

SCHEDULE 2
METHODS OF ANALYSIS

PART I

7.

DETERMINATION OF BIURET IN UREA

SCOPE

1. This method is for the determination of biuret in urea.

FIELD OF APPLICATION

2. The method is applied exclusively to urea.

PRINCIPLE

3. In an alkaline medium, in the presence of potassium sodium tartrate, biuret and bivalent copper from a violet cupric compound, the absorbance of which is measured at 546 mm.

REAGENTS

- 4.—(4.1) Methanol.
- (4.2) Sulphuric acid solution, approximately 0.1 N.
- (4.3) Sodium hydroxide solution, approximately 0.1 N.
- (4.4) Alkaline solution of potassium sodium tartrate:

In a 1 litre graduated flask dissolve 40 g of sodium hydroxide in 500 ml of water and leave to cool. Add 50 g of potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$). Make up to the mark and mix. Leave standing 24 hours before use.

Copper sulphate solution:

- (4.5) In a litre graduated flask dissolve 15 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in 500 ml of water. Make up to the mark and mix.

Biuret standard solution:

- (4.6) In a 250 ml graduated flask, dissolve 0.250 g of pure biuret(1) in water. Make up to the mark and mix. 1 ml of this solution contains 0.001 g of biuret. This solution should be freshly prepared.

Methyl red indicator solution:

- (4.7) Dissolve 0.1 g methyl red in 50 ml 95% ethanol and make up to 100 ml with water. Filter if necessary.

(1) Biuret can be purified beforehand by washing with ammoniacal solution (10%), then with acetone and drying in a vacuum.

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APPARATUS

5.—(5.1) Spectrophotometer.

PREPARATION OF SAMPLE

6. See Method 1.

PROCEDURE

Preparation of the standard curve

Preparation of the standard curve

7.—(7.1) Transfer 2, 5, 10, 20, 25 and 50 ml aliquot parts of biuret standard solution (4.6) into a series of six 100 ml graduated flasks. Make up the volumes to about 50 ml with water, add one drop of indicator solution (4.7) and neutralise, if necessary, with 0.1 N sulphuric acid (4.2). Add with swirling 20.0 ml of the alkaline tartrate solution (4.4) and then 20.0 ml copper sulphate solution (4.5). Make up to the mark with water, mix and allow to stand at $30 \pm 2^\circ\text{C}$ for fifteen minutes.

At the same time prepare a reagent blank as follows. Place 50 ml water in a 100 ml graduated flask and proceed as described above from "...add one drop of indicator solution..."

Measure the absorbance of each solution at 546 nm against the reagent blank as reference, using cells of a suitable thickness. Plot the calibration curve, using the absorbances as the ordinates and the corresponding quantities of biuret in milligrams, as the abscissae.

Preparation of solution for analysis

(7.2) Weigh to the nearest 0.001 g, 10 g of the prepared sample; dissolve in about 150 ml of water in a 250 ml graduated flask and make up to the mark and mix. Filter if necessary.

Note 1 If the sample for analysis contains more than 0.015 g of ammoniacal nitrogen, dissolve it in 50 ml methanol (4.1) in a 250 ml beaker. Reduce by evaporation to a volume of about 25 ml. Transfer quantitatively to a graduated 250 ml flask. Make up to the mark with water. Filter, if necessary, through a dry fluted filter into a dry receiver.

Note 2 Elimination of the opalescence: if any colloidal substance is present difficulties may arise during filtering. In that case the solution for analysis is prepared as follows:

dissolve the sample in 150 ml of water, add 2 ml 1 N hydrochloric acid, and filter the solution through two flat very fine filters into a 250 ml graduated flask. Wash the filters with water and make up to volume. Continue the process according to the method described in 7.3.

Determination

(7.3) According to the presumed biuret content, transfer with a pipette 25 or 50 ml from the solution prepared in 7.2, to a 100 ml graduated flask and neutralise if necessary with 0.1 N sulphuric acid or sodium hydroxide solution (4.2 or 4.3) as required, using methyl red indicator (4.7). Add 20.0 ml of the alkaline solution of potassium sodium tartrate (4.4) and 20.0 ml of the copper solution (4.5). Make up to volume, mix thoroughly and leave standing for 15 minutes at $30^\circ\text{C} \pm 2$. Measure the absorbance of the solution as described in 7.1

EXPRESSION OF RESULTS

$$\% \text{ biuret} = \frac{C \times 2.5}{V}$$

where

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C = weight, in milligrams, of biuret read from the standard curve;

V = volume of the aliquot used for the determination.