistically guided studies are excellent for determining the intricate range of interactions present they are complex (the original DoE required 66 experiments per drug [25], which limits application in a drug development setting and they are also potentially not biorelevant due to the statistically constructed media recipes. In order to remove the biorelevant limitation a recent study [34] has performed a multidimensional analysis of FaHIF samples [36] to calculate eight FaHIF compositions that cover greater than 90% of the variability present in the sampled fasted intestinal fluid and can be applied to create bioequivalent FaSSIF media. This has been coupled with a central distribution point, based on the FaHIF samples to create a set of nine bioequivalent FaSSIF media recipes [2] that can be used to explore fasted intestinal solubility. Whilst this approach substantially reduces the experimental load and improves the biorelevance of the test media by removing the statistical aspects associated with DoE generated media, the lower number of samples [30] along with the media structure derivation [2] limits the ability to asses individual media components' contribution to solubility.

1.3. Bioequivalent fasted simulated intestinal media solubility

A recent paper [2] has compared the equilibrium solubility distribution of naproxen, indomethacin, phenytoin, piroxicam, zafirlukast, aprepitant, carvedilol, tadalafil, fenofibrate, griseofulvin, felodipine, and probucol measured using the nine bioequivalent fasted media against results from the original fasted DoE [25] and other DoE references which included the fasted state [3,30,33]. There were no statistically significant differences between the data sets indicating that all the systems were examining an identical solubility space. In addition, the measured solubility range for the nine bioequivalent media was, due to the elimination of non-biorelevant statistical media compositions, smaller than the DoE range.

A further paper [1] has utilised the equilibrium solubility measured in the nine bioequivalent fasted media for ibuprofen, mefenamic acid, furosemide, dipyridamole, griseofulvin, paracetamol and acyclovir to expand the original Developability Classification System grid [10]. The results indicate that the single point based on measurement in a FaSSIF recipe [9] sits within a range of points extending from the highest to the lowest measured solubility based on the nine bioequivalent fasted media. This indicates that for some drugs (mefenamic acid for example) the range crosses DCS boundaries, which indicates that development and formulation strategies should be adapted to suit the range and not the single measurement [26].

1.4. Solubility behaviour and measurement reduction

In this paper using the nine bioequivalent fasted media [2,34], we have examined the equilibrium solubility of the twelve drugs (naproxen, indomethacin, phenytoin, piroxicam, aprepitant, carvedilol, zafirlukast, tadalafil, fenofibrate, griseofulvin, felodipine, probucol) investigated in the original DoE study [25] with the additional drugs (ibuprofen, mefenamic acid, furosemide, dipyridamole, griseofulvin, paracetamol, and acyclovir) investigated in order to replicate the Developability Classification System study [1,10] along with posaconazole, atazanavir and carbamazepine. The aim is to investigate the solubility behaviour of the nine bioequivalent fasted media recipes to determine if this is consistent between the drugs and drug categories. Consistent solubility behaviour might permit a further reduction or refinement of the number of media required to determine a FaHIF solubility range in vitro using FaSSIF media. Determination of an intestinal solubility range, with minimum addition of required media would be useful during early drug development, when API material is limited but crucial decisions concerning for example API physical form and formulation are made [7,16,17].

2. Materials and methods

2.1. Materials

Sodium taurocholate, cholesterol, sodium chloride (NaCl), sodium oleate, ammonium formate, formic acid, potassium hydroxide (KOH), hydrochloric acid (HCl), furosemide, dipyridamole, naproxen, phenytoin, piroxicam, fenofibrate, probucol, griseofulvin, carvedilol, tadalafil, carbamazepine, indomethacin and acyclovir (see Table 1 for basic physicochemical data) were purchased from Merck Chemicals Ltd. Aprepitant and felodipine were provided through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark. Zafirlukast was purchased from Stratech Scientific Ltd. Ibuprofen was obtained from BSAF chemical company, paracetamol was from Mallinckrodt Pharmaceuticals and mefenamic acid from Sigma Aldrich. Atazanavir and posaconazole were purchased from Chemshuttle company. Phosphatidylcholine from soybean (PC S) was purchased from Lipoid company. See Table 1 for physicochemical properties and molecular structures. Chloroform was from Rathburn Chemical Company, and sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$) was purchased from Fisher Scientific. All acetonitrile (ACN) and methanol (MeOH) solvents were HPLC gradient (VWR). All water is ultrapure Milli-Q water.

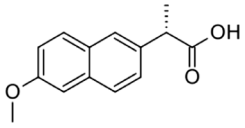
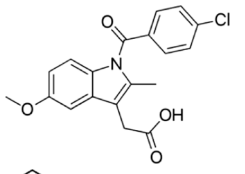
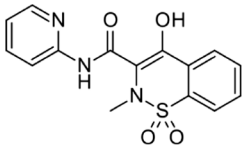
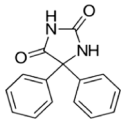
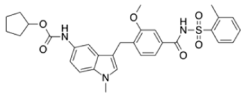
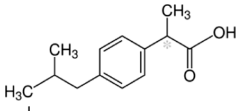
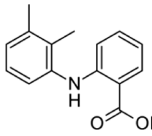
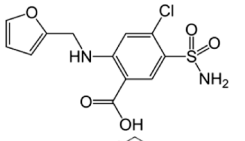
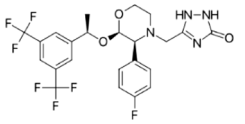
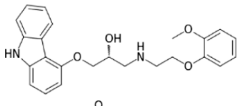
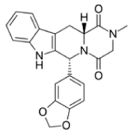
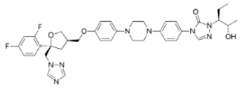
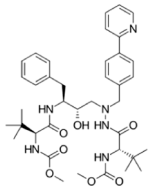
2.2. Methods

2.2.1. Solubility media preparation

Bioequivalent media stock solutions.

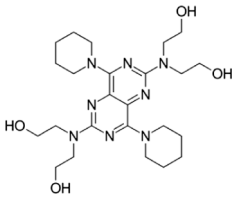
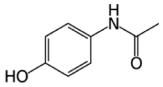
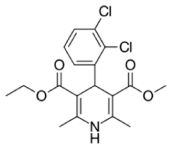
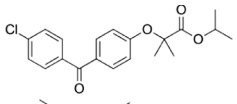
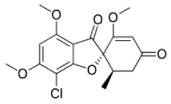
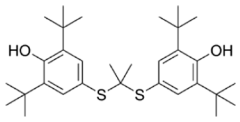
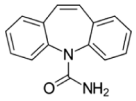
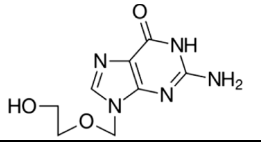
For each media recipe (Table 2), a concentrated lipid stock was prepared as follows. The required (x15) weight of bile salt (sodium taurocholate), phospholipid (soyabean lecithin) and free fatty acid

Table 1
Physicochemical properties and molecular structures of drugs.

