Biological Buffers

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Introduction

The buffer concept

Many biochemical processes are markedly impaired by even small changes in the concentrations of free H^+ ions. It is therefore usually necessary to stabilise the H^+ concentration in vitro by adding a suitable buffer to the medium, without, however, affecting the functioning of the system under investigation. A buffer keeps the pH of a solution constant by taking up protons that are released during reactions, or by releasing protons when they are consumed by reactions. The observation that partially neutralised solutions of weak acids or bases are resistant to changes in pH when small amounts of strong acids or bases are added led to the concept of the 'buffer'.

Buffer capacity

Buffers consist of an acid and its conjugated base. The quality of a buffer is determined by its buffer capacity, i.e. its resistance to changes in pH when strong acids or bases are added. In other words: the buffer capacity corresponds to the amount of H^+ or OH^- ions that can be neutralised by the buffer. The buffer capacity is related to the buffer concentration. The graph described by the relation of the pH to the addition of H^+/OH^- ions is called the titration curve. The point of inflection of the curve corresponds to the pK_a value. At this point, the buffer capacity is at its maximum at the pK_a value. This point therefore corresponds to the mid-point of the pH range covered by the buffer and is where the concentration of acid and base is the same. In the area of this pH range, therefore, relatively large amounts of H^+/OH^- ions result in only small changes in pH.

A basic principle is that a buffer that has a pH value of one pH unit above or below the pK_a value loses so much buffer capacity that it no longer has any real buffer function. Based on the Henderson-Hasselbalch equation

$$\mathbf{pH = pK_a + \log [A^-]/[HA]}$$

for the calculation of the pH of a weak acid or alkaline solution, the ions in the water must also be taken into account when working in pH ranges below 3.0 and above 11.0. Most biochemical reactions, however, take place in the pH range between 6.0 and 10.0.

The pH value

Conductivity can also be detected in highly purified water due to the OH^- and H_3O^+ ions resulting from the autoprotolysis of water. This intrinsic dissociation of the water is an equilibrium reaction and the product of the concentrations of the two ions represents a constant:

$$\mathbf{K = [H_3O^+] \times [OH^-].}$$

This value is depends on the temperature only and is 10^{-14} for purified water at 22°C. Depending on which of the two ions is present in a higher concentration in a solution, the solution is called to be acidic or alkaline. To express this fact in terms of a simple number, the negative exponent of the easily measurable hydronium ion concentration $[H_3O^+]$ was chosen. This dimensionless number is called the pH value. The pH can also be described as the negative, decadic logarithm of the hydronium ion concentration of a solution:

$$\mathbf{pH = - \log [H_3O^+]}$$

The hydronium ion concentration of pure water is 10^{-7} mol/L, as can be derived from the above equation for the ion product. Therefore, the pH value is 7.

In an acidic solution, the concentration of H_3O^+ ions is increased (e.g. from 10^{-7} mol/L to 10^{-2} mol/L) and the pH is therefore < 7. In an alkaline solution, the hydronium ion concentration is decreased and this results in a pH > 7.

The pK_a value

To change the pH value of a solution, substances are dissolved in the solution which release H^+ ions into the water (acids), thus raising the H_3O^+ ion concentration and lowering the pH value, or which decrease the H_3O^+ ion concentration and thus increase the pH value by taking up H^+ ions (bases). As always in chemistry, this reaction is an equilibrium reaction, and the capacity of a substance to shift this equilibrium in one direction or another is determined by the potency of the acid. It is calculated from the equilibrium constants using the following equation

$$\mathbf{K = [A^-] \times [H_3O^+] / [HA],}$$

as the negative decadic logarithm of the constants and, in analogy to the pH value, is termed the pK_a value. The pK_a value is therefore a simple number that describes the acid potency of a substance.
This calculation shows that hydrochloric acid, as one of the most potent acids, has a pK_a value of -6, and all HCl molecules form hydronium ions with water. For a weak acid, such as acetic acid, a pK_a value of only 4.75 is calculated (i.e. only very few molecules form an H_3O^+ and a CH_3COO^- ion), and the alkaline HPO_4^{2-} ion has a pK_a value of 12.32.

Biological buffers

Different inorganic substances were originally used as buffers (e.g. phosphate, cacodylate, borate, bicarbonate), and later weak organic acids were also used. Many of these buffer substances, however, have the disadvantage that they are not inert and have lasting effects on the system under investigation (e.g. inhibition of enzymes, interactions with enzyme substrates etc.). Most biological buffers in use today were developed by NE Good and his research team (Good *et al.* 1966, Good & Izawa 1972, Ferguson *et al.* 1980; "Good buffers") and are N-substituted taurine or glycine buffers. These zwitterionic buffers meet most of the requirements that biological buffers have to fulfil.
Buffer systems described in the literature are usually used for experiments to enable direct comparison of results. Again and again, it is shown that the conditions in experiments – even in standard systems – could be optimised (Spectrophotometric Assessment of Nucleic Acid Purity: Wilfinger *et al.* 1997, p*K*-Matched Running Buffers for Gel Electrophoresis: Liu *et al.* 1999, Buffer Effects on *Eco*RV Kinetics: Wenner & Bloomfield 1999).
We have put together the information from the literature that we believe will be of assistance to you in solving your everyday problems and in the development and optimisation of your test systems.

Requirements of biological buffers

Solubility

The buffer should be freely soluble in water and poorly soluble in other solvents. The higher the water solubility, the simpler it is to prepare concentrated stock solutions (frequently 10X, 50X or 100X stock solutions). The pH of concentrated stock solutions may change on dilution. For example, the pH value of an 100 mM sodium phosphate buffer increases from 6.7 to 6.9 with 10fold dilution and to 7.0 with 100fold dilution (Tipton & Dixon 1979). The pH value of a Tris solution decreases by 0.1 pH units per 10fold dilution.

Permeability

The buffer should not be able to permeate biological membranes to prevent concentration in the cell or organelles. Tris has a relatively high degree of fat solubility and may therefore permeate membranes. This also explains its toxicity for many mammalian cells in culture.

