Use of biorelevant dissolution media in dissolution tests as a predictive method of oral bioavailability

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Dissolution is a key step in the uptake of oral drugs. In order to compare the behaviour of the dissolution of two formulations, the dissolution profile test was used. This assay must be discriminative and should mimic in vivo conditions. Many dissolution media described in pharmacopoeias are not predictive of bioavailability. Due to this, biorelevant media are used as an alternative to solve this problem. The objective of this work is to evaluate the relevance of biorelevant dissolution media to predict in vivo drug dissolution. For this, a bibliographic search was carried out in scientific databases. The search was first performed for articles verifying the physicochemical properties of human gastrointestinal fluids. Subsequently, a comparison was made between the properties of gastrointestinal fluids and those of biorelevant and pharmacopoeial media. Finally, the results of bioequivalence studies and dissolution profile tests in biorelevant media described in the literature were compared. The results revealed that there are a few publications that have analysed some physicochemical properties of gastrointestinal fluids. In addition, high variability was observed for some properties. Regarding the comparison of these properties with pharmacopoeial media and biorelevant media, the analysis showed that the biorelevant media are more similar to gastrointestinal fluids than the pharmacopoeial media. Finally, the in vitro dissolution profile results were similar to the results obtained in vivo. Thus, biorelevant media may be useful for analysing dissolution profiles.

Keywords: Dissolution profile. Biorelevant media. Human gastrointestinal fluids. Pharmacopoeia media.

INTRODUCTION

Dissolution is an important step in the absorption of orally administered drugs. At this stage, a drug is dissolved in physiological media and is available to be absorbed by the gastrointestinal mucosa (Almukainzi *et al.*, 2014). To compare the behaviour of the in vitro dissolution of two formulations (tablets, capsules, powders for suspensions, and suspensions), the dissolution profile test can be used (ANVISA, 2011; EMA, 2010; FDA, 2015; WHO, 2017).

The dissolution profile can be defined as an in vitro assay that allows a curve of drug percentage

dissolved *vs* time to be constructed, and the conditions established in the dissolution test are generally described in pharmacopoeias. In the absence of a pharmacopoeial dissolution method, the drug product manufacturer must develop and validate the appropriate analytical method for the product, in accordance with the respective legislation of each country. The dissolution profile test is used in the development of new formulations, in the evaluation of post-registration changes and, in some cases, in biowaivering studies (ANVISA, 2011; ANVISA, 2016). This test must be discriminative, allowing the detection of significant changes in formulations and manufacturing processes (Almukainzi *et al.*, 2014).

One of the major challenges in dissolution profile testing is to mimic the in vivo conditions in the body to differentiate the dissolution of two distinct formulations (Klein, 2010).

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Most of the dissolution media described in pharmacopoeias do not have this predictive capacity, since their physicochemical characteristics differ greatly from the conditions found in vivo. Thus, during the development of generic drug products, a similar dissolution profile may lead to a false impression of bioequivalence and thus to a future failure in this test (Klein, 2010; Mudie, Amidon, Amidon, 2010; Honório *et al.*, 2013).

There are several factors that may impact the dissolution of drugs related to dissolution medium, including the presence of food, as well as changes in pH in the fed or fasting state, the type and concentration of surfactants and enzymes, the ionic strength of the dissolution medium, surface tension, volume of medium, osmolarity of medium, and type and concentration of buffer. Thus, it is necessary to develop dissolution media that are more predictive of bioavailability and that can differentiate the release profiles of different formulations (Grignard *et al.*, 2016).

Biorelevant media appear to be an alternative to this problem, since they are described in the literature as more predictive and discriminative than pharmacopoeial dissolution media (Grignard *et al.*, 2016).

Dressman *et al.* (1998) and Jantratid *et al.* (2008) developed dissolution media to simulate dissolution in the small intestine, FaSSIF (fasted-state simulated intestinal fluid) and FeSSIF (fed-state simulated intestinal fluid), which mimic the conditions in the small intestine in fasting and postprandial states, respectively. Vertzoni *et al.* (2005) and Jantratid *et al.* (2008) developed dissolution media to simulate stomach conditions, FaSSGF (fasted-state simulated gastric fluid) and FeSSGF (fed-state simulated gastric fluid), which simulate the conditions in the stomach in fasting and postprandial states, respectively.

The objective of this work is to evaluate the ability of biorelevant dissolution media to predict in vivo dissolution based on technical scientific literature.

MATERIAL AND METHODS

In the present study, bibliographic research was carried out with the scientific databases Scopus (scopus.com), Scielo (scielo.org), Science Direct (sciencedirect.com), Capes Periodics (periodicos.capes.gov.br) and PubMed (ncbi.nlm.nih.gov/pubmed). The articles were selected based on the summary of the articles searched. The articles were not restricted based on the publication year, and articles published until May 2019 were considered.

