The AAPS Journal (2020) 22: 34
DOI: 10.1208/s12248-020-0417-8

Selection of *In Vivo* Predictive Dissolution Media Using Drug Substance and Physiological Properties

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Received 7 October 2019; accepted 4 January 2020; published online 27 January 2020

ABSTRACT. The rate and extent of drug dissolution in the gastrointestinal (GI) tract are highly dependent upon drug physicochemical properties and GI fluid properties. Biorelevant dissolution media (BDM), which aim to facilitate *in vitro* prediction of *in vivo* dissolution performance, have evolved with our understanding of GI physiology. However, BDM with a variety of properties and compositions are available, making the choice of dissolution medium challenging. In this tutorial, we describe a simple and quantitative methodology for selecting practical, yet physiologically relevant BDM representative of fasted humans for evaluating dissolution of immediate release formulations. Specifically, this methodology describes selection of pH, buffer species, and concentration and evaluates the importance of including bile salts and phospholipids in the BDM based upon drug substance log D, pK_a, and intrinsic solubility. The methodology is based upon a mechanistic understanding of how three main factors affect dissolution, including (1) drug ionization at gastrointestinal pH, (2) alteration of surface pH by charged drug species, and (3) drug solubilization in mixed lipidic aggregates comprising bile salts and phospholipids. Assessment of this methodology through testing and comparison with literature reports showed that the recommendations correctly identified when a biorelevant buffer capacity or the addition of bile salts and phospholipids to the medium would appreciably change the drug dissolution profile. This methodology can enable informed decisions about when a time, complexity, and/or cost-saving buffer is expected to lead to physiologically meaningful *in vitro* dissolution testing, *versus* when a more complex buffer would be required.

KEY WORDS: bicarbonate; biorelevant; buffer; dissolution; solubility.

INTRODUCTION

One of the many challenging tasks facing formulators developing and testing drug candidates is the selection of the optimal dissolution medium with which to conduct *in vitro* tests. The goal is to select a medium and test protocol that produces *in vitro* results that accurately reflect the rate and extent of drug dissolution *in vivo*—an increasingly difficult task, given the quantity of complex molecules in drug pipelines and the variety of media types and

Electronic supplementary material The online version of this article (https://doi.org/10.1208/s12248-020-0417-8) contains supplementary material, which is available to authorized users.

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ABBREVIATIONS: α, pK_a-log S_o; A_s, Surface area of the dissolving drug particles; *BCS*, Biopharmaceutics Classification System; *BDM*, Biorelevant dissolution medium; β, pK_w-pK_a-log S_o; *BS*, Bile salts; C_b, Measured bulk concentration; C_s, Saturation solubility (concentration of non-ionized + ionized drug at saturation); C_s/S_o, Relative solubility; D_{eff}, Effective drug diffusivity in the dissolution medium; *FaHIF*, Fasted-state human intestinal fluid; *FeHIF*, Fed-state human intestinal fluid; *FaSSIF*, Fasted-state simulated intestinal fluid; *FeSSIF*, Fed-state simulated intestinal fluid; *GI*, Gastrointestinal; *Log D*, Logarithm of pH-adjusted partition coefficient between octanol and water; *Log* $D_{6.5}$, Logarithm of pH-adjusted partition coefficient between octanol and water at pH = 6.5; *Log P*, Logarithm of the partition coefficient between octanol and water for a completely non-ionized molecule; *pH*, Negative logarithm of the hydrogen ion concentration; pK_a, Negative logarithm of the acid dissociation constant; pK_w, Negative logarithm of the water dissociation constant; *PL*, Phospholipids; *R*, Effective particle radius; *SGF*, Simulated gastric fluid; *Sh*, Sherwood number; *SIF*, Simulated intestinal fluid; S_o, Intrinsic solubility; *t*, Time; *USP*, US Pharmacopeia; V_b, Volume of bulk dissolution medium.

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compositions available. Selection of the optimum dissolution medium depends strongly on the physicochemical properties of the drug and the fluid properties of the gastrointestinal (GI) tract.

Conventional dissolution media, such as simple US Pharmacopeia (USP) buffers (e.g., hydrochloric acid, 50-mM phosphate, acetate, and citrate) have been used for solubility and dissolution assessment for decades and are referenced in the majority of USP monographs (1,2). These media can be valuable and provide simple, reasonably accurate assessments of *in vivo* solubility and dissolution rate in some cases, such as for highly soluble, highly permeable compounds (Class 1 Biopharmaceutics Classification System (BCS) compounds) (3,4). However, these media do not mimic the properties and composition of GI fluids, which vary along the length of the intestine and exhibit high inter- and intra-subject variability (5–12) (see Table I for key GI fluid properties).

To address the need for more accurate *in vitro/in vivo* correlations for the variety of conditions along the GI tract for poorly soluble (i.e., BCS 2 and 4) drug compounds, biorelevant dissolution media (BDM) have been developed. These BDM have evolved significantly with our knowledge of GI physiology (13–19) and include versions representative of fasted and fed states along the entire length of the GI tract (6–8,20,21). These media vary in pH, buffer species, buffer concentration, osmolality, viscosity, and surface tension, as well as the concentration and type of bile components. These media have been shown to accurately predict solubility values measured in aspirated intestinal fluid for many drug substances (22). While this diversity of available buffers and simulated media enables investigation of the dissolution sensitivity of a compound to medium composition, it can also make selection of the most practical, yet biorelevant, medium challenging for pharmaceutical scientists.

This tutorial describes a methodology for selecting the simplest and most practical BDM expected to provide physiologically relevant *in vitro* dissolution performance of immediate release (IR) formulations in the upper GI tract (stomach, duodenum, and jejunum) of fasted healthy humans. This section of the GI tract was chosen because it is often where most drug absorption occurs. Our recommendations are designed primarily to guide formulation selection and optimization by screening formulations in media comprising the key physiological parameters expected to impact dissolution. Therefore, these recommendations are suited for biorelevant dissolution testing, which typically commences during early development and may continue through clinical testing and beyond (23). However, these recommendations can also be useful for development of some quality control (QC) or clinically relevant methods. While in some cases, QC, biorelevant, and clinically relevant dissolution methods may be different, in other cases, a single dissolution method may meet the purpose and requirements of all three (23).

