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Research paper

Micelle dynamic simulation and physicochemical characterization of biorelevant media to reflect gastrointestinal environment in fasted and fed states

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ABSTRACT

The characterization of biorelevant media simulating the upper part of the gastrointestinal tract in the fasted and fed states was investigated by classical determination of physicochemical parameters such as pH, osmolality, surface tension and results were compared to *in vivo* physiological data. Incorporation of fatty material, in order to better simulate the influence of high fat meal was also performed. Stability and characterization of this medium was studied and compared to classical FeSSIF. Micelle characterization and computer dynamic simulation were performed in order to understand the interaction between lecithin and taurocholate and possible interactions between mixed micelle and drugs. The addition of NaTc, lecithin, and/or fatty materials has no influence on pH and osmolality, whereas the presence of fatty material modifies the surface tension. Values of FaSSIF and FeSSIF are in accordance with *in vivo* parameters and the presence of micelles can simulate the gastrointestinal environment. Modelization of micelles by computer simulation led to a model of mixed micelles in which structures of NaTc interact either by their hydrophilic or hydrophobic phase to give a bilayer stable model in which the lecithin molecule can insert its long carbon chain. The micelle structure is stable and can enhance dissolution of hydrophobic molecules by hydrophobic interaction with the numerous hydrophobic spaces available in the multilayer hydrophilic/hydrophobic layer.

1. Introduction

Dissolution testing of solid dosage form is a well-established quality control method. Different apparatuses described in pharmacopeias and officially approved by authorities, as well as various dissolution media, can be used to perform *in vitro* dissolution tests [1–7]. Along with the determination of physicochemical properties (i.e. solubility, p*K*a, particle size, Log*P*, etc.), dissolution studies in different conditions related to animal or human *in vivo* data are of high interest to develop *in vitro in vivo* relations (IVIVR) or correlations (IVIVC) and predict as early as possible the *in vivo* behavior of active pharmaceutical ingredients (API) after oral administration [8]. Dissolution can be used to select potential drug candidates in preformulation studies and optimize formulation afterward. In preformulation, one of the main challenges is to mimic the *in vivo* limiting process associated with API and formulation and the possible influence of the gastrointestinal environment (i.e. pH, composition of secretions, etc.) under various conditions. For anticipating the behavior of the drug in fasted and fed conditions, classical buffers defined in the pharmacopeias, which cover the physiological pH range, are too simple to simulate environmental conditions, especially in the fed state [9,10]. To achieve a dissolution environment that can reflect *in vivo*

Abbreviations: API, active pharmaceutical ingredients; BCS, biopharmaceutical classification system; IVIVR, *in vitro in vivo* relations; IVIVC, *in vitro in vivo* correlations; FaSSGF, fasted state simulated gastric fluid; FaSSIF, fasted state simulated intestinal fluid; FaSSIFsl, fasted state simulated intestinal fluid *sine* lecithin; FaSSIFsnl, fasted state simulated intestinal fluid *sine* NaTc *sine* lecithin; FeSSGF, fed state simulated gastric fluid; FeSSIF, fed state simulated intestinal fluid; FeSSIFsl, fed state simulated intestinal fluid *sine* lecithin; FeSSIFsnl, fed state simulated intestinal fluid *sine* NaTc *sine* lecithin; NaTc, sodium taurocholate; SGF, Simulated Gastric Fluid; SIF, Simulated Intestinal Fluid; TEM, transmission electron microscope.

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conditions, lots of studies have been done to adjust dissolution media to physiological conditions. One approach consists of addition of enzyme such as pepsin to reflect stomach conditions (Simulated Gastric Fluid, SGF) or pancreatin (Simulated Intestinal Fluid, SIF) to reflect the intestinal compartment [1,2]. Another approach is to simulate, based on physiological data and human aspirates, the dissolution environment by the addition of sodium taurocholate (NaTc) and lecithin. Different biorelevant media (fasted state simulated gastric fluid, FaSSGF and fed state simulated gastric fluid FeSSGF; fasted state simulated intestinal fluid, FaSSIF and fed state simulated intestinal fluid, FeSSIF), have been proposed to reflect better the stomach and proximal small intestine conditions in fasted and fed state [9–11].

These biorelevant media have been widely used to predict the *in vivo* behavior of drug products; especially the BCS (biopharmaceutical classification system) class II and IV APIs [12–16]. The micelles, composed of NaTc and lecithin that mimic the human bile salts in fasted and fed state, allow the biorelevant media to better estimate the solubility and/or dissolution rate of poorly soluble drugs [17–20].

