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Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs

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ABSTRACT

Upon oral administration the solubility of a drug in intestinal fluid is a key property influencing bioavailability. It is also recognised that simple aqueous solubility does not reflect intestinal solubility and to optimise in vitro investigations simulated intestinal media systems have been developed. Simulated intestinal media which can mimic either the fasted or fed state consists of multiple components each of which either singly or in combination may influence drug solubility, a property that can be investigated by a statistical design of experiment technique. In this study a design of experiment covering the full range from the lower limit of fasted to the upper limit of fed parameters and using a small number of experiments has been performed. The measured equilibrium solubility values are comparable with literature values for simulated fasted and fed intestinal fluids as well as human fasted and fed intestinal fluids. The equilibrium solubility data range is statistically equivalent to a combination of published fasted and fed design of experiment data in six (indomethacin, phenytoin, zafirlukast, carvedilol, fenofibrate and probucol) drugs with three (aprepitant, tadalafil and felodipine) drugs not equivalent. In addition the measured equilibrium solubility data sets were not normally distributed. Further studies will be required to determine the reasons for these results however it implies that a single solubility measurement without knowledge of the solubility distribution will be of limited value. The statistically significant media factors which promote equilibrium solubility (pH, sodium oleate and bile salt) were in agreement with published results but the number of determined significant factors and factor interactions was fewer in this study, lecithin for example did not influence solubility. This may be due to the reduction in statistical sensitivity from the lower number of experimental data points or the fact that using the full range will examine media parameters ratios that are not biorelevant. Overall the approach will provide an estimate of the solubility range and the most important media factors but will not be equivalent to larger scale focussed studies. Further investigations will be required to determine why some drugs do not produce equivalent DoE solubility distributions, for example combined fasted and fed DoE, but this simply may be due to the complexity and individuality of the interactions between a drug and the media components.

1. Introduction

Dissolution and solubility are essential parameters in the absorption process of orally administered drugs and especially for poorly soluble drugs (BCS class II and IV). Over the last two decades there has been an increasing development of molecules with low aqueous solubility due to the application during development of high throughput screening systems (Lipinski et al., 2001). Therefore, it is necessary to develop new formulation techniques in order to address this issue (Savjani et al., 2012) along with in vitro methods to predict drug solubility in gastrointestinal fluids (Lennernas et al., 2014). High throughput solubility screening is possible (Alsenz and Kansy, 2007) but a low aqueous solubility does not automatically mean poor gastrointestinal solubility. The solubilizing potential of the gastrointestinal environment can improve the bioavailability for some drugs over that predicted on the basis of simple aqueous solubility (Sunesen et al., 2005). For example, it has been reported that mixtures of bile salts increase the solubility of steroid formulations (Mithani et al., 1996; Wiedmann et al., 2002) and the interaction of lecithin with bile salts yields an even greater positive solubility effect (Naylor et al., 1993). Solubilisation can be further influenced by the formation of mixed micelles with other lipid digestion products such as monoglycerides and the interaction of monoglycerides

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; GIT, gastrointestinal tract; API, active pharmaceutical ingredient

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with bile salt was demonstrated to increase the solubility of alpha-tocopherol in comparison to bile salt alone (Nielsen et al., 2001). To address the problem of poor aqueous solubility and bioavailability for oral drug formulations, it is therefore essential to use solubility and dissolution test conditions which closely reproduce key parameters of human gastrointestinal physiology (Dressman et al., 2007).

Over the past two decades simulated gastrointestinal media for the human fasted and fed states have been developed to assist in vitro drug development and formulation studies (Markopoulos et al., 2015; Stappaerts et al., 2014). These media were based around available literature data on the detailed composition and physicochemical parameters of human GI fluid however, the gastrointestinal tract and the interactions of all its constituents is very complex. To assess these interactions and improve the determination of the pivotal factors influencing the intestinal solubility of BCS class II drugs, a statistical design of experiment (DoE) approach was applied to investigate the influence of simulated gastrointestinal media composition in the fasted (Khadra et al., 2015) and fed state (Zhou et al., 2017) on the equilibrium solubility of BCS II compounds. This illustrated the utility of this approach, provided solubility values that are in agreement with literature values and highlighted the differences in solubility between the fasted and fed state (Augustijns et al., 2014; Bevernage et al., 2010; Clarysse et al., 2011). In addition, the approach simulated the inherent solubility variability and determined the key parameters controlling a drug's solubility. For acidic compounds pH was the most significant factor. For basic and neutral drugs the combination of pH and concentration of sodium oleate, bile salt and lecithin was significant. Various interactions between media components and unusual drug specific solubility behaviour were also identified. For neutral drugs solubilisation in fed simulated media was a more complicated interplay since seven (pH, oleate, bile salt, lecithin, monoglyceride, buffer and pancreatin) out of the eight single factors were significant along with more than half of the factor interactions.

