

Physicochemical Properties of Buffers Used in Simulated Biological Fluids with Potential Application for In Vitro Dissolution Testing: A Mini-review

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ABSTRACT

This literature review focuses on the physicochemical properties of buffers used in biorelevant media for in vitro dissolution testing. Because biorelevant dissolution systems have been previously reviewed, this review is primarily related to the buffers used in biorelevant media and not the media, per se. It is based on a survey of the literature in areas including analytical chemistry; biochemistry; clinical, solution, and physical chemistry; dissolution science and technology; pharmaceutical sciences; simulated biological fluids; and buffer-related articles from the National Institute of Standards and Technology with the intent of identifying challenges in formulating buffered biorelevant dissolution media and also identifying general attributes that are useful in selecting a buffer for a particular dissolution application. Other topics include: (1) key physicochemical properties of buffers that affect experimental conditions and dissolution results; (2) buffer compatibility with a diverse array of ingredients found in biorelevant media; and (3) the potential to supplement the library of traditional buffers used for in vitro dissolution testing with zwitterionic buffers that were designed for biological application.

KEYWORDS: Buffers; physicochemical buffer properties; in vitro dissolution; biorelevant media; zwitterionic buffers.

INTRODUCTION

The pH of buffered dissolution media is a singularly important parameter for dissolution testing because pH influences drug solubility and dissolution rate (1). Additionally, lack of pH control negatively influences the reproducibility of the results (2). The history of dissolution media over the past 20 years has been the subject of a recent review (2), which concluded that “dissolution testing has been and will continue to be the paradigm in which the ability of the product to release the drug under physiologically relevant conditions is increasingly emphasized.” Marques et al. (3) support this conclusion in their review of simulated biological fluids that have application for in vitro dissolution testing.

Buffers have maintained a central role in the evolution of dissolution media. Standardized buffers, exemplified by those in the *USP* (4), are commonly used as in vitro dissolution media. These buffers are chemically defined, cover a useful pH range, and have known ionic strength and buffer capacity. The bicarbonate buffer system has been studied as a physiologically relevant medium (5), and a recent development in the evolution of biorelevant dissolution media is the use of diluted phosphate buffers as a surrogate for the bicarbonate buffer (5, 6).

Developing buffered systems that simulate biological fluids is an active area of research in several biomedical areas (7, 8). These buffers share many of the challenges encountered with buffers used for in vitro dissolution testing including requirements for ionic strength, buffer capacity, pH control, biological relevancy, and avoidance of incompatibilities. These simulated biological fluids exhibit the following general characteristics: (1) the buffer capacity reflects the function at a biological site, such as the eye; (2) even at relatively low concentrations, the buffer contributes significantly to the physicochemical properties of the simulated biological fluid; and (3) sodium chloride and other salts contribute substantially to the ionic strength of the simulated biological fluid.

Although aqueous buffers alone cannot be expected to fulfill all the requirements of biorelevant media, buffers will likely continue to play a central role in controlling the pH of complex formulations simulating biological fluids (3, 9). The use of biorelevant dissolution media to forecast in vivo drug performance has been reviewed (9). This mini-review focuses on the physicochemical factors related to buffers used in these media and not the biorelevant dissolution media, per se. This mini-review is based on a survey of the literature associated with buffers in general

and, specifically, biologically relevant buffers, with the intent of identifying challenges in formulating buffered dissolution media and identifying general attributes that are useful in selecting a buffer for a particular application.

Challenges Associated with Formulating Buffered Biorelevant Dissolution Media

Recent developments in buffered dissolution media point to several challenges due, in part, to having significantly reduced buffer capacity in comparison with traditional buffered dissolution media. For example, the currently used USP phosphate buffer, pH 6.8, has a total phosphate concentration of 50 mM and a buffer capacity of 29 mM/ Δ pH (6). By comparison, the average concentration of the bicarbonate buffer system in the small intestine is 6–20 mM, which corresponds to a buffer capacity of 2.48–13 mM/ Δ pH (6). Krieg et al. (6) note that external control of pH may be necessary to maintain the pH of the bulk solution due to the low buffering capacity of surrogate phosphate buffers. Therefore, maintaining a stable pH with a buffer having relatively low buffer capacity is one challenge for a biorelevant dissolution medium that simulates in vivo conditions. Other challenges and factors affecting buffer performance include the potential for incompatibilities between the buffer and other ingredients in a complex biorelevant dissolution media formulation (3, 9); the effect of ionic strength contributed by the buffer on enzymes and bovine serum albumin (10–12); and an interaction between the buffer, metals, and polyvalent cations (13–15).

These challenges draw attention to the need to identify general physicochemical characteristics and attributes that are useful for selecting a buffer for a specific biorelevant dissolution medium.

Identifying General Characteristics Useful for Selecting Buffers for Biorelevant Dissolution Media

Good et al. (16, 17) discussed characteristics and attributes for buffers used in biological and biochemical research that have proved to be useful in selecting a buffer for a specific application. Good's original list can be modified to describe general buffer characteristics and attributes applicable to biorelevant media used for in vitro dissolution testing. These include: (1) buffer pKa within ± 1 pH unit of the target pH, (2) sufficient buffer capacity to maintain pH control, (3) minimal buffer-related ionic strength effects and minimal buffer-pKa dependence on temperature and ionic strength, (4) compatibility with other dissolution media components, (5) low absorbance spectra in UV and visible regions, (6) buffer effect on drug solubility, (7) nonreactivity with atmospheric gases, and compliance with standards (8) for safety and toxicity.

A brief outline of buffer definitions and terminology will be provided as a preface to a review of these characteristics.

