University of Ahel AL-Bait
College of Pharmacy
Practical of Biochemistry

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Proteins Precipitation

Applications of Protein precipitation by organic and inorganic acids

Milk protein precipitation by Tungstic Acid

Reagent & compounds :

Milk sample.

Sodium tungstate 10%.

Sulfuric Acid 2\3 N .

Work Method :

Place 5ml of milk in small cup then add 10ml of D.W then add 5ml of sodium tungstate 10% mix well then add 5ml of sulfuric acid 2\3N little by little with shaking, note milk proteins precipitation filtrate , examine proteins absence from filtrated solution by biuret test (test should be negative).

Questions :

1- **When we need to precipitate milk proteins why we add sodium tungstate 10% first then we add sulfuric acid 2\3N?**
2- **How you make sure that proteins precipitation has been done by tungstic acid? Mention one test?**

Blood proteins precipitation by Tungstic Acid

Reagents & compounds :

Sodium tungstate 10%.
Sulfuric Acid 2\3N.
Blood sample.
Work Method :

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Add 1ml of blood to 7ml of D.W. then add 1ml of sodium tungstate 10% mix well. Add then 1ml of sulfuric acid 2\3N … note the precipitated blood proteins , filtrate then examine the filtrant by biuret test to detect blood proteins absence (-VE) .

Proteins precipitation by saturated mineral salts

Reagent & Compounds

1. **Egg albumen sol^{n} 3%.**
2. **Gelatin sol^{n} 3%.**
3. **Casein sol^{n} 3%.**
4. **Ammonium sulfate.**
5. **Sodium chloride.**
6. **Magnesium sulfate.**
7. **Sodium hydroxide 10% Soln.**
8. **Copper sulfate 1% Soln.**

Work Method:

Try to precipitate the given samples of proteins solution using half & full saturation by following salts:

Ammonium sulfate.

Sodium chloride.

Magnesium sulfate.

Put your observation in table:

IN SALT HALF SATURATION PRECIPITATION CASE : take definite volume of protein solution then add similar volume of saturated salt solution … filter the solution mixture . precipitating proteins by this manner the filtrated give negative result with biuret test. If the protein doesn't precipitate by salt half precipitation, then filtrated solution give positive biuret test.

IN SALT FULL SATURATION PRECIPITATION CASE : take definite volume of protein solution then add salt in solid form till full saturation ,this can note by the remaining non-dissolved salt in tube bottom , and to know if protein precipitation occur or not , filter the solution and apply biuret test on filtrated.

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Notes :

The excess of ammonium sulfate conflict with protein test, thus to execute biuret test in ammonium sulfate presence , excess of sodium hydroxide must added then boil the mix to decompose ammonium sulfate and release ammonia after that additional amount of sodium hydroxide should added then add one drop of copper sulfate 1% …if violate or pink color appear this indicate protein presence.
Proteins that precipitate by half saturation of ammonium sulfate precipitate also by full saturation of sodium chloride & magnesium sulfate on the other hand the proteins precipitate by full saturation of ammonium sulfate doesn't precipitate by magnesium sulfate or sodium chloride therefore ammonium sulfate considering stronger in their precipitation ability than sodium chloride & magnesium sulfate.

protein	Sodium Chloride		Magnesium Sulfate		Ammonium Sulfate	
	Half Saturation	Full Saturation	Half Saturation	Full Saturation	Half Saturation	Full Saturation
Egg white Gelatin Casein						

Question :

1. **What is the difference between precipitating proteins by mineral salts and alcohol?**
2. **What is the most efficient salt that you been used to precipitate proteins?**
3. **Describe how you can precipitate such protein by full saturation of mineral salt?**

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Method of Identifying unknown protein

To identify unknown sample follow the following steps :

Physical properties

Describe the compound through the following physical properties :

1- **External appearance (crystalline – powder – non crystalline … etc).**
2- **the effect of heating Proteins matter in dry test tube: note burned feather smell.**
3- **Smell : note peptone & casein smell.**
4- **Solubility : note sample solubility in cold D.water – worm D.water – NaCl 1%- hydrochloric acid 0.1N – sodium hydroxide 0.1N – note that commercial gelatin dissolve slimly in cold water.**

Chemical properties

After complete determining compound physical properties, apply the suitable chemical tests

First : dissolve the protein substance in cold D.W , if sample dissolved… proceed with following tests :

A- Biuret test.

1. **If test negative , this indicate protein absence.**
2. **If test positive , this indicate protein presence therefore :**

B- Test coagulation

1. **If coagulation happen, this indicate to albumin or globulin presence thus proceed with insuring tests (chromatic tests : zanthoprotein – Millon – Rosenheim – lead acetate), distinguish between them by ammonium sulfate precipitation (globulin precipitate with half saturation with ammonium sulfate while albumin precipitate with full saturation).**
2. **If coagulation didn't happen , there is probability to peptone or gelatin presence . distinguish between them as follows :**

- **peptone give pink color by biuret test while gelatin give violate color.**
- **Peptone give positive chromic test (Millon – Rosenheim – lead acetate) while gelatin doesn't give these tests.**

3. **Peptone doesn’t precipitate by ammonium sulfate saturation while Gelatin precipitate.**
4. **Gelatin dissolve completely with hot water, peptone dissolve in cold water.**
5. **Hot concentrated Gelatin solution solidify when cooling … this solidifying doesn’t happen to peptone.**

Second : if protein substance show weak solubility in cold D.water try to dissolve it in hot water :

1. **If substance dissolved in hot water detect gelatin as previously.**
2. **If not dissolve in hot water , dissolve it in diluted sodium hydroxide and detect casein as follow :**

A. Casein give all chromatic tests except lead acetate and phosphor.

B. Casein precipitate in alkaline solution by adding excess of acids.

C. Not coagulating by heat.

D. Precipitate by full saturation of ammonium acetate.

phosphor presence can detect in casein by boiling 3ml of casein solution with 3ml of sodium hydroxide 2N for 3 minutes, add 1ml of molybdic acid reagent (20g ammonium mlybidate + 85ml of conc.sulfuric acid then complete up to 1 litter) then add 1ml of sodium sulfate20% then 1ml of hydroquinone solution 0.5% , blue color will from if phosphor presence.