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Biological Buffers

BioUltra Reagents

Introduction

pKa Value and Buffer Range of important Biological Buffers

List of Zwitterionic Buffers

List of Buffer Solutions

List of all buffers

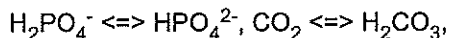
References

To view a complete list of buffers, please visit the Buffer Explorer.

To meet highest demands for quality, we offer a selection of BioUltra Biological Buffers

Introduction

A buffer, as defined by Van Slyke [1], is "a substance which by its presence in solution amount of acid or alkali that must be added to cause unit change in pH". Buffers are the components in experiments designed to study biological reactions by maintaining a constant concentration of hydrogen ions within the physiological range. The pH of mammalian blood is maintained close to 7.38 by buffer systems such as



In living plants, the normal range of pH in tissues is about 4.0-6.2. It is not as narrowly defined as mammalian tissues.

Universally applicable buffers for biochemistry must display:

- water solubility
- no interference with biological processes
- known complex-forming tendency with metal ions
- non-toxicity
- no interference with biological membranes (penetration, solubilisation, adsorption)
- very low U.V. absorption at wavelength >260 nm

"BioUltra" Zwitterionic (Good's) Buffers

The use of buffers based on inorganic or organic salts is limited because of the interference of cations and anions with the biological reaction under study. The development and introduction of Zwitterionic Biological Buffers by Good [2] did much to change this situation. This type of buffer has the desired characteristics: Low interference with biological processes is due to the fact that cationic sites are present as non-interacting carboxylate or sulfonate and anionic sites are present as non-interacting ammonium or trimethylammonium groups. The pK and the buffer range of the zwitterionic substances lie within the physiological range (pKa 6.0-9.5). Moreover the zwitterionic nature of these buffers makes them very water soluble in the one-molar range. Physical constants of the buffer substance (pKa, D pKa/°C, solubility, UV-range) are included under the product entry in the alphabetical list.

"BioUltra" Buffer Salts and other Buffer Components

Buffers based on organic and inorganic salts, acids and bases are widely used in biochemistry.

biological research. The statements regarding the effect of anions and cations on biology also apply here.

Primary Standards

N.B.S.* Standard Buffer Substances [4]

Primary Standards

Composition and properties of the five primary standard buffers at 25°C (see notes on below).

Buffer solution

	Tartrate	Phthalate	Phosphate D	Phosphate E	Borate
Buffer substance	$\text{KHC}_4\text{H}_4\text{O}_6$	$\text{KHC}_8\text{H}_4\text{O}_4$	$\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$	$\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$
g/l soln. at 25 °C	Saturated at 25°C	10.12	3.39 [b] 3.53 [c]	1.179 [b] 4.30 [c]	3.80
Molality (m)	0.0341	0.05	0.025 [a]	0.008695 [b] 0.03043 [c]	0.01
Molarity (M)	0.034	0.04958	0.02490 [a]	0.008665 [b] 0.03032 [c]	0.009971
Density (g/ml)	1.0036	1.0017	1.0028	1.0020	0.9996
pH at 25°C	3.557	4.008	6.865	7.413	9.180
Dilution value, D pH½	+0.049	+0.052	+0.080	+0.07 [d]	+0.01
Buffer value, b, equiv./pH	0.027	0.016	0.029	0.016	0.020
Temp. coeff., dpH (S)/dt, units/°C	-0.0014	+0.0012	-0.0028	-0.0028	-0.0082

[a] Concentration of each phosphate salt. [b] KH_2PO_4 . [c] Na_2HPO_4 . [d] Calculated value.

Recommended standard values of pH(S) for primary standard buffers (+/- 0.005 at 0 - 10°C, +/- 0.008 from 60-90°C).

Buffer pH

Temp. (°C)	Tartrate	Phthalate	Phosphate D	Phosphate E	Borate
0		4.003	6.984	7.534	9.46
5		3.999	6.951	7.500	9.39
10		3.998	6.923	7.472	9.33
15		3.999	6.900	7.448	9.27
20		4.002	6.881	7.429	9.22
25	3.557	4.008	6.865	7.413	9.18
30	3.552	4.015	6.853	7.400	9.13
35	3.549	4.024	6.844	7.389	9.10
38	3.548	4.030	6.840	7.384	9.08
40	3.547	4.035	6.838	7.380	9.06
45	3.547	4.047	6.834	7.373	9.03
50	3.549	4.060	6.833	7.367	9.01

55	3.554	40.75	6.834	8.984
60	3.560	4.091	6.836	8.962
70	3.580	4.126	6.845	8.927
80	3.609	4.164	6.859	8.884
90	3.650	4.205	6.877	8.884
95	3.674	4.227	6.886	8.834

*N.B.S. National Bureau of Standards

Secondary Standards

Composition and properties of the two secondary standard buffers at 25°C.

