Preparation of a Buffered Solution

A buffer solution is an aqueous solution that resists changes in *p*H[1] upon the addition of small amouts of acid or base.

The Hendersen-Hasselbalch equation,

$$pH = pK + \log(\text{base/acid}),$$

is used to calculate the **ratio** of the base concentration to the acid concentration for a desired *p*H. The absolute concentrations of the base and acid are unimportant, assuming a constant *p*K.

Example

Tris(base) is supplied as a 2 M "stock" solution. The desired "working" concentration of tris is 100 mM at *p*H 7.8. Tris has a *p*K of 8.1. The *p*H will be adjusted with 1 N HCl. To prepare 25 ml of extraction cocktail:

Step 1: Calculate and make the required dilution from the stock.

$$\frac{2\text{ M } \textit{(stock)}}{100\text{ mM } \textit{(working)}} = \frac{2000\text{ mM}}{100\text{ mM}} \quad (= 20\text{x dilution})$$

Thus, to a 25-ml mixing cylinder, add some H_2O, then add 1.25 ml of 2 M stock Tris(base) (1.25 ml = 1/20 x 25 ml). Mix.

Step 2: Calculate the required ratio of base to acid, using the Hendersen-Hasselbalch equation.

$$7.8 = 8.1 + \log(\text{base/acid})$$

$$\log(\text{base/acid}) = -0.3$$

$$\log(\text{acid/base}) = 0.3$$

$$\text{acid/base} = 2$$

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[1] $pH = -\log_{10}[H^+]$. As a refresher, $\log_b N = L$ and $b^L = N$, e.g., $\log_{10} 100 = 2$ and $10^2 = 100$.

As the *p*H will be adjusted with HCl,

$$\frac{\text{tris-Cl}}{\text{tris(base)}} = 2$$

For a total tris concentration of 100 mM,

$$\frac{[2\ \textit{parts tris-Cl}\,]}{[2\ \textit{part tris-Cl}] + [1\ \textit{part tris (base)}]} \times 100\text{ mM} = 2/3 \times 100\text{ mM} = 67\text{ mM tris-Cl}$$

Similarly,

$$\frac{[1\ \textit{part tris(base)}]}{[2\ \textit{part tris-Cl}] + [1\ \textit{part tris(base)}]} \times 100\text{ mM} = 1/3 \times 100\text{ mM} = 33\text{ mM tris (base)}$$

Step 3: Calculate and make the dilution required to achieve the desired tris-Cl concentration

$$\frac{1\text{ N}(\textit{stock } H^+)}{67\text{ mM } (\textit{working})} = \frac{1000\text{ mM}}{67\text{ mM}} \quad (= 15\text{x dilution})$$

Thus, to the mixing cylinder containing the tris(base), add 1.67 ml HCl (1.67 ml = 1/15 x 25 ml).

Step 4: To the mixing cylinder, add some water and mix. (Do not exceed ca. 20 mL total volume.)

Step 5: Add calculated aliquots of other stock reagents ($MgCl_2$, EDTA).

Although mixing between additions of these particular reagents is not necessary, it is "good lab practice," as it can be important in other cocktails.

Step 6: Bring the final volume to 25 ml with water and mix.

Step 7: Remove a small aliquot and check the p*H by electrode.*

Typically, the *p*H estimate obtained on a *p*H meter for Tris buffer is 0.2 units lower than the actual *p*H.

Exercises

(a) Complete Table 2.1 (on the following page).

(b) Which is the weakest buffer/*p*H combination for buffering against acid addition or formation?

Answer: Buffer X, 50 mM, *p*H 7

(c) Which is the weakest buffer/*p*H combination for buffering against base addition or formation?

Answer: Buffer Y, 100 mM, pH 8.5.

(d) At pH 8, why would you use Buffer X at 50 mM instead of Buffer Y at 100 mM? To answer this question, consider the effect of adding or forming 5 mM acid or base in each solution.

Answer:
Buffer X + 5 mM acid = 30 mM acid: 20 mM base; pH = 7.8
Buffer X + 5 mM base = 20 mM acid: 30 mM base; pH = 8.2
Buffer Y + 5 mM acid = 29 mM acid: 71 mM base; pH = 7.9
Buffer Y + 5 mM base = 19 mM acid: 81 mM base; pH = 8.1
Thus, the pH change in Buffer X is less than the pH change in Buffer Y

Acid:base ratios for different buffers at a range of pHs.

pH	7.0	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.5
Acid (A) or Base (B) in mM	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B
Buffer X, pK 8.0 100 mM										
Buffer X, pK 8.0 50 mM										
Buffer Y, pK 7.5 100 mM										