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Research paper

Structured solubility behaviour in fed simulated intestinal fluids

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A B S T R A C T

Intestinal drug solubility is a key parameter controlling absorption after the administration of a solid oral dosage form. The ability to measure fed state solubility in vitro is limited and multiple simulated intestinal fluid recipes have been developed but with no consensus which is optimal. This study has utilised nine bioequivalent simulated fed intestinal media recipes that cover over 90% of the compositional variability of sampled fed human intestinal fluid. The solubility of 24 drugs (Acidic; furosemide, ibuprofen, indomethacin, mefenamic acid, naproxen, phenytoin, piroxicam, valsartan, zafirlukast: Basic; aprepitant, atazanavir, bromocriptine, carvedilol, dipyridamole, posaconazole, tadalafil: Neutral; acyclovir, carbamazepine, felodipine, fenofibrate, griseofulvin, itraconazole, paracetamol, probucol) has been assessed to determine if structured solubility behaviour is present. The measured solubility behaviour can be split into four categories and is consistent with drug physicochemical properties and previous solubility studies. For acidic drugs (category 1) solubility is controlled by media pH and the lowest and highest pH media identify the lowest and highest solubility in 90% of cases. For weakly acidic, basic and neutral drugs (category 2) solubility is controlled by media pH and total amphiphile concentration (TAC), a consistent solubility pattern is evident with variation related to individual drug media component interactions. The lowest and highest pH × TAC media identify the lowest and highest solubility in 70% and 90% of cases respectively. Four drugs, which are non-ionised in the media systems (category 3), have been identified with a very narrow solubility range, indicating minimal impact of the simulated media on solubility. Three drugs exhibit solubility behaviour that is not consistent with the remainder (category 4). The results indicate that the use of two bioequivalent fed intestinal media from the original nine will identify in vitro the maximum and minimum solubility values for the majority of drugs and due to the media derivation this is probably applicable in vivo. When combined with a previous fasted study, this introduces interesting possibilities to measure a solubility range in vitro that can provide Quality by Design based decisions to rationalise drug and formulation development. Overall this indicates that the multi-dimensional media system is worthy of further investigation as in vitro tool to assess fed intestinal solubility.

1. Introduction

The most popular choice to administer medication is through the oral route, which enables patients to self-medicate and enhances patient compliance and tolerance of treatment [1]. For the pharmaceutical industry, this route has advantages since it allows the preparation of stable solid formulations that are cost effective. However, to achieve systemic therapeutic effects the drugs in oral formulations need to be absorbed from the gastrointestinal tract and enter the bloodstream [2,3]. Dissolution is therefore a crucial step in oral administration that can be influenced by the drug's physicochemical properties, formulation, gastrointestinal tract physiology and patient's food intake and clinical condition [2]. Since drugs cannot be absorbed in their solid form, dissolution is a vital step and solubility is known to play a significant role in this process [4]. The importance of solubility was highlighted in the Biopharmaceutics Classification System [5] and further refined in the Developability Classification System (DCS) [6–8] where intestinal solubility and permeability were linked to in vivo absorption.

Administering drugs with poor solubility may lead to incomplete and inconsistent drug absorption therefore, measuring in vivo intestinal solubility in vitro is a key stage in drug development [9,10]. Drug related factors such as pKa, logP, chemical structure and gastrointestinal factors such as tract physiology and anatomy along with patient related factors such as age, lifestyle and disease state, can affect intestinal solubility [9,11]. Therefore, simple aqueous and buffer solubility approaches may not always reflect the gastrointestinal solubility. To address this issue, two options are available. One involves measuring solubility in human intestinal fluid (HIF) samples [12–14]. The other uses simulated intestinal fluids (SIF) [10,15,16] to assess intestinal solubility in vitro and to simulate either the fasted (FaSSIF) or fed (FeSSIF) states.

When fed state simulated intestinal fluids (FeSSIF) were introduced [17], the aim was to simulate critical aspects of the gastrointestinal environment that were not considered when measuring solubility in aqueous buffer systems. The recipes were based on available HIF composition data and included important elements such as bile salts,

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lecithin and pH [18,19]. More complex recipes with free fatty acid, monoglyceride and enzyme components (FeSSIF-V2) were also developed and intended to help understand the intricate interactions of drugs in the GI tract especially after food consumption. Several in vitro - in vivo correlations are available [9,12,20,21], however, different FeSSIF recipes are applied without a consensus on which is optimal [22]. The fed conditions also present specific challenges that can hamper a comparison between studies. The type of meal (solid or liquid), its composition and calorie content, the amount of fluid ingested and the collection technique are among the factors that can affect comparisons [23,24]. The fed state is associated with large HIF variability that is not simulated with single FeSSIF media and approaches where only one solubility value is determined are not covering the full in vivo fed solubility range.

In recent statistical design of experiment studies (DoE) multiple combinations and concentrations (high and low) of FeHIF media components[1] were tested in order to study the solubility variability [25–28]. These approaches are great tools to study the key media components affecting solubility and the complex interactions between them, highlighting that intestinal solubility is a range. Although useful, their application to early drug development is limited by the heavy experimental resource required (the published fed DoE required 92 experiments per drug [25]) and their statistically constructed media recipes may not be biologically relevant. A subsequent study performed a multidimensional mathematical analysis of fasted and fed HIF composition [29] (pH, bile salts, phospholipid, free fatty acid, and cholesterol) obtained from twenty human volunteers [30]. This analysis resulted in eight media recipes for the fasted and fed states that statistically characterised over 95% of the HIF samples' component variation plus a calculated centre point through a Euclidean approach. This approach potentially generates solubility data with improved bioequivalence using fewer experiments and could be an alternative to current FeSSIF media for biopharmaceutical studies.

A recent paper [31] compared the equilibrium solubility in fed simulated intestinal media systems of a group of 13 drugs (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast, aprepitant, carvedilol, tadalafil, bromocriptine, fenofibrate, felodipine, probucol, itraconazole) using two approaches, either a multidimensional analysis [29] (9 media system) or DoE (92DoE [25], 10DoE [26], 9DoE [26]). Statistical differences between the data sets highlighted that larger scale DoE (92DoE) approaches generate FeSSIF compositions with excessive component concentration ranges and combinations not likely to be equivalent to FeHIF. The 9 media system recipes, which are derived from FeHIF compositions, are more likely to represent fed intestinal media than statistical DoE approaches and therefore could be considered to provide a bioequivalent solubility measurement. It should be noted that there is a limitation since the fed state in the original study [30] used to derive the fed 9 media system was obtained via the administration of the liquid feed Ensure Plus™ which is not equivalent to solid meals.

The equilibrium solubility of a further group of drugs (furosemide, dipyridamole, mefenamic acid, ibuprofen, griseofulvin, acyclovir and paracetamol) was measured using the multidimensional 9 media system (Table 2) and applied to the original Developability Classification System grid [32]. The inclusion of nine fed intestinal solubility values instead of the traditional single measurement approach (eg FeSSIF value) resulted in more information regarding the solubility behaviour of drugs, including the lowest solubility value that represents the worst case solubility scenario. This could be applied to risk assessment or Quality by Design (QbD) approaches in early development and formulation.

In this paper we have measured the equilibrium solubility of additional drugs piroxicam, carbamazepine, atazanavir and posaconazole (see Table 1). In combination with the equilibrium solubility values from previous studies (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast, aprepitant, carvedilol, tadalafil, bromocriptine, fenofibrate, felodipine, probucol, itraconazole) [31] (furosemide, dipyridamole, mefenamic acid, ibuprofen, griseofulvin, acyclovir and paracetamol) [32] our aim is to examine the solubility behaviour and determine patterns that can be applied to define drug categories. If present this would permit a reduction in the number of simulated intestinal media measurements required to establish a fed state solubility range. The determination of an in vitro maximum and minimum solubility would provide additional solubility information with less resource and could be applied in early drug development when API material is limited.

2. Materials and methods

2.1. Materials

Merck Chemicals Ltd supplied sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl), and formic acid. Lipoid® Germany supplied Lecithin S PC, which is phosphatidylcholine derived from Soybean with a purity of 98%. Rathburn Chemical® supplied chloroform, and Biorelevant.com Ltd supplied FeSSIF-v2 media. Fisher Scientific provided sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$). The active pharmaceutical ingredients carvedilol, tadalafil, valsartan, piroxicam, naproxen, griseofulvin, fenofibrate, bromocriptine, phenytoin, itraconazole, indomethacin, probucol, ibuprofen, furosemide, dipyridamole, carbamazepine, and acyclovir were purchased from Merck Chemicals Ltd. Aprepitant and felodipine were provided through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark and zafirlukast was purchased from Stratech Scientific Ltd. Paracetamol was obtained from Mallinckrodt Pharmaceuticals and mefenamic acid from Sigma Aldrich. Posaconazole and atazanavir were purchase from ChemShuttle. All active pharmaceutical ingredients were $>$ 98% pure based on certificates of analysis. The physicochemical properties of the drugs in this study are displayed in Table 1. The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

2.2. Methods

2.2.1. Stock media solutions for fed solubility experiments

To prepare stock solutions with 2.5 times higher concentrations than required for each of the 9 recipes in Table 2, the following method was used.

