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Research Paper

Advances in the design of fasted state simulating intestinal fluids: FaSSIF-V3

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ABSTRACT

Biorelevant media are commonly used to simulate the physiological composition of human intestinal fluids (HIF) in *in vitro* solubility and dissolution investigations. In comparison with the surfactant solutions or blank buffers, these media are able to better reflect the physiological solubility and dissolution behavior of poorly soluble active pharmaceutical ingredients (APIs). The aim of this investigation was to review the composition of FaSSIF and FaSSIF-V2 according to recently summarized data about the physiological composition of fasted state human intestinal fluid and propose an updated version, FaSSIF-V3. Furthermore the surface tension was considered as a possible surrogate parameter to gauge the physiological correctness of new versions of biorelevant media.

Various prototypes of FaSSIF-V3 were prepared with each of the following five bile salts: taurocholate (TC), glycocholate (GC), tauroursodeoxycholate (TUDC), taurochenodeoxycholate (TCDC) and glycochenodeoxycholate (GCDC) as well as replacing lecithin with its hydrolysis products, lysolecithin and sodium oleate. Two additional media consisting of a mixture of glycocholate (GC) and taurocholate (TG), with or without 0.2 mM cholesterol, were also investigated.

Solubilities of ten model compounds in various prototypes of FaSSIF-V3 were measured using HPLC-UV and compared to the solubilities in the existing biorelevant media (FaSSIF and FaSSIF-V2), fasted HIF, blank buffer and a 0.5% sodium dodecyl sulfate (SDS) solution. Additionally, the influence on the surface tension properties of various combinations of bile salts, phospholipids and their hydrolysis products and cholesterol in these media was investigated and an attempt was made to calculate the CMC of the various generations of FaSSIF.

The results demonstrated that the amount and the type of phospholipids as well as the type of bile salt had a significant influence on the solubility and surface tension in the various FaSSIF-V3 prototypes and existing biorelevant media. In contrast to results with biorelevant media, it was demonstrated that blank buffers generally underestimate and SDS solutions highly overestimate the physiological relevant solubilities of all investigated APIs.

The prototype containing FaSSIF-V3-GC/TC_Chol was able to better reflect the solubilities of the most investigated APIs in fasted HIF than the existing media, and it also matched the physiological surface tension reported for the fasted human gut, and was designated FaSSIF-V3.

1. Introduction

Over the last decade, biorelevant media have become increasingly important in pharmaceutical research and development. The aim of these media is to reflect the conditions in the human gastrointestinal tract, enabling investigations of active pharmaceutical ingredients (APIs) in terms of their physiological solubility and dissolution behavior *in vitro* to forecast *in vivo* behavior.

To reflect the entire human gastrointestinal tract, media have been introduced to simulate gastric juice (e.g. FaSSGF [1] and FeSSGF [2]), small intestinal (e.g. FaSSIF and FeSSIF [3]) and colonic fluids (e.g. FaSSCoF and FeSSCoF [4]) under pre- and postprandial conditions.

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The first two biorelevant media simulating the fasted and fed state small intestine (FaSSIF and FeSSIF; fasted and fed state simulated intestinal fluid) were introduced by Dressman et al. [5] and Galia et al. [3] in 1998. The aim of these media was to reflect not only the pH, buffer capacity and osmolarity of human intestinal fluid (which were not adequately reflected at that time by compendial media, bearing in mind that in 1998 the pH of SIF was 7.5 [6]) but also include physiologically relevant surface active species such as bile salts and phospholipids to improve the biorelevance of these media. Galia et al. [3] were able to show that, particularly in the case of BCS class II drugs, dissolution behavior is strongly influenced by these natural surfactants. It was concluded that in cases where the dissolution of an API is the rate limiting step to its absorption, using this new approach of testing in biorelevant media should enable construction of IVIVCs for poorly soluble drugs [3,7].

In 2008, Jantratid et al. introduced FaSSIF-V2 and FeSSIF-V2 [2]. In these media the composition of FaSSIF and FeSSIF was updated according to a review of data by Porter et al. [8] about the composition of human intestinal fluids in different nutritional states which had been published shortly beforehand. The aim of Jantratid et al.'s investigation was twofold: to better reflect the composition of the fluids in the human small intestine and to attain better stability of the media during dissolution tests and short term storage [2].

Both the original and updated versions of the biorelevant media reflecting conditions in the small intestine are commonly used for pharmaceutical development purposes [9] and a recent informal survey has indicated that almost a third of industrial scientists now rely more on results in biorelevant media than in animal testing to forecast performance in clinical studies [10].

