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Wool — Determination of cysteic acid content of wool hydrolysates by paper electrophoresis and colorimetry

Laine — Détermination de la teneur en acide cystéique dans les hydrolysats de laine par électrophorèse sur papier et colorimétrie

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FOREWORD

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

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Wool — Determination of cysteic acid content of wool hydrolysates by paper electrophoresis and colorimetry

0 INTRODUCTION

This International Standard is based on the IWTO test method 23-70, drawn up by the International Wool Textile Organization.

Cysteic acid is one of the oxidation products of the amino acids cystine and cysteine. The cysteic acid content of raw wool is normally very low; it increases with photochemical degradation (weathering). Finishing processes such as bleaching or chlorination always result in an increase in cysteic acid.

The severity of any kind of oxidation may be determined directly by quantitative determination of cysteic acid. In certain instances it may be useful to make comparative tests on corresponding samples of untreated material, or material not subject to complaint.

1 SCOPE AND FIELD OF APPLICATION

This International Standard specifies a method for the determination of the cysteic acid content of wool hydrolysates using paper electrophoresis and colorimetry.

The method is applicable to all-wool textiles in any form before or after dyeing — for example, loose wool, sliver, slubbing, yarn or fabric. When testing blends, the proportion of wool must be known exactly. The method is also applicable to wool after carbonizing.

2 PRINCIPLE

Hydrolysis of the wool, followed by separation of the cysteic acid present in the resultant hydrolysate from the other amino acids by paper electrophoresis. Staining of the paper strip and elution of the zone containing the cysteic acid. Colorimetric determination of the cysteic acid content by comparison with a known amount of cysteic acid applied to the paper strip at the same time.

3 REAGENTS

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

3.1 Methanol.

3.2 Hydrochloric acid, 5,7 N solution made from concentrated hydrochloric acid, ρ 1,19 g/ml, azeotropically distilled.

3.3 Ninhydrin-cadmium reagent.

Dissolve 100 mg of cadmium acetate in 10 ml of water, and add consecutively 5 ml of glacial acetic acid, 100 ml of acetone and 1 g of ninhydrin. The reagent may be kept for one week in a brown bottle in a refrigerator. Each portion of reagent used for staining shall be used once only.

3.4 Buffer solution, pH 3,5.

Dissolve 10 ml of pyridine and 100 ml of glacial acetic acid in 890 ml of distilled water. The buffer solution must be renewed after five separations.

3.5 Cysteic acid standard solution.

Dissolve 110,6 mg of cysteic acid monohydrate (corresponding to 100 mg of anhydrous cysteic acid) in distilled water in a 100 ml measuring flask. Before weighing, dry the cysteic acid for 24 h at approximately 70 °C in a drying pistol over phosphorus pentoxide.

3.6 Barium chloride solution.

Dissolve 2,5 g of barium chloride dihydrate ($BaCl_2.2H_2O$) in distilled water in a 100 ml measuring flask.

4 APPARATUS

4.1 Weighing bottles.

4.2 Analytical balance, accurate to 0,000 2 g.

4.3 Ventilated drying oven for drying and hydrolysing the test specimens at 105 ± 2 °C and for drying the paper strips at 70 °C.

4.4 Desiccator.

4.5 Thick-walled glass tube, diameter approximately 2 cm, length 30 cm.

4.6 Forceps and glass rod.

4.7 Blow-pipe.

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4.8 Glass cutter.

4.9 Pipette, 20 ml.

4.10 Pipettes, 2 X 1 ml.

4.11 Calibrated precision pipettes, 0,01 ml.

4.12 Round-bottomed flasks, 100 ml and 250 ml.

4.13 Measuring flask, 100 ml.

4.14 Measuring flasks, 2 X 5 ml.

4.15 Beaker, 250 ml.

4.16 Rotary evaporator.

4.17 Electrophoresis chamber and power supply equipment.

4.18 Filter paper strips or sheets (Whatman No. 1, Schleicher and Schull No. 2043 aMgl or other paper of similar quality).

4.19 Test tubes with stand.

4.20 Glass dishes.

4.21 Spectrophotometer or **filterphotometer** with a filter having maximum absorption at 500 ± 10 nm.

The instrument shall be capable of measuring the optical density over the range 0 to 0,7 correct to 0,01, the next decimal place being estimated.

4.22 Thermostatically controlled oil bath (high boiling point) or **hot-plate** fitted with asbestos plate (see 6.3).

4.23 Reflux condenser.

5 SAMPLING AND PREPARATION OF TEST SPECIMENS

Take a sample representative of the material to be tested, and sufficiently large to provide for the following test specimens :

- two test specimens each of mass approximately 1 g for determining dry mass;
- one test specimen of mass approximately 1 g for preparing the hydrolysate.

