WI 160802.02

T 504

DRAFT NO. 04 SARG

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SUBJECT CATEGORY Chemical Properties

RELATED METHODS See "Additional Information"

CAUTION:
This Test Method may include safety precautions which are believed to be appropriate at the time of publication of the method. The intent of these is to alert the user of the method to safety issues related to such use. The user is responsible for determining that the safety precautions are complete and are appropriate to their use of the method, and for ensuring that suitable safety practices have not changed since publication of the method. This method may require the use, disposal, or both, of chemicals which may present serious health hazards to humans. Procedures for the handling of such substances are set forth on Safety Data Sheets (SDS) which must be developed by all manufacturers and importers of potentially hazardous chemicals and maintained by all distributors of potentially hazardous chemicals. Prior to the use of this method, the user must determine whether any of the chemicals to be used or disposed of are potentially hazardous and, if so, must follow strictly the procedures specified by both the manufacturer, as well as local, state, and federal authorities for safe use and disposal of these chemicals.

Glue in paper (qualitative and quantitative determination)

(Reconfirmation of T 504 cm-07 as a Classical Method)

(Minor editorial changes incorporated from Draft 3)

1. Scope and significance

1.1 This method deals with the qualitative and quantitative determination of animal glue in paper in quantities that are used for sizing high grade papers (*1*). It is applicable to glue hardened with formaldehyde, alum, or amino-formaldehyde resins, and to photographic papers. This method can also be used in the determination of gelatin (a more highly refined form of animal protein).

1.2 The method is based on the color reaction of Ehrlich's reagent (*p*-dimethyl-aminobenzaldehyde) with hydroxyproline, one of the amino acids derived from collagen. It is specific for glue and gelatin; other nitrogenous

materials do not interfere.

1.3 Two qualitative procedures are given, which are essentially the same: a macromethod where at least 100 mm^2 of paper is available and a micromethod where only 10 mm^2 can be used.

1.4 The scope of the quantitative procedure is given in 3.1.

NOTE 1: *Safety precautions* - This method requires the use of hazardous chemicals. Please refer to specific Material Safety Data Sheets for personal protective equipment, safe handling precautions, and disposal requirements.

2. Qualitative determination

2.1 *Apparatus*

2.1.1 *Boiling water bath*, a 600- or 100-mL beaker heated on an electric hot plate is satisfactory.

2.1.2 *Clamps*, to hold test tubes in the bath. Thermometer clamps attached to the rim of the beaker may be used for the micromethod.

2.1.3 *Glassware*, volumetric flasks, 100-, 500-, and 1000-mL. For macromethod: test tubes, 16 x 150 mm; pipets, two each, 5 and 1 mL with 0.1-mL graduations; one 0.2-mL with 0.01-mL graduations. For micromethod: test tubes, 10 x 75 mm; pipets, two 1-mL with 0.1-mL graduations; three 0.1-mL with 0.01-mL graduations.

2.2 *Reagents*

2.2.1 *Sodium hydroxide.* For the macromethod, approximately 12.5*N* NaOH: dissolve 50 g NaOH in water and dilute to 100 mL. For the micromethod, 6*N* NaOH: dissolve 24 g NaOH in water and dilute to 100 mL.

2.2.2 *Copper sulfate*, approximately 0.01*M* $CuSO_4$: dissolve 1.25 g $CuSO_4 \cdot 5H_2O$ in water and dilute to 500 mL.

2.2.3 *Hydrogen peroxide*, approximately 4% H_2O_2: dilute 13 mL of 30% H_2O_2 with water to 100 mL.

2.2.4 *Sulfuric acid*, approximately 3*N* H_2SO_4. Add 83 mL concentrated H_2SO_4, slowly and with stirring, to about 800 mL water, cool, and dilute to 1000 mL.

2.2.5 *1-Propanol* (reagent, b. p. 96-98°C).

2.2.6 *p-Dimethylaminobenzaldehyde* reagent, purified grade. Obtain in 10- or 25-g bottles and refrigerate after opening. The crystals should be white and the solution practically colorless.

2.2.7 *Ehrlich's reagent*: dissolve 1 g of *p*-dimethylaminobenzaldehyde in 20 mL of 1-propanol. The solution may be used as long as it is colorless or yellowish but should be discarded if it develops a green, brown, or red color.

