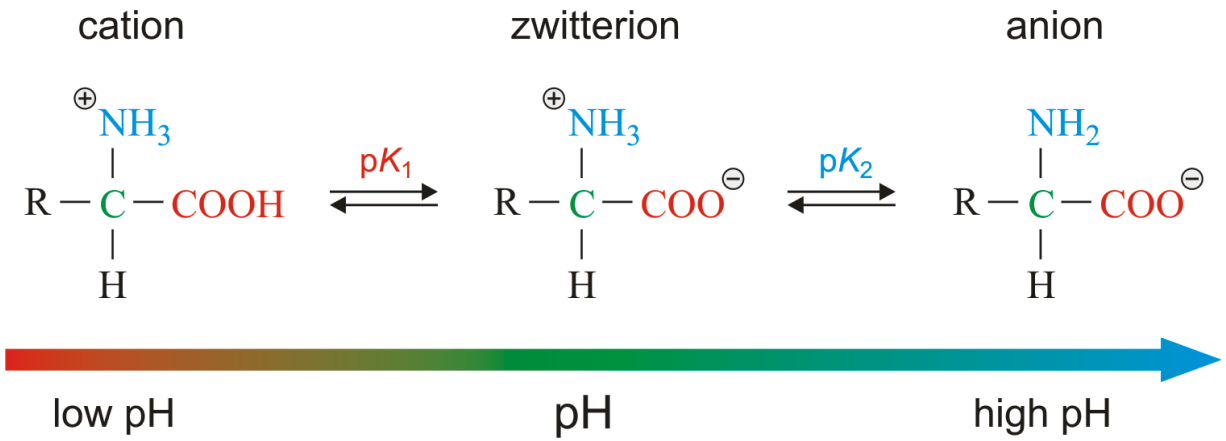
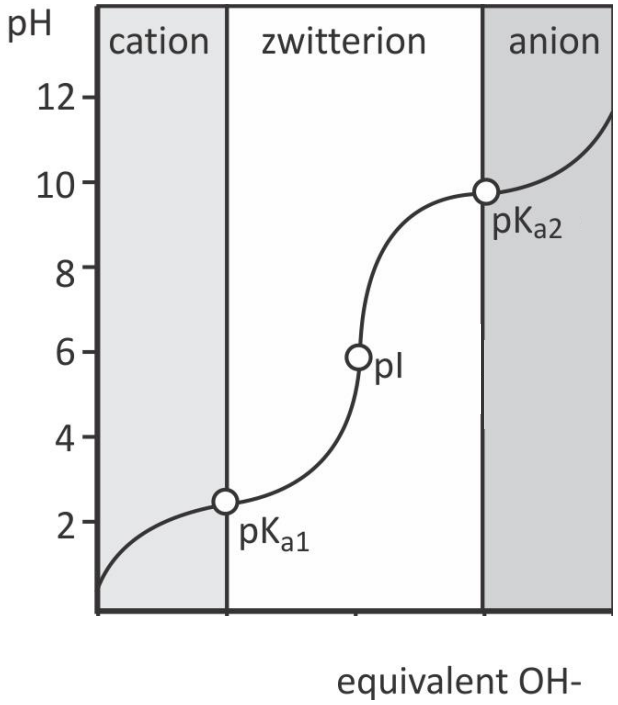
EXPERIMENT 1

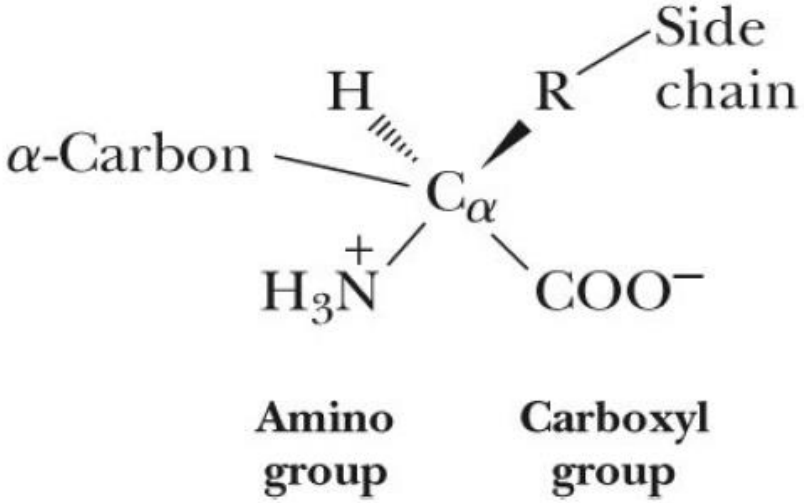
DETERMINATION OF ISOELECTRIC POINT OF AN AMINO ACID





Amino Acids

A compound that contains an amino group, a carboxyl group and a side-chain that is specific to each amino acid.

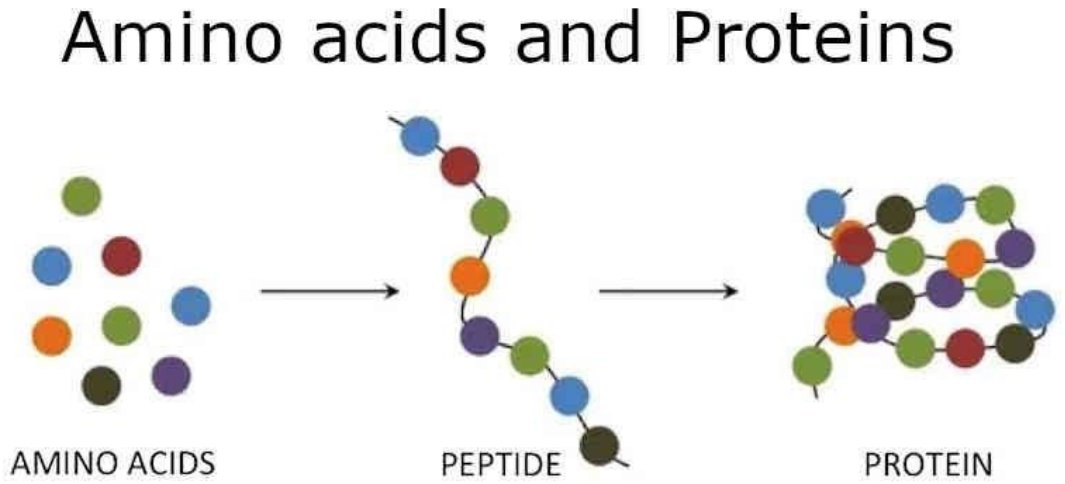


α-Amino acid:

An amino acid in which the amino group is on the carbon adjacent to the carboxyl group.