Main Stock Solution: For each media recipe the required amount of bile salt (sodium taurocholate) and phospholipid (soybean lecithin) was dissolved in 3 mL of chloroform, which was designated as Solution A. In a separate flask, the required amount of cholesterol was dissolved in 10 mL of chloroform, which was designated as Solution B. Then, 100 µl of Solution B was added to Solution A, stirred, and the chloroform was evaporated using a stream of nitrogen gas until a dry film formed.

The dry lipidic film was resuspended with water and then transferred to a 5 mL volumetric flask, and made up to volume with water. A stock solution of 120 mM sodium oleate in water was prepared using sonication and an elevated temperature to aid solubilization, and the solution was kept at 50 °C. Additionally, stock solutions of sodium phosphate monobasic monohydrate (28.4 mM) and sodium chloride (105.9 mM) were prepared in water.

2.2.2. Equilibrium solubility measurement

In a centrifuge tube (15 mL Corning® tubes), an excess amount of drug, exceeding its solubility limit, was weighed, followed by the addition of fed biorelevant media stock, buffer stock, salt stock, FFA stock, and water according to Table 3. The pH of each tube was adjusted to ± 0.02, using KOH or HCl if necessary, and shaken for an hour at

[1] pH is not a component per se however the terminology will be applied for simplicity.

Table 1
Physicochemical properties and molecular structures of drugs.

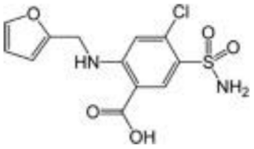
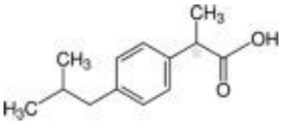
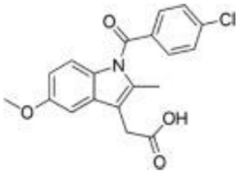
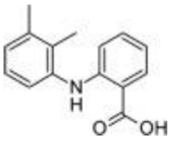
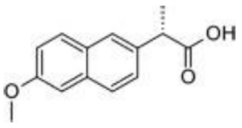
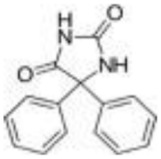
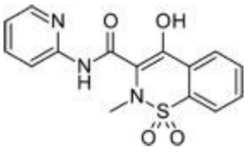
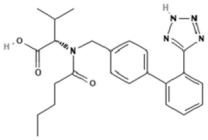
Acidic Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Furosemide	a	3.9	2.03	
Ibuprofen	a	5.3	3.97	
Indomethacin	a	4.5	4.27	
Mefenamic Acid	a	4.2	5.12	
Naproxen	a	4.15	3.18	
Phenytoin	a	8.33	2.47	
Piroxicam	a	6.3	3.06	
Valsartan	a	3.9	1.5	

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Table 1 (*continued*)

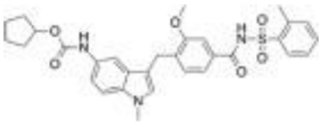
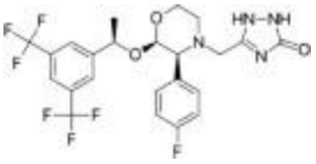
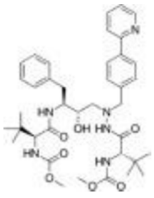
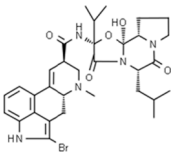
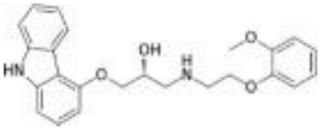
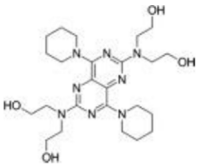
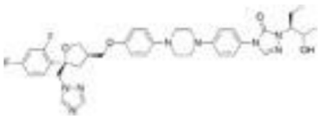
Acidic Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Zafirlukast	a	4.94	2.3	
Basic Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Aprepitant	b	9.7	4.5	
Atazanavir	b	4.7	5.9	
Bromocriptine	b	6.68	3.2	
Carvedilol	b	7.8	4.19	
Dipyridamole	b	6.2	3.77	
Posaconazole	b	3.6 & 4.6	4.6	

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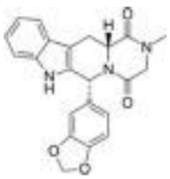
Table 1 (*continued*)

Acidic Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Tadalafil	b	3.5	1.7	

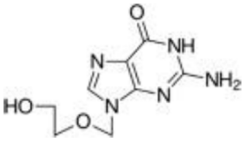
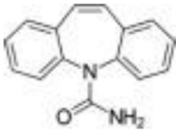
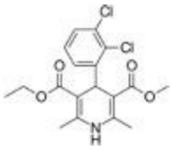
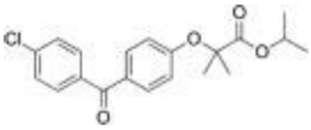
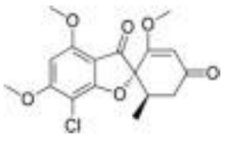
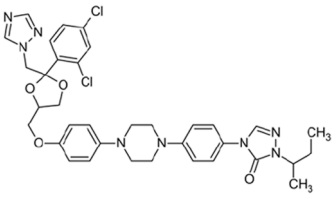
Neutral Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Acyclovir	n	2.52/9.35	−1.56	
Carbamazepine	n	–	2.45	
Felodipine	n	–	3.86	
Fenofibrate	n	–	5.2	
Griseofulvin	n	–	2.18	
Itraconazole	n	–	5.66	

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Table 1 (*continued*)

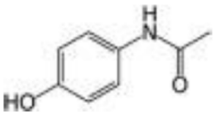
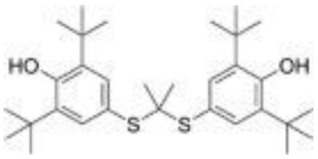
Acidic Drugs				
Compound	**a/b/n**	**pKa**	**Log P**	**Structure**
Paracetamol	n	–	0.46	
Probucol	n	–	11.3	

Table 2
Fed Media Compositions.

Media	Bile Salt (mM)	Phospholipid (mM)	Free Fatty Acid (mM)	Cholesterol (mM)	pH	pH × Total Amphiphile Concentration
1	4.94	2.02	10.5	0.95	5.97	109.91
2	19.04	7.94	47.51	0.34	6.59	493.13
3	5.65	2.43	18.06	0.1	6.13	160.85
4	16.65	6.59	27.63	3.45	6.42	348.73
5	15.66	5.1	10.92	0.5	6.24	200.80
6	6.00	3.14	45.68	0.65	6.32	350.57
7	7.34	6.17	21.82	0.57	5.97	214.32
8	12.81	2.6	22.85	0.58	6.59	255.96
9 (Centre)	10.94	4.02	23.38	0.32	6.26	242.03

Table 3
Fed Media Preparation.

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

room temperature. The pH was readjusted if needed. The tubes were then placed in an orbital shaker (Labinco L28 Orbital Shaker) and incubated for 24 h at 37 °C and 240 rpm.

Following the 24-hour incubation period, the contents of all tubes were inspected for the presence of solid drug. Then, 1 mL of each solution was transferred to 1.5 mL Eppendorf tubes and centrifuged at 10,000 rpm (RCF approx. 14,000) for 15 min using the Hettich Zentrifugen Mikro 20. The supernatant from each tube was analysed for drug content using HPLC. Three measurements were taken for each media point to ensure accuracy [25,31,33].

2.2.3. HPLC analysis

HPLC analysis was performed using a Shimadzu High Performance Liquid Chromatography Prominence-I LC-2030C system with the conditions outlined in Table 4. The HPLC method was previously validated to accurately quantify the concentration of the specified drug [27]. Six point calibration curves (lowest standard below lowest measured solubility, highest standard greater than highest measured solubility) were generated for each drug, and the equation of the line was applied to calculate the drug concentration.

2.2.4. Data analysis

Data analysis and comparison was conducted using GraphPad Prism 9 and DataGraph 5.0 for MacOSX.

3. Results and discussion

3.1. Solubility analysis

The 9 fed state simulated media recipes applied in this paper resulted from a multidimensional analysis of five FeHIF components (bile salts, cholesterol, lecithin, free fatty acid and pH). The impact of these components on solubility can be studied in combination using a DoE approach [25,27,28] or as the sum of all component concentrations [34] (TAC, total amphiphile concentration in mM). In this paper, to present the solubility data on an x-y coordinate system, each fed media recipe was simplified to a single value. This is achieved by either calculating the product of the total amphiphile concentration and media pH (Table 2) or using pH alone. A published fed DoE [25] studied the influence of media composition on solubility for acidic, basic, and neutral drugs. For acidic drugs, the average standardised effect of pH on solubility behaviour was more than three times larger when compared to the other amphiphilic media components. Whilst for basic and neutral drugs

Table 4
HPLC Method Detail.