This tutorial complements other published decision trees in the area of *in vitro* dissolution testing and is also unique in certain aspects. For example, Andreas and coworkers have published a paper introducing the OrBiTo WP2 Decision Tree, which provides guidance for selecting *in vitro* methods for aiding oral formulation development of IR, delayed release and extended release formulations (24–28). The decision tree directs the user to different "levels" of dissolution media composition as proposed by Markopolous and coworkers (27). They present general concepts for medium selection for a range of dosage forms in the fasted and fed GI tract based upon the Developability Classification System (DCS) (26,28). This tutorial complements the general framework of Markopoulos by giving the reader tools to select a medium based on drug pK_a, intrinsic solubility, and log D for IR dosage forms in the fasted state. While this

Table I. Relevant Properties of Fasted-State Human Gastric Fluid (FaHGF) and Human Intestinal Fluid (FaHIF) (jejunum) that Affect Dissolution

Property	Value		
	FaHGF (stomach)	FaHIF (duodenum)	FaHIF (jejunum)
pH	2.5 (median)[a], 1.7–3.3 (range)[a], 2.3 (median)[b], 1.1–7.5 (range)[b], 2.0 (median)[c], 1.1–3.9, (range)[c]	6.3 (median)[a], 5.6–7.0 (range)[a], 4.9 (median)[b], 1.7–7.6 (range)[b]	6.9 (median)[a], 6.5–7.8 (range)[a], 5.6 (median)[b], 2.2–6.8 (range)[b]
Buffer capacity (mM/ΔpH)	17.9 (average)[c], 1 to 160 (range)[c]	1.7 (average)[d], 0.4–6.3 (range of averages)[d]	2.3 (average)[e], 0.3–6.3 (range of averages)[e] 2 to 13[f]
Buffer concentration (mM)/species	~0.5–20 mM (range)/HCl[a]	6–20 at pH 6.5/bicarbonate[g]	6–20 at pH 6.5/bicarbonate[g]
Bile salts (mM)[a]	0.28 (median), 0.0 to 0.8 (range)	3.25 (median), 2.5–5.9 (range)	2.52 (median), 1.4 to 5.5 (range)
Phospholipids (mM)[a]	0.029 (median)	0.26 (median)	0.19 (median)
Osmolality (mOsmol)[a]	202 (median), 119 to 221 (range)	197 (median), 137–224 (range)	280 (median), 200 to 300 (range)
Surface tension (mN/m)[a]	36.8 (median), 31 to 45 (range)	34–41 (range)	25–34 (range)

[a] From ref. (38)
[b] From refs. (7,8)
[c] From ref. (39)
[d] Personal communication with author of reference (7)
[e] From ref. (7)
[f] From refs. (54,55)
[g] From ref. (29)

tutorial is most applicable to poorly soluble (i.e., BCS/DCS 2 and 4) compounds, knowing BCS/DCS class is not a prerequisite. In addition, the recommendations differ from Markopolous and coworkers in the selection of buffer capacity. Markopolous defines biorelevant buffer capacity as a BDM with a buffer capacity of the bulk solution within the range of the bulk buffer capacity reported *in vivo* (i.e., for FaHIF). In contrast, we define biorelevant buffer capacity to be drug property dependent. It refers to the buffer capacity at the surface of the dissolving drug that results in surface pH and dissolution rate similar to that of physiological bicarbonate.

DISSOLUTION MEDIUM SELECTION METHODOLOGY

Introduction

Drug-substance and drug-product dissolution is the result of a complex interplay between dissolution medium, physiological, drug substance, formulation, and product properties. The BDM selection methodology described in this tutorial accounts for the interplay between the most important properties impacting *in vivo* performance. The methodology is based upon a mechanistic understanding of how three main factors affect dissolution: (1) drug ionization at the pH levels of the stomach and small intestine, (2) alteration of surface pH by charged drug species, and (3) drug solubilization in mixed lipidic aggregates composed of bile components (i.e., bile salts, phospholipids, and cholesterol). These three phenomena are a result of the interplay between BDM and drug-substance properties, as shown in Table II.

BDM selection recommendations based upon these phenomena are summarized in Fig. 1 and described in detail in subsequent sections of this tutorial. Because dissolution can also be affected by properties such as osmolality, surface tension, viscosity, and the ionic strength of GI fluids, the recommended BDM properties were chosen to align with physiological values. While other drug-substance properties and solid-state characteristics may also affect dissolution (5,10), these properties were not the focus herein. In addition, potential impacts of excipients and dosage form design on drug release were not considered. For example, any acidic or basic excipients present in the formulation could impact the GI region/pH at which the drug is released and modulate bulk and surface pH (29–31). Therefore, recommendations are most applicable to IR dosage forms containing standard tableting excipients.

Below, we describe a methodology to select the optimum (1) pH, (2) buffer species and concentration, and (3) bile salt (BS)/phospholipid (PL) content for the BDM. We then describe confirmation of this methodology through testing and literature reviews. This evaluation showed that the recommendations correctly identified when a biorelevant buffer capacity or the addition of BS/PL to the BDM would appreciably change the dissolution profiles of the compounds. Finally, we discuss additional considerations related to *in vitro* test methods and *in silico* modeling.

Selection of pH

This section addresses the selection of medium pH based on two main variables—drug-substance pK_a and acid/base character. Specifically, we examine how these two variables affect drug ionization at gastric and small-intestinal pH levels.

Theory

The rate of dissolution of a collection of drug particles in solution can be described by

$$\frac{dC_b(t)}{dt} = \frac{1}{V_b} \cdot \mathrm{Sh} \cdot \frac{D_{\mathrm{eff}}}{R(t)} \cdot A_s(t) \cdot (C_s(t) - C_b(t)) \quad (1)$$

where C_b is the measured bulk drug concentration, t is time, V_b is the volume of the bulk solution (i.e., the BDM), Sh is the Sherwood number (i.e., the non-dimensional flux of molecules/mass from the particle surface into the surrounding fluid, equal to the effective particle radius (R) divided by the diffusion-layer thickness), D_{eff} is the effective drug diffusivity in the dissolution medium, A_s is the surface area of the dissolving particles, and C_s is the saturated concentration (i.e., saturation solubility) at the surface of the dissolving drug particle (32). As is described below, the pH at the surface of the dissolving particle, defined as the "surface pH" influences the value of C_s.