2.3 *Test specimens*

2.3.1 *Macromethod*: From each test unit obtained in accordance with TAPPI T 400 "Sampling and Accepting a Single Lot of Paper, Paperboard, Containerboard, or Related Product," take a specimen having a total area of about 100 mm^2 of the paper and cut into small pieces.

2.3.2 *Micromethod*: Take a specimen approximately 3 mm square. If the paper is thicker than 0.125 mm (5 mils), peel off and use only the surface layer or layers.

2.4 *Procedure*

2.4.1 *Macromethod*

2.4.1.1 Carefully pipet 0.20 mL of 12.5*N* NaOH into the bottom of a 16 x 150-mm dry test tube, touching the bottom of the tube with the tip of the pipet to remove the last drop. Hold the tube in a vertical position and add the test specimen. Place the test tube in the boiling water bath for at least 10 min. Remove from the bath, cool for a few seconds with tap water, add 1 mL of 0.01*M* $CuSO_4$, mix and add 0.5 mL of 4% H_2O_2, without allowing it to contact the upper part of the tube. Shake occasionally until most of the foaming stops and return the tube to the boiling water for 5 min. The peroxide must be completely decomposed before the H_2SO_4 is added.

2.4.1.2 Again cool with tap water for a few seconds, add 2.5 mL of 3*N* H_2SO_4 and 1.5-2.0 mL of Ehrlich's reagent and mix well. Cool the water bath to 80-90°C by adding cool water and return the test tube to the bath. If a rose-red or pink color develops within about 10 min, the presence of glue is indicated.

2.4.2 *Micromethod*: Carefully pipet 0.015 mL of 6*N* NaOH onto the bottom of a 10 x 75-mm dry test tube, touching the bottom of the tube as before to remove the last drop. Hold the tube in a vertical position, add the test specimen, and proceed as above, except use 0.05 mL of $CuSO_4$, 0.025 mL of H_2O_2, 0.125 mL of H_2SO_4 and 0.1 mL of Ehrlich's reagent.

2.5 *Report.* Report the presence or absence of glue as indicated above.

3. Quantitative determination

3.1 *Scope and summary*

3.1.1 This procedure (*2*) determines glue in the presence of melamine resin. It may be used in the presence of other additives such as glycerin, alum, and rosin. The recovery of glue from papers made from rag pulps is almost complete. Recovery from wood pulp papers and from papers containing groundwood is not complete, but the data are possibly sufficiently accurate for most purposes.

3.1.2 The glue is extracted from the paper by refluxing with 0.1*N* HCl. An aliquot of the extract is hydrolyzed with 6*N* HCl at 110°C. The hydroxyproline in the hydrolyzate is reacted with Ehrlich's reagent to give a colored compound with an absorbance maximum of 560 nm. The absorbance is a measure of the amount of glue on the paper.

3.1.3 The procedure is a specific test for glue. It is not intended to replace TAPPI T 418 “Organic Nitrogen in Paper” when interfering materials are absent.

3.2 *Apparatus*

3.2.1 *Spectrophotometer,* for making absorbance measurements of 560 nm, and equipped with quartz or glass absorption cells. A filter colorimeter may be used, but it is not as satisfactory as a spectrophotometer.

3.2.2 *Reflux assembly,* all glass, consisting of a 250-mL Erlenmeyer flask and water-cooled condenser with standard taper connections.

3.2.3 *Oil bath,* maintained at a temperature of 110 ± 2°C.

3.2.4 *Water bath,* maintained at a temperature of 70 ± 0.2°C.

3.2.5 *Volumetric flasks,* at least five 25-mL, glass-stoppered, made of acid-resistant glass with provision for clamping the stopper against the pressure generated during hydrolysis in 6*N* HCl at 110°C. Also at least five 100-mL, one 200-mL and one 500-mL stoppered flasks.

3.2.6 *Pipets*: 1, 2, 3, 4, 10, 20, 30, 40, and 50 mL.

3.2.7 *Graduated cylinders*: 25, 100, 500, and 1000 mL.

3.2.8 *Nitrogen,* cylinder of compressed gas, water pump.