Drug	Mobile Phase	Column	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
Aprepitant	Mobile Phase A: 10 mM Ammonium Formate pH 3 in H20	a	1	10	254	2.2
Tadalafil	Mobile Phase B: 10 mM Ammonium Formate in ACN:H20	a	1	10	291	1.5
Zafirlukast	(9:1 V/V)	a	1	10	254	2.5
Carvedilol		a	0.7	10	254	1.6
Phenytoin		a	1	10	254	1.0
Piroxicam		a	1	10	254	1.1
Indomethacin		a	1	10	254	2.0
Felodipine		a	1	10	254	2.6
Fenofibrate		a	1	10	291	3.2
Ibuprofen		a	1	10	254	2.1
Probucol		a	1	10	254	4.4
Valsartan		b	1	10	254	1.3
Itraconazole		b	1	10	254	2.6
Carbamazepine		a	0.7	10	291	1.4
Posaconazole		a	1	10	254	2.1
Atazanavir		a	1	10	254	1.9
Dipyridamole		b	1	10	291	1.6
Naproxen		a	1	10	254	1.5
Mefenamic Acid		b	1	10	291	1.7
Paracetamol		b	1	10	254	1.1
Acyclovir		b	0.5	10	254	2.2
Griseofulvin		a	1	10	291	1.5
Furosemide		b	1	10	254	1.1
Bromocriptine	Isocratic method ACN and 0.1% w/v acetic acid (50:50 v/v)	a	1	10	291	0.6

a- Column: XBridge C18 5 µm 2.1 × 50 mm, 30 °C.
b- Column: ACE 5 C18 150 × 3.0 mm, 30 °C.
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.

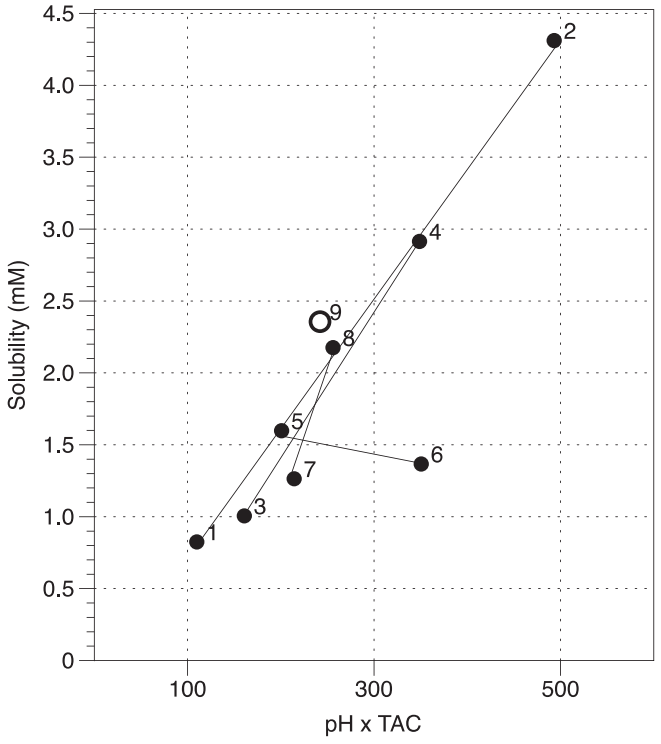


Fig. 1. Representative plot of solubility vs (pH × TAC). Point label indicates media number, see Table 2.

the average standardised effect values for pH and amphiphilic components were broadly equivalent with no single component dominant. In addition, a previous SIF study using a four-component mixture design [35] determined that as both pH and TAC increased a general increase in solubility is measured. Therefore, a plot of solubility vs media pH was applied to analyse acidic drugs. Whilst for basic and neutral drugs a plot of solubility versus pH × TAC was used. This latter analysis was also applied to acidic drugs in BCS Class II or IV with higher dose/solubility values.

A representative pH × TAC plot is presented in Fig. 1 that highlights how the media recipes were structured in pairs along the axes of an ellipse by the multidimensional analysis. Media 1 and 2 were based on the major axis of the multi-dimensional ellipse that characterised the FeHIF data cloud [29], while media points 3 and 4 were based on the minor axis. Media points 5 and 6, as well as 7 and 8, were calculated based on additional major and minor axes in other dimensions. The eight media points collectively account for > 95% of the compositional variability observed in the HIF samples for the analysed media components.

3.2. Acidic drugs

3.2.1. Solubility behaviour

Fig. 2 presents solubility plots for the acidic drugs. An easily spotted characteristic is that overall solubility increases with increasing media pH (Fig. 2a) and for the majority of drugs media 2 with the highest pH (6.59) presents the highest solubility value. The lowest solubility is measured in media 1 or 7 with the lowest pH value, both at 5.97. The pKa values for most drugs in this study (Table 1) are lower than the lowest media pH (Table 2), confirming that the solubility measured is controlled by the ionised form. This is reinforced by the fitting of a mono-exponential curve through the data and the generally high correlation coefficient for each drug (Fig. 2a). The exceptions are phenytoin (see Table 2 and Fig. 2b) with a pKa above the highest media pH value and piroxicam with a pKa higher than the lowest media pH but still within the media pH range. Although pH is clearly the driving force for solubility (Fig. 2a), there are minor variations in the solubility of points with close pH values, probably due to the influence of other media components present at high concentrations in the fed state. In some cases the solubility ranking of media 5, 6, and 9 varies despite having similar pH values (6.24, 6.32, and 6.26, respectively). Analogous

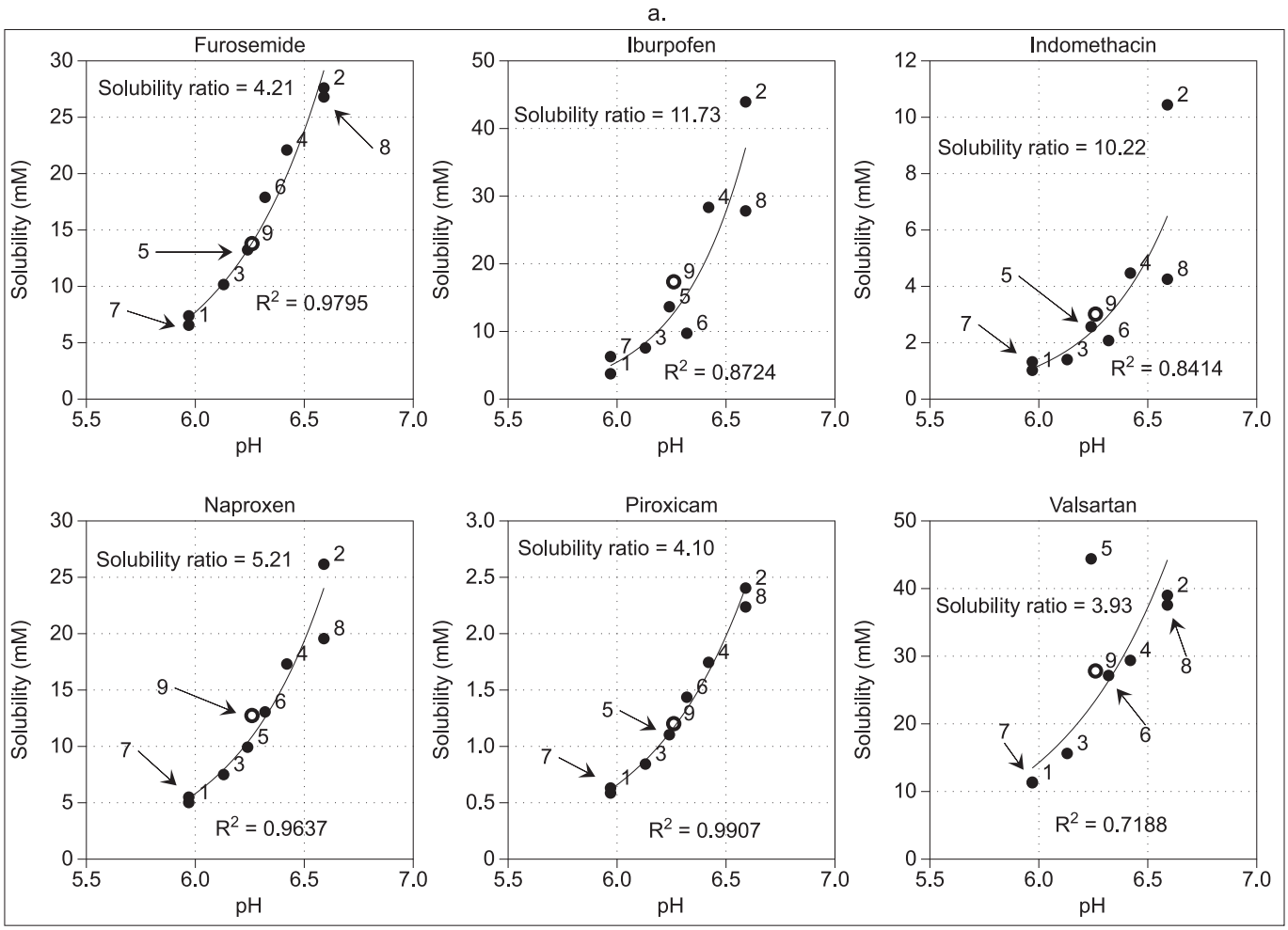


Fig. 2. (a) Acidic Drug Solubility vs Media pH. Point label indicates media number, see Table 2. Line mono-exponential best fit of solubility vs pH, solubility = $Ae^{k \times pH}$ where A = constant, k = power value and pH = media pH value. R^2 = correlation coefficient of fitted exponential. (b). Acidic Drug Solubility vs Media pH and Media pH × TAC. Point label indicates media number, see Table 2. pH figures (top graphs) Line mono-exponential best fit of solubility vs pH, solubility = $Ae^{k \times pH}$ where A = constant, k = power value and pH = media pH value. R^2 = correlation coefficient of fitted exponential.

