
**Photography — Processing waste —
Determination of total amino nitrogen
(microdiffusion Kjeldahl method)**

*Photographie — Effluents de traitement — Détermination de l'azote amino
total (méthode de microdiffusion Kjeldahl)*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established 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. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6851 was prepared by Technical Committee ISO/TC 42, *Photography*.

This second edition cancels and replaces the first edition (ISO 6851:1987), of which it constitutes a technical revision.

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Introduction

This International Standard is one of a series devoted to the analysis of photographic wastes; it encompasses the field of analysis of the total amino nitrogen (microdiffusion Kjeldahl method) in a photographic effluent.

This International Standard is intended for use by individuals with a working knowledge of analytical techniques. Some of the procedures use caustic, toxic or otherwise hazardous chemicals. Safe laboratory practice for the handling of chemicals requires the use of safety glasses or goggles and, in some cases, other protective apparel such as rubber gloves, face masks or aprons. Normal precautions for the safe performance of any chemical procedure must be exercised at all times, but specific details have been provided for hazardous materials. Hazard warnings are designated by a letter enclosed in angle brackets "< >." These are defined in clause 5 and then used throughout the text. More detailed information on hazards, handling and use of these chemicals may be available from the manufacturer.

Photographic laboratories can establish conformity to effluent regulations only by chemical analysis. If this cannot be done in-house, an outside laboratory should be used.

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Photography — Processing waste — Determination of total amino nitrogen (microdiffusion Kjeldahl method)

1 Scope

This International Standard specifies a method for the determination of the total organic nitrogen, as well as any ammoniacal nitrogen, present in photographic processing wastes by the Kjeldahl method.

Total Kjeldahl nitrogen can be determined in photographic processing wastes in the range of 10 mg/l to 200 mg/l as ammonia, or in the range of 8 mg/l to 160 mg/l in terms of nitrogen. If ammoniacal nitrogen (see ISO 6853) is determined separately and subtracted, the concentration of organic amino nitrogen can be established.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 648:1977, *Laboratory glassware — One-mark pipettes*.

ISO 1042:1998, *Laboratory glassware — One-mark volumetric flasks*.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*.

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 6353-1:1982, *Reagents for chemical analysis — Part 1: General test methods*.

ISO 6353-2:1983, *Reagents for chemical analysis — Part 2: Specifications — First series*.

ISO 6353-3:1987, *Reagents for chemical analysis — Part 3: Specifications — Second series*.

ISO 6853:2001, *Photography — Processing waste — Determination of ammoniacal nitrogen (microdiffusion method)*.

ISO 10349-1:1992, *Photography — Photographic-grade chemicals — Test methods — Part 1: General*.

3 Principle

In the presence of sulfuric acid and/or potassium sulfate with mercuric sulfate as a catalyst, the amino nitrogen of most organic substances is converted into ammonium hydrogen sulfate. The ammonia is then liberated by treatment with a suitable alkali and absorbed into a solution of boric acid. The liberation and absorption of the ammonia is carried out in a microdiffusion cell. The ammonia absorbed into the boric acid is then determined by titration with standard sulfuric acid.

4 Reactions

... NH_2

... $\text{NHR} + \text{H}_2\text{SO}_4 \rightarrow (\text{NH}_4)\text{HSO}_4$

... NR_2

$(\text{NH}_4)\text{HSO}_4 + 2\text{OH}^- \rightarrow \text{NH}_3 + 2\text{H}_2\text{O} + \text{SO}_4^{2-}$

5 Safety and operational precautions

5.1 Hazard warnings

Some of the chemicals specified in the test procedures are caustic, toxic or otherwise hazardous. Safe laboratory practice for the handling of chemicals requires the use of safety glasses or goggles, and in some cases other protective apparel such as rubber gloves, face masks and aprons. Specific danger notices are given in the text for particularly dangerous materials, but normal precautions are required during the performance of any chemical procedure at all times.

The first time that a hazardous material is noted in the procedures, the hazard will be indicated by the word "**DANGER**" followed by a symbol consisting of angle brackets "< >" containing a letter that designates the specific hazard. A double bracket "<< >>" will be used for particularly perilous situations. In subsequent statements involving handling of these hazardous materials, only the hazard symbol consisting of the brackets and letter(s) will be displayed. Furthermore, for a given material, the hazard symbols will be used only once in a single paragraph.

Hazard warning symbols will not be used for common organic solvents when used in quantities of less than 1 litre, unless they are particularly hazardous.

Detailed warnings for handling chemicals and their diluted solutions are beyond the scope of this International Standard.

Employers shall provide training and health and safety information in conformance with legal requirements.