3.2.9 *Timer,* stop watch or electric timer.

3.2.10 *Other equipment*: glass-stoppered weighing bottles, 25 mL; porcelain crucible, 25 mL, with lid; filter funnels; hardened, fast filter paper; powder funnel with short wide stem.

3.3 *Reagents*

3.3.1 *Glue standard.* If possible, use the same glue for reference standards as used in the paper. If this is not possible, use a good grade of hide glue or purified calfskin gelatin. (See Appendix, Table A1.)

3.3.2 *Hydrochloric acid,* concentrated HCl, approximately 12*N*, also approximately 0.1*N* HCl, not standardized (8 mL concentrated HCl diluted to 1000 mL).

3.3.3 *Sodium hydroxide,* 2.5*N* NaOH: dissolve 100 g of NaOH pellets in about 800 mL of water, cool and dilute to 1000 mL in a graduated cylinder.

3.3.4 *Sulfuric acid,* 3*N* H_2SO_4: pour (slowly) 83 mL concentrated H_2SO_4 into 800 mL of water with stirring, cool and dilute to 1000 mL.

3.3.5 *Hydrogen peroxide,* 4% H_2O_2 prepared fresh daily by diluting 10 mL of 30% H_2O_2 to 75 mL in a graduated cylinder.

3.3.6 *Copper sulfate,* 0.02*M* $CuSO_4$. Dissolve 5 g of $CuSO_4 \cdot 5H_2O$ in water and dilute to 1000 mL in a graduated cylinder.

3.3.7 *1-Propanol,* reagent, b.p. 96-98°C.

3.3.8 *p-Dimethylaminobenzaldehyde.* Use only purified material packed under inert atmosphere or purified by one of the methods in 3.3.8.1 and 3.3.8.2 below. Obtain in 10-or 25-g bottles and refrigerate after opening. The crystals should be white and the solution should be practically colorless. The *p*-dimethylaminobenzaldehyde may be purified by recrystallizing, using one of the following methods:

3.3.8.1 Dissolve 25 g in 140 mL of approximately 2*N* HCl. Precipitate by slowly adding 4*N* NaOH and stirring. If a brown color develops, add a little of the HCl solution. Filter and wash with water. Repeat until crystals are white. Vacuum dry at room temperature.

3.3.8.2 Dissolve the *p*-dimethylaminobenzaldehyde in 95% ethyl alcohol (2 mL of alcohol for each gram) at 70°C. Cool; add water as long as a precipitate forms. Filter and wash with water. If necessary, repeat until crystals are white. Vacuum dry at room temperature.

3.3.8.3 For the reagent: dissolve 5 g *p*-dimethylaminobenzaldehyde in 1-propanol and dilute to 100 mL with 1-propanol. Prepare the solution fresh daily. Store the dry solid in a refrigerator after the bottle has been opened.

3.3.9 *L-hydroxyproline,* reagent grade.

3.3.10 *Ethyl alcohol,* 95% C_2H_5OH.

3.4 *Test specimens*

3.4.1 From each test unit obtained in accordance with TAPPI T 400, take a 1-g specimen, tear or cut into pieces approximately 5 mm square, and weigh it to the nearest 0.1 g.

3.4.2 Determine the moisture content of another specimen in accordance with TAPPI T 412 "Moisture in Paper and Paperboard."

3.5 *Procedure*

3.5.1 Reflux the weighed specimen with 100 mL of 0.1*N* HCl for 1 h, filter through the hardened filter paper into a 200-mL volumetric flask, wash the filter paper well with hot 0.1*N* HCl, dilute the filtrate in the volumetric flask to the mark with water and mix. If the extract is to be used for the determination of melamine resin also, dilute it with 0.1*N* HCl.

3.5.2 Pipet an aliquot of this solution containing 0.2 mg to 0.4 mg of glue, into a 25-mL volumetric flask. With a pipet, add water to make exactly 4 mL.

NOTE 2: Use 4 mL of the extract from the paper if the paper contains less than 2% glue; 2 mL if between 2 and 4% glue; and 1 mL if the glue content is greater than 4%.