Compound	a/b/n	pKa	Log P	Structure
Naproxen	a	4.15	3.18	
Indomethacin	a	4.5	4.27	
Piroxicam	a	6.3	3.06	
Phenytoin	a	8.33	2.47	
Zafirlukast	a	4.94	2.3	
Ibuprofen	a	5.3	3.97	
Mefenamic Acid	a	4.2	5.12	
Furosemide	a	3.9	2.03	
Aprepitant	b	9.7	4.5	
Carvedilol	b	7.8	4.19	
Tadalafil	b	3.5	1.7	
Posaconazole	b	3.6 & 4.6	4.6	
Atazanavir	b	4.7	5.9	

(*continued on next page*)

Table 1 (*continued*)

Compound	a/b/n	pKa	Log P	Structure
Dipyridamole	b	6.2	3.77	
Paracetamol	n	–	0.46	
Felodipine	n	–	3.86	
Fenofibrate	n	–	5.2	
Griseofulvin	n	–	2.18	
Probucol	n	–	11.3	
Carbamazepine	n	–	2.45	
Acyclovir	n	2.52/ 9.35	−1.56	

(sodium oleate) for each media recipe were dissolved in chloroform (3 mL) – Stock A. The required weight of cholesterol (x1500) for each media recipe was dissolved in chloroform (10 mL) – Stock B. An aliquot of Stock B (0.1 mL) was added to each Stock A, mixed and the Stock A chloroform solution evaporated under a stream of dry nitrogen gas. The dry lipid film was resuspended in water, quantitatively transferred to a volumetric flask (5 mL) and made to volume with water. Stock aqueous 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*

The method is based on previous papers [25], aliquots (267 μL) of the lipid, buffer and salt stock solutions, an excess of the solid drug and water (3.199 mL) were added into a centrifuge tube (15 mL Corning® tubes) to make a final aqueous system volume of 4 mL. The pH was adjusted to the required value (Table 2) using 1 M KOH or HCl as required. The required bioequivalent FaSSIF medium (4 mL) was added to the tube along with an excess of the solid drug and pH was adjusted if required. Tubes were capped and placed into a shaker (Labinco L 28 Orbital shaker) for 1 h at room temperature and the final pH was re-adjusted as required. Tubes were then placed in the shaker at 37 °C for 24 h. Post incubation, an aliquot (1 mL) of each tube was transferred to a 1.5 mL Eppendorf tube and then centrifuged for 15 min, 10,000 rpm. The supernatant was analysed by HPLC for drug content. For each

Table 2
Compositional values of the 8 points, centre point and FaSSIF.

Media	Bile Salt (mM)	Phospholipid (mM)	FFA (mM)	Cholesterol (mM)	pH	Total Amphiphile Concentration (mM) × pH
1	1.06	0.16	1.04	0.01	6.64	15.07
2	11.45	2.48	2.88	0.38	7.12	122.4
3	3.56	1.18	1.04	0.06	5.72	33.40
4	3.4	0.33	2.88	0.09	8.04	53.87
5	3.35	0.31	0.87	0.17	6.62	31.11
6	3.62	1.25	3.43	0.03	7.14	59.48
7	2.27	0.96	1.01	0.08	7.34	31.71
8	5.33	0.4	2.96	0.07	6.42	56.24
centre point (9)	3.46	0.52	1.64	0.032	6.54	36.96
FaSSIFv1	3	0.75	1.64	–	6.5	35.04

Values from [34].

Table 3
HPLC conditions.

Drug	Injection volume (μL)	Wave-length (nm)	Retention time (min)
Naproxen	10	254	1.6
Indomethacin	10	254	2.1
Phenytoin	20	254	1.1
Piroxicam	20	254	1.07
Mefenamic acid	10	291	2.3
Furosemide	10	291	2.5
Ibuprofen	100	254	2
Zafirlukast	25	254	2.6
Aprepitant	50	254	2.27
Carvedilol	10	254	1.6
Tadalafil	50	291	1.4
Dipyridamole	10	291	2.5
Posaconazole	10	254	1.9
Atazanavir	10	254	1.7
Fenofibrate	10	291	3
Felodipine	10	254	2.4
Griseofulvin	10	291	1.5
Probucol	100	220	4.9
Paracetamol	10	254	1.07
Acyclovir	10	254	1.52
Carbamazepine	10	291	1.9

drug, this process was repeated three times and the average value was used.

2.2.3. HPLC analysis

Analysis was performed on a Shimadzu Prominence-i LC-2030C HPLC system using a gradient method for all the drugs except probucol. Gradient Mobile phases A 10 mM ammonium formate pH 3 (adjusted with formic acid) in water, and mobile phase B 10 mM ammonium formate pH 3 (adjusted with formic acid) in acetonitrile:water (9:1) flow rate 1 mL/min (except for acyclovir and carbamazepine 0.5 mL/min, and carvedilol 0.7 mL/min), time start 70:30 (A:B), 3 min 0:100, 4 min 0:100, 4.5 min 70:30, total run time 8 min. For probucol an isocratic method was used [25] mobile phase ACN, MeOH, and water 45:45:10. The following columns were used (all at 30 °C): Speck and Burke, ODS-H optimal 5 μm (30 × 150 mm) for acyclovir, furosemide, probucol, and dipyridamole, Kromasil 60–5-SIL (3 mm, 15 cm) for paracetamol, and

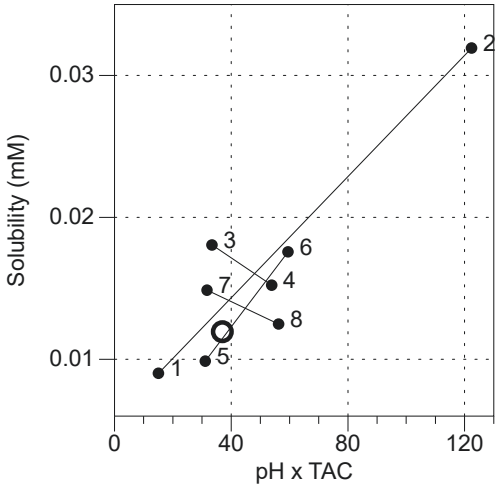


Fig. 1. Representative plot of Solubility vs pH × TAC. Legend: Point label indicates media number (see Table 2). Media 1 and 2 are based on the major axis of the multi-dimensional ellipse that described the FaHIF data cloud [34] and 3 and 4 on the minor axis. Media points 5 and 6 and 7 and 8 are based on further major and minor axes in other dimensions. The statistical analysis means that media are arranged in approximately two columns of three, based on pH × TAC values (3, 5 and 7 and 4, 6 and 8).

the rest of drugs were analysed by Xbridge® C18 5 μm (2.1 × 50 mm), the retention time, detection wavelength and injection volume for each drug are provided in Table 3. For each drug, a concentration curve was prepared using five or six standards that bracketed all the measurement concentrations. For all drugs, the correlation coefficient of the calibration curve was >0.99.

2.2.4. Data analysis

Data analysis and comparison was conducted using Graphpad Prism 9 for MacOSX.