Amino Acids

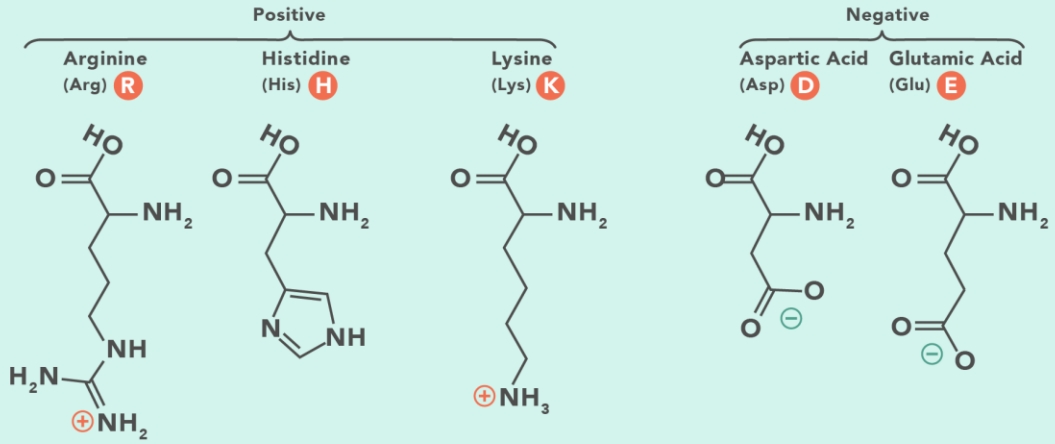
- The building blocks of proteins



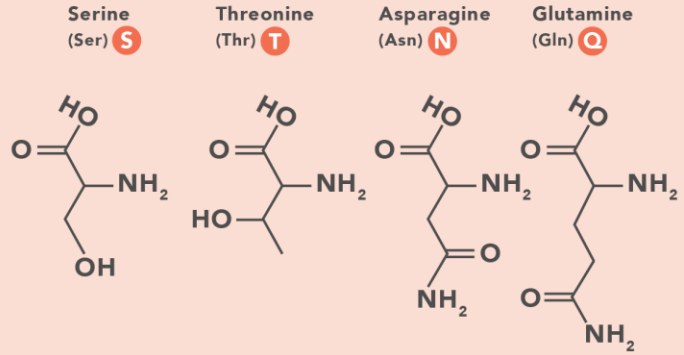
- **20 amino acids** are naturally incorporated into polypeptides and are called proteinogenic or standard amino acids. These 20 are encoded by universal genetic code.

- 10 standard amino acids (Lys, Met, His, Leu, Ile, Thr, , Tyr, Phe, Val & Arg) are called "essential" for humans because they cannot be created from other compounds by the human body, and so must be taken in as food.

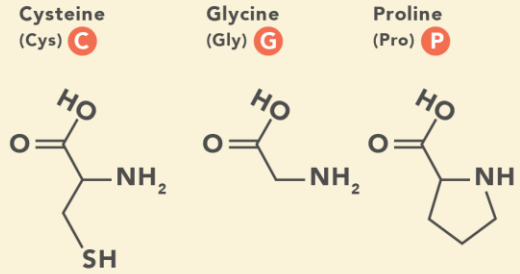
A. Amino Acids with Electrically Charged Side Chains



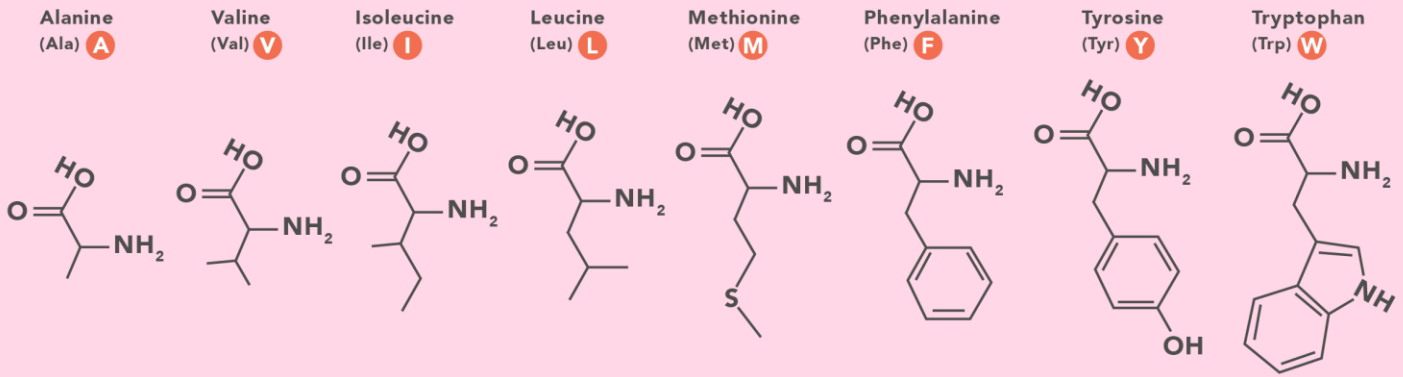
B. Amino Acids with Polar Uncharged Side Chains



