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Update on Gastrointestinal Biorelevant Media and Physiologically Relevant Dissolution Conditions

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ABSTRACT

Dissolution testing constitutes one of the most widely used in vitro performance tests during drug development and routine quality control testing. It monitors the rate and extent of in vitro drug release (batch release test), and it is often used to ensure consistent in vivo performance. The purpose of this review is to summarize and update the many physiologically adapted media and buffers proposed over the years, focusing on the upper gastrointestinal tract because this is where most drug absorption occurs. Emphasis will be given on the application of bicarbonate-based media because this is the major buffering species in the human intestinal lumen. Due to the pragmatical difficulties of using bicarbonate-based dissolution media, surrogate media with simpler buffer systems are desirable. Herein we describe some of the proposed models and different approaches to develop such substitutes. Special consideration has to be taken when dealing with enteric coated (delayed release) formulations because the interaction of coating polymer with bicarbonate is very complex. All factors considered, using physiologically relevant conditions can ameliorate the risks and enable drug development with increased likelihood to select formulations with the desired in vivo performance.

KEYWORDS: Bicarbonate buffer, dissolution, physiologically relevant, biorelevant, enteric coating

BACKGROUND

Dissolution testing constitutes one of the most widely used in vitro performance tests during drug product development and routine quality control testing. It monitors the rate and extent of in vitro drug release (batch release test), and it is often used to ensure consistent in vivo performance (*1*, *2*). The description of standard dissolution apparatus by the United States Pharmacopeia (USP) in the 1970s together with guidance from the United States Food and Drug Administration (FDA) in the late 90s propelled its broad application during the various stages of drug development (*1*, *3*). Alongside that, the introduction of the Biopharmaceutics Classification System (BCS) in 1995 provided a simple but robust way to mechanistically describe the biopharmaceutical behavior of a drug (*4*). Under this system, drugs are classified based on their solubility and permeability. These parameters may be used to predict the fraction of dose absorbed and consequently its chances to become bioavailable (*5*, *6*).

At the time when USP apparatus 1 and 2 were introduced and FDA guidances were published, most of the molecules under development presented good aqueous solubility (BCS classes 1 and 3), and conventional dosage forms (capsules and tablets) were the most common. Hence, establishing in vitro dissolution conditions with presumed in vivo relevance was reasonably simple (*1*, *7*). The development scenario has changed to molecular entities that are more potent accompanied with lower aqueous solubility (BCS classes 2 and 4). Although these drug substances have enhanced many therapies by acting on new molecular targets, they also present significant formulation and process development challenges, especially regarding the biopredictive power of previous traditional in vitro performance test methods (*8*). Hence, there was a need for advancement in the field of dissolution testing (e.g., development of biorelevant and physiologically relevant dissolution methods) to address the shortfalls of traditional methods.

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Accordingly, the use of dissolution testing has expanded beyond the routine end-product release application to a comprehensive analysis that can be implemented at the various stages of the product life cycle (*1*, *5*). Changes in the regulatory landscape, such as the introduction of quality by design (QbD) concepts, have also contributed to the progression of dissolution methodology, linking quality tests to product performance in patients and ultimately therapeutic outcomes. Hence, there was a push to develop dissolution media and apparatus that may mimic the human gastrointestinal (GI) tract to further understand in vivo dissolution mechanisms. The innovation in this field has also evolved to the integration of in vitro dissolution data, applying different methods and analytical techniques with modeling and simulation, and correlating with in vivo data (*9*–*12*). This approach is a robust way to select the best formulation with the desired in vivo performance.

The purpose of a particular dissolution test varies at the different stages of development (*1*). As first introduced by Azarmi et al., there might be a need for more than one dissolution test for the same product (*13*). For example, a quality control (QC) dissolution test is usually used to identify possible variations during product manufacturing and/or changes in product storage that could have an impact on the product's performance. This method needs to be simple to be used in a typical routine QC environment, such as conventional USP apparatus 1 or 2 and simple buffer media. At the same time, this method has to demonstrate an appropriate level of discriminatory power to confirm product consistency. On the other hand, a biorelevant/ physiologically relevant dissolution method applies conditions that mimic the different physiological environments. These usually consist of non-compendial media and apparatus, such as bicarbonate-based buffers, biphasic dissolution to assess the impact of concurrent drug absorption and multiple compartmental apparatuses (*14*–*16*). This methodology is mostly used to guide formulation selection and optimization. It typically starts during early development and may continue through clinical testing and beyond. Lastly, a clinically relevant dissolution method is any particular method in which a link between in vitro dissolution data with in vivo pharmacokinetic (PK) data can be established, creating an in vitro-in vivo correlation or relationship (IVIVC or IVIVR), which is important for lifecycle management. The different methods used may or may not overlap with each other. However, they are useful during the research and development stage where in vivo insight is desirable. The information retrieved from such methods can then be used to set specifications for the QC method to be used for regulatory applications, meeting the specified criteria by the regulatory agency.

In vivo drug dissolution depends on the drug's physicochemical properties and the GI fluid environment. The current understanding of the human GI physiology allowed biorelevant dissolution media (BDM) to evolve, facilitating the in vitro prediction of in vivo dissolution performance (*12*, *17*–*20*). The many proposed BDM include various properties of the human GI tract, such as pH, buffer species, buffer concentration, osmolality, viscosity, surface tension, concentration, type of bile salts and lipolysis products, and physiological state, such as fasted and fed states (*21*–*24*). Evidently conventional dissolution media, such as simple USP buffers, fall short in mimicking the properties and composition of GI fluids (*17*). At the same time, the compendial methods that are approved by a given regulatory agency for products marketed in that region cover different types of drug substances and dosage forms.

