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INTERNATIONAL STANDARD ISO 4274

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Urea for industrial use — Determination of biuret content — Flame atomic absorption and photometric absorption methods

Urée à usage industriel — Dosage du biuret — Méthodes par absorption atomique dans la flamme et par photométrie d'absorption

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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 4274 was developed by Technical Committee ISO/TC 47, *Chemistry,* and was circulated to the member bodies in November 1975.

It has been approved by the member bodies of the following countries :

Austria
Belgium
Brazil
Chile
Czechoslovakia
France
Germany
Hungary
India
Israel
Mexico
Netherlands
Poland
Romania
South Africa, Rep. of
Thailand
Turkey
United Kingdom
Yugoslavia

The member body of the following country expressed disapproval of the document on technical grounds :

U.S.S.R.

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

Printed in Switzerland

Urea for industrial use — Determination of biuret content — Flame atomic absorption and photometric absorption methods

1 SCOPE

This International Standard specifies a flame atomic absorption method and a photometric absorption method for the determination of the biuret ($H_2N.CO.NH.CO.NH_2$) content of urea for industrial use.

2 FIELD OF APPLICATION

These methods are applicable to products having biuret contents equal to or greater than 0,05 % (*m/m*) for the flame atomic absorption method and equal to or greater than 0,1 % (*m/m*) for the photometric absorption method.

3 PRINCIPLE

Formation of a complex between the biuret and copper(II) sulphate in alkaline medium. Fixation of this complex on an anion exchange resin; elution of the copper, first with a potassium nitrate solution and then with a nitric acid solution. Determination of the copper content by one of the following two methods :

— *Flame atomic absorption method*

Aspiration of the solution into an air-acetylene flame and measurement of the absorption of the 325 nm line emitted by a hollow-cathode copper lamp, using an atomic absorption spectrophotometer.

— *Photometric absorption method*

Formation of a coloured complex between the copper and zinc dibenzyldithiocarbamate. Extraction of this complex with carbon tetrachloride and measurement at a wavelength of approximately 435 nm, using a spectrophotometer or a photoelectric absorptiometer.

4 REAGENTS

During the analysis, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

4.1 Carbon tetrachloride, redistilled.

4.2 Biuret ($H_2N.CO.NH.CO.NH_2$).

4.3 Copper(II) sulphate, approximately 0,05 M solution.

Dissolve 12,5 g of copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$) in water, dilute to 1 000 ml and mix.

4.4 Ammonia solution, approximately 9 M.

Dilute 700 ml of ammonia solution, ρ approximately 0,91 g/ml, about 24 % (*m/m*) solution, to 1 000 ml with water and mix.

4.5 Sodium hydroxide, approximately 3 N solution.

Dissolve 120 g of sodium hydroxide in about 500 ml of water and, after cooling, dilute to 1 000 ml and mix.

4.6 Alkaline washing solution.

Mix 100 ml of the ammonium hydroxide solution (4.4) and 100 ml of the sodium hydroxide solution (4.5), dilute to 1 000 ml and mix.

4.7 Potassium nitrate, approximately 2 M solution.

Dissolve 202,2 g of potassium nitrate in water, dilute to 1 000 ml and mix.

4.8 Nitric acid, approximately 0,2 M solution.

Dilute 13,3 ml of nitric acid solution, ρ approximately 1,40 g/ml, about 68 % (*m/m*) solution, with water. Dilute to 1 000 ml and mix.

4.9 Hydrochloric acid, approximately 1 M solution.

4.10 Zinc dibenzyldithiocarbamate, 0,5 g/l solution in carbon tetrachloride.

Dissolve 0,05 g of zinc dibenzyldithiocarbamate, $[(C_6H_5CH_2)_2NCSS]_2Zn$, in a little of the carbon tetrachloride (4.1), dilute to 100 ml with the same carbon tetrachloride and mix.