C. Special Cases



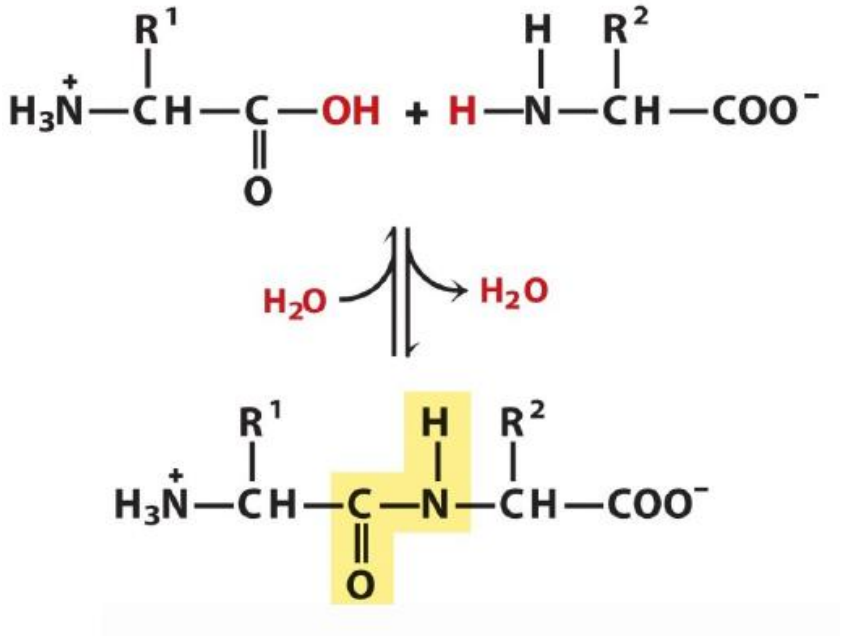
D. Amino Acids with Hydrophobic Side Chains



Amino Acids

All amino acids (except glycine) are optically active (chiral).

Two amino acids can react with loss of a water molecule to form a covalent bond.



Amide linkage is planar NH and CO are anti.

Classification of Amino Acids

Classification based on side-chain structure:

- Non-polar amino acids.
- Polar, uncharged amino acids.
- Acidic amino acids.
- Basic amino acids.

Other side chain structural classifications:

- Aromatic, cyclic, hydroxyl, and thiol amino acids.

Nonpolar side chains (predominant form at pH 7.0

R =

glycine (gly, G)

H-

alanine (ala, A)

CH_3-

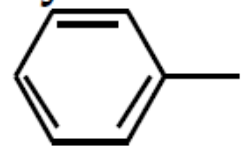
valine (val, V)

$(CH_3)_2CH$—

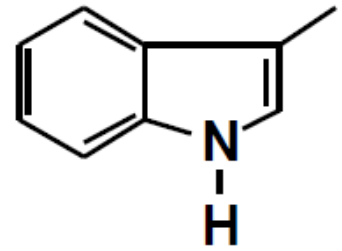
leucine (leu, L)

$(CH_3)_2CHCH_2$-

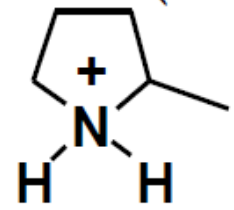
phenylalanine (phe, F



tryptophan (trp, W)



proline (Pro, P)



isoleucine (ile, I)

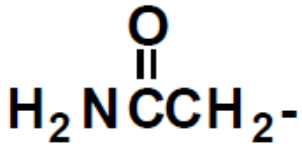
$CH_3CH_2CH(CH_3)$-

methionine (met, M)

$CH_3SCH_2CH_2$-

Polar side chains (predominant form at pH 7.0)

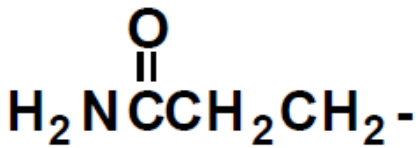
asparagine (asn, N)



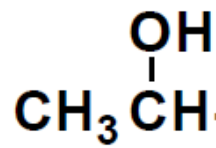
serine (ser, S)

$HOCH_2$-

glutamine (glu, G)

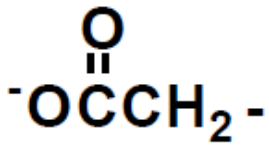


threonine (thr, T)

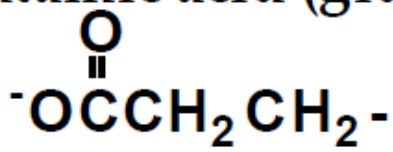


Acidic side chains (predominant form at pH 7.0)

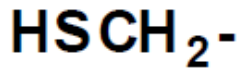
aspartic acid (asp, D)



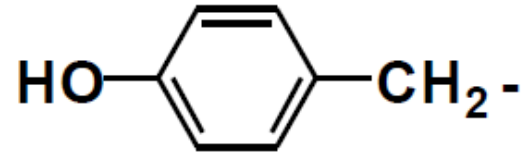
glutamic acid (glu, E)



cysteine (cys, C)

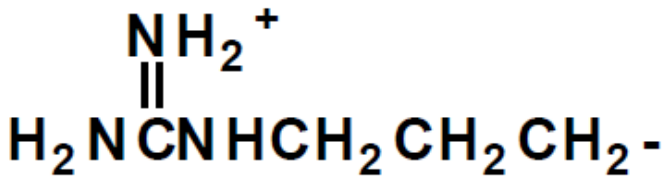


tyrosine (tyr, Y)

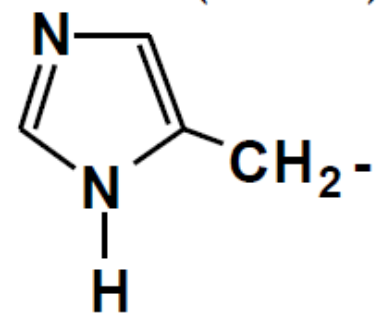


Basic side chains (predominant form at pH 7.0)

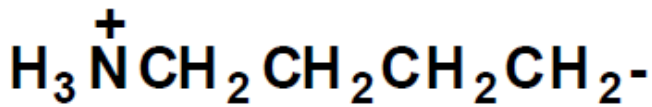
arginine (arg, R)



histidine (his, H)



lysine (lys, K)