Buffer Definitions and Terminology

For buffers with a single ionization or well-separated pKa values, the following definitions and terminology, according to Ellis et al. (18), follow for the dissociation of a weak acid, HA^{z+1} :



where z is the charge on the conjugate base, A^z . The thermodynamic dissociation constant, K_a , is given by:

$$K_a = (a_{H^+} a_{A^z}) / (a_{HA^{z+1}}); a_i = [c_i] \gamma_i \quad (2)$$

where the activity of each ion, a_i , is the product of its molar or molal concentration $[c_i]$ and activity coefficient, γ_i . Equation 1 can now be expressed in terms of molar or molal concentrations, denoted by square brackets $[]$, with each concentration term being multiplied by its respective activity coefficient, γ_i :

$$K_a = (\gamma_{H^+} [H^+] \gamma_{A^z} [A^z]) / (\gamma_{HA^{z+1}} [HA^{z+1}]) \quad (3)$$

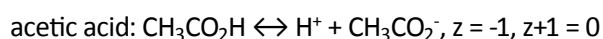
A commonly used definition of the apparent dissociation constant, K_a' , is given by the following expression (18):

$$K_a' = (\gamma_{H^+} [H^+] [A^z]) / [HA^{z+1}] \quad (4)$$

and the logarithmic form of Equation 4 is:

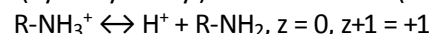
$$pH = -\log (\gamma_{H^+} [H^+]) = pK_a' + \log([A^z] / [HA^{z+1}]) \quad (5)$$

Examples of common buffer systems that follow the general form of Equation 5 are:

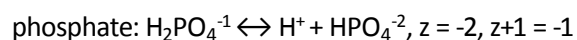


$$pH = pK_a' + \log([CH_3CO_2^-] / [CH_3CO_2H])$$

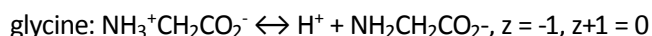
tris-(hydroxymethyl)-aminomethane (TRIS):



$$pH = pK_a' + \log([R-NH_2] / [R-NH_3^+])$$



$$pH = pK_{a2}' + \log([HPO_4^{-2}] / [H_2PO_4^{-1}])$$



$$pH = pK_{a2}' + \log([NH_2CH_2CO_2^-] / [NH_3^+CH_2CO_2^-]).$$

Equation 5 can be expressed in a more general form as:

$$pH = pK_a' + \log([\text{conjugate base}] / [\text{weak acid}]) \quad (6)$$

Equating [conjugate base] and [weak acid] with their analytical concentrations, C_{cb} , and C_{wa} , respectively, and substituting into Equation 6 yields:

$$\text{pH} = \text{pKa}' + \log (C_{cb}/C_{wa}) \quad (7).$$

Equation 7, commonly referred to as the buffer equation, is valid in the pH range of 3–11 where the concentration of the hydronium and hydroxyl ions is much less than the concentration of the buffer species. A complete derivation of the buffer equation, with assumptions and constraints related to its use, is provided by Butler (19).

The ionic strength, I , for the buffer systems described by Equation 1 is expressed by (18):

$$I = [\text{HA}^{z+1}]\{(z + 1)^2 + |z + 1|\}/2 + [\text{A}^z]\{(z^2 + |z|)/2\} \quad (8).$$

The factors in curly brackets { } associated with $[\text{HA}^{z+1}]$ and $[\text{A}^z]$ as a function of z , and as constructed by Ellis et al. (18), are useful to calculate I and are found in Table 1.

Table 1. The Factors in { } Associated with $[\text{HA}^{z+1}]$ and $[\text{A}^z]$ as a Function of z , and as Constructed by Ellis et al^a

z	Factor { } associated with	
	HA^{z+1}	A^z
2	6	3
1	3	1
0	1	0
-1	0	1
-2	1	3

^aEllis, K. J.; Morrison, J.F. Buffers of Constant Ionic Strength for Studying pH-Dependent Processes. *Methods Enzymol.* **1982**, *87*, 405–426. DOI: 10.1016/S0076-6879(82)87025-0 (18).

Discussion of Characteristics Potentially Useful in Selecting Buffers for Biorelevant Dissolution Media

Relationship Between the Target Dissolution Media pH And Buffer pKa

For a buffer to have sufficient buffer capacity to be effective, its pH must be within a range of $\text{pKa}_{\text{buffer}} \pm 1$ or preferably within $\text{pKa}_{\text{buffer}} \pm 0.5$ (20). Using a criterion of $\text{pKa}_{\text{buffer}} \pm 0.5$ assures that the effectiveness of the buffer will not be less than 73% of the maximum buffer capacity for a specified buffer concentration. Thermodynamic pKa data ($I = 0$) are available for buffers commonly used in biorelevant dissolution media (see Table 2) (21–32); however, these values do not reflect the electrolyte environment where buffer concentration is relatively low and salts, such as sodium chloride, contribute significantly to the ionic strength of the media. For example, the thermodynamic pKa_2 for a phosphate buffer system

at 37 °C is 7.181 (Table 2) (21–32) while pKa_2' is 6.75 at 37 °C under conditions where the buffer concentration is low and sodium chloride contributes significantly to the ionic strength of the buffer solution (33). Selecting a pKa' value measured at a temperature close to test conditions is another factor because pKa-temperature dependence for buffers such as TRIS may be significant (34).

The pH required for simulated biological fluids ranges from 1.6 for fasting simulated gastric fluid to 7.7 for simulated semen (3, 9). The pH of the distal colon of fed healthy adults is reported to be approximately 8.1 (35).

Table 3 provides pKa' data for buffers relative to this target pH range (3, 9, 16, 17, 25, 34, 36–47). The buffers listed first are those included in the formulations for the simulated biological fluids provided in Marques et al (3) and Klein (9). Buffers listed second under “Buffers for Biological Research” heading are those described by Good et al. (16, 17) that were developed with specific biocompatibility criteria in mind and are potentially useful for buffered dissolution media for liposomal drug formulations as described by Xu et al. (48).

The pKa' values in Table 3 differ significantly from the thermodynamic pKa values in Table 2, suggesting that thermodynamic values are potentially less useful in selecting a buffer to meet the target pH for biorelevant dissolution media. The data in Table 3 also show that buffers currently used in simulated biological fluids adequately cover the pH range from 1.2 to 8.4 while the zwitterionic buffers developed for biological research supplement the current buffer library in the pH range of 5.5–8.3. The zwitterionic buffer 2-(*N*-morpholino) ethanesulfonic acid (MES) is currently being used for in vitro dissolution testing of liposomes (48). Not all pKa' data in Table 3 were measured in either biologically relevant media or at temperatures related to dissolution test conditions. In the case of lactic and citric acids, stoichiometric or concentration-based pKa data (which differ from the definition given by Equation 5) were only available at 25 °C. A pKa_2' value for maleic acid was estimated using data from Marques et al. (3) for fed state simulated intestinal fluid (FeSSIF) middle, pH 5.8, where no temperature is stated.

