Expt. BL 302

Determination of pK\textsubscript{a} by pH Titration Method

Objective
To determine the pK\textsubscript{a} values and buffering capacity of buffer solutions.

Requirements
Buffer stock solution (concentrated), pH meter, distilled water, wash bottle, volumetric flasks, measuring cylinders, beakers, and pipettes.
Glycine-NaOH buffer system:
1. 20 mM glycine
2. 200 mM NaOH
Glycine-HCl buffer system
1. 20 mM glycine
2. 200 mM HCl

Theory
The observation that partially neutralized solutions of weak acids or weak bases are resistant to pH changes on addition of small amounts of strong acid or strong base leads to the concept of "buffering". Buffers consist of an acid and its conjugate base, such as carbonate and bicarbonate, or acetate and acetic acid. The quality of a buffer is dependent on its buffering capacity (resistance to change in pH by addition of strong acid or base) and ability to maintain a stable pH upon dilution or addition of neutral salts. Because of the following equilibria, addition of small amounts of strong acid and strong base result in removal of only small amounts of the weakly acidic or basic species, therefore there is little change in the pH:

\[
HA(\text{acid}) \leftrightarrow H^+ + A^- (\text{conjugate base})
\]

\[
B (\text{base}) + H^+ \leftrightarrow BH^+ (\text{conjugate acid})
\]
The pH of a solution of a weak acid or base may be calculated from the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[\text{basic species}]}{[\text{acidic species}]}$$  \hspace{1cm} (1)

The $pK_a$ of a buffer is that pH where the concentrations of basic and acidic species are equal, and this basic form of equation is accurate between the pH ranges of 3 to 11. Below pH 3 and above pH 11 the concentration of the ionic species of water must be included in the equation. Since the pH range of interest to the biochemical engineer is 3 - 11 ranges, this can be ignored. From the Henderson-Hasselbalch equation an expression for buffer capacity ($\beta = d[A^-]/d[pH]$) may be deduced.

**Procedure**

1. **pH Measurement:** Mix all solutions thoroughly. The pH measurement may be made in original beakers. Do not change any control on the pH meter except as directed. With the meter on stand by, rinse the electrode with deionized water, gently shake off the excess water, and immerse the electrode in the sample solution. Switch the meter to the pH mode, allow the reading to stabilize, and record the pH. Switch the meter back to the standby mode, rinse the electrode again, and leave the electrode immersed in deionized water. Repeat this procedure for all samples.

2. **Glycine-NaOH buffer system:** Prepare 50 ml 20 mM glycine solution and 100 ml of 200mM NaOH solution. Calibrate the pH meter with standard buffer solution at room temperature. Take 50 ml of glycine solution in a beaker and add 0.5 ml of NaOH solution and shake well to mix. Note the change in pH. Add subsequent quantities of NaOH with an increment of 0.5 ml each time and note observed pH at regular intervals. Take about 30-35 readings and generate the following observation table:

<table>
<thead>
<tr>
<th>Volume of NaOH added (ml)</th>
<th>Observed pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results and Discussion**

1. Plot the titration curve for the given buffer system.

2. Estimate the $pK_a$ for the different ionic species.

3. Derive Equation 1. Derive an equation for the buffering capacity in terms of $K_a$ and [H$^+$].

4. When is $\beta$ maximum and what is its value at that condition?
5. List a few biological buffers and their range of operation. How do biological systems (cells tissues etc.) maintain pH?

Further Reading

1. Read up the theory on buffering of blood.
2. How does the pH meter work?