Zwitterions

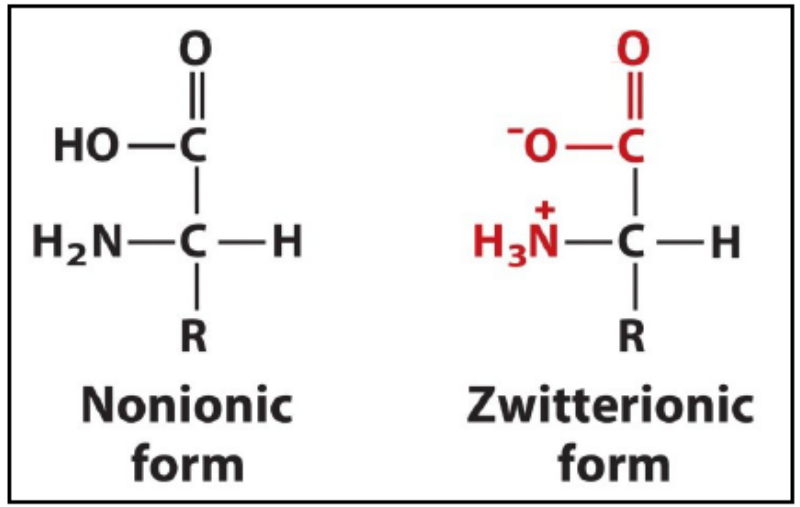
- Although α-amino acids are commonly written in the unionized form, they are more properly written in the zwitterion (internal salt) form (Germ. Zwitter means hybrid)
- Both the $-NH_2$ and the –COOH groups in an amino acid undergo ionization in water.
- At physiological pH (7.4), a zwitterion forms

– Both + and – charges

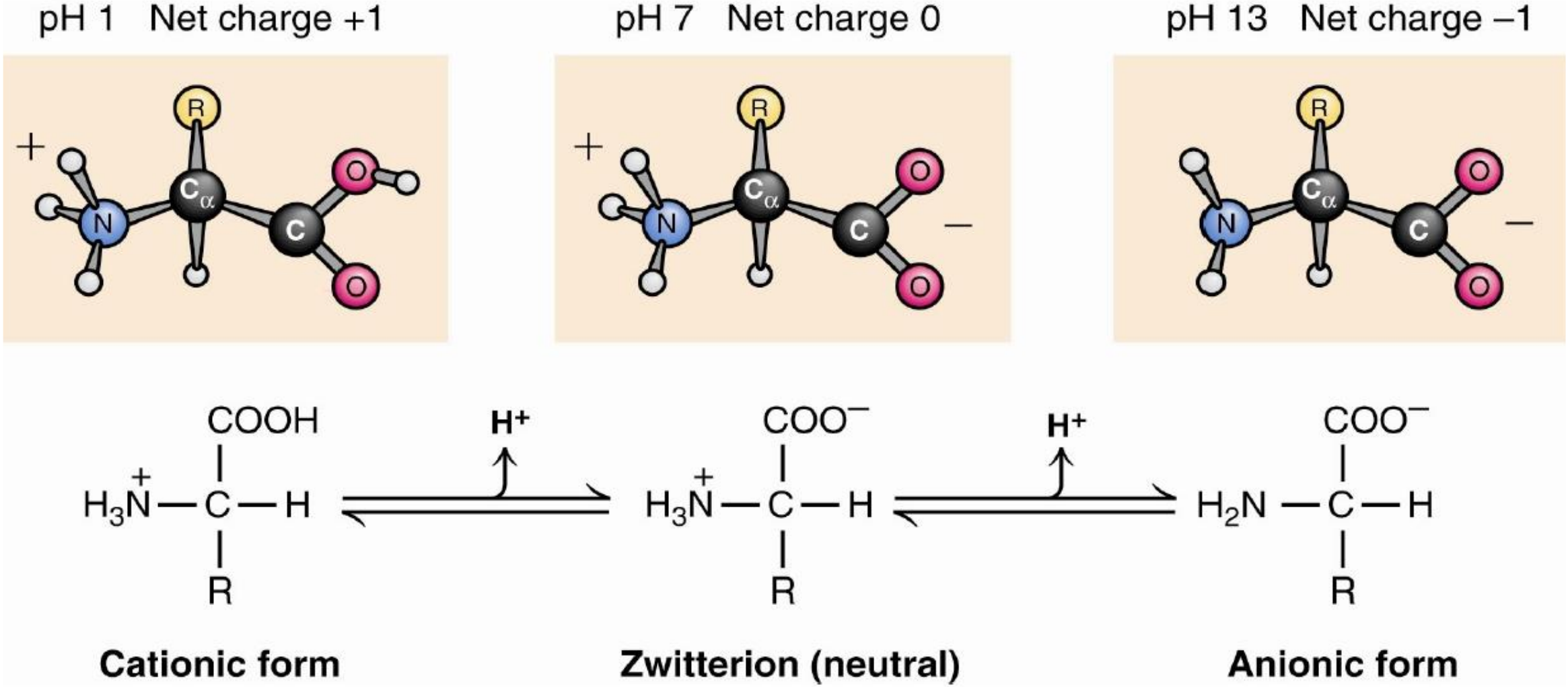
– Overall neutral

– Amphoteric

  - Amino group is protonated
  - Carboxyl group is deprotonated
  - **Soluble** in polar solvents due to ionic character



Acid-Base Properties of Amino Acids



Isoelectric Point (pI) of an Amino Acid

• The **isoelectronic point** or **isoionic point** is the pH at which the amino acid does not migrate in an electric field.

• This means it is the pH at which the amino acid is neutral, *i.e.* the zwitterion form is dominant.

The equations that define acidity and basicity are:

$$K_a = \frac{[H^+][A^-]}{[HA]} \qquad pK_a = -\log_{10} K_a$$

The lower the pK_a, the stronger the acid.