This work was divided into three phases. First, a search was performed in the scientific literature to verify the physicochemical properties of human gastrointestinal fluids. The pH, buffering capacity, osmolality, surface tension, bile acid concentration and concentration of total proteins in the stomach, duodenal and jejunal fluid were investigated in both the fasted and fed states. For this search, the following keywords were used: human duodenal fluids, human gastric fluids and human gastrointestinal fluids. All these terms were crossed with the Boolean operator "and" and the word "composition" or with the word "properties". For this first topic, an interval was established for each physicochemical property searched based on the standard deviation of the means of the studies found in the literature.

For the second topic, the mean and the respective standard deviation of the analysed studies of the physicochemical properties of human gastrointestinal fluids were compared with the physicochemical properties found in the studies with biorelevant media and pharmacopoeias. In cases where only one article that verified a certain physicochemical property in the gastrointestinal fluids was found, the lower and upper limits of the property analysed in the study were compared.

The biorelevant media selected for comparison were FaSSIF, FeSSIF, FaSSGF and FeSSGF, which are the most described media in the literature. To search for the properties of biorelevant media, the keyword "biorelevant medium" was used. This word was crossed with the Boolean operator "and" and the word "composition" or with the word "properties". To search for the properties of the pharmacopoeial media, the following keywords were used: pharmacopeial media and phosphate buffer 6.8. These terms were crossed with the Boolean operator "and" and the words "composition" or "properties". The pharmacopoeial media used in the comparison were 0.1 M hydrochloric acid (HCl) and phosphate buffer pH 6.8, which are used for dissolution profile tests in biowaiver studies.

Finally, the results of the bioequivalence studies described in the literature were compared with the

dissolution profile test results with biorelevant media. For this, the keywords "in vitro-in vivo correlations" and "biorelevant media" were used. These words were crossed with the Boolean operator "and" and the terms "bioequivalence studies" or "clinical studies".

RESULTS AND DISCUSSION

Physicochemical characteristics of human gastrointestinal fluids

Fasting gastrointestinal fluids

Table I shows the comparison of physicochemical characteristics in different studies found in the literature for fasting gastrointestinal fluids.

TABLE I - Comparison of physicochemical characteristics of fasting gastrointestinal fluids in human beings

Physicochemical Characteristics	Stomach	Duodenum	Jejunum
pH	1.79 - 2.90 (n= 7)	6.49 - 7.37 (n= 11)	6.69 - 7.37 (n= 7)
Buffer capacity ($mmol.L^{-1}. \Delta pH^{-1}$)	13.56 – 18.61 (n= 2)	3.61 - 12.05 (n=3)	1.85 - 6.30 (n= 3)
Osmolality ($mOsm.Kg^{-1}$)	73.44 - 226.06 (n= 6)	156.96 - 241.43 (n=9)	195.30 - 262.70 (n=5)
Superficial tension ($mN.m^{-1}$)	31.96 - 42.45 (n= 3)	31.33 - 39.87 (n=3)	26.84 - 34.76 (n=2)
Total bile acids (mM)	0.06 - 0.68 (n= 4)	1.92 - 6.31 (n=10)	1.58 - 3.40 (n= 4)
Total proteins ($mg.mL^{-1}$)	0.14 – 4.66 (n= 3)	2.30 (n= 1)	0.77 - 2.33 (n= 2)

n= number of studies found
The stipulated range for each physicochemical characteristic was calculated based on the standard deviation of the means found in each individual study.
References: EVANS *et al*., 1988; ARMAND *et al*., 1996; LINDAHL *et al*., 1997; GISOLFI, 1998; PEDERSEN *et al*., 2000; PERSSON *et al*., 2005; BROWERS, 2006; KALANTZI *et al*., 2006a; KALANTZI *et al*., 2006b; MORENO *et al*., 2006; CLARYSSE *et al*., 2009; ANNAERT *et al*., 2010; LU *et al*., 2010; FADDA *et al*., 2010; HOLMSTOCK *et al*., 2013; PEDERSEN *et al*., 2013; WUYTS *et al*., 2013; FOLTZ *et al*., 2015; RABBIE *et al*., 2015; WUYTS *et al*., 2015; LITOU *et al*., 2016; RIETHORST *et al*., 2016

The pH range in the three regions analysed was narrow, and a large number of studies were found, mainly for the duodenal region. The pH of gastrointestinal fluids impacts dissolution, as this property is correlated with drug ionization. Thus, the solubility of a drug with basic characteristics can be increased by lowering the pH of the dissolution medium, while the solubility of an acidic drug can be increased by increasing the pH (Ashford, 2005).

The buffering capacity, as well as the pH, in the verified studies also presented low variability in each region analysed. However, a small number of articles were found for buffering capacity. Buffering capacity is related to the ability of a buffer to maintain pH after the addition of acids and bases. The higher this resistance, the greater the ability to maintain the pH of this solution. Thus, dissolution media with a higher buffering capacity

may have a lower change in pH after the dissolution of acidic/basic drugs compared to dissolution media with a lower buffering capacity. The buffering capacity of the dissolution medium, together with its pH, has a great impact on dissolution, since a change in the pH of a medium can impact the ionization of drugs and consequently their solubility (Jantratid *et al*., 2008).