Sh in Eq. (1) accounts for potential enhancements in dissolution rate over pure diffusion, which can occur as a result of factors such as fluid shear, convection, or effects

Table II. Effect of Interplay Between BDM and Drug-Substance Properties on Dissolution

BDM property	Drug-substance property	Effect of BDM-drug-substance interplay
pH	• pK_a[a] • Acid/base character	Extent of drug ionization across pH range of GI tract
Buffer capacity	• pK_a • Acid/base character • Intrinsic solubility	Extent of surface pH alteration by charged drug species
Concentration of bile salts and phospholipids	• Log P[b]/log D[c]	Extent of drug solubilization in mixed lipidic aggregates

pK$_a$, negative log of the acid dissociation constant (K_a); *log P*, logarithm of the partition coefficient between octanol and water for a completely non-ionized molecule; *log D*, logarithm of the pH-adjusted partition coefficient of a molecule between octanol and water

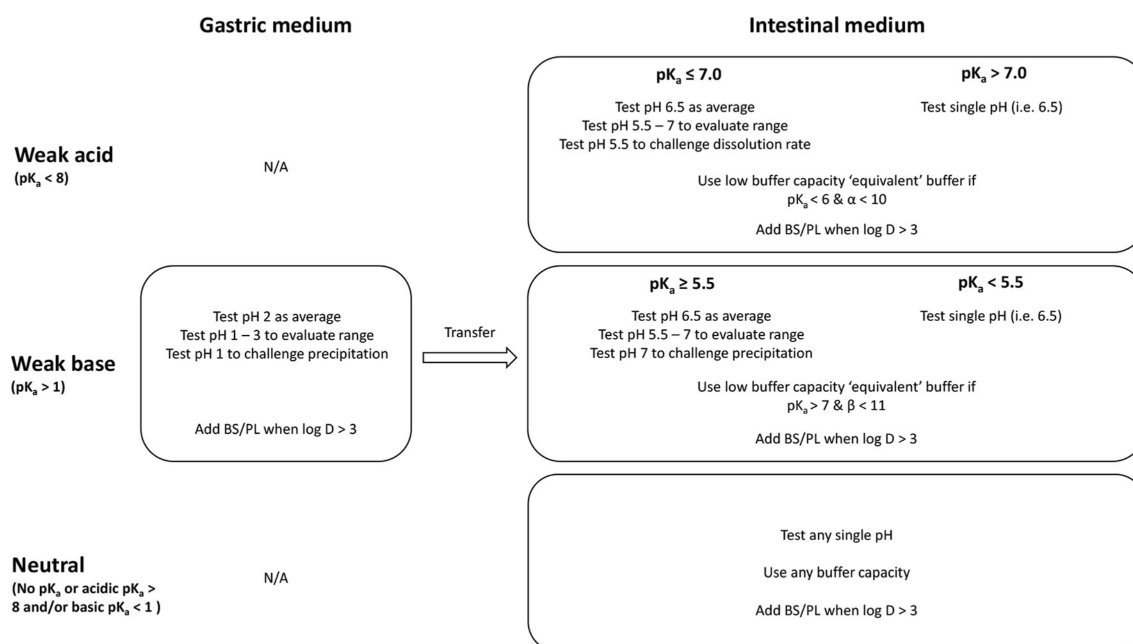

Fig. 1. Medium types recommended for *in vivo* predictive dissolution measurements. For p$K_a \leq 7$ (acids) and p$K_a \geq 5.5$ (bases), three different options are provided to accommodate different dissolution testing goals. BS/PL, bile salts and phospholipids; log D, logarithm of the pH-adjusted partition coefficient between octanol and water. α, pK_a-log S_o; β, pK_w-pK_a-log S_o. All buffers should be adjusted to an ionic strength of 0.15 M

from neighboring drug molecules in a concentrated system. When dissolution occurs as a result of pure diffusion, Sh is equal to unity and effective diffusion layer thickness equals R (32–34).

The extent of ionization of a weak acid or weak base drug can significantly affect C_s and, therefore, the rate and extent of dissolution. The extent of ionization is dependent upon the pH of the medium at equilibrium and the drug pK_a value(s) (35,36). As pH varies between different regions of the GI tract, C_s and the rate and extent of dissolution can also vary. While weak acids tend to have low solubility in the acidic stomach and increased solubility in the small intestine, weak bases tend to have high solubility in the stomach and decreased solubility in the small intestine, where supersaturation and/or precipitation may occur (37). However, for free acids and bases, the solubility from one region to another depends upon the pK_a of the drug together with physiological factors such as pH, buffer species and concentration. The relative solubility, defined as C_s/S_o can be calculated, as described in Sect. 1 and Fig. S1 of the Electronic Supplementary Materials. This value is useful for estimating the difference in solubility between the stomach and small intestine for weak acids and bases.

It is also important to consider differences in solubility within a given region due to pH variations within that region. A pH range of 1–3 brackets the median values reported in the stomach, and a pH range of 5.5–7 brackets the median pH values reported in the fasted human jejunum (see Table I) (8,38,39). Within the gastric pH range of 1–3, C_s for a weak acid is estimated to be relatively constant for drugs with a pK_a above 2.5. However, C_s is expected to vary 2-fold or greater for weak bases with p$K_a \geq 1$ across this pH range. For example, for a weak base with a p$K_a \geq 4.5$, C_s is calculated to vary 100-fold between pH 1 and 3. Therefore, gastric pH variation can be more impactful to solubility and dissolution rate of weak bases compared with weak acids.

As the pK_a of a weak acid decreases, the extent of ionization and therefore solubility becomes more sensitive to pH in the jejunal pH range (see Fig. S2 in the Electronic Supplementary Materials). For example, when p$K_a = 5$, there is a calculated 24-fold difference in C_s between pH 5.5 and 7. However, when p$K_a = 7$, there is only a 2-fold difference in C_s between pH 5.5 and 7. The opposite is true for weak bases. As the pK_a of a weak base increases, the extent of ionization and therefore C_s is more sensitive to pH in the range of 5.5–7. When p$K_a = 8$, there is a calculated 29-fold difference in C_s between pH 5.5 and 7. When p$K_a = 5.5$, there is only a 2-fold difference in C_s between pH 5.5 and 7.

Recommendations

We recommend testing weak acids in dissolution medium representative of the small intestine since a limited relative extent of dissolution is expected in the stomach. We recommend testing weak bases in a sequential gastric to intestinal dissolution medium, since a high relative extent of dissolution is expected in the stomach followed by supersaturation/precipitation in the small intestine (See Fig. 1). A "pH-dilution" method such as that performed by Gao and coworkers (40) or multicompartment methods could be employed when testing bases in a sequential dissolution transfer test, for example, from pH 2.0 to 6.5 medium (40–53). Since neutral drugs do not ionize over the intestinal pH range, they can be tested in any single pH medium.

For weak acids with p$K_a \leq 7$ and weak bases with p$K_a \geq$ 5.5, the reader can choose to study dissolution and/or precipitation at (1) an average pH, (2) over a pH range, or (3) at either the low or high end of the range. Selection of one

or multiple options may depend on whether the reader is seeking to understand performance over a range (i.e., option 2) or wants to develop a discriminating test (i.e., option 3).

A pH of 2 was selected as the average gastric pH. A range of ~1 to 3 has been reported for gastric pH in fasted healthy humans after they have ingested a glass of water (7,11,38). A pH of 6.5 was selected as the average intestinal pH, as it falls between the values reported in two recent publications and is the pH of *in vitro* surrogates of small-intestinal fluids (e.g., the pH of original FaSSIF and FaSSIF-V2) (13,27). We recommend both gastric and intestinal buffers to be adjusted to an ionic strength of 0.15 M using NaCl to reflect average ionic strength in the GI tract (9).