Buffer solution

	Tetraoxalate	Calcium hydroxide
Buffer substance	$\text{KH}_2(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$	$\text{Ca}(\text{OH})_2$
g/l of soln. at 25°C	12.61	Saturated at 25°C
Molality (m)	0.05	0.0203
Molarity (M)	0.04962	0.02025
Density (g/ml)	1.0032	0.9991
pH at 25°C	1.0032	12.454
Dilution value, D pH _{1/2}	1.679	- 0.28
Buffer value, b, equiv./pH	+ 0.186	0.09
Temp. coeff., dpH (S)/dt, units/°C	0.070	- 0.033

Recommended standard values of pH(S) for the secondary buffer standards

Buffer pH

Temp (°C)	Tetraoxalate	Calcium hydroxide
0	1.666	13.423
5	1.668	13.207
10	1.670	13.003
15	1.672	12.810
20	1.675	12.627
25	1.679	12.454
30	1.683	12.289
35	1.688	12.133
38	1.691	12.043
40	1.694	11.984
45	1.700	11.841
50	1.707	11.705
55	1.715	11.574
60	1.723	11.449
70	1.743	
80	1.766	
90	1.792	
95	1.806	

Note. See below for remarks on drying potassium tetraoxalate dihydrate.

Product Number	Primary N.B.S.* Standard Buffer Substances
60219	Potassium dihydrogen phosphate
60359	Potassium hydrogen phthalate
60366	Potassium hydrogen D-tartrate
71639	di-Sodium hydrogen phosphate anhydrous
71999	Sodium tetraborate Decahydrate
Secondary N.B.S.* Standard Buffer Substances	
21187	Calcium hydroxide
60589	Potassium tetraoxalate Dihydrate

Notes

Notes on the preparation

The primary and secondary standard buffer solutions are prepared from the standard b as indicated in the table. The standard pH [pH(S)] of the buffer solution in the temperat C are indicated and can be used for calibration purposes. Buffer compositions are on tl Only freshly prepared solutions should be used, as the initial buffer composition may cl rapidly. Tartrate, phthalate, and phosphates may be dried at 110°C for 1-2 hours prior l Potassium tetraoxalate should not be dried above 60°C, and borax should not be dried

Molality (m)	dimension: mole per kilogram of sol
Molarity (M)	dimension: mole per litre of solution
Dilution value ($DpH_{1/2}$)	change of pH value observed by dil buffer solution with an equal volume It is positive when pH increases anc when pH decreases with increasing
Buffer value b (equiv./pH)	(also Buffer capacity, Van Slyke Bul $d[B]/dpH$, where $d[B]$ is the increme equivalents) of a strong base requir a certain pH change of the buffer so acids effect negative ($-d[B]$) increme. lower the pH
Temperature coefficient (pH unit/°C)	$dpH(S)/dT$ standard change of pH v degree centigrade. It can be positiv

General Aspects regarding Buffer Applications

With few exceptions, studies of biochemical systems require the use of a buffer in orde pH value. Therefore the action of the buffer is of prime importance. Factors influencing buffer solutions and pH are [5, 6]:

- activity effects: concentration and electrical charge of the species involved
- salt effects: added "indifferent" electrolytes
- dilution effect: pH-variation on dilution of buffer solutions buffer capacity: added b
- temperature dependence

The choice of the correct buffer for a particular biochemical system or technique depen of additional factors. For example: undesired interaction of the buffer with the biopolym

stability, metal ion complexing properties and purity. One way to solve the difficult problem of the right buffer is to evaluate as many buffers as possible. Reviews on the use of buffer areas are available [7-9]. However they do not provide detailed information and a comprehensive treatise on the subject should be consulted.

Practical Aspects of Buffer Application

- Activity and salt effects have a marked influence on the pH value of a solution according to the following equation

$$\text{pH} = \text{pKa}' + \log\left[\frac{[\text{B}]}{[\text{BH}]}\right] \quad (1)$$

where

$$\text{pKa}' = \text{pKa} + \text{correction factor}$$

The factors for different ionic strengths are tabulated in [5] and range from 0.015 for $I = 0.001$ to 0.159 for $I = 0.5$.

- Ionic strength is defined as $I = \frac{1}{2} \sum (c_i \cdot z^2)$ (2)

where c_i is the concentration of species i , and z is the corresponding charge. I can be calculated easily from the experimental parameters.