From these expressions it is possible to derive the important **Henderson-Hasselbalch equation** :

$$pK_a = pH + \log [HA] / [A^-]$$

$$pK_a = pH + \log [HA] / [A^-]$$

- It tells us that when the pH = pK_a then log [HA] / [A^-] = 0 therefore [HA] = [A^-], *i.e.* ***equal amounts of the two forms, the acid and the conjugate base****.*

- If we make the solution more acidic, *i.e.* lower the pH, so pH < pK_a, then log [HA] / [A^-] has to be > 0 so [HA] > [A^-].
  A stronger acid will cause the formation of HA, the protonated form**.**

- If instead we make the solution more basic, *i.e.* raise the pH, so pH > pK_a and log [HA] / [A^-] has to be < 0 so [HA] < [A^-].
  A stronger base will cause the formation of A^- , the deprotonated form**.**

There are 3 cases to consider

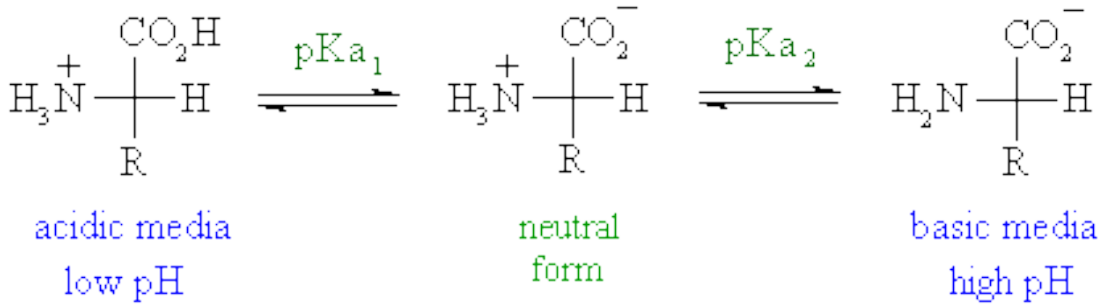
- **neutral side chains**

These amino acids are characterised by two pK_as : pK_a1 and pK_a2 for the carboxylic acid and the amine respectively.

The isoelectronic point will be halfway between, or the average of, these two pK_as,
i.e., **$pI = 1/2 (pK_{a1} + pK_{a2})$**.

At very acidic pH (below pK_a1) the amino acid will have an overall +ve charge and at very basic pH (above pK_a2) the amino acid will have an overall -ve charge.

For the simplest amino acid, glycine, pK_{a1}= 2.34 and pK_{a2} = 9.6, pI = 5.97.

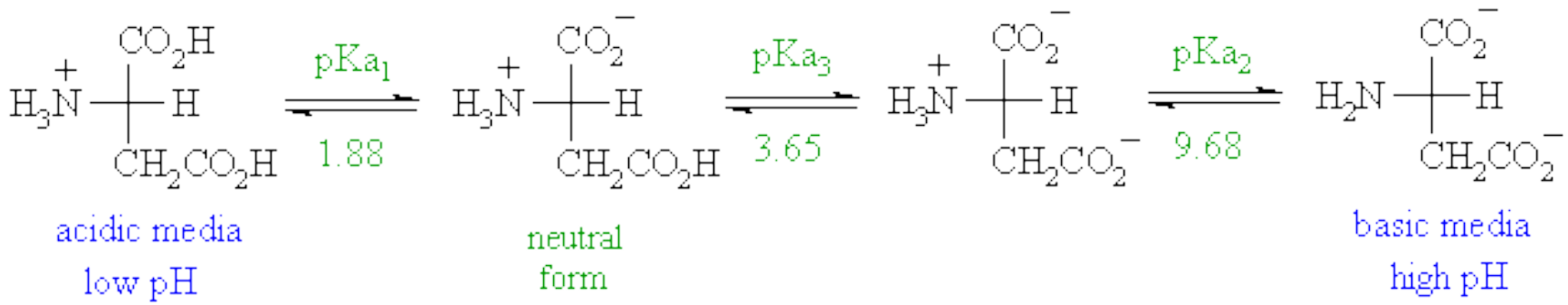


• **acidic side chains**

The pI will be at a lower pH because the acidic side chain introduces an "extra" negative charge.

So the neutral form exists under more **acidic** conditions when the extra -ve has been neutralised.

For example, for aspartic acid shown below, the neutral form is dominant between pH 1.88 and 3.65, pI is halfway between these two values, *i.e.* **pI = 1/2 (pK_{a1} + pK_{a3})**, so pI = 2.77.



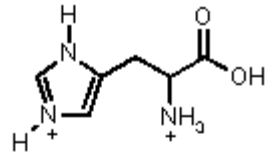
• **basic side chains**

The pI will be at a higher pH because the basic side chain introduces an "extra" positive charge.

So the neutral form exists under more **basic** conditions when the extra +ve has been neutralised.

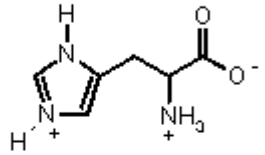
For example, for histidine, the neutral form is dominant between pH 6.00 and 9.17, pI is halfway between these two values, *i.e.* **pI = 1/2 (pK_{a2} + pK_{a3})**, so pI = 7.59.

A



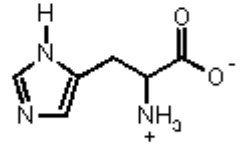
1.82 pK_{a1}

B



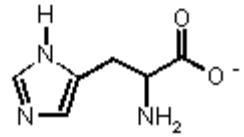
6.00 pK_{a3}

C



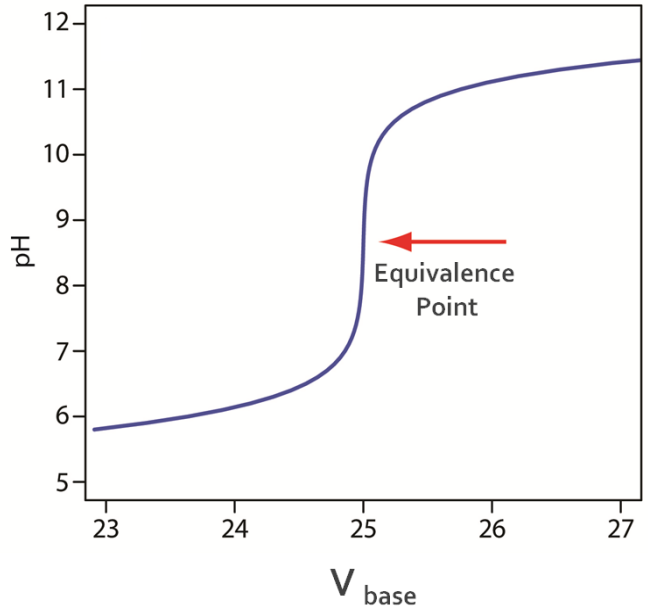
9.17 pK_{a2}

D



The Experiment to Determine Isoelectric Point

1. At the beginning, i.e., before base is added, the pH of the solution is fairly low because it contains acid.
2. As titration proceeds, acid is neutralized by the added base and pH rises.
3. Addition of base after all of the acid is neutralized produces a basic solution with a high pH.
4. The pH of the solution at each interval is monitored by a **pH meter**.
5. A plot of **pH versus the volume of titrant added to the solution** gives the "S" shaped titration curve.