A more accurate prediction of the drug product's in vivo performance is expected the closer the in vitro conditions are to the in vivo environment. However, depending on the information one is seeking or on the physicochemical properties of the API (e.g., BCS class I), simulating all aspects of the GI tract may or may not be necessary to evaluate the drug product performance. Based on this, Markopoulos et al. have suggested levels of simulation of luminal composition, as follows: Level 0 (pH); Level I (pH and buffer capacity); Level II (pH, buffer capacity, bile components, dietary lipids, lipid digestion products, and osmolarity) and Level III (pH, buffer capacity, bile components, dietary lipids, lipid digestion products, osmolarity, proteins, enzymes, and viscosity effects) (*25*).

The purpose of this review is to summarize and update the many physiologically adapted media and buffers proposed over the years, focusing on the upper GI tract because this is where most drug absorption occurs. Emphasis will be given on the application of bicarbonate-based media because this is the major buffering species in the human intestinal lumen.

PHYSIOLOGICALLY RELEVANT MEDIA

Gastric Environment

The composition, pH, and surface tension are important aspects to be considered when simulating the gastric fluid (Table 1). The composition of the stomach fluid is not merely hydrochloric acid (HCl); it also contains saliva, digestive enzymes (pepsin and gastric lipase), food, and refluxed fluids from the duodenum (*26*). The pH of gastric fluids can vary greatly depending on the physiological

state (fed vs fasted), health-related conditions (such as achlorhydria), and pharmacological treatments (such as anti-acid agents). The reported pH range of gastric fluids is 1.5–1.9 under fasted conditions and 3.0–7.0 under fed conditions (the rate in which the pH changes is strongly related to the type and size of the meal) (*27–30*). The reported surface tension in gastric fluids ranges from 30–46 mN/m (*28*, *31*, *32*). This could be indicative of the presence of surface-active agents, such as lecithin and lysolecithin (*33*).

One of the earliest proposed media to simulate the stomach in the fasted state was the artificial gastric fluid (AGF), described by Ruby at al. in 1996 (*34*). The compendial simulated gastric fluid (SGF) and its version without pepsin (SGFsp) described in the USP presents a different composition than AGF, but a similar pH (pH 1.2), as shown in Table 1 (*35*). Many aspects of the gastric juice are addressed in these media, but qualities such as pH, surface tension and pepsin concentration could be more reflective of the in vivo values.

To mimic in vivo conditions as closely as possible, Vertzoni et al. designed a fasted state simulating gastric fluid (FaSSGF) including compounds found in the intragastric environment, such as pepsin and sodium taurocholate (*19*). Even though the use of physiologically relevant surfactants is desirable to mimic in vivo conditions as closely as possible, these media can be unstable, difficult to prepare, and costly. Hence, synthetic surfactants, such as sodium lauryl sulphate (SLS) and polyethylene glycol tert-octylphenyl ether (Triton X 100) are often used as an alternative. These surfactants are added into compendial simulated gastric fluid without pepsin to form SGFSLS and SGFTriton, respectively (Table 1). This can be an interesting approach, but on the other hand it is important to be aware that different types of surfactants can impact the product's performance, leading to erroneous predictions of drug dissolution (*19*, *36*).

Another important aspect to consider is the difference of fasted versus fed physiological states. Macheras et al., proposed the use of milk as a medium that can simulate gastric components in the fed state because it contains similar ratios of fat, protein, and carbohydrates present in the western diet (*37, 38*). However, there are some drawbacks with the use of milk, such as batch- to-batch variability in the milk composition (contributing to variable dissolution data), the tendency of lipophilic compounds to bind to lipidic components of the milk, and the source of milk (goat vs. cow) (*38*).

Table 1. Composition of Biorelevant Media to Simulate Gastric Fluid Under Fasted and Fed Conditions (12, 19, 34, 36)

	AGF	SGF	SGFsp	SGF_{SLS}	SGF Triton X	FaSSGF	FeSSGF Early	FeSSGF Middle	FeSSGF Late
Acetic acid	500 µL (for 1L)	-	-	-	-	-	-	17.12 mM	-
Lactic acid (µL)	420 (for 1L)	-	-	-	-	-	-	-	-
Lecithin (µM)	-	-	-	-	-	20	-	-	-
Pepsin (g)	1.25	3.2	-	-	-	0.1	-	-	-
Sodium chloride (mM)	-	34.22	34.22	34.22	34.22	34.22	148	237.02	122.6
Sodium citrate (mM)	2.34	-	-	-	-	-	-	-	-
Sodium lauryl sulphate (mM)	-	-	-	8.57	-	-	-	-	-
Sodium malate (mM)	2.81	-	-	-	-	-	-	-	-
Sodium taurocholate (µM)	-	-	-	-	-	80	-	-	-
Triton X 100 (mM)	-	-	-	-	1.55	-	-	-	-
Sodium acetate (mM)	-	-	-	-	-	-	-	29.75	-
Ortho-phosphoric acid (mM)	-	-	-	-	-	-	-	-	5.5
Sodium dihydrogen phosphate (mM)	-	-	-	-	-	-	-	-	32
Milk/buffer	-	-	-	-	-	-	1:0	1:1	1:3
Hydrochloric acid	qs	qs	qs	qs	qs	qs	qs	qs	qs
pH	1.2	1.2	1.2	1.2	1.2	1.6	6.4	5	3

AGF: artificial gastric fluid; SGF: simulated gastric fluid; SGFsp: SGF without pepsin; SGF_{SLS}: sodium lauryl sulphate; SGF TritonX: polyethylene glycol tert-octylphenyl ether; FaSSGF: fasted state simulating gastric fluid; FeSSGF: fed state gastric fluid. Dash (-) indicates not applicable.

Another approach was proposed by Jantratid et al. in 2008 (*12*). The authors proposed a "snapshot" approach to capture the changes in the composition of the gastric fluid associated with digestion and gastric emptying process (*12*). Table 1 describes the composition of early, middle, and late fed state gastric environments (FeSSGF). The early stage media corresponds to the first 75 min after meal ingestion, the middle stage to 75–165 min, and the late stage is beyond 165 min.

