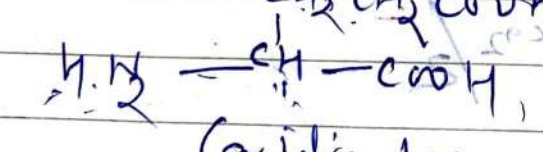
Seperation of Amino acids

Electrophoresis is a method used for the separation & analysis of amino acids. This method is based upon pH control & electric charge as amino acids differ in their isoelectric point.

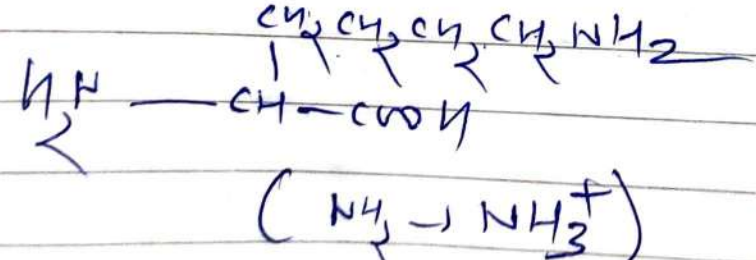
→ The mixture of amino acid is placed on the center of paper strip (Cellulose acetate) at certain pH. The pH is maintained by saturating the paper strip with buffer soln. The paper strip is attached to two electrodes. On passing electric current through the strip, amino acid migrate towards electrodes, depending upon the net charge on them

Eg: Mix of Lysine, alanine, glutamic acid
(basic, neutral, acidic amino)

at pH = 6.0, when placed on a paper strip, On passing current results in the movement of glutamic acid towards Anode (Positive electrode)



Lysine will move towards Cathode



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The alanine has no net charge & therefore doesnot move.

Generally :-

The amino acids with iso-electric point greater than buffer pH, gains a proton and becomes positively charged (lysine, PI - 9.7) & thus move towards cathode. On the other hand, the amino acids with isoelectric point lower than buffer pH, looses a proton & becomes negatively charged (Glutamic acid, PI - 3.2) & thus move towards anode.

The amino acids with isoelectric point comparable to buffer pH, doesnot migrate towards any electrode.

Alanine, PI - 6.0

chemical properties of Amino Acids

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Amino acids give reactions characteristics of both Carboxylic acids & Amines.

Reaction of the Carboxyl group

→ Esterification

α-Amino acids reacts with alcohols in the presence of HCl. The acid acts as catalyst & also reacts with amino group. The free ester can be obtained by the action of the aq. Na_2CO_3 or Moist Ag_2O or AgOH on the ester salt.

$$H_2N-CH_2-COOH + C_2H_5OH \xrightarrow{HCl} Cl^-H_3\overset{+}{N}-CH_2-COOC_2H_5 + H_2O$$

$$\xrightarrow{AgOH} (-AgCl, -H_2O)$$

$$H_2N-CH_2-COOC_2H_5$$

Ethyl α-amino acetate.

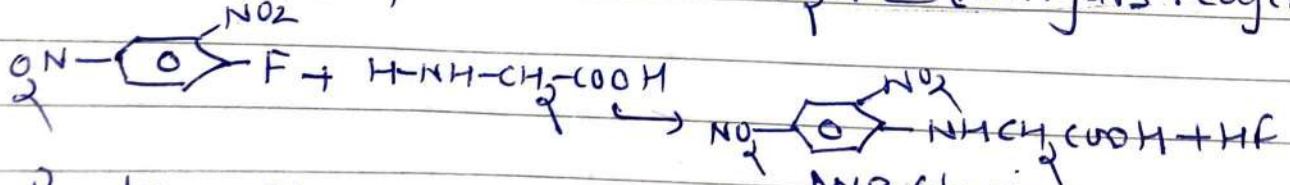
For benzyl esters

Benzyl esters are prepared using benzene Sulphonic acid as the catalyst. (Water, by product is removed using azeotropic distillation)

$$H_2N-CH_2-COOH + C_6H_5CH_2OH \xrightarrow{C_6H_5SO_3H} CH_2(NH_3^+)-COOCH_2-C_6H_5 + C_6H_5SO_3^- + H_2O$$

