STANDARD TESTING METHODS FOR EDIBLE GELATIN

Official procedures of the Gelatin Manufacturers Institute of America, Inc.

Revised: January 2019

http://www.gelatin-gmia.com

Table of Contents

1. General Information

The Standard Methods for the Sampling and Testing of Edible Gelatin contained in this booklet are the results obtained by a co-operative testing program conducted by the Technical Staffs of the entire membership of the Gelatin Manufacturers Institute of America. These methods have been found to give accurate and consistent results.

1.1 Member Companies

The Gelatin Manufacturers Institute of America (GMIA) consists of five member companies who are leading gelatin manufacturers in North America. For more information on member companies, please follow link below.

GMIA Member Companies

1.2 Definition

Gelatin is the product obtained from the acid, alkaline, or enzymatic treatment of collagen, the chief protein component of the skins, bones, and connective tissues of animals, including fish and poultry. (These animal sources shall have not been exposed to pentachlorophenol.)

Two types of processes are most commonly used to produce gelatin:

Type A gelatin: Produced by the acid processing of collagenous raw materials and exhibits an isoelectric point between pH = 6.0 and pH = 9.5.

Type B gelatin: Produced by the alkaline or lime processing of collagenous raw materials and exhibits an isoelectric point between pH = 4.7 and pH = 5.6.

Mixtures of Types A and B as well as gelatins produced by modifications of the above mentioned processes might exhibit isoelectric points outside of the stated ranges.

For additional information regarding the processing, characteristics, use and functionality of gelatin, please refer to the *GMIA Gelatin Handbook*.

1.3 Description

Gelatin is nearly tasteless and odorless. It is a vitreous, brittle solid that is faintly yellow to light tan. It is supplied in various physical forms such as coarse granules, fine powders and leaves.

1.4 Stability

Gelatin is very stable when stored in its original, sealed container in ambient warehouse conditions. The shelf life of gelatin is generally recognized as five (5) years (minimum) when stored under these conditions. After five years, it is recommended that physical/chemical properties such as Gel Strength (Bloom) and Loss on Drying (Moisture) are reanalyzed to reestablish these values. Gelatin that is stored as described above will remain safe and suitable for use.

1.5 Identification A (Biuret Method)

Principle

Reactions between proteins and copper ions in an alkaline medium produce a violet colored solution.

References

1. US Pharmacopoeia (current edition)

Reagents and Solutions

1. Dry gelatin
2. Distilled/deionized (purified) water
3. Copper sulfate pentahydrate ($CuSO_4.5H_2O$) 125-g/L purified water
4. Sodium hydroxide (NaOH) 85-g/L purified water

Apparatus

1. Standard laboratory glassware
2. Water bath at approximately 55°C

Procedure

1. Dissolve 1 g of sample in purified water at about 55°C. Dilute to 100 ml with the same solvent and hold the solution at this temperature.
2. To 2 mL of solution add 0.05 mL of a 125-g/L solution of copper sulfate pentahydrate. Mix, and add 0.5 mL of an 85-g/L solution of sodium hydroxide.

Result

A violet color is produced.

1.6 Identification B (Gel-test)

Principle

Gelling-grade gelatins form a gel under the described test conditions. Non-gelling gelatins (e.g. gelatin hydrolysate, hydrolyzed collage, collagen peptides, etc.) and other proteins do not form a gel.

References

1. US Pharmacopoeia (current edition)

Reagents and Solutions

1. Dry gelatin
2. Distilled/deionized water

Apparatus

1. Standard laboratory glassware
2. Water bath at approximately 60°C
3. Refrigerator

Procedure

1. Place the *Sample* in a test tube of about 15 mm internal diameter and add 10 mL of water. Allow to stand for 10 min.
2. Heat at 60°C for 15 min.
3. Keep the tube upright at 0°C (or in refrigerator 0-10°C) for 6 hours. Invert the tube.

Result

The contents do not flow out immediately for gelling grades. The contents immediately flow out for non-gelling grades.

1.7 Identification C (Hydroxyproline Content – Qualitative Method)

Principle

Gelatin contains a high amount of the amino acid hydroxyproline. Hydroxyproline is liberated through acid hydrolysis, oxidized, and then identified with Erlich's reagent (5% *p*-dimethylaminobenzaldehyde in *n*- propanol).

References

1. US Pharmacopoeia (current edition)

Reagents and Solutions *(All reagents must be analytical grade)*

1. 0.05N $CuSO_4$
2. 2.5N NaOH
3. 6% H_2O_2
4. 3N H_2SO_4
1. Erlich's Reagent – 5% *p*-Dimethylaminobenzaldehyde in *n*-Propanol (make fresh each time).

Apparatus

1. Oil bath, capable of 145°C.
2. Water bath, 40°C
3. Ice bath
4. Bunsen burner
5. 18 x 150 mm test tubes
6. 500 mL volumetric flask
7. Aluminum foil
8. Distilled/deionized water
9. Concentrated HCl

Procedure

1. Dissolve 1.0 gram of material in 200 mL of water.
2. Add 3 mL of this solution and 3mL of conc. HCl to an 18 x 150 mm test tube. Seal by melting the top in a Bunsen burner. Hydrolyze at 145°C for 1.5 hours in an agitated oil bath.
3. Cool the hydrolysate, cut off the top of the tube, transfer contents to the volumetric flask and dilute to 500 mL.
4. Transfer 1 mL to an 18 x 150 mm test tube. Add 1 mL 0.05N $CuSO_4$, 1 mL 2.5N NaOH, and place in a 40°C water bath for 5 minutes.
5. Add 1 mL 6% H_2O_2 and mix immediately.