Small Intestinal Environment

Biorelevant Dissolution Media

The composition of biorelevant dissolution media for simulating human small intestine fluids are presented in Table 2. The bicarbonate ions secreted into the intestinal lumen neutralize the gastric fluid that is emptied in the intestines. The reported pH range under fasted conditions is 5.8–6.5 in the duodenum, 5.3–8.1 in the jejunum, and 6.8–8.0 in the ileum (*31*). Bile salts are also secreted into the intestines, and the formation of micelles results in a much lower surface tension compared to the gastric fluids. The surface tension of the intestinal fluids is even lower under fed conditions due to the higher concentration of bile (*39*).

Based on this, biorelevant media, e.g., USP simulated intestinal fluids, were developed to simulate the pH including components present in the human GI tract, such as bile salts and lecithin. Osmolality, pH, and surface tension were adjusted to physiological values. According to FDA guidance and other sources, simulated intestinal fluid with pancreatin (USP-SIF) and without enzyme (SIF-blank) reflect the physiologic conditions of the small intestine better than other simpler buffer systems (*16*, *40*, *41*).

Another example of biorelevant media is the fasted and fed simulated intestinal fluid (FaSSIF and FeSSIF) proposed by Dressman in 1998 and its many adaptations (*30*). The human intestinal lumen is buffer by bicarbonate; however, due to pragmatical reasons, other buffers are typically used to mimic the physiological pH of intestinal fluids (*30*). For example, FeSSIF uses acetate buffer to adjust the pH to 5.0. Moreover, the prevalent bile salt in the human bile is cholic acid, but sodium taurocholate (conjugate of cholic acid with taurine) was chosen to be the most representative bile salt in vitro. Biorelevant media contain bile salts and phospholipids, and when

Table 2. Composition of Biorelevant Media to Simulate Human Small Intestine Fluids in the Fasted and Fed State and Canine Gastric and Intestinal Biorelevant Media (5, 12, 30, 21, 54)

Media	pH	Components
USP SIF	6.8	NaOH (qs pH); KH2PO4 (6.8 g); Pancreatin (10.0 g); Deionized water qs 1L
FaSSIF	6.5	NaOH (qs pH); KCl (103.29 mM); Bile salt (Sodium taurocholate) (3 mM); Phospholipid (lecithin) (0.75 mM); Potassium dihydrogen orthophosphate (28.66 mM); Deionized water qs 1L
FeSSIF	5.0	NaOH (qs pH); KCl (203.89 mM); Bile salt (Sodium taurocholate) (15 mM); Phospholipid (lecithin) (3.75 mM); Acetic acid (144.05 mM); Deionized water qs 1L
FaSSIF V2	6.5	NaOH (34.8 mM); NaCl (68.62 mM); Bile salt (Sodium taurocholate) (3 mM); Phospholipid (lecithin) (0.2 mM); Maleic acid (19.12 mM); Deionized water qs 1L
FeSSIF V2	5.8	NaOH (81.65 mM); NaCl (125.5 mM); Bile salt (Sodium taurocholate) (10 mM); Phospholipid (lecithin) (2 mM); Maleic acid (55.02 mM); Glyceryl monooleate (5 mM); Sodium oleate (0.8 mM); Deionized water qs 1L
FeSSIF Early	6.5	NaOH (52.5 mM); NaCl (145.2 mM); Bile salt (Sodium taurocholate) (10 mM); Phospholipid (lecithin) (3 mM); Maleic acid (28.6 mM); Glyceryl monooleate (6.5 mM); Sodium oleate (40 mM); Deionized water qs 1L
FeSSIF Middle	5.8	NaOH (65.3 mM); NaCl (125.8 mM); Bile salt (Sodium taurocholate) (7.5 mM); Phospholipid (lecithin) (2 mM); Maleic acid (44 mM); Glyceryl monooleate (5 mM); Sodium oleate (30 mM); Deionized water qs 1L
FeSSIF Late	5.4	NaOH (72 mM); NaCl (51 mM); Bile salt (Sodium taurocholate) (4.5 mM); Phospholipid (lecithin) (0.5 mM); Maleic acid (55.09 mM); Glyceryl monooleate (1 mM); Sodium oleate (0.8 mM); Deionized water qs 1L
SEIF	6.5	NaN3 (6 mM); NaCl (98 mM); Bile salts* (4 mM); Phospholipid (Lyso-phosphatidylcholine) (1 mM); Cholesterol (0.25 mM); Sodium dihydrogen phosphate (18 mM); Sodium hydrogen phosphate (12mM)
FaSSGFc I	1.2–2.5	HCl (~3.6 - 82 mM); NaCl (14.5 mM);Sodium taurocholate (0.1 mM); Sodium taurodeoxycholate (0.1 mM); Lecithin (0.025 mM); Lysolecithin (0.025 mM); Sodium oleate (0.025 mM)
FaSSGFc II	2.5–6.5	NaOH (~14.5 - 40 mM); NaCl (18.81 mM); Sodium taurocholate (0.1 mM); Sodium taurodeoxycholate (0.1 mM); Lecithin (0.025 mM); Lysolecithin (0.025 mM); Sodium oleate (0.025 mM); Maleic acid (21.68 mM)
FaSSIFc	7.5	NaOH (21.66 mM); NaCl (59.63 mM); Sodium taurocholate (5.0 mM); Sodium taurodeoxycholate (5.0 mM); Lecithin (1.25 mM); Lysolecithin (1.25 mM); Sodium oleate (1.25 mM); Sodium dihydrogen phosphate (28.65 mM)

**Sodium salts of the following conjugates: glycocholate (1 mM), glycodeoxycholate (0.7 mM), glycochenodeoxycholate (1 mM), taurocholate (0.5 mM), taurodeoxycholate (0.3 mM), taurochenodeoxycholate (0.5 mM).*
SIF: simulated intestinal fluid with pancreatin; FaSSIF: fasted simulated intestinal fluid; FeSSIF: fed simulated intestinal fluid: V2: version 2; SEIF: simulated endogenous intestinal fluid; FaSSGFc: canine fasted-state simulated gastric fluid; FaSSIFc: canine fasted-state simulated intestinal fluid.

simulating the fed state, also monoglycerides and free fatty acids. The composition of FaSSIF and FeSSIF are given in Table 2.