The goal of these recommendations is to minimize the number of pH values that must be tested, while still gaining information about potential variations in dissolution rate for pH-sensitive drugs. In addition, exposing an acidic or neutral drug to gastric medium could be important if the formulation comprises excipients whose disintegration or dissolution may be impacted differently in acidic compared to moderate pH medium. While the approach above is specific to monoprotic weak acids and bases, it can also be applied to drugs that have multiple pK_a values as described in Sect. 4 in the Electronic Supplementary Materials.

Selection of Buffer Species and Concentration

This section addresses the selection of buffer species and concentration, specifically examining the effects of drug pK_a, acid/base character, and intrinsic solubility (S_o) on surface pH.

Theory

As an ionizable drug dissolves and goes into solution, it can decrease the fluid pH (acid) or increase the fluid pH (base) when the buffer capacity of the fluid is not sufficiently high. Both cases would lead to a lower percentage of drug ionization. Therefore, solubility and dissolution rate would be lower compared to a case where the buffering capacity was high enough to withstand a potential pH change caused by dissolution of a weak acid or base.

This resulting decrease in driving force for dissolution would be reflected as a lower C_s value in Eq. (1). The buffer capacity of fluids aspirated from different regions of the GI tract from human subjects has been reported to be low. Recently, Hens and coworkers reported measured buffer capacities of aspirated fasted human intestinal fluid (FaHIF) of healthy volunteers in the fasted and fed states in the range of 2 to 6 mM/ΔpH (7). Other researchers have shown bicarbonate buffer concentrations ranging from about 6 to 20 mM in the upper small intestine, with corresponding buffer capacities ranging from 2.5 to 13 mM/ΔpH (6,38,54–57). In contrast, the buffer capacities of several BDM are at the upper end of those measured *in vivo* (see Table III), with the commonly used USP SIF (50 mM phosphate, 18 mM/ΔpH) being considerably higher. These relatively high *in vitro* buffer capacities may lead to higher *in vitro* dissolution rates than expected *in vivo* (58,59).

To what extent a drug may change the fluid pH as it goes into solution depends not only on the pH and buffer capacity of the fluid, but also on the pK_a and S_o of the drug. For an acid, the lower the pK_a relative to the starting pH of the buffer and the higher its S_o, the higher its propensity to lower the pH as it dissolves. For a base, the higher its pK_a relative to the starting pH of the buffer and the higher its S_o, the higher its propensity to increase pH as it dissolves. In both cases, the concentration of ionized drug in solution can become high relative to the concentration of buffer and result in a pH change. The terms pK_a-log S_o (designated α) for a weak acid and pK_w-pK_a-log S_o (designated β)[1] for a weak base provide a means of determining the combined contribution of drug pK_a and drug S_o on the capacity of a drug to alter surface pH.

Figure 2 (acids) and Fig. 3 (bases) show surface-area-normalized relative dissolution rates in a pH 6.5 phosphate buffer as a function of α or β. The relative dissolution rate is the calculated dissolution rate at high buffer concentration (50 mM) divided by the calculated dissolution rate at low buffer concentration (1 mM). When the relative dissolution rate equals unity, no differences in rate are expected over this range in buffer concentration. As shown in Fig. 2, for a monoprotic weak acid, when the drug pK_a is ≤ 6, up to a 2-fold or greater difference in dissolution rate could occur when α is in the range of 5 to 10. As shown in Fig. 3, for a weak base, when drug p$K_a \geq 7$, up to a 2-fold or greater difference in dissolution rate could occur when β is in the range of 5 to 11. When α or β is outside of this range, the effect of buffer concentration on dissolution rate is insignificant. Above a value of 10 (acids) or 11 (bases), the drug S_o is low enough that the concentration of ionized drug in solution at the starting pH is too low relative to the buffering capacity to change surface pH. Therefore, the dissolution rate is near its maximum at that pH in both buffer concentrations. At low values of α or β (such as p$K_a = 5$ and $\alpha < 6$), S_o is high enough that surface pH has changed to a similar extent in both buffer systems and dissolution rate is near its minimum. Section 2 in the Electronic Supplementary Materials provides plots of surface-area-normalized relative dissolution rates in pH 5.5 and 7 phosphate buffers for monoprotic weak acids and bases as a function of α or β, respectively. For a weak acid, the propensity to decrease surface pH at a given S_o is higher at pH 7 compared with pH 6.5 since pH-pK_a increases. For a weak base, the propensity to increase surface pH at a given S_o is higher at pH 5.5 compared to pH 6.5 since pK_a-pH increases. Section 2 in the Electronic Supplementary Materials also provides a plot of calculated surface area normalized dissolution rate in a given buffer system relative to infinite buffer capacity. This plot can be used to estimate when a drug may have a propensity to alter surface pH over a range of buffer species, buffer concentrations, and pH values.

Recommendations

Based upon the impact of alteration of surface pH on dissolution, we make the recommendations for selection of buffer capacity shown in Fig. 1. If a drug substance has a propensity to alter surface pH, we recommend using buffer concentrations/capacities lower than what is achieved using a

[1] pK_w value of 13.6 at 37°C and saturated vapor pressure used in this tutorial (60).

Table III. Buffer Capacities and Compositions of Bile Components and Phospholipids in of Some Common *In Vitro* Biorelevant Media and USP SIF, TS

BDM property	Value						
	FaSSGF [a,b]	FaSSIF[c]	FaSSIF-V2[d]	FaSSIF-V3[e]		Bicarbonate[f]	USP SIF, TS[g]
Buffer species	–	Phosphate	Maleate	Maleate	Phosphate	Bicarbonate	Phosphate
Buffer pK_a	–	6.69[c]	6.00[e]	6.00[e]	6.69[c]	6.04	6.69
Buffer concentration (mM)	–	28.7	19.1	10.26	13.51	16.2	50
pH	1.6	6.5	6.5	6.7		6.5	6.8
Osmolarity (mOsmol/kg)	120.7	270	180	215		Not measured	113
Experimental buffer capacity (mM/ΔpH)	–	12	10	5.6		7	18.4
Bile salt(s) (mM)	0.08 (TC)	3 (TC)	3 (TC)	1.4 (TC), 1.4 (GC)		–	–
Phospho-lipid(s) (mM)	0.020 (PC)	0.75 (PC)	0.2 (PC)	0.035 (PC), 0.315 (LPC)		–	–
Sodium oleate (mM)	–	–	–	0.315		–	–
Cholesterol (mM)	–	–	–	0.2		–	–
Average surface tension (mN/m)	42.6	54.7	54.2	35.1		Not measured	Not available

TC, taurocholate; *GC*, glycocholate; *PC*, phosphatidylcholine (lecithin); *LPC*, lysophosphatidylcholine (lysolecithin)
[a] From ref. (19)
[b] Medium also contains 0.1 mg/mL pepsin
[c] From ref. (14)
[d] From ref. (16)
[e] From ref. (13)
[f] From ref. (59)
[g] From ref. (15)

typical (i.e., USP 50-mM) buffer. The values of pK_a, α and β were selected because they delineate when a greater than 2-fold difference would be expected between a 50-mM phosphate buffer concentration (i.e., USP SIF) and a 1-mM concentration with a calculated Van Slyke buffer capacity (0.5 mM/ΔpH at pH 6.5) at the lower end of the range in FaHIF. Although a difference in dissolution rate between 50 mM and 1 mM phosphate buffers is not expected at low values of α or β (for example, p$K_a = 5$ and $\alpha < 6$) despite alteration of surface pH, a lower threshold is not specified since these cases would mainly be relevant for high solubility (i.e., BCS 1 or 3) drugs with $S_o > 1$ M.