6. Keep the sample at 40°C for 10 minutes. Shake and rotate to remove all excess H_2O_2.
7. Cool rapidly in an ice bath. Add 4 mL 3N H_2SO_4, mix, then add 2 mL Ehrlich's reagent, mix, cover with aluminum foil and hold at room temperature for 15 minutes.

Result

The development of an intense red color shows the presence of hydroxyproline.

1.8 Sampling

Principle
Aseptic gelatin samples are required for all testing. The square root plus one method should be followed to determine the number of samples per lot.

Reagents and Solutions
None

Apparatus
1. Sterile gloves
2. Scoop
3. Suitable container

Procedure
1. Take aseptic sample by scooping out a cone several inches below surface of the gelatin in the container.
2. Pull the scoop up, across the vertical surface of the cone to obtain a representative sample.
3. Place samples as drawn in clean, suitable containers.
4. Proportion the amount taken from each container selected to at least 120g of sample per container.
5. Blend or mix samples thoroughly.
6. Withdraw and retain at least 500g as the final sample.

2. Physical / Chemical Testing

2.1 Gel Strength

Principle

The gel strength of gelatin is a measure of the rigidity of a gel formed from a 6.67% solution and prepared according at 60°C, cooled to 10.0°C for 17 h. Bloom is a measure of force (weight) required to depress a standard plunger into the gel sample a distance of 4 mm.

References

BSI	British Standards Institution, BS 757:1975
AOAC	Association of Official Analytical Chemist, Official Methods of Analysis 23.007
Eur. Ph.	European Pharmacopoeia, (current edition)
AFNOR	Norme Francaise, NF V 59-001
JIS	Japanese Industrial Standard, K 6503-1970
PAGI	Methods for Testing Photographic Gelatine; Photographic and Gelatine Industries, Japan; Seventh Edition 1992
USP/NF	United States Pharmacopoeia – National Formulary (current edition)

Reagents and Solutions

1. Dry gelatin
2. Distilled/deionized water

Apparatus

1. For measurement of gel strength the following gelometers can be used
    a. Lloyd TA Plus
    b. LFRA Texture Analyser (Brookfield)
    c. LFRA Texture Analyser CT3 (Brookfield)
    d. Texture Analyser TA-XTplus (Stable Micro Systems)
    e. Texture Analyser TA-XT2i (Stable Micro Systems)
    f. Texture Analyser TAXT Express (Stable Micro Systems)
    g. Zwick /Roell.
2. Plunger (AOAC) with 12.70 mm (0.500 inches) diameter, plane surface and sharp edge, no measurable radius. **The GMIA recommends replacing this plunger every six months.**
3. Stir rods (plastic, stainless steel or brass) of sufficient length and thickness (e.g. approximately 3 mm diameter and 15 cm in length, tapered at one end).

4. Bloom jar [available from Schott or Brookfield Engineering(Type TA-GBB-2)]: capacity 150-155 mL, overall height 85 mm, inside diameter 59 mm, outside diameter 66 mm, neck inside diameter 41 mm, shoulder height 65 mm. All linear dimensions are ± 1.0 mm. Bottle must have flat bottom to ensure it does not rock on a flat surface. Uneven bottles should be corrected by grinding or rejected.
5. Single-hole stoppers appropriate in size to fit Bloom jar.
6. Analytical balance with 0.0 1g sensitivity.
7. Thermostatic water bath capable of uniform temperature at 65 ± 2°C with agitation. The bath should be provided with platform at such height that the water level is approximately 1 cm above the surface of the test gelatin solution in the Bloom bottle.
8. Thermostatic water bath, same as point 7 above, held at 45 ± 2°C with agitation (optional)
9. Refrigerated water bath with agitation, with heating and cooling units, capable of maintaining water at 10 ± 0.1°C throughout the bath. The bath should be provided with a platform at such height that the water level is approximately 1 cm above the surface of the test gelatin solution in the Bloom bottle. The platform must be level ensuring sample test surface is level. *Note: the design of the chill bath should be such that it will recover to 10 ± 0.1°C within one hour after the bloom samples are placed in the bath. This may limit the number of sample jars that can be placed in the water bath.*
10. Dummy bloom strip device available from Brookfield Engineering or internal gelatin standards.

Procedure

1. Weigh 7.50 ± 0.01 g of gelatin into the bloom bottles using an analytical balance.
2. Add 105.0 ± 0.2 g of distilled water at 25 ± 2°C. Stir with a stirring rod while adding the water ensuring that **all** of the gelatin particles are moistened. Wash any gelatin adhering to the rod into the bottle with the last portion of water as needed.
3. Insert a perforated stopper or cover bottle with a watch glass and allow the sample to hydrate 1-4 hours at room temperature.
4. Place the sample bottle in the 65°C bath for dissolving of the sample. Stir or swirl periodically. After 8-10 minutes the bottle may be removed from the bath and swirled. Replace the bottle in the bath (if removed). Continue to swirl or gently stir the bottles until all gelatin is in solution. Once all of the gelatin is clearly in solution, the sample bottle should be removed from the water bath. For most gelatins, the total time in the bath should not exceed 15 minutes. Depending on manufacturing conditions some gelatins may require more time in the water bath. Care should be taken so that the total time does not exceed 25 minutes. **It is critical that all of the gelatin is in solution before proceeding to the next step.**
5. Temper the sample by letting it stand for 15-20 minutes at room temperature. Alternatively, temper the sample by placing the bottle in the 45°C bath for 30-45 minutes.