behaviour is also noted in the ranking of media 2, 8 and 4 (6.59, 6.59, and 6.42, respectively) where for some drugs media 4 with slightly lower pH but highest TAC, is exhibiting higher solubility values than media 8. The lower correlation values calculated for ibuprofen, indomethacin and valsartan might be an interesting indicator of the influence of amphiphilic media content on their solubility, see next paragraph. The behaviour of valsartan is anomalous, since media 5 displays the highest solubility, indicating that amphiphilic component solubilisation is important for this drug, see next section. This pH solubility dependent behaviour is consistent with a published fed state DoE [25], similar to the fasted state [33,36] and for the purposes of this paper described as Category 1 in Table 5.

Fig. 2b presents the solubility plots for the acidic drugs with higher dose/solubility values (mefenamic acid, phenytoin and zafirlukast) in the DCS II or IV range. A comparison of the pH and pH × TAC plots (Fig. 2b) for these drugs indicates that amphiphile content and composition might be influencing the solubility behaviour indicated by a lower mono-exponential pH correlation coefficient for these drugs than those presented in Fig. 2a. This solubility behaviour is similar to the fasted state [33,36] and for the purposes of this paper has been described as Category 2 in Table 5.

3.2.2. Solubility behaviour analysis

The solubility behaviour in this study is in line with previous literature [37] and in fed DoE studies [25,27,28] reporting acidic drug solubility in FeSSIF. The most significant factor found to affect solubility was pH with a clear trend that solubility increased with media pH. Similar behaviour was registered for the fasted state [36] which is consistent with the fasted DoE study [33] that determined the impact of pH on solubility to be twenty times greater than any media amphiphilic component. The fed state DoE [25] found the impact of pH on acidic drugs to be dominant but less predominant than in the fasted state. Media pH still plays a major role in fed state, but oleate, bile salts and their interactions also play an important secondary role for certain acidic drugs [25]. This variation between fasted and fed states is probably related to the higher concentrations of amphiphilic components present in the fed state and the impact of these components might explain the variations in media ranking that were observed for some acidic drugs in this paper. In this study phenytoin and zafirlukast (Fig. 2b) were more affected by media amphiphilic components than other acid drugs with media 2 presenting higher solubility values than expected by pH alone. For these drugs the fed DoE [25] found that their solubility was positively affected by pH, oleate and lecithin which is consistent with this study's observations. Ibuprofen, indomethacin and valsartan also present a slight variation in the media ranking which is in line with the DoE analysis since their solubility was also found to be affected by pH and bile salts. Mefenamic acid and furosemide were not studied in the DoE study and their solubility cannot be analysed in a

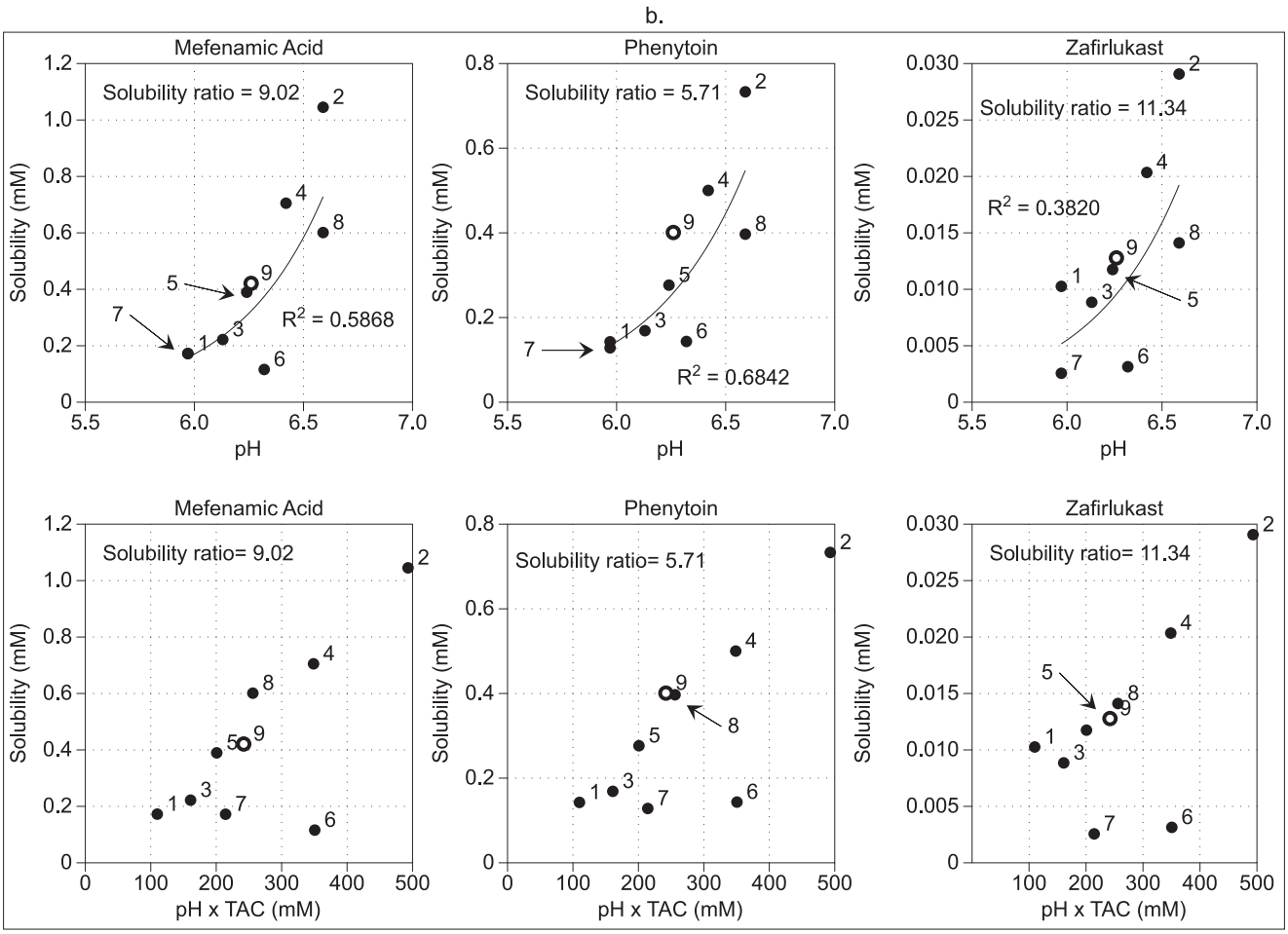


Fig. 2. (*continued*).

similar fashion. Overall, the dominant impact of pH on acidic drug solubility is evident and the exceptions regarding the impact of amphiphilic content on the drugs studied are consistent with the fed DoE [25]. Of note is that the average solubility ratio in this fed state study (Category 1 drugs Table 5: 7.28 ± 3.27, mean ± standard deviation, n = 9) is lower than the comparable fasted study (23.4 ± 11.8, n = 7) [36], reflecting the narrower media pH range in the fed (pH 5.97 – 6.59, Δ = 0.62) compared with the fasted state (pH 5.72 – 7.34, Δ = 1.62). If this effect is present in vivo then the pharmacokinetic variability for acidic drugs in the fasted state might be larger than in the fed state.

3.2.3. *Media frequency analysis*

Fig. 3 presents the frequency of each media recipe as the highest and lowest solubility value for acidic drugs. The highest solubility was provided by the media with highest pH, media 2 (Table 2), in 8 out of 9 drugs (89%). The only exception was valsartan where the highest solubility was measured in media 5. The pH difference between the media is not large (Table 2) and the solubility difference, (media 5 = 44 ± 0.74 mM and media 2 = 39 ± 1.6 mM: all values mean ± standard deviation n = 3) if compared using a non-parametric Mann Whitney test is not significant (P = 0.10), see below.