4.11 Anion exchange resin, particle size between 0,15 and 0,30 mm, strongly basic, consisting of styrene-divinylbenzene copolymers, having a functional group $N(CH_3)_3^+$ in the form of chloride.

4.12 Copper, standard solution, corresponding to 1,000 g of Cu per litre.

Weigh, to the nearest 0,000 1 g, 1,000 g of electrolytic copper (purity greater than 99,9 %), place in a beaker of suitable capacity (for example 400 ml) and dissolve in 100 ml of an approximately 8 M nitric acid solution. Heat the solution on a hot-plate until the fumes are no longer brown, cool and add about 250 ml of water. Transfer the solution quantitatively to a 1 000 ml one-mark volumetric flask, dilute to the mark and mix.

1 ml of this standard solution contains 1,000 mg of Cu.

4.13 Copper, standard solution, corresponding to 0,100 g of Cu per litre.

Transfer 50,0 ml of the standard copper solution (4.12) to a 500 ml one-mark volumetric flask, dilute to the mark and mix.

1 ml of this standard solution contains 0,100 mg of Cu.

4.14 Copper, standard solution, corresponding to 0,010 g of Cu per litre.

Transfer 50,0 ml of the standard copper solution (4.13) to a 500 ml one-mark volumetric flask, dilute to the mark and mix.

1 ml of this standard solution contains 0,010 mg of Cu.

Prepare this solution at the time of use.

5 APPARATUS

Ordinary laboratory apparatus and

5.1 Chromatography column with ground joints, effective height 350 mm, internal diameter 12 mm, fitted with a sintered glass disc of porosity between 150 and 200 μm and with a stopcock at its lower end and a graduated cylindrical dropping funnel of capacity 100 ml at its upper end (see the figure).

5.2 Atomic absorption spectrophotometer, fitted with a burner fed with compressed air and acetylene and a hollow-cathode copper lamp, or

5.3 Apparatus for photometric measurement :

5.3.1 Spectrophotometer, or

5.3.2 Photoelectric absorptiometer, fitted with suitable filters.

6 PROCEDURE

6.1 Test portion

Weigh, to the nearest 0,000 1 g, 5 ± 0,01 g of the test sample.

6.2 Washing of the resin

Place about 50 g of the resin (4.11) on a sieve having a mesh aperture of 0,15 mm and wash thoroughly with a jet of water. Use the portion of the resin remaining on the sieve for the preparation of the column.

6.3 Preparation of the column

Place the washed resin (6.2) in the column (5.1) up to a height of 200 mm.

Insert a silica wool plug in the upper part and fill the column with water to a level 5 mm above the top of the resin.

Some portions of the resin may be capable of absorbing the copper-biuret complex irreversibly. Hence, before its use for the first time, treat the resin twice as specified in 6.4, replacing the test portion by 0,050 g of the biuret (4.2) and discarding the eluates.

After each use of the resin (including the treatment specified in the preceding paragraph), regenerate it by washing, first with 100 ml of the potassium nitrate solution (4.7), then with 100 ml of the nitric acid solution (4.8) and finally with water until the pH of the final washings is between 4,5 and 5.

6.4 Preparation of the test solution

Place the test portion (6.1) in a conical flask of suitable capacity (100 ml for example) and dissolve in a little water. Add 5 ml of the copper(II) sulphate solution (4.3), 10 ml of the ammonia solution (4.4) and 10 ml of the sodium hydroxide solution (4.5), dilute to about 100 ml and mix.

Pass the solution quantitatively through the column (6.3) at a rate of 2 to 3 ml/min. Then wash the column with about 120 ml of the alkaline washing solution (4.6). Discard all the liquids.

Recover the copper-biuret complex from the resin by elution, first with 100 ml of the potassium nitrate solution

(4.7) and then with 100 ml of the nitric acid solution (4.8), collecting the eluates quantitatively in a 1 000 ml one-mark volumetric flask. Dilute to the mark with water and mix.

To determine the copper content of the test solution (6.4), follow one of the two procedures specified in 6.5 and 6.6.

