

Experiment-4: Qualitative determination of Amino acids

Aim: To determine the presence of Proteins in the given sample qualitatively

Principle: Following tests are used to determine the amino acids qualitatively

a) Solubility test

Polar amino acids are more soluble in water than non-polar, due to presence of amino and carboxyl group which enables amino acids to accept and donate protons to aqueous solution.

b) Ninhydrin test for alpha L amino acids

Ninhydrin (tri ketohydrindene hydrate) degrades amino acids into aldehydes, ammonia and CO₂ (on pH range 4-8) through a series of reactions. The net result is ninhydrin in a partially reduced form from hydrindantin. Ninhydrin then condenses with ammonia and hydrindantin to produce an intensely blue or purple pigment, sometimes called Ruhemann's purple. The color varies slightly from acid to acid. Proline and hydroxy-proline (amino acids) give yellow color. Many substances other than amino acids, such as amines will yield a blue color with ninhydrin, particularly if reaction is carried out on filter paper.

c) Xanthoproteic test for Aromatic amino acids

Conc. HNO₃ reacts with the aromatic rings that are derivatives of benzene giving the characteristic nitration reaction. Amino acids tyrosine and tryptophan contain activated benzene rings which are easily nitrated to yellow colored compounds. The aromatic ring of phenyl alanine does not react with nitric acid despite it contains a benzene ring, but it is not activated, therefore it will not react.

d) Millon's test for amino acids containing hydroxy phenyl group

The phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury ion in the solution to form a brick-red solution or precipitate of nitrated tyrosine, in all cases, appearance of red color is positive test.

e) Sakaguchi Test

In alkaline solution, arginine reacts with alpha-naphthol and sodium hypobromite /chlorite as an oxidizing agent, to form red complexes as a positive result.

Materials and Reagents

Hydrochloric acid

Sodium hydroxide

Chloroform

Ninhydrin

Nitric acid

Phenol solution

10% NaOH

α-naphthol in 10% ethanol

5% sodium hypobromate (or sodium hypochlorite)

Test tubes

Water bath

Procedure

Solubility Test

Place 0.5 mL of amino acid sample in 4 test tubes clean, dry containing 4 mL of different solvents (chloroform and hot water), Shake the tubes thoroughly, and then leave the solution for about one minute, Record your result.

Ninhydrin Test

- i. Place 1 mL of each of the solutions in a test tube and add 2 drops of Ninhydrin solution.
- ii. Boil the mixture over a water bath for 2 min.
- iii. Allow to cool and observe the blue color formed
- iv. Complete the below table

Xanthoproteic Test

- i. Label five tubes (1 - 5), then add 0.5 mL of each amino acid solution and phenol solutions to those test tubes each alone.
- ii. Add a few drops of concentrated HNO₃.
- iii. Compare the color with that given by blank using water instead.
Now COOL THOROUGHLY under the tap and CAUTIOUSLY add 5 drops of 10M NaOH to make the solution strongly alkaline.

Millons Test

- i. Label 4 test tubes (1 - 5).
- ii. Add 1 mL of test solutions in separate tubes and the phenol solution in one tube.
- iii. Add to each tube 2 mL Millon's reagent and shake it well.
- iv. Place the test tubes in the boiling bath with care, for 10 min.
- v. Write your observation in the following table.

Sakuguchi Test

- i. Label 3 test tube and put in each one 1 mL of the aminoacid solution.
- ii. Add to each tube 2 mL of NaOH solution. Mix well.
- iii. Add to each tube 2 mL of α -naphthol solution. Mix well.
- iv. Add to each tube 3 drops of sodium hypobromite solution, and record your result.

Table 2.1: Qualitative reactions of Amino acids

S. No	Name of the Test	Observation

Result:

The amino acids in the given sample are qualitatively determined and results were tabulated in Table 2.1

Reference:

- 1) Sadasivam S and Balasubramanian T (1985). Practical Manual (Undergraduate), Tamil Nadu Agriculture University, Coimbatore, p.2.
- 2) Sadasivam S, Manickam A (2008). Biochemical Methods, New Age International Publishers, 3rd Edition, ISBN: 978-81-224-2140-8.