The lowest solubility in 5 out of 9 drugs (56%) was measured in media 1. For 3 (33%) drugs phenytoin (media 1 = 0.14 ± 0.0072 mM and media 7 = 0.13 ± 0.018 mM), piroxicam (media 1 = 0.63 ± 0.015 mM and media 7 = 0.59 ± 0.0095 mM) and zafirlukast (media 1 = 0.0010 ± 0.00051 mM and media 7 = 0.0026 ± 0.00039 mM) it was media 7. In one case mefenamic acid the lowest solubility is measured in media 6 (media 1 = 0.17 ± 0.0062 mM and media 6 = 0.12 ± 0.017 mM). As above a non-parametric Mann Whitney comparison of the solubility data sets for the four drugs is not significant (P = 0.10), see below. The lowest solubility media is predominated by the lowest pH media (media 1 56%) but other media (media 7 and 6) contribute 4 out of 9 (44%) results. These media have very similar pH values (1 = 5.97; 3 = 6.13; 6 = 6.32; 7 = 5.97) and the major difference between them is the total concentration of amphiphiles present (1 = 18.4 mM; 3 = 26.2 mM; 6 = 55.5 mM; 7 = 35.9 mM). This can be visualised in Fig. 2b with the pH × TAC plots where media 1, 3, 6 and 7 all have a low solubility. A previous study [35] noted that for indomethacin a high amphiphile concentration depressed solubility, albeit at a higher media pH of 7. The result noted in this study is similar and may indicate that high amphiphile concentrations can depress solubility for acidic drugs a behaviour that was not evident in the DoE study.

The statistical comparison of the data above does not detect any significant difference in the measured solubility values. This indicates that the use of media 1 to determine the lowest solubility is appropriate however, this result requires a cautious interpretation. In previous studies with larger data sets [26,27] SIF measured solubility values were not normally distributed and therefore a non-parametric statistical comparison was valid. Application of non-parametric analysis in this study to compare two individual media might not be appropriate, however with only 3 measurements calculation of the solubility distributions statistical properties is not feasible. Further examination of this issue is required to fully assess individual media solubility behaviour and comparison.

Table 5
Biorelevant Fed Simulated Intestinal Fluids - Solubility Behaviours.

Category	1 pH controlled TAC variation evident	2 pH & TAC controlled	3 Minimal pH & TAC control	4 pH & TAC & Drug controlled
Solubility Behaviour	Solubility increases with increasing pH, impact from amphiphilic media components at solubility extremes	Solubility increases with increasing pH and total amphiphile content, solubility behaviour controlled by individual drug interactions with media components	Minimal impact of media components on solubility	No evident solubility relationship between pH and total amphiphile content, drug dependent behaviour, increasing pH and total amphiphile content might reduce solubility
Description	Acidic drugs pKa < 8.33[A]	Basic and neutral drugs weak acidic drugs pKa > 8[B]	Neutral drugs[C] Solubility Ratio < 3	Basic and neutral drugs – categorisation based on solubility behaviour
Drugs	Furosemide, ibuprofen, indomethacin, mefenamic acid, naproxen, phenytoin, piroxicam, valsartan, zafirlukast	Aprepitant, carbamazepine, carvedilol, dipyridamole, felodipine, griseofulvin, itraconazole, phenytoin, posaconazole, tadalafil,	Acyclovir, atazanavir, fenofibrate, paracetamol	Atazanavir, bromocriptine, probucol
Comment	Five out of nine examples from non-steroidal anti-inflammatory therapeutic category, expansion into other therapeutic modalities required	Varied physicochemical properties, increased drug examples required	Increased drug examples required	Insufficient data for conclusive analysis, increased drug examples required
Lowest Solubility Media[D] Number and Frequency	1 or 7 (pH = 5.97) 89% 8 out of 9 examples	1 70% 7 out of 10 examples	1 25% 1 out of 4 examples	Not assigned
Highest Solubility Media[D] Number and Frequency	2 (pH = 6.59) 89% 8 out of 9 examples	2 90% 9 out of 10 examples	2 75% 3 out of 4 examples	Not assigned
Mean Solubility Ratio[E] (Highest/Lowest) ± Standard Deviation/ Ratio Range	7.28 ± 3.27/7.79 (n = 9)	5.78 ± 1.87/5.45 (n = 10)	1.76 ± 0.92/1.96 (n = 4)	6.43 ± 3.76/7.41 (n = 3)

TAC Total Amphiphile Concentration. A: Based on highest pKa of acidic drugs measured – phenytoin. 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. D: Values not equal to Fig. 4 or 9, consult drugs list for values included in each category. E: Calculated solubility ratio (highest solubility/lowest solubility).

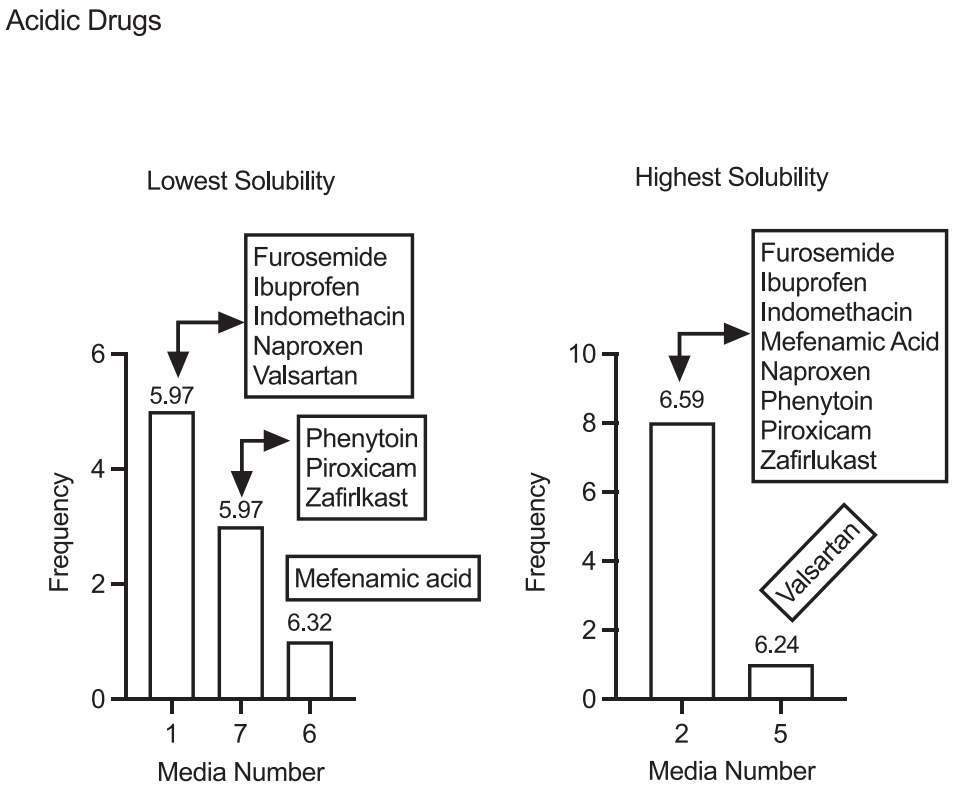
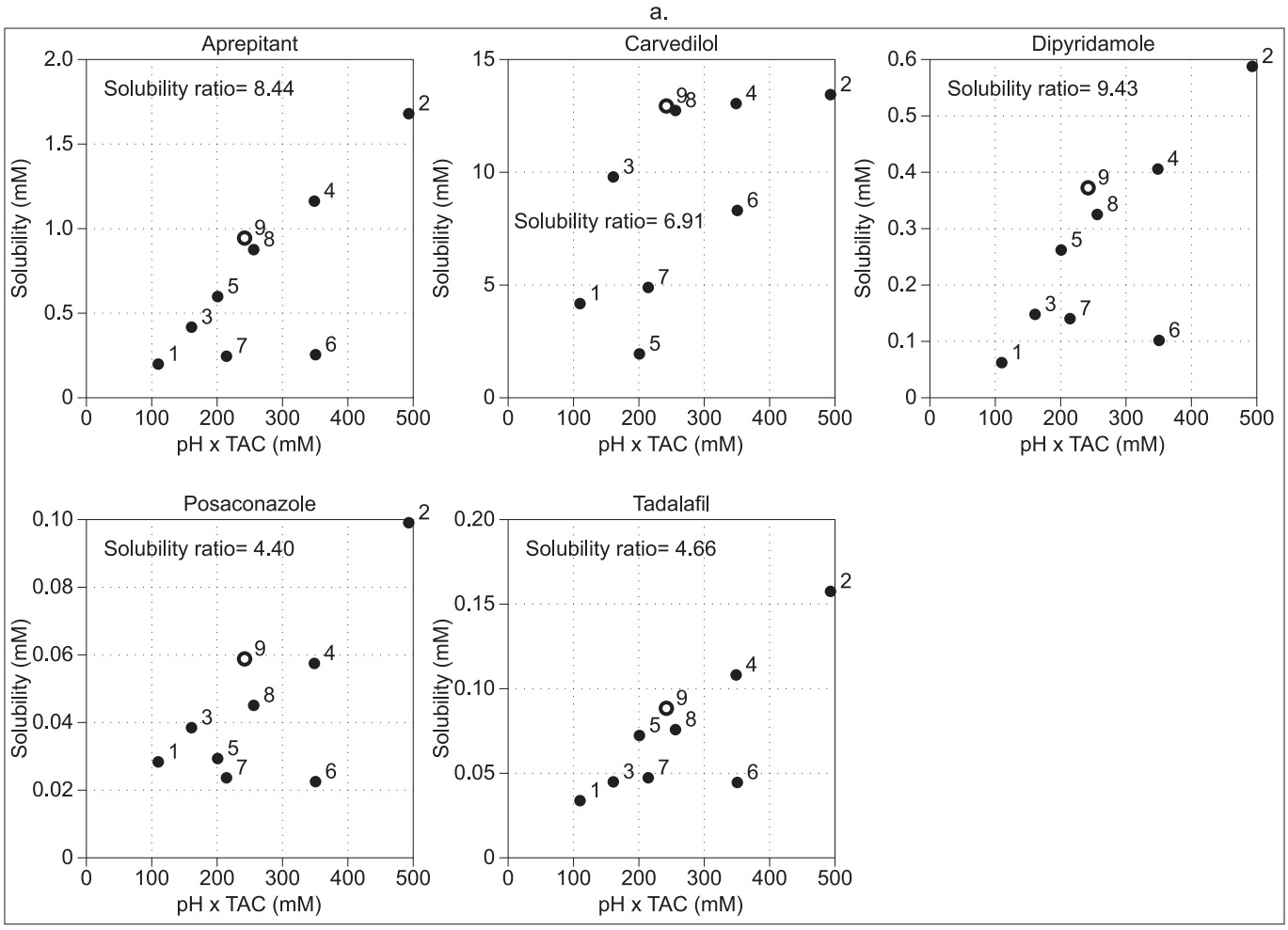