Remove all vegetable matter and other foreign substances from samples of loose wool, roving, etc. Dissect samples of yarn or cloth before extraction into short lengths (approximately 1 cm) of yarn. Felted materials that cannot be dissected into yarn must first be cut up into small pieces. Extract the sample with dichloromethane for 1 h in a Soxhlet apparatus, at a minimum rate of 6 cycles per hour and evaporate the dichloromethane from the cleaned sample.

6 PROCEDURE

6.1 Weighing of test specimens

Weigh successively, to an accuracy of 0,000 2 g, the three test specimens described in clause 5. Use two test specimens for determining the dry mass (see 6.2) and the other test specimen for preparing a hydrolysate (see 6.3).

6.2 Determination of dry mass

Transfer each test specimen to a weighing bottle (4.1) and dry in the drying oven (4.3) at 105 ± 2 °C. Stopper the weighing bottles, place them in the desiccator (4.4), allow to cool and weigh. Repeat these drying and weighing operations until constant mass is attained.[1)]

Remove the test specimens from the weighing bottles, weigh the weighing bottles and hence determine the dry mass of the test specimens. Calculate, by proportion, the dry mass of the hydrolysate test specimen.

6.3 Hydrolysis

The hydrolysis shall be carried out according to one of the following methods :

a) Place the weighed test specimen for hydrolysis (6.1) and 20 ml of 5,7 N hydrochloric acid solution (3.2) in a 100 ml round-bottomed flask (4.12) fitted with the reflux condenser (4.23). Heat the flask for 4 h at 105 °C in the thermostatically controlled oil bath (4.22). A hot-plate, in conjunction with an asbestos plate, may be used instead of an oil bath. Insert the round-bottomed flask through a hole in the asbestos plate so that the liquid level is still visible above this plate; the bottom of the flask must not touch the hot-plate.

b) Place the weighed test specimen for hydrolysis (6.1) in a thick-walled glass tube (4.5) and add 20 ml of 5,7 N hydrochloric acid solution by pipette (4.9). Seal the tube with the blow-pipe (4.7), and heat at 105 °C in the drying oven for 24 h. Shake the tube thoroughly at intervals so that no fibre residue remains in the hydrolysate.

1) Constant mass is attained when the mass of a specimen, after repeated drying for at least 30 min, does not change by more than 0,000 2 g.

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Transfer the hydrolysate obtained by method a) or b), including the rinsings with distilled water :

– into a 250 ml round-bottomed flask (4.12), where the sample is uncarbonized wool; or

– to a 250 ml beaker (4.15), where the sample is from carbonized wool[1]), and heat to boiling point. When the solution boils, add 1 ml of barium chloride solution (3.6) drop by drop by pipette (4.10) while stirring with distilled water.

Finally, in both cases, concentrate the solution to a syrupy consistency in the rotary evaporator (4.16) at 50 °C. Mix the syrup three times with 10 ml of distilled water to eliminate excess hydrochloric acid and concentrate each time in the rotary evaporator. Transfer the residue completely with distilled water into a 5 ml measuring flask (4.14) and dilute to the mark.

6.4 Electrophoresis

A low-voltage apparatus can be used for electrophoretic separation. For this purpose any equipment with an output of approximately 300 V is suitable. To speed up the analysis, an apparatus with a higher output voltage (up to about 1 500 V) may be used. The separation chamber must be able to accept paper strips at least 30 cm long (distance between electrodes).

Mark a vertical line with a pencil across the centre of the paper (4.18). Then, using a precision pipette (4.11), apply 0,01 ml of each test hydrolysate (6.3) in the form of a stripe onto the pencil lines of each of two dry paper strips, for tests in duplicate.

Using another paper strip or another part of the line marked on the sheet, apply 0,01 ml of the cysteic acid standard solution (3.5). Care must always be taken to ensure that the contents of the pipette are completely and evenly applied 0,5 cm away from the edge of the paper. After inserting the two paper strips into the electrode chamber, allow the filter paper to absorb the buffer solution (3.4) uniformly. The time required for this can be shortened by spraying the paper with buffer.

When the paper is completely saturated with buffer, apply a potential of approximately 5 to 8 V/cm (that is 200 to 300 V, depending on the paper length). Electrophoresis is completed after 4 to 5 h.

Hang the paper strips (or sheets) in the drying oven (4.3) with the anode end of the paper uppermost and dry for 30 min at 70 °C.

6.5 Colour development and elution

Carefully impregnate the dried paper strips (or sheets) with a small amount of the ninhydrin-cadmium solution (3.3) and place in a flat glass dish (4.20). The paper should merely absorb the reagent and on no account shall it be immersed in it.

Hang the papers, anode end uppermost, in the drying oven and dry for 30 min at 70 °C. Depending on the voltage applied and the duration of electrophoresis, the cysteic acid zone moves 4 to 6 cm in the anode direction from its original position.

Cut out equal areas of paper containing the two cysteic acid "spots" from the hydrolysate and that from the standard solution and, after chopping into small pieces, place the paper from each of the three spots in separate test tubes (4.19). In a fourth test tube, place an equal area of paper, taken from between the cysteic acid zone and the anode end of the paper (see diagram) and showing the same background colour as the paper in general. The elute from this paper gives a reference value.