The hazard code system used in this International Standard is intended to provide information to the users and is not meant for compliance with any legal requirements for labelling, as these vary from country to country.

It is strongly recommended that anyone using these chemicals obtain pertinent information from the manufacturer about the hazards, handling, use and disposal of these chemicals.

5.2 Hazard information code system

 Harmful if inhaled. Avoid breathing dust, vapour, mist or gas. Use only with adequate ventilation.

- ⟨C⟩ Harmful if contact occurs. Avoid contact with eyes, skin or clothing. Wash thoroughly after handling.
- ⟨F⟩ Will burn. Keep away from heat, sparks and open flame. Use with adequate ventilation.
- ⟨O⟩ Oxidizer. Contact with other material may cause fire. Do not store near combustible materials.
- ⟨S⟩ Harmful if swallowed. Wash thoroughly after handling. If swallowed, obtain medical attention immediately.
- ⟨⟨S⟩⟩ May be fatal if swallowed. If swallowed, obtain medical attention immediately.

5.3 Safety precautions

ALL PIPETTE OPERATIONS SHALL BE PERFORMED WITH A PIPETTE BULB OR PLUNGER PIPETTE. THIS IS A CRITICAL SAFETY WARNING!

Safety glasses shall be worn for all laboratory work.

6 Materials and reagents

6.1 General

6.1.1 Handling and labelling

Reagents shall be handled in conformity with health and safety precautions as shown on containers, or as given in other sources of such information. Proper labelling of prepared reagents includes the chemical name, date of preparation, expiration date, restandardization date, name of preparer, and adequate health and safety precautions. The discharge of reagents shall conform to applicable environmental regulations.

6.1.2 Purity

Reagents used in the test procedures shall be certified reagent-grade chemicals and shall meet appropriate standards, or be chemicals of a purity acceptable for the analysis. For details, see ISO 6353-1, ISO 6353-2 and ISO 6353-3.

6.1.3 Water

Whenever water is specified without other qualifiers in the test procedures, only distilled water or water of equivalent purity shall be used.

6.1.4 Strength of solutions

6.1.4.1 Acids and ammonium hydroxide are full strength unless otherwise specified.

6.1.4.2 When a standardized solution is required, its amount-of-substance concentration is expressed in moles per litre. The number of significant figures to which the molarity is known shall be sufficient to ensure that the reagent does not limit the reliability of the test method.

6.1.4.3 When a standardized solution is not required, its concentration is expressed in grams per litre (g/l) to the appropriate number of significant figures.

6.1.4.4 When a solution is to be diluted, its dilution is indicated by $(X + Y)$, meaning that X volumes of reagent, or concentrated solution, are to be diluted with Y volumes of water (6.1.3).

6.2 Reagents

6.2.1 Acidified mercury(II) sulfate/potassium sulfate solution (DANGER: $\langle\langle C \rangle\rangle\langle\langle S \rangle\rangle$), $\rho(\text{HgO}) = 2 \text{ g/l}$, $\rho(\text{K}_2\text{SO}_4) = 134 \text{ g/l}$.

Weigh $134 \text{ g} \pm 0,1 \text{ g}$ of potassium sulfate, K_2SO_4 , and transfer it quantitatively to a 1 litre volumetric flask conforming to Class A of ISO 1042. Add about 650 ml of water and dissolve the potassium sulfate. Carefully add 200 ml of sulfuric acid, H_2SO_4 (DANGER: $\langle\langle C \rangle\rangle$) $\rho \approx 1,84 \text{ g/ml}$, from a graduated measuring cylinder and mix.

Weigh $2 \text{ g} \pm 0,1 \text{ g}$ of red mercury(II) oxide, $\text{HgO}^{(1)}$ (DANGER: $\langle\langle B \rangle\rangle\langle\langle C \rangle\rangle\langle\langle S \rangle\rangle$), and dissolve it in 25 ml of 3 mol/l sulfuric acid solution (6.2.4) (DANGER: $\langle C \rangle$) in a small beaker. Add the contents of the beaker to the acidified potassium sulfate solution, and rinse the beaker into the flask. Dilute the solution to volume when cool, and mix well. Store this solution at a temperature above 14°C to prevent crystallization.