6.5 Flame atomic absorption method

6.5.1 *Preparation of the calibration graph*

6.5.1.1 PREPARATION OF THE STANDARD MATCHING SOLUTIONS

Into a series of four 1 000 ml one-mark volumetric flasks, place the volumes of the standard copper solution (4.12) shown in table 1.

TABLE 1

Standard copper solution (4.12)	Corresponding mass of Cu
ml	mg
5,0	5
10,0	10
15,0	15
20,0	20

To each flask, add 100 ml of the potassium nitrate solution (4.7) and then 100 ml of the nitric acid solution (4.8). Dilute to the mark and mix.

6.5.1.2 SPECTROPHOTOMETRIC MEASUREMENTS

Switch on the atomic absorption spectrophotometer (5.2) a sufficient time in advance to ensure stabilization. Adjust the wavelength to about 325 nm, and the sensitivity and the slit aperture according to the characteristics of the apparatus.

Adjust the pressure of the air and of the acetylene according to the characteristics of the atomizer-burner in order to obtain a clear, oxidizing flame.

Adjust the flow rate according to the characteristics of the apparatus.

Aspirate the series of standard matching solutions (6.5.1.1) in succession in the flame, and measure the absorbance for each. Keep the rate of aspiration constant throughout the preparation of the calibration graph.

Spray water through the burner after each measurement.

6.5.1.3 PLOTTING OF THE CALIBRATION GRAPH

Plot a graph having, for example, the values of the masses, in milligrams, of Cu contained in 1 000 ml of the standard matching solutions as abscissae, and the corresponding values of the absorbance as ordinates.

6.5.2 *Determination*

6.5.2.1 SPECTROPHOTOMETRIC MEASUREMENTS

6.5.2.1.1 *Preliminary measurement*

Carry out a first orientation measurement on the test solution (6.4) as specified in 6.5.1.2, at the same time as the spectrophotometric measurements are carried out on the standard matching solutions (6.5.1.1).

6.5.2.1.2 *Bracketing measurement*

Carry out a second measurement on the test solution (6.4) by bracketing between two standard matching solutions differing by only 0,5 mg of Cu, prepared as specified in 6.5.1.1, but using appropriate quantities of the standard copper solution (4.12).

6.5.3 *Expression of results*

The concentration (c), in milligrams per litre of the test solution (6.4), of copper (Cu) is given by the formula

$$c = c_1 + (c_2 - c_1)\frac{A_0 - A_1}{A_2 - A_1}$$

where

c_1 is the concentration, in milligrams per litre, of the weaker bracketing solution used in the determination (6.5.2.1.2);

A_1 is the value of the corresponding absorbance;

c_2 is the concentration, in milligrams per litre, of the stronger bracketing solution used in the determination (6.5.2.1.2);

A_2 is the value of the corresponding absorbance;

A_0 is the value of the absorbance corresponding to the test solution (6.4).

The biuret content, expressed as a percentage by mass, is given by the formula

$$\frac{3{,}244\,8 \times c \times 100}{1\,000 \times m}$$

$$= \frac{0{,}324\,48\,c}{m}$$

where

c is the concentration of Cu, in milligrams per litre, of the test solution (6.4);

m is the mass, in grams, of the test portion (6.1);

3,244 8 is the mass, in milligrams, of biuret corresponding to 1 mg of Cu.

6.6 Photometric absorption method

6.6.1 *Preparation of the calibration graph*

6.6.1.1 PREPARATION OF THE STANDARD COLORIMETRIC SOLUTIONS for photometric measurements with cells of 2 cm optical path length

Into a series of six 250 ml separating funnels, each containing 10 ml of the hydrochloric acid solution (4.9), place the quantities of the standard copper solution (4.14) shown in table 2.

TABLE 2

Standard copper solution (4.14)	Corresponding mass of Cu
ml	mg
0 *	0
1,00	0,010
2,50	0,025
5,00	0,050
7,50	0,075
10,00	0,100

* Compensation solution.