The literature search for pKa' values for buffers used in biorelevant dissolution media resulted in locating a database of pH values measured at 37 °C under conditions where the ratio of conjugate base to weak acid is defined (e.g., 1:1); the total concentration of the buffer is known; and the total ionic strength is fixed and held constant by

Table 2. Thermodynamic pKa Values, Charge on Conjugate Base and Weak Acid, Ionization Enthalpies, and pKa-Temperature Differential for Buffers Listed in Table 1

Buffer	Thermodynamic pKa (I = 0)		Charge on conjugate base (z) and weak acid (z+1)	Ionization Enthalpy, ΔH°, kJ/mole at 298.15 °C	dpKa/dT ¹ = -ΔH° / (2.303RT ²); T = 298.15 K R = 8.314472J K ⁻¹ mol ⁻¹
Lactate ^a					
CH ₃ CHOHCO ₂ H ↔ H ⁺ + CH ₃ CHOHCO ₂ ⁻	3.862 (25 °C)	3.872 (37.5 °C)	-1,0	-0.251 ^a	+1.48E-04
Citrate ^b					
H ₃ L ↔ H ⁺ + H ₂ L ⁻	3.128 (25 °C)	3.105 (37 °C)	-1,0	+4.170 ^b	-2.45E-03
H ₂ L ⁻ ↔ H ⁺ + HL ⁻²	4.761 (25 °C)	4.750 (37 °C)	-2,-1	+2.437 ^b	-1.43E-03
HL ⁻² ↔ H ⁺ + L ⁻³	6.396 (25 °C)	6.429 (37 °C)	-3,-2	-3.359 ^b	+1.97E-03
H ₃ L = C ₆ H ₈ O ₇					
Acetate ^c					
CH ₃ CO ₂ H ↔ H ⁺ + CH ₃ CO ₂ ⁻	4.756 (25 °C)	4.766 (37 °C)	-1,0	-0.469 ^c	+2.76E-04
Carbonate ^d					
H ₂ CO ₃ ↔ H ⁺ + HCO ₃ ⁻	6.3514 (25 °C)	6.303 (37 °C)	-1,0	+9.15 ^k	-5.38E-03
Maleate ^e					
H ₂ L ↔ H ⁺ + HL ⁻¹	1.932 (25 °C)	1.892 (37 °C)	-1,0	+5.462 ^e	-3.21E-03
HL ⁻¹ ↔ H ⁺ + L ⁻²	6.139 (25 °C)	6.166 (37 °C)	-2,-1	-3.736 ^e	+2.20E-03
H ₂ L = C ₄ H ₄ O ₄					
Phosphate ^f					
H ² PO ₄ ⁻¹ ↔ H ⁺ + HPO ₄ ⁻²	7.198 (25 °C)	7.181(37 °C)	-2,-1	+4.10 ^l	-2.41E-03
TRIS ^g					
HL ⁺ ↔ H ⁺ + L	8.075 (25 °C)	7.752 (37 °C)	0,1	+47.5 ^k	-2.79E-02
L = C ₄ H ₁₁ NO ₃					
MES ^h					
HL [±] ↔ H ⁺ + L ⁻	6.270 (25 °C)	6.170 (37 °C)	-1,0	+14.8 ^k	-8.70E-03
HL = C ₆ H ₁₃ NO ₄ S					
MOPS ⁱ					
HL [±] ↔ H ⁺ + L ⁻	7.184 (25 °C)	7.041 (37°C)	-1,0	+21.1 ^k	-1.24E-02
HL = C ₇ H ₁₅ NO ₄ S					
HEPES ^h					
HL [±] ↔ H ⁺ + L ⁻	7.565 (25 °C)	7.427 (37 °C)	-1,0	+20.4 ^k	-1.20E-02
HL = C ₈ H ₁₈ N ₂ O ₄ S					
HEPPS ^j					
HL [±] ↔ H ⁺ + L ⁻	7.957 (25 °C)	7.810 (37 °C)	-1,0	+21.3 ^k	-1.25E-02
HL = C ₆ H ₂ ON ₂ O ₄ S					

^aNims, L. F.; Smith, P. K. The Ionization of Lactic Acid from Zero to Fifty Degrees. *J. Biol. Chem.* **1936**, *113*, 145–152 (22).

^bBates, R. G.; Pinching, G. D. Resolution of the Dissociation Constants of Citric Acid at 0 to 50°, and Determination of Certain Related Thermodynamic Functions. *J. Am. Chem. Soc.* **1949**, *71*, 1274–1283. DOI: 10.1021/ja0117a039 (23).

^cHarned, H. S.; Ehlers, R. W. The Dissociation of Acetic Acid from 0 to 60° Centigrade. *J. Am. Chem. Soc.* **1933**, *55* (2), 652-656. DOI: 10.1021/ja01329a027 (24).

^dHarned, H. S.; Bonner, F. T. The First Ionization Constant of Carbonic Acid in Aqueous Solutions of Sodium Chloride. *J. Am. Chem. Soc.*, **1945**, *67* (6), 1026–1031. DOI: 10.1021/ja01222a037 (25).

^eDas, R. C. Thermodynamics of DL-Malic, Maleic and Fumaric Acids in Water and Water + Dioxan Mixtures. *J.C.S. Faraday I.* **1980**, *76*, 2152–2158 (26).

^fBates, R. G.; Acree, S. F. pH of Aqueous Mixtures of Potassium Dihydrogen Phosphate and Disodium Hydrogen Phosphate at 0° to 60 °C. *J. Res. Natl. Bur. Std. (U.S.)* **1945**, *34*, 373–394 (28).

