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Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs

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ABSTRACT

Gastrointestinal fluid is a complex milieu and it is recognised that gut drug solubility is different to that observed in simple aqueous buffers. Simulated gastrointestinal media have been developed covering fasted and fed states to facilitate in vitro prediction of gut solubility and product dissolution. However, the combination of bile salts, phospholipids, fatty acids and proteins in an aqueous buffered system creates multiple phases and drug solubility is therefore a complex interaction between these components, which may create unique environments for each API. The impact on solubility can be assessed through a statistical design of experiment (DoE) approach, to determine the influence and relationships between factors. In this paper DoE has been applied to fed simulated gastrointestinal media consisting of eight components (pH, bile salt, lecithin, sodium oleate, monoglyceride, buffer, salt and pancreatin) using a two level D-optimal design with forty-four duplicate measurements and four centre points. The equilibrium solubility of a range of poorly soluble acidic (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast), basic (aprepitant, carvedilol, tadalafil, bromocriptine) and neutral (fenofibrate, felodipine, probucol, itraconazole) drugs was investigated. Results indicate that the DoE provides equilibrium solubility values that are comparable to literature results for other simulated fed gastrointestinal media systems or human intestinal fluid samples. For acidic drugs the influence of pH predominates but other significant factors related to oleate and bile salt or interactions between them are present. For basic drugs pH, oleate and bile salt have equal significance along with interactions between pH and oleate and lecithin and oleate. Neutral drugs show diverse effects of the media components particularly with regard to oleate, bile salt, pH and lecithin but the presence of monoglyceride, pancreatin and buffer have significant but smaller effects on solubility. There are fourteen significant interactions between factors mainly related to the surfactant components and pH, indicating that the solubility of neutral drugs in fed simulated media is complex. The results also indicate that the equilibrium solubility of each drug can exhibit individualistic behaviour associated with the drug's chemical structure, physicochemical properties and interaction with media components. The utility of DoE for fed simulated media has been demonstrated providing equilibrium solubility values comparable with similar in vitro systems whilst also providing greater information on the influence of media factors and their interactions. The determination of a drug's gastrointestinal solubility envelope provides useful limits that can potentially be applied to in silico modelling and in vivo experiments.

1. Introduction

The current trend in drug discovery towards molecules with a higher molecular weight and increased lipophilicity continues to result in a greater number of drug candidates with decreasing aqueous solubility (Sugano et al., 2007), (Lipinski, 2000). Aqueous solubility is a key parameter influencing biological activity (Stegemann et al., 2007), formulation (Pouton, 2006) and in vitro and in vivo biopharmaceutical performance (Lipinski, 2000). Aqueous solubility may be determined in vitro using a number of experimental techniques (Sugano et al., 2007). Intrinsic solubility is a measure of the neutral (non-ionised) molecule's maximum solubility (Yalkowsky, 1999) in aqueous solution, whilst equilibrium solubility includes both un-ionised and ionised forms using a defined aqueous system (pH and presence of other salts) and employing the drug's most stable solid form in contact with the solution. Either value can be measured using classical shake-flask methods (Dittert et al., 1964) where an excess of solid drug is mixed with a buffered solution phase until equilibrium is achieved. During oral administration and absorption, an equilibrium concentration is unlikely to exist due to the competing processes of dissolution and

Abbreviations: BCS, Biopharmaceutics Classification System; DoE, Design of Experiment; FASSIF, Fasted Simulated Intestinal Fluid; FESSIF, Fed Simulated Intestinal Fluid; IVIVC, In vitro In vivo correlation.

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absorption although equilibrium aqueous solubility is demonstrably still a key parameter controlling rate and extent of absorption (Sugano and Terada, 2015). This is recognised in the Biopharmaceutics Classification System where drugs are allocated to categories based on solubility with respect to dose either high or low and gastrointestinal permeability (Amidon et al., 1995). Low solubility drugs present problems during formulation and development (Butler and Dressman, 2010) and in order to avoid solubility related failures during drug discovery, an early and comprehensive assessment of a drug's solubility is essential (Bergstrom et al., 2014).

Peroral drug administration is the most convenient and popular method for drug therapy covering a range of diseases and applications from acute through to chronic dosing. The normal function of the gastrointestinal tract is to provide efficient nutrition from a range of food matrices, coupled with excretion of metabolic waste products. This is accomplished by a dynamic, responsive secretion of fluids, and appropriate muscular activity to mix food, extract nutrients with the residues being pushed forward. It is appreciated therefore that the dynamic and complex physiology of the gastrointestinal tract influences drug absorption (Varum et al., 2013). Two major features of the gut are the inherent physicochemical conditions within the tract which vary with position along the tract (Bergstrom et al., 2014; Mudie et al., 2010) and the effect of ingested food (Yasuji et al., 2012) on these conditions, both of which exhibit intra- and inter-subject variability. Simple aqueous drug solubility therefore cannot reflect gastrointestinal solubility (Dressman et al., 2007) and in order to improve this determination in vitro, either sampled human fluids can be employed (Augustijns et al., 2014) or simulated gastrointestinal media prepared (Vertzoni et al., 2004). Human gastrointestinal fluids are expensive and problematical to sample, variable in composition (Bergstrom et al., 2014; Riethorst et al., 2016), unstable in air and therefore not an ideal material for in vitro experimental studies. Simulated gastrointestinal media are more easily prepared and two initial recipes simulating the fed state were published in 1998 (Dressman et al., 1998; Galia et al., 1998) see Table 1. Several adaptations have been investigated, for example changing the buffer to citrate (Vertzoni et al., 2004) or maleate (Jantratid et al., 2008a) and modification of the bile salt and lecithin concentration and ratio plus the inclusion of additional components such as monoglyceride or fatty acid (Jantratid et al., 2008b; Kleberg et al., 2010). However, a fixed composition simulated media reflects a single physicochemical state usually based around the average of measured parameters. As already discussed, gastrointestinal fluid composition is highly variable (Riethorst et al., 2016) and the situation is further confounded by changes in fluid composition as the mass passes along the small intestine (Bergstrom et al., 2014).