6.6.1.2 COLOUR DEVELOPMENT

Dilute the contents of each separating funnel to about 90 ml with water and mix. Add 10 ml of the zinc dibenzyldithiocarbamate solution (4.10), shake the funnel vigorously for 1 min and allow the two phases to separate. Run off the lower (organic) phase, filtering through a dry filter paper, collecting the filtrate in a 50 ml dark glass one-mark volumetric flask. Add 10 ml of the zinc dibenzyldithiocarbamate solution to the aqueous phase, shake vigorously for 1 min and allow the phases to separate. Run off the lower (organic) phase and filter through the same filter paper, collecting the filtrate in the same volumetric flask. Repeat the extraction of the aqueous phase, using 10 ml of the zinc dibenzyldithiocarbamate solution and collecting the filtered organic phase in the same volumetric flask. Finally wash the filter paper three times with 5 ml portions of the carbon tetrachloride (4.1), collecting the filtrate each time in the volumetric flask. Dilute to the mark with the carbon tetrachloride and mix.

6.6.1.3 PHOTOMETRIC MEASUREMENTS

Carry out the photometric measurements using the spectrophotometer (5.3.1) adjusted to a wavelength of approximately 435 nm, or the photoelectric absorptiometer (5.3.2), fitted with suitable filters, after having adjusted the apparatus to zero absorbance against the compensation solution.

6.6.1.4 PLOTTING OF THE CALIBRATION GRAPH

Plot a graph having, for example, the values of the masses, in milligrams, of Cu contained in 50 ml of the standard colorimetric solutions (6.6.1.1) as abscissae, and the corresponding values of the absorbance as ordinates.

6.6.2 *Blank test*

Carry out a blank test at the same time as the determination, following the same procedure and using the same quantities of all the reagents as used for the determination, but omitting the test portion.

6.6.3 *Determination*

6.6.3.1 COLOUR DEVELOPMENT

Take a quantity of the test solution (6.4) containing 0,01 to 0,10 mg of copper, place in a 250 ml separating funnel, containing 10 ml of the hydrochloric acid solution (4.9), and carry out the colour development as specified in 6.6.1.2.

6.6.3.2 PHOTOMETRIC MEASUREMENTS

Carry out the photometric measurements of the solution 6.6.3.1 and of the corresponding blank test solution as specified in 6.6.1.3, after having adjusted the instrument to zero absorbance against the carbon tetrachloride (4.1).

6.6.4 *Expression of results*

By means of the calibration graph (6.6.1.4), determine the mass of copper corresponding to the absorbance of the solution 6.6.3.1 and that corresponding to the absorbance of the blank test solution.

The biuret content, expressed as a percentage by mass, is given by the formula

$$\frac{(m_1 - m_2) \times 3{,}244\,8 \times D \times 100}{1\,000 \times m_0}$$

$$= \frac{(m_1 - m_2) \times 0{,}324\,48 \times D}{m_0}$$

where

m_0 is the mass, in grams, of the test portion (6.1);

m_1 is the mass, in milligrams, of copper found in the solution (6.6.3.1);

m_2 is the mass, in milligrams, of copper found in the blank test solution (6.6.2);

D is the ratio of the volume of the test solution (6.4) to the volume of the aliquot portion taken for the colour development;

3,244 8 is the mass, in milligrams, of biuret corresponding to 1 mg of copper.

7 TEST REPORT

The test report shall include the following particulars :

a) the reference of the method used;

b) the results and the method of expression used;

c) any unusual features noted during the determination;

d) any operation not included in this International Standard, or regarded as optional.