Revised versions of FaSSIF and FeSSIF (FaSSIF-V2 and FeSSIF-V2, respectively) were developed to address some of the shortcomings of the initially proposed media. For example, Persson et al. reported that cyclosporine, danazol, griseofulvin, and felodipine presented between 2–5-times higher solubility values in fed human intestinal fluid (HIF) compared to FeSSIF (*42*). This could be due to the lack of neutral lipids in the FeSSIF composition. Additionally, the purity of bile salts can also have an impact on the solubility of poorly soluble drugs. Wei and Löbenberg reported the solubility of glyburide in biorelevant media with crude bile salts to be over 2-fold higher than when pure bile salts were used in FaSSIF (*43*). Additionally, the reported in vivo bile salt concentration is lower than the concentration used previously (*12*, *32*).

Psachoulias et al. proposed a method to predict the concentration and potential precipitation of lipophilic weak bases using an upgraded version of FaSSIF-V2 (FaSSIF-V2plus) (*44*). The proposed in vitro method was composed of a gastric and duodenal compartment along with a reservoir. FaSSIF-V2plus was used in the duodenal compartment. The composition of FaSSIF-V2plus is similar to FaSSIF-V2; in addition to all FaSSIF-V2 components, the "plus" version contains free fatty acid (sodium oleate, 0.5 mM) and cholesterol (0.2 mM). The authors concluded that for some weak bases, such as ketoconazole, FaSSIF-V2plus is a superior fluid for investigating the drug's intraluminal precipitation.

Later, Fuchs et al. further proposed an updated version of the fasted state biorelevant media based on the up to date physiological composition of fasted HIF at that time (*18*). The proposed media was named FaSSIF-V3. The surface tension was considered as a surrogate parameter in establishing the medium's correctness. Several prototypes were investigated containing five different bile salts (taurocholate, glycocholate, tauroursodeoxycholate, taurochenodeoxycholate, and glycochenodeoxycholate), as well as replacing lecithin with its hydrolysis products (lysolecithin and sodium oleate). Additionally, a mixture of glycocholate and taurocholate, with or without 0.2 mM cholesterol, were investigated. The authors assessed the solubility of 10 model compounds and observed that the amount and type of phospholipids and bile salt significantly impacted the solubility and surface tension in the various prototypes. Additionally, the authors reported that blank buffers tend to underestimate the physiological solubility of the investigated active pharmaceutical ingredient (API), whereas the sodium dodecyl sulfate (SDS) solutions overestimated solubility. Finally, the proposed FaSSIF-V3 composition contained glycocholate and taurocholate with 0.2 mM cholesterol (*18*).

Cristofoletti and Dressman used FaSSIF-V3 with reduced phosphate buffer concentration (5.0 mM) (*45*, *46*). The rationale behind this approach was to use a buffer system that would match the pH at the particle's surface, utilizing physiologically relevant bicarbonate buffer (BCB). For this purpose, ibuprofen was used as the model drug. The authors reported that the proposed 5.0-mM phosphate buffer FaSSIF-V3 was able to predict in vivo differences in peak and extent of exposure between test and reference ibuprofen formulations (*46*).

When analyzing the fed state, as shown in Table 2, the main differences between FeSSIF and FeSSIF-V2 are the concentrations of bile salts and lecithin, the replacement of phosphate for maleate buffer resulting in lower osmolality and buffer capacity values, and the addition of glyceryl monooleate and sodium oleate to reflect the presence of lipolysis products (*32*).

Similarly to SGF, Jantratid and Dressman developed a snapshot media to simulate the intestinal fluids in the fed state. The authors proposed the inclusion of lipolysis products and changes in parameters such as bile salts concentration, osmolality, buffer capacity, and fluid pH according to the early, medium, and late stages after food intake (*12*).

Biorelevant media have been shown to be very useful in assessing the in vivo solubility of compounds. Söderlind et al. studied the solubility of 24 molecules in FaSSIF, FaSSIF-V2, and HIF. FaSSIF-V2 solubilities correlated better with solubilities in HIF for neutral compounds, whereas for acidic and basic compounds the solubility in FaSSIF and FaSSIF-V2 were similar (*47*). A similar trend was observed by Fagerberg et al. (*48*). The authors reported that the estimation of the in vivo solubility of poorly soluble compounds was more accurate in biorelevant media. This was particularly true for bases and neutral molecules, which display higher solubility in FeSSIF compared to FaSSIF. The opposite was observed for acidic drugs (*48*). Biorelevant media have also been widely used to forecast the in vivo performance drugs, achieving good IVIVC in some cases, but not always (*49–53*). Other biorelevant media have also been proposed to simulate fluids in the fasted state small intestine, such as the simulated endogenous intestinal fluid (SEIF), described by Kossena

et al. (Table 2) (*54*). Since the focus of this review is on the upper GI tract, colonic fluids are not included in Table 2.

The use of bicarbonate-based biorelevant media has been proposed in the literature (*55*, *56*). Litou and colleagues assessed a level II biorelevant media based on BCB to simulate the contents of upper small intestine under conditions of reduced acid secretion in the stomach (*56*). The authors reported that bicarbonates were not important in estimating drug precipitation and that level II biorelevant media underestimated the concentration of the given compounds in intestinal human aspirates; however, more data are needed to confirm this finding as the usefulness of bicarbonate in biorelevant dissolution testing may be compound specific (*56*). For example, Jede et al. investigated the supersaturation and precipitation kinetics of weak bases using a transfer model with biorelevant BCB (*55*). The authors compared bicarbonate- and phosphate-based FaSSIF and found that bicarbonate-based FaSSIF had better predictive power compared to phosphate-based FaSSIF. They concluded that the proposed model is a promising approach to increase the predictive power of in vitro tests, thus contributing to a more biorelevant drug development process (*55*).