Ionic strength

The buffer should not alter the ionic strength of the system as far as possible. The physiological ionic strength is between 100 – 200 mM KCl or NaCl. This can be very important, especially when investigating enzymatic reactions, because the ionic strength of the solution is a measure of the ionic milieu, which may also affect the catalytic activity of an enzyme. The protonisation and deprotonisation depending on the ionic composition of the surrounding medium in the reaction set-up affects the binding and conversion of an enzyme substrate by the enzyme. Under non-physiological conditions in altered protonised and deprotonised forms, both the amino acid residues in proteins that interact with the substrate and the substrate itself will not be able to interact in the same way as under physiological conditions (Ellis & Morrison 1982). At a pH of 7.5, for example, phosphate buffers add about 7x more ions to the medium than zwitterionic Tricine buffers at the same pH (Good & Izawa 1972). The Tris buffers for the preparation of the separation and stacking gels for SDS-PAGE are made from Tris base and HCl because of the ionic strength. If Tris · HCl is used and the pH value is adjusted using NaOH, NaCl forms, resulting in an increased salt concentration that causes abnormal migration of protein and diffuse bands (Ausubel *et al.* 1995).

Dependence of the pK_a value

The pK_a value of a buffer should be influenced as little as possible by the buffer concentration, the temperature and the ion composition of the medium. Amongst the buffers with temperature dependent pK_a values, for example, are the amine buffers, whilst carboxylic acid buffers generally react less sensitively to changes in temperature. The pH value of a Tris solution set at a pH of 7.8 at room temperature is 8.4 at 0°C and 7.4 at 37°C.

Complex formation

When a buffer forms complexes with metal ions, protons are released, which causes the pH value to decrease. The formation of insoluble precipitates usually represents a greater problem, however. If enzymes need the metal ions for their activity, these would be inhibited. Complexes should therefore be soluble and their binding constant should be known. Phosphates, for example, form insoluble salts with bivalent metals and precipitate. Phosphate buffered salt solution (PBS) is never autoclaved with Ca^{2+} or Mg^{2+} for this reason. Good buffers, such as PIPES, TES, HEPES and CAPS have very low metal-binding constants and are therefore particularly suited to investigate metal-dependent enzymes (Good & Izawa 1972, Blanchard 1984).

Inert substances

The buffer should not be subject to either enzymatic or non-enzymatic changes, i.e. it should not be an enzyme substrate or enzyme inhibitor and should not react with metabolites or other components. The buffer should therefore be inert. Phosphate and pyrophosphate are both substrates and inhibitors of different enzymatic reactions (inhibition of carboxypeptidase, urease, various kinases, various dehydrogenases). Borate forms covalent complexes with mono- and oligosaccharides, ribose subunits of nucleic acids, glycerol and pyridine nucleotides. Bicarbonate is in equilibrium with CO_2 and therefore needs a closed system. Tris and other primary amines can form Schiff's bases with aldehydes and ketones. They also interfere with the Bradford protein assay (e.g. Tris and glycine). Tricine is photo-oxidised by flavins, and daylight is therefore sufficient to reduce the activity of flavone enzymes. HEPES, HEPPS and Bicine interfere with Lowry (Folin) protein assays. Buffers that are chemically based on the piperazine ring may form radicals under certain circumstances (see below).

UV absorption

Buffers should not absorb any light at wave-lengths longer than 230 nm, since many spectrophotometric investigations are performed in this range (determination of the concentrations of DNA, RNA and proteins). ADA, for example, has an absorption of 0.1 at 260 nm. If buffers interfere with photometric analyses, they should be neutralised or set at the pH optimum for the test system used (Lowry pH 10; BCA pH 11; Bradford pH 1; colloidal gold pH 3). If this is not possible, proteins can be precipitated with trichloroacetic acid, perchloric acid or acetone, for example, and can then be redissolved in a solvent that does not interfere.

Purity – simple method of manufacture

Buffers should be as easy to manufacture and purify as possible. Purity is extremely important, since contaminations (e.g. heavy metals) can easily interfere with sensitive biological systems.

Costs

When purifying proteins, large amounts of buffer are often need for centrifugation, chromatography steps or dialysis. The costs for materials therefore affect the planning of an experiment.

Overview of the most important properties of buffers

(Good & Izawa 1972, Scopes 1994):

1.) Solubility
2.) Permeability through biological membranes
3.) pK_a value at the mid-point of the range of the test system
4.) Change in pK_a value dependent on temperature
5.) Change in pK_a value dependent on dilution
6.) Interaction with other components (e.g. metal ions, enzymes)
7.) UV absorption
8.) Non-toxic
9.) Costs

Recommendations for the setting of the pH value of a buffer

Temperature

Depending on the buffer substance, its pH may vary with temperature. It is therefore advisable, as far as possible, to set the pH at the working temperature to be used for the investigation. The physiological pH value for most animal cells at 37°C is between 7.0 and 7.5. One buffer particularly susceptible to changes in temperature is Tris (see above). If set at 7.5 at 37°C, it increases to about 8.5 if a test system with a temperature of 0°C is used. *In vitro* tests on cell extracts are often performed at 0°C (Scopes, 1994). Good buffers generally have a low degree of temperature sensitivity, and carboxylic acid buffers (citrate, formate, succinate) are even less sensitive. For practical work, this means that the buffer should be brought to the working temperature (Scopes 1994, Chapter 12.3) and that the pH electrode should also be calibrated at the working temperature. Nowadays, many pH meters have an integrated function that enables setting of the pH value at room temperature and allows for different working temperatures (e.g. +4°C or +37°C). A limitation on this function, however, is that the dpK_a/dT value, i.e. the value for the change in the pK_a value (dpK_a) dependent on the temperature change (dT), is not the same for all buffers. For example, the change in the pK_a value for Tris with an increase of 1°C amounts to 0.028 units, whilst the value for HEPES changes by only 0.014. Imprecision is unavoidable with this approach, since such changes should actually be accounted for with the pH meter.

Titration

Generally, the pH value is set using NaOH/KOH or HCl. Slow addition of the acid or base whilst stirring vigorously avoids local high concentrations of H^+ or OH^- ions. If this is not done, the buffer substances may undergo chemical changes that inactivate them or modify them so that they have an inhibitory action (Ellis & Morrison 1982). If a buffer is available in the protonised form (acid) and the non-protonised form (base), the pH value can also be set by mixing the two substances.
If monovalent cations interfere with the reaction or are to be investigated, the pH value can be set with tetramethyl or tetraethylammonium hydroxide. Acetate, sulphate or glutamate can be used instead of HCl, although here the risk of interference with an enzyme is particularly high.