In contrast to pH and buffer capacity, osmolality presented high variability among the analysed studies, mainly for the stomach fluid region. Thus, similar to pH, a considerable number of articles analysed osmolality in fasted gastrointestinal fluids. The high difference between the values found in various studies may have occurred due to the individual variability of the volunteers and different aspiration and sample storage techniques (Bergstrom *et al*., 2014). The osmolality of the dissolution medium may affect the dissolution of drugs by changing the swelling of the formulation. This property is related to the penetration of water into the formulation. When the difference in the osmotic pressure between the interior of the formulation and the exterior (dissolution media) decreases, the water penetration also decreases, negatively affecting drug release (Rudolph *et al*., 2001).

The surface tension presented a low variability among the analysed studies; however, few articles analysed this physicochemical property in gastrointestinal fluids. The surface tension can also influence the dissolution of drugs since this parameter is related to the wetting of particles. Thus, a high surface tension results in a lower wettability (Dahan, Amidon, 2009). The surface tension of gastrointestinal fluids is considerably lower than that of water due to the presence of surfactants in the region (Dressman *et al*., 1998).

A large number of studies have analysed the concentration of total bile acids in gastrointestinal fluids, mainly in the duodenal region. The concentration of total bile acids is higher in the duodenum and jejunum than in the stomach. The presence of bile acids may change the bioavailability of poorly water-soluble drugs by enhancing the rate of dissolution. This may occur by increasing the rate of dissolution through a decrease in the interfacial energy barrier between the solid drug and the dissolution medium (enhanced wetting), leading to an effective increase in surface area or an increase in solubility via micellar solubilization (Horter, Dressman, 2001; Baxevanis, Kuiper, Fotaki, 2016).

A small number of articles that studied the total protein concentration were found. Proteins can degrade drugs such as nucleotides and fatty acids and may affect the release of drugs containing lipid substances (Ashford, 2005).

Gastrointestinal fluids in the fed state

Table II shows the comparison of physicochemical characteristics in different studies found in the literature search for gastrointestinal fluids in the fed state.

TABLE II - Comparison of physicochemical characteristics of gastrointestinal fluids in the fed state in human beings

Physicochemical Characteristics	Stomach	Duodenum	Jejunum
pH	4.90 - 5.47 (n=1)	5.85 - 6.22 (n=6)	6.10 (n=1)
Buffer capacity (mmol.L^{-1}. ΔpH^{-1})	22.50 - 30.00 (n=1)	19.05 - 26.90 (n=2)	14.60 (n=1)
Osmolality (mOsm.Kg^{-1})	475.00 - 515.00 (n=1)	332.09 - 528.17 (n=5)	-
Superficial tension (mN.m^{-1})	-	26.91 - 35.50 (n=2)	27.00 (n=1)
Total bile acids (mM)	-	5.14 - 11.32 (n=7)	8.00 (n=1)

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TABLE II - Comparison of physicochemical characteristics of gastrointestinal fluids in the fed state in human beings

Physicochemical Characteristics	Stomach	Duodenum	Jejunum
Total proteins (mg.mL^{-1})	11.10 - 24.60 (n=1)	-	5.00 (n=1)

n= number of studies found
The stipulated range for each physicochemical characteristic was calculated based on the standard deviation of the means found in each individual study.
References: ARMAND *et al.*, 1996; PERSSON *et al.*, 2005; KALANTZI *et al.*, 2006b; CLARYSSE *et al.*, 2009; DIAKIDOU *et al.*, 2009; HOLMSTOCK *et al.*, 2013; WUYTS *et al.*, 2013; WUYTS *et al.*, 2015; RIETHORST *et al.*, 2016.

A large number of papers was found for the duodenum region. For the stomach and jejunum, only one study carried out an evaluation of the physicochemical characteristics in the fed state.

The pH range of the stomach fluid in the fed state (4.90-5.47) was greater than that in the fasting state, which presented pH values between 1.70 and 2.90. Thus, it was observed that the stomach pH was high in the presence of food. In contrast, it was verified that the mean pH of the duodenal fluid in the fed state, with values between 5.85 and 6.22, and the pH of the jejunum fluid, with a value of pH 6.10, were lower than the pH of the duodenal fluid in the fasted state, with a pH range of 6.49 to 7.37; the pH range of the fasting jejunum fluid was 6.69 to 7.37. Therefore, it was concluded that the pH of the small intestine decreases in the presence of food.

In the stomach, the dissolution of acidic drugs will increase in the fed state compared to the fasting state; in the gut, the opposite occurs (Jones *et al.*, 2006).