Moles of acid in the original aliquot is calculated as :

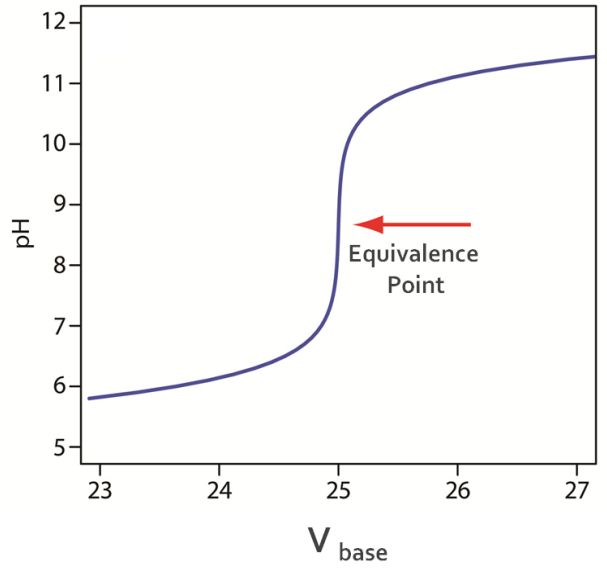
Moles of acid
= V_{base} at inflection point x M_{base}

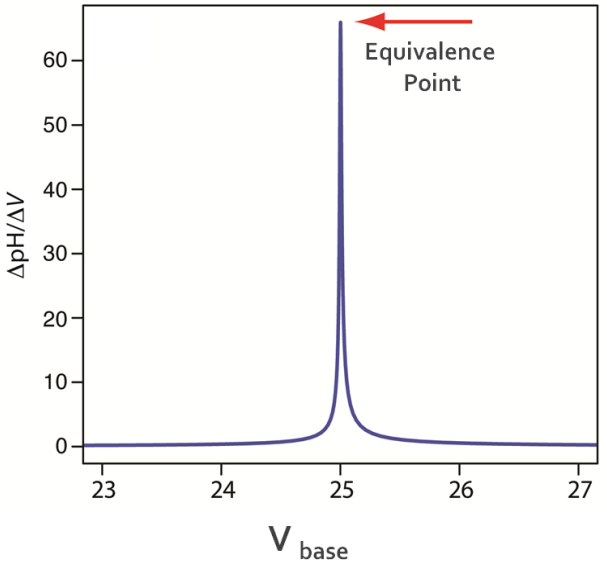
Successively add small volumes of base, measure pH after each addition, and plot the titration curve, from which we may find V_{base} at the inflection point (the equivalence point).

A **derivative plot** needs to be created to determine the pK_a values accurately.

The steps are as follows:

1. Calculate $\Delta pH/\Delta V$ from the collected pH data for each addition of the titrant.
2. Plot $\Delta pH/\Delta V$ against V (volume of titrant added).
3. The plot gives sharp peaks at the equivalence points corresponding to the sharp jumps in the titration plot.





APPARATUS: pH meter, beaker, burette, pipette, glass rod, spatula.

REAGENT AND MATERIALS: Potassium hydrogen phthalate, glycine, alanine, HCl, NaOH, phenolphthalein.

EXPERIMENTAL PROCEDURE:

i) Standardisation of NaOH solution

a) Prepare 100 mL 0.1 *M* KHP (Potassium hydrogen phthalate) solution.

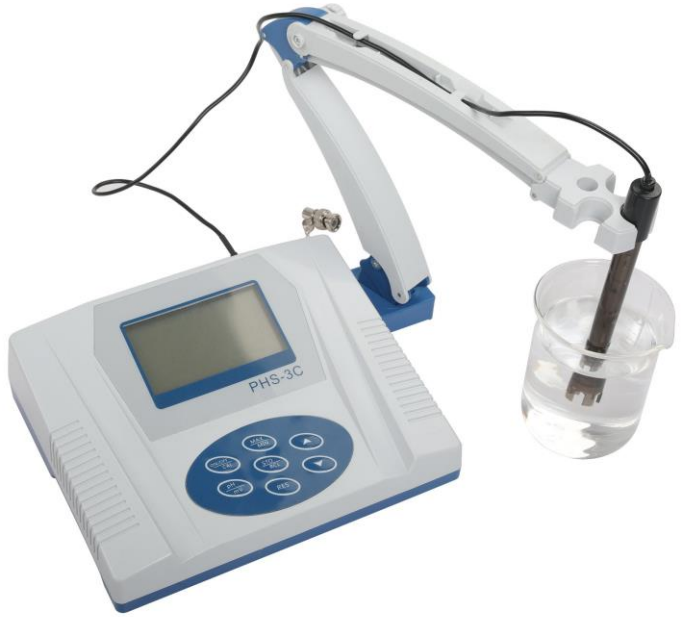
b) Standardize the supplied (~0.1 *M)* NaOH solution against KHP solution using phenolphthalein indicator (three results).