Ionic strength

The setting of the ionic strength of a buffer solution (if necessary) should be done in the same way as the setting of the pH value when selecting the electrolyte, since this increases depending on the electrolyte used. The salts of tetramethylammonium or tetraethylammonium are suitable for the setting of the ionic strength, since the larger cations do not interact so well with the negative charges of the enzymes. Acetate, as a large anion, has a poor interaction with the alkali metals (Ellis & Morrison 1982). The following example with the buffer triethanolamine (20 mM, pH 7.5) illustrates how the different setting of a buffer can affect the ionic strength (I). If 20 mM triethanolamine are set at a pH of 7.5 with HCl, the resulting ionic strength is I = 0.012, with the ions H-triethanolamine$^+$ and Cl^-. However, if 20 mM triethanolamine hydrochloride are set at a pH of 7.5 with NaOH, the resulting ionic strength is I = 0.020, since the buffer solution also contains 8 mM NaCl (Scopes 1994). A further example is the electrophoresis buffer for SDS-PAGE, which is prepared using Tris base with HCl and not Tris hydrochloride and NaOH (Ausubel *et al.* 1995).

Buffer additives

If other components are added to the buffer (e.g. EDTA, DTT, Mg^{2+}), changes in the pH should also be expected and it should be retested. In living cells, particularly the oxidisation of proteins by different substances is inhibited by glutathione. Usually, therefore, if cells are being disrupted, a reducing agent such as β-mercaptoethanol (5 – 20 mM) or DTT (1 – 5 mM) has to be added. β-mercaptoethanol is oxidised within 24 hours after addition to the buffer (Bollag & Edelstein 1992, Scopes 1994. It is therefore advisable to add this substance only to the buffer while the proteins are being processed and to use DTT for longer storage periods of proteins.
To prevent the growth of bacteria or fungi, particularly in buffers in the pH range of 6.0 – 8.0, sterile filtration (0.22 µm) and/or the addition of 0.02 % (3 mM) sodium azide is recommended. If added to concentrated stock solutions, the latter is diluted to such an extent in the working solution that it usually does not interfere with the reaction.

pH meter control

Nowadays, accurate pH meters with a digital display are usually available for the setting of the pH value of a buffer. The pH meter is calibrated using two pH standards which cover the range of the buffer to be set. If there are any doubts about the precision of the device, this can simply be resolved by standardising the pH meter using 50 mM phosphate buffer, which is then diluted 10fold. The pH value should then be 0.2 pH units higher (Scopes 1994).

Criteria for the selection of a buffer

As already described, buffers can also have a decisive influence on the activity of an enzyme. Together with other factors, such as the ionic strength and the salt concentration, the activity of the restriction enzyme *Eco*RV can also be improved (Wenner & Bloomfield, 1999). We shall therefore take a closer look at a range of factors at this point.

Selection of the buffer for the correct pH range

The pK_a value of the buffer should be in the range of the pH optimum for the test system, as far as possible. If the pH is likely to increase during the experiment, then a buffer should be chosen with a pK_a value that is slightly higher than the optimum at the beginning of the experiment. Conversely, if the pH value is expected to decrease during the experiment, a buffer with a slightly lower pK_a value should be chosen.

Determination of the pH optimum of an enzyme

If an enzyme is to be investigated, the first step is usually to determine the conditions under which the enzyme will show the highest possible degree of stability and activity. The determination of the pH optimum is important as the initial step in this. It is advisable to test chemically similar buffers first of all, which cover overall a wide pH spectrum, e.g. MES, PIPES, HEPES, TAPS, CHES and CAPS for the pH range ~5.5 – 11.0 (Viola & Cleland 1978, Cook *et al.* 1981, Blanchard 1984). Once the pH optimum has been found, different buffers (e.g. for the pH value 7.5: TES, TEA or phosphate; Blanchard 1984) can be tested, in order to be able to rule out or minimise non-specific buffer effects for later investigations. The pK_a value of a buffer, i.e. the mid-point of its pH range, should be as near as possible to the desired pH value for the buffer being used, in other words, it should correspond to the pH optimum of the enzyme under testing. The protonised (ionised) forms of amine buffers have less inhibitory effects than the non-protonised forms. For Tris and zwitterionic buffers, therefore, a working range slightly lower than the pK_a value is usually more suitable, whilst in contrast to this, carboxylic acid buffers with a working range slightly above their pK_a values are better suited, since these buffers consist mainly of the ionised form (Good & Izawa 1972).

Determination of the optimum buffer concentration

An adequate buffer capacity is often only reached at concentrations higher than 25 mM. However, higher buffer concentrations and related high ionic strengths can inhibit enzyme activity. Suitable initial concentrations are therefore between 10 and 25 mM. If, after addition of the protein or enzyme, the pH value changes by more than 0.05 units, the concentration of the buffer can first be increased to 50 mM. Up to this concentration, no interference was observed with the Good buffers in cell culture experiments, for example (Ferguson *et al.* 1980). In order to form complexes with heavy metals, EDTA can be added in small amounts to buffers, if desirable (10 – 100 µM; Stoll & Blanchard 1990). Between 0.1 and 5.0 mM chelating agents can be added to achieve the complete removal of multivalent cations.

Application-dependent choice of buffer substances

The decision for or against a buffer is also dependent on the method for which it is used. In addition to the measurement of activity, the concentration is also usually determined when proteins or enzymes are undergoing purification. In assays using reagents for protein determination, many of the buffer substances based on amino acids can lead to false-positive results due to interactions with the reagents or absorption of the buffer substance itself in the range above 230 nm. For example, various buffers interfere with the Lowry protein assay (see below). Such interference can, however, usually be relatively easily abolished by inclusion of the buffer in the blank control (Peterson 1979).

Many buffers are basically suitable for gel filtration. Cationic buffers such as Tris are preferred for anion exchange chromatography. Anionic buffers (such as phosphate or MES) should be preferred for cation exchange chromatography or hydroxylapatite chromatography, i.e. the buffer should have the same charge as the ion exchange material, to prevent it binding itself to the ion exchanger (Blanchard 1984; Scopes 1994). The buffer requirements for ion exchange chromatography are discussed in detail by Scopes (1984).

For example, borate is not suitable for the isolation of glycoproteins or systems that include nucleotides, since it interacts with the cis-hydroxyl group of sugars. If electrophoresis is performed subsequent to dissolution of the protein in protein purification systems, a buffer with a low ionic strength should be used, since a high ionic strength would heat up the gel (Hjelmeland & Chrambach, 1984).

The Good buffers based on the piperazine ring – HEPES, HEPPS, HEPPSO and PIPES – are not suitable for the investigation of redox processes, since, in the presence of H_2O_2, oxygen radicals, autooxidising iron or, under certain electrolytic conditions, they easily form radicals. In contrast to this, the Good buffer based on a morpholine ring, MES, does not form any radicals (Grady *et al.* 1988).

