Julia Elisabeth Boni (Autor)

Improvements to biorelevant dissolution testing: lyophilized media, buffer alternatives and miniaturized apparatus

https://cuvillier.de/de/shop/publications/1016

3 Biorelevance in dissolution testing

3.1 The GI Tract

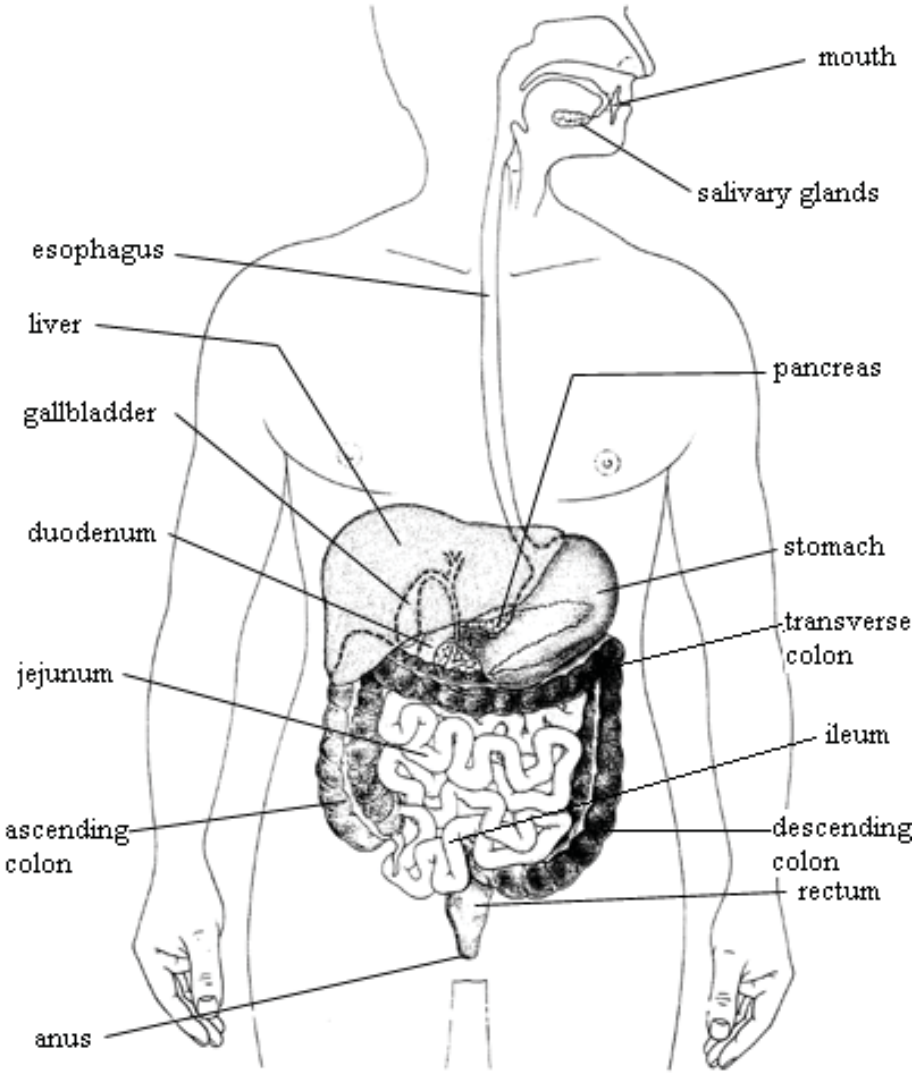

Figure 3.1 Anatomy of the human digestive tract

Figure 3.1 schematically shows the human digestive tract. The stomach and intestines are referred to as the gastrointestinal tract (GIT), in which digestion and assimilation of the aliment takes place. Organs supporting the digestive process are teeth, tongue, salivary glands, pancreas, liver and gall bladder. Secretion of the bile and the digestive enzymes produced in the pancreas occurs continuously but is more pronounced after intake of food. The majority of ingested food and xenobiotics is absorbed in general in about the first 80 cm

of the small intestine.[14]

Orally administered drugs can pass through the GIT en bloc (e.g. matrix tablets), can become dispersed or can dissolve. Fluids are mainly presented to the dosage form / drug intermittently in the intestine (as opposite to the continous contact in standardized dissolution tests).[9]

The processes of digestion and absorption of foodstuffs are enormously efficient.