By observing the results, it was suggested that the presence of food enhances the buffering capacity of gastrointestinal fluids. For example, the comparison of fasting stomach fluid with an average buffering capacity of 13.56 to 18.61 mmol.L^{-1}.ΔpH^{-1} and fed fluid in the fed state with a mean buffering capacity of 22.50 to 30.00 mmol.L^{-1}.ΔpH^{-1}; the comparison of fasting duodenal fluid with a mean buffering capacity of 3.61 to 12.05 mmol.L^{-1}.pH^{-1} and duodenal fluid in the postprandial state with a mean buffer capacity of 19.05 to 26.90 mmol.L^{-1}.pH^{-1}; and the comparison of fasting jejunum fluid in with an average buffer capacity of 1.80 to 6.30 mmol.L^{-1}.pH^{-1}

and jejunum fluid in the postprandial state with a mean buffering capacity of 14.60 mmol.L^{-1}.pH^{-1}.

As the pH of the medium influences the dissolution of a drug and the buffering capacity is related to the ability of the pH of this medium to be maintained after the addition of acids and bases, it can be suggested that the presence of a certain type of food can increase the buffering capacity of the gastrointestinal fluids and consequently alter the dissolution of drugs (Horter, Dressman, 2001; Baxevanis, Kuiper, Fotaki, 2016).

The results of osmolality studies suggest that the presence of food increases the osmolality of gastrointestinal fluids; for example, the comparison of fasting stomach fluid with a mean osmolality of 73.44 to 226.06 mOsm.kg^{-1} and stomach fluid in the fed state with mean osmolality of 475.00 to 515.00 mOsm.kg^{-1} and the comparison of fasting duodenal fluid with a mean osmolality of 156.96 at 241.34 mOsm.kg^{-1} and duodenal fluid in the postprandial state with a mean osmolality of 332.09 to 528.17 mOsm.kg^{-1}.

Osmolality can influence the dissolution of a drug due to the change in swelling of the formulation, since this physicochemical property is correlated with the degree of water penetration (Baxevanis, Kuiper, Fotaki, 2016).

Surface tension values were similar in the fasted and fed states. However, few studies that examined both nutritional states evaluated this physicochemical property.

In relation to the concentration of total bile acids, it was observed that there was a small increase in the concentration of total bile acids in the fed state compared to that of the fasting for the intestinal region. This fact

is correlated with increased fatty acid secretion after feeding (Almukainzi *et al.*, 2014).

Finally, the concentration of total proteins in the fed state is dependent on the type of feed consumed; therefore, the concentration of total proteins is highly variable, so it cannot be defined in a single study (Baxevanis, Kuiper, Fotaki, 2016).

Comparison of the physicochemical properties of gastrointestinal fluids with the biorelevant and pharmacopoeial dissolution media

Stomach fasting

Table III compares the physicochemical properties of fasting stomach fluid with the biorelevant medium FaSSGF and with the 0.1 M HCl pharmacopoeial media. Fasting stomach fluid data were drawn from the ranges in Table I for the fluid stomach. On the other hand, the HCl 0.1 M medium was chosen because it is the dissolution medium used to analyse biowaivering, according to ANVISA (Brazilian National Surveillance Agency), EMA (European Medicines Agency), FDA (Food and Drug Administration) and WHO (World Health Organization) (ANVISA, 2011; EMA, 2010; FDA, 2015; WHO, 2017). The FaSSGF medium developed by Vertzoni *et al.* (2005) was selected, since this was the first biorelevant medium developed to simulate the stomach fluid and because it is one of the most widely used biorelevant media.

TABLE III - Comparison of the physicochemical properties of fasting stomach fluid, the biorelevant dissolution medium FaSSGF and the dissolution medium 0.1 M HCl pH 1.2

Property	Stomach fluid in fasting	FaSSGF[a]	HCl 0,1 M pH 1,2[b]
pH	1.79 - 2.90 (n= 7)	1.60	1.20
Buffer capacity (mmol.L^{-1}. ΔpH^{-1})	13.56 – 18.61 (n= 2)	-	-
Osmolality (mOsm.Kg^{-1})	73.44 - 226.06 (n= 6)	120.70 ($\pm$2.50)	188.00 ($\pm$0.30)
Superficial tension (mN.m^{-1})	31.96 - 42.45 (n= 3)	42.60	54.60 ($\pm$0.20)
Total bile acids (mM)	0.06 - 0.68 (n= 4)	0.08	0.00
Total proteins (mg.mL^{-1})	0.14 – 4.66 (n= 3)	0.10	0.00

Reference: [a] VERTZONI *et al.*, 2005; [b] PEDERSEN *et al.*, 2013.

It can be observed that the pH of the biorelevant medium FaSSGF is closer to the reported pH range of fasting stomach fluid than that of the 0.1 M HCl medium. The more acidic pH value of the pharmacopoeial medium may influence the degree of drug ionization and thus influence drug dissolution (Ashford, 2005).