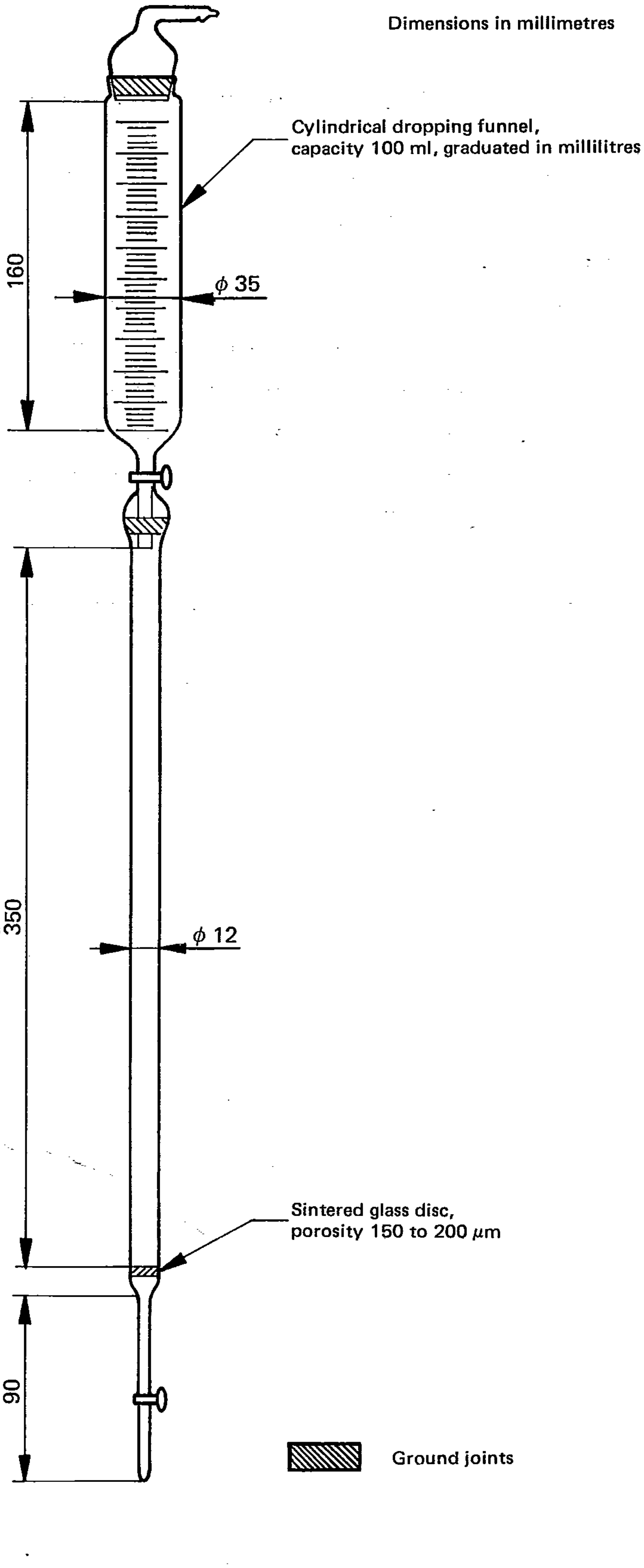

FIGURE — **Chromatography column**

ANNEX

ISO PUBLICATIONS RELATING TO UREA FOR INDUSTRIAL USE

ISO 1592 – Determination of nitrogen content – Titrimetric method after distillation.

ISO 1593 – Determination of alkalinity – Titrimetric method.

ISO 1594 – Determination of ash – Gravimetric method.

ISO/R 1595 – Determination of iron content – 2,2′-Bipyridyl photometric method.

ISO 2749 Measurement of the pH of a solution of urea of conventional concentration (100 g/l) – Potentiometric method.

ISO 2750 – Measurement of colour in Hazen units (platinum-cobalt scale) of a urea-formaldehyde solution.

ISO 2751 – Determination of the buffer coefficient – Potentiometric method.

ISO 2752 – Measurement of the variation of pH in the presence of formaldehyde – Potentiometric method.

ISO 2753 – Determination of water content – Karl Fischer method.

ISO 2754 – Determination of biuret content – Photometric method.

ISO 4274 – Determination of biuret content – Flame atomic absorption and photometric absorption methods.