Physical Biochemistry Lab

Experiment no. 2:

Preparation of buffer solutions

Objective:

To study the nature of the buffers.

Introduction and principle:

Adding a 0.01 ml droplet of 1M HCl to 1L of pure water changes the pH of the water from 7 to 5, which represents a 100 fold increase in [H\(^+\)]. Such a huge change in pH would be intolerable to most biological systems, since even small changes in pH can dramatically affect the structures and functions of biological molecules. Regulation of the pH of the body fluids and tissues within limits consistent with life and normal function is provided by buffers. Buffers are defined as solutions that resist changes in the pH of a system upon addition of limited amounts of either acid or alkali. Buffers mixtures contain two substances, a conjugate acid and conjugate base. An "acidic" buffer contains a weak acid and its salt of a strong base. A "basic" buffer contains a weak base and its salt of strong acid. The action of buffers and their role in maintaining the pH of a solution are explained with the aid of the Henderson–Hasselbalch equation.

\[
pH = pK_a + \log \frac{[\text{salt}]}{[\text{acid}]}
\]

Together the two species (conjugate acid plus conjugate base) resist large changes in pH by partially absorbing addition of H\(^+\) or OH\(^-\) ions to the system. If H\(^+\) ions are added to the buffered solution, they react partially with the conjugate base present to form the conjugate acid. Thus, the H\(^+\) ions are taken out of circulation. If OH\(^-\) ions are added to the buffered solution, they react partially with the conjugate acid present to form water and conjugate base. Thus, the OH\(^-\) ions are taken out of circulation.

Buffered solutions do change in pH upon the addition of H\(^+\) or OH\(^-\) ions. However, the change is much less than that which would occur if no buffer were present. The amount of change depends on the strength of the buffer and the conjugate base / conjugate acid ratio. The ability of a buffer to resist changes in pH is referred to as the "buffer capacity". Buffer capacity can be defined as the number of moles of H\(^+\) or OH\(^-\) that must be added to one liter of the buffer in order to change the pH by one unit. The buffering capacity of the buffer solution is maximal when \(pH = pK_a\), when the concentrations of conjugate acid and conjugate base are equal. In general, buffers should not be used at a pH greater or lower than 1 unit from their pKa.
In the laboratory, many biochemical reactions including those catalyzed by enzymes require pH control which is provided by buffers. The desired pH of the buffered solution determines which buffering compound is selected.

There are three practical methods to prepare a buffer:

I. If there is only one of the buffer components available, the buffer preparation is done by titration method.
   a) In case of availability of weak acid or weak base only. An acidic buffer is made by titrating the weak acid by strong base, and a basic buffer is made by titrating the weak base by strong acid until the desired pH is obtained.
   b) In case of availability of the salt of weak acid or base only. An acidic buffer is made by titrating the salt of weak acid by strong acid, and a basic buffer is made by titrating the salt of weak base by strong base until the desired pH is obtained.
   Note: In this method, it can be calculating mathematically the volume of titrant required to prepare the desired buffer.

II. If both forms are available, using the buffer pKa, calculate the amounts (in moles) of acid/salt or base/salt present in the buffer at the desired pH. In calculation, convert the amount required from moles to grams using the molecular weight of that component, and then weight out the correct amounts. Or convert moles to volume if the stock is available in the liquid form.

III. Find a table of the correct amounts of acid/salt or base/salt required for different pH's. Dissolve the components in slightly less water than is required for the final solution volume. Check that the pH and correct it if necessary. Add water to the final volume.