3. Results and discussion

3.1. Solubility analysis

The multi-dimensional analysis of FaHIF composition included five factors pH, bile salt, phospholipid, free fatty acid and cholesterol [34] and in order to plot solubility data on x-y co-ordinates each media recipe has been reduced to a single value by either by calculating the total amphiphile concentration (mM) multiplied by the media pH value (Table 2) or using pH alone. The rationale for the former unusual data manipulation is based on three behaviour properties of these media systems. Previous studies have used the total amphiphile concentration (TAC) to correlate solubility, either individually [32], or in combination [23]. The fasted DoE study [25] indicated that for basic and neutral drugs the media components' (pH, free fatty acid, bile salt and phospholipid) standardised effect values on solubility were similar and that the majority of significant two way interactions involved pH along with an ionisable amphiphile. Finally, a topographical analysis of solubilisation in simulated fluids that employed a four component (bile salt, phospholipid, free fatty acid and monoglyceride) mixture design with varying pH and TAC [20] noted that solubility generally increased as both TAC and pH increased. The pH X TAC manipulation is not completely applicable to acidic drugs where in the fasted DoE the standardised effect value for pH dominated solubility behaviour by a factor of twenty fold greater than any of the amphiphilic media factors. Therefore for acidic drugs a plot using media pH only is presented for the more soluble drugs and comparison (pH and pH × TAC) plots for poorly soluble.

A representative plot of solubility against pH × TAC is presented in Fig. 1, which illustrates the data structure of the media compositions induced by the multidimensional analysis. Media 1 and 2 are based on the major axis of the multi-dimensional ellipse that described the FaHIF data cloud [34] and 3 and 4 on the minor axis. Media points 5 and 6 and 7 and 8 are based on further major and minor axes in other dimensions and the eight points cover 96.4% of the variability in the HIF samples. The statistical analysis means that media are arranged in approximately two columns of three, based on pH × TAC values (3, 5 and 7 and 4, 6 and 8) with the solubility measured in each media dependent upon the drug under investigation.

3.2. Acidic drugs

3.2.1. Solubility behaviour

The solubility plots for the acidic drugs are presented in Fig. 2a and b. Visual analysis indicates that there is a relationship of increasing solubility with media pH and the drugs generally have a very similar behaviour with variation linked in the main to pKa but with minor influences from other amphiphilic media components. For example the solubility ranking of media 2, 6 and 7 is not identical for all the drugs even although the pH values (7.12, 7.14 and 7.34 respectively) are very similar. For the majority of the drugs the pKa value (Table 1) is below the pH of the lowest media (Table 2) and therefore the measurement is of ionised drug solubility in the media. For piroxicam the pKa value is within the media range and for phenytoin the pKa is greater than the highest media pH value. This pH dependent solubility behaviour is

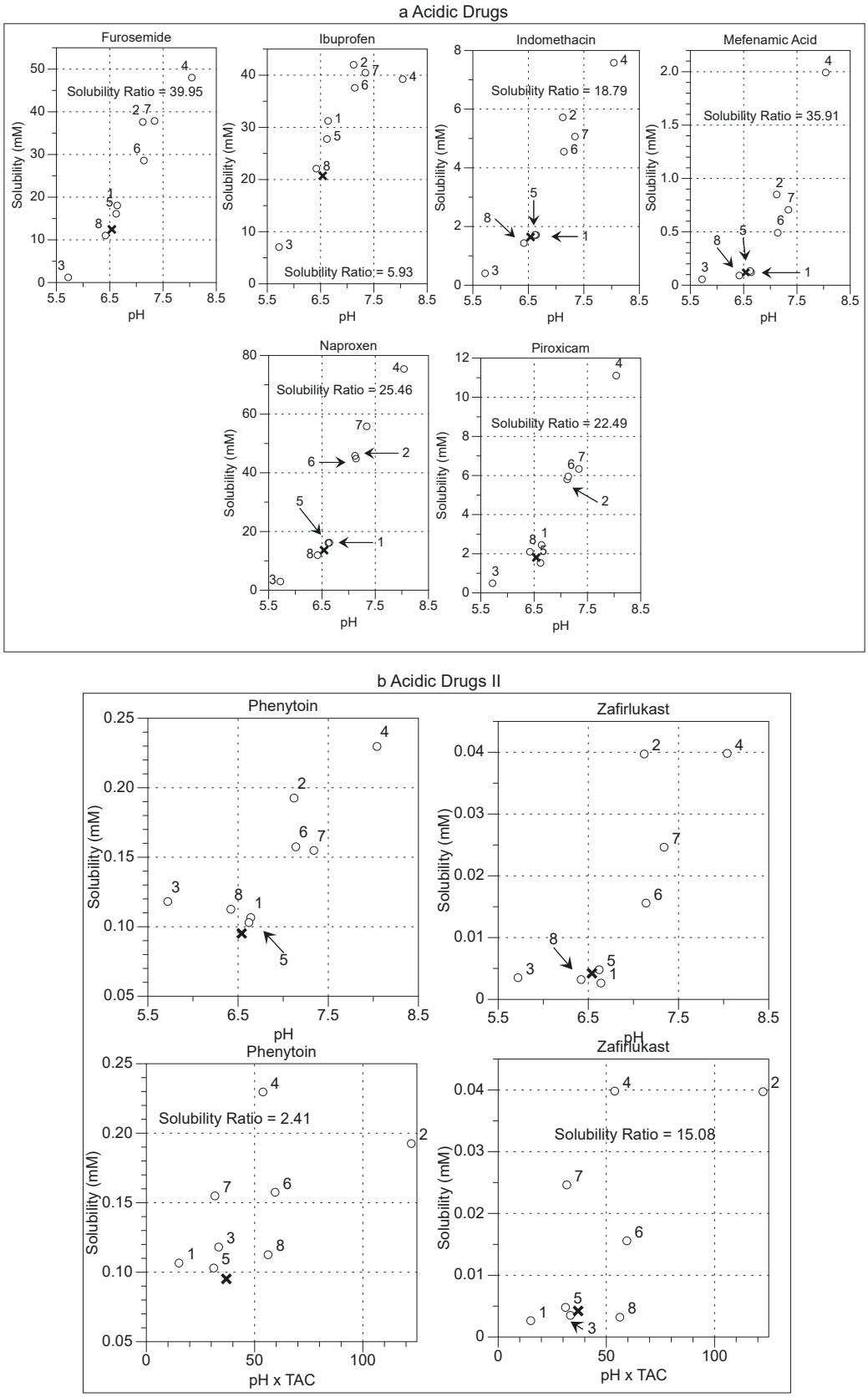


Fig. 2. a and b. Acidic Drugs – Solubility Plot Legend: Point label indicates media number (see Table 2); x centre point - media 9. Calculated solubility ratio (highest solubility/lowest solubility) value in text.

Table 4
Biorelevant fasted simulated intestinal fluids – solubility behaviours.

Category	1 pH controlled TAC minimal variation	2 pH × TAC controlled	3 Minimal pH × TAC control	4 Non-categorised solubility behaviour Drug controlled
Solubility Behaviour	Solubility increases with increasing pH, minimal impact from amphiphilic media components	Gross solubility increases with increasing pH and total amphiphile content, solubility granularity controlled by individual drug interactions with media components	Minimal impact of media components on solubility	No gross solubility relationship between pH and total amphiphile content, drug dependent behaviour, increasing pH and total amphiphile content might reduce solubility
Description	Acidic drugs pKa < 6.3[A]	Basic, neutral drugs, and weak acidic drugs pKa > 8[B]	Neutral drugs[C]	Basic and neutral drugs – categorisation based on solubility behaviour
Drugs	Naproxen, piroxicam, indomethacin, zafirlukast, ibuprofen, mefenamic acid, furosemide	Aprepitant, carvedilol, tadalafil, dipyridamole, posaconazole, fenofibrate, felodipine, griseofulvin, phenytoin	Paracetamol, carbamazepine, acyclovir, griseofulvin[D], phenytoin[D]	Probucol, atazanavir
Comment	Five out of seven examples from non-steroidal anti-inflammatory therapeutic category, expansion into other therapeutic modalities required	Examples varied physicochemical properties, increased drug examples required	Increased drug examples required	Insufficient data for conclusive analysis, increased drug examples required
Lowest Solubility Media[E] Number and Frequency	3 71% 5 out of 7 examples	1 89% 8 out of 9 examples	1 40% 2 out of 5 examples	Not assigned
Highest Solubility Media[E] Number and Frequency	4 86% 6 out of 7 examples	2 78% 7 out of 9 examples	2 80% 4 out of 5 examples	Not assigned
Mean Solubility Ratio (Highest/Lowest) ± Standard Deviation/Ratio Range	23.4 ± 11.8/34 (n = 7)	7.34 ± 5.96/17.37 (n = 9)	1.74 ± 0.596/1.26 (n = 5)	–