6. After tempering, gently swirl the solution in the Bloom bottle to remove condensation from the sides of the bottle as needed. Remove the stopper and gently remove all foam from the center of the gelatin solution surface with a spoon or other convenient means to ensure a smooth surface for the gelometer plunger. Replace the stopper and place the sample bottle in the 10.0 ± 0.1°C water bath for 17 ± 1 h .
7. Set the gelometer according to the manufacturer's manual. ***Settings are: Distance 4mm, Speed 0.5 mm/sec (Alternative Speed: 1.0 mm/sec).***
8. Remove the jelled sample from the 10°C bath and quickly wipe the water from the exterior of the bottle. Remove the stopper and center the bottle on the gelometer platform so that the plunger contacts the sample as close its midpoint as possible.
9. Start the measurement [e.g. press "Start" (LFRA) or "Run" (TA.XT2)] to run the analysis. (If there is any foam in the area of the sample where the plunger makes contact, start the determination over.) (If using the LFRA, check the zero weight often.) Repeat as needed until all bloom determinations are complete.

Note: The sample prepared for this analysis may also be used for Viscosity analysis. In these cases, during step 4 above, a thermometer should be inserted into the sample. Once the temperature of the solution reaches 61°C and the sample is completely dissolved and thoroughly mixed, remove the thermometer and transfer to a viscometer and determine the viscosity according to the designated procedure (see Viscosity Procedure). Collect all of the effluent from the viscometer in the original bottle and immediately replace the stopper. Proceed with step 5 above to continue Gel Strength analysis.

Result

1. The value given by the gelometer is the gel strength (grams Bloom) for the test gelatin (not corrected for moisture).
2. ***Moisture Correction:*** An approximate Bloom value that corresponds to a certain moisture content can be calculated using the following formula:

$$B2 = B1 + B1 \times 0.02 \times (M1 - M2)$$

(B2 = Bloom at moisture M2, B1 = Bloom at moisture M1)

Quality Control

1. Check calibration with standard weights at least monthly per manufacturer's instructions.
2. Monitor daily operation with the use of the Dummy Bloom strip or internal gelatin standards of determined bloom strength. The Dummy Bloom Strip must read ± 1 g to accept test results. Internal gelatin standards must fall within acceptable SPC ranges determined during development of the standard(s) to accept test results.
3. Repeatability within one lab of 1.5% standard deviation is realistic.
4. Reproducibility between labs using the same procedure: Ring test results indicate 3% or better standard deviation is achievable.

Example Texture Analyzer Set-Up

TA.XT2	**LFRA** (Power on – allow 15 minute warm-up).
Speed = 0.5 or 1.0 mm/sec	Penetration Speed = 0.5 or 1.0 mm/sec
Distance = 4 mm	Penetration Distance = 4 mm
Trigger = 5	Cycle = Normal
Pre = 2.0	Adjust the test platform , assuring it is level and the probe is at least 10
Post = 5.0	mm above the surface of the sample.
Penet = 1.0	Set the digital readout to "0"
PHT = 1.0	
Test Output = Final	

2.2 Viscosity

Principle

The viscosity of a 6.67% gelatin solution is determined at 60°C by measuring the flow time of 100 mL of the solution through a standard pipette.

For hydrolyzed collagen, the viscosity of a 20% solution is determined at 25°C by measuring the flow time of 100 ml of the solution through a 100 ml pipette.

Viscosity results are typically expressed in mPa.s or mP.

References

1. GME Monograph, February 2018, Version 13

Reagents and Solutions

1. Calibration Oils: Two standard (reference) viscosity oils calibrated at 60°C, having viscosities within the approximate range 2-10 cS. One oil should have a viscosity at least twice that of the other oil.

Apparatus

1. PIPETTE: 100 ml pipette with a precision capillary outlet and an upper and lower mark on the glass. (See "Viscosity Pipette" below)
2. THERMOSTATIC BATH: for the pipette with a thermostatic device for stirring and heating (60°C or 25°C, ± 0.1°C).
3. PRECISION THERMOMETER: graduated in 0.01°C with a long slim stem for measuring the temperature inside the pipette.
4. STOP WATCH: accurate to 0.1 seconds.
5. BALANCE: with 0.01 g sensitivity
6. THERMOSTATIC WATER BATH: for sample preparation, held at 65°C ± 2 or 40°C ± 2 (hydrolyzed collagen).

Procedure (Gelatin)

1. Weigh 7.50 g (± 0.01) gelatin into a 150 ml bottle, add 105 ml (± 0.2) water, stir, so that all the gelatin is moistened, cover the bottles with a rubber stopper or a watch glass and allow the sample to stand at room temperature for 1 - 4 h. Place the bottles in the 65°C waterbath for about 20 min. for dissolving the samples. To dissolve the gelatin completely and to achieve a homogeneous solution, the bottles should be shaken (when covered with stoppers) or stirred gently with a glass rod (when covered with watch glasses) occasionally. Take care not to build up too much froth.
2. When the temperature of the solution reaches 61 – 62°C and the sample is completely dissolved, remove the thermometer and transfer the solution to the viscosity pipette as quickly as possible without entrapping air.
3. Sufficient solution is poured into the pipette to bring its level about 1 cm above the upper mark while a finger closes the capillary end. The bottle with the residual solution is placed directly under the exit tube.
   The temperature of the solution in the pipette can be checked with the thermometer and when it has reached 60°C the measurement can be started by removing the finger and determining the flow time between upper mark and lower mark.
4. Read and record the time required for the 100 ml of solution to pass through the capillary tube of the pipette.
5. After each determination, and before use, the pipette is washed out with about 25 ml of water heated to about 62°C and allowed to drain thoroughly.