Tris buffer: not always the best choice! (Sambrook & Russell 2001)

Tris (tris-(hydroxymethyl)-aminomethane) is probably the most frequently used buffer substance in biological experiments. The reasons for this are that Tris is comparatively inexpensive, very freely soluble in water, is inert in many enzymatic systems (no interactions with other components) and has a high buffer capacity. Since, however, Tris may have a series of negative characteristics, these are presented here in detail.

1.) The pK_a value of Tris is 8.06 at 25°C. This means that it is already at the upper end of the pH range of many biological systems (pH 6.0 – 8.0) and that it has a relatively low buffer capacity in the actual physiological pH range (7.0 – 7.5).

2.) Tris buffers have a significantly high degree of temperature sensitivity. The effects are therefore very different when Tris is used in the cold room, at room temperature, or at 37°C. This means that the pH value has to be set for the ambient temperature at which it is used.

3.) Tris reacts with many pH electrodes that have a linen-fibre junction. This results in high liquid-junction potentials, electromotive force drifts (emf) and long equilibration times. This means that only electrodes with ceramic or glass junctions can be used which are declared as suitable by the manufacturer.

4.) The pH value of a Tris solution is concentration-dependent. On dilution, the pH value decreases by 0.1 pH unit, when diluted from 100 mM to 10 mM.

5.) Tris is toxic for many mammalian cells, since it penetrates cells due to its relatively good fat solubility.

6.) Tris is a primary amine. It cannot be used with fixation reagents such as glutaraldehyde or formaldehyde. It also reacts with glyoxal and DEPC. In such cases, phosphate, HEPES or MOPS buffers are used instead.

Preparation of Tris solutions in a concentration of 50 mM

for 100 ml 50 mM Tris solution*				for 1 liter 50 mM Tris solution	
50 ml 100 mM Tris (12.11 g Tris base per liter dH_2O) x ml 0,1 N HCl y ml dH_2O ad 100 ml				6.057 g Tris base/900 ml liter dH_2O z ml 1.0 N HCl dH_2O ad 1 liter	
pH	100 mM Tris	x ml 0.1 N HCl	y ml dH_2O	pH	z ml 1,0 N HCl
7.10	50 ml	45.7	4.3	7.10	45.7
7.20	50 ml	44.7	5.3	7.20	44.7
7.30	50 ml	43.4	6.6	7.30	43.4
7.40	50 ml	42.0	8.0	7.40	42.0
7.50	50 ml	40.3	9.7	7.50	40.3
7.60	50 ml	38.5	11.5	7.60	38.5
7.70	50 ml	36.6	13.4	7.70	36.6
7.80	50 ml	34.5	15.5	7.80	34.5
7.90	50 ml	32.0	18.0	7.90	32.0
8.00	50 ml	29.2	20.8	8.00	29.2
8.10	50 ml	26.2	23.8	8.10	26.2
8.20	50 ml	22.9	27.1	8.20	22.9
8.30	50 ml	19.9	30.1	8.30	19.9
8.40	50 ml	17.2	32.8	8.40	17.2
8.50	50 ml	14.7	35.3	8.50	14.7
8.60	50 ml	12.4	37.6	8.60	12.4
8.70	50 ml	10.3	39.7	8.70	10.3
8.80	50 ml	8.5	41.5	8.80	8.5
8.90	50 ml	7.0	47.0	8.90	7.0

* Dawson, R.M.C. *et al.* (1986) Data for Biochemical Research. p. 436. Clarendon Press, Oxford.

pH value of a 50 mM Tris solution

5°C	25°C	37°C
9.5	8.9	8.6

Temperature dependency of the pH value of a 50 mM Tris solution
(pH value set at 25°C)
The pH values to be expected at +4°C and +37°C are given.

4°C	25°C	37°C
7.79	7.20	6.86
7.89	7.30	6,96
7.99	7.40	7,06
8.09	7.50	7.16
8.19	7.60	7.26
8.29	7.70	7.36
8.39	7.80	7.46
8.49	7.90	7.56
8.59	8.00	7.66
8.69	8.10	7.76
8.79	8.20	7.86
8.89	8.30	7.96
8.99	8.40	8.06
9.09	8.50	8.16
9.19	8.60	8.26
9.29	8.70	8.36

Preparation of 1 M Tris solutions (1 liter)

121.14 g Tris base in 800 ml dH_2O set pH with concentrated hydrochloric acid dH_2O ad 1 liter

pH	for 1 liter 1 M Tris x ml conc. HCl
7.2	76.10
7.5	69.10
8.0	48.30
8.5	23.90
9.0	8.25

TE buffer

10 mM Tris · HCl (pH 7.4, 7.5 or 8.0)
1 mM EDTA (pH 8.0)

This buffer has become the standard buffer for the storage of nucleic acids. It is used at different pH values. It is generally prepared by mixing Tris buffer stock solutions (1 M) with an EDTA stock solution (0.5 M; pH 8.0). The prepared buffer can also be stored at room temperature following autoclaving. TE stock solutions are prepared in concentrations of 100X to 1X.

Volatile buffer systems

effective pH range	Description	Counter ion	pK-value
3.3 – 4.3	Formic acid	H^+	3.75
3.3 – 4.3	Pyridine / formic acid	$HCOO^-$	3.75
3.3 – 4.3	Trimethylamine / formic acid	$HCOO^-$	4.75
3.3 – 4.3	Ammonia / formic acid	$HCOO^-$	3.75
4.3 – 5.3	Trimethylamine / acetic acid	CH_3CO^-	4.75
4.3 – 5.3	Ammonia / acetic acid	CH_3COO^-	4.75
4.3 – 5.3	N-ethylmorpholine / acetate	$HCOO^-$	4.75
4.3 – 5.8	Pyridine / acetic acid	CH_3COO^-	4.75; 5.25
4.8 – 5.8	Pyridine / formic acid	$HCOO^-$	5.25
5.9 – 6.9	Trimethylamine / carbonate	CO_3^{2-}	6.35
5.9 – 6.9	Ammonium bicarbonate	HCO_3^-	6.35
5.9 – 6.9	Ammonium carbonate / ammonia	CO_3^{2-}	6.35
5.9 – 6.9	Ammonium carbonate	CO_3^{2-}	6.35
6.8 – 8.8	Trimethylamine / hydrochloric acid	Cl^-	9.25
7.0 – 8.2	N-ethylmorpholine / acetate	$HCOO^-$	7.72
8.8 – 9.8	Ammonia / formic acid	$HCOO^-$	9.25
8.8 – 9.8	Ammonia / acetic acid	CH_3COO^-	9.25
8.8 – 9.8	Ammonium bicarbonate	HCO_3^-	9.25
8.8 – 9.8	Ammonium carbonate / ammonia	CO_3^{2-}	9.25
8.8 – 9.8	Ammonium carbonate	CO_3^{2-}	9.25
9.3 – 10.3	Trimethylamine / formic acid	$HCOO^-$	9.81
9.3 – 10.3	Trimethylamine / acetic acid	CH_3COO^-	9.81
9.3 – 10.3	Trimethylamine / carbonate	CO_3^{2-}	9.81

