General Comments on Buffers

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Abstract

The major factor in biological pH control in eukaryotic cells is the carbon dioxoide-biocarbonate-carbonate buffer (Scheme I) system[1,2,3,4]. There other biological buffers such as bulk protein and phosphate anions which can provide some buffering effect, metabolites such as lactic acid which can lower pH and tris(hydroxylmethylaminomethyl) methane, THAM®) has been used to treat acid base disorders[5,6,7]. pH control in prokaryotic cells is mediated by membrane transport of various ions including hydrogen, potassium and sodium[8,9,10].

Figure 1

$CO_2 + H_2O = H_2CO_3$; $H_2CO_3 + H_2O = HCO_3^{-1} + H_3O^+$ (pKa 6.15) ; $HCO_3^{-1} + H_2O = CO_3^{-2}$ (pKa 10.3)

See Jungas, R.L., Best literature values for the pK of carbonic and phosphoric acid under physiological conditions, *Anal. Biochem.* 349, 1-15, 2006

In the laboratory, the bicarbonate/carbonate buffer system can only be used in the far alkaline range (pH 9-11) and unless “fixed” by a suitable cation such as sodium, can be volatile.

A variety of buffers, most notably the “Good” buffers which were developed by Norman Good and colleagues[[[10a]]], have been developed over the years to provide pH control in in vitro experiments. While effective in controlling pH , the numerous non-buffer effects that buffer salts have on experimental systems are somewhat less appreciated. Some effects, such as observed with phosphate buffers, are based on biologically significant interactions with proteins and, as such, demonstrate specificity. Other effects, such as metal ion chelation, can be considered general. However, the binding of metal ions by a specific buffer must be carefully evaluated considering the recent controversy regarding the ability of MOPS buffer to bind magnesium ions[11]. There are some effects where the stability of a reagent is dependent on both pH and buffer species. One example is provided by the stability of phenylmethylsulfonyl fluoride(PMSF)[12]. PMSF was less stable in Tris buffer than in either HEPES or phosphate buffer; PMSF is less stable in HEPES than in phosphate buffer. Activity was measured by the ability of PMSF to inhibit chymotrypsin; all activity was lost in Tris (10 mM; pH 7.5) after one hour at 25°C while activity was fully retained in phosphate (10 mM, pH 7.5). This is likely a reflection of the nucleophilic property of Tris[13,14] which appears to be enhanced in the presence of divalent cations such as zinc[15]. The loss of activity, presumably the result of the hydrolysis of the fluoride to hydroxyl function, is more marked at more alkaline pH. Tris can also function as phosphoacceptor in assays for alkaline phosphatase but was not as effective as 2-amino-2-methyl-1,3-propanediol[16]. The various nitrogen-based buffers such as Tris, HEPES, CAP, and BICINE influence colorimetric protein assays [17,18,19].

Other specific examples are presented in Table 1.

Figure 2

Table 1: Effects of Buffers

Buffer	Observation
ACES	Competitive inhibitor of γ-aminobutyric acid receptor binding[1]
ADA	Competitive inhibitor of γ-aminobutyric acid receptor binding[1]; chelation of calcium ions[2]
BES	Interacts with DNA yielding distortion of DNA electrophoretograms[3]
BICINE	Chelation of calcium ions[2]; protects liver alcohol dehydrogenase from inactivation by iodoacetic acid[4]
Borate	Anomalous complex formation with nucleic acids[5]; complex formation with carbohydrates[6,7]; participant in the modification of arginine residues by 1,2-cyclohexanedione[8].
Cacodylic Acid	Reaction with sulfydryl compounds[9].
Carbonate	Enhances rate of reaction of phenylglyoxal with arginine residues in proteins[10]; modulation of peroxynitrite reactions with proteins[11, 12]; modulation of Cu^{2+} oxidation reactions[13-15].
Citrate	Chelation of calcium ions[2].
HEPES	Free radical generation[16,17] and complexation of copper ions[18]; reported adverse effects in tissue culture[19,20]
MES	Complexes copper ions[21]
MOPS	Adverse effect on smooth muscle contraction[22]; Oxidation of metal ions[22]; formation of nitric oxide donors on incubation with peroxynitrite[24]; slow reaction with hydrogen peroxide[25].
Phosphate	Catalysis of the racemization of 5-phenylhydantoins[26,27]
PIPES	Binding to bile salt-stimulated lipase[28]; variation in physiological response based on vendor source[29]; inhibition of a K^+-activated phosphatase[30].
TES	Interaction with extracellular matrices[31]; inhibition of the interaction of proteoglycans with type 1 collagen[32].
Tricine	Chelating agent[2]; tricine radicals have been reported in the presence of peroxide-forming enzymes[33].
Tris	Nucleophile[34,35] and enzyme inhibitor[36]

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