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^jRoy, R. N.; Moore, C. P.; Lord, P.; Mrad, D.; Roy, L. N.; Good, W. S.; Niederschmidt, J.; Kuhler, K. M. Thermodynamic Constants of N-(2-hydroxyethyl) piperazine-N'-3-propanesulfonic acid (HEPPS) and (3-[N-morpholino]-2-hydroxypropanesulfonic acid (MOPSO) from Temperatures 278.15 K to 328.15 K. *J. Chem. Thermodyn.* **1997**, *29*, 1323–1331 (31).

^kIonization enthalpy values taken from Goldberg, R. N.; Kishore, N.; Lennen, R. M. Thermodynamic Quantities for the Ionization Reactions of Buffers. *J. Phys. Chem. Ref. Data.* **2002**, *31* (2), 231–370. DOI: 10.1063/1.1416902 (21).

^ldpKa/dT values were calculated using the van't Hoff Equation as described in Roig, T.; Bäckman, P.; Olofsson, G. Ionization Enthalpies of Some Common Zwitterionic Hydrogen-Ion Buffers for Biological Research. *Acta Chemica Scandinavica*, **1993**, *47*, 899–901 (32).

Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; HEPPS, N-2-hydroxyethylpiperazine-N'-propanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; TRIS, tris-(hydroxymethyl) aminomethane.

Table 3. Buffer pKa' Values for Buffers Currently Use in Simulated Biological Fluid Formulas^{a,b} or for Biological Research^{c,d}

Buffers Currently Used in Simulated Biological Fluid Formulas ^{a,b}			
Buffer System	pKa'	Buffer Range Based on pH = pKa ± 0.5	Comments
HCL/KCL		1.2-2.2 (based on USPHCL/ KCLBuffer)	Bower & Bates' provide compositions and buffer values for solutions of pH 1.0 to 2.2
Lactic Acid ^e	3.63	3.1-4.1	pKa' (based on hydrogen ion concentration) ^m at I = 0.15 (molal scale) NaCl, 25 °C
Citric Acid ^f	pKa ₁ ' = 2.87	2.4-3.4	pKa' (based on hydrogen ion concentration) ^m at I = 0.15 (concentration scale not given) NaCl, 25 °C
	pKa ₂ ' = 4.28	3.8-4.8	
	pKa ₃ ' = 5.58	5.1-6.1	
Acetic Acid ^g	4.62	4.1-5.1	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C
Carbonate ^h	pKa ₁ ' = 6.07	5.6-6.6	pKa' at I = 0.1 (molal scale) NaCl, 35 °C
Maleic Acid ⁱ	pKa ₂ ' = 5.83	5.3-6.3	pKa ₂ ' estimated ⁱ
Phosphate ^g	pKa ₂ ' = 6.78	6.3-7.3	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C
Tris/TRIS-HCL ^j	7.91	7.4-8.4	pKa' estimated from pH data for a buffer composed of 0.05 molal TRIS, 0.05 molal TRIS-HCL and made isotonic with NaCl at 37 °C
Buffers for Biological Research ^{c,d}			
MES ^{g,k}	pKa ₂ ' = 5.98	5.5-6.5	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C
MOPS ^{g,k}	pKa ₂ ' = 6.96	6.5-7.5	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C
HEPES ^{g,k}	pKa ₂ ' = 7.33	6.8-7.8	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C
HEPPS ^{g,k}	pKa ₂ ' = 7.75	7.3-8.3	pKa' measured at I = 0.1 (molar scale) KCL, 35 °C

^aMarques, M. R. C.; Loebenberg, R.; Almukainzi, M. Simulated Biological Fluids with Possible Application in Dissolution Testing. *Dissolution Technol.* **2011**, *18* (3), 15–28. DOI: 10.14227/DT180311P15 (3).

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^hHarned, H. S.; Bonner, F. T. The First Ionization Constant of Carbonic Acid in Aqueous Solutions of Sodium Chloride. *J. Am. Chem. Soc.*, **1945**, *67* (6), 1026–1031. DOI: 10.1021/ja01222a037 (25).

ⁱpKa' value estimated using data given in Marques et al. Table 7 for FeSSIF middle, temperature not stated (3).

^jData from Durst, R. A.; Staples, B. R. Tris/Tris-HCL: A Standard Buffer for Use in the Physiologic pH Range. *Clin. Chem.* **1972**, *18* (3), 206–208 (34).

^kAdditional pH data for zwitterionic buffers available in:

Roy, L. N.; Roy, R. N.; Wollen, J. T.; Harmon, M. A.; Stegner, J. M.; Shah, A. A.; Henson, I. B. Buffer Standards for the Biological pH of the Amino Acid N-[2-hydroxyethyl]piperazine-N'-[3-propanesulfonic acid], HEPPS, From (278.15 to 328.15) K. *J. Chem. Eng. Data*. **2011**, *56* (11), 4126–4132. DOI: 10.1021/je200629y (36)

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Roy, L. N.; Roy, R. N.; Wollen, J. T.; Bwashi, A.; Stegner, J. M.; Downs, Z. M.; Merhoff, C. J.; Bodendorfer, B. M.; Allen, K. A.; Harmon, M. A.; Henson, I. B. 3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic Acid (HEPPS) and the pH of Its Buffer Solutions from (278.15 to 328.15) K. *J. Chem. Eng. Data*. **2010**, *55*, 1327–1332. DOI: 10.1021/je9006508 (41)

^mBower, V. E.; Bates, R. G. pH Values of the Clark and Lubs Buffer Solutions at 25 °C. *J. Res. Natl. Bur. Stand. (U.S.)* **1955**, *55* (4), 197–200 (45).

ⁿA review of the definition of the dissociation constant based on hydrogen ion concentration is found in Kennedy, C. D. Ionic Strength and the Dissociation of Acids. *Biochem. Educ.* **1990**, *18* (1), 35–40 (46) and Partanen, J. I.; Kärki, M. H. Determination of the Thermodynamic Dissociation Constant of a Weak Acid by Potentiometric Acid-Base Titration. *J. Chem. Educ.* **1994**, *71* (5), A120–A122 (47).