TAC Total Amphiphile Concentration; A: Based on highest pKa of acidic drugs measured – piroxicam; B: Based on the single example of phenytoin.
C: Category could include acidic and basic drugs that have pKa values outside of the media pH ranges – see drugs section; D: Added to category based on solubility ratio < 3 – phenytoin and griseofulvin therefore in category 2 and 4; E: Values not equal to Fig. 4 or 9, consult drugs list for values included in each category.

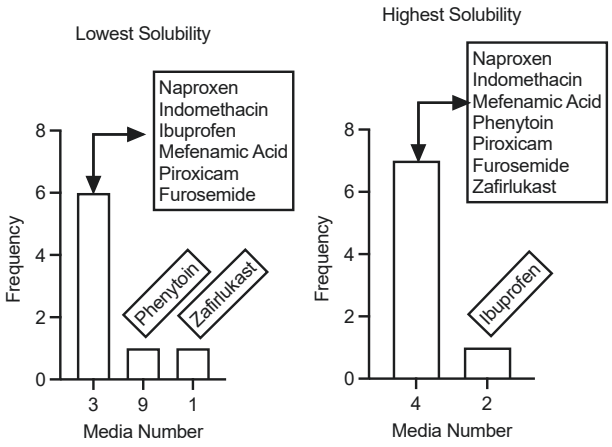


Fig. 3. Acidic Drugs – Lowest and Highest Solubility Media Frequency. Legend: Frequency of lowest and highest solubility media for drugs in Fig. 2a and b. Drugs as listed in boxes.

described as Category 1 in Table 4. The solubility of phenytoin and zafirlukast in Fig. 2b is also presented based on the media pH × TAC value, since for both these drugs media 2 the highest pH × TAC value is providing a solubility greater than expected based simply on pH. This indicates that for these two drugs solubilisation by the amphiphilic components is important, see Catergory 2 in Table 4.

3.2.2. Solubility behaviour analysis

This solubility behaviour is consistent with previous literature for acidic drugs in intestinal media [12] and with the various DoE studies [3,25,30,33] in fasted simulated media that identify pH as the major media component driving solubility. The initial DoE study [25] reported that for acidic drugs the average standardised effect value of pH on solubility was twenty times greater than any of the individual amphiphilic media components an observation that is replicated by the behaviour in this study with these different simulated media recipes. The DoE also identified two way interactions between media components [25], however for acidic drugs the largest interaction was between pH and free fatty acid, but with a magnitude around a tenth of pH alone. Therefore, variations in concentrations and interactions between the amphiphilic media components are not interfering with the major solubility driver pH. The initial data analysis performed by applying a DoE structure to the media did detect pH as a significant factor for naproxen indomethacin and piroxicam [2], but with only nine data points the significance of other factors is, due to statistical limitations, not detected [30].

Mefenamic acid, furosemide and ibuprofen have not been assessed in the large sized DoE protocol, therefore it is not possible to analyse their solubility behaviour with respect to known standardised effect values for the media components. However, the consistent distribution of the points in Fig. 2a indicate that these drugs are behaving in a similar manner to the drugs that have been subjected to a DoE investigation. The point distribution may therefore represent a useful tool for determining major features of drug solubility behaviour without conducting a DoE.

3.2.3. Media frequency analysis

In Fig. 3 the frequency of each media recipe as either the highest or lowest solubility value for an acidic drug is presented. In 7 out of 8 cases (87%) media number 4 provides the highest solubility value and is not surprisingly the media with the highest pH value (Table 2). The one exception is ibuprofen, where media 2 provides the highest solubility, examination of the data indicates that there is minimal solubility difference (media 2 = 42 mM, media 4 = 39 mM) between the media and for ibuprofen media numbers 7, 4, 6 and 2 are very similar. In 6 out of 8 cases (75%) media number 3 provides the lowest solubility value and again this is not surprising since this media has the lowest pH value. The