Even though biorelevant media have been extensively used, its preparation can be time-consuming, costly, and it may present a short-shelf life for utility. Furthermore, the buffering species in the human intestinal lumen is bicarbonate, whereas FaSSIF uses phosphate, FeSSIF uses acetate, FaSSIF-V2 and FeSSIF-V2 use maleate. Simpler and more physiologically relevant dissolution media are therefore desired.

Moreover, in addition to FaSSGF, FeSSGF, FaSSIF-V2, and FeSSIF-V2, there are several biorelevant media described in the USP general chapter <1236> Solubility Measurements, such as human simulated colonic fluid—proximal colon; human simulated colonic fluid—distal colon, canine fasted-state simulated gastric fluid (FaSSGFc pH 1.2–2.5); canine fasted-state simulated gastric fluid (FaSSGFc pH 2.5–6.5); canine fasted-state simulated intestinal fluid (FaSSIFc), and bovine simulated ruminal fluids. Biorelevant canine media are described in Table 2. For the composition of the other media, refer to USP general chapter <1236> Solubility Measurements.

Physiologically Relevant Dissolution Media – Bicarbonate Buffer (BCB)

At present, the most widely applied dissolution media are phosphate-based buffers (*16*, *57*). However, the concentration of phosphates in the intestinal luminal fluids is insignificant. This makes phosphate-based dissolution media poorly representative of the in vivo environment, failing to reflect in vivo characteristics such as ionic strength, buffer capacity, fluid volume, and viscosity (*16*).

The pH along the GI tract is maintained by bicarbonate ions, which are present in pancreatic, hepatic, and intestinal secretions (*58*, *59*). Hence, the development of suitable in vitro dissolution media based on BCB has gained much attention because it closely mimics the environment of the intestinal fluids and can thus improve in vitro-in vivo correlations compared to phosphate buffers (*60*).

In vivo, the pH is held stable by the constant supply of bicarbonate-containing secretions in the intestines. On the other hand, the application of BCB as an in vitro dissolution medium is challenging due to the evaporation of $CO_{2(g)}$ from the aqueous phase causing the pH to rise. This can lead to changes in the buffer strength and poor reproducibility of the dissolution test. Hence, the first step in establishing a stable BCB is to maintain $CO_{2(aq)}$ and $CO_{2(g)}$ at equilibrium (Eq. 1).

$$\begin{array}{c} CO_{2(g)} \\ \uparrow\downarrow \\ H_2O_{(l)} + CO_{2\,(aq)} \leftrightarrows H_2CO_{3\,(aq)} \leftrightarrows H^+_{(aq)} + HCO_3^-{}_{(aq)} \end{array} \qquad (1)$$

One of the ways to stabilize the BCB pH is to purge the medium with CO_2 gas, thus supplying $CO_{2(g)}$, which compensates its loss from the aqueous medium. Automated systems have been developed to adjust the pH by sparging gas according to the pH shift and were reviewed by Amaral Silva et al. and others (*16*). However, bubbling gases into the dissolution medium can be problematic due to the hydrodynamic disturbances in the dissolution vessel, which can affect the dissolution rate of certain drugs and lead to failure in meeting compendial requirements. Another concern is the possible foaming when surfactant-containing media are used.

Preventing the escape of CO_2 instead of purging the medium has been proposed as an alternative to control the medium pH. Approaches such as sealing the dissolution vessel or using a liquid paraffin layer on top of the dissolution medium have been effective in stabilizing the media pH (*61*, *62*). Nevertheless, these were closed systems, so dynamic pH regulation was not possible.

To circumvent this, Scott and colleagues have recently studied the use of a novel bicarbonate-based dissolution system that supplies N_2 (pH increasing) and CO_2 (pH

decreasing) gases above the dissolution medium without purging into the solution (see schematic of the device in ref *60*). The system is composed of an enclosure device with two inlets that supply N_2 and CO_2. The gases are distributed through a ring-shaped diffuser and released through outlets pointing towards the surface of the dissolution medium. The authors report that this method regulated the pH of the BCB without substantial disruption to the surface of the media and that no foaming was observed when surfactant-containing medium was used.

The approach taken by Scott et al. is similar to the one reported by Boni et al., in which the $CO_{2(g)}$ was supplied above the medium to maintain the pH throughout the dissolution test (*57, 60*). However, the setup proposed by Boni et al. was not effective because the dissolution vessel only had a conventional lid (open system) that did not prevent the escape of the supplied gas. Hence, the enclosure method is a superior design in the sense that it prevents gas escape, thus improving the efficiency of gas supply. The authors concluded that this novel system is a step towards the application of physiologically relevant BCBs as dissolution media that meets compendial requirements.

Sakamoto et al. proposed a simple and facile method that allows the use of BCB for dissolution testing (see schematic of the device in ref. *63*). The authors developed a floating lid system that prevents the escape of CO_2 from the BCB solution. The lid is made of a 5-mm-thick styrofoam that covers the surface of the medium almost completely but not in a tight-sealing configuration. The buffer is added to the dissolution vessel and the lid is placed on top of it. The medium pH was adjusted by adding HCl via a small hole. The authors investigated the suitability of this method for a 6.0–7.5 pH range and 2–50-mM BCB concentration. In all cases, the pH change was less than 0.1 pH unit after 3.5 h when the floating lid method was used, whereas without the lid, the pH increased by more than 1 pH unit within 3.5 h. The authors concluded that the floating lid method would be useful for formulation development while covering the physiological intestinal and colonic conditions in terms of pH and buffer concentration.