6.2.2 Potassium tetraborate solution, 514 g/l.

Weigh $673 \text{ g} \pm 0,1 \text{ g}$ of potassium tetraborate, $\text{K}_2\text{B}_4\text{O}_7 \cdot 4\text{H}_2\text{O}$, and dissolve it in 550 ml of water in a 1 litre beaker. Then weigh $247 \text{ g} \pm 0,1 \text{ g}$ of potassium hydroxide, KOH (DANGER: $\langle\langle C \rangle\rangle$), and dissolve it in the tetraborate solution. Boil on a hotplate for 5 min, cool and add 5 ml of a 10 % aqueous solution of nonylphenoxypoly (6-10) ethylene oxide, NPPO, or similar wetting agent²⁾. Transfer the mixture to a 1 litre volumetric flask, rinsing the beaker into the flask several times. When cool, dilute to volume and mix well. Note that the wetting agent will separate out on standing; therefore, the flask must be shaken vigorously before each use.

6.2.3 Boric acid absorbent solution

Add about 800 ml of water to a 1 litre volumetric flask. Stir, using a magnetic stirrer, and add 2 mg to 3 mg of xylene cyanole FF, weighed to the nearest 1 mg, followed by 0,5 ml of NPPO, followed by 5,0 ml of methyl red indicator solution prepared by dissolving 0,125 g of methyl red in 250 ml of methanol (DANGER: $\langle F \rangle\langle S \rangle$). Add $6 \text{ g} \pm 0,1 \text{ g}$ of boric acid, H_3BO_3 , keeping the contents of the flask stirred until all the constituents are dissolved. Dilute to within about 15 ml of the mark and mix. Place 1,5 ml of this solution in the centre of a microdiffusion cell and observe the colour.

If the colour in the cell is pink, add just sufficient 0,1 mol/l sodium hydroxide (6.2.9) to the solution in the 1 litre flask to obtain a neutral colour when 1 ml is viewed in the microdiffusion cell.

Check that excess sodium hydroxide is absent by adding 0,10 ml of 0,002 50 mol/l sulfuric acid (6.2.7) to 1 ml of the solution, at which point a pink colour should be produced. Note that the solution in the 1 litre flask will appear red, even when the 1 ml in the microdiffusion cell looks neutral.

6.2.4 Sulfuric acid solution, $c(\text{H}_2\text{SO}_4) \approx 3 \text{ mol/l}$ (DANGER: $\langle C \rangle$).

Carefully add 170 ml of sulfuric acid ($\langle\langle C \rangle\rangle$), $\rho \approx 1,84 \text{ g/ml}$, to 500 ml of water in a 1 litre volumetric flask while mixing and cooling. Dilute to the mark with water.

6.2.5 Sulfuric acid solution, $c(\text{H}_2\text{SO}_4) \approx 0,5 \text{ mol/l}$.

Carefully add 28,3 ml of sulfuric acid ($\langle\langle C \rangle\rangle$), $\rho \approx 1,84 \text{ g/ml}$, to 500 ml of water in a 1 litre volumetric flask while mixing and cooling. Dilute to the mark with water.

1) Due to the toxicity of mercuric oxide, it is being replaced with cupric sulfate in some reactions. The use of cupric sulfate in this microdiffusion standard has not yet been established.

2) Non-ionic detergent with a hydrophilic lipophilic balance in the range of 13 to 14.

6.2.6 Sulfuric acid solution, $c(\text{H}_2\text{SO}_4) \approx 0,05 \text{ mol/l}$.

Pipette 100,0 ml of 0,5 mol/l sulfuric acid (6.2.5) into a 1 litre volumetric flask and dilute to the mark with water.

NOTE This solution is not the standard volumetric solution that is required in 6.2.7.

6.2.7 Sulfuric acid solution, $c(\text{H}_2\text{SO}_4) = 0,002 50 \text{ mol/l}$.

Pipette 50,0 ml of standard volumetric 0,050 00 mol/l sulfuric acid into a 1 litre volumetric flask and dilute to the mark with water.

The solution prepared in 6.2.6 is not a standard volumetric solution. A commercial volumetric standard solution is recommended. If a volumetric standard solution needs to be prepared, see an analytical chemistry text.

6.2.8 Sodium hydroxide solution, $c(\text{NaOH}) \approx 1 \text{ mol/l}$.

Dissolve $40 \text{ g} \pm 0,5 \text{ g}$ of sodium hydroxide (DANGER: <<C>>) in 500 ml of water in a 1 litre volumetric flask. Mix, cool, and dilute to the mark with water.

6.2.9 Sodium hydroxide solution, $c(\text{NaOH}) \approx 0,1 \text{ mol/l}$.

Dissolve $4,0 \text{ g} \pm 0,1 \text{ g}$ of sodium hydroxide (<<C>>) in 500 ml of water in a 1 litre volumetric flask. Mix, cool, and dilute to the mark with water.

