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Identification Tests for Albumin and Casein

Experiment 1 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To recognise protein reactions and distinguish albumin from casein using approved protein solutions.

Principle

Peptide bonds form a violet complex with copper in alkaline biuret reagent. Heat denatures many proteins; casein instead separates prominently near its isoelectric pH, about 4.6. These tests describe protein behaviour and are not species-specific identification.

Requirements

- Dilute albumin solution, skim-milk or assigned casein dispersion and water blank.
- Ready-to-use biuret reagent, dilute acetic acid, pH paper, tubes, pipettes and water bath.

Procedure

1. Biuret reaction

Place equal small volumes of albumin, casein dispersion and water in labelled tubes. Add the assigned biuret reagent volume, mix and compare the violet colour with the blank.

2. Albumin heating

Heat an instructor-approved albumin aliquot in a water bath and compare with an unheated tube. Record turbidity or coagulation; protein concentration and pH influence the result.

3. Casein precipitation

Add dilute acetic acid to a separate milk/casein aliquot gradually while checking pH. Near pH 4.6, collect the precipitate; retain an untreated comparison.

4. Distinguish the findings

Record peptide-bond evidence, heating behaviour and acid precipitation separately. Do not identify a protein solely from its colour.

Observations and Record

Test	Albumin	Casein / milk
Biuret	Violet expected	Violet expected
Heating	Coagulation may occur	Different heat behaviour; record it
Acidification to about pH 4.6	Record response	Casein precipitation expected

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If 1 mL protein solution is mixed with 4 mL reagent, the reaction mixture is five times the original volume. This dilution is a setup calculation, not a protein concentration assay.

Result

Record the observed protein reactions and the evidence used to distinguish the assigned albumin and casein preparations.

Precautions

- Use dilute acid gradually and record actual pH. Do not confuse milk fat turbidity with protein precipitation.
- Use clean tubes and a water blank. Do not heat closed tubes or point them towards another person.

Viva Questions and Answers

1. What does biuret detect?

The coordination complex formed by peptide bonds with copper under alkaline conditions.

2. Why does casein precipitate near pH 4.6?

Its net charge and electrostatic stabilisation are reduced near the isoelectric point.

3. Does a positive biuret reaction prove albumin?

No. Many proteins give a positive reaction.

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