Procedure (Hydrolyzed Collagen)

1. The same equipment described in viscosity method is used, with the following modifications: The viscosity pipette must be held at 25°C (± 0.1). The water bath should be held at 40°C.
2. Weigh 100 g (± 0.1) of water into a 250 ml bottle, add either 11.1 g (± 0.01), for a 10 % solution, or 25 g (± 0.01), for a 20 % solution of hydrolyzed gelatin, stir, so that all is moistened, cover the bottles with a rubber stopper or a watch glass.
3. Place the bottles in the 40°C water bath for about 20 min. for dissolving the samples. Dissolve the hydrolyzed gelatine completely. To achieve a homogenous solution the bottles should be shaken (when covered with stoppers) or stirred gently with a glass rod (when covered with watch glasses) occasionally. Take care not to build up too much froth.
4. Reduce the temperature to about 26.5°C and then proceed as described in 6.3 -6.5 except for temperature of solution which should be 25°C.
5. Read and record the time required for the 100 ml of solution to pass through the capillary tube of the pipette.

Result

The viscosity of any sample (V) with the efflux time t may be calculated from the equation:

$$V = (A \times t - B / t) \times d$$

V = dynamic viscosity in mPa.s

For gelatin at 6,67 %, 60°C d = 1.001

For hydrolyzed collagen at 20 %, 25°C d = 1.060

Calibration

1. Pipettes can be calibrated using two standard oils of different viscosity (determination of constants A and B).
2. The pipette must be thoroughly cleaned before use and dried with reagent grade acetone.
3. Preheat each oil in a constant temperature bath set at 63 – 64°C by stirring and transfer to the pipette, holding a finger over the pipette outlet until the oil temperature is exactly 60°C as read on the immersed thermometer.
4. Measure the efflux time of the oil between the upper and lower graduation of the pipette using the stopwatch. Repeat the measurement at least three times with each oil.
5. Clean the pipette thoroughly between different oils using suitable organic solvents for removing the oil, wash out the solvent with acetone and dry.
6. Calculation of the constants A and B:
   (t = efflux time in sec; d = oil density at 60°C; A and B = pipette constants)

$$B = \frac{t_1 t_2 (V_2 t_1 - V_1 t_2)}{t_2^2 - t_1^2}$$

$$A = \frac{V_1 + B/t_1}{t_1} = \frac{V_2 + B/t_2}{t_2}$$

V1 = kinematic viscosity of lower viscosity oil in cSt

V2 = kinematic viscosity of higher viscosity oil in cSt

t1 = average efflux time of lower viscosity oil in sec

t2 = average efflux time of higher viscosity oil in sec

Remarks

1. **Internal standard gelatins**
   To ensure that the equipment and procedure is satisfactory, it is recommended that standard gelatins of established viscosity are prepared and tested regularly as reference gelatins.

   For hydrolyzed gelatins, a reference with known viscosity should also be used.

   The standard gelatins must be stored under suitable conditions so that the moisture does not change with the time.

2. **Correction of the results regarding to the moisture:**
   Moisture content of the samples influences the viscosity result for a 6.67 % solution, so that with all viscosity results also the moisture content should be indicated.

   The viscosity value that corresponds to a certain moisture content can be calculated approximately using the following formula:

   $V2 = V1 + V1 \times 0.02 \times (M1 - M2)$

   (V1 = viscosity at moisture M1, V2 = viscosity at moisture M2)

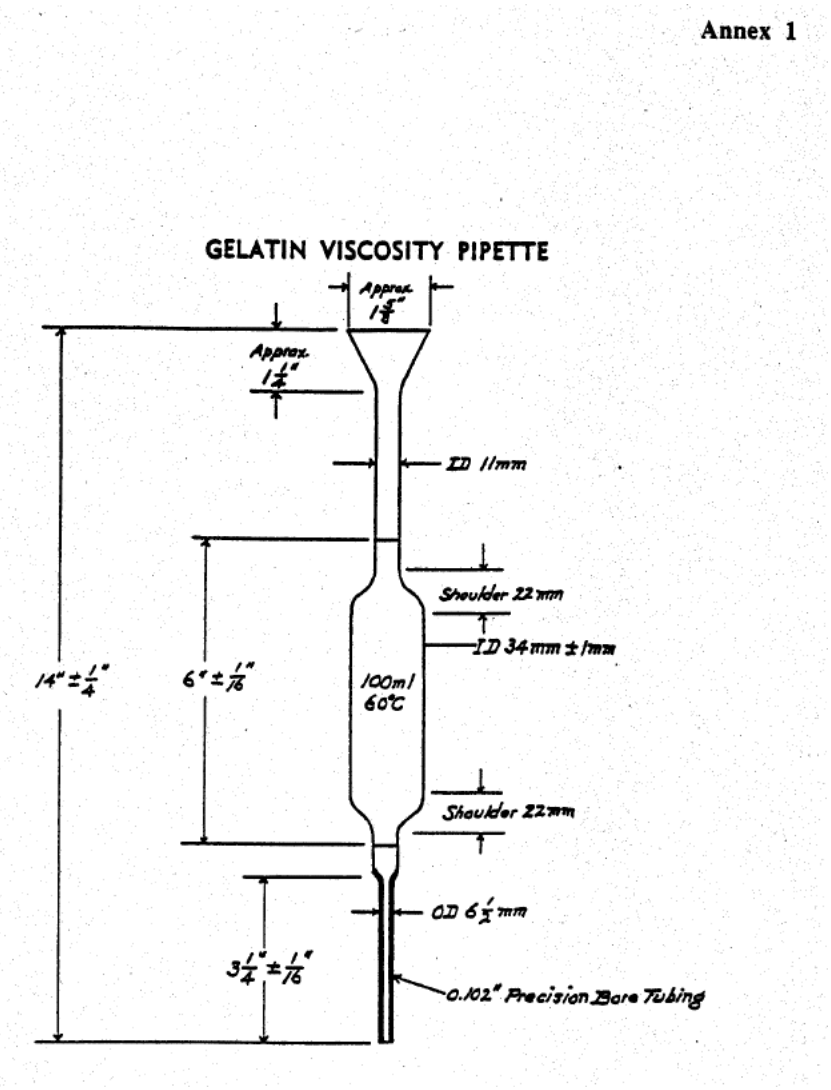
3. **Cleaning of pipette:**
   The pipette must be thoroughly cleaned before use, preferably by washing with soap and hot water, and rinsed with distilled water. When not in use it is best to keep the pipette filled with distilled water.