from Dawson *et al.* 1986 and Stoll & Blanchard 1990

Volatile buffers

A number of buffers are available that can be easily and completely removed. These buffers are used particularly when subsequent reactions must not contain any disturbing components. They are useful for electrophoresis, ion exchange chromatography, or for digestion of proteins with subsequent removal of peptides and amino acids. These buffer substances include: formic acid, ammonia, ammonium carbonate, acetic acid, pyridine and triethanolamine. A pH range of 1.9 – 8.9 can be covered with appropriate mixtures of these substances.

Buffer mixtures

Since the maximum buffer range of a weak acid or base is relatively narrow, namely one pH unit above and below the pK_a value, it is necessary under certain circumstances to prepare mixtures of buffers that cover a wider pH range and therefore have a constant buffer capacity in this range. For such mixtures, it is advisable to use buffers with a similar structure that have overlapping optimal buffer ranges (pH ranges) (e.g. MES/acetate/Tris, pH 4.0 – 9.0). The pK_a values should not be separated more than 1 – 2 pH units (Williams & Morrison 1981, Blanchard 1984, Stoll & Blanchard 1990). The buffer capacities are additive where the ranges overlap.
These systems do, however, have disadvantages in some situations. Since each of the components of the buffer only buffers in a very narrow pH range, it is also present outside the buffer range in its ionised form, and this ionised form may have inhibitory effects. In addition, the presence of different additional buffer substances increases the ionic strength.

Buffer series or multicomponent buffers are used for the determination of the pH-dependency of enzyme activity, for example. Examples for the use of buffer series are assays on hexokinase from yeast (Viola & Cleland 1978), muscle creatine kinase from rabbits (Cook *et al.* 1981), dihydrofolate reductase from S. faecium (Williams & Morrison 1981), chymase from humans (McEuen *et al.* 1995) and trehalase from silkworm moths (Ando *et al.* 1995).

If the ionic strength is important, it can be reduced by choosing the appropriate buffer substances. The amount of acid or alkali (electrolyte) that has to be added to set the pH value can be reduced by combining a weak acid with a weak base (Ellis & Morrison 1982). And the ionic strength can also be maintained constant over wide pH ranges by choosing the right buffers. Ellis & Morrison (1982) describe examples of three-component buffers of this type that can cover up to 4 pH units.

Buffer mixtures are also used for high performance chromatofocusing. This chromatographic method enables the separation, also of protein isoforms, for example, according to the surface charge of the protein in pH gradients which are created by applying an electrical field. The focussing buffers used can have a very complex composition (31 components; Hutchens *et al.* 1986).

Buffers for gel electrophoresis

Gel electrophoresis has become one of the most important methods in the analysis of nucleic acids and proteins. Three principal buffers have established themselves as the standards for the techniques of polyacrylamide gel electrophoresis and agarose gel electrophoresis: TAE buffer (Tris-acetate-EDTA), TBE buffer (Tris-borate-EDTA) and Tris-glycine buffer. Depending on the application, other substances may be added to these, such as urea and SDS. Since there are many derived methods based on these electrophoresis techniques, there is a correspondingly high number of modified buffers.

Electrophoresis buffers

TAE buffer (Tris-acetate-EDTA) Order No. A1691

50X stock solution (usual working concentration 0.5X–1X)	
242 g	Tris
57.1 ml	Glacial acetic acid
37.2 g	EDTA – disodium salt - dihydrate
	set pH to 8.5
add 1 liter	dH_2O

TBE buffer (Tris-borate-EDTA) Order No. A0972

10X stock solution (usual working concentration 1X)	
108 g	Tris (890 mM)
55 g	Boric acid (890 mM)
40 ml	0.5 M EDTA – disodium salt – dihydrate (pH 8.0)
	set pH to 8.3
add 1 liter	dH_2O

Tris-glycine buffer (TG) Order No. A1418

10X stock solution	
15.1 g	Tris
72.0 g	Glycine
add 5 liter	dH_2O
	Storage for up to 1 month at +4°C

SDS-Tris-glycine buffer (Laemmli buffer) Order No. A1415

10X stock solution		
30.29 g	Tris	(0.25 M)
144.13 g	Glycine	(1.92 M)
10.00 g	SDS	(1 %)
	pH should be 8.3!	
add 1 liter	dH_2O	

The following buffers are also used for this electrophoresis system (Laemmli):

4X Tris/SDS pH 6.8 stacking gel buffer

	1. dissolve 6.05 g Tris-base in 40 ml H_2O
	2. adjust pH to 6.8 with 1 N HCl
	3. add H_2O to 100 ml
	4. add 0.4 g SDS
	store at room temperature

4X Tris/SDS pH 8.8 resolving gel buffer

	1. dissolve 91 g Tris-base in 300 ml H2O
	2. adjust to pH 8.8 with 1 N HCl
	3. add H_2O to 500 ml
	4. add 2 g SDS
	store at room temperature

6X SDS sample buffer

	• 7 ml 4X Tris/SDS pH 6.8 stacking gel buffer
	• 3.0 ml glycerol
	• 1 g SDS
	• 0.93 g DTT (dithiothreitol)
	• 1.2 mg bromophenol blue
	• add dH_2O to 10 ml
	store in 1 ml aliquots at -20 °C

Tris-Tricine buffer

Working concentration (do not adjust pH)	
12.11 g	Tris (0.1 M)
17.92 g	Tricine (0.1 M)
1 g	SDS ultrapure (0,1 %)
add 1 liter	dH_2O
	Storage for up to 1 month at +4°C

Technical tips

How can microbial contamination of buffer solutions be prevented?