It is interesting to note that the pH of the medium can be adjusted either by adding HCl/NaOH or by sparging gases or a combination of both (*60, 63, 64*). In the case of sparging, when CO_2 gas is supplied and diffused into the medium the $CO_{2(aq)}$ interacts with water, generating carbonic acid, which in turn dissociates, releasing hydrogen ion, culminating in the pH decrease (equilibrium shown in Eq. 1 shifts to the right). Reversely, the sparging of a pH-increasing gas (e.g., N_2 or He) has an indirect effect in increasing the medium pH (*65, 66*). As the pH-increasing gas is supplied, the partial pressure of CO_2 is reduced, decreasing the dissolved $CO_{2(aq)}$ in the medium, thus increasing the pH (equilibrium in Eq. 1 shifts to the left). Scott et al. observed that the CO_2 supply was much more efficient in decreasing the medium pH than N_2 was in increasing the pH (*60*). This is most likely due to the indirect effect of N_2, thus taking longer for the pH change to be observed.

BCB Kinetics and Mathematical Models

Although BCB is physiologically relevant, its application has been limited because of the pragmatical hurdles, and some authors have doomed it as a medium with restricted suitability for dissolution testing (*57*). Matching the effective buffering pKa of bicarbonate at the solid-liquid surface (diffusion layer) of a dissolving solid with a surrogate buffer system is a way to simplify the dissolution conditions while maintaining physiologic relevance in terms of buffering capacity at the diffusion layer (*67, 68*).

When the whole system is at equilibrium (Eq. 1), the pKa of the BCB system is 6.04, which is the situation in the bulk solution in a dissolution vessel (*16, 67*). However, in the diffusion layer around dissolving solutes the interconversion, $H2O_{(l)} + CO_{2(aq)} \leftrightarrows H_2CO_{3(aq)}$, does not equilibrate very rapidly compared to the fast diffusion processes. Therefore, BCB behaves as having an effective pKa in the diffusion layer that is different from that in bulk. This value is lower than 6.04 (bulk), but higher than the intrinsic pKa of 3.30 ($H_2CO_{3(aq)} \leftrightarrows H^+_{(aq)} + HCO^-_{3(aq)}$). As a result, the ability of BCB in buffering the diffusion layer against incoming ionizable solute is weakened and the in vivo dissolution rate is slower compared to highly concentrated compendial buffers.

Based on this, investigators have proposed the reduction in molarity of nonbicarbonate-based surrogate buffers as a possible approach to increase its biopredictability, thus matching the typically slower in vivo dissolution (*15, 67, 68*). For example, Tsume et al. showed that ibuprofen tablets had slower in vitro dissolution in phosphate 10 mM compared to 50 mM at a starting pH of 6.0 (*69*). This can be explained by Mooney's stagnant film-based dissolution model, i.e., more diluted buffers have a reduced buffer capacity, which translates into a lower ability to counter the acidifying effect of the dissolving ibuprofen at the diffusion layer pH (*70*). In highly concentrated buffer systems, such as compendial buffers, an abundance of the buffer's conjugate base species surrounds the drug particle. This leads to prompt neutralization in the

diffusion layer, i.e., the buffer species readily consumes the ions formed on the dissolving drug surface. Hence, the pH in the diffusion layer is similar to the bulk, resulting in a higher dissolution rate (*15*, *45*, *71*, *72*). Conversely, when the buffer system is less concentrated (as in vivo), the neutralization is slower.

Different models have been proposed to predict the drug flux, thus enabling calculation of the surrogate buffer molarity to determine a good match to physiological bicarbonate in terms of drug dissolution. This includes but is not limited to the equilibrium model (which assumes that H_2CO_3 and CO_2 are at equilibrium), the carbonic acid ionization (CAI) model (hypothetical situation where neither hydration or dehydration is assumed), the irreversible reaction (IRR) transport model, and the reversible non-equilibrium (RNE) model.

Krieg et. al. proposed the IRR transport model to develop more physiologically relevant buffer systems for dissolution testing (*73*). This model assumes the dehydration process ($H_2CO_{3(aq)} \rightarrow H_2O(l) + CO_{2(aq)}$) is an irreversible chemical reaction because it is approximately 500 times faster than the hydration rate. This approximate model yielded improved predictions for the intrinsic dissolution rates of ibuprofen, ketoprofen, and indomethacin in BCBs. However, Al-Gousous et al showed that this assumption was not as accurate (*74*). They proposed the RNE model, which does not make any equilibrium assumptions. It not only includes both the hydration and dehydration rates ($H_2O(l) + CO_{2(aq)} \rightleftharpoons H_2CO_{3(aq)}$) but also accounts for the fluxes of all species involved in the mass transfer process. The RNE model predicted the flux values obtained in the intrinsic dissolution experiments more accurately compared to the other models (*74*).

It is of crucial importance to understand the kinetics of bicarbonate at the diffusion layer of a dissolving particle. For example, in the equilibrium model, BCB would have a pKa close to the bulk pH, resulting in effective buffering at the surface of the dissolving drugs (overestimation). In the CAI model, the assumption that hydration and dehydration reactions do not happen would mean that the buffer pKa would be much lower than the bulk pH, resulting in a very poor ability to buffer the surface of the dissolving drug (underestimation). Similarly, the IRR transport model would also underestimate the drug flux, but not to the extent as of the CAI model because it includes an irreversible dehydration reaction. The RNE model represents an intermediate situation in which the reactions occur but do not reach equilibrium. In this case, as previously mentioned, this situation results in BCB not behaving as having a pKa exceeding 6 in terms of promoting the dissolution of ionizable solids. The RNE model has been shown to successfully estimate the pH on the surface of a solid particle in BCB, and the Mooney model can be used to estimate the phosphate concentration that would give the same surface pH (pH0) (*67*, *74*). Thus, a proper surrogate buffer molarity can be used that would give good matches to physiological bicarbonate in terms of drug dissolution. This shows that in some cases it is feasible to develop surrogate buffers for bicarbonate.

Furthermore, Salehi et. al. incorporated into the RNE model other properties such as medium hydrodynamic effect and drug particle size distribution (*75*). The authors described it as a hierarchical mass transfer (HMT) model that considers drug properties (intrinsic solubility, acid/ base character, pKa, particle size, and particle polydispersity) as well as GI fluid properties and fluid hydrodynamics (bulk pH, buffer species concentration, fluid shear rate, and convection).