4. **Thermal viscosity breakdown:**
   Due to the possibility of thermal viscosity breakdown samples should not be held longer than 60 min at 60°C.

Viscosity pipette

Supplier: Wilmad Glass (wilmad-labglass.com)

Annex 1



2.3 Moisture Content (Loss on Drying)

Principle
A weighed sample of gelatin is maintained for 16 to 18 hours at 105 ± 2°C and is then reweighed. The moisture content is defined as the percentage loss in weight of the sample.

References
2. GME Monograph, February 2018, Version 13

Reagents and Solutions
None

Apparatus
1. Pyrex evaporating dishes, 45 mm in diameter and 30 mm high, or other suitable moisture pan
2. Drying oven, set at 105 ± 2°C.
3. Desiccator containing $CaCl_2$ or sillacagel
4. Analytical balance, capable of weight to 0.001 grams

Procedure
1. Wash the evaporating dish very carefully in hot water.
2. Place the dish in the drying oven at 105ºC for at least one hour. Cool dish in the desiccator until room temperature is reached.
3. Weigh approximately 5.0 g of gelatin to the nearest milligram and note the weight of the test sample (m0) and the weight of the sample together with the evaporating dish (m1).
4. Place the evaporating dish containing the sample in the drying oven at 105 ± 2ºC for 16 to 18 hours.
5. Cool the dish in the desiccator until room temperature is reached and weigh to the nearest milligram (m2), weigh and calculate the percentage of residue.

Result
The moisture content, expressed as a percentage by weight, is equal to:

$$\% \textit{ Moisture} = [(m1) - m2) / m0] \text{ X } 100\%$$

m0 = weight in grams of the test sample
m1 = weight in grams of the test sample and the evaporating dish before drying
m2 = weight in grams of the test sample and the evaporating dish, after drying

2.4 pH

Principle

The pH of a 6.67% gelatin solution is determined by potentiometry at a temperature of 60 ± 1°C using a pH meter. The pH may also be determined on 1% solution at 55°C as described in the United States Pharmacopoeia.

References

1. GME Monograph, February 2018, Version 13
2. US Pharmacopoeia (current edition)

Reagents and Solutions

1. pH 4 Buffer Solution
2. pH 7 Buffer Solution
3. Deionized Water

Apparatus

1. BALANCE: with 0.01 g sensitivity
2. WATER BATH: constant temperature at 65 ± 0.5°C
3. WATER BATH: constant temperature at 60 ± 0.5°C
4. pH Meter: conventional pH meter with at least two decimal place display
5. pH Electrode: combination pH electrode with temperature compensation

Procedure

1. Weigh 7.50 ± 0.01g of gelatin into a bloom jar or 150 mL beaker and add 105.0 ± 0.2g of deionized water, stirring often to suspend all gelatin particles. Cover and let stand 1 – 3 hours at room temperature.
   Note that the solution prepared for gel strength, viscosity and clarity may be used for this test.
2. Dissolve the sample in a 65°C water bath for 10 – 15 minutes, stirring or swirling as required.
3. Transfer the sample to the 60°C water bath and temper to 60ºC.
4. Perform a two-point calibration on the pH meter, using pH 4 and pH 7 buffers, at 60°C.
5. Determine the pH of the gelatin solution according the pH-meter instructions.
6. Swirl the solution well using the pH probe to ensure the electrode is sufficiently saturated.

7. Rinse the electrode with warm distilled water once testing is complete.

Calibration

Calibration should be done at the same temperature as the pH measurement.

The calibration is performed by using two reference solutions at a pH of 7 and a pH of 4.

Calibration is performed according the pH meter-instructions. Swirl the solution well to fully saturate the electrode with buffer and rinse well with distilled water or equivalent afterwards. Electrodes should be regularly cleaned with an enzyme solution.

2.5 Granulation (Particle Size Distribution)

Principle

A weighed sample of gelatin is placed on a sieve shaker to determine the particle size distribution of the sample.