In Table 1, the composition of various media currently used to simulate the fasted state human small intestine is summarized [2,3,11–13].

Recently, Fuchs et al. published a commentary in which relevant literature data about the composition and physicochemical properties of fasted state human intestinal fluid were summarized and evaluated [14]. In addition to addressing the pH, buffer capacity and osmolarity of these fluids, the commentary focused on the composition and concentration of bile components. Lysolecithin was identified as the main phospholipid while taurocholate, glycocholate and glycochenodeoxycholate were identified as the main bile salts in fasted human intestinal fluid. Further, the surface tension was identified as an important surrogate parameter for the qualitative composition of these components [14]. In consideration of these data, the composition of FaSSIF and FaSSIF-V2 was reviewed and various prototype media that may be able to better reflect the *in vivo* range of data were proposed. The prototypes were compared with fasted state aspirated human intestinal fluids according to the solubility of ten poorly soluble drugs (seven neutral compounds, one weak acid and two weak bases) and surface tension to identify the most biorelevant composition for the third version of FaSSIF, FaSSIF-V3. Additionally, the CMC of the proposed final version of FaSSIF-V3 was evaluated and compared with the forerunner media, FaSSIF and FaSSIF-V2.

2. Materials

Maleic acid was purchased from Merck Schuchardt OHG, Hohenbrunn, Germany. Sodium dihydrogen phosphate dihydrate was purchased from Merck KGaA, Darmstadt, Germany. Sodium hydroxide and sodium chloride were purchased from VWR International bvba/sprl, Haasrode, Belgium. Hydrochloric acid solution 1 N, hydrochloric acid solution 0.1 N, sodium hydroxide solution 1 N and Orthophosphoric acid 85% were purchased from VWR International S.A.S, Fontenay-sous-Bois, France. Sodium taurocholate (⩾99% pure, lot PHA S 1306 026 and PHA S 1204 029), sodium glycocholate (⩾99% pure, lot PHA S 130627 and PHA S 1204 023), lysophosphatidylcholine (85.7% pure, lot PHA S 1104027 and lot PHA S 1306028), sodium oleate (Carl Roth, 90.31% pure, lot 461166580), egg phosphatidylcholine (Lipoid S100, lot 790637), SIF Powder original (lot PHA S 1003012/06 and lot 01-1402-03) and SIF Powder FaSSIF-V2 (lot 02-1302-01 and lot 02-1302-04) were donated from biorelevant.com, Croydon, UK. Sodium oleate (82.7% pure, lot 51110) was obtained from Diedel-de Haen, Seelze, Germany. Sodium taurocholate (lot 2011040152) was obtained from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. Egg phosphatidylcholine (97.5% pure, lot 108072-04/065) was donated from Lipoid GmbH, Ludwigshafen, Germany. Diethylamine was purchased from Fluka Chemie GmbH, Buchs, Suisse. Dichloromethane, acetonitrile and methanol were of analytical grade and purchased from Merck KGaA, Darmstadt, Germany. All modified SIF Powder versions of FaSSIF-V3 were produced according to the prescribed composition

Table 1
Composition of various media which have been proposed to simulate fluids in the fasted state small intestine.

Medium		FaSSIF [3]	FaSSIF-V2 [2]	FaSSIF-V2plus [13]	Copenhagen fasted [11]	SEIF [12]
BS (mM)	GC					1
	GDC					0.7
	GCDC					1
	TC	3	3	3		0.5
	TDC					0.3
	TCDC					0.5
	Crude[a]				2.5	
PL (mM)	PC	0.75	0.2	0.2	0.625	
	LPC					1
Ratio BS/PL		4/1	15/1	15/1	4/1	4/1
SO				0.5		
Chol				0.2		0.25
pH		6.5	6.5		6.5	6.5
Buffer		Phosphate	Maleate	Maleate	Trizma maleate	Phosphate
Osmolarity		270	180	181.2[b]	270	289.25[b]
Stabilizer	NaN_3					6

FaSSIF = Fasted State Simulated Intestinal Fluid; FaSSIF-V2 = Fasted State Simulated Intestinal Fluid Version 2; FaSSIF-V2plus = Fasted State Simulated Intestinal Fluid Version 2plus; SEIF = Simulated Endogenous Intestinal Fluid; Copenhagen fasted.
BS = bile salts; PL = phospholipids; SO = sodium oleate; Chol = cholesterol; GC = glycocholate; GDC = glycodeoxycholate; GCDC = glycochenodeoxycholate; TC = taurocholate; TDC = taurodeoxycholate; TCDC = taurochenodeoxycholate.
[a] Crude porcine bile extract or taurocholate.
[b] Calculated.

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