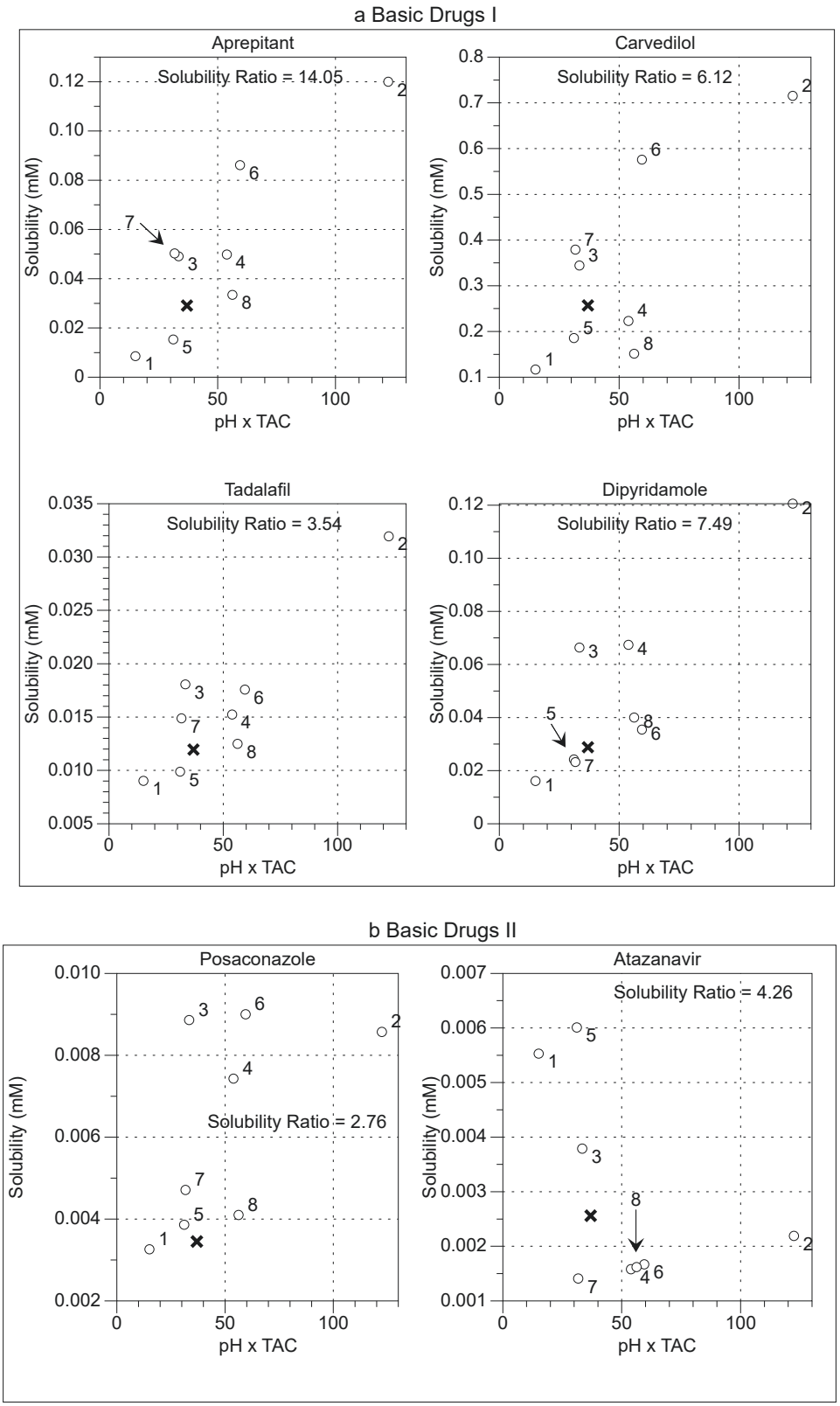


Fig. 4. a and b. Basic Drugs – Solubility Plot Legend: Point label indicates media number (see Table 2); x centre point - media 9. Calculated solubility ratio (highest solubility/lowest solubility) value in text.

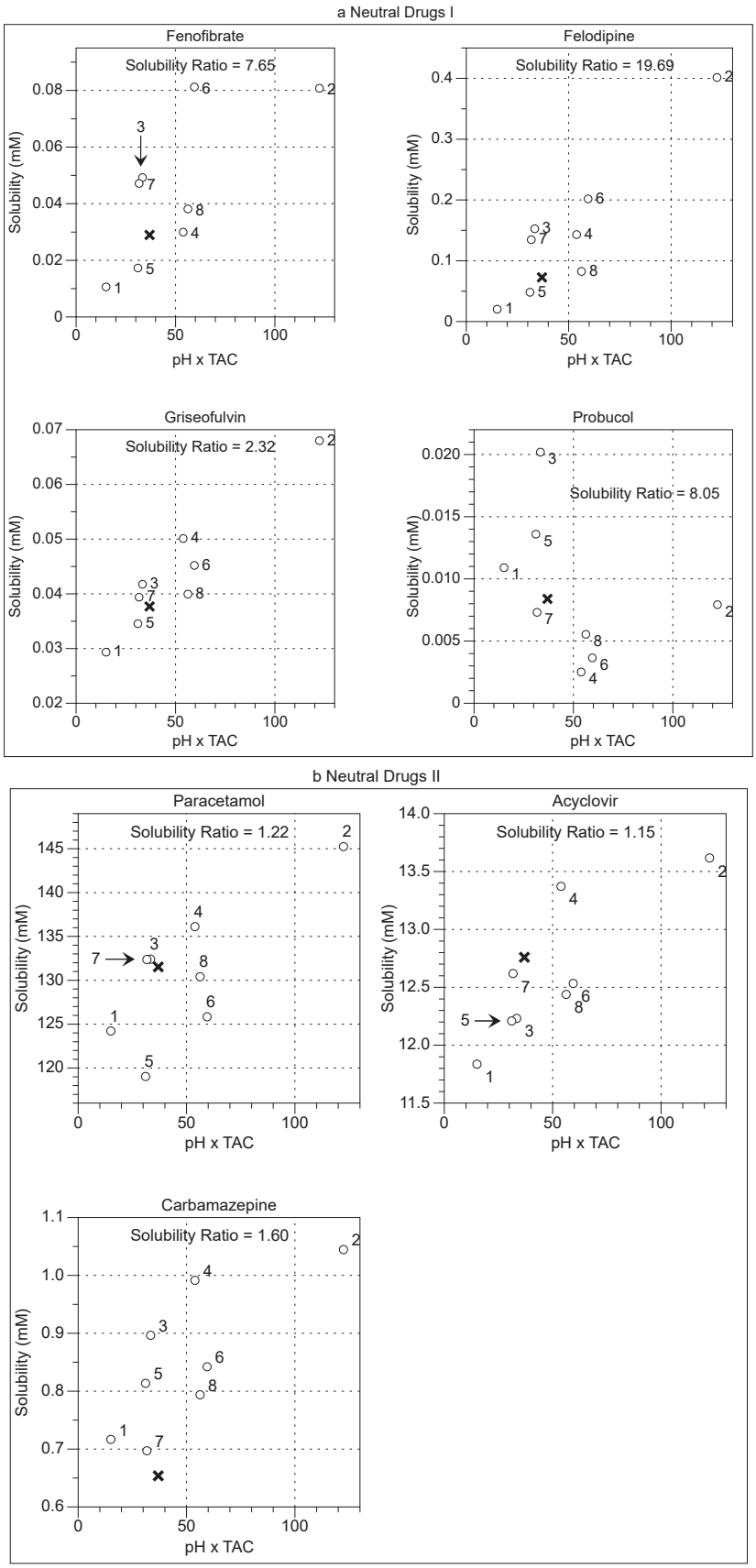


Fig. 5. a and b. Neutral Drugs – Solubility Plot. Legend: Point label indicates media number (see Table 2); x centre point - media 9. Calculated solubility ratio (highest solubility/lowest solubility) value in text.