Apparatus

1. Ro-Tap testing sieve shaker (e.g. W.S. Tyler Company)
2. Assorted Sieves

8 mesh (0.0937 in)	40 mesh (0.0167 in)
10 mesh (0.0787 in)	60 mesh (0.0098 in)
16 mesh (0.0469 in)	80 mesh (0.0070 in)
20 mesh (0.0335 in)	100 mesh (0.0059 in)
30 mesh (0.0234 in)	

3. Sieve Cover
4. Sieve Receiver
5. Balance (readability to 0.1g)
6. Brushes (bristle and steel)
7. Large tray or basin, plastic or non-stick freezer paper

Procedure

1. Weigh a homogeneous sample of gelatin to be tested.
2. Select the sieves to be used. The selection should cover the full range of expected particle sizes.
3. Arrange the sieves with the coarsest screen on top and a catch tray on the bottom.
4. Weigh out 100 ± 0.1 grams of gelatin.
5. Pour gelatin sample into the top screen, place the cover on, position in the shaker, and place hammer on top of the cover.
6. Turn the shaker on for five minutes.
7. When the shaking is completed, remove from the shaker; brush out all granules from each sieve.
8. Use the freezer paper to capture all gelatin from each screen. Weigh the sample retained on each screen and that which passed through the finest screen (material in catch tray).
9. Record the actual weight retained on each screen.
10. Calculate the percentages of gelatin retained and/or passed through the sieves.

2.6 Ash Content (Residue on Ignition)

Principle

Gelatin is incinerated in a crucible at 550°C using a muffle furnace. The residue is determined by differential weighing and the result expressed as a weight percentage of the original sample.

References

1. Current USP/NF and FCC.

Reagents and Solutions

1. Paraffin

Apparatus

1. Muffle furnace, capable of heating to 550 ± 25°C
2. Analytical balance, capable of weight to 0.001 grams

Procedure

Sample Preparation

1. Weigh approximately 5.0 g of gelatin to 0.001 g in a suitable crucible that has previously has been ignited, cooled and weighed.
2. Add 1.5 to 2.0 g of paraffin to avoid loss due to swelling.
3. Heat, gently at first on an electric hot plate or in a muffle furnace, until the substance is thoroughly charred.
4. Finish ashing in a muffle furnace at 550°C for 15 to 20 hours.

Determination

1. Cool the sample in a desiccator.
2. Weigh the sample and calculate the percentage of residue

Note: Do not handle dried crucibles without gloves or crucible tongs

Result

The ash content, expressed as % ash, is equal to:

$$\%\ ash = [\text{weight of ash}] / [\text{weight of sample}] \times 100\%$$

2.7 Sulfur Dioxide

Principle

Sulfur dioxide content can be determined using the modified Monier-Williams test method in which sulfur dioxide is set free from a gelatin sample by boiling it with hydrochloric acid. The created sulphur dioxide is distilled into a hydrogen peroxide solution with a stream of carbon dioxide. Sulfuric acid is formed that can be titrated with sodium hydroxide.

References

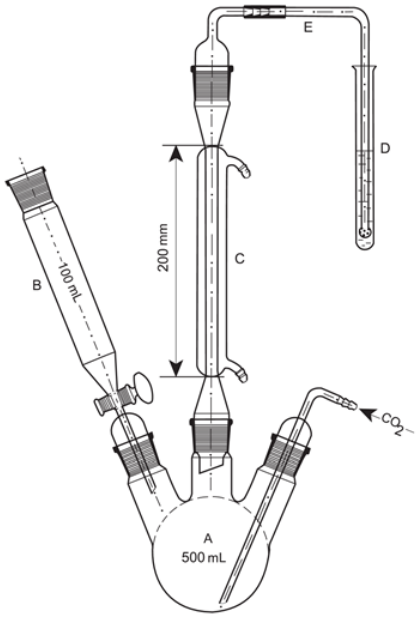
1. AOAC Method 962.16 as stated in Food Chemicals Codex, General Tests and Assays, Appendix X.
2. Current USP/NF Gelatin Monograph
3. GME Monograph, February 2018, Version 13

Reagents and Solutions

1. Carbon dioxide : CO_2 > 99.5% V/V
2. Dilute Hydrogen peroxide solution : H_2O_2, 3% in water (+/- 0.5%)
3. Bromophenol blue solution : 1 g/L in ethanol (20% V/V) Ethanol: 96 % C_2H_5OH (V/V)
4. Dilute hydrochloric acid : HCl, 2 mol/l (73 g/l)
5. Sodium hydroxide : NaOH, 0.1 mol/l or 0.01 mol/l
6. Water: Purified water

Apparatus

1. Monier-Williams apparatus:



Procedure

1. Introduce 150 ml of water into flask A (see Apparatus) and pass carbon dioxide through the whole system for 15 min. at a rate of 100 ml +/- 5 ml/min.
2. To 10 ml of dilute hydrogen peroxide solution add 0,15 ml of a 1 g/L solution of bromophenol blue in ethanol (20 % V/V). Add 0,1M sodium hydroxide until a violet-blue color is obtained, without exceeding the end-point. Place the solution in the test-tube (D).
3. Without interrupting the stream of carbon dioxide, remove the funnel (B) and introduce through the opening into the flask 25.0 g (m in g) of the gelatine sample with the aid of 100 ml water. Replace the funnel
4. Close the tap of the funnel and add 80 ml of dilute hydrochloric acid to the funnel. Open the tap of the funnel to allow the hydrochloric acid solution to flow into the flask, making sure that no sulfur dioxide escapes into the funnel by closing the tap before the last few milliliters of hydrochloric acid solution drain out. Boil for 1 h.
5. Open the tap of the funnel and stop the flow of carbon dioxide and also the heating and the cooling water. Transfer the contents of the test-tube with the aid of a little water to a 200 ml wide-necked, conical flask. Heat on a water-bath for 15 min and allow to cool.