1.) Sterilisation by filtration or autoclaving
2.) addition of 0.02 % (3 mM) sodium azide
3.) Storage at +4°C
4.) High-concentration stock solutions

Special note for buffers containing sodium hydrogen carbonate (sodium bicarbonate): this buffer substance requires a closed system. In aqueous solutions, sodium hydrogen carbonate degrades into CO_2 and sodium carbonate above 20°C. Complete degradation occurs at 100°C. Solutions containing sodium hydrogen carbonate cannot therefore be autoclaved, but have to be sterile filtered. When preparing, they should not be stirred too vigorously and too long. The pH of a freshly prepared 100 mM solution is 8.3 at 25°C.

How can precipitation in concentrated TBE buffers be prevented?

Precipitation tends to occur in concentrated TBE buffer solutions (usually 10X) very soon after they are prepared. This can be prevented by filtering the solution using a cellulose acetate or cellulose nitrate filter (0.2 – 0.45 µm). The vessels into which the buffer is filtered must be dust-free. Salt crystals appear to be responsible for the precipitation, which form as crystallisation buds on dust particles or other microscopically small particles. Concentrated TBE buffer solutions that have become turbid can also be autoclaved (Mayeda & Krainer 1991).

What is the best way of obtaining solutions of the free acids of PIPES, POPSO or ADA?

The free acid of PIPES is very poorly soluble in water (only 1 g/L; see Good *et al.* 1966 [page 469]). By conversion to the sodium salt with NaOH, the pH of the solution increases to higher than 6 and the salt is easily soluble. The same applies to POPSO and ADA, which are very poorly soluble and are not soluble until converted to the sodium salt.

What is the importance of water as a solvent?

The buffer substances that are commercially available today are usually of the highest quality. For example, they are tested for low heavy metal content, absence of endotoxins and enzyme contamination (DNases, RNases, proteases, phosphatases). The water in which the buffer substances are dissolved is usually from the user's laboratory where the buffer solutions are prepared. Here, too, attention must be paid to using only the highest quality. Water that stands too long in pipes increases the risk of contamination of the buffer solution. Gases may be dissolved into the water and contaminating agents may adhere to the taps. The water should therefore be run for a short time before using it to prepare the buffer solution.

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[a] **BCA** Kaushal, V. & Barnes, L.D. (1986) *Anal. Biochem.* **157**, 291-294 – Bicinchoninic Acid – protein detection: the buffers were used in a concentration of 50 mM.

[b] **Lowry** Peterson, G.L. (1979) *Anal. Biochem.* **100**, 201-220 – with recommendations on how to minimise and rule out disturbing factors and information on tolerable final concentrations. In some cases it is sufficient to include the substance concerned as a control.

[c] **Radical formation** Grady, J.K. *et al.* (1988) Anal. Biochem. 173, 111-115. Under certain conditions, the piperazine ring system forms radicals. These buffers are therefore not suitable for the investigation of redox processes in biochemistry.

[d] absence of any comments does not indicate that there is no influence on results.

(Disturbing) effects of biological buffers in different assays*

Buffer substance	BCA [a, d]	Lowry [b, d] (folin)	Comments
ACES		+	significant absorption of UV light at 230 nm, binds Cu^{2+}
ADA	+	+	marked absorption in UV range below 260 nm; binds metal ions
AMP			
BES	–	+	binds Cu^{2+}
Bicarbonate			limited solubility; needs closed system, since in equilibrium with CO_2
Bicine	+	+	slowly oxidised by ferricyanide; strongly binds Cu^{2+}
Bis-Tris	+		substitute for cacodylate
Bis-Tris-Propane			
Borate			forms covalent complexes with mono- and oligosaccharides, ribose subunits of nucleic acids, pyridine nucleotides, glycerol
Cacodylate			very toxic; nowadays usually replaced by MES
CAPS	–	+	
CAPSO			
CHES		+	
Citrate			binds to some proteins, forms complexes with metals; replaced by MES
DIPSO		+	
Glycine		+	interferes with Bradford protein assay
Glycylglycine		+	binds Cu^{2+}
HEPES	–	+	can form radicals, not suitable for redox studies
HEPPS, EPPS	–	+	can form radicals, not suitable for redox studies
HEPPSO	–	+	can form radicals, not suitable for redox studies
Imidazole			forms complexes with Me^{2+}, relatively instable
Maleic acid			absorbs in the UV range; replaced by MES or Bis-Tris
MES	–	+	substitute for cacodylate
MOPS	–	+	partly degraded on autoclaving in the presence of glucose; negligible metal ion binding
MOPSO		+	
Phosphate			substrate/inhibitor of various enzymes (inhibits many kinases and dehydrogenases, enzymes with phosphate esters as substrate; inhibits carboxypeptidase, fumarase, urease); precipitates/binds bivalent cations; pK increases on dilution;
PIPES	–	+	can form radicals, not suitable for redox studies
POPSO		+	
TAPS		+	
TAPSO		+	
TEA			
TES	–	+	binds Cu^{2+}
Tricine	+	+	strongly binds Cu^{2+}; addition of Cu^{2+} in the Lowry assay enables it to be used; is photooxidised by flavines; substitute for barbital (Veronal)
Tris	+	+	high degree of temperature-sensitivity; pH decreases by 0.1 unit with each 10fold dilution; inactivates DEPC, can form Schiff's bases with aldehydes/ketones, as it is a primary amine; is involved in some enzymatic reactions (e.g. alkaline phosphatase)

* partly taken from Bollag, D.M. & Edelstein, S.J. (1992) Protein Methods, Chapter 1, II (pp. 3-9). Wiley-Liss, New York.

Concentration limits for buffers in protein assays *

Buffer substance	Lowry (Folin)	BCA	Bradford	Colloidal Gold	UV 280 nm	UV 205 nm
Acetate		0.2 M	0.6 M		0.1 M	10 mM
Borate		10 mM				>100 mM
Citrate	2.5 mM	< 1 mM	50 mM		5 %	<10 mM
Glycine	2.5 mM	1 M	0.1 M	100 mM	1 M	5 mM
HEPES	2.5 µM	100 µM	100 mM	20 mM		<20 mM
Phosphate	250 mM	250 µM	2 M	100 mM	1 M	50 mM
Tris	250 mM	0.1 M	2 M		0.5 M	40 mM

*according to Stoscheck, C.M. (1990) *Methods Enzymol.* **182**, 50-68 – The values given correspond to the final concentration. In case of the UV absorption, the final concentration of the chemical corresponds to an absorption value smaller than 0.5 above water. Bradford, M.M. (1976) *Anal. Biochem.* **72**, 248-254

Saturated concentrations of buffers in solution at 0°C
(according to Good *et al.* 1966, Good & Izawa 1972, Ferguson *et al.* 1980)