In the fed state, for poorly soluble drugs (class II or IV), bioavailability can be influenced by either secretion associated with the meal intake and/or the lipid content. In the fed state, for example in the standard meals used in bioequivalence studies, fatty materials are always present and can clearly influence drug dissolution and absorption [21]. The presence of fatty component in a meal could largely modify the *in vivo* behavior of the hydrophobic molecules not only by forming micelles with bile salts but also by dissolving the drug molecules in the lipophilic phase itself. Therefore simulation of influence of fatty materials is very critical and addition of such compounds in biorelevant media could improve the predictions in the fed state as long as the stability of the medium and its main physicochemical properties remain constant [12,22–23].

The aim of this work was (i) to study the influence of NaTc and lecithin amount on physicochemical properties of micelles, by comparing the experimental results to reported physiological data, (ii) to perform molecular modeling of micelles composed of NaTc and lecithin in order to investigate drug/micelle interaction and (iii) to investigate the influence of incorporation of fatty material in FeSSIF biorelevant media on its physicochemical properties.

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloric acid (HCl), monopotassium phosphate (KH_2PO_4), sodium hydroxide (NaOH), sodium chloride (NaCl), acetic acid (HAc), monosodium phosphate (NaH_2PO_4), and disodium phosphate (Na_2HPO_4) were purchased from Fisher Scientifique, France. Soybean phosphatidylcholine (S100, lecithin) was purchased from Lipoid, Switzerland. Sodium taurocholate (NaTc) was bought from Prodotti Chimici e Alimentari SpA, Basalozzo, Italy. Capmul MCM EP (Glyceryl Caprylate/Caprate, lot: 100902-6) was provided by ABITEC Corporation.

2.2. Dissolution media

FaSSIF was composed, as described by Dressman et al. [9], using 3 mM NaTc and 0.75 mM lecithin and the pH value was 6.5. A dissolution medium of FaSSIFsl pH 6.5 (*sine* lecithin) and a dissolution medium of FaSSIFsnl pH 6.5 (*sine* NaTc *sine* lecithin) were prepared for a comparative purpose.

FeSSIF was prepared with 15 mM NaTc and 3.75 mM lecithin and the pH value was 5.0 [9]. A dissolution medium of FeSSIFsl pH 5.0 (*sine* lecithin) and a dissolution medium of FeSSIFsnl pH 5.0 (*sine* NaTc *sine* lecithin) were prepared for a comparative purpose.

A series of dissolution media based on FaSSIFsnl and FeSSIFsnl with an increasing NaTc (from 1 mM to 7 mM and from 5 mM to 20 mM for FaSSIF and FeSSIF respectively) and NaTc–lecithin (3–7 mM for NaTc and 0.75–1.8 mM for lecithin for FaSSIF and from 5 mM to 20 mM for NaTc and 1.3 mM to 5.2 mM for lecithin for FeSSIF) were prepared to study the influence of NaTc/lecithin concentration on physicochemical parameters of micelles.

A new dissolution medium with fatty material was prepared. The FeSSIF with Capmul® MCM added was prepared by adding Capmul® MCM at a constant rate (3 mL/min) into FeSSIF under intensive stirring, followed by a stabilization time of at least 4 h before using. Different quantities of Capmul® MCM were added to obtain a series of media of 0.1%, 0.3%, 0.5%, 0.7%, 1.0%, and 5% (v/v) fatty material, simulating fat meal content. According to the FDA standard [24], the fatty material in a standardized high fat meal represents more than 50% of total calories (i.e. 5% represents 50 g/L of fatty material). Physical stability of this new medium (lack of phase separation) as well as the need to create stable micelle limited the amount of fatty material incorporated in FeSSIF to 5%.

All dissolution media were prepared in a volume of 1 L minimum and all the experiments were performed on freshly prepared media.

2.3. Physicochemical characterization of media

2.3.1. pH

A freshly calibrated pH-meter (Inolab pH Level 2, WTW GmbH, Germany) was used for media pH determinations.

2.3.2. Osmolarity

Osmolarity values were measured with an osmometer (Osmometer type 6, Löser Messtechnik, Germany) by determining the freezing-point depression of the media.

2.3.3. Buffer capacity

Buffer capacity was determined according to the USP 30, in which a potentiometric titration method is described [2]. Briefly, the buffer capacity was estimated by dropwise addition of 1 M NaOH, measuring the volume required to change the pH by one unit.

The buffer capacity (β) was calculated using equation:

$$\beta = \frac{\Delta AB}{\Delta pH}$$

where AB is the amount of NaOH added.

2.3.4. Surface tension

Surface tension measurements of the media were conducted by tensiometer (Lauda TD1, Germany) with a platinum lame (19.60 mm top side length, 19.54 mm bottom side length) at 37 °C with Wilhelmy plate method. 10 mL media were sampled after the preparation to determine the surface tension. The determination was realized by detecting the modification of force between platinum plate and liquid samples.

2.4. Micelle characterization

The micelle size and surface charge of micelle were measured with a Zetasizer® nano ZSP (Malvern, France) at room temperature.