For drugs that do not have the propensity to alter surface pH (i.e., they have values outside the range described above), any convenient buffer-capacity buffer can be used. It is unnecessary to select an "equivalent" buffer to bicarbonate, since the buffer capacity of a maleate or phosphate buffer, for example, will have little to no effect on the resulting

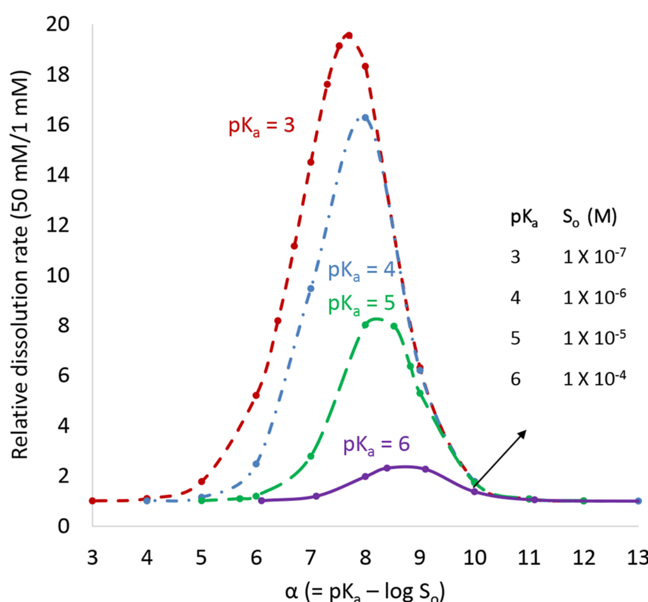

Fig. 2. Relative predicted surface-area-normalized dissolution rate for a monoprotic weak acid in phosphate buffer at pH 6.5. Assumes different drug pK_a values at a high (50 mM) and low (1 mM) buffer concentration. Drug diffusivity = 7.9×10^{-6} cm^2/s. Effective diffusion layer thickness = 30 μm

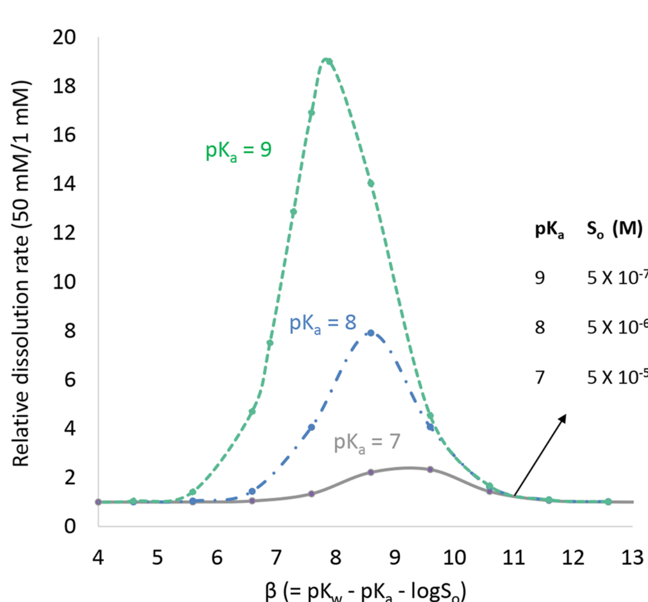

Fig. 3. Relative predicted surface-area-normalized dissolution rate for a monoprotic weak base in phosphate buffer at pH 6.5. Assumes different drug pK_a values at a high (50 mM) and low (1 mM) buffer concentration. Drug diffusivity = 7.9×10^{-6} cm^2/s. Effective diffusion layer thickness = 30 μm

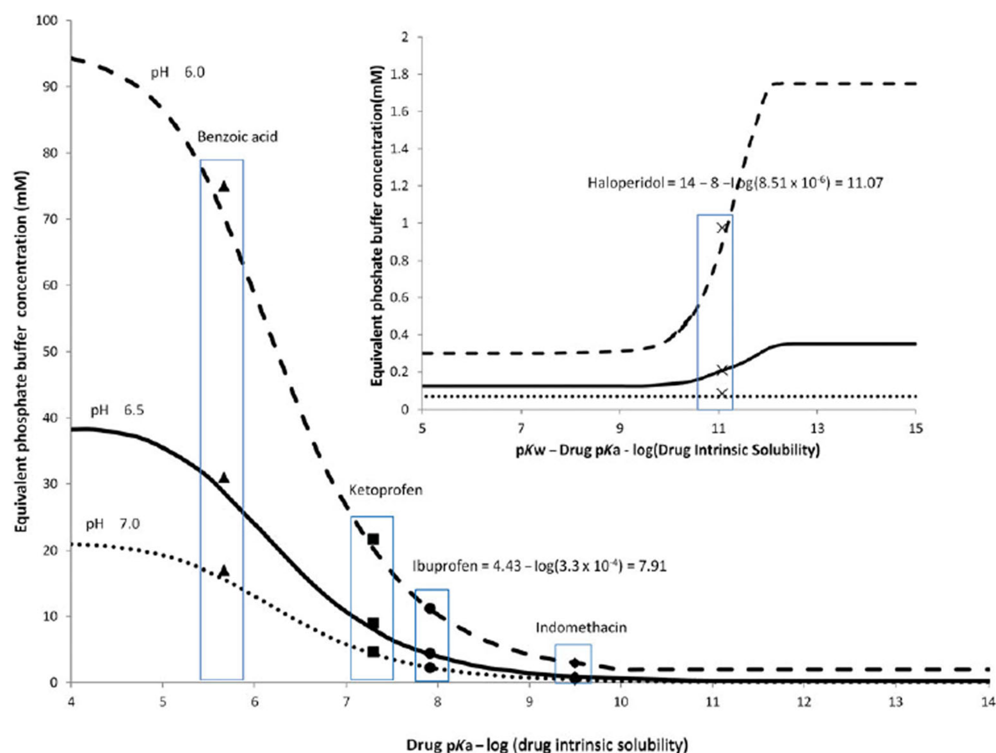

Fig. 4. Predicted equivalent phosphate buffer concentration needed to match physiological bicarbonate buffer for weak acids and weak bases (reproduced with permission from Krieg *et al.* (59)

dissolution rate, provided properties such as ionic strength are held constant. These recommendations can be applied to polyprotic drugs as described in Sect. 4 in the Electronic Supplementary Materials.