Abbreviations: HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; HEPPS, N-2-hydroxyethylpiperazine-N'-propanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; TRIS, tris-(hydroxymethyl)-aminomethane.

a known salt concentration that is biologically relevant, as with isotonic saline (36–41). pH data measured under these conditions may be useful as a surrogate for pKa' values because the pH of a buffer solution is an estimate of the buffer pKa' when the ratio of conjugate base to weak acid is 1:1.

Avdeef et al. (49) observed that reliable published pKa values measured at 37 °C and with physiologically relevant ionic strengths are usually not available. This observation, made in connection with weak electrolyte drug molecules, also applies to buffers and underscores the importance of accumulating a body of reliable buffer pKa data relevant to dissolution test conditions and the physicochemical properties of biorelevant dissolution media.

Sufficient Buffer Capacity to Maintain pH Control Throughout the Dissolution Test

Buffer capacity, β , as defined by Van Slyke (50), provides a relationship between the total buffer concentration, C_b , and the pH and pKa of the buffer. The maximum buffer capacity, β_{max} , occurs when buffer pH = buffer pKa:

$$\beta_{max} = 0.576 C_b \quad (9).$$

For a specific buffer concentration, C_b , the buffer capacity can be expressed as a percentage of the maximum buffer capacity for units of pH = pKa \pm pH. The data in Table 4 exemplify this relationship (20).

Table 4. Percent β_{max} as a Function of pH

pH = pKa \pm pH	Percent β_{max} ^a
1.0	33.0
0.7	55.4
0.5	73.0
0.3	88.9
0.0	100.0

^aData from Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*; Chapman and Hall Ltd.: London, 1974; p 21 (20).

Based on Equation 9, there are two approaches to maximizing buffer capacity: (1) increasing total buffer concentration and (2) selecting a buffer where buffer pH = buffer pKa.

While a calculated value for the intrinsic buffer capacity of the buffer is a useful parameter, it does not reflect buffer pH and buffer capacity of the simulated biological fluid where sugars (51), salts (52), and other ingredients that affect pH and buffer capacity are present in the formula.

An estimate of the buffer capacity for the complete formula can be made by:

$$\beta \approx \Delta A/\Delta pH \text{ or } \beta \approx \Delta B/\Delta pH \quad (10)$$

where ΔA and ΔB are small increments of strong acid or base added to the buffer to produce a pH change, ΔpH . Methods to experimentally measure β have been discussed by Stippler et al. (53), Ramtoola et al. (54), and Levis et al. (1).

Of ultimate importance is whether the buffer capacity of the simulated biological fluid is sufficient to withstand changes in bulk pH related to the solubility of the drug substance and any pH effect from the dosage form excipients (54, 55). A practical method to test buffer capacity and pH control is to measure the pH of a suspension/solution of the dosage form, including drug and excipients, in a volume of simulated biological fluid that will be used for dissolution test conditions (55).

A significant change in pH is indicative of an insufficient buffer capacity to maintain pH control during the dissolution test, which, in turn, can affect the reproducibility of the test results (2). One method of increasing buffer capacity is to increase buffer concentration; however, this approach may be inconsistent with the physicochemical properties of the simulated biological fluid. Garbacz et al. (56) reported on the use of an automated pH-stat system for monitoring and regulating the pH of bicarbonate buffers; however, the use of a pH-stat adds complexity and requires optimizing additional experimental parameters (57).

Based on this literature review, pH control is potentially a challenge for biorelevant dissolution media having relatively low buffer capacity (6), and approaches for pH control are needed that are consistent with the physicochemical properties of the simulated biological fluid, do not contribute to changes in dissolution test conditions that affect test results, and do not add complexity to dissolution test conditions.

Buffer Contribution to the Ionic Strength of the Dissolution Media and Buffer pKa Dependence on Temperature and Ionic Strength

As noted by Ellis et al. (18), both ionic strength and temperature modify buffer pKa. The correction of the thermodynamic pKa₁ = 0 for both ionic strength and temperature has been expressed by Beynon et al. (58) as:

$$pKa' = pKa_1 = 0 + \text{Ionic Strength Correction} + \text{Temperature Correction} \quad (11)$$

where each correction term may be positive, negative or zero. The effect of each correction term is discussed in the following sections.

Ionic strength affects the diffusional process for drug dissolution (49) as well as sensitivity to changes in buffer pH due to dilution effects and the effects of adding salts (52). Additionally, ionic strength is interrelated with the solubility of other dissolved solutes including the drug substance (59).

The ionic strength of the buffer is dependent on the pH of the solution relative to the pKa of the buffer, the charge on the buffer species, and buffer concentration. Table 5 shows the ionic strength for various buffers at a constant total molar concentration of 50 mM and where the concentration of each buffer species is 25 mM. An analysis of these data shows that ionic strength for each buffer varies over the useful buffer pH range of $\text{pH} = \text{pKa} \pm 1$, the greatest buffer-related contribution to ionic strength is by the phosphate buffer where the ionic strength at $\text{pH} = \text{pKa}$ is greater than the total molar concentration of the buffer by a factor of two ($I = 1 \times [\text{KH}_2\text{PO}_4] + 3 \times [\text{Na}_2\text{HPO}_4]$), and the smallest contribution to ionic strength is from buffers where one of the buffer species has a charge of zero (e.g., acetate and TRIS, for the acetate buffer at $\text{pH} = \text{pKa}$, $I = 0 \times [\text{CH}_3\text{CO}_2\text{H}] + 1 \times [\text{CH}_3\text{CO}_2\text{Na}]$). Therefore, buffers having an uncharged species will minimize buffer contribution to ionic strength across the useful buffer pH range. Strictly speaking, neutral species such as acetic acid have a finite ionic strength where $I \approx (\text{Ka}'M)^{1/2}$ (60).

Table 5. Ionic Strength as a Function of Buffer pH Relative to Buffer pKa (total molar buffer concentration = 50 mM, with equimolar concentration of each buffer species)

Buffer System	Charge on the acid (z+1)	Charge on the conjugate base (z)	Ionic Strength ^a (mM)		
			pH = pKa - 1	pH = pKa	pH = pKa + 1
CH ₃ CO ₂ H/ CH ₃ CO ₂ Na	0	-1	4.55	25	45.5
MES±/ NaMES	0	-1	4.55	25	45.5
TRIS/TRIS- HCL	+1	0	45.5	25	4.55
KH ₂ PO ₄ / Na ₂ HPO ₄	-1	-2	59.1	100	140.9

^aIonic strength calculated using Equation 8.

