Buffers

What is a Buffer?

Buffers are used to maintain the pH of an aqueous solution at a desired constant range when this is subject to small pH variations. Buffers are a mixture of a weak acid, proton donor, and its conjugate base, proton acceptor, in equilibrium with each other. This equilibrium is dependent on the pK_a of the acid, which is an intrinsic feature of the buffer, and is correlated to the buffering capacity, defined as the capacity of the buffer to resist to pH variations when acids or bases are added.

When a small amount of strong base is added to the buffer solution, the acid form of the buffer releases a proton, neutralising the base and keeping the pH to its initial value. In a similar way, when a small amount of strong acid is added, the conjugated base of the buffer picks up a proton. Effectively, the buffer removes undesired strong acids or bases by accepting or releasing protons, in order to respond to pH variations.

Addition of base (B) to the buffer (BuH): $B^- + BuH \rightarrow BH + Bu^-$

Addition of acid (H^+) to the buffer (Bu^-): $H^+ + Bu^- \rightarrow BuH$

Ideal Features of a Buffer

Buffers are widely used in biology and biochemistry, where the pH is a crucial factor determining activity of enzymes, solubility and even structural characteristics of biological macromolecules. The pH changes of solutions can therefore have a dramatic influence on biological molecules and systems. The characteristics of suitable buffers to be used in biological and biochemical experiments were defined by Norman Good and co-workers. An ideal buffer would have the following features:

- Water soluble: as biological experiments take place in an aqueous environment, an ideal buffer should be water soluble, has low or no solubility in non-polar media and absent or no biological membrane permeability.
- Buffering capacity in the physiological pH range: since most biological reactions take place at a pH close to neutrality, the ideal buffer should have a maximum buffering capacity in the range of pH 6-8.
- Concentration, temperature, and ionic composition of the medium should have little or no influence on the buffer pH, and the buffer should have minimal salt effects.
- None of the buffer components should be volatile at the temperature range of 15-40 °C, so that the pH of the aqueous solution will not change due to evaporation of any component.
- Stable to temperature variations, chemical reactions, should not take part in, or have an influence on, any enzymatic reaction, should not inhibit growth and/or metabolic activity of tested organism in applied buffer concentration.

- Should not form complexes with ions present in solutions, and if they do, these complexes should be water-soluble and not precipitate.
- Available in sufficient purity to avoid negative side effects or assay interference by buffer impurities (heavy metals, endotoxins etc.).
- Easily synthesised using inexpensive and widely available starting materials, non toxic.
- Not absorb visible or UV light at 230 nm or longer wavelengths to not interfere with spectrophotometric assays.

Practical Tips When Choosing a Buffer

The choice of the appropriate buffer is dictated by the desired pH range and the application. The key factors to take into consideration when choosing and preparing a buffer are:

- **Good buffering capacity**: it is crucial to choose a buffer with good buffering capacity at the pH used in the experiment. In fact, if the buffer pK_a differs greatly from the pH used in the experiment, its ability to respond to pH variation is negligible, and therefore the experimental conditions are not optimal as they might not keep well controlled. The pH used in the experiment should be in the middle of the pH range optimal for the buffer. If a pH increase or decrease is expected to occur during the experiment, it is advised to take this into consideration and choose a buffer with slightly higher or lower buffering capacity respectively.
- **Temperature effect**: since the pH is temperature dependent, it is strongly advised to double check and adjust the pH at the desired temperature of use. In certain applications such as lyophilisation pH changes during freezing can have a big influence and need to be taken into consideration.
- **Other factors influencing pH**: The pH is also influenced by the presence of organic solvents, such as ethanol, methanol or DMSO, which are often added to increase solubility of compounds added to the media.
- **Interfering compounds**: it is extremely important to make sure the chosen buffer does not interact with metal ions in solution affecting the biological system and with enzymes influencing their reactivity. In fact, some buffers interact with metal ions as metal chelating agents. Examples are citrate buffer, which form calcium chelators, and phosphate buffer, which forms insoluble salts in presence of calcium ions. It is not advised to use citrate and phosphate buffers in highly calcium-dependent systems.