The findings reported by Álvarez et al. further reinforce that the current compendial buffers concentrations seem to be too high to correlate with the in vivo carbonate concentration (*76*). The authors investigated the in vitro dissolution of ibuprofen tablets in different pharmacopeial media at both 50 and 75 rpm. The media investigated by the group included 130-mM HCl pH 1.2, 540-mM acetate buffer pH 4.5, and 70-mM phosphate buffer pH 6.8. In all media, the dissolution profiles showed similarity at both rotation speeds. However, the in vivo bioequivalence studies revealed that only one out of the three test formulations was bioequivalent to the reference. Hence, these in vitro tests were not able to detect differences regarding the rate of absorption. Based on this finding, the authors concluded that there remains a need to develop dissolution conditions that can predict bioequivalence outcomes and that the application of biowaivers to BCS class IIa drugs would not be feasible.

In contrast, Hofmann et. al. studied the dissolution of ibuprofen in physiologically relevant BCB and reported that the in vitro dissolution profiles in bicarbonate compared reasonably well with the in vivo intestinal dissolution of the tested suspensions (*67*). They concluded that this demonstrates the possible potential toward extending biowaivers to BCS class IIa compounds.

Amaral Silva et. al. tested a 5-mM phosphate buffer as the surrogate buffer for ibuprofen based on the IRR model described by Krieg et. al (*15*). The authors also observed a slower dissolution rate of ibuprofen immediate-release

tablets in low buffer capacity (5 mM) compared to compendial buffer (50 mM) and that compendial buffer lacked discriminatory power (*15*, *67*, *76*). The authors pointed out that the rapid in vitro dissolution rate cannot be translated to the observed in vivo dissolution rate of ibuprofen. In contrast from the methodology used by Álvarez at al., in which different absorption rates could not be detected in vitro, Amaral Silva and colleagues utilized the low-capacity surrogate buffer in a biphasic dissolution system (*76*). This system is composed of an organic layer on top of the aqueous medium, thus mimicking the concurrent in vivo processes of drug dissolution and absorption. The addition of the organic phase works as a sink to the aqueous layer, assisting the medium pH maintenance by the removal of the dissolved drug from the aqueous medium. Hence, the pH changes that are expected when a low buffer capacity medium is used are reduced. This is a valuable approach to investigate the drug product performance with improved physiological relevance (*15*).

Based on this, we herein suggest the use of a biphasic system with the aqueous layer composed of BCB. Adding paraffin on top of the buffer has been previously proposed to prevent the CO_2 escape, but drugs do not typically partition to the liquid paraffin layer (*61*). We believe that the use of BCB coupled with an organic layer (octanol) would not only prevent the escape of CO_2 – thus taking away the need to sparge the medium – but it would also allow assessment of the drug partitioning (absorption). This would be a very robust physiologically relevant approach and we suggest that future in vitro studies along this line be conducted.

Application to Enteric-Coated Formulations and Design of Surrogate Buffers

Oversimplification of the dissolution conditions, for example using a surrogate buffer instead of BCB, may not be relevant or proper for certain formulations. This is the case for delayed release drug products. Formulations coated with pH-responsive polymers have been shown to have poor in vivo performance (*77*). One of the reasons for this is the lack of biopredictability of the buffers used for in vitro performance testing, preventing suitable in vitro product evaluation (*78*). The great discrepancy in the performance of delayed release (enteric-coated [EC]) products in physiologically relevant BCB vs. phosphate buffer is well recognized in the literature, as highlighted by Amaral Silva et al. (*77*). This performance problem persists today, and recent reports by Scott et al. and Sakamoto et al. have corroborated these findings (*60*, *63*).

Scott and colleagues investigated the release of EC prednisolone microparticles, pellets, and tablets (*60*). They observed that in phosphate buffer the drug release was immediate after the 2-h acid exposure for the all the tested dosage forms with no significant difference among the dissolution profiles. On the other hand, in BCB there was a long lag time for the onset of drug release. An interesting observation highlighted by the authors was a shorter lag time for the microparticle formulation compared to pellets and tablets, which could be explained by the larger surface area available for polymer dissolution. Similarly, Sakamoto et al. reported a 30-min disintegration time and similar release profiles for EC 5-ASA tablets in a phosphate-based buffer, whereas in BCB the disintegration time was about 4–8 h, with large variation (*63*).

With this in mind, the most biorelevant dissolution media for EC formulations would be a bicarbonate-based one. As highlighted before, the routine use of BCB is technically difficult and even unfeasible for disintegration testing and dissolution apparatuses, such as reciprocal cylinder (*78*). Therefore, similarly to small drug molecules, developing a non-volatile surrogate buffer for EC products is of great interest. However, EC polymers, being poly-acids with ionizable carboxylic groups, are much more complex than small molecules as its dissolution includes different phases (*31*, *77*, *79–81*). In an environment with low pH values (such as the stomach) the carboxyl groups are not ionized, so the polymer is insoluble, resisting disintegration and dissolution which prevents drug release. When the EC dosage form is exposed to the intestinal fluids (higher pH and buffered by bicarbonate) and when the pH0 (surface pH) of the polymer is above its pKa (dissolution pH threshold), its ionization is promoted (*77*). Due to electrostatic repulsion, the polymer relaxes, swells, and undergoes chain disentanglement allowing further ionization of other polymer chains, which diffuse away to the bulk solution (*79*, *81*). This consists of the dissolution phases of pH-responsive polymers, ultimately leading to disintegration and dissolution of the dosage form.

Recently, Blechar et al. proposed a mechanistic approach to enable the development of surrogate buffers for EC products with little bench work. As described before, the effective pKa of BCB in the diffusion layer (pKa_{eff}) is different from other buffers such as phosphate and maleate (pKa of 6.8 and 5.8, respectively) and different from the bulk where everything is at equilibrium (*78*). For small molecules under regular hydrodynamic conditions the pKa_{eff} of bicarbonate lies between 4 and 5 (*78*, *82*). However, the complex behavior of EC polymers makes it difficult for a direct calculation.