Fig. 3. Frequency of lowest and highest solubility media for acidic drugs. Drugs as listed in boxes, number on bar = media pH.



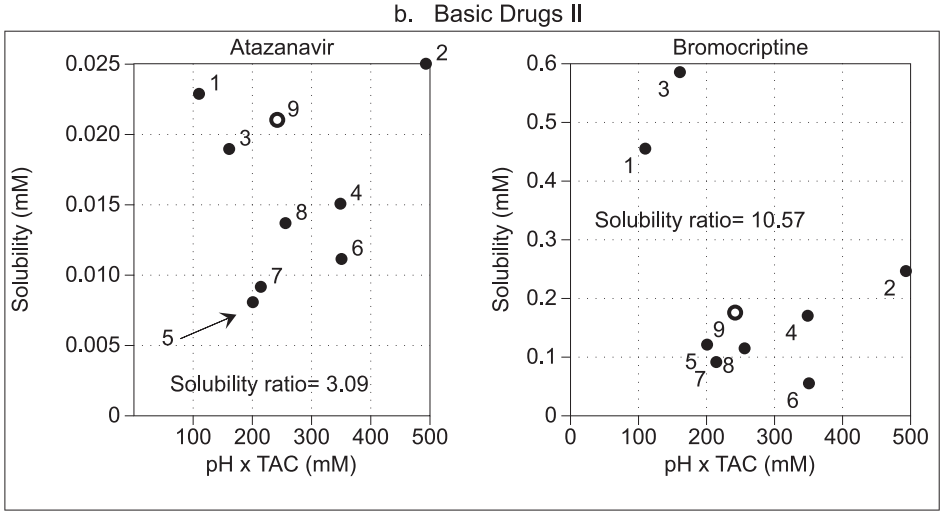


Fig. 4. (a). Basic Drug Solubility vs Media pH × TAC. Point label indicates media number, see Table 2. (b). Basic Drug Solubility vs Media pH × TAC. Point label indicates media number, see Table 2.

3.3. Basic and neutral drugs

3.3.1. Solubility behaviour

Fig. 4a and b and 5a and b, present the solubility plots for the basic and neutral drugs respectively. Figs. 6 and 7 re-present the data as a spider or polar plot where solubility values have been normalised to the highest value (set to 100) and arranged in a clockwise order starting at 12o'clock with the lowest pH × TAC media value (Table 2, media 1) and continuing to the highest value (media 2).

Based on a visual analysis of Fig. 4a and 5a, it can be observed that for these drugs (basic - aprepitant, carvedilol, dipyridamole, posaconazole, tadalafil; neutral - carbamazepine, felodipine, fenofibrate, griseofulvin, itraconazole, and paracetamol) there is a solubility pattern with media 1 generally providing the lowest solubility values and media

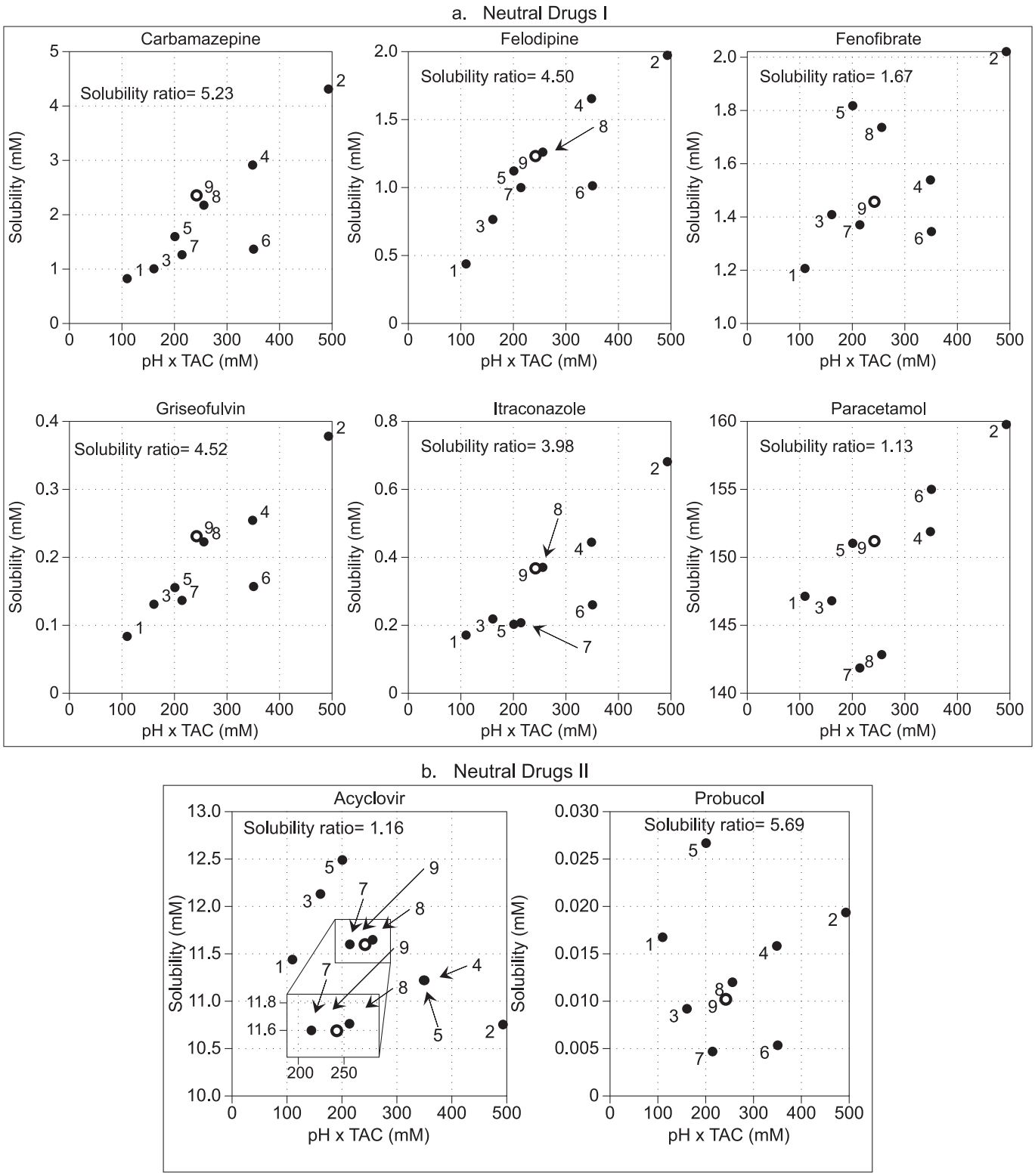


Fig. 5. (a). Neutral Drug Solubility vs Media pH × TAC. Point label indicates media number, see Table 2. (b). Neutral Drug Solubility vs Media pH × TAC. Point label indicates media number, see Table 2.

2 the highest. In some cases (basic - carvedilol, posaconazole; neutral - paracetamol) media 5, 6, or 7 provide the lowest solubility. The intermediate media have an increasing solubility and similar but not a consistent pattern across all drugs. Minor variations in the intermediate media ranking can be observed for the majority of drugs highlighting the influence of media composition on solubility [25,38]. In Fig. 4b and 5b the drugs do not exhibit this pattern and a complicated solubility behaviour is evident.