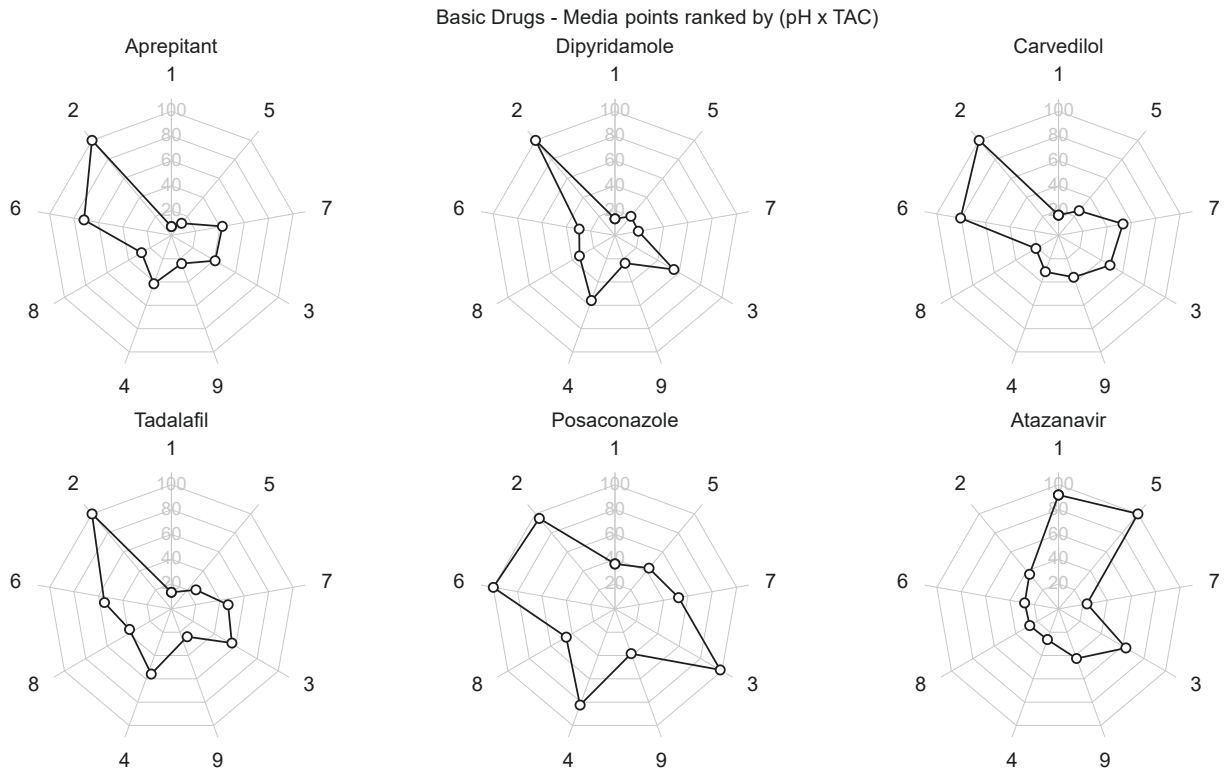


Fig. 6. Basic Drugs – Spider Plot Legend:Highest solubility value normalised to 100; point label indicates media number (see Table 2) arranged in a clockwise order of increasing pH × TAC – lowest pH at 12o'clock.

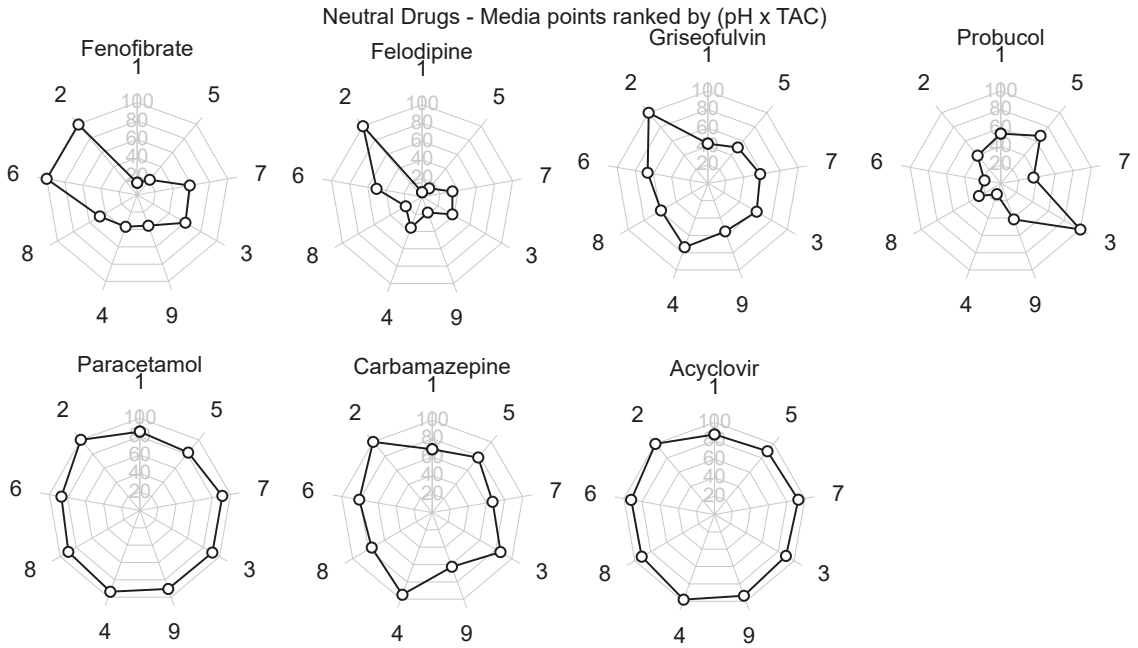


Fig. 7. Neutral Drugs – Spider Plot Legend: Highest solubility value normalised to 100; point label indicates media number (see Table 2) arranged in a clockwise order of increasing pH × TAC – lowest pH at 12o'clock.

exceptions are phenytoin and zafirlukast, where the lowest solubility media is number 9 (centre point) and 1 respectively. For phenytoin due to pKa this represents the interaction of the un-ionised molecule with the media components and overall is an unusual result since media 9 or the centre point usually is located within the point cloud, see other figures. However, phenytoin is a class of drug that has a very narrow solubility distribution (solubility ratio = highest solubility/lowest solubility) in FaSSIF systems [2,25] and the solubility values for media systems 1, 3, 5 and 8 are very similar, see category 3 Table 4. Zafirlukast is acidic but is known to be very poorly soluble in aqueous systems [15] therefore the lowest solubility in media 1 even although it's pH is higher than the pKa (pH 6.64 vs pKa 4.94) can be rationalised due to the low pH × TAC value

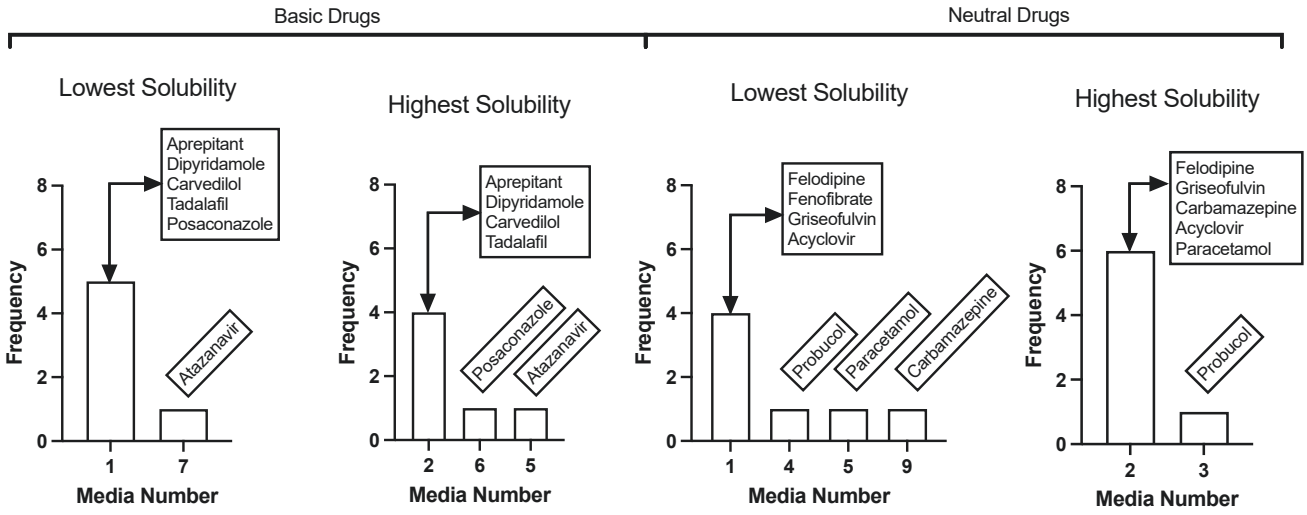


Fig. 8. Basic and Neutral Drugs – Lowest and Highest Solubility Media Frequency. Legend:Frequency of lowest and highest solubility media for drugs in Fig. 4a, b, 5a and 5b. Drugs as listed in boxes.

of the media, see next section. In a similar manner to phenytoin for zafirlukast the solubility in media systems 1, 3, 5 and 8 are very similar, therefore even if the lowest pH value media (number 3) was applied a low solubility value would be determined.