Besides the diffusion layer, a viscoelastic gel layer is formed on a polymer's surface (Fig. 1), as opposed to only a diffusion layer on a particle's surface. The gel layer presents an increased diffusional resistance which reduces the diffusion rate of the buffer species. Consequently, the time available for the interconversion between CO_2 and H_2CO_3 is increased, allowing the equation, $H_2O(l) + CO_{2(aq)} \leftrightarrows H_2CO_{3(aq)}$, to approach equilibrium. As a result, the pKa of bicarbonate in the gel layer is increased compared to the pKaeff in the diffusion layer. Finally, both the pKaeff (diffusion layer) and higher pKa in the gel layer will control the polymer's surface pH. Therefore, the gel layer increases the effective interfacial buffering pKa of bicarbonate.

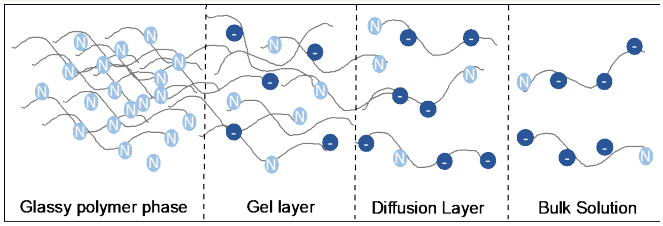

Figure 1. Representation of the solid-liquid interface of a dissolving enteric polymer. From the gel layer to the bulk solution, the pH increases and the viscosity decreases. N: neutral (unionized carboxylic acid, -COOH); Minus (-) indicates negative charge (ionized carboxylic acid, -COO-). Adapted from Blechar et al (79) under the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

The authors performed dissolution experiments in maleate (pKa 5.8), citrate (pKa 5.7), succinate (pKa 5.2), and acetate (pKa 4.6) buffers to find a buffer species that would promote similar dissolution as bicarbonate (*78*). The time taken for 5% release ($t_{5\%}$) was used for comparison because it is most representative of the coat dissolution as opposed to the whole dissolution profile. The observed trend of dissolution based on $t_{5\%}$ was that succinate matched BCB well for relatively fast dissolving formulations, and citrate was a good estimate for relatively slow dissolving ones. These media could be used as good starting points. Based on these findings, the authors proposed a decision tree for establishing a surrogate buffer (Fig. 2).

A physiologically relevant approach is of primary importance not only to predict the in vivo performance of a formulation under development, but also to assess the similarity of reference and test formulations in a bioequivalence (BE) study. Our group assessed clinical data of a failed BE study for EC pantoprazole tablets (*83*). The formulations used in the dissolution study were from the same batch as those used in the BE study. Both formulations complied with the USP specifications and had a somewhat similar performance in phosphate buffer, but when tested in vivo they did were not bioequivalent. Solely satisfying the in vitro standard for drug dissolution does not guarantee similar in vivo behavior. On the other hand, when these formulations were tested in BCB, a great discrepancy was observed, where the test formulation had a much more delayed onset of dissolution than the reference. The use of non-physiologically relevant dissolution media during the drug product development phase can be misleading, causing poor selection of prototype formulations. Therefore, it was further evidenced that using BCB can de-risk the development of generic EC formulations, increasing the likelihood for a successful BE.

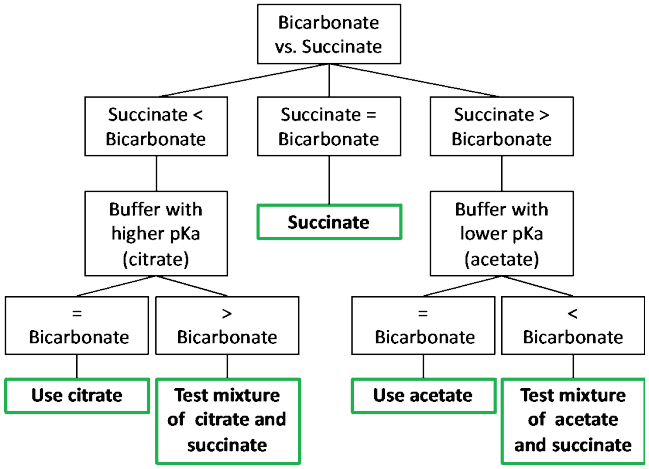

Figure 2. Decision tree for establishing a surrogate buffer for enteric coated products. Adapted from Blechar et al (79) under the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

CONCLUSION

The evolution of media and buffers used in dissolution testing to achieve physiological relevance was reviewed. There are many important factors to be considered when developing a biorelevant dissolution method such as pH, buffer species, buffer concentration, osmolality, viscosity, surface tension, concentration and type of bile salts, lipolysis products, as well as physiological state, such as fasted and fed states. Physiologically relevant methods usually do not apply compendial conditions and its use is most meaningful in the development phase, rather than in a QC environment for batch release, for example. At the same time, the information retrieved from such methods can be used to set specifications for QC and regulatory purposes. One of the majors disconnects between the in vivo environment and in vitro conditions is the buffer species and concentration. The human intestinal lumen is buffered by bicarbonate at low molarities, but highly concentrated phosphate buffers are often used in dissolution testing, which can give misleading results during the drug product development phase. This is

especially true for delayed release (EC) formulations. For these formulations, using bicarbonate-based buffers would be the most ideal in terms of physiological relevance. On the other hand, a biorelevant test will not necessarily be a clinically relevant dissolution test. The pragmatical hurdles of using BCB makes it desirous to develop a surrogate method with simpler buffer systems. This can be achieved on a case-by-case study by comparing the drug flux in BCB and other buffer solutions (which are often more diluted systems compared to compendial buffers). Precise mechanistic understanding of the in vivo and in vitro dissolution processes is imperative to provide physiological relevance for the dissolution method. Using such conditions can aid the drug product development process by increasing the likelihood to select formulations with improved in vivo performance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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