In relation to the buffer system, no studies were found with buffering capacity results, since no buffer system was used to prepare the media (Farmacopeia Brasileira 5ª Ed, 2010; Vertzoni *et al.*, 2005). The buffering capacity is dependent on the pH of the dissolution medium, the pK$_a$ of the buffer and the concentration of the buffer (Mudie,

Amidon, Amidon, 2010). Thus, as no buffer system is used in the biorelevant medium FaSSGF or in the pharmacopoeial medium, there is a possibility that a change in the pH of these media occurs after the dissolution of basic drugs, thus causing a change in the degree of drug ionization and, consequently, drug dissolution thereof. This event may not occur in gastric fluid because a buffer system is present. Thus, a different dissolution behaviour can be observed in the biorelevant medium and the pharmacopoeial medium compared to the fasting stomach fluid.

It was found that the osmolarity values of both the biorelevant medium and the 0.1 M HCl medium are similar to that of fasting gastric fluid. Osmolality in the stomach fluid is mainly determined by Cl^-, Na^+, K^+ and Ca^{2+} electrolytes (Abeele *et al.*, 2017). The difference in osmolality between the pharmacopoeial medium and the FaSSGF medium can cause a greater osmotic difference between the interior of the formulation and the dissolution medium and thus cause a greater penetration of water into the formulation, resulting in a difference in dissolution behaviour of the same formulation in these two media (Rudolph *et al.*, 2001).

It can be verified that the surface tension one of the pharmacopoeial media is greater than that of the biorelevant medium and that of the fasted gastrointestinal fluid. The difference between the pharmacopoeial medium and the biorelevant medium is due to the presence of sodium taurocholate, a bile acid, and lecithin, a phospholipid (Vertzoni *et al.*, 2005). This physicochemical property can influence the wetting of a drug particle. Dissolution medium with low surface tension results in further wetting of a drug and, as a result, greater dissolution (Dahan, Amidon, 2009). Thus, a difference in the dissolution profile behaviour of a tested formulation in 0.1 M HCl medium compared

to that in FaSSGF medium and fasted gastric fluid can be expected.

It was found that the pharmacopoeial media did not contain bile acids. Sodium taurocholate was present in the biorelevant media at a concentration of 0.08 mM, a value in the concentration range found in fasting stomach fluid. Bile acids, as already mentioned, are correlated with a decrease in the surface tension of media and are therefore important constituents in drug dissolution, especially for poorly soluble drugs (Almukainzi *et al.*, 2014).

Finally, an absence of proteins in the 0.1 M HCl medium was observed. The concentration of total proteins in the biorelevant media was found to be somewhat below that in gastric fluid. The concentration of 0.10 mg.mL^{-1} protein in the FaSSGF medium is due to the addition of pepsin. The presence of enzymes in the gastrointestinal tract may impact drug dissolution and stability (Grignard *et al.*, 2016).

It can be concluded that although some differences between the FaSSGF medium and the fasted stomach fluid exist, the FaSSGF medium is much more similar to the fasted stomach fluid than the media recommended by pharmacopoeias. Thus, this biorelevant medium is an alternative to 0.1 M HCl medium for dissolution profile evaluation.

Stomach in the fed state

Table IV shows a comparison between the physicochemical properties of the stomach fluid in the fed state with the biorelevant medium FeSSGF. Data about stomach fluid in the fed state were collected from the intervals in Table II. The FeSSGF medium, developed by Jantratid *et al.* (2008), was selected since it is one of the most widely used biorelevant media.

TABLE IV - Comparison of the properties of the stomach fluid in the fed state with the FeSSGF dissolution medium

Property	Stomach Fluid Postprandial	FeSSGF[a]
pH	4.90 - 5.47 (n=1)	5.00[a]
Buffer capacity (mmol.L^{-1}. ΔpH^{-1})	22.50 - 30.00 (n=1)	25.00[a]
Osmolality (mOsm.Kg^{-1})	475.00 - 515.00 (n=1)	400.00[a]
Superficial tension (mN.m^{-1})	-	52.30[b]
Total bile acids (mM)	-	0.00
Total proteins (mg.mL^{-1})	11.10 - 24.60 (n=1)	-

Reference: [a]JANTRATID *et al.*, 2008; [b] KOZIOLEK *et al.*, 2013.

It can be observed that the pH and buffer capacity values of the biorelevant medium FeSSGF are within the range found for those of the stomach fluid in the fed state. The osmolality value of the biorelevant medium is below the that of the stomach fluid in the fed state. It was not possible to compare the surface tension and total bile acid values, as no study presented data about these properties in the postprandial stomach fluid. The total protein concentration could also not be compared because in the studies analysed, the concentration of total protein in the biorelevant media was not measured.

It can be concluded that despite the physicochemical differences between stomach fluid and the biorelevant medium FeSSGF, the latter appears to be an alternative medium that mimics the in vivo conditions of stomach fluid; unlike the pharmacopoeial media, FeSSGF medium simulates the presence of food in the stomach. In addition, few studies have analysed the physicochemical properties of the fed gastric fluid, making it difficult to compare the properties of fed gastric fluid with those of biorelevant media.