Abbreviations: MES, 2-(N-morpholino)ethanesulfonic acid; TRIS, tris-(hydroxymethyl)-aminomethane.

For example, the ionic strength of a 0.01 M solution of acetic acid ($K_a = 10^{-4.76}$) is approximately 4.2×10^{-4} compared with the ionic strength of a 0.01 M solution of sodium acetate, which is 0.01. According to Roy et al. (36) and Stellwagen et al. (61), zwitterionic species, such as MES±, do not contribute to the ionic strength of an

aqueous solution. As noted by Blanchard (10) and Stoll (11), the lowest possible buffer concentration is preferable to avoid nonspecific ionic strength effects on enzymes and other dissolved solutes in the media.

Svensson (62) discussed a classification of buffers known as isoelectric buffers, with histidyl-glycine being an example. These buffers have negligible ionic strength at their isoionic point, pI, while having finite buffer capacity if they meet the criterion that $(\text{pI} - \text{pKa}_1) < 1.5$ (63).

The ionic strength of the buffer affects the buffer pKa' via the following relationship (18):

$$\text{pKa}' = \text{pKa}_{i=0} + (2z + 1) \left[\frac{(A \times I^{1/2})}{(1 + I^{1/2})} - 0.1 \times I \right] \quad (12)$$

where $\text{pKa}_{i=0}$ is the thermodynamic pKa at a specified temperature and A is a temperature-dependent constant that is close to 0.5 for the temperature range of 25–37 °C. The exact value for A is dependent on the dielectric constant of water, ϵ , at temperature T (Kelvin) where $A = 1.825 \text{E}06(\epsilon T)^{-3/2}$ (64). At 25 °C, $A = 0.5115$ and $\epsilon = 74.3$; at 37 °C, $A = 0.5233$ and $\epsilon = 74.2$. The equation relating dielectric constant with temperature is given by:

$$\epsilon = 87.740 - 0.40008t + 9.398 \times 10^{-4}t^2 - 1.410 \times 10^{-6}t^3 \quad (t \text{ °C}) \quad (65)$$

For example, the estimated pKa₂' for a phosphate buffer, $z = -2$, at 25 °C with $I = 0.1$ is:

$$\text{pKa}_2' = 7.198 + (-3) \left[\frac{(0.5115 \times 0.1^{1/2})}{(1 + 0.1^{1/2})} - 0.1 \times 0.1 \right] = 6.86.$$

Based on this estimate of pKa₂', the expected pH for a buffer with equal concentrations of H₂PO₄⁻¹ and HPO₄⁻² and $I = 0.1$ is 6.86. By comparison, the measured pH is 6.865 for a primary standard phosphate buffer containing 0.025 molal H₂PO₄⁻¹ and 0.025 molal HPO₄⁻² ($I = 0.1$) at 25 °C (66).

Two other factors involving ionic strength affect the pH of biorelevant dissolution media. The first factor is related to diluting stock solutions of buffer to a lower biorelevant concentration and the second factor is related to the addition of salts to adjust the osmotic pressure of the buffered media. First, an example showing the effect of dilution: 50 mM phosphate buffer, initial pH 6.772, increases to pH 7.018 when diluted to 5 mM at 25 °C (28). Therefore, the pH of USP 50 mM phosphate buffer can be expected to change when diluted for use as a surrogate buffer (6). And second, an example showing the effect of adding a neutral salt to a phosphate buffer: a 50 mM phosphate buffer, $I = 0.1$, when changed to $I = 0.2$ by the addition of sodium chloride, results in a change in pH of -0.88 pH units (52). Both the dilution and salt addition effects are primarily related to ionic strength (52) and

can be minimized by fixing the total ionic strength at a constant and biologically relevant level, such as isotonic conditions, with a salt.

Ellis et al. (18) provide a temperature-pKa correction via the following relationship:

$$pK_{aRT} = pK_{aT} + (RT - T)DT \quad (13)$$

where RT is the required temperature, T is the temperature at which the measurement is made, and DT is the change in pKa per degree change in temperature, dpKa/dT.

Values for dpKa/dT for the buffers in Table 3 are given in Table 2. These values were calculated using the van't Hoff Equation (32) at T = 298.15 K (25 °C), with literature values for the enthalpy of ionization measured at T = 298.15 K and at I = 0 for each buffer system.

A comprehensive listing of enthalpy of ionization and other thermodynamic quantities for the ionization reaction of buffers is available in Goldberg et al. (21). An analysis of these data show that the rank order of temperature dependence by buffer grouping is:

(TRIS) > zwitterionic buffers > phosphate buffer > carboxylic acid buffers.

The pKa of carboxylic acid buffers is nearly invariant with temperature. By comparison, the pKa for TRIS buffers is more temperature sensitive than the acetate buffer by approximately two orders of magnitude.

A review of the literature points to physicochemical considerations related to the selection of a buffer. Buffers with an uncharged species will minimize buffer contribution to ionic strength across the useful buffer pH range; primary amine buffers, such as TRIS, have a significant change in pKa with a change in temperature while carboxylic acid buffers have a negligible pKa-temperature dependence; and buffers are susceptible to pH change due to dilution and the effect of added salts primarily because of ionic strength effect.

Compatibility Between the Buffer and Other Components in the Biorelevant Dissolution Media

Compatibility is an important buffer characteristic made more complex because of the diversity of ingredients in dissolution media including salts of divalent cations, sugars, bovine serum albumin, enzymes, and surfactants. Table 6 lists the buffer ingredient, the potential incompatibility or interaction with other components of the biorelevant buffer, and the literature evaluation (4, 13–15, 58, 67–70). The literature points to the compatibility of zwitterionic buffers with metal ions and to potential incompatibilities

between polyvalent cations with phosphate and citrate buffers.