Buffer substance	Concentration (M)
ACES	0.22
ADA	0.09
BES	3.20
Bicine	1.10
CAPS	0.47
CHES	1.14
DIPSO	0.24
Glycylglycine	1.10
HEPES	2.25
HEPPS	4.58

Buffer substance	Concentration (M)
HEPPSO	2.20
Potassium phosphate	2.50
MES	0.65
MOPS	3.00
MOPSO	0.75
PIPES	2.30
TES	2.60
TAPSO	1.00
Tricine	0.80
Tris	2.40

"Old" buffers replaced by buffers with better properties
(according to Scopes 1994)

"Old" Buffer	Unwanted properties	Recommended substitute
Veronal (5,5-Diethylbarbituric acid; Barbital)	toxic	Tricine, Tris
Cacodylic acid, Cacodylate	toxic	MES, Bis-Tris
Citric acid, Citrate	complexes metal ions	MES, Bis-Tris
Maleic acid	UV-Absorption	MES, Bis-Tris

t i p s

Alphabetical list of biological buffers

Trivial name	Name
ACES	*N*-(2-Acetamido)-aminoethanesulfonic acid
Acetate	Salt of acetic acid
ADA	*N*-(2-Acetamido)-iminodiacetic acid
AES	2-Aminoethanesulfonic acid, Taurine
Ammonia	–
AMP	2-Amino-2-methyl-1-propanol
AMPD	2-Amino-2-methyl-1,3-propanediol, Ammediol
AMPSO	*N*-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid
BES	*N,N*-Bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid
Bicarbonate	Sodium hydrogen carbonate
Bicine	*N,N'*-Bis(2-hydroxyethyl)-glycine
BIS-Tris	[Bis-(2-hydroxyethyl)-imino]-tris-(hydroxymethylmethane)
BIS-Tris-Propane	1,3-Bis[tris(hydroxymethyl)-methylamino]propane
Boric acid	–
Cacodylate	Dimethylarsinic acid
CAPS	3-(Cyclohexylamino)-propanesulfonic acid
CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid
Carbonate	Sodium carbonate
CHES	Cyclohexylaminoethanesulfonic acid
Citrate	Salt of citric acid
DIPSO	3-[*N*-Bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid
Formate	Salt of formic acid
Glycine	–
Glycylglycine	–
HEPES	*N*-(2-Hydroxyethyl)-piperazine-*N'*-ethanesulfonic acid
HEPPS, EPPS	*N*-(2-Hydroxyethyl)-piperazine-*N'*-3-propanesulfonic acid
HEPPSO	*N*-(2-Hydroxyethyl)-piperazine-N'-2-hydroxypropanesulfonic acid
Imidazole	–
Malate	Salt of malic acid
Maleate	Salt of maleic acid
MES	2-(*N*-Morpholino)-ethanesulfonic acid
MOPS	3-(*N*-Morpholino)-propanesulfonic acid
MOPSO	3-(*N*-Morpholino)-2-hydroxypropanesulfonic acid
Phosphate	Salt of phosphoric acid
PIPES	Piperazine-*N,N'*-bis(2-ethanesulfonic acid)
POPSO	Piperazine-*N,N'*-bis(2-hydroxypropanesulfonic acid)
Pyridine	–
Succinate	Salt of succinic acid
TAPS	3-{[Tris(hydroxymethyl)-methyl]-amino}-propanesulfonic acid
TAPSO	3-[*N*-Tris(hydroxymethyl)-methylamino]-2-hydroxypropanesulfonic acid
Taurine	2-Aminoethanesulfonic acid, AES
TEA	Triethanolamine
TES	2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid
Tricine	N-[Tris(hydroxymethyl)-methyl]-glycine
Tris	Tris(hydroxymethyl)-aminomethane

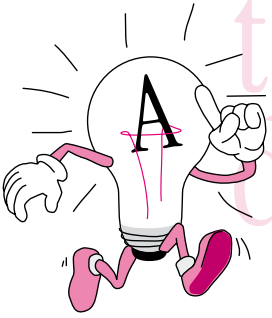
Temperature dependence of the pK_a value of biological buffers (100 mM)

effective pH range	Description	$d(pK_a)/dT$	pK_a (0°C)	pK_a (4°C)	pK_a (20°C)	pK_a (25°C)	pK_a (37°C)
1.2 – 2.6	Maleate (pK_1)					1.97	
1.7 – 2.9	Phosphate (pK_1)	0.0044				2.15	
2.2 – 3.6	Glycine (pK_1)					2.35	
2.2 – 6.5	Citrate (pK_1)					3.13	
2.5 – 3.8	Glycylglycine					3.14	
2.7 – 4.2	Malate (pK_1)					3.40	
3.0 – 4.5	Formate	0.0				3.75	
3.0 – 6.2	Citrate (pK_2)	-0.0016		4.79	4.77	4.76	4.74
3.2 – 5.2	Succinate (pK_1)	-0.0018				4.21	
3.6 – 5.6	Acetate	0.0002				4.76	
4.0 – 6.0	Malate (pK_2)					5.13	
4.9 – 5.9	Pyridine	-0.014				5.23	
5.0 – 7.4	Cacodylate					6.27	
5.5 – 6.5	Succinate (pK_2)	0.0				5.64	
5.5 – 6.7	MES	-0.011	6.38	6.33	6.15	6.10	5.98
5.5 – 7.2	Maleate (pK_2)				6.15	6.24	
5.5 – 7.2	Citrate (pK_3)	0.0				6.40	
5.8 – 7.2	BIS-Tris	-0.017		6.82	6.54	6.46	6.25
5.8 – 8.0	Phosphate (pK_2)	-0.0028		7.26	7.21	7.20	7.16
6.0 – 7.2	ADA	-0.011	6.85	6.80	6.60	6.59	6.45
6.0 – 8.0	Carbonate (pK_1)	-0.0055			6.30	6.35	
6.1 – 7.5	PIPES	-0.0085	7.02	6.94	6.80	6.76	6.66
6.1 – 7.5	ACES	-0.020	7.32	7.20	6.90	6.78	6.56
6.2 – 7.6	MOPSO	-0.015			6.95	6.87	
6.2 – 7.8	Imidazole	-0.020		7.37	7.05	6.95	6.71
6.3 – 9.5	BIS-Tris-Propane					6.80	
6.4 – 7.8	BES	-0.016	7.50	7.41	7.15	7.09	6.90
6.5 – 7.9	MOPS	-0.011		7.41	7.20	7.14	6.98
6.8 – 8.2	TES	-0.020	7.92	7.82	7.50	7.40	7.14
6.8 – 8.2	HEPES	-0.014	7.85	7.77	7.55	7.48	7.31
7.0 – 8.2	DIPSO	-0.015			7.60	7.52	
7.0 – 8.2	TAPSO	-0.018			7.70	7.61	
7.0 – 8.3	TEA	-0.020				7.76	
7.1 – 8.5	HEPPSO	-0.010			7.90	7.85	
7.2 – 8.5	POPSO	-0.013			7.85	7.78	
7.4 – 8.8	Tricine	-0.021	8.60	8.49	8.15	8.05	7.80
7.5 – 8.9	Glycylglycine	-0.025	9.00	8.85	8.40	8.25	7.90
7.5 – 9.0	Tris	-0.028	8.90	8.80	8.30	8.06	7.70
7.6 – 8.6	HEPPS. EPPS	-0.015		8.18	8.10	8.00	7.81
7.6 – 9.0	Bicine	-0.018	8.70	8.64	8.35	8.26	8.04

