Exp.2 Determination of isoelectric point

Introduction：

All amino acids contain at least one amino and one carboxyl group, are classified as amphoteric substances (act as either an acid or as a base).

If both positive and negative charges present in the molecules, are called **zwitterions**. The ionic form of the amino acid can become more positively or negatively(fig 2.1) in an aqueous solution depends on the pH of the solution. **Isoelectric point** (pI) is at the pH making zwitterions with the net charges of zero.

For an amino acid with only one amine and one carboxyl group, the pI can be calculated from the pK_{a1} and pK_{a2} of this molecule.

$$\mathbf{pI = (pK_{a1} + pK_{a2})\div 2}$$

where K_{a1} and K_{a2} are the dissociation constants of the carboxyl and amino groups, respectively.

For amino acids with more than two ionizable groups, the same formula is used, but the two pK_a's used are those of the two groups that lose and gain a charge from the neutral form of the amino acid.

This experiment will determine the pK_a value for each dissociable group of an amino acid by extrapolating the midpoint of each buffering region in the titration curve.

Reagents:

1. 0.1M solutions of amino acids
2. 0.5M NaOH
3. 6M HCl

Procedure：

Pipet 40ml of amino acid solution into a clean 100ml beaker, adding 6M HCl slowly to the solution by mixing until pH to ~2 and recording the pH value before you begin titrating, then titrate with 0.5M NaOH until the pH to ~11. Record the pH and volume.

Results：

1. Plot of pH versus added volume of NaOH.
2. Mark pK_{a1} and pK_{a2}=?.
3. Calculate pI value and error(%).
4. Record your results.

Discussions：