Buffer capacity. The maximum buffer capacity b_{max} of a monovalent species is found at pKa' , the practical pK-value. b_{max} in the pH range 3-11 is calculated according to equation (3)

$$b_{\text{max}} = 0.576 c \quad (3)$$

where c is the total concentration of the buffer substance. Thus the useful buffer capacity range is $\text{pKa}' \pm 1$ unit. If more than 50% of the maximum buffer capacity must be reached, the corresponding range is only $\text{pKa}' + 0.75$ units.

The Practical Buffer Range

b , the buffer capacity, is defined as given in (4)

$$b = \frac{d[\text{B}]}{d\text{pH}} \quad (4)$$

where $[\text{B}]$ is the amount of base added to the buffer component BH. The buffer capacity of a weak acid-base buffer system is greater, the closer the individual pKa values lie. b values of buffers are additive.

From equation (5) it is possible to calculate the molar ratio [basic species]/[acidic species] to a desired pH within the practical buffer range, $\text{pKa}' \pm 1$ unit.

$$\text{pH} = \text{pK} + \log\left[\frac{[\text{basic species}]}{[\text{acid species}]}\right] \quad (5)$$

From the diagram on page ...
 $[\text{B}]/[\text{BH}]$ - pH - % buffer capacity can quickly be estimated.

Temperature effects on the pH of a given solution may be considerable. TRIS has a pKa of 8.06 at 25° and 7.22 at 37° (mean $d\text{pH}/dT = 0.03$ pH units/°C). Salt buffers, such as the

Standards show dpH/dT of about 0.002 pH-units/ $^{\circ}C$. The change can be either positive

Dilution effects depend mainly on the charge of the buffer species; dilution of a 0.1 M system (total concentration) with an equal volume of water results in a pH-value change whereby the pH is lowered in the case of basic buffers and increased when acidic ones. pH variation of HA-/A²⁻ buffer systems are increased by a factor of approximately three

Use of diagram: Determine from experimental parameters the molar concentration ratio of acidic species in the buffer system.

$$\frac{(1 \text{ to } 10)}{10 \text{ to } 1}$$

Read the pH of the Solution from the upper diagram of pH deviation and from the lower diagram of **maximum buffer capacity** (% b_{max}).

pK_a Value and Buffer Range of important Biological Buffers

sorted by buffer range

sorted alphabetically

pKa Value and Buffer Range of important Biological Buffers sorted by buffer range

effective pH range	pKa 25 $^{\circ}C$	buffer
1.2-2.6	1.97	maleate (pK1)
1.7-2.9	2.15	phosphate (pK1)
10.0-11.4	10.70	CABS
10.5-12.0	11.12	piperidine
2.2-3.6	2.35	glycine (pK1)
2.2-6.5	3.13	citrate (pK1)
2.5-3.8	3.14	glycylglycine (pK1)
2.7-4.2	3.40	malate (pK1)
3.0-4.5	3.75	formate
3.0-6.2	4.76	citrate (pK2)
3.2-5.2	4.21	succinate (pK1)
3.6-5.6	4.76	acetate
3.8-5.6	4.87	propionate
4.0-6.0	5.13	malate (pK2)
4.9-5.9	5.23	pyridine
5.0-6.0	5.33	piperazine (pK1)
5.0-7.4	6.27	cacodylate

5.5-6.5	5.64	succinate (pK2)
5.5-6.7	6.10	MES
5.5-7.2	6.40	citrate (pK3)
5.5-7.2	6.24	maleate (pK2)
5.5-7.4	1.70, 6.04, 9.09	histidine
5.8-7.2	6.46	bis-tris
5.8-8.0	7.20	phosphate (pK2)
6.0-12.0	9.50	ethanolamine
6.0-7.2	6.59	ADA
6.0-8.0	6.35	carbonate (pK1)
6.1-7.5	6.78	ACES
6.1-7.5	6.76	PIPES
6.2-7.6	6.87	MOPSO
6.2-7.8	6.95	imidazole
6.3-9.5	6.80, 9.00	BIS-TRIS propane
6.4-7.8	7.09	BES
6.5-7.9	7.14	MOPS
6.8-8.2	7.48	HEPES
6.8-8.2	7.40	TES
6.9-8.3	7.60	MOBS
7.0-8.2	7.52	DIPSO
7.0-8.2	7.61	TAPSO
7.0-8.3	7.76	triethanolamine (TEA)
7.0-9.0	0.91, 2.10, 6.70, 9.32	pyrophosphate
7.1-8.5	7.85	HEPPSO
7.2-8.5	7.78	POPSO
7.4-8.8	8.05	tricine
7.5-10.0	8.10	hydrazine
7.5-8.9	8.25	glycylglycine (pK2)
7.5-9.0	8.06	Trizma (tris)
7.6-8.6	8.00	EPPS, HEPPS
7.6-9.0	8.26	BICINE
7.6-9.0	8.30	HEPBS