continued

effective pH range	Description	$d(pK_a)/dT$	pK_a (0°C)	pK_a (4°C)	pK_a (20°C)	pK_a (25°C)	pK_a (37°C)
7.7 – 9.1	TAPS	+0.018		8.02	8.31	8.40	8.62
7.8 – 9.7	AMPD	-0.029				8.80	
8.3 – 9.7	AMPSO				9.10	9.00	
8.4 – 9.6	Taurine (AES)	-0.022				9.06	
8.5 – 10.2	Boric acid (pK_1)	-0.008				9.23	
8.8 – 9.9	Ammonia	-0.031				9.25	
8.6 – 10.0	CHES	-0.011		9.73	9.55	9.50	9.36
8.7 – 10.4	AMP	-0.032				9.69	
8.8 – 10.6	Glycine (pK_2)	-0.025		10.30	9.90	9.78	9.48
8.9 – 10.3	CAPSO					9.60	
9.5 – 11.1	Carbonate (pK_2)	-0.009				10.33	
9.7 – 11.1	CAPS	-0.009				10.40	
	Phosphate (pK_3)	-0.026				12.33	
	Boric acid (pK_2)					12.74	
	Boric acid (pK_3)					13.80	

$d(pK_a)/dT$ from Ellis & Morrison 1982 and Good & Izawa 1972 and Dawson *et al.* 1986
pK_a 25°C from Stoll & Blanchard 1990 and Dawson *et al.* 1986
pK_a 20°C from Good *et al.* 1966 and Good & Izawa 1972 and Ferguson *et al.* 1980
pK_a 0°C and 37°C from Good *et al.* 1966
Depending on the author small differences may occur!



temperature dependence

pK_a values of biological buffers (25°C, 100 mM), alphabetical list

Description	pK_a (25°C)	effective pH range
ACES	6.78	6.1 – 7.5
Acetate	4.76	3.6 – 5.6
ADA	6.59	6.0 – 7.2
Ammonia	9.25	8.8 – 9.9
AMP	9.69	8.7 – 10.4
AMPD	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	6.3 – 9.5
Boric acid (pK_1)	9.23	8.5 – 10.2
Boric acid (pK_2)	12.74	
Boric acid (pK_3)	13.80	
Cacodylate	6.27	5.0 – 7.4
CAPS	10.40	9.7 – 11.1
CAPSO	9.60	8.9 – 10.3
Carbonate (pK_1)	6.35	6.0 – 8.0
Carbonate (pK_2)	10.33	9.5 – 11.1
CHES	9.50	8.6 – 10.0
Citrate (pK_1)	3.13	2.2 – 6.5
Citrate (pK_2)	4.76	3.0 – 6.2
Citrate (pK_3)	6.40	5.5 – 7.2
DIPSO	7.52	7.0 – 8.2
Formate	3.75	3.0 – 4.5
Glycine (pK_1)	2.35	2.2 – 3.6
Glycine (pK_2)	9.78	8.8 – 10.6
Glycylglycine	3.14	2.5 – 3.8
Glycylglycine	8.25	7.5 – 8.9
HEPES	7.48	6.8 – 8.2
HEPPS, EPPS	8.00	7.6 – 8.6
HEPPSO	7.85	7.1 – 8.5
Imidazole	6.95	6.2 – 7.8
Malate (pK_1)	3.40	2.7 – 4.2
Malate (pK_2)	5.13	4.0 – 6.0
Maleate (pK_1)	1.97	1.2 – 2.6
Maleate (pK_2)	6.24	5.5 – 7.2
MES	6.10	5.5 – 6.7
MOPS	7.14	6.5 – 7.9
MOPSO	6.87	6.2 – 7.6
Phosphate (pK_1)	2.15	1.7 – 2.9
Phosphate (pK_2)	7.20	5.8 – 8.0
Phosphate (pK_3)	12.33	
PIPES	6.76	6.1 – 7.5
POPSO	7.78	7.2 – 8.5
Pyridine	5.23	4.9 – 5.9
Succinate (pK_1)	4.21	3.2 – 5.2
Succinate (pK_2)	5.64	5.5 – 6.5
TAPS	8.40	7.7 – 9.1
TAPSO	7.61	7.0 – 8.2
Taurine (AES)	9.06	8.4 – 9.6
TEA	7.76	7.0 – 8.3
TES	7.40	6.8 – 8.2
Tricine	8.05	7.4 – 8.8
Tris	8.06	7.5 – 9.0

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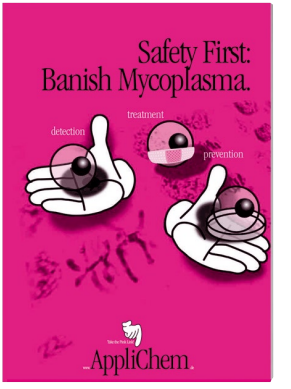

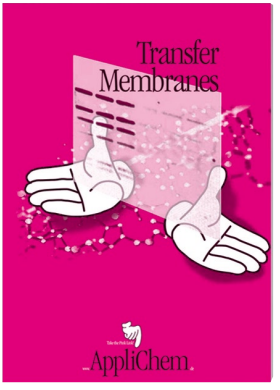

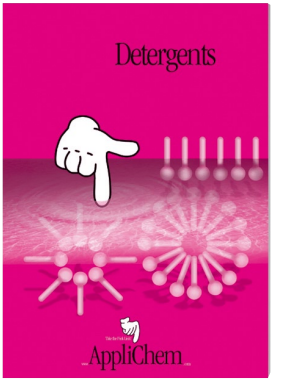

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