Intestinal fasting

Table V presents a comparison between the physicochemical properties of fasting duodenal fluid, fasting jejunum fluid, the biorelevant medium FaSSIF and pharmacopoeial buffer pH 6.8. Data from fasted intestinal fluids were drawn from the intervals in Table I for duodenal fluid and fasting jejunum. The phosphate buffer medium pH 6.8 of the European Pharmacopoeia 3rd edition was chosen, as the physicochemical properties of this dissolution medium were found in articles in the literature. The FaSSIF medium, developed by Dressman *et al.* (1998), was selected, since this was the first biorelevant medium developed to simulate fasting intestinal fluid and because it is one of the most widely used biorelevant media.

TABLE V - Comparison of the physicochemical properties of fasting duodenal fluid, fasting jejunum fluid, dissolution medium FaSSIF and the pharmacopoeic dissolution medium pH 6.8 phosphate buffer

Property	Fasting duodenal fluid	Fasting jejunum fluid	FaSSIF	Phosphate buffer pH 6,8
pH	6.49 - 7.37 (n= 11)	6.69 - 7.37 (n= 7)	6.80	6.80[b.c]

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TABLE V - Comparison of the physicochemical properties of fasting duodenal fluid, fasting jejunum fluid, dissolution medium FaSSIF and the pharmacopoeic dissolution medium pH 6.8 phosphate buffer

Property	Fasting duodenal fluid	Fasting jejunum fluid	FaSSIF	Phosphate buffer pH 6,8
Buffer capacity (mmol.L^{-1}. ΔpH^{-1})	3.61 - 12.05 (n=3)	1.85 - 6.30 (n= 3)	8.00 - 12.00	18.60[b]
Osmolality (mOsm.Kg^{-1})	156.96 - 241.43 (n=9)	195.30 - 262.70 (n=5)	280.00 -310.00	99.30 - 116.10[b.c]
Superficial tension (mN.m^{-1})	31.33 - 39.87 (n=3)	26.84 - 34.76 (n=2)	-	-
Total bile acids (mM)	1.92 - 6.31 (n=10)	1.58 - 3.40 (n= 4)	5.00	0.00
Total proteins (mg.mL^{-1})	2.30 (n= 1)	0.77 - 2.33 (n= 2)	0.00	0.00

Reference: [a]DRESSMAN *et al.*, 1998; [b]STIPPLER; KOPP; DRESSMAN, 2004; [c]DE SPIEGELER *et al.*, 2007.

Both the FaSSIF biorelevant medium and the phosphate buffer pH 6.8 medium exhibit pH values within the reported pH ranges of fasting duodenal fluid and fasting jejunum fluid.

The buffering capacity of the dissolution medium FaSSIF was within the range of the buffering capacity of duodenal fluid. Jejunum fluid, however, has a lower buffer capacity than the biorelevant medium FaSSIF. The pharmacopoeial media had a higher buffer capacity than the intestinal fluids. The higher buffer capacity of phosphate buffer pH 6.8 may lead to a difference in the dissolution profile compared to intestinal fluids, since a higher resistance in maintaining the pH in the pharmacopoeial media may occur after the dissolution of acidic and basic drugs (Jantratid *et al.*, 2008).

It has been verified that the osmolality values of FaSSIF medium are higher than those of intestinal fluids. In contrast, the osmolality of the pharmacopoeial media was lower than those of intestinal fluids. This discrepancy between the osmolality values may cause a greater osmotic difference between the interior of the formulation and the dissolution medium and thus cause a difference in the amount of water penetration in the formulation, causing a difference in the dissolution profile of the formulation in those media (Rudolph *et al.*, 2001).

It was not possible to compare the surface tension values because no study analysed the surface tension of FaSSIF or pharmacopoeial media.

In relation to the concentration of bile acids, the absence of these acids in the pharmacopeial media was verified. The biorelevant medium contained sodium taurocholate at a concentration of 5.00 mM, close to the concentration of bile acids found in the intestinal fluids (Lindahl *et al.*, 1997).

Finally, the absence of proteins in the FaSSIF media and in the phosphate buffer medium pH 6.8 was observed.

Thus, properties of both media differ compared to those observed in intestinal fluids. However, compared to the pharmacopoeial media, the biorelevant medium still exhibits properties more similar to those of intestinal fluids.

Intestine in the fed state

Table VI shows the comparison of the physicochemical properties of duodenal fluid in the fed state, postprandial jejunum fluid and the biorelevant medium FeSSIF. Data on intestinal fluid in the fed state were collected from the intervals in Table II. The FeSSIF medium, developed by Jantratid *et al.* (2008), was selected because it is one of the most widely used biorelevant media in dissolution profile testing because it mimics the postprandial state (Almukainzi *et al.*, 2014).

