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**Urea for industrial use — Determination of biuret content —
Photometric method**

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FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO Member Bodies). The work of developing International Standards is carried out through ISO Technical Committees. Every Member Body interested in a subject for which a Technical Committee has been set up has the right to be represented on that Committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work.

Draft International Standards adopted by the Technical Committees are circulated to the Member Bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 2754 was drawn up by Technical Committee ISO/TC 47, *Chemistry*, and circulated to the Member Bodies in June 1972.

It has been approved by the Member Bodies of the following countries :

Austria	Italy	Switzerland
Belgium	New Zealand	Thailand
France	Poland	Turkey
Germany	Portugal	United Kingdom
Hungary	Romania	U.S.S.R.
India	South Africa, Rep. of	
Ireland	Sweden	

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

The Member Body of the following country expressed disapproval of the document on technical grounds :

Netherlands

Urea for industrial use – Determination of biuret content – Photometric method

1 SCOPE

This International Standard specifies a photometric method for the determination of the biuret ($\text{H}_2\text{N.CO.NH.CO.NH}_2$) content of urea for industrial use.

2 FIELD OF APPLICATION

The method is applicable to the determination of the biuret content of urea for industrial use. The presence, in the aliquot taken for the determination, of quantities of ammoniacal salts exceeding 15 mg, expressed as NH_3 , interferes with the determination and should first be eliminated.

2.1 Special case

Presence, in the aliquot on which the determination is carried out, of quantities of ammoniacal salts exceeding 15 mg, expressed as NH_3 (see section 8).

3 PRINCIPLE

Formation of a violet-red coloured complex between biuret and copper sulphate in the presence of an alkaline solution of potassium sodium tartrate. Photometric measurement of the coloured complex at a wavelength of about 550 nm.

4 REAGENTS

Distilled water, or water of equivalent purity, recently boiled and cooled, shall be used in the test.

4.1 Copper(II) sulphate 15 g/l solution.

Dissolve 15 g of copper(II) sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water, dilute to 1 000 ml and mix.

4.2 Potassium sodium tartrate, alkaline 50 g/l solution.

Dissolve 50,0 g of potassium sodium tartrate tetrahydrate ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) in water, add 40 g of sodium hydroxide, previously washed to remove the outer layer, dilute to 1 000 ml and mix.

4.3 Sulphuric acid, approximately 0,1 N solution.

4.4 Sodium hydroxide, approximately 0,1 N solution.

4.5 Biuret, 2,00 g/l standard solution.

4.5.1 Purification of the biuret

Wash the biuret [$(\text{NH}_2\text{CO})_2\text{NH}$] with a 100 g/l ammonia solution and then with water until the ammonia is removed. Eliminate the water by washing with acetone and finally dry the product at about 105 °C.

4.5.2 Preparation of the solution

Weigh, to the nearest 0,001 g, 1 g of the purified biuret (4.5.1). Dissolve it in approximately 450 ml of water and bring the solution to pH 7 by the addition of either the sulphuric acid solution (4.3) or the sodium hydroxide solution (4.4), checking the pH with the aid of the indicator paper (4.6). Transfer the solution quantitatively to a 500 ml one-mark volumetric flask, dilute to the mark and mix.

1 ml of this standard solution contains 2,00 mg of biuret.

4.6 Indicator paper, bromothymol blue, or indicator paper covering the pH range 6 to 8.

5 APPARATUS

Ordinary laboratory apparatus and

5.1 Water bath, regulated at 25 ± 1 °C.

5.2 Spectrophotometer or

5.3 Photoelectric absorptiometer.

6 PROCEDURE

6.1 Test portion

Weigh, to the nearest 0,01 g, 50 g of the test sample.

6.2 Blank test

Carry out a blank test in parallel with the determination, following the same procedure and using the same quantities of all the reagents used for the determination.

6.3 Preparation of the calibration curve

6.3.1 Preparation of the standard matching solutions for photometric measurements with 4 or 5 cm cells.

Into a series of six 100 ml one-mark volumetric flasks, transfer the volumes of the standard biuret solution (4.5) indicated in the following table :

Standard biuret solution (4.5)	Corresponding mass of biuret
ml	mg
0 ¹⁾	0
0,50	1
5,0	10
15,0	30
25,0	50
40,0	80

1) Compensation solution.

Add to each flask, first the quantity of water necessary to make up the volume to approximately 50 ml, then in turn, mixing after each addition, 20 ml of the potassium sodium tartrate solution (4.2) and 20 ml of the copper sulphate solution (4.1). Dilute to the mark and mix. Immerse the flasks in the water bath (5.1), regulated at 25 ± 1 °C and leave them there for about 20 min, stirring from time to time.

6.3.2 Photometric measurements

After a period of not more than 30 min carry out the photometric measurements with the spectrophotometer (5.2) at a wavelength of about 550 nm, or with the photoelectric absorptiometer (5.3) fitted with suitable filters, after having adjusted the instrument to zero absorbance against the compensation solution.

6.3.3 Preparation of the calibration chart

Plot a graph having, for example, the number of milligrams of biuret contained in 100 ml of standard matching solution as abscissae and the corresponding values of the absorbance as ordinates.