3.5.3 Pipet 4 mL of concentrated HCl into the volumetric flask. Close with a ground glass or Teflon stopper, securing the stopper with a spring clamp. Submerge the flask to a point 15 or 20 mm below the stopper in an oil bath held at 110°C. Allow the flask to remain overnight in the oil bath. Remove the stopper, return the flask to the oil bath, and evaporate the contents in a current of nitrogen flowing through the flask at a rate of about 150 mL/min. A small glass tube, bent at right angles where it enters the flask, with the outlet about 10 mm above the liquid level is satisfactory for the purpose. A thermometer clamp may be used to hold the flask in the bath.

NOTE 3: The flow of nitrogen may be checked by displacement of water from an inverted graduated cylinder, with its open end submerged.

NOTE 4: It is important that all the acid, including that which condenses in the neck of the flask, be completely removed. Otherwise, part of the NaOH will be neutralized and NaCl formed in a subsequent step. Both interfere seriously with determination.

3.5.4 With a pipet, add 2 mL of water and dissolve the residue. Pipet into the flask 1 mL each of 0.02*M* $CuSO_4$, 2.5*N* NaOH, and 4% H_2O_2, mixing after each addition. Allow to stand for 30 min at room temperature, then destroy the excess peroxide by heating in a water bath at 70°C for 5 min. Shake well and cool to room temperature. Pipet 3 mL of 3*N* H_2SO_4 and 3 mL of the 5% *p*-dimethylaminobenzaldehyde reagent into the flask, shaking after each addition, and place in a water bath at 70°C for 50 min. During the procedure heat every flask, including the reference solution, for exactly the same length of time at the same temperature, and arrange for the same time intervals between removing each from the 70° bath and reading the absorbance of its solution. Cool at room temperature (an ice bath is desirable), dilute to the mark with water, mix, transfer a portion to an absorption cell, and measure the absorbance at 560 nm, using a reagent blank as a reference solution.

3.6 *Calculations*

3.6.1 Calculate the glue content of the paper from one of the following formulas, depending on which material was used in the preparation of the calibration curve. Convert micrograms to grams before calculating.

3.6.1.1 If glue is used as a standard,

$$\text{Glue, \%} = \frac{GV}{AW} \times 100$$

3.6.1.2 If hydroxyproline is used as a standard,

$$\text{Glue, \%} = \frac{HV}{0.126 \times AW} \times 100$$

3.6.1.3 If purified calfskin gelatin is used as a standard,

$$\text{Glue, \%} = \frac{0.145 \times PV}{0.126 \times AW} \times 100$$

where:

G	=	glue in the test solution, indicated by calibration curve, g
H	=	hydroxyproline in test solution, indicated by calibration curve, g
P	=	purified gelatin equivalent in the test solution, indicated by calibration curve, g
V	=	volume of extract, mL
A	=	aliquot taken, mL
W	=	weight of moisture-free specimen, g

NOTE 5: The factor 0.126 represents the fraction by weight of hydroxyproline in a typical hide glue. The factor 0.145 represents the fraction by weight of hydroxyproline in purified calfskin gelatin.

3.7 *Report.* Report the average of two determinations of glue content of the paper as a percentage of the oven-dry paper to the nearest 0.1%. Indicate in the report which standard was used in the calibrations.

3.8 *Precision*

3.8.1 Repeatability (within laboratory) = 0.2%.

3.8.2 Reproducibility (between laboratories) = not known, in accordance with definitions of these terms in TAPPI T 1200 “Interlaboratory Evaluation of Test Methods to Determine TAPPI Repeatability and Reproducibility.”

4. Keywords

Analysis, Glue, Paper, Gelatin, Hydroxyproline

5. Additional information

5.1 Effective date of issue: to be assigned.

5.2 The quantitative procedure described here is empirical and possible variables are not all completely understood. Accordingly, the analyst should rigidly standardize the procedure and check it by testing at least three reference standards in triplicate each time a group of tests is made.

5.3 The production of color complex is very sensitive to the slightest change in reagents or experimental conditions, so that a calibration curve on one occasion can serve only as a rough guide for the next time.