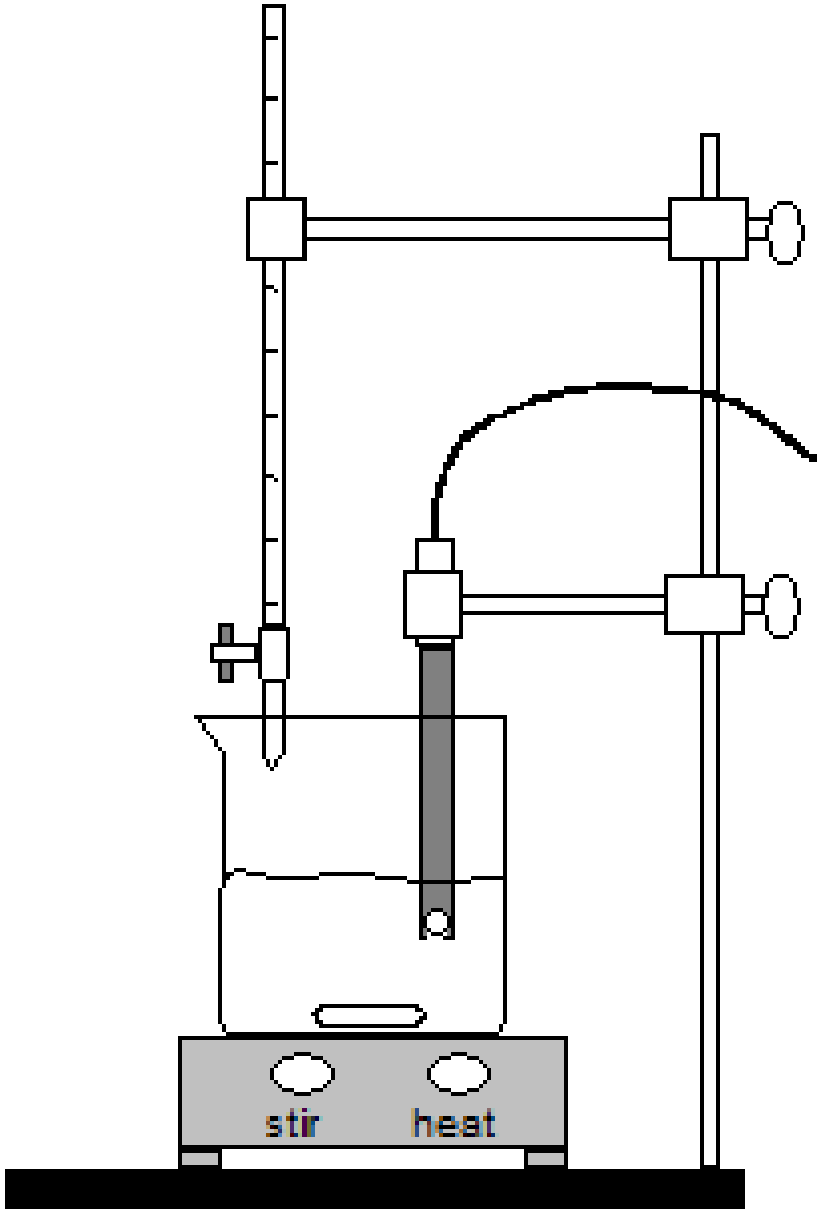
Table 1. Preparation of 100 mL standard 0.1 *N* KHP solution

Weight taken (g)	Weight to be taken (g)	Strength of KHP solution

Table 2. Standardization of NaOH solution using standard KHP solution

Sl. No.	Volume of KHP (mL)	Burette reading (mL)			Average volume (mL)	Strength of NaOH solution
		Initial	Final	Difference		


PHS-3C


stir
heat

i) Amino acid titration and estimation of equivalence point

a) Transfer exactly 10 mL of the supplied protonated amino acid solution to a clean 100 mL beaker.

b) Add 15 mL of distilled water to the beaker so that the total volume of the amino acid solution is 25 mL.

c) To homogenize the solution, place the beaker on the top plate of a magnetic stirrer and place a 1-inch stir bar in the beaker. Rinse the pH electrode and submerge it in the solution containing protonated amino acid. Make sure that the tip of the electrode is clear of the magnetic stir bar in the beaker before starting the stirrer. The rotation rate should be reasonably fast, but not so vigorous that splashing of the solution occurs.

d) Record the initial pH of the solution. Initiate the pH titration by adding 0.5 mL of NaOH solution from burette.

e) On each addition of base solution, note the pH of the solution. Continue this addition until you find larger gaps between two subsequent pH values. This indicates approach of the equivalence point. Reduce the volume of addition of the alkali solution to 0.1 mL until you comfortably cross the sudden jump in pH, indicating the equivalence point. After the equivalence point is passed, increase each volume of addition to 0.5 mL. Repeat this process if you expect more than one equivalence points.

f) Discard the solution on completion. Rinse the pH electrode with distilled water till pH meter reading is approximately equal to that of distilled water. Leave the pH electrode in beaker of distilled water and turn the meter off.