6.2.10 Cleaning solutions for microdiffusion cells**6.2.10.1 Cleaning solution A**

From a graduated measuring cylinder, add about 750 ml of water and about 750 ml of 0,5 mol/l sulfuric acid (6.2.5) to a 2 litre beaker and stir. Continue stirring, and add 2 ml to 3 ml of household liquid dishwashing detergent and 1 ml of methyl red indicator solution.

NOTE Avoid the use of detergents containing ammonia.

6.2.10.2 Cleaning solution B

Add about 1,5 litres of water and 2 ml to 3 ml of the detergent to a 2 litre beaker and mix. Add 10 ml of 1 mol/l sodium hydroxide (6.2.8), and then add enough methyl red indicator solution, while stirring, to produce a yellow colour.

6.2.10.3 Cleaning solution C

Add about 1,5 litres of water and about 5 ml of the detergent to a 2 litre beaker and stir. Continue to stir, and add about 10 ml of 0,05 mol/l sulfuric acid (6.2.6) and enough methyl red indicator to produce a pink colour.

6.2.11 Standard nitrogen samples, to check the ammonia liberation technique.**6.2.11.1 Nitrogen stock solution, $\rho_{\text{N}} = 1 000 \text{ mg/l}$ ($c_{\text{N}} = 0,071 4 \text{ mol/l}$).**

Dry ammonium chloride for 2 h in an oven at 100 °C and allow it to cool in a desiccator before weighing. Weigh $3,819 \text{ g} \pm 0,001 \text{ g}$ of this ammonium chloride and quantitatively transfer it to a 1 litre volumetric flask. Dissolve it in water and dilute to volume. This stock solution is stable for at least 3 months.

6.2.11.2 Standard nitrogen solution, $\rho_{\text{N}} = 100 \text{ mg/l}$ ($c_{\text{N}} = 0,007 14 \text{ mol/l}$).

Pipette 10 ml of the nitrogen stock solution (6.2.11.1) into a 100 ml volumetric flask and dilute to the mark with water.

6.2.11.3 Standard nitrogen solution, $\rho_N = 20 \text{ mg/l}$ ($c_N = 0,001\,428 \text{ mol/l}$).

Pipette 2 ml of the nitrogen stock solution (6.2.11.1) into a 100 ml volumetric flask and dilute to the mark with water.

7 Apparatus

7.1 General

All glassware subject to heating shall be of heat-resistant borosilicate glass.³⁾

Pipettes and other volumetric glassware shall meet the requirements specified in ISO 10349-1.

7.2 Kjeldahl digestion rack.

7.3 Kjeldahl flasks, of 100 ml capacity.

7.4 Macro-set pipette, capable of delivering 4,00 ml.

7.5 Glass beads, 4 mm in diameter.

7.6 One-mark volumetric flask, of capacity 25 ml, equipped with a suitable ground-glass stopper and conforming to ISO 1042.

7.7 Microdiffusion cell, 83 mm, Obrick microdiffusion.

7.8 Micropipette, of capacity 0,500 ml and conforming to ISO 648.

7.9 Syringe pipette, of capacity 1,00 ml.

7.10 Syringe microburette.

7.11 Syringe, capable of delivering 1,50 ml.

7.12 Polytetrafluoroethylene-coated magnetic bar, 7 mm $\times$ 2 mm.

8 Sampling and sample preparation

It is necessary that the analysis be carried out on a representative sample. The sampling of a process effluent or a plant effluent can encompass many difficulties and due care shall be exercised. See ISO 5667-1, ISO 5667-2 and ISO 5667-3.

Sampling shall be carried out in conformance with regulatory requirements. Sampling should be done under typical operating conditions and normally should be representative of the overall plant effluent. Daily samples that are truly representative of the effluents require sampling over 24 h and sampling that is proportional to the flow rate.

Samples taken during a sudden discharge or during another non-routine operation will not yield results representative of the normal operation.

3) Pyrex ® is an example of suitable glassware available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.

9 Procedure

9.1 Digestion of sample

Ensure that the digestion-rack fume duct is connected to an aspirator through a water trap with water running down the sink while the apparatus is in use. Turn the aspirator on fully. Pipette 25 ml of the sample into a 100 ml Kjeldahl digestion flask (7.3), and add 4,00 ml of the acidified mercuric sulfate/potassium sulfate solution (6.2.1) (DANGER: <<C>> <<S>>) using the macro-set pipette (7.4). Add two glass beads (7.5), mix well, and position the flask in the digestion rack (7.2), ensuring that the neck is correctly positioned in the fume duct.