Low Absorbance Spectra in UV and Visible Regions

Zwitterionic buffers were designed to not absorb light in the visible or ultraviolet region of the spectrum. Good et al. (16) report that 0.05 M solutions of MES and N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (HEPES) absorbed a negligible amount at wavelengths greater than 240 nm and very little light at 230 nm. Similar properties are expected for 3-(N-morpholino) propanesulfonic acid (MOPS) and N-2-hydroxyethylpiperazine -N'-propanesulfonic acid (HEPPS) given the chemical similarity of MOPS to MES and HEPPS to HEPES. Xu (48) used the HEPES buffer in a two-stage reverse dialysis method for in vitro release testing and did not report any interference from the buffer with a high-performance liquid chromatography (HPLC) assay where the analyte was detected at 260 nm. Ment et al. (71) report that a low assay value for an antihistamine maleate salt was due to the chromophoric properties of maleic acid.

Perrin et al. (20) provide a table of buffers that have been developed for use in spectrophotometry, which includes those commonly used in simulated biological fluids, such as acetate, phosphate, citrate, carbonate, and TRIS. Singh et al. (72) describe the successful development and validation of a new HPLC method for the estimation of fluconazole in different simulated biological fluids (e.g., gastric, vaginal, and blood plasma).

Buffer Effect on Drug Solubility

The buffer may affect drug solubility from pH-solubility relationships (1), common ion effect (73), and buffer concentration/ionic strength effects (59). Additionally, other ingredients in the bioequivalent dissolution media, such as surface-active agents and bile salts, may affect drug solubility. Any influence that the buffer or bioequivalent dissolution media has on drug solubility will be reflected in the dissolution rate as shown by Equation 14:

$$\text{Dissolution Rate} = k(C_{\text{sat}} - C_t) \quad (14)$$

where C_t is the concentration of dissolved drug at time t, C_{sat} is the concentration of a saturated solution of the drug at a specified temperature and k is a constant (74). Therefore, C_{sat} , the equilibrium solubility at a specified temperature, is an important parameter needed to estimate the effect of the buffer and the dissolution media on drug solubility and dissolution rate.

In summary, Dressman et al. (75) emphasize the equilibrium solubility of the drug in biorelevant dissolution media is dependent on the properties of the media, and

Table 6. Evaluation of Potential Incompatibilities and Interactions Between Buffer Ingredients and Other Components of Biorelevant Dissolution Media

Buffer/Buffer Ingredient	Potential Interaction/Incompatibility	Literature Evaluation
TRIS	Reactive primary amine with potential for Schiff Base formation with aldehydes and ketones	A reaction that can be responsible for an incompatibility with reducing sugars (e.g., glucose, lactose) ^a
TRIS	Interaction with metal ions (Mg ⁺² , Ca ⁺² , Ba ⁺² , Mn ⁺² , Co ⁺² , Ni ⁺² , Cu ⁺² , Zn ⁺² , Cd ⁺² and Pb ⁺²)	Reservation should be exercised in employing TRIS as a buffer system which also contains metal ions ^b
Phosphate	Interaction with divalent ions (e.g., Ca ⁺²) to form insoluble precipitate	When CaCl ₂ is added to a phosphate buffer a phosphate salt often precipitates, affecting free Ca ⁺² ion concentration, phosphate ion concentration, and pH ^c
MES, MOPS, HEPES, HEPPS	Interaction with metals	Evaluated as being suitable to use with metals (Mg ⁺² , Ca ⁺² , Cr ⁺³ , Mn ⁺² , Fe ⁺³ , Co ⁺² , Ni ⁺² , Cu ⁺² , Zn ⁺² , Cd ⁺² , Pb ⁺²) ^d
Potassium salt of the phosphate buffer	Interaction with sodium dodecyl sulfate (SDS)	Precipitate forms when the potassium salt of the phosphate buffer is used at a concentration of 0.5M in combination with SDS; the problem is avoided by using the sodium phosphate salt when preparing the media with SDS ^e
MES, MOPS	Interaction with macromolecules (e.g., bovine serum albumin)	Interacts with peptide backbone of bovine serum albumin leading to a net stabilization of the protein ^f
Citric acid	Interaction with Ca ⁺²	Half neutralized citric acid interacts with CaCl ₂ resulting in a significant decrease in pH due to released hydrogen ions: ΔpH = 5.93(initial) to 4.65(final) ^g . The solubility of calcium citrate is pH dependent ^h
Maleate	Interaction with proteins	The –SH bonds of proteins or free thiols can add across the double bond of maleic acid to produce proteins that are irreversibly modified by this reagent ⁱ

^aWu, Y.; Levons, J.; Narang, A. S.; Raghavan, K.; Rao, V. M. Reactive Impurities in Excipients: Profiling, Identification and Mitigation of Drug-Excipient Incompatibility. *AAPS PharmSciTech*. **2011**, *12* (4), 1248–1263. DOI: 10.1028/s12249-011-9677-z (67).

^bFisher, B. E.; Haring, U. K.; Tribolet, R.; Sigel, H. Metal Ion/Buffer Interactions. Stability of Binary and Ternary Complexes Containing 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and Adenosine 5'-Triphosphate (ATP). *Eur. J. Biochem.* **1979**, *94*, 523–530 (68).

^cPark, Y.; Kim, S. H.; Matalon, S.; Wang, N-H. L.; Franses, E. I. Effect of Phosphate Salt Concentrations, Supporting Electrolytes, and Calcium Phosphate Salt Precipitation on the pH of Phosphate Salt Solutions. *Fluid Phase Equilib.* **2009**, *278*, 76–84. DOI: 10.1016/j.fluid.2009.01.005 (15).

^dFerreira, C. M. H.; Pinto, I. S. S.; Soares, E. V.; Soares, H. M. V. M. (Un)Suitability of the Use of pH Buffers in Biological, Biochemical, and Environmental Studies and Their Interaction With Metal Ions-A Review. *RCS Adv.* **2015**, *5*, 30989–31003. DOI: 10.1039/c4ra15453c (13).

^e*The United States Pharmacopeia and National Formulary USP 39 –NF 34*; The United States Pharmacopeial Convention, Inc.: Rockville, MD, USA, 2016 (4).

