The Gut in a Beaker: More Challenges for *In Vitro* Testing

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1. Introduction

The *in vitro* testing of drug formulations is an essential safety/quality test in formulation assessment, process evaluation and batch release. In addition, it is desirable that the media be physiologically relevant, assist in the prediction of IVIVC and provide data that could be used in computer modelling. The number of relevant chemical variables has expanded with the adoption of biorelevant fluid compositions for fasted and fed states and the expansion of simulated gastric and intestinal fluids into simple colonic media. Dissolution in variants of these media is justified on the grounds of human variability and diet, but the number of permutations possible eventually becomes prohibitively large for the pharmaceutical industry. A compromise between a minimum set of compositions and the chance of missing what would be clinically significant effect has to be balanced. Over a number of iterative processes based on the design of experiments approach, we have attempted to progress towards a minimized matrix of compositions.

2. Sequential approaches to the problem

A key statement in one of the seminal papers in the development of biorelevant media identifies the merits of this line of research: there is a clear need to mimic the conditions for dissolution in vitro which are relevant for the human gut. This would allow the adoption of media in fed and fasted conditions which would show the enhanced (usual) or decreased (less common) dissolution effects in clinical trials (1). Gastrointestinal media are of course complex, variable and to a certain extent unstable and there was a need to develop robust formulae for both fed and fasted conditions. With better access to data provided by industry-academic collaboration through European initiatives such as OrBiTo in and individual efforts in the USA, there was a diversity in the number of formulae developed which eventually provided a resource issue for industry. In our laboratories as part of an OrBiTo group, we have employed a gradually changing Design of Experiments approach to the problem which allowed the collection of average significant factors and identification of interactions over a wider small molecule design space. Compounds included basic, acidic, zwitterionic and non-ionisable motifs, the chief limitation being a certified supply of a compound allowing accumulation of data using different apparatus across national groups. The approach used initially was to identify seven factors in equilibrium solubility measurements namely the influence of bile salt, sodium taurocholate, lecithin , egg-derived phospholipid, buffer, tonicity (sodium chloride), pH , pancreatin and fatty acids. All factors were studied at high and low levels resulting in 66 experiments per run for each of the panel of 12 compounds (2).

3. Results

The first attempts revealed that for small molecules, the influence of pancreatin was minimal and the component could be removed. Significant media interactions between multiple factors – for example between bile acid and pH existed and for acids pH effects were clearly important. Nevertheless over the panel of 12 compounds, no overall effect dominated and generally the effects were more subtle. For example for neutral compounds the pH shifts exerted effects on the ionization of the media components. Our next papers attempted to map out the two dimensional space and begin the use of phase diagrams and a four component matrix design to reduce the number of per-
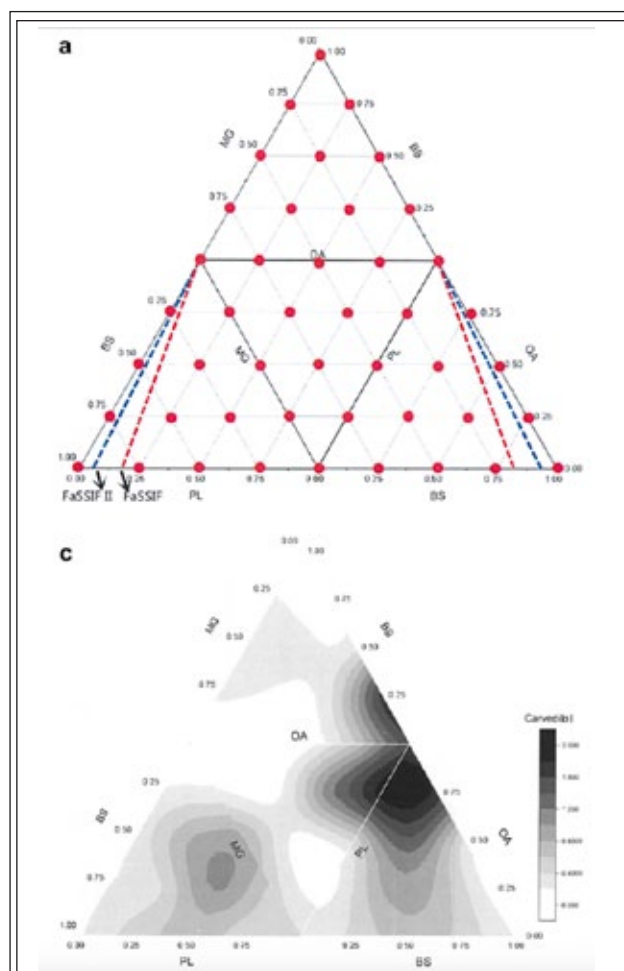
Figure 1 *Equilibrium solubility space for carvedilol as a function of bile salt (BS), phospholipid (PL), oleate (OA) and monoglyceride (MG) at a particular pH. (From Zhou et al., 2017).*