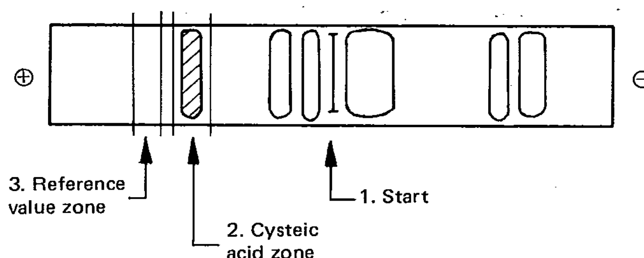

FIGURE — **Diagram of electrophoretic separation**

Pipette 5 ml of methanol (3.1) into each of the four test tubes. After light shaking several times, the red colour is dissolved from the paper within 30 min.

6.6 Colorimetry

Make the optical density measurements at a wavelength of 500 ± 10 nm in 10 mm cells. The optical density value should lie in the range 0,2 to 0,7. If higher values are obtained or if a very high cysteic acid content is anticipated, either use a smaller test specimen or make the hydrolysate up to volume in a 10 ml measuring flask.

Filter the cysteic acid eluates obtained from the cysteic acid standard solution and from the hydrolysate to remove paper residues. Carry out the photometric measurements each using the eluate from the ground shade of the paper as a "blank".

1) The modified procedure for samples from carbonized wool serves merely to remove residual sulphuric acid by precipitation as barium sulphate. If the residual sulphuric acid is not removed, it can bring about the formation of serine sulphate during concentration. Serine sulphate behaves similarly to cysteic acid during electrophoresis and its presence may lead to inaccurate values for cysteic acid content.

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7 CALCULATION OF THE CYSTEIC ACID CONTENT

The cysteic acid content, C, expressed as a percentage by mass, is given by the formula :

$$C = \frac{C_S \times V_T}{V_H \times 10^6} \times \frac{D_H}{D_S \times m} \times 100$$

where

C_S is the mass, in micrograms, of cysteic acid contained in the volume of standard solution applied;

V_T is the volume, in millilitres, of the measuring flask in which the hydrolysate was made up;

V_H is the volume, in millilitres, of hydrolysate applied;

D_H is the optical density of the cysteic acid zone from the hydrolysate;

D_S is the optical density of the cysteic acid zone from the standard solution;

m is the dry mass, in grams, of the analysis specimen.

If the same quantities are always used for C_S, V_T and V_H, the above formula is simplified as follows :

$$C = \text{factor} \times \frac{D_H}{D_S \times m} \times 100$$

Express the result to two decimal places.

8 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.

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ANNEX

ACCURACY OF THE METHOD

Following a preliminary interlaboratory trial, a second trial involving ten laboratories was carried out.

Each laboratory prepared two hydrolysates (one 24 h hydrolysate and one 4 h hydrolysate) from two wools of differing cysteic acid content, duplicate determinations being made on each hydrolysate.

The results for the two different hydrolysis times agreed well; hence, the table below gives only the values for 24 h hydrolysis.

	Wool A	Wool B
Mean cysteic acid content of wool, %	0,20	0,75
Estimated value for the components of standard deviation s_L between laboratories, % cysteic acid	not significant	0,066
Estimated value for the components of standard deviation s_H between the hydrolysates, % cysteic acid	0,013	0,026
Estimated value for the components of standard deviation s_R between the repeats, % cysteic acid	0,012	0,015

These data make possible the calculation of the confidence limits that may be expected in a cysteic acid content determination by the method described.

A.1 CONFIDENCE LIMITS FOR THE RESULTS OF ONE LABORATORY

If the standard deviation between laboratories is not taken into account (comparison within a laboratory), the confidence limits, T_i, of the mean values for a laboratory at 95 % statistical significance (5 % probability of error) are given approximately by the formula

$$T_i = \pm 2 \sqrt{\frac{s_H^2}{j} + \frac{s_R^2}{j \times k}} \text{ \% cysteic acid,}$$

where j is the number of hydrolysates and k the number of determinations per hydrolysate.

Approximate values for s_H and s_R should be taken from the above table.

A.2 CONFIDENCE LIMITS TAKING ACCOUNT OF STANDARD DEVIATION BETWEEN LABORATORIES

If the standard deviation between laboratories is taken into account (comparison between different laboratories), then the confidence limits, T_L, at 95 % statistical significance (5 % probability of error) are given approximately by the formula

$$T_L = \pm 2 \sqrt{\frac{s_L^2}{i} + \frac{s_H^2}{i \times j} + \frac{s_R^2}{i \times j \times k}} \text{ \% cysteic acid}$$

when each of the i participating laboratories carry out j hydrolysates with k tests on each.

When $i = 1$, the confidence limits for individual laboratories with interlaboratory variance taken into account are obtained.

Approximate numerical values for s_L, s_H and s_R should be taken from the table.

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