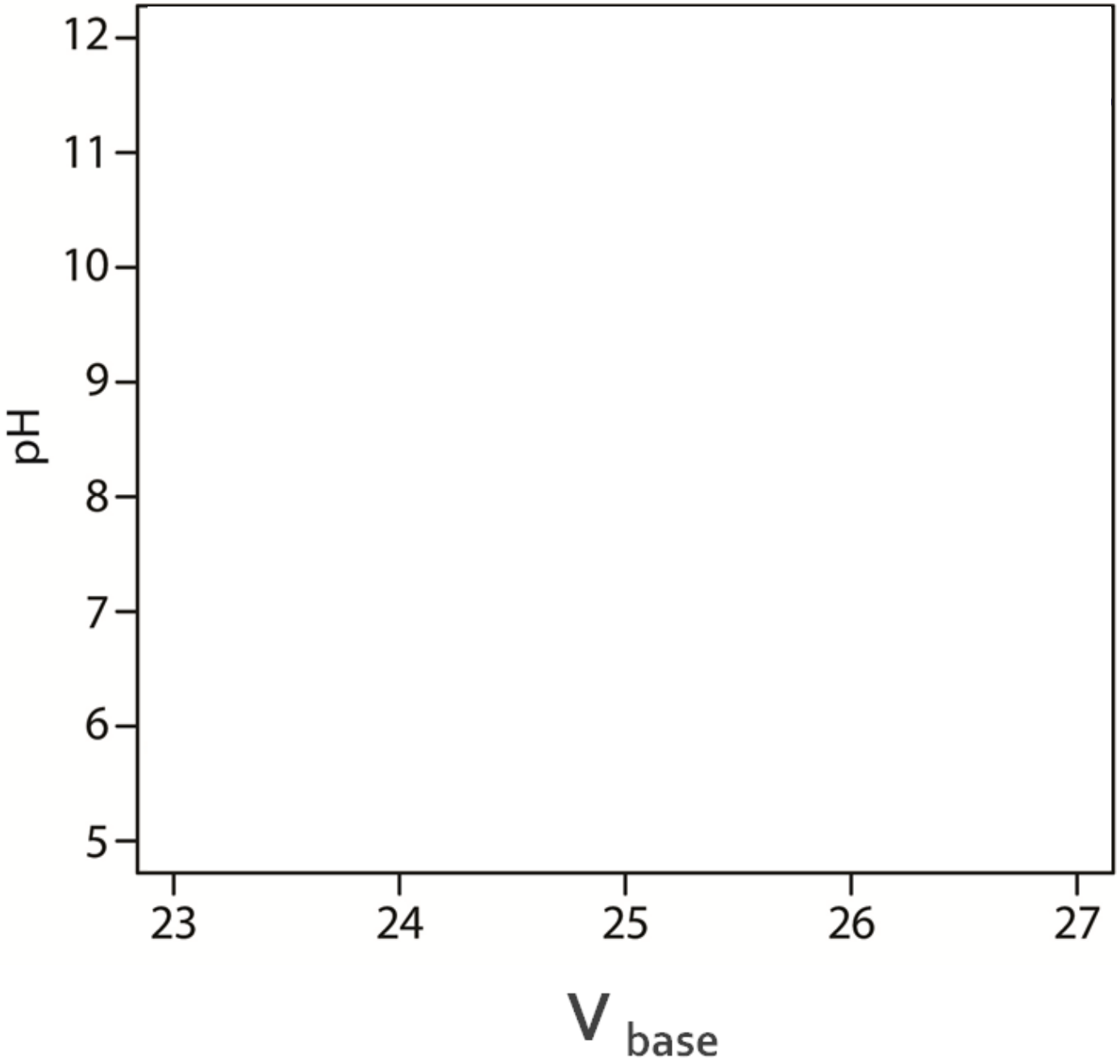
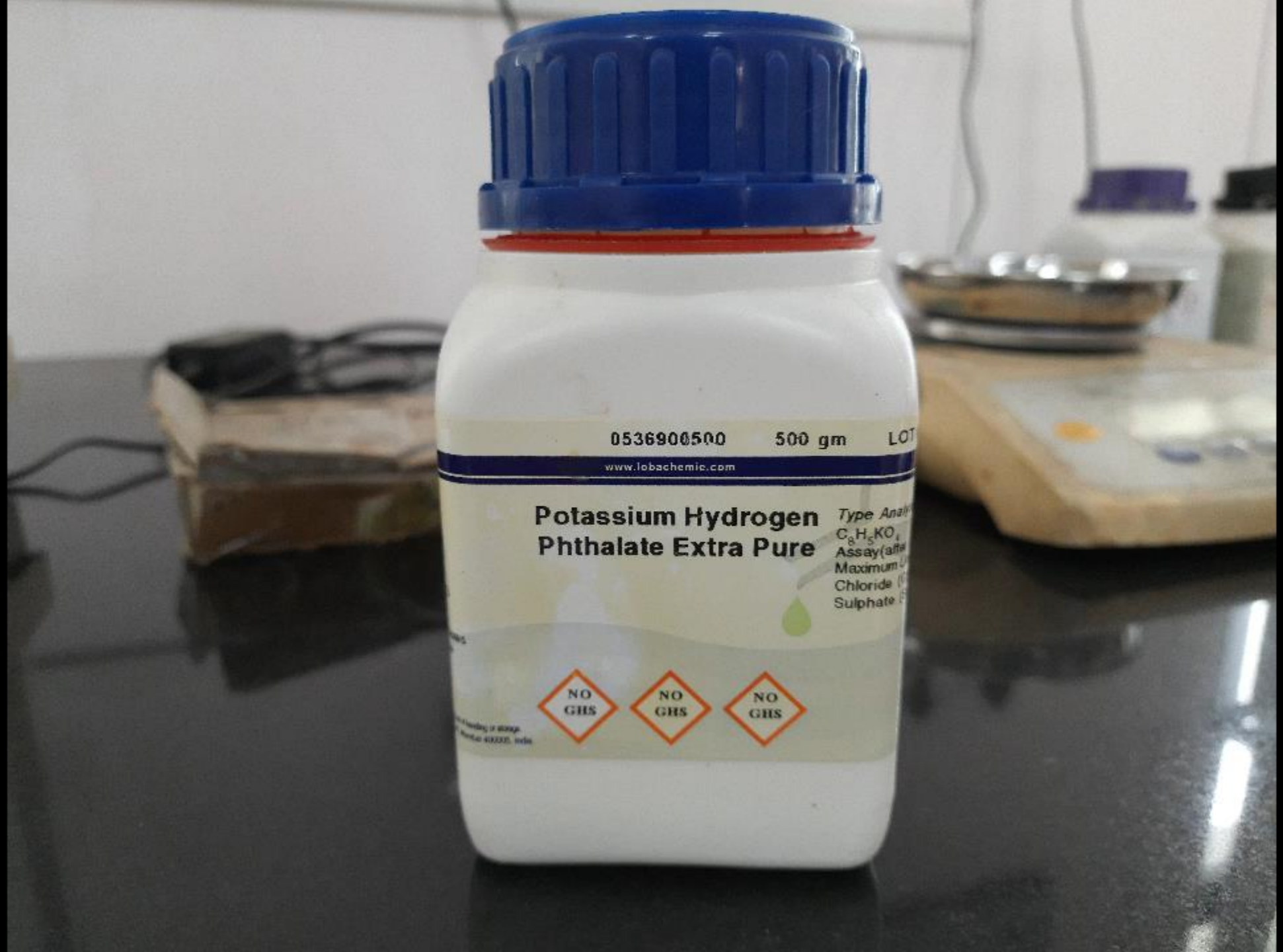

12
11
10
9
8
7
6
5
pH
23
24
25
26
27
V base

Table 3. Titration of amino acid solution using standard NaOH solution

Volume of amino acid (mL) =

Sl. No.	Volume of NaOH (mL)	pH	ΔV (mL)	ΔpH	ΔpH/ΔV

Results: Isoelectric point (pI) of the supplied amino acid = _____ at _____ ^{o}C.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www.lobachemie.com
Potassium Hydrogen Phthalate Extra Pure
NO GHS
NO GHS
NO GHS