Digest at moderate to high temperature until all the water is driven off, and continue digestion for at least 30 min after the evolution of sulfur trioxide, SO_3 , commences. If the residue is coloured after this treatment, continue the digestion until it becomes colourless and then for a further 20 min. Any material spattered onto the walls or neck should be removed by swirling the flask.

When digestion is complete, allow the residue to cool and then quantitatively transfer the contents to a 25 ml volumetric flask (7.6). Dilute to the mark with water and insert the glass stopper. Mix well by inverting.

9.2 Cleaning microdiffusion cells and covers

Soak the cells and covers in cleaning solution A (6.2.10.1) for 1 h; remove and soak in cleaning solution B (6.2.10.2) for 1 h; then remove and soak in cleaning solution C (6.2.10.3) for 1 h.

Remove the cells and covers, shake them as dry as possible and invert them to dry on a clean towel. Do not touch the insides of the clean cells and covers.

If the cleaned cells and covers have not been washed on the day that they are to be used, rinse them with water and place them on a clean towel to dry before using.

9.3 Liberation of ammonia from samples

Using a syringe (7.11), add 1,50 ml of the boric acid absorbent solution (6.2.3) to the centre of the microdiffusion cell (7.7). Using the 1,00 ml syringe pipette (7.9), add 1,00 ml of the potassium tetraborate solution (6.2.2) to the outer sealing chamber and 1,00 ml to the sample chamber, i.e. the second chamber from the outside.

The tetraborate solution should be vigorously shaken before sampling in order to distribute the NPPO. Great care shall be taken not to splash any tetraborate solution into the centre chamber. If this occurs, a green coloration will be produced and the sample shall be discarded. The tetraborate solution should be deposited on only one part of the sample chamber. Leave enough space for the sample to be added without mixing until the cell is sealed.

Using the calibrated 0,500 ml micropipette (7.8), carefully add 0,500 ml of the sample to the empty side of the sample chamber. Cover the cell immediately and rotate the cover to spread the tetraborate solution and form a good seal. Using a rotary tipping motion, mix the sample with the tetraborate solution for 30 s, making certain that the sample and absorbent chambers are fully covered and that the contents of the separate chambers do not mix. Let the cell stand for 2 h. Longer standing times will not introduce any error.

9.4 Titration

Remove the lid carefully from the cell and place the cell on a magnetic stirrer. Place the magnetic bar (7.12) in the centre chamber without splashing. Using the syringe microburette (7.10), titrate the solution in the centre chamber with 0,002 50 mol/l sulfuric acid (6.2.7) to the first pink colour. Keep the burette tip immersed while titrating. Stir for about 15 s. If the colour fades to light green, continue to titrate until the pink colour remains for at least 15 s. Identify the volume used for titration as V_1 .

9.5 Blank reading and a calibration curve check

Concurrently with the sample to be analysed, run procedures 9.1, 9.2 and 9.3 on 0,500 ml of water, on 0,500 ml of the 100 mg/l standard nitrogen solution (6.2.11.2), and on the 20 mg/l standard nitrogen solution (6.2.11.3). Identify the volume used for the titration on the blank as V_2 ; on the 100 mg/l standard nitrogen solution as V_3 ; and on the 20 mg/l standard nitrogen solution as V_4 .

10 Expression of results

10.1 Method of calculation

The total Kjeldahl nitrogen concentration ρ_N , in milligrams per litre, is given by the formula

$$\rho_N = \frac{72,6(V_1 - V_2)}{V_s}$$

where

V_1 is the volume, in millilitres, used for the titration of the sample;

V_2 is the volume, in millilitres, used for the titration of the water blank;

V_s is the volume, in millilitres, of the sample taken for diffusion.

If V_s is 0,500 ml, the formula becomes

$$\rho_N = 145(V_1 - V_2)$$

NOTE The above constants (72,6 and 145) are experimental values and slightly less than theoretical. They were obtained by a linear regression analysis of a calibration curve and take into account a small loss of amino nitrogen in the diffusion step. Laboratories may wish to generate their own constants for more accurate results.

If the digested sample (25 ml) must be further diluted, multiply the result above by:

ml final dilution volume/ml sample diluted

As a check on the precision, perform similar calculations on the results ($V_3 - V_2$) and ($V_4 - V_2$) for the two standard nitrogen samples. These should be carried out for each set of effluent samples or at least daily. The agreement of these results indicates the probable reliability of the results on unknown samples.

Total organic nitrogen (N) = Total Kjeldahl nitrogen – ammoniacal nitrogen

10.2 Precision

The 95 % confidence limits for a single determination are expected to be between 4 mg and 8 mg of nitrogen per litre. The stoichiometric yield by the microdiffusion method is about 96 %.