Cas no	Product Code	Buffer	pK_a	pH range	Features
56-40-6	FG02717 FG71510 FG175750	Glycine	2.35	2.2-3.6	Used in electrophoresis for protein samples
4432-31-9	FM31183 W-106203	MES	6.10	5.5-6.7	Morpholinic buffer, used in culture media, does not coordinate metal ions, used in capillary electrochromatography
21416-85-3 6131-99-3	FP158656 FS10852	Potassium cacodylate Sodium cacodylate trihydrate	6.27	5.0-7.4	Toxic, oxidises thiols at low pH, used in electron microscopy and histology
6976-37-0	FB15824	Bis-Tris	6.46	5.8-7.2	Bis(2-hydroxethyl) amine buffer used for applications involving both protein and nucleic acids, forms metal chelates

26239-55-4 7415-22-7	FA15758 FA37505	ADA ADA sodium salt	6.59	6.0-7.2	Acetamido buffer, coordinates to metal ions
5625-37-6	FP11594	PIPES	6.76	6.1-7.5	Piperazinic buffer, non-coordinating to metal ions, used in culture media, protein crystallisation, electrophoresis, chromatography
7365-82-4	FA08304	ACES	6.78	6.1-7.5	Acetamido buffer used in culture media and protein extractions, forms complex with metal ions
68399-77-9	FM37206	MOPSO	6.87	6.2-7.6	Morpholinic buffer used for culture media, proteins electrophoresis, chromatography and capillary electrochromatography, poor coordination with metal ions
10191-18-1	FB11592	BES	7.09	6.4-7.8	Bis(2-hydroxethyl) amine buffer, forms a complex with DNA and certain metal ions
1132-61-2	FM09059	MOPS	7.14	6.5-7.9	Morpholinic buffer, used in cell culture, nucleic acid agarose separation, protein electrophoresis and chromatography, does not coordinate to metal ions
	FP157114	Phosphate Buffered Saline (PBS)	7.4	7.3-7.5	Non-toxic, mimics human physiological conditions (pH, ion concentration, osmolarity). Used in cell culture, immunoassays, protein purification
7365-44-8	FT33482	TES	7.4	6.8-8.2	Used in protein assays and protein purification, good pH range for most biological applications, forms complexes with DNA and copper ions
7365-45-9	FH31182 FH35404	HEPES HEPES sodium salt	7.48	6.8-8.2	Piperazinic buffer widely used in cell culture, good pH range for most biological applications, forms hydrogen peroxide when exposed to light, therefore solutions must be in the darkness
68399-78-0	FH15210	HEPPSO	7.84	7.1-8.5	Piperazinic buffer, used as ampholytic separator, binds to copper ions
16052-06-5	FH15209	HEPPS (EPPS)	8.0	7.3-8.7	Piperazinic buffer similar to HEPES. The pH range is suitable for phosphorylation reaction studies
77-86-1 1185-53-1	FT15751 FT45361	Tris Tris HCl	8.07	7.1-9.1	Good pH range for most biological processes, used in western blot and nucleic acid agarose electrophoresis, incompatible with Ag electrodes, can form metal chelates inhibiting enzyme activity
5704-04-1	FT15752	Tricine	8.15	7.4-8.8	Widely used in electrophoresis and ATP assays
150-25-4	FB11031	Bicine	8.26	7.6-9.0	Bis(2-hydroxethyl) amine buffer used for protein crystallisation, electrophoresis and study of enzymatic reactions, forms metal complexes
29915-38-6	FT39128	TAPS	8.40	7.7-9.1	Used in capillary electrophoresis with DNA, forms metal complexes
103-47-9	FC55871	CHES	9.5	8.6-10.0	Cyclohexylamino buffer used to study enzymatic processes at pH above physiological, poor metal ion coordination
1135-40-6	FC09379 FC55870	CAPS CAPS sodium salt	10.4	9.7-11.1	Cyclohexylamino buffer used to study enzymatic processes at pH above physiological, poor metal ion coordination

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References

Good *et al.* (1966). Hydrogen Ion Buffers for Biological Research. Biochemistry 5 (2):467-477.

Good *et al.* (1972). Hydrogen ion buffers. Methods in Enzymology 24:53-68.

Ferguson *et al.* (1980). Hydrogen Ion Buffers for Biological Research. Analytical Biochemistry 104 (2):300-310.