TABLE VI - Comparison of the properties of the duodenal fluid in the fed state, jejunum fluid in the fed state, FeSSIF dissolution medium

Propriedade	Duodenal fluid in the fed state	Jejunum fluid in the fed state	FeSSIF
pH	5.85 - 6.22 (n=6)	6.10 (n=1)	5.80[a]
Buffer capacity (mmol.L^{-1}. ΔpH^{-1})	19.05 - 26.90 (n=2)	14.60 (n=1)	25.00[a]
Osmolality (mOsm.Kg^{-1})	332.09 - 528.17 (n=5)	-	380.00 -400.00[a]
Superficial tension (mN.m^{-1})	26.91 - 35.50 (n=2)	27.00 (n=1)	32.20 - 33.20[b]
Total bile acids (mM)	5.14 - 11.32 (n=7)	8.00 (n=1)	7.50[a]
Total proteins (mg.mL^{-1})	-	5.00 (n=1)	0[a]

Reference: [a]JANTRATID *et al.*, 2008; [b] JANTRATID, DRESSMAN, 2009.

It can be observed that the biorelevant medium FeSSIF presents pH values close to the pH values of duodenal fluid and jejunum fluid in the fed state.

The buffering capacity of FeSSIF medium is within the buffering capacity range found for duodenal fluid in the postprandial state. However, the value of this physicochemical property was higher in the FeSSIF medium than in fluid of the jejunum in the fed state. The high buffering capacity observed in the FeSSIF medium mimics what occurs in vivo, since food intake may cause a greater buffering effect compared to the fasted state (Fotaki, Vertzoni, 2010).

The FeSSIF dissolution medium showed osmolality values within the range found in the duodenal fluid. It was not possible to carry out the comparison between the osmolarity of biorelevant medium and jejunum fluid, as no articles were found in the literature analysed this property in the postprandial jejunum fluid.

The surface tension of the biorelevant medium FeSSIF was close to the surface tension of duodenal fluid in the fed state. The surface tension values of the biorelevant medium are higher than those of jejunum fluid.

It was observed that the total bile acids of the biorelevant medium were within the range of the total bile acids in intestinal fluids. The small intestine in the fed state tends to have an increased concentration of bile acids, increasing the solubility of poorly soluble drugs (Fotaki, Vertzoni, 2010; Lindalh *et al.*, 1997).

The concentration of total proteins could not be compared due to the absence of proteins in the biorelevant medium.

Thus, it can be concluded that despite the physicochemical differences between the duodenal fluid and the biorelevant medium FeSSIF, the physicochemical properties of this medium are similar to those of duodenal fluid. It was difficult to conclude how similar the jejunum fluid was to the biorelevant medium, since only the article of Persson and collaborators (2005) presented data on the physicochemical properties in this region of the small intestine.

Therefore, it is possible to suggest that the biorelevant medium FeSSIF is a suitable means for simulating dissolution occurring in vivo in the duodenum.

In vivo prediction

In vitro-in vivo correlation (IVIVC) is the establishment of a relationship between an in vitro measurement for a pharmaceutical product and a response generated by it in an in vivo condition. IVIVC refers to the establishment of a rational relationship between the behaviour of a physicochemical assay, such as in vitro dissolution, and the biological properties produced by a particular dosage form, such as the maximum concentration (C_{max}) and the area under the curve (AUC) (Chiann, 2009).

If a strong relationship can be established between an in vitro response and in vivo measurement, it may be possible to exempt a particular drug from a bioequivalence study. Bioequivalence studies are often costly and time consuming and expose healthy volunteers to risks caused by the use of a drug (Chiann, 2009). It is mainly possible to establish an IVIVC for class II drugs of the biopharmaceutical classification system because in vitro dissolution is the limiting stage for IVIVC. For class III and IV drugs, the drug permeability influences the assessment of IVIVC (Amidon *et al.*, 1995).

It is important to consider that in some cases, no IVIVC is established, although a prediction of in vivo behaviour can be made by in vitro results. This is the case when in vitro dissolution discriminates some formulations, and these variations are also observed in vivo. The IVIVC must be considered when evaluating the use of biorelevant media.

Literature on the use of biorelevant media in IVIVC is lacking, and most of the studies are dedicated to using these media as a discriminative system to choose formulations before in vivo studies. Some examples are mentioned below.

In a study with 100 mg danazol (BCS class II) capsules, the dissolution profile of commercial Danatrol® samples was compared in the following dissolution media: water, FeSSIF, FaSSIF and simulated intestinal fluid (SIF) (Dressman, Reppas, 2000). In SIF and water, where no bile salts were used, there was no drug release. In FeSSIF and FaSSIF, which contain bile salts, the dissolution detected was low. It was also observed that the dissolution of this drug in FeSSIF medium was higher than that in FaSSIF medium, concluding that there was an increase in the dissolution of this drug in the presence of a higher concentration of bile salts (Dressman, Reppas, 2000; Lindahl *et al.*, 1997).

A lipid emulsion formulation was tested in vivo, and C_{max} and AUC increased threefold compared to the fasting state (Charman *et al.*, 1993; Dressman, Reppas, 2000). There was a higher concentration of bile salts in the fed state than in the fasting state. This fact is fundamental to increase the dissolution of poorly soluble drugs (Fotaki, Vertzoni, 2010). Therefore, FeSSIF dissolution medium may be an alternative for the evaluation of danazol

drug dissolution profiles since the in vivo results were consistent with the in vitro results.