Materials and apparatus:

1. Acetic acid (0.1M)
2. Sodium acetate solid (m.wt. = 82.03 g/mole)
3. Hydrochloric acid (0.1M)
4. Sodium hydroxide (0.1M)
5. Distilled water
6. Burettes
7. Beakers
8. Glass rode
9. Measuring cylinder (50ml)
10. Pipette (10ml)
11. Pipette bulb
12. Pasteur pipette  
13. Balance  
14. pH meter

Methods:

1) You have provided with 0.1 M acetic acid and solid sodium acetate with molecular weight of 82.03 g/mole. Prepare 50 ml of the buffer solution by mixing the two components. Use the following information to calculate the composition of the buffer.
   - pH of the buffer = 4.86
   - pKa of acetic acid at 25 °C = 4.76
   - Concentration of the buffer = 0.1 M
   - To check the buffering capacity of your buffer, add 1 ml of 0.1 M HCl to the buffer and find out the pH of the mixture.
   - Take out 50 ml distilled water, find out its pH value, add to it 1 ml of 0.1 M HCl and measure the pH.
   - Compare between the change in pH of the buffer after adding HCl and the change in pH of the distilled water after adding HCl.
   - Record your result in the following table.

<table>
<thead>
<tr>
<th>Solution</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td></td>
</tr>
<tr>
<td>Buffer + 1 ml of 0.1 M HCl</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>Distilled water + 1 ml of 0.1 M HCl</td>
<td></td>
</tr>
</tbody>
</table>

2) Pipette 10 ml of acetic acid solution (0.1 M) into a 100 ml beaker. Standardize the pH meter and determine the pH of the solution. Titrate the acetic acid solution with 0.1 M NaOH to pH 9.5 . During titration, add 0.5 ml of sodium hydroxide aliquot each time and read the pH. Plot a titration curve for acetic acid and determine the pKa value.
   - pKa value can be obtained from titration data by the following methods:
     a) The pH at the point of inflection is the pKa value and this may be read directly.
     b) By definition, the pKa value is equal to the pH at which the acid is half titrated. The pKa can therefore be obtained from a knowledge of the end point of the titration.
Titration result:

<table>
<thead>
<tr>
<th>Volume of 0.1 M NaOH added</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

From the curve, pKa value of acetic acid =
3) You have provided with 0.1 M acetic acid and 0.1 M sodium hydroxide solutions. Prepare an acetate buffer by titrate a 10 ml of the acetic acid with the sodium hydroxide until a volume of base added equal to 6.3 ml. Determine the pH of the acetic acid solution before starting. During titration, add 0.5 ml of sodium hydroxide aliquot each time and read the pH. Plot a titration curve.

- To check the buffering capacity of your buffer, add 1 ml of 0.1 M HCl to the buffer and find out the pH of the mixture.
- Record your result in the following tables.

Titration result:

<table>
<thead>
<tr>
<th>Volume of 0.1 M NaOH added</th>
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</table>
A student needed to prepare an acetate buffer of $pH = 5.0$, $0.08$ M, $volume = 50 ml$ from a $0.1$ M acetic acid solution by titration with $0.4$ M NaOH solution. Do the proper calculations and describe the preparation of the required buffer?
Using of burette for titration:

• Burettes are used primarily for titration, to deliver one reactant until the precise end point of the reaction is reached.

• To fill a burette, close the stopcock at the bottom and use a funnel, you may need to lift up on the funnel slightly, to allow the solution to flow in freely.

• Before titrating, condition the burette with titrant solution and check that the burette is flowing freely. To condition a piece of glassware, rinse it so that all surfaces are coated with solution, then drain. Conditioning two or three times will insure that the concentration of titrant is not changed by a stray drop of water.

• Check the tip of the burette for an air bubble. To remove an air bubble, whack the side of the burette tip while solution is flowing.

• If an air bubble is present during a titration, volume readings may be in error.

• Rinse the tip of the burette with water from a wash bottle and dry it carefully. After a minute, check for solution on the tip to see if your burette is leaking. The tip should be clean and dry before you take an initial volume reading.

• When your burette is conditioned and filled, with no air bubbles or leaks, take an initial volume reading. A burette reading card with a black rectangle can help you to take a more accurate reading. Read the bottom of the meniscus. Be sure your eye is at the level of meniscus, not above or below. Reading from an angle, rather than straight on, results in an error.

• Deliver solution to the titration flask by turning the stopcock. The solution should be delivered quickly until a couple of ml from the endpoint.

• The endpoint should be approached slowly, a drop at a time. Use a wash bottle to rinse the tip of the burette and the sides of the flask.