Bile secreted by the liver is released directly into the gallbladder and into the GIT at a lecithin to bile salt ratio of about 1:1. Concentrations of bile salts in the hepatic bile are low, but a 10 to 25 fold concentration takes place in the gall bladder. Whereas 50 % of hepatic bile is continuously secreted into the GIT, the gallbladder bile is stored until emptying is induced by food intake.[15,16] The major difference between the composition of the hepatic bile and the luminal contents is the higher concentrations of Na^+, Cl^- and HCO_3^-.[17] The typical composition of gall bladder bile is given in Table 3.1.

Table 3.1 Composition of gall bladder bile (percentage of lipid [$^{mol}/_{mol}$])

concentration	ion		
200 mM	Na^+		
10 mM	K^+		
3.5 mM	Ca^{2+}		
50 mM	Cl^-		
150 mM	bile salts	65 mM	glycine conjugated
		34 mM	taurine conjugated
15 g/dl	total lipid	72 %	bile salts (54% cholic acid, 25% chenodeoxycholic acid, 21% deoxycholic acid, 0.2% lithocholic acid)
		17 %	phospholipids
		9 %	cholesterol
50 mM	phospholipids (7 types of lecithin, whereof 90% phosphatidylcholine)		

The metabolic half-life of cholic acid and taurocholic acid is three times higher than that of the glycine conjugate. However, synthesis of the glycine conjugate is higher, leading to relatively higher concentrations of this conjugate.

Bile composition of the postprandial upper intestinal contents is roughly equivalent to that of the gallbladder bile, with a ratio of lecithin to bile salt of 1 to 3.5. In the fasted state however, due to the constant secretion of hepatic bile, the relative concentrations of lecithin to bile salt are about 1 to 5.[18] The most prevalent bile salts NaGC (sodium salt of glycocholic acid) and NaTC (sodium salt of taurocholic acid), are both soluble at intestinal pH values. The bile salts are dissociated due to their low pk_a values of about 4 for glycine and about 2 for taurine

conjugated cholic acid.[19] Mixed micelles are formed consisting mainly of bile salts and lecithin, which, due to the highly lipophilic core, are able to incorporate lipophilic substances like lipids (e.g. cholesterol and acylglycerides) or lipophilic drugs.[16]

Exact determination of the intralumenal composition is difficult. Human data can only be obtained by aspiration of the intestinal fluids. The process of aspiriation however, causes irritation to the GIT, which again can lead to increased secretion and therewith overestimation of concentrations. Also challenging is the handling of these highly complex samples, e.g. in terms of the qualitative and quantitative identification of salts or the concentration of gases like CO_2 and subsequently their ionized counterparts like bicarbonate.[20] In fact, bicarbonate is the predominant buffer in the fasted small intestine, secreted continuously to neutralize acidic gastric juice and to protect the epithelium against digestion[21]. Secretion from epithelial cells and glands is regulated in order to maintain a pH[22] which is more or less stable against changes in acid or base concentration entering the small intestine. Postprandially, a variety of buffer species originating from food components as well as indigenous ones contribute to the overall buffer capacity.[23]

The complex and changing composition combined with high interindividual variations of human fluids leads to differing data about physiological composition in the literature. In most studies glycocholic acid was measured to be the most prevalent bile salt, wheras in 2006 Perez de la Cruz Moreno et al.[24] calculated a much higher concentrations of taurocholic acid in intestinal aspirates. Human studies conducted in the early 70s generally report higher concentrations of various components compared to recent studies.[25,26] In the intestine pH values in the fasted state were expected to be high (near neutral), whereas in the fed state the lower pH was assumed to reflect the influence of chyme emptying from the stomach.[27,28] Table 3.2 summarizes some of the most important studies about intestinal fluid composition.