3.3. Basic and neutral drugs

3.3.1. Solubility behaviour

The solubility plots for the basic drugs are presented in Fig. 4a and b with the neutral drugs in Fig. 5a and b. Visual analysis indicates that there is a general structure with the lowest solubility measured in media 1, the highest in media 2 and spatial arrangement of the intermediate media similar although unlike the acids, this is not identical or consistent between drugs. There are also noticeable exceptions in probucol and atazanavir. The data has been transformed into a spider or polar plot in Figs. 6 and 7, where solubility is normalised to the highest value (set to 100) and arranged in a clockwise order around the plot starting at 12o'clock with the lowest pH × TAC media value (media 1, see Table 2) and running to the highest (media 2). This also highlights further noticeable exceptions in addition to atazanavir and probucol, with paracetamol, carbamazepine and acyclovir displaying an almost circular polar plot. A universal solubility behaviour is not evident, but three categories can be identified.

For aprepitant, dipyridamole, carvedilol, tadalafil, posaconazole, fenofibrate, felodipine, and griseofulvin there is a general increase in solubility from media 1 around the plot to media 2, but the increase is not smooth and there are variations in the profile. This variation is evident in Figs. 4 and 5 where for example in Fig. 4a the solubility rank (highest to lowest) of media 4, 6 and 8 is for aprepitant, carvedilol and tadalafil 6, 4 then 8 but for dipyridamole is 4, 8 then 6. A similar variation analysis can be applied to posaconazole, fenofibrate, felodipine, griseofulvin, paracetamol, acyclovir and carbamazepine and the other media numbers 3, 5, and 7. This solubility behaviour pH × TAC dependent is described in Category 2 in Table 4.

The circular polar plots for paracetamol, carbamazepine and acyclovir indicate that there is minimal variation in solubility with changing media (see solubility range values in Fig. 5b compared to other similar Figs. 2a/b, 4a), a feature that has been previously highlighted for griseofulvin, phenytoin and tadalafil [2]. This behaviour was also evident in the original DoE [25] and a comparison drugs (see Figs. 2a, 4a and 5a) also indicates a small solubility range is measured. As reported [1,2] the identification of this solubility property is a feature of measurement using multiple media and there is no direct literature comparison available. However, the reported standard deviation for fenofibrate solubility in multiple pooled FaHIF samples is one hundred and thirty two percent [11] whilst for griseofulvin it is twenty nine percent [5]. An arbitrary ratio cut off at phenytoin (solubility ratio 2.41) has been applied and this solubility behaviour, which is a subset of category 2, with a low solubility ratio is described in Category 3 in Table 4. This subset categorisation might be excessive, since it is based on a limited number of examples.

For probucol media 1 has a higher solubility than media 2 and the polar plot shape is unique amongst the neutral drugs with a flat top (between media 1 and 5) and a point out to media 3. Atazanavir has a very similar shape and although it is basic, has a pKa value (4.7) below the pH of the lowest pH media and therefore the solubility measurements are on the neutral molecule. This behaviour is counter intuitive, probucol has the highest log P (Table 1) and solubility would be expected to increase with TAC, see next section. The solubility is therefore not linked to pH × TAC and is described in Category 4 in Table 4. With only two examples, further research and examples are required.

3.3.2. Solubility behaviour analysis

For basic and neutral drugs, the average standardised effect values from the original DoE studies for pH, free fatty acid, bile salt and phospholipid[1] were equal indicating that these media components all impact solubility. For the basic drugs (tadalafil, carvedilol and aprepitant) the standardised effect fingerprint is variable, pH and free fatty acid were generally not as significant as bile salt and phospholipid with only aprepitant displaying a positive value for the four factors. For fenofibrate, felodipine, and griseofulvin the standardised effect values for pH, free fatty acid, bile salt and phospholipid were positive [25]. There is therefore a generally increasing solubility with increasing pH × TAC. The variation of standardised effect value for media components for each drug is the reason behind the individual drug changes in media number solubility behaviour discussed above, within the background of an overall solubility increase related to increasing pH × TAC [20]. There will therefore be a gross solubility trend of increasing solubility with increasing pH × TAC for the media, hence why point 1 universally has a lower solubility than point 2, atazanavir and probucol excepted. This average behaviour will be modified by each drug's individual

[1] [1] NB Cholesterol was not examined as a media factor in the original DoE.

fingerprint of standardised effect values, hence the inconsistent solubility ranks previously mentioned. This behaviour is consistent with the original DoE [25] and topographical analysis [20]. The original DoE also identified for basic and neutral drugs two way interactions between media components were equivalent contributors to solubility as the components acting alone. For example, for both drug categories pH with free fatty acid was the third and bile salt with free fatty acid the sixth most significant solubility drivers along with free fatty acid, pH, bile salt and phospholipid. These interactions will influence the analysis presented above and highlight that the media (Table 2) due to the method of calculation are not optimised for a DoE, therefore only gross single component effects can be determined [2]. Dipyridamole and posaconazole, have not been measured in any of the fasted DoE protocols, therefore analysis with respect to media component standardised effect values is not possible. However, dipyridamole and posaconazole have a similar shape to the other basic drugs (aprepitant, carvedilol and tadalafil) and therefore indicates that these drugs are behaving in a comparable manner to the DoE drugs.

The behaviour of paracetamol, acyclovir and carbamazepine with essentially circular polar plots represent a category that has been previously recognised for phenytoin and griseofulvin and for these drugs media variation has very limited solubility impact. Paracetamol, acyclovir and carbamazepine have not been measured in the fasted DoE, and with only 5 examples it is difficult to determine which parameters are involved in the property. However, it is interesting that these drugs have the simplest molecular structures amongst all the drugs in the study and are obviously different to probucol and atazanavir described below (Table 1). Possibly indicating that molecular structure somehow has to considered as a property over and above the total molecule physicochemical measurements of melting point, intrinsic solubility, pKa, and log P. A narrow solubility range might also be a useful solubility property to design into oral drug candidates [1] and would provide resistance to variation in human intestinal fluid composition.

For probucol in the DoE [25] bile salt and phospholipid had no significant standardised effect value on solubility and free fatty acid and pH were only just significant. This maybe the reason behind the unusual and paradoxical solubility behaviour (Fig. 5a or 7) with no correlation between solubility and pH × TAC, media 1 (lowest pH × TAC) has a higher solubility than media 2. This behaviour is also present for atazanavir, which as discussed will be behaving as a neutral molecule. With only two examples it is very difficult to rationalise this solubility behaviour, but it does indicate for these drugs a complex solubility behaviour across the fasted intestinal media space. It is also interesting that the solubility ratio for atazanavir is relatively low (4.26), which in a similar manner to category 3 is indicating that it is not interacting with the media components, but has a complex molecular structure. For poorly soluble drugs the use of micellar surfactant solutions as a surrogate for intestinal fluids has been suggested with bile salt one of the initial systems examined [9,31]. For a range of drugs solubility in D-α-tocopheryl polyethylene glycol 1000 succinate increased with increasing amphiphile concentration [11]. A recent paper has published a similar relationship with atazanavir [24] using sodium dodecyl sulphate. However, the result for atazanavir in Fig. 4b indicates that in the multi component bioequivalent media systems the relationship between solubility and TAC is not applicable. This is only a single example but potentially indicates that for some drugs use of single surfactant systems could provide misleading results, a situation that can only be discovered if a multiple FaSSIF media measurement is conducted.