In this paper the design of experiment approach has been applied to explore the equilibrium solubility of BCS class II drugs in simulated media spanning the full range of both fasted and fed intestinal states in a single experiment. The purpose is to examine the feasibility of merging the individual fasted and fed studies into one reduced experiment in order to obtain comparable results from a smaller experimental load. In this full range DoE the simulated intestinal fluid consists of seven factors or parameters (sodium oleate, bile salt, pH, lecithin, buffer, salt and monoglyceride) with phosphate buffer used instead of maleic acid. A fractional factorial design with two levels (upper and lower limit) was applied requiring a total of thirty two measurements and conducted in duplicate. This gives a total of 64 measurements for the statistical analysis. The lower limit values are derived from the lower limits of the literature fasted study (Khadra et al., 2015) and the upper limits are from the upper limits of fed study (Zhou et al., 2017) (Table 1). A smaller scaled DoE was selected in order to assess the utility of this systematic approach with a limited number of measurements. The equilibrium solubility of nine BCS class II drugs was investigated, two acids (indomethacin and phenytoin), four bases (aprepitant,[1] tadalafil, zafirlukast and carvedilol) and three neutral drugs (felodipine, fenofibrate, probucol) and compared to the previous fasted and fed DoE studies.

[1] Aprepitant has been classified as a basic drug in order to assist comparison with the two previous Design of Experiment studies (Khadra et al., 2015; Zhou et al., 2017), it is recognised that with a reported pKa of 9.7 (Liu et al., 2015) at the pH values in this study it will be predominantly un-ionised.

Table 1
Composition and concentration levels employed in full range design of experiment.

Parameter	Substance	Lower limit fasted	Upper limit fed
Bile salt (mM)	Sodium taurocholate	1.5	24
Lecithin (mM)	Phosphatidylcholine	0.2	4.8
Fatty acid (mM)	Sodium oleate	0.5	52
pH	Sodium hydroxide/ hydrochloric acid	5	7
Salt (mM)	Sodium chloride	68	203
Buffer (mM)	Phosphate[a]	15	45
Monoglyceride (mM)	Glyceryl mono-oleate	0.5	6.5

[a] Monophosphate buffer (KH_2PO_4).

2. Materials and methods

2.1. Materials

Sodium taurocholate, ammonium formate, sodium chloride (NaCl), chloroform, formic acid, monosodium phosphate (NaH_2PO_4), fenofibrate, indomethacin and phenythoin were purchased from Sigma Aldrich Poole, Dorset UK. Lecithin S PC (phosphatidylcholine from Soybean “98%”) was purchased from Lipoid. Glycerol mono oleate was obtained from CRODA Healthcare. The active pharmaceutical ingredients felodipine, probucol, aprepitant, tadalafil, carvedilol and zafirlukast were provided through OrBiTo by Dr. R. Holm Head of Preformulation, Lundbeck, Denmark. Sodium oleate was obtained from BDH Chemical Ltd. Poole England. The analytical solvents methanol and acetonitrile were of HPLC grade (VWR, UK). All water was ultra pure Milli-Q water.

2.2. Design of experiment and data analysis

A quarter of the full factorial design of experiment with 7 factors (either a component concentration or a system parameter such as pH) and 2 levels (upper and lower limits) was constructed and analysed using Minitab®17.2.1. Minitab generated 32 different experiments by various combinations of the upper and lower limits of the 7 factors based on Table 1 (no centre point and no replicate). When designing and analysing the DoE assumptions were made. 1. Only main effects and 2-way interactions are considered in the analysis and 3-way interactions or more were not considered. 2. The single factors and factor interactions are confounded with 3 to 6-way interactions which were not included. There are three confounded 2-way factor interactions, sodium oleate and salt with buffer and monoglyceride, sodium oleate and buffer with salt and monoglyceride, sodium oleate and monoglyceride with salt and buffer. For these interactions if the result is significant then any conclusions must be drawn with caution as it might be the result of the four factors together or only one of the 2 way interactions. 3. The main effect can be positive (+) or negative (−), but when it is involved in an interaction, the conclusion will be considered with the interactions (±).

The Kolmogorov normality test was used in Minitab® to assess the distribution of each data set, based on the result that all data sets have a non-normal distribution the Mann-Whitney test was applied to evaluate differences between two data sets.

2.3. Equilibrium solubility measurements

2.3.1. Preparation of lipid stock mixtures

Sodium taurocholate, monoglyceride and lecithin were weighed into a flask and 2 ml of chloroform was added to dissolve all the solid material. A stream of nitrogen gas was used to remove the chloroform ensuring a dry film was produced. Water was added to reform the dried film, stirred to obtain a homogenous mixture, transferred to a