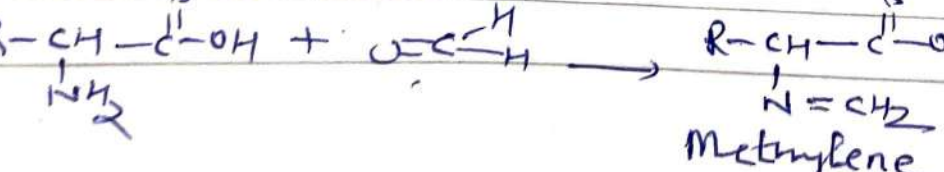
[The esters doesnot posseses the dipolar structure found in Unsubstituted amino acids & therefore more Volatile

→ Reaction with 2,4-Dinitrofluorobenzene (Sangar's reagent)



DNP Glycine

→ Reaction with HCHO



Methylene Amino acid.

Methylene amino acid is a strong acid and can be directly titrated with base, this method is Sorensen's formol Titration, used for estimation of A.A's

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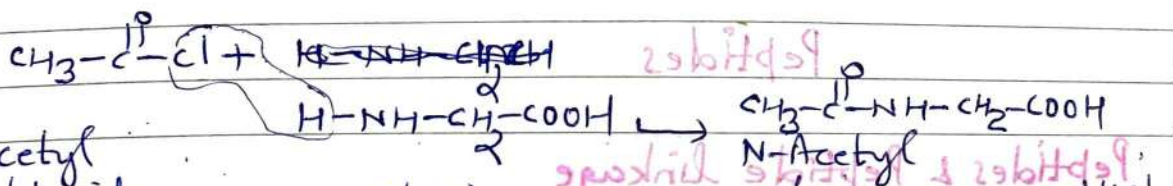
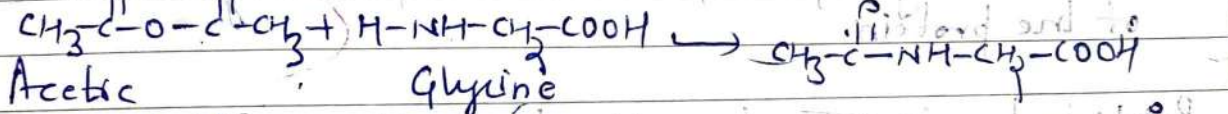
Reaction of $-NH_2$ group

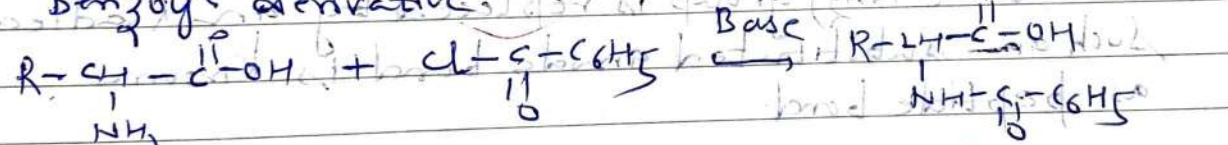
→ Acetylation of $-NH_2$ group

α-Amino acids reacts with acid chlorides or anhydrides to form N-acylamino acids





→ Benzoyl derivative



→ Reaction with metal ions (Cu^{2+})

Amino acids forms salts with metal ions. Heavy metal ions such as Cu^{2+}, Ag^{+}, Hg^{2+}, forms insoluble complexes involving the Amino acids

Eg. The copper salt of glycine which has deep blue colour, is formed by heating copper Oxide with an aq. soln of glycine. These metal ion also precipitates proteins

$CuSO_4(aq)$ + Glycine