Table 3.2 Characterization of intestinal fluids originating from the duodenum (duo.) or jejunum (jej.)

sample source	Kalantzi[29] *3	Perez de la Cruz[30]		Pedersen[31]		Lindahl[32]	Rune[21]	Dam[25] *2	Fordtran[33]	Evans[34]
sampling area (distal to pylorus [cm])	30	5 - 10	100	60		60	10	30-60	40	30-60
intestine	duo.	duo.	jej.	jej.		jej.	duo.	jej.	jej.	jej.
fasted state				fresh	frozen					
pH	5.7	7	6.8	6.1	6.7	7.1	6.2			6.63
buffer cap [mM/ΔpH]	7.8									
osmol [mosmol/kg]	188.2	137	200	272	278	271.0				
γ[mN/m]	32.9				33.7					
BS concn [mM]	2.1	2.6	3.5	2.0	1.5	2.9				
lecithin concn [mM]					<0.2 *1					
Na^+ concn [mM]						142.0				
fed state										
pH	6.6						5.7 - 5.8	5.5	7.3	
buffer cap [mM/ΔpH]	27.7								280	
osmol [mosmol/kg]	406.1						200- 375			
γ[mN/m]	28.5									
BS concn [mM]	8.7							38.9	11.9	
lecithin concn [mM]								11.1		
Na^+ concn [mM]										

*1 not determined in fresh fluid, only frozen -> as bile salt concentration decreased, probably lecithin concentration did as well

*2 collected after injection of cholecystikinin

*3 30 min after administration of water or nutrient drink

Knowledge about the GIT physiology is necessary to understand formulation behaviour in man, since dissolution conditions which do not reflect *in vivo* behaviour might lead to choice of suboptimal formulation. Physiological dissolution testing e.g. using biorelevant media is one way of gaining better prediction of *in vivo* behaviour of the probable formulations and hence to better results in development.

Even so, not only the composition but also movements of the gut wall and further factors such as enzymatic activity will cause interindividual variations, making a one to one copy *in vitro* to *in vivo* impossible. A general prediction of *in vivo* data from dissolution testing will therefore always be limited to estimating the average result.

3.2 Simulation of *in vivo* conditions

Many efforts have been made to establish systems simulating *in vivo* behaviour of formulations. Some systems, like the TIM model of TNO Pharma[35], attempt to exactly mimic *in vivo* conditions. But the high complexity of the TIM model makes it impractical for routine dissolution testing. More simple and correspondingly easier to set into practice is the use of simulated gastrointestinal media in a standard dissolution apparatus. Knowledge of the composition and physicochemical properties of gastrointestinal (GI) fluids combined with consideration of motility in the upper GI tract in the design of the dissolution test should lead to a better prediction of *in vivo* performance. These arguments apply especially to compounds belonging to Class II & IV of the BCS (Biopharmaceutical Classification System), since the absorption of these compounds is likely to be limited by dissolution[1]. Oversimplification of the dissolution conditions, as for example with compendial media, often leads to profiles which do not reflect the formulation's *in vivo* behaviour.

To simulate the *in vivo* conditions more nearly than just approximating the pH and volume, substances that occur in the GI fluids can be added to the media. Several media simulating the GI fluids have been proposed, including FaSSIF and FeSSIF[12], fed HIF[36] or SEIF[37].