The method developed by Krieg and coworkers (see Fig. 4) can be used to tailor the buffer concentration of a phosphate buffer to achieve an "equivalent" buffer representative of average performance in physiological bicarbonate (48). For acids, Krieg and coworkers recommend phosphate concentrations in the range of 1 to 25 mM, depending on the value of α. For bases, they recommend extremely low buffer concentrations (< 2 mM) to match physiological bicarbonate buffer (59). To study the range of the expected variation in dissolution rate as a function of buffer capacity, the concentration could be expanded above and below the calculated "equivalent concentration" rather than simply relying on an average bicarbonate buffer capacity as suggested by Krieg. Because the recommendations by Krieg and coworkers are based upon experiments performed under sink conditions, it may be necessary to use a titrant to maintain bulk pH if desired when drug-substance solubility and the dose-to-volume ratio in the dissolution experiment are high (59). Since a pH of 5.5 is greater than 1 pH unit below that of phosphate, equivalent maleate concentrations for monoprotic weak acids and bases at pH 5.5 are provided in Sect. 3 in the Electronic Supplementary Materials.

The analysis described in this tutorial represents a static situation in which a drug is dissolving in a buffer within a well-mixed, closed container. In contrast, the *in vivo* situation is dynamic. In the intestinal lumen, the fluid composition and resulting buffer capacity at a given location is impacted by factors such as secretion of bicarbonate and digestive enzymes, fluid absorption, fluid transit and hydrodynamics (58). In addition, the concentration of dissolved drug is affected by absorption into the intestinal membrane or transit down the GI tract. While closed container *in vitro* dissolution devices cannot mimic this situation, using a pH-stat or multicompartmental devices that incorporate both transit and secretion move closer to capturing the dynamic situation *in vivo* (40–53).

When considering dissolution in the intestine, selecting an equivalent buffer is likely more important for weak acids than for weak bases. Whereas weak acids tend to dissolve to a much greater extent in the small intestine, weak bases tend to first dissolve in the stomach and then potentially supersaturate and or/precipitate in the small intestine. Therefore, the implication of buffer capacity on dissolution rate for bases in the small intestine is less important, particularly for small precipitates, which may re-dissolve in the intestine. In this case, the effect of bulk buffer capacity on the extent of supersaturation/precipitation/re-dissolution of bases is likely of greater importance.

While this tutorial focuses on the impact of surface pH on intestinal dissolution, surface pH in the stomach can be important, particularly for weak bases. Pepin and coworkers demonstrated the impact of surface pH on the solubility and dissolution rate of the weak base, acalabrutinib (61). They demonstrated that surface pH increased to 4.0 at a bulk pH of 2 in hydrochloric acid. Inputting surface solubility calculated from surface pH in an *in silico* model provided better predictions of experimental dissolution rate compared to overpredictions arising from using bulk solubility. These authors provide a methodology for predicting surface pH of monoprotic and diprotic weak bases (61).

Selection of Bile Salts and Phospholipids

This section addresses the inclusion or exclusion of BS and PL in the BDM and their concentrations, focusing on the effect of drug-substance lipophilicity on solubilization in mixed lipidic aggregates.

Theory

Solubilization of lipophilic drug substances in mixed lipidic aggregates has the potential to increase C_s and, therefore, the rate and extent of dissolution. Intestinal mixed lipidic aggregates are mixed micelles or vesicles composed of BS, PL, and cholesterol. The aggregates present in aspirated FaHIF have been found to vary significantly between individuals and prandial state in terms of composition, size, and form (62). Riethorst and coworkers provide recent updates on the human inter-subject variability of the composition (20). In simulated intestinal media, mixed lipidic aggregates can be present as vesicles or swollen micelles with approximate diameters of 45 nm (in fasted-state media) or micelles with diameters near 6.5 nm (in fed-state media) (5,63–66).

The extent of solubilization in mixed lipidic aggregates depends on colloidal properties such as lipid concentration, BS and PL composition, ratio of BS to PL, and structure. Further, drug properties such as size, charge, polarity, flexibility, and lipophilicity impact the partitioning into these colloids (5). Based on solubility profiling of more than 100 drug substances, Fagerberg and Bergström showed that molecules with a pH-dependent partition coefficient between octanol and water (log D) greater than 3 at pH 6.5 showed significantly higher solubility in FaSSIF than in blank buffer (i.e., same buffer species/concentration and pH but excluding BS and PL) (5). This result is intuitive; because the volume of FaSSIF lipidic aggregates/vesicles is about 0.1% of aqueous volume, 1000-fold partitioning into these structures enhance solubility by at least 2-fold. Based on these data, the solubility of lipophilic compounds (log $D > 3$) should always be assessed in a FaSSIF (any version) rather than a simple buffer to estimate solubilization that occurs *in vivo* (14,55,67–72).

For cases where the dose of drug exceeds C_s in blank buffer, then the increased solubilization in mixed lipidic aggregates would increase the extent of dissolution due to the increased solubilization capacity of the fluid. However, when the dose is less than C_s in blank buffer, the extent of dissolution with and without mixed lipidic aggregates would be expected to be similar. The rate of dissolution in the presence of mixed lipidic aggregates is influenced by a competing effect of D_{eff} and C_s. While solubilization of drug in lipidic aggregates increases C_s, it decreases D_{eff} due to an increase in the effective size of drug associated with mixed lipidic aggregates over that of unbound drug (73–76).