6. Add 0.1 ml of a 1 g/L solution of bromophenol blue R in ethanol (20 per cent V/V) R and titrate with 0.1 M sodium hydroxide until the color changes from yellow to violet-blue (V1 mL). Carry out a blank titration (V2 mL).

Result

Calculate the content of sulfur dioxide in mg / kg gelatine using the following expression:

32030 x (V1 – V2) x n /m

N = molarity of the sodium hydroxide solution used as titrant

M = mass of gelatin sample

7. Remark: The sensitivity of the described method is improved by increasing the sample weight to 50 g and the use of a 0,01 mol/L NaOH as the titrant.

Note: This method is commonly performed by contract laboratories per the above referenced methods.

2.8 Clarity

Principle

The clarity of a 6.67% gelatin solution is determined at 45ºC by measuring the percent transmittance through a 1 cm cuvette at 620 nm.

References

3. GME Monograph, February 2018, Version 13

Apparatus

1. Spectrophotometer
2. 1 cm cuvettes, optically matched
3. Automatic pipette, capacity 105.0 ± 0.2
4. Bloom jar or 150 mL beaker
5. Balance with 0.01 g sensitivity
6. Water Bath: constant temperature at 65 ± 0.5°C
7. Water Bath: constant temperature at 45 ± 0.5°C

Procedure

1. Weigh 7.50 ± 0.01 g gelatin into bloom jar or 150 mL beaker
2. Add 105.0 ± 0.2 g deionized water, stirring often to suspend all gelatin particles
3. Cover and let stand 1 – 3 hours at room temperature
4. Dissolve the sample in a 65°C water bath for 10 – 15 minutes, stirring or swirling as required.
5. Remove the sample to a 45°C water bath and hold until sample temperature is 45 ± 1°C.
6. Calibrate the spectrophotometer to 100% transmittance with deionized water blank at 620 nm according to the manufacturer's instructions.
7. Transfer an aliquot of the sample solution to the cuvette and record the percent transmittance value at 620 nm.
8. Results of spectrophotometric measurements can be expressed as absorbance (E) or transmission (T). Conversion of results is possible using the following formulas:

$$E = \log 1 / T \quad T = 1 / 10^{E}$$

Note: Sample preparation for the clarity test is the same as for gel strength, viscosity and color tests; those samples may be utilized. The clarity determination may be conducted on samples after the gel strength test by melting down and tempering to 45ºC as previously described.

2.9 Color

Principle

The color of a 6.67% gelatin solution is determined at 45ºC by measuring the percent transmittance through a 1 cm cuvette at 450 nm.

References

4. GME Monograph, February 2018, Version 13

Apparatus

1. Spectrophotometer
2. 1 cm cuvettes, optically matched
3. Automatic pipette, capacity 105.0 ± 0.2
4. Bloom jar or 150 mL beaker
5. Balance with 0.01 g sensitivity
6. Water Bath: constant temperature at 65 ± 0.5°C
7. Water Bath: constant temperature at 45 ± 0.5°C

Procedure

1. Weigh 7.50 ± 0.01 g gelatin into bloom jar or 150 mL beaker
2. Add 105.0 ± 0.2 g deionized water, stirring often to suspend all gelatin particles
3. Cover and let stand 1 – 3 hours at room temperature
4. Dissolve the sample in a 65°C water bath for 10 – 15 minutes, stirring or swirling as required.
5. Remove the sample to a 45°C water bath and hold until sample temperature is 45 ± 1°C.
6. Calibrate the spectrophotometer to 100% transmittance with deionized water blank at 450 nm according to the manufacturer's instructions.
7. Transfer an aliquot of the sample solution to the cuvette and record the percent transmittance value at 450 nm.
8. Results of spectrophotometric measurements can be expressed as absorbance (E) or transmission (T). Conversion of results is possible using the following formulas:

$$E = \log 1 / T \quad T = 1 / 10^{E}$$

Note: Sample preparation for the clarity test is the same as for gel strength, viscosity and color tests; those samples may be utilized. The clarity determination may be conducted on samples after the gel strength test by melting down and tempering to 45ºC as previously described.

2.10 Hydrogen Peroxide

Principle

Peroxidase transfers oxygen from peroxides to an organic redox indicator which is converted to a blue oxidation product. The intensity of the color obtained is proportional to the quantity of peroxide, and can be compared with a color scale provided with the test strips to determine the peroxide concentration.

References

5. GME Monograph, February 2018, Version 13
6. European Pharmacopoeia (current edition)
7. US Pharmacopoeia (current edition)

Apparatus

1. Balance with 0.01 g sensitivity
2. Water Bath with constant temperature at 65 ± 0.5°C
3. Peroxide Test Strips, 0 – 25 mg/L (ppm) range

Procedure

1. Weigh 20.0 ± 0.1 g of gelatin into a glass beaker. Add 80.0 ± 0.2 mL of water and stir to moisten the gelatin. Cover the beaker with a watch glass and allow the gelatin to soak at room temperature for 1- 3 hours.
2. Place the beakers into a water bath at 65°C ± 2°C for 20 ± 5 minute to fully dissolve the sample.
3. Dip the test strip into the beaker with gelatin to be tested for 1 second
4. Remove the test strip, shake off excess liquid, and after 15 s compare the reaction zone with the color scale provided.
5. Multiply the concentration read from the color scale by a factor of 5 to calculate the concentration, in ppm, of peroxide in the test substance.