6.4 Determination

6.4.1 Preparation of the test solution

Place the test portion (6.1) in a beaker of suitable capacity (400 ml, for example) and dissolve it in about 100 ml of water.

Bring the solution to pH 7 by the addition of either the sulphuric acid solution (4.3) or the sodium hydroxide solution (4.4), checking the pH by means of the indicator paper (4.6).

Transfer the solution quantitatively to a 250 ml one-mark volumetric flask, dilute to the mark and mix.

6.4.2 Colour development

Transfer an aliquot of the test solution (6.4.1) containing 10 to 80 mg of biuret to a 100 ml one-mark volumetric flask. Then add, with stirring after each addition, 20 ml of the potassium sodium tartrate solution (4.2) and 20 ml of the copper sulphate solution (4.1). Dilute to the mark and mix. Immerse the flask in the water bath (5.1) regulated at 25 ± 1 °C and keep at this temperature for approximately 20 min.

6.4.3 Photometric measurements

Carry out the photometric measurements on the test and blank solutions, following the procedure described in 6.3.2, after having adjusted the instrument to zero absorbance against water.

NOTE – If the test solution (6.4.1) is turbid and/or coloured, take an aliquot corresponding to that used for the development of the colour and submit it to the same procedure, omitting, however, the addition of the copper sulphate solution. Use this solution to adjust the instrument to zero absorbance for the measurement of the test solution.

Alternatively, if the test solution (6.4.1) is turbid, it is possible, before adjusting the pH as described in 6.4.1, to proceed as follows :

Add to the solution 2 ml of approximately N hydrochloric acid solution, shake vigorously and filter under vacuum through a sintered glass crucible (porosity of between 5 and 15 µm). Wash the filter and beaker with a little water, collect the filtrates quantitatively in a beaker and carry out the adjustment of the pH and the dilution as indicated in paragraph 2 of 6.4.1.

7 EXPRESSION OF RESULTS

By reference to the calibration chart (see 6.3.3), read the biuret content corresponding to the values of the photometric measurements. The biuret [(NH₂CO)₂NH] content is given, as a percentage by mass, by the formula

$$\frac{(m_1 - m_2) \times D \times 100}{m_0}$$

where

D is the ratio of the volume of test solution to the volume of the aliquot taken for the colour reaction;

*m*₀ is the mass, in grams, of the test portion;

*m*₁ is the mass, in grams, of biuret found in the aliquot of the test solution;

*m*₂ is the mass, in grams, of biuret found in the corresponding aliquot of the blank test solution.

8 SPECIAL CASE

Presence, in the aliquot on which the determination is carried out, of ammoniacal salts exceeding 15 mg, expressed as NH_3 .

NOTE – The approximate content of ammoniacal salts can be estimated by measuring the variation in pH in the presence of formaldehyde (see ISO 2752). When this measurement leads to a consumption of 0,05 N sodium hydroxide solution exceeding 90 ml it is necessary, if the quantity of urea contained in the aliquot on which the determination of the biuret is carried out exceeds 10 g, to proceed according to the technique described in this special case.

8.1 Principle

Removal of the ammoniacal nitrogen by a cation exchange resin.

Determination of the biuret content in the eluate by the preceding method.

8.2 Reagents

As in section 4 and

8.2.1 Cation exchange resin, strong.

NOTE – DOWEX 50 and AMBERLITE MB3 mixed bed ion exchange resins, for example, comply with this requirement.

8.3 Apparatus

As in section 5 and

8.3.1 Resin column, fitted with a stopcock at the lower end. Height : 320 mm; internal diameter : 8 mm.

8.4 Procedure

8.4.1 Test portion

Weigh, to the nearest 0,01 g, 20 g of the test sample.

8.4.2 Blank test

See 6.2.

8.4.3 Preparation of the calibration curve.

See 6.3.

8.4.4 Determination

8.4.4.1 CONDITIONING OF THE RESIN

Treat 100 g of the resin (8.2.1) by decantation with four portions of 250 ml each of hydrochloric acid about 4 N solution and then with 1 000 ml of water.

8.4.4.2 PREPARATION OF THE COLUMN

Place the conditioned resin (8.4.4.1) in the column (8.3.1) up to a height of 200 mm, wash and fill with water up to a level 5 mm above the resin.

8.4.4.3 PREPARATION OF THE TEST SOLUTION

Place the test portion (8.4.1) in a beaker of suitable capacity (200 ml, for example) and dissolve it in about 50 ml of water.

Pass the solution through the column (8.4.4.2) at an approximate speed of 150 ml/h. Collect the eluate in a 250 ml one-mark volumetric flask. Wash the resin with water until a total volume of approximately 220 ml is obtained. Correct any acidity of the eluate by addition of the sodium hydroxide solution (4.4) until the pH is shown to be 7 by the indicator paper (4.6). Dilute to the mark and mix.

8.4.4.4 COLOUR DEVELOPMENT

See 6.4.2.

8.4.4.5 PHOTOMETRIC MEASUREMENTS

See 6.4.3.

8.5 Expression of results

See section 7.

9 TEST REPORT

The test report shall include the following particulars :

- the reference of the method used;
- the results and the method of expression used;
- any unusual features noted during the determination;
- any operation not included in this International Standard or regarded as optional.

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