Analysis of the spider plots highlights that the majority of basic (aprepitant, carvedilol, dipyridamole, posaconazole and tadalafil) and neutral (carbamazepine, felodipine, fenofibrate, griseofulvin and itraconazole) drugs display a similar shape profile and increasing solubility

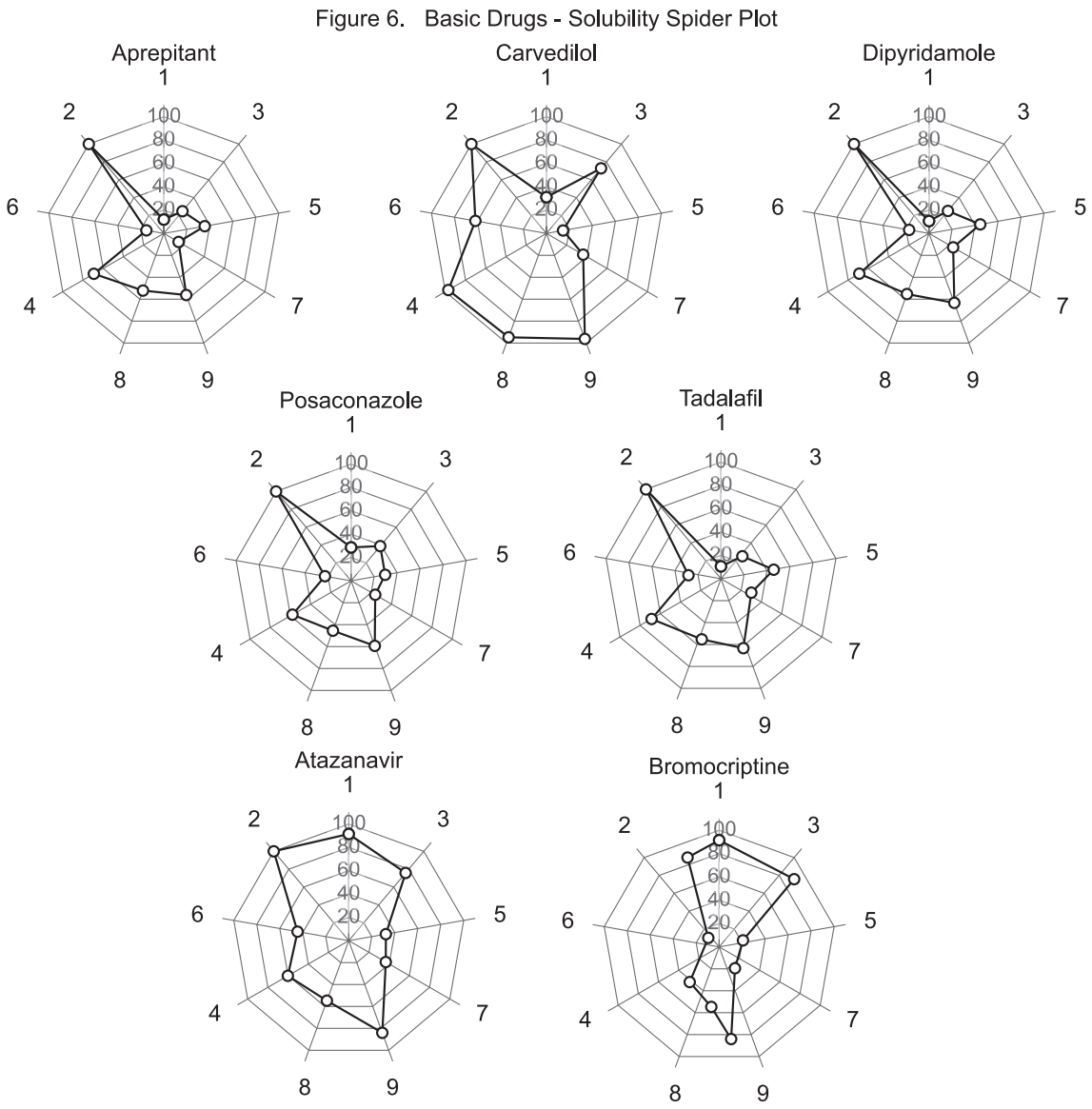


Fig. 6. Basic Drugs – Solubility Spider Plot. Highest solubility value normalised to 100; points correspond to media number (Table 2) arranged in a clockwise order of increasing pH × TAC – lowest value at 12o'clock.

clockwise around the web from media 1 to media 2. The increase is not smooth and there is variation in the intermediate media as discussed above, but the shape highlights their pH × TAC solubility dependency. Atazanavir and bromocriptine (Fig. 6) exhibit different behaviours with a distinctive waisted plot shape and for neutral drugs, exceptional behaviour is registered for probucol, acyclovir and paracetamol with the latter two displaying almost circular spider plots.

3.3.2. *Solubility behaviour analysis*

The solubility behaviour registered for the basic and neutral drugs in this experiment is in broad agreement with the behaviour in previous fed DoE studies [25–28]. When examining the solubility versus pH × TAC distributions for basic drugs it is clear the impact of pH is not as prominent as with acidic drugs. The previous DoE study [25] found a more intricate solubility relationship is apparent for basic drugs where on average pH, oleate, and bile salts display comparable effects on solubility, with lecithin having a lower effect. This finding is consistent with the behaviour displayed by the majority of basic drugs in this study. Neutral drug solubility behaviour was found on average to be influenced primarily by oleate and bile salts and to a lesser extent by lecithin and pH [25]. Since pH cannot influence neutral drug ionization, the effect is conveyed through the ionization of media components. This mechanism is similar to the one observed in the fasted DoE [33].

For both basic and neutral drugs, there is an overarching trend towards increased solubility with increasing media pH × TAC, which is why media 1 generally has lower solubility compared to media 2 (Fig. 4a and Fig. 5a). However, for each drug this trend is subject to modification by the standardised effect of each media component on individual drug solubility [25]. For aprepitant, carvedilol and tadalafil, oleate and bile salt were media components significantly positively influencing solubility, pH was only significant for aprepitant and carvedilol whilst lecithin was minimally significant for these drugs. This explains the similar polar plot shapes for these drugs. For bromocriptine no media components significantly influenced solubility, which explains the different solubility profiles in Fig. 4b and 6. Dipyridamole and atazanavir were not studied in the fed DoE therefore no comparative analysis regarding the significant effect of media components on solubility is available. However, dipyridamole seems to behave in a similar manner to the majority of basic drugs with its solubility appearing to be linked to pH × TAC. Atazanavir presents an unusual behaviour with media 1