Figure 2 *Constructed pyramid from Figure 1*

ponents with an adjustment of range and encompassing fed and fasted states (5). In our latest attempt, we have attempted to still further reduce the range of media composition but inevitably have run into the inevitable situation that the reduced DoE only identified single and in some cases none of the major components influencing solubility in contrast to the larger published DoE studies which as noted, identified multiple individual components and component interactions.

The general problem of handling multidimensional analysis in a statistically relevant manner is difficult as the equilibrium solubility approach cannot capture phenomena such as supersaturation and the physical state of dissolution structure – mixed micelles for example – may rapidly convert at the concentration of key components predominates. New approaches are currently under discussion with OrBiTo colleagues.

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mutations. (3). An example is shown in **Figure 1**. We noted through the next series of iterations that for some compounds at the same amphophile concentrations, the proportion of each component can have a remarkable influence on solubility with, in some cases, the highest and lowest solubility points close to each other.

Exploring the topographical descriptors of oleate, monoglycerides, bile salts and phospholipid and the influence of pH (4) are difficult to picture without a holographic projector leading me to attempt to make the pyramid as shown in **Figure 2**. And of course for each pH and each compound the solidus is different.

A key issue is that solubility in the gut is a dynamic process and the concentrations of components are dramatically altered along the gastrointestinal tract. Thus working within concentration envelopes for each parameter becomes more valuable which led us towards a reduced set of com-
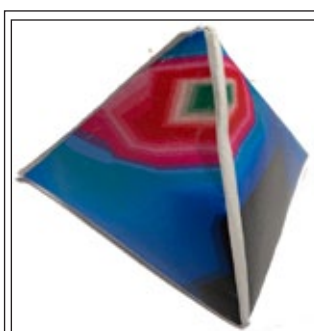
References

1. Dressman, J.B., Amidon, G.L., Reppas, C. and Shah, V.P.: Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharmaceutical research*, 15 (1): 11-22 (1998).
2. Khadra, I., Zhou, Z., Dunn, C., Wilson, C.G. and Halbert, G.: Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs. *European Journal of Pharmaceutical Sciences*, 67: 65-75 (2015).
3. Zhou, Z., Dunn, C., Khadra, I., Wilson, C.G. and Halbert, G.W., Influence of physiological gastrointestinal surfactant ratio on the equilibrium solubility of BCS class II drugs investigated using a four component mixture design. *Molecular pharmaceutics*, 14(12): 4132-4144 (2017).
4. Dunn, C., Perrier, J., Khadra, I., Wilson, C.G., Halbert, G.W.: Topography of Simulated Intestinal Equilibrium Solubility. *Molecular pharmaceutics*, 16(5): 1890-1905 (2019).
5. Perrier, J., Zhou, Z., Dunn, C., Khadra, I., Wilson, C.G. Halbert, G.: Statistical investigation of the full concentration range of fasted and fed simulated intestinal luid on the equilibrium solubility of oral drugs. *European Journal of Pharmaceutical Sciences*, 111: 247-256 (2018).