5.4 The hydroxyproline content of various samples of glue and gelatin was found to be as follows:

Material	*Hydroxyproline, %*
Hide glue	12.4-12.8, average 12.6
Bone glue	10.8-11.8, average 11.4
Purified calfskin gelatin	14.4-14.8, average 14.5

5.5 Two qualitative detection procedures are included in order to cover all situations. The micro procedure is particularly applicable in cases where the amount of sample is small and insufficient to permit the use of the macro procedure. The macro procedure is, however, more accurate and is to be preferred in all instances where adequate sample is available.

5.6 This method was reclassified as a Classical Method in 1997. A classical method is one that is no longer in common use or has been superseded by advanced technology. Such methods are technical sound, have a history of use, and contain a body of references that make their preservation valuable.

Appendix A. Calibration

A.1 Prepare a calibration curve from pure L-hydroxyproline, from purified calfskin gelatin (Eastman No. 1099 or equivalent), or preferably the glue that was used to size the paper. See Table A1 for approximate dilutions required in preparing a calibration curve.

A.1.1 *Calibration with pure L-hydroxyproline.* Dry the hydroxyproline 12 to 16 h in a desiccator. Prepare a stock solution by dissolving in water exactly 100 mg of L-hydroxyproline, weighed to the nearest 0.2 mg, and diluting to 1000 mL in a volumetric flask. Prepare solutions containing 10, 20, 30, 40, and 50 g/mL by pipetting 10, 20, 30, 40, and 50 mL of the stock solution into 100 mL volumetric flasks and diluting to the mark with water. Pipet 2 mL of each of these solutions into 25-mL glass-stoppered volumetric flasks, add 1 mL each of 0.02*M* $CuSO_4$, 2.5*N* NaOH, and 4% H_2O_2 and complete as described under procedure above. Plot the absorbance at 560 nm against the hydroxyproline concentration.

A.1.2 *Calibration with glue.* Weigh about 0.50 g of glue in a glass-stoppered weighing bottle, heat in an oven at 105 ± 2°C for at least 2 h or overnight to drive off the moisture. Cool in a desiccator, and weigh to 0.2 mg.

Using a powder funnel, transfer the bulk of the glue to a 500-mL volumetric flask. Reweigh the weighing bottle and calculate the glue concentration from the difference, correcting for the ash found by ashing a 2-g dried specimen at 450°C in a weighed crucible. Add about 100 mL water to the volumetric flask, washing any glue on the funnel into the funnel into the flask, and allow to swell at least 2 h or overnight. Heat to about 60°C to effect solution, cool to room temperature, dilute to mark, and mix.

Table A1. Approximate dilutions required for calibration curve

Stock solution, mg/1000 mL	*Volume of stock solution diluted to 100 mL, mL*	*Concentration in 100 mL of solution, mg/1000 mL*	*Taken for analysis*		*Approximate glue equivalent, g*
			mL	*g*	
		Hydroxyproline			
100	10	10	2	20	160
	20	20	2	40	320
	30	30	2	60	480
	40	40	2	80	640
	50	50	2	100	800
		Glue			
800	10	80	2	160	—
	20	160	2	320	—
	30	240	2	480	—
	40	320	2	640	—
	50	400	2	800	—

A.1.2.1 Pipet 10, 20, 30, 40, and 50 mL of this solution into 100-mL volumetric flasks, dilute each to the mark with water and mix. These solutions contain approximately 80, 160, 240, 320, and 400 mg of glue per mL. Calculate the exact concentration from the concentration of the stock solution.

A.1.2.2 Pipet 2 mL of each of these solutions and 2 mL of concentrated HCl into 25-mL glass-stoppered volumetric flasks and carry through as described under procedure above. Plot the absorbance at 560 nm against the glue concentration. Run duplicates on each solution.

A.1.3 *Calibration with purified calfskin gelatin* (Eastman No. 1099 or equivalent). Use the same procedure as given above for glue; however, a correction for ash is unnecessary.

References

1. Tutt, R., Jr., and Lane, L. B., "Animal Glue in Coated Paper," *Synthetic and Protein Adhesives for Paper Coating*, Chapter X, TAPPI Monograph Series No. 22 (1961).
2. Hutterer, F., and Singer, E. J., "A Modified Method for Hydroxyproline Determination," *Anal. Chem.* **32**:556 (1960).

Your comments and suggestions on this procedure are earnestly requested and should be sent to the TAPPI Standards Department. ■