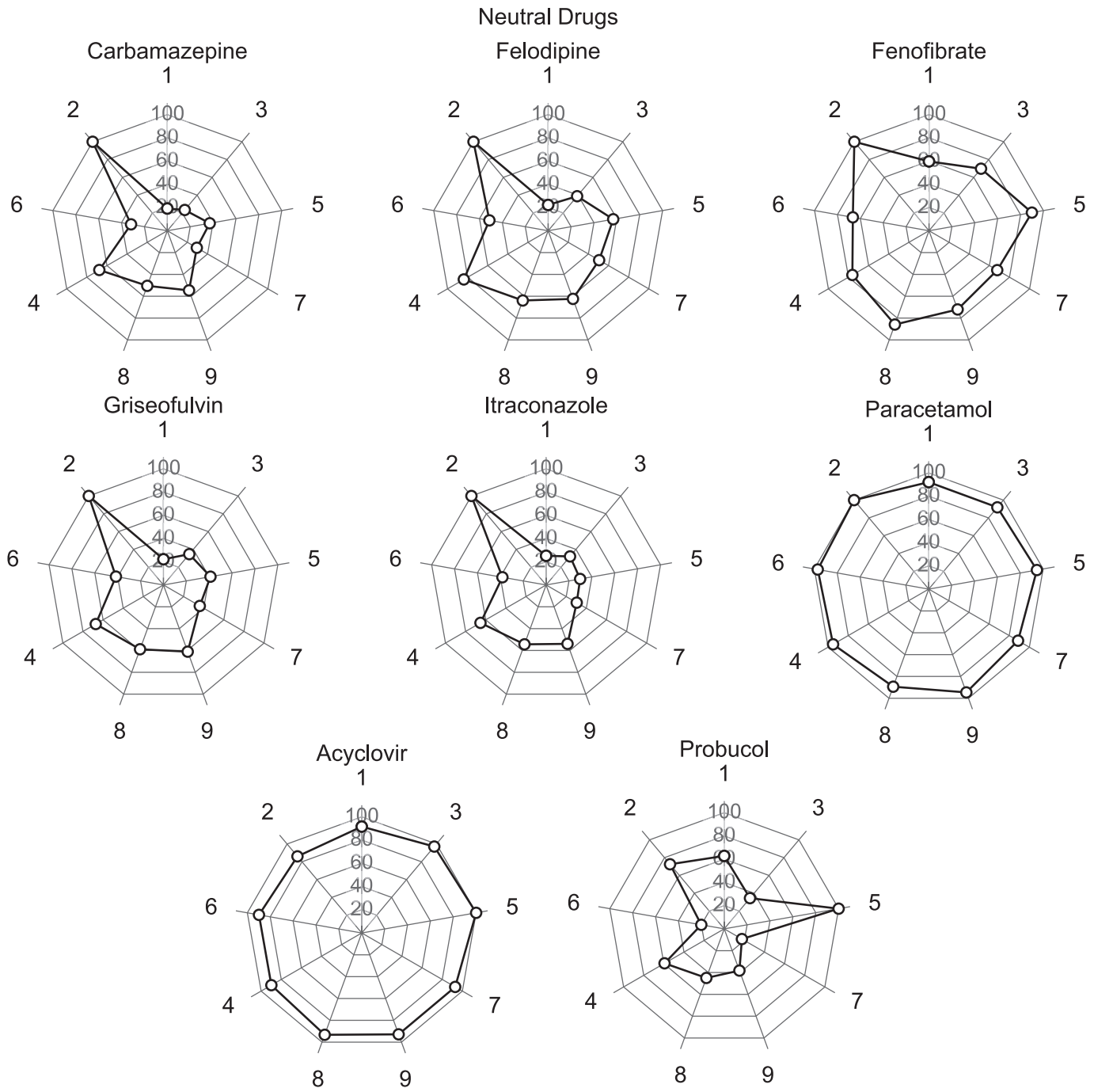


Fig. 7. Neutral Drugs – Solubility Spider Plot. Highest solubility value normalised to 100; points correspond to media number (Table 2) arranged in a clockwise order of increasing pH × TAC – lowest value at 12o'clock.

(lowest pH × TAC) exhibiting a similar solubility value to media 2 (highest pH × TAC) revealing no correlation between solubility and pH × TAC. Atazanavir's polar plot shape is similar to bromocriptine and this implies that for atazanavir none of the media components have a significant influence on solubility. For felodipine, fenofibrate, itraconazole and probucol, oleate, bile salt and lecithin were media components significantly positively influencing solubility, but to very different magnitudes. The exception was fenofibrate where bile salt had a negative impact on solubility [25]. The impact of pH was variable, being negative for itraconazole and probucol solubility, not significant for fenofibrate and positive for felodipine. Felodipine, fenofibrate and itraconazole have very similar solubility profiles, whilst probucol has a different distinctive profile. Probucol's behaviour can be rationalised based on the very high solubilisation effect of bile salt in the DoE, and media bile salt concentration in combination with other components. Acyclovir, carbamazepine, griseofulvin and paracetamol were not studied in the fed DoE and no analysis of media components on solubility is available. Carbamazepine and griseofulvin display congruent solubility behaviour to felodipine, fenofibrate and itraconazole and therefore can be assumed to show similar interaction with media components.

For acyclovir and paracetamol (also fenofibrate) the spider plots (Fig. 7), display solubility that does not vary with media composition and therefore measured solubility ratios are low at 1.16, 1.13, and 1.67 respectively. A previous fasted study [36] also registered similar solubility behaviour for acyclovir and paracetamol (solubility ratios 1.15

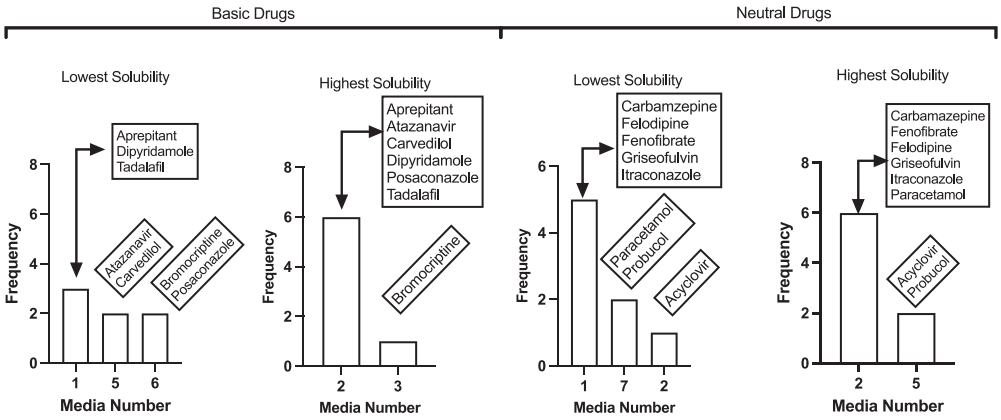


Fig. 8. Frequency of lowest and highest solubility media for basic and neutral drugs. Drugs as listed in boxes.

and 1.22) thus confirming that solubility is not influenced by the different composition of these fasted and fed media. Fenofibrate's solubility ratio has decreased from the fasted to fed state (7.65 vs 1.67), whilst for griseofulvin the reverse is evident (2.32 vs 4.52). This is an interesting biopharmaceutical observation that may be responsible for differences in pharmacokinetic behaviour between the fasted and fed states and worthy of further investigation.

Overall, for basic and neutral drugs three solubility behaviours are identifiable: solubility dependent on the variation of pH × TAC (Category 2, Table 5), limited solubility impact of media variation where solubility ratio is ≤ 3 (Category 3, Table 5) and no correlation between pH × TAC variation and solubility (Category 4, Table 5).

3.3.3. *Media frequency analysis*

Fig. 8 presents the frequency of each media recipe as the lowest and highest solubility value for basic and neutral drugs. For basic drugs the highest solubility was measured in media 2 (Table 2) in 6 out of 7 drugs (86%). The only exception was bromocriptine, discussed previously, where the highest solubility was provided by media 3. The lowest solubility was measured in media 1 (Table 2) for 3 drugs (aprepitant, dypridamole and tadalafil) (43%), in media 5 for 2 (atazanavir and carvedilol) and media 6 for 2 (bromocriptine and posaconazole) out of 7 (29%). For carvedilol and posaconazole the solubility difference between media 1 and the measured lowest media is small (carvedilol 1 = 4.17 mM ± 0.236; 5 = 1.95 mM ± 0.0641: posaconazole media 1 = 0.0284 mM ± 0.00257; 6 = 0.0225 mM ± 0.00229), As above a non-parametric Mann Whitney comparison of the solubility data sets for the four drugs is not significant (P = 0.10). Therefore for these drugs media 1 would represent an approximate value for the lowest fed state solubility. Atazanavir and bromocriptine were discussed above (Section 3.3.1) as they present a very different solubility behaviour from the other basic drugs. For these drugs there is no obvious signal to this behaviour other than the polar plot shape and for these drugs identifying the lowest solubility media in the fed state may require measurement of all media. For neutral drugs the lowest solubility was registered in media 1 for 5 out of 8 drugs (63%), in media 7 for probucol and paracetamol and in media 2 for acyclovir. Paracetamol and acyclovir have very low solubility ranges (see above), with almost circular polar plots with minimal media impact on solubility. Therefore media 1 would represent the lowest solubility for 7 out of 8 drugs (88%) with minimal error. A similar argument will apply for these drugs to the identification of the highest solubility media, which was identified as media 2 for 7 out of 8 drugs (88%). The behaviour of probucol as discussed above is individualistic and only identifiable via the polar plot shape.

4. Conclusions

In this study 24 drugs were examined to assess solubility behaviour in 9 fed state simulated intestinal media with a biorelevant composition determined by a multi-dimensional analysis of sampled fed human intestinal fluid. The caveat mentioned in the introduction regarding the use of a liquid meal, Ensure Plus™ which is not equivalent to solid meals, to attain the fed state in the original study [30] utilised to derive the media in this study is worthy of repetition. The solubility behaviour for the three categories of drugs acidic, basic and neutral is consistent with previous Design of Experiment studies examining simulated fed state intestinal media [25–27]. For acidic or category 1 drugs (furosemide, ibuprofen, indomethacin, mefenamic acid, naproxen, phenytoin, piroxicam, valsartan and zafirlukast) solubility is pH dependent. For the majority of basic, neutral and weakly acidic drugs (aprepitant, carbamazepine, carvedilol, dipyridamole, felodipine, griseofulvin, itraconazole, phenytoin, posaconazole and tadalafil) solubility is controlled by media pH × TAC (category 2), with generally increasing solubility as pH × TAC increases. Solubility variation is evident due to the diversity of individual drug interactions with media components [25,35,38]. For some drugs (acyclovir, atazanavir, fenofibrate, paracetamol) there is a very low solubility variation (category 3) across all the measured media. Three drugs (atazanavir, bromocriptine, probucol, category 4) exhibit an unusual solubility behaviour that does not conform with previous categories.

Overall a structured solubility behaviour has been identified for 18 of the 24 drugs studied with media 1 identifying the lowest solubility in 80% of cases and media 2 the highest solubility in almost 90% of cases. For 4 of the remaining drugs their minimal solubility variation means that media 1 and media 2 would still provide a realistic solubility assessment. The remaining 3 drugs present individualistic solubility behaviour that is at this stage not simply characterised. This study demonstrates for the majority of drugs the fed solubility range can be identified in vitro through application of only 2 media. In combination with the previous fasted study [36] this provides very interesting possibilities during drug discovery and development to determine fasted and fed solubility envelopes and indicates that the multi-dimensional media system [29] is worthy of further investigation.

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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