Buffer Preparation
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Purpose and Principles

Monobasic and dibasic phosphate (respectively potassium dihydrogen phosphate and potassium hydrogen phosphate) pH activity is calculated and experimentally tested in aqueous solution involving both the acid and its conjugate. The concentration ratio of proton-accepting base to proton-donating acid sharing a common conjugate ion is accurately determined using the Henderson-Hasselbalch equation. The inputs into the equation in this case are a target concentration for the acid and its conjugate in the solution as well as target pH. To be sure that the experimental solution is within effective buffer range, the target pH is kept within one unit of pKa, or a proton concentration within $10^{\pm 1}$ M of the proton concentration given for a fifty-percent dissociated aqueous buffering agent, as indicated by Ka. A different set of calculations to the same effect are carried out using the free amine form of TRIS, buffered with a concentration of the conjugate acid obtained by addition of hydrochloric acid, which has a negligibly low pKa.

Buffered solutions are primarily useful because they resist changes in pH, a necessity in living systems maintaining dynamic equilibrium among pH among many other factors. This capability is the direct result of the presence of both proton-donating and proton-accepting forms of a weak acid or base in the buffer solution. In the example of monobasic phosphate, this acid and the corresponding conjugate base concentrations in a solution are added such that the solution pH is near the known pKa for the acid. Both the acid and base forms remain in equilibrium so that any protons removed from or added to the solution change the ratio of these forms in the solution *before* solution pH changes, because water is both a weaker proton donor and acceptor than the buffer components in solution. The pH does begin to change as the ratio becomes less than 1-to-10 or more than 10-to-1. This is the reason why the effective buffer pH range of any buffer system is only plus or minus one unit from the pKa of either component. In the phosphate system, the pKa of the monobasic form is 6.86; the TRIS base has a pKa of 8.10. A pH at which both are effective buffers is 7.50. This is the target pH used for both sets of calculations.

Results

Calculations are for 0.250 L of a 0.50 M buffer at pH 7.5 in both of the following systems. Variables *b* and *a* are base and acid components of the corresponding buffer system.

In general, the Henderson-Hasselbalch equation is pH= pKa + log{[base] / [acid]}

250.0 ml · 0.5000 mol / 1000. ml = 0.1250 mol total ion concentration in both solutions with target concentration of 0.5M

In the phosphate buffer system, concentration ratio is given by:

$7.50 = 6.86 + \log \{ [K_2HPO_4] / [KH_2PO_4] \}$
$[K_2HPO_4] / [KH_2PO_4] = 4.37$
0.1250 mol = 4.37a + a

Converting to target buffer solution concentration and units,

a = 0.0233 mol KH_2PO_4 = 0.0233 mol · 136.086 g/mol = 3.17g
b = 0.1016 mol K_2HPO_4 = 0.1016 mol · 174.176 g/mol = 17.70g

Actual amounts used were 3.1706g monobasic phosphate and 17.6904g dibasic phosphate in 250.0 ml deionized water, producing a solution at an experimental pH 7.58.

In the TRIS buffer system, concentration ratio is given again by the Henderson-Hasselbalch equation:

7.5 = 8.1 + log {[TRIS base] / [HCl] }
[TRIS base] / [HCl] = 0.2512

But this is technically incorrect, because the calculation finds [TRIS base] / [TRIS acid]. Converting to target amounts and units,

0.1250 = 0.2512b + 1.000a
a = 0.0999 mol HCl **(incorrect)**
b = 0.1250 – 0.0999 = 0.0251 mol TRIS base remaining
0.0999 mol HCl / {6 mol / 1000 ml} = 16.65 ml

Correcting the above requires using acid and base values taken directly from the base-to-acid ratio given by the Henderson-Hasselbalch equation:

(.1250 mol total TRIS needed) / (.0251 mol base + 1.000 mol acid) is the factor to use times 0.2512 mol TRIS and 1 mol TRIS acid (b and a) to reach a 250 ml solution of 0.500 M TRIS buffer.

0.1250 mol=0.2512· 0.1219 mol TRIS base remaining + 0.1219 mol TRIS acid

Total TRIS used should be **0.1250 mol** while strong acid added should be 0.1219 mol / (6 / 1000 ml), or 20.3 ml 6M HCl.

Interpretation:

Extra figures were retained in calculating the hydrochloric acid needed to adjust the TRIS buffer solution to target pH, even though this assumes greater precision of the ~6M HCl solution in fact used. A serious error was made while preparing this buffer in that the moles remaining base (0.025 mol) were used to calculate the dry weight of TRIS measured, instead of the total moles TRIS (0.125 mol). Therefore, the measured pH of the resulting buffer solution was far below the target, at 0.86, using 16.6 ml HCl and ~0.0251 mol, or 3.04g TRIS (121.14g/mol). Isolating the problem to the an insufficient amount of base used is possible by finding a close match between the pH expected for strong acid remaining and the measured pH:

$$pH = - \log [(0.0999 - 0.0251) \text{ mol} / 0.25 \text{ L}] = pH\ 0.52$$

This figure is within the same unit pH experimentally measured, 0.57. In turn, this is experimental evidence that a more accurate acid strength of the particular preparation of 6M hydrochloric acid is slightly less than 6.0 M. This consideration, of course, is minor relative to the failure to use enough TRIS base to give the full target molarity of the desired buffer. Considering only pH independent of molarity, pH 7.5 could have been reached by adding the missing base, exactly 0.125 - 0.025, or 0.100 moles TRIS to the solution already prepared.

The phosphate buffer prepared matched the predictions of the Henderson-Hasselbalch model, and presumably had buffering capacity of about 0.5M. This could be tested in a titration against either strong acid or base. The buffering capacity of a 7.5 pH monobasic-dibasic phosphate system must be effectively greater when titrated against a strong acid rather than a base, because the concentration of base is five times greater than the concentration of conjugate acid with which it remains nonetheless in equilibrium. Thus, it becomes apparent that the Henderson-Hasselbalch model is based on the fact that the pKa of any given weak acid or base tells us the center of the effective buffer range of any given buffer system involving the conjugate, and therefore, pH=pKa when the ratio of that base or acid to the corresponding conjugate is 1. More simply put, pKa is the basis of these buffer system calculations because when it has been measured accurately, it indicates the theoretical and actual inflection point as the center of effective buffer range for any buffer system in dynamic equilibrium with factors that would otherwise influence pH.