2.11 Hydroxyproline Content (Quantitative Method)

Principle

The sample is first hydrolyzed in acid medium to liberate hydroxyproline from the sample. The hydrolysate is oxidised with chloramine-T. The oxidised hydroxyproline is measured by colourimetry using p-dimethylaminobenzaldehyde.

Reference(s)

1. ISO - TC 34 - 3496 (1978); Meat and meat products - Determination of L-hydroxyproline content (Reference method).
2. US Pharmacopoeia (current edition) – Identification test C

Reagents and solutions

1. All reagents are analytical grade. Water is purified water or equivalent.
2. Hydrochloric acid 6N, alternatively sulfuric acid 6N
3. Sodium hydroxide 10N
4. 1-Propanol
5. 2-Propanol
6. Buffer solution pH 6: dissolve in water 50 g citric acid monohydrate, 12 ml glacial acetic acid, 120 g sodium acetate trihydrate, 34 g sodium hydroxide. Make up to volume 1 litre with water. Mix with 200 ml water and 300 ml 1-propanol.
7. Oxidant reagent: dissolve 1.4 g chloramine-T in 10 ml water, add 10 ml 1-propanol and 80 ml buffer solution pH 6.
8. Colorimetric reagent: (*Prepare fresh each day*) dissolve 10 g p-dimethylaminobenzaldehyde in 35 ml perchloric acid 60%, add slowly65 ml 2-propanol .
9. Hydroxyproline standard solutions: dissolve 100 mg in water, add 1 drop HCl 6N and dilute to 100 ml. For use, dilute 5 ml of the solution to 500 ml. Prepare three standard solutions by diluting 10, 20 and 40 ml of this solution to 100 ml with water.

Apparatus

1. Digestion vessels
2. Paper filters
3. Water bath at 60 ± 0.5 °C.
4. Spectrophotometer and optical cells (path 1 cm).
5. Analytical balance.

Procedure

1. Accurately weigh, in duplicate, 1g of sample and introduce into digestion vessels. Add 30 ml HCl 6N (or H2SO4 6N) and hydrolyze in a drying oven for 22 to 24 hours at 105°C (do not screw the caps of the digestion vessels tightly).
2. Cool the hydrolysate to room temperature and transfer to a 1000 ml volumetric flask through a funnel containing a filter. Wash the digestion vessel with water. Make up to 1000 ml with water.
3. Dilute the hydrolysate with a factor of 1/10 : put 10 ml of the hydrolysate solution in a 100 ml flask and make up until 100 ml with water.
4. Pipette 2 ml of diluted hydrolysate sample solution from step 3 into a test tube and add 2 ml of oxidant reagent (Chloramine-T solution). Mix and allow to stand for 20 minutes at room temperature.
5. Add 2 ml of colorimetric reagent, mix and close the tube. Place the tube in a water bath at 60°C for 15 minutes. Cool to room temperature within 3 minutes (cooling water bath).
6. Make up to 10 ml with water. Allow to stand for 30 min at room temperature.
7. Measure the absorbance at 558 nm against a blank.
8. Blank preparation: proceed in the same way from step 4 using 2 ml of water instead of the hydrolysate.
9. Calibration curve: proceed in the same way from step 4 using 2 ml of the three standard solutions instead of the hydrolysate. Plot the curve.

Result

1. **h** = hydroxyproline content in µg/ml read from the calibration curve,
   m = weight of the sample in mg

$$\textbf{\% hydroxyproline} = \frac{1000 \text{ x } h}{m}$$

Express the result to 0.1 %.

2. Repeatability: the difference between two determinations should not be larger than 10%

2.12 Cations

The GMIA recommends the methods below for the analysis of cations:

Cation	Recommended Method	Alternative
Iron	Atomic Absorption Spectrometry	ICP-OES
Chromium	Atomic Absorption Spectrometry	ICP-OES
Zinc	Atomic Absorption Spectrometry	ICP-OES
Lead	Atomic Absorption Spectrometry	ICP-OES

References

1. US Pharmacopoeia (current edition)
2. Food Chemicals Codex (current edition)

3. Microbiological Testing

Principle
Several methods are available for determining the microbiological characteristics of gelatin.

Typical expectations for pharmaceutical grade gelatin follow the National Formulary (NF) monograph for gelatin requirements:

Total Aerobic Microbial Count (TAMC)	≤1000 cfu/g
Total Yeast and Mold Count (TYMC)	≤100 cfu/g
Escherichia coli	Negative/1 g
Salmonella	Negative/10 g

Typical expectations for food grade gelatin follow the Food Chemicals Codex (FCC) monograph for gelatin requirements:

Escherichia coli	Negative/25 g
Salmonella	Negative/25 g

The GMIA follows the methods specified by the US Pharmacopeia or the FDA Bacteriological Analytical Manual (BAM) for the testing of gelatin and hydrolyzed gelatin products (see references below).

References

1. US Pharmacopeia (current edition)
    a. General Chapter <61> **Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests**
    b. General Chapter <62> **Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms**
2. FDA Bacteriological Analytical Manual (BAM) (current edition)