^fTaha, M.; Gupta, B. S.; Khoiroh, I.; Lee, M-J. Interactions of Biological Buffers: The Ubiquitous “Smart” Polymer PNIPAM and the Biological Buffers, MES, MOPS and MOPSO. *Macromolecules*. **2011**, *44*, 8575-8589. DOI:10.1021/ma201790c (69).

^gGreenwald, I. The Dissociation of Some Calcium Salts. *J. Biol. Chem.* **1938**, *124*, 437–452 (70).

^hGoss, S. L.; Lemons, K. A.; Kerstetter, J. E.; Bogner, R. H. Determination of Calcium Salt Solubility with Changes in pH and P_{CO2}, Simulating Varying Gastrointestinal Environments. *J. Pharm. Pharmacol.* **2007**, *59*, 1485–1492. DOI: 10.1211/jpp.59.11.0004 (14).

ⁱBeynon, R. J.; Easterby, J. S. *Buffer Solutions – The Basics*; IRL PRESS at Oxford University Press: Oxford, 1996; p 77 (58).

Abbreviations: HEPPS, N-2-hydroxyethylpiperazine-N'-propanesulfonic acid; HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; MOPS, 3-(N-morpholino)propanesulfonic acid; TRIS, tris-(hydroxymethyl)-aminomethane.

aqueous solubility is not predictive of the solubility in these simulated fluids. This statement applies to the need to determine drug solubility in buffered bioequivalent dissolution media when evaluating experimental conditions for dissolution testing.

Nonreactivity with Atmospheric Gases

According to Perrin et al. (20), a difficulty related to using bicarbonate/CO₂ buffers is the need to use a closed system to maintain pH control. Persat et al. (76) point to a problem with weak base buffers such as TRIS where the pH of a diluted buffer can be affected by dissolved CO₂. Persat et al. (76) also note that CO₂ can react with uncharged primary and secondary amine components of buffers to form carbamate ions. The extent to which carbamate ions affect the dissolution process is unknown.

Safety and Toxicity

All buffers listed in Tables 2 and 3 are available commercially with material data safety sheets. Several reagents used to prepare the buffers listed in Tables 2 and 3 are referenced in the Select Committee on GRAS Substances Database (<http://www.accessdata.fda.gov/scripts/fdcc/?set=SCOGS>). Most of the anionic buffers listed in Table 3 (e.g., acetate, citrate, bicarbonate/carbonate, lactate, maleate, and phosphate) have U.S. Food and Drug Administration approval as counter-ions for pharmaceutical salts (77), with safety and toxicology information for these anions being available in the *Handbook of Pharmaceutical Salts* (78).

A Note on Reporting Buffers

The protocol for reporting the buffer component of the dissolution media varies as evidenced by the following reporting formats: (1) “pH 4.5 acetate buffer” (79), and (2) “Sorenson PBS pH 7.4 buffer, NaH₂PO₄·2H₂O 13.46 mM, Na₂HPO₄·12H₂O 53.31 mM, NaCl 74.27 mM” (1). The second example is consistent with the recommendation of Mooney et al. (80) to report both the buffer composition and concentration in addition to pH.

Pfannkoch (81) comments on the importance of having completely documented information to be able to reproduce the buffer including the grade of water used, the pH of the starting water source, and whether the pH adjustment was done before or after the solution was brought to final volume. Persat et al. (76) recommend reporting the following items: (1) the temperature at which the pH was measured, (2) the pH after dilution if the buffer was prepared from a stock solution, and (3) the manufacturer and model of the pH meter. When reporting pKa values, Prankerd (82) notes the importance of data reliability and reviews the system used by the

International Union of Pure and Applied Chemistry. This system describes pKa values as being “very reliable” (pKa error < ±0.005); “reliable” (pKa error ±0.005 to ±0.02); “approximate” (pKa error ±0.02 to ±0.04); and “uncertain” (pKa error > ±0.04).

The USP format (4) provides a standardized protocol that includes the chemical composition, concentration of each buffer species, and pH; the chemical composition and concentration of other buffer reagents such as salts; the method of preparation so that the final concentration of each reagent is known; and water-related specifications. USP <791> also provides information about calibration and operation of the pH measurement system.

CONCLUSIONS

This literature review confirms that buffers play a central role in pH control and that pH stability is particularly important to dissolution test conditions because it affects data reproducibility and interpretation. The literature points to several practical considerations related to buffer selection, which are summarized as follows:

1. Maintaining pH stability due to low buffer capacity and potential compatibility problems between the buffer and reagents in the simulated biological fluid are among the challenges that can be identified for buffers used with biorelevant dissolution media.
2. Attributes for selecting buffer components for biorelevant dissolution media, like those originally outlined by Good et al. (16, 17), may prove useful in selecting a buffer for a dissolution-related application.
3. Buffer-related ionic strength effects can be minimized by selecting a buffer where one of the buffer species has a zero charge, such as acetic acid or a zwitterionic buffer.
4. Buffers containing a primary amine, such as TRIS, can interact with reducing sugars in biorelevant dissolution media.
5. Phosphate, citrate, and TRIS buffers can interact with polyvalent cations to form insoluble precipitates.
6. Buffer pKa-temperature effects can be minimized by avoiding primary amine buffers, such as TRIS.
7. Zwitterionic buffers like MES, MOPS, HEPPS, and HEPES have been characterized as having negligible reactivity with metals.
8. Except for maleic acid, other buffers commonly used in dissolution media have low absorbance in the UV and visible region of the spectrum.
9. TRIS may interact with bicarbonate/CO₂ buffer systems.

The literature suggests three areas that merit consideration in the continuing evolution of buffers used for biorelevant dissolution media: (1) development of methods to maintain pH control for biorelevant dissolution media having low buffer capacity, (2) development of a literature base containing pH and pKa values of buffers measured under conditions related to biorelevant dissolution media and under dissolution test conditions where the temperature is 37 °C, and 3) continued evaluation of zwitterionic buffers designed for biological application that provide an opportunity to enlarge the library of buffers traditionally used for pH control of biorelevant dissolution media.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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