Biorelevant media, which are designed to closely simulate physiological effects, aim to better link *in vitro* with *in vivo* performance. Dressman et al.[12] created dissolution media through consideration of the main factors which are generally expected to influence dissolution *in vivo*. Values of lecithin, taurocholic acid, osmolality, surface tension, buffer capacity and pH were adjusted to physiological values. To reflect differences between the fasted and fed state, different media were established: FaSSIF (Fasted State Simulating Intestinal Fluid), FeSSIF (Fed State Simulating Intestinal Fluid) and FeSSIFplus (similar composition to fed HIF)[31]. These fluids simulate intestinal composition by using concentrations of key substances similar to those given in Table 3.2. Usually taurocholic acid and phosphatidylcholine from egg are used as bile salts and lecithin components, respectively. To represent triglycerides and fatty acids glycerol monooleate and sodium oleate are commonly used. Osmolality is adjusted to physiological values with NaCl. But even though the media simulate most relevant characteristics, such as concentration of solubilising substances, buffer capacity, pH and solubilisation capacity of drugs[38], they are not a one-to-one copy of gastric or duodenal juice. For example, pH and buffer capacity vary considerably as a function of digestion and as a function of location within the intestine. Additionally, FaSSIF is based on a non-physiological buffer system, phosphate buffer (0.029 M, pH 6.5) whereas FeSSIF and FeSSIFplus are based

on a partly physiological acetate buffer (0.144 M, pH 5.0). In FeSSIFplus, citrate buffer is used as an alternative to acetate buffer.

Interest in using biorelevant media in dissolution testing has increased about 10fold (based on number of publications) over the last ten years (see Figure 3.2). Establishment of biorelevant dissolution testing has made prediction of or at least a ranking of *in vivo* formulation behaviour possible[12].

However, this "simple" idea of improving predictability shows some disadvantages: The conventional preparation method of these fluids is time-consuming, requires organic solvents, requires daily preparation and sometimes does not result in a usable medium.

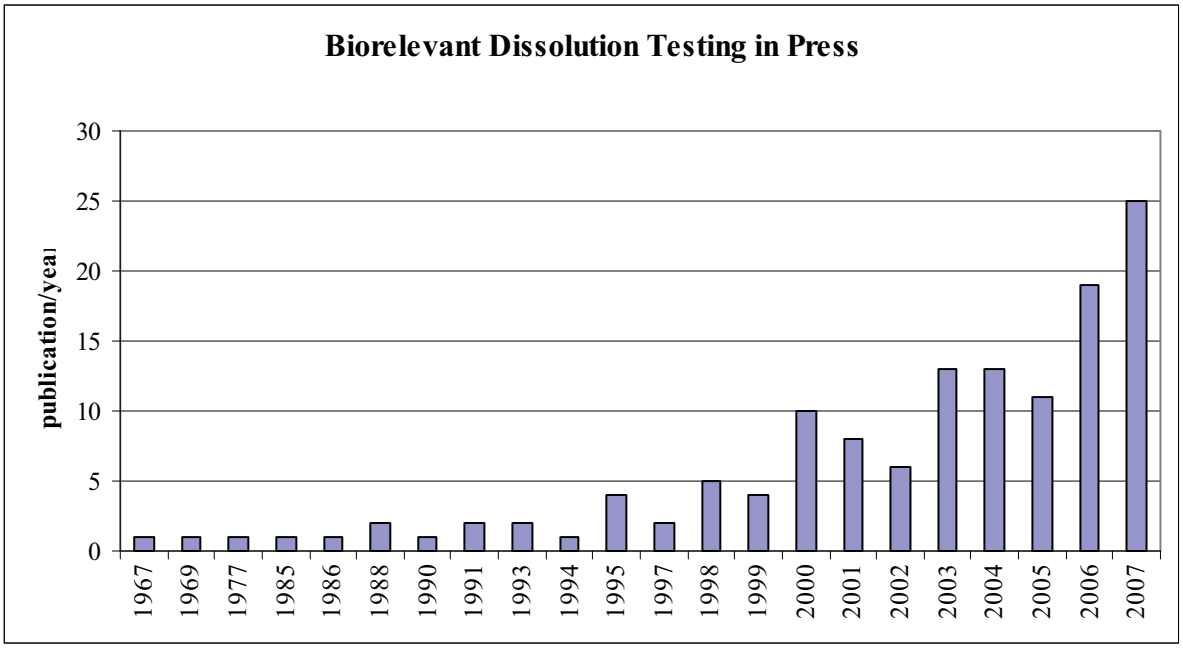

Figure 3.2 Number of publications per year relating to biorelevant dissolution testing (publications listed in this figure are referenced in Table 5.1 in appendix)