Figure 5 shows the predicted relative dissolution rate in FaSSIF *versus* blank buffer as a function of the relative solubility in FaSSIF *versus* blank buffer. The basis for these calculations is described in Sect. 5 in the Electronic Supplementary Materials. As shown, a 10-fold increase in C_s compared with blank buffer is expected to lead to only a

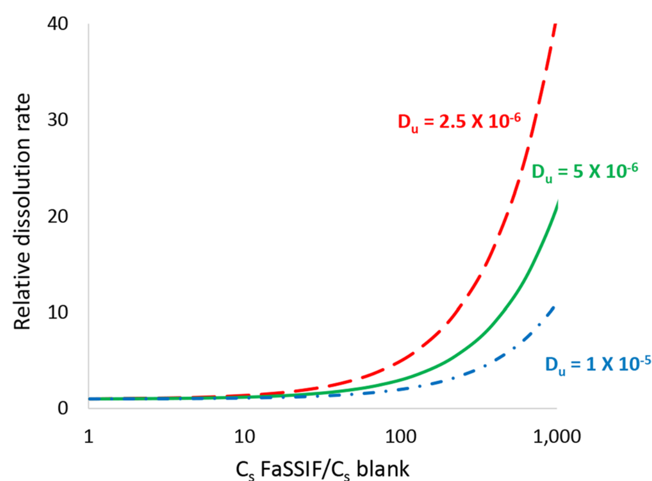

Fig. 5. Calculated relative dissolution rate in FaSSIF *versus* blank buffer as a function of solubility ratio (e.g., solubility in FaSSIF/solubility in blank buffer) for drugs with different aqueous diffusion coefficients. Blank buffer comprises the same buffer species and concentration as FaSSIF, but excludes BS and PS. Assumes micelle diffusion coefficient = 1×10^{-7} cm^2/s

1.2-fold increase in dissolution rate under sink conditions assuming $D_{eff} = 5 \times 10^{-6}$ cm^2/s. Fagerberg and Bergström showed a fold increase in FaSSIF exceeding 10 only for compounds with log $D_{6.5}$ values > 3. Therefore, only a minor difference in dissolution rate between FaSSIF and blank buffer would be expected for compounds with log $D < 3$ and $D_{eff} = 5 \times 10^{-6}$ cm^2/s. More significant differences in dissolution rate between FaSSIF and blank buffer would be expected for compounds with much higher extents of solubilization in mixed lipidic aggregates. Assuming sink conditions and $D_{eff} = 5 \times 10^{-6}$ cm^2/s, it would take a 50-fold increase in C_s compared to blank buffer to produce a 2-fold increase in dissolution rate, and a 450-fold increase in C_s compared with blank buffer to produce a 10-fold increase in dissolution rate.

Recommendations

We provide recommendations in Fig. 1 regarding addition of BS and PL in the dissolution medium. We select a log $D > 3$ as a minimum value for when to add BS and PL to the medium. While log D is not the sole determinant of solubilization in mixed lipidic aggregates, it serves as a convenient estimate (5). The extent of solubilization in mixed lipidic aggregates is also influenced by physicochemical properties as previously described. While some compounds with log $D < 3$ may have a fold increase in C_s compared to blank buffer higher than what was observed by Fagerberg and Bergström (i.e., > 10-fold), a fold increase below 50 still would not be expected to significantly impact dissolution rate assuming $D_{eff} \geq 5 \times 10^{-6}$ cm^2/s. BS and PL have been shown to improve wetting by lowering the interfacial tension between the dissolution medium and the drug. Therefore, inclusion of these components in the medium could be considered for compounds with log $D < 3$ that demonstrate poor wetting characteristics (77).

No single type and concentration of bile salts and phospholipids is expected to provide the best forecast of

solubility in FaHIF for all drugs. Therefore, we recommend using the BS and PL types and concentrations of one of the standard fasted-state media, e.g., FaSSIF, FaSSIF-v2, or FaSSIF-v3 for intestinal media or FaSSGF for gastric media. While the original FaSSIF composition and FaSSIF-v2 contain only two bile components (lecithin and taurocholate), FaSSIF-v3 more closely resembles luminal composition with the incorporation of additional components, such as lecithin hydrolysis products and cholesterol (see Table III). For acids and bases, FaSSIF and FaSSIF-V2 both provide solubility values similar to those obtained in aspirated FaHIF (72). For neutral compounds, the FaSSIF seems to over predict the effect of solubilization, whereas the solubility values obtained in FaSSIF-V2 and the aspirated FaHIF are in better agreement (72). Fuchs and coworkers compared the solubility values of ten different model compounds in FaSSIF, FaSSIF-v2, and FaSSIF-v3, as well as in FaHIF. They found the differences in solubility values across *in vitro* media vary as a function of the drug, with some having equal solubility values in all three media and others showing increased solubility in one or two media. FaSSIF-v3 provided an equal or better forecast of solubility in FaHIF for eight of the compounds compared with FaSSIF and FaSSIF-v2. Evaluating the dissolution rate using all three compositions of bile components—as reflected by FaSSIF, FaSSIF-v2, and FaSSIF-v3—would provide an early indication of differences within and among individuals in dissolution screens.

CONFIRMATION OF RECOMMENDATIONS

To confirm the recommendations, we performed in-house tests on eight model compounds and evaluated the recommendations against multiple reports in the literature. The study was narrowed to the impacts of buffer concentration and inclusion of BS and PL on dissolution rate since these factors have been less studied than pH impacts (4,26). In all cases, the recommendations were in good agreement with the results.

In-house Experimental Evaluation

In vitro measurements were performed in house to confirm the recommendations. We determined the dissolution rate for eight model compounds that reflected different categories (e.g., acid/base/nonionizable; highly lipophilic *versus* modestly lipophilic). Experiments were performed under sink conditions using a μDISS Profiler (Pion Inc., Billerica, Massachusetts) in 10 to 20 mL of dissolution medium at 37 °C. Methods and a detailed explanation of the experimental confirmation are provided in Sect. 6 in the Electronic Supplementary Materials.

The in-house experimental evaluation showed that the recommendations correctly identified when a biorelevant buffer capacity or the addition of the bile salts/phospholipids to the medium would appreciably change the dissolution profiles of the compounds. For compounds for which biorelevant buffer capacity was expected to show no change in dissolution rate (i.e., <2-fold difference), the difference in initial dissolution rate was 0.9–1.8-fold, whereas when a change was expected, the difference was 2.7- to 8.1-fold. For compounds for which the addition of BS and PL was expected to show no change in dissolution rate, the difference in initial dissolution rate was 1.0- to 1.1-fold, whereas when a change was expected, the difference in initial dissolution rate was 1.3- to 1.7-fold.

Literature Evaluation

Literature evaluations assessing the recommendations herein were focused on studies that probed the effect of (1) buffer concentration on dissolution rate and (2) BS and PL on dissolution rate. As described below, both evaluations revealed good agreement with recommendations.

Effect of Buffer Capacity on Dissolution Rate

We evaluated multiple literature studies to determine the effect of buffer capacity on dissolution rate. In the first study, the dataset of acids published by Krieg and coworkers confirms the recommended guidelines for selection of biorelevant buffer capacity (59). They studied the effect of phosphate buffer concentration on dissolution rate for five different acids and one base in the rotating-disk dissolution apparatus at a pH of 6.5. All six compounds had α (acids) or β (bases) values less than 10 and showed an increase in flux at higher buffer concentrations within the range of ~3 to 50 mM. "Equivalent" phosphate buffer concentrations for these compounds ranged from less than 1 mM to 30 mM.