In order to investigate the influence of simulated fasted gastrointestinal media composition on the equilibrium solubility of twelve test drugs (four acidic, four basic and four neutral), we have employed a design of experiment (DoE) (Myers et al., 2009) type approach using published literature composition values for fasted gastrointestinal fluid (Khadra et al., 2015). This study illustrated that the DoE approach was feasible, simulated the inherent solubility variability associated with fasted gastrointestinal fluid and identified the key media components controlling solubility. For acidic drugs, pH was the major factor, whilst for basic and neutral compounds a combination of pH and the concentrations of fatty acid, bile salt and lecithin were important. The DoE also highlighted interactions between media components, for example pH and fatty acid, an interdependence that would otherwise have been undetected and also identified drugs where solubility behaviour was unusual or influenced by media components or interactions.

In this paper we have extended the DoE approach (Khadra et al., 2015) to simulated fed gastrointestinal media using the same components at higher concentrations and with the addition of monoglyceride as an additional fed media component, Table 1 (Jantratid et al., 2008a; Kleberg et al., 2010). The lower and upper concentration values of the experiment are presented in Table 2 and are based on published measured fed intestinal fluid ranges as reviewed by Bergstrom and colleagues (Bergstrom et al., 2014) (see Figs. 1, 6, 9 and 10) and typical concentrations employed by previously published simulated fed media, see Table 1. The addition of a factor to a fractional factorial DoE would double the number of required test conditions if the power of the experiment was to remain constant. In order to limit the number of conditions tested, the experimental design has been changed to a D-optimal design, which accommodates the same number of factors with less experiments. The D-optimal design provides an increased resolution of the main effects but with a reduced resolution of two way interactions. Finally, the HPLC method has been simplified to a single method accommodating all tested drugs.

2. Materials and methods

2.1. Materials

Hydrochloric acid (HCl), potassium hydroxide (KOH), acetic acid, sodium taurocholate, lecithin S PC (phosphatidylcholine from Soybean "98%") from Lipoid, Germany and Pancreatin from porcine sources, monosodium phosphate (NaH_2PO_4), sodium chloride (NaCl), chloroform, fenofibrate, and indomethacin were purchased from Sigma-Aldrich, Poole, Dorset UK. The active pharmaceutical ingredients aprepitant, carvedilol, felodipine, probucol, tadalafil and zafirlukast were kindly provided through OrBiTo (see Acknowledgements) by Dr. R. Holm Head of Preformulation, Lundbeck, Denmark. Itraconazole, bromocriptine, valsartan and phenytoin were purchased from Sigma, Poole, Dorset, UK. Sodium oleate was obtained from BDH Chemical Ltd. Poole England. All water used was ultrapure Milli-Q water. The analytical solvents methanol and acetonitrile were of HPLC grade (VWR, UK). Other materials used in this study included trifluoroacetic acid (Merck Schuchardt OHG, Germany) and ammonium acetate (Merck, Germany).

2.2. Design of experiment and data analysis

A D-Optimal DoE with 8 factors (either a component concentration or a system parameter such as pH) and 2 levels was constructed and analysed using MODDE (Umetrics) with the design selected using G-efficiency, which required 92 different experiments (44 conditions each measured in duplicate and 4 repeating centre points). Two assumptions

Table 1
Composition of literature fed simulated intestinal media (FeSSIF).

	Dressman et al., 1998 (FeSSIF)	Galia et al., 1998 (FeSSIF)	Vertzoni et al., 2004	Jantratid et al., 2008a (FeSSIF-V2)	Kleberg et al., 2010
pH	5	5	5	5.8	6.5
Buffer	Acetate	Acetate	Citrate	Maleate	Maleate
Sodium taurocholate	15 mM	15 mM	15 mM	10 mM	5–20 mM
Lecithin	4 mM	3.75 mM	3.75 mM	2 mM	1.25–5 mM
BS/PL	3.75	4	4	5	4
Salt	0.19 M (KCl)	0.20 M (KCl)			
Sodium oleate	–	–	–	0.8 mM	0–45 mM
Mono-oleate	–	–	–	5 mM	0–10 mM