In a clinical study with nifedipine (BCS class II), the effect of 60 mg ADALAT XL® was influenced by food (observed by variations in AUC and C_{max}), which did not occur for 30 mg ADALAT Eins® (Schug *et al.*, 2009). In an in vitro study, both products were evaluated in different dissolution media (Andreas *et al.*, 2016). Through in vitro-in vivo correlation studies, the C_{max} ratio of the fed/fasted state of the in vitro study was compared with the food/fasting ratio of the in vivo tests.

Therefore, it was possible to differentiate the formulations through the use of biorelevant media, as well as to verify the effect of food on drug release. It was also possible to correlate the in vitro results with clinical studies because the fasting food ratio of the in vivo study was similar to that of the in vitro study. The dissolution profile of the pharmacopoeial medium showed a strong discrepancy with the results of biorelevant media due to an excessive release of the drug in the pharmacopoeial medium caused by the presence of sodium dodecyl sulfate. It is known that this surfactant can excessively increase the dissolution of poorly soluble drugs and thus generate a false impression of bioequivalence (Andreas *et al.*, 2016).

Wei & Loberberg (2006) showed that the absorption of glibenclamide (BCS class II) is formulation dependent. These authors evaluated the dissolution profile of two commercial glibenclamide tablets in different media. They also performed permeability analysis using Caco-2 cells and a computer simulation using GastroPlus™ software. The results obtained were compared with a bioequivalence study. The dissolution medium FaSSIF LQ showed the best IVIVC, demonstrating a linear correlation of 0.94 for the reference drug product and 0.93 for the test medicine (Wei, Lobenberg, 2006).

In an in vitro study with the drug pantoprazole, two products were evaluated in the biorelevant media FaSSIF (pH = 6.5) and FeSSIF (pH = 6.0) (Campos, 2008). The calculated F2 values were 78.87 (for FaSSIF) and 58.25 (for FeSSIF); therefore, the formulations were considered to have equivalent dissolution profiles. In the same work, a bioequivalence study was conducted in the fed and fasting states. The formulations were not

considered bioequivalent in the postprandial study. Thus, FeSSIF medium is not an appropriate medium for the in vitro evaluation of pantoprazole, and in vivo studies of postprandial are not included due to the high variability of the drug under these conditions. Therefore, FaSSIF medium is a suitable medium to mimic gastrointestinal fluids for pantoprazole.

CONCLUSION

After searching the literature for articles that analysed the physicochemical properties of gastrointestinal fluids, it can be concluded that there are only a few references relative to human beings. We also observed a smaller number of studies that investigated various properties in the fed state. In addition, it was found that most studies were performed with a small number of volunteers. Despite the limitations of these studies, the present paper verified the range of physicochemical properties in gastrointestinal fluids, and the results will be of paramount importance for the development of biorelevant media that are more predictive of bioavailability.

Thus, there is a need for more research investigating the various properties of gastrointestinal fluids, both in the fasted and fed states. In addition, these studies should be conducted with a large number of volunteers due to the high variability in gastrointestinal fluids. These works will be of great importance for the development of biorelevant media with physicochemical properties more similar to in vivo conditions.

A great difference was observed between the physicochemical properties of gastrointestinal fluids and pharmacopoeial media, mainly for surface tension and the concentration of bile acids. These properties are fundamental in drug dissolution, especially for poorly soluble drugs. This fact may lead to a difference in the behaviour of drug dissolution between these two media, thus leading to a false idea of bioequivalence when analysing the dissolution profile in a pharmacopoeial medium.

In addition, few studies in the literature have analysed the physicochemical properties of pharmacopoeial media. Due to the ease of carrying out these analyses, it is suggested that future studies should investigate these properties in dissolution media.

The comparative analysis of the physicochemical properties of biorelevant media with gastrointestinal fluids demonstrated that the properties of the biorelevant medium were more similar to those of the gastrointestinal fluids in comparison to those of the pharmacopoeial media studied. However, significant differences were observed in these properties, mainly in relation to protein concentration, which was higher in gastrointestinal fluids.

Finally, results from in vitro dissolution profile assays using biorelevant media were compared to the results obtained in vivo, demonstrating, in some cases, that the biorelevant media present similar results to those observed in vivo. However, few articles found in the literature made this comparison. In addition, the bioequivalence studies found used few volunteers in their analysis. The most robust bioequivalence studies have been performed by the pharmaceutical industry and are not published. Thus, a larger number of studies are needed to compare drug dissolution profiles in biorelevant media with the results of bioequivalence studies. This will give greater certainty that biorelevant media can more accurately predict bioavailability than pharmacopoeial media.

Thus, biorelevant media may be useful for dissolution profile analysis in the development of generics, new drug products, and post-registration changes. However, more studies are required to verify the effectiveness of such media in the prediction of bioavailability. In addition, the cost of employing these dissolution media as well as the greater complexity in the preparation of these media and quantification techniques should be considered.

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