In the second study, Cristofoletti and Dressman used the approach of calculating an equivalent phosphate (5.0 mM) or equivalent maleate (2.2 mM) buffer to match dissolution rate of ibuprofen tablets in a physiological bicarbonate buffer at a pH of 6.7 (78–80). They found that a 5.0-mM phosphate concentration resulted in slower dissolution compared to a 13.5-mM phosphate concentration (i.e. FaSSIF-v3 buffer concentration), as well as better predicted *in vivo* performance differences of two different tablet formulations of ibuprofen.

In the third study, Hamed and coworkers demonstrated an increase in the dissolution rate of a weak acid, valsartan, as a function of buffer concentration in phosphate buffer (81). Within the first 5 min of the experiment, 36.3, 55.2, 72.3, and 82.9% of valsartan was released in 12.5-, 25-, 50-, and 100-mM buffer concentrations, respectively, despite maintaining a bulk pH of 6.8 throughout the duration of the experiment. Valsartan has a pK_a of 4.37 and an α value of 5.0 (82). These values meet the criteria for an expected effect of buffer capacity/concentration (pK_a <6 and $\alpha<10$). The same authors showed an increase in the dissolution rate of a weak base, carvedilol, as a function of buffer concentration in phosphate buffer (83). The authors showed that approximately 34% of the dose was released after 60 min for a 6.25-mM buffer, whereas approximately 52 to 58% of the dose was released after 60 min for buffer concentrations ranging from 12.5 to 100 mM. The bulk pH of 6.8 was maintained within ± 0.05 units through 60 min. Carvedilol has a pK_a of 7.8 and β value of 5.7, which again meets the methodology criteria for bases showing buffer capacity-dependent dissolution (p$K_a>7$ and $\beta<11$) (83).

Effect of Bile Salts/Phospholipids on Dissolution Rate

For this evaluation, we looked at a literature report by Okazaki and coworkers, who performed dissolution experiments using suspensions of two neutral drugs—griseofulvin (Admet Predictor log $P = 2.5$) and Danazol (log $P = 4.5$)—in a USP 2 apparatus (76). They compared dissolution rates for each drug in buffers containing bile salts and lipids in the medium (sodium taurocholate concentrations of 3 (FaSSIF), 15 (FeSSIF), and 30 mM with sodium taurocholate:lecithin 4:1) to blank buffer. They found the increase in solubilization due to added bile salts and lipids in the medium was higher than the increase in initial dissolution rate in five out of six cases due to the decrease in effective diffusion coefficient. Griseofulvin showed a modest 1.5-fold increase in dissolution rate in FaSSIF compared to blank buffer, whereas the more lipophilic Danazol showed a 2.5-fold increase in dissolution rate. Additional researchers have reported similar results for other types for media, for example, using FaSSIF-dog and sodium dodecyl sulfate (75,84,85).

IN VITRO TEST METHOD AND *IN SILICO* MODELING CONSIDERATIONS

While this tutorial focuses on selection of the optimal BDM, it is important to consider the impact of *in vitro* test methods and usefulness of *in silico* modeling. *In vitro* dissolution testing can serve a multitude of purposes through drug development and commercialization (23). The recommendations outlined in this tutorial are well suited to biorelevant dissolution testing. However, in some cases, they can also be useful for development of a QC or clinically relevant methods. QC methods typically require standard apparatuses with one compartment, commonly used buffers that do not contain BS or PL (but may contain synthetic surfactants) and standard medium volumes. In addition, QC methods for IR products tend to specify sink conditions with 80% release within a relatively short time frame, such as within 30 min. These methods must be low in cost, complexity, time, and labor. Since our recommendations suggest the simplest, yet accurate dissolution medium/media based upon drug properties, they work toward selection of a "QC-relevant" medium whenever possible; that is, when drug properties suggest that added medium cost and complexity are not critical for achieving biorelevant dissolution results. In this way, these guidelines provide a preliminary analysis in terms of understanding when a medium may meet the requirements of both a QC medium and a biorelevant medium. In addition, since our recommendations suggest inclusion of key properties and components expected to impact *in vivo* performance, they may also be useful for the development of dissolution media for clinically relevant dissolution methods.

The choice of dissolution apparatus such as single compartment, multicompartmental, or a system comprising an absorption compartment can significantly impact results (86). In addition, associated parameters such as fluid volume(s), stirring and related shear and hydrodynamics and transfer rates (if applicable) are important considerations (10,23,25).

In silico modeling can complement *in vitro* dissolution testing in facilitating understanding of the range in important physiological, drug substance and formulation properties impacting *in vivo* performance (87). For example, commercial software packages or in-house models can be used to study the impact of factors such as pH, buffer concentration, and mixed lipidic aggregate concentration on *in vitro* dissolution performance. Modeling can complement the approach of minimizing the number and complexity of media selected for *in vitro* dissolution testing by predicting these impacts *in silico*. Further, these *in vitro* predictions can be coupled with physiologically based modeling to further predict *in vivo* performance of the drug product of interest.

In addition, modeling could be used to further refine the recommendations in this tutorial. For example, one could integrate drug pK_a, intrinsic solubility and log *D*/extent of solubilization into a dissolution model. This model could be solved for various types of dissolution media, providing relative dissolution rates as a function of several physiological and drug substance factors simultaneously.

CONCLUSION

This tutorial presents a methodology to select a practical yet physiologically relevant dissolution medium for assessing dissolution of standard IR dosage forms administered to fasted humans. Recommendations are primarily suited toward biorelevant dissolution testing, which typically commences during early development and may continue through clinical testing and beyond. However, in some cases, they can also be useful for development of QC or clinically relevant methods.

This methodology is based upon the mechanisms by which drug substance physicochemical properties and human physiological characteristics influence *in vivo* dissolution of drug substances. The drug physicochemical properties needed to employ these recommendations can be predicted *a priori* using in-house models or commercial tools or be measured *in vitro* if further refinement is needed. While using a dissolution medium that mimics *in vivo* human intestinal fluid as closely as possible would be expected to provide the most biorelevant dissolution results, simplifying the medium has several advantages, such as reducing time and cost and increasing medium robustness. Finally, for biorelevant dissolution testing to be fully realized, these recommendations must be coupled with a physiologically relevant dissolution apparatus and associated testing parameters.

ACKNOWLEDGMENTS

We are grateful for the discussions on pK_a and buffer capacity with Dr. Alex Avdeef. We would also like to acknowledge Dr. Brian Krieg for discussions regarding calculations of predicted dissolution rate of acidic and basic drugs in bicarbonate and "equivalent" buffers and access to his MATLAB script. We thank Simulations Plus (Lancaster, California) for providing the Drug Delivery Group at the Department of Pharmacy, Uppsala University, with a reference site license for the software

ADMET Predictor. The authors declare no conflicts of interest.

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