3.3.3. Media frequency analysis

In Fig. 8 the frequency of each media recipe as either the highest or lowest solubility value for the basic and neutral drugs is presented. For the basic drugs media number 2 provides the highest solubility in 4 out of the 6 (66%) drugs analysed with posaconazole registering media number 6 and atazanavir number 5. With posaconazole the highest measured solubility in media 6 was very close to the value of media 2 (see Fig. 4b and 5a) indicating that the difference is minor. For the neutral drugs media number 2 provides the highest solubility in 6 out of 7 (86%) cases, with probucol registering media number 3. For the basic drugs the lowest solubility is measured in media number 1 in 5 out of 6 (83%) drugs analysed with atazanavir registering in media number 7. For the neutral drugs the lowest solubility occurs in 4 out of 7 cases (57%) in media 1 with probucol registering media 4, paracetamol media 5 and carbamazepine media 9. Due to the low solubility variability exhibited by paracetamol and carbamazepine the difference in solubility measurement between media 1 and the lowest solubility media will be low. Probucol and atazanavir are also two of the three drugs that do not register media number 1 for the lowest solubility and as discussed above exhibit solubility behaviour that is very different from the other drugs.

3.4. Solubility behaviour categorisation

The grouping of the drugs analysed in this study, as discussed above, is based on a simple classification around ionisation for comparability with previous studies [25]. Based on the results in this study the categorisation can be modified to reflect the drugs solubility behaviour in the bioequivalent fasted simulated intestinal media but also utilising the ionisation properties, see Table 4. This provides four categories of solubility behaviour, with two defined by physicochemical properties, a third as a subset based on behaviour in the bioequivalent fasted simulated intestinal media, with the final category based on solubility behaviour alone.

The first category is acidic drugs with a pKa value <6.3 (defined by the highest pKa in the sample set) where pH is the main solubility driver, resulting in a consistent pH dependent solubility behaviour and the lowest and highest solubility is measured in media number 3 and 4 respectively in over 70% of cases. This category also exhibits a high solubility ratio and range of solubilities, reflective of the impact of pH on ionisation and solubility. Further studies would be required to expand this set and refine the behaviour pattern, especially since the majority of examples are derived from the non-steroidal analgesic therapeutic category. The second category includes, weakly acidic drugs with a pKa value greater than 8, and basic and neutral drugs where the main solubility driver is a combination of pH and TAC resulting in a general trend of increasing solubility with increasing pH × TAC. The solubility behaviour in the bioequivalent fasted simulated intestinal media will be drug dependent but the lowest and highest solubility is measured in media number 1 and 2 respectively in almost 80% of cases. With only 9 examples it would be sensible to expand the data set as for category 1. Based on physicochemical principles an acidic drug with a pKa between 6.3 and 8 and with a low solubility (comparable to zafirlukast) would be likely to exhibit solubility behaviour that is associated with both categories.

Category 3 drugs have a very low solubility ratio, which is logical based on physicochemical properties and present on polar plots as an almost circular distribution. An arbitrary (based on phenytoin) solubility ratio of<3 has been applied for this category, which means that in this analysis it includes neutral and acidic drugs. Solubility measurement in media number 2 would identify the highest solubility in 80% of cases, but the lowest solubility if media 1 was applied would only be identified in 40% of cases. However, the low solubility ratio would assist in identification of the categorisation and the low range would indicate that the low value solubility error is likely to be small. In Category 4 drug solubility does not increase with pH and TAC and both drugs (probucol and atazanavir) in this category exhibit different solubility behaviour to all other measured drugs. With only two examples in this category it is not possible to fully define properties and the categorisation could be an artefact of the inclusion of these two drugs in the study. However, if media numbers 1 and 2 where applied to measure solubility the signature of lower solubility in media 2 than 1 would identify the behaviour.

3.5. Drug solubility and media component interactions

The solubility behaviour analysis above is predicated on the results from the original fasted design of experiment study [25] which applied a quarter fraction factorial design and required 66 experiments per drug. This measured a standardised effect value for the impact of each media component and two way interactions between components on solubility, although in the latter case some of these were conflicted due to the reduced (quarter fraction) design. The DoE focus was to understand the importance of the media components on drug solubility whereas the current study has applied bioequivalent media derived from a mathematical analysis of FaHIF samples to determine fasted intestinal fluid solubility limits. The two approaches are complementary, with the current (only three media required, see Conclusions) less resource intensive than the DoE. Simple statistical issues around sample numbers indicate that this low media number approach [30] will never provide the depth of information available from a fully structured DoE. During drug development a combination approach would be sensible, solubility screen with limited media numbers with exemplar candidates investigated by DoE, to link the statistical with the bioequivalent to guide development and reduce the possibility for solubility surprises.

4. Conclusions

This is the first study that has examined the solubility behaviour of a range of drugs in a structured set of bioequivalent fasted intestinal fluid media and no consistent solubility behaviour that covers all the drugs tested is evident [6,9]. However, the measured solubility behaviours can be categorised into two types using physicochemical properties and two further categories based on media solubility behaviour.

For acidic drugs (pKa $<$ 6.3) (Category 1, Table 4) equilibrium solubility is directly linked to media pH, an identical solubility behaviour is present for all drugs and the lowest and highest solubility can be determined in the lowest (media 3) and highest (media 4) pH media with a greater than 70% frequency.

For weakly acidic (pKa $>$ 8), basic and neutral drugs (Category 2, Table 4) equilibrium solubility is correlated to increasing media pH $\times$ TAC but the solubility behaviour is not consistent between drugs. The lowest and highest solubility can be determined in the lowest (media 1) and highest (media 2) pH $\times$ TAC media with a greater than 70% frequency. Category 3 is a subset of category 2 including neutral or drugs not ionised within the media pH range and characterised by solubility behaviour that is not sensitive to media composition, leading to a very narrow solubility range. The lowest and highest solubility can be determined in the lowest (media 1) with a 40% certainty and highest (media 2) with an 80% certainty. Any possible error in the low solubility measurement would be easily spotted by the narrow solubility ratio and likely to be minimal due to the narrow range.

The final category with only two drug examples is not well defined but would be detected if media numbers 1 and 2 were applied since the solubility in media 1 would be higher than 2, which is opposite to categories 2 and 3. This category requires further examples to fully define and for all categories increased example numbers and analysis would be prudent.

This indicates that if a drug can be categorised as an acid or weak acid, base or neutral then two simulated intestinal fluid media, either 3 and 4 (representing minimum and maximum pH values for for acidic drugs) or 1 and 2 (representing mininum and maximum pH $\times$ TAC values for weak acid, basic and neutral drugs) can be used to determine in vitro the fasted intestinal solubility range (minimum to maximum). The measurement can then be applied to refine the categorisation and relate solubility behaviour to previous DoE studied examples [25]. Coupled with a central point measure, three media can provide limits and information on the potential BCS or DCS classification and position with respect to the boundaries [1]. If three media are too onerous then the lowest solubility media can be applied as a worst case scenario. This fasted intestinal solubility measurement will provide more information than a single FaSSIF value and can be applied to assess the suitability during drug discovery [16] and development strategies [7] for oral administration.

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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