7.7-9.1	8.40	TAPS
7.8-9.7	8.80	2-amino-2-methyl-1,3-propanediol (AMPD)
8.2-9.6	8.90	TABS
8.3-9.7	9.00	AMPSO
8.4-9.6	9.06	taurine (AES)
8.5-10.2	9.23, 12.74, 13.80	borate
8.6-10.0	9.50	CHES
8.7-10.4	9.69	2-amino-2-methyl-1-propanol
8.8-10.6	9.78	glycine (pK2)
8.8-9.9	9.25	ammonium hydroxide
8.9-10.3	9.60	CAPSO
9.5-11.1	10.33	carbonate (pK2)
9.5-11.5	10.66	methylamine
9.5-9.8	9.73	piperazine (pK2)
9.7-11.1	10.40	CAPS
	12.33	phosphate (pK3)

pKa Value and Buffer Range of important Biological Buffers sorted alphabetically

buffer	pKa 25°C	effective pH
ACES	6.78	6.1-7.5
acetate	4.76	3.6-5.6
ADA	6.59	6.0-7.2
ammonium hydroxide	9.25	8.8-9.9
AMP (2-amino-2-methyl-1-propanol)	9.69	8.7-10.4
AMPD (2-amino-2-methyl-1,3-propanediol)	8.80	7.8-9.7
AMPSO	9.00	8.3-9.7
BES	7.09	6.4-7.8
BICINE	8.26	7.6-9.0
bis-tris	6.46	5.8-7.2
BIS-TRIS propane	6.80, 9.00	6.3-9.5
borate	9.23, 12.74, 13.80	8.5-10.2

CABS	10.70	10.0-11.4
cacodylate	6.27	5.0-7.4
CAPS	10.40	9.7-11.1
CAPSO	9.60	8.9-10.3
carbonate (pK1)	6.35	6.0-8.0
carbonate (pK2)	10.33	9.5-11.1
CHES	9.50	8.6-10.0
citrate (pK1)	3.13	2.2-6.5
citrate (pK2)	4.76	3.0-6.2
citrate (pK3)	6.40	5.5-7.2
DIPSO	7.52	7.0-8.2
EPPS, HEPPS	8.00	7.6-8.6
ethanolamine	9.50	6.0-12.0
formate	3.75	3.0-4.5
glycine (pK1)	2.35	2.2-3.6
glycine (pK2)	9.78	8.8-10.6
glycylglycine (pK1)	3.14	2.5-3.8
glycylglycine (pK2)	8.25	7.5-8.9
HEPBS	8.30	7.6-9.0
HEPES	7.48	6.8-8.2
HEPPSO	7.85	7.1-8.5
histidine	1.70, 6.04, 9.09	5.5-7.4
hydrazine	8.10	7.5-10.0
imidazole	6.95	6.2-7.8
malate (pK1)	3.40	2.7-4.2
malate (pK2)	5.13	4.0-6.0
maleate (pK1)	1.97	1.2-2.6
maleate (pK2)	6.24	5.5-7.2
MES	6.10	5.5-6.7
methylamine	10.66	9.5-11.5
MOBS	7.60	6.9-8.3
MOPS	7.14	6.5-7.9
MOPSO	6.87	6.2-7.6

phosphate (pK1)	2.15	1.7-2.9
phosphate (pK2)	7.20	5.8-8.0
phosphate (pK3)	12.33	
piperazine (pK1)	5.33	5.0-6.0
piperazine (pK2)	9.73	9.5-9.8
piperidine	11.12	10.5-12.0
PIPES	6.76	6.1-7.5
POPSO	7.78	7.2-8.5
propionate	4.87	3.8-5.6
pyridine	5.23	4.9-5.9
pyrophosphate	0.91, 2.10, 6.70, 9.32	7.0-9.0
succinate (pK1)	4.21	3.2-5.2
succinate (pK2)	5.64	5.5-6.5
TABS	8.90	8.2-9.6
TAPS	8.40	7.7-9.1
TAPSO	7.61	7.0-8.2
taurine (AES)	9.06	8.4-9.6
TES	7.40	6.8-8.2
tricine	8.05	7.4-8.8
triethanolamine (TEA)	7.76	7.0-8.3
Trizma (tris)	8.06	7.5-9.0

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