# Gelatin

Food-Grade Gelatin; Edible Gelatin

CAS: [9000-70-8]

## DESCRIPTION

Gelatin is the product obtained from the acid, alkaline, or enzymatic hydrolysis of collagen, the chief protein component of the skin, bones, and connective tissues of animals. These animal sources shall not have been exposed to pentachlorophenol.

*Type A* Gelatin is produced by the acid processing of collagenous raw materials and exhibits an isoelectric point between pH 7 and pH 9. *Type B* Gelatin is produced by the alkaline or lime processing of collagenous raw materials and exhibits an isoelectric point between pH 4.6 and pH 5.2. Mixtures of *Types A* and *B* as well as Gelatins produced by modifications of the above mentioned processes may exhibit isoelectric points outside of the stated ranges.

Gelatin is nearly tasteless and odorless. It is a vitreous, brittle solid that is faintly yellow. When Gelatin granules are immersed in cold water, they hydrate into discrete, swollen particles. On being warmed, Gelatin disperses into the water, resulting in a stable suspension. Water solutions of Gelatin will form a reversible gel if cooled below the specific gel point of Gelatin. The gel point is dependent on the source of the raw material. Gelatin extracted from the tissues of warm-blooded animals will have a gel point in the range of 30° to 35°. Gelatin extracted from the skin of cold-water ocean fish will have a gel point in the range of 5° to 10°. Gelatin is soluble in aqueous solutions of polyhydric alcohols such as glycerin and propylene glycol. It is insoluble in most organic solvents.

**Functional Use in Foods** Firming agent; formulation and processing aid; stabilizer and thickener; surface-active agent; surface-finishing agent.

## REQUIREMENTS

**Identification**

A. Gelatin forms a reversible gel when tested as follows: Dissolve 10 g in 100 mL of hot water in a suitable flask, and cool in a refrigerator at 2° for 24 h. A gel forms. Transfer the flask to a water bath heated to 60°. Within 30 min, upon stirring, the gel reverts to the original liquid state.

B. To a 1 in 100 solution of the sample add trinitrophenol TS or a 1 in 1.5 solution of potassium dichromate previously mixed with about one-fourth its volume of 3 *N* hydrochloric acid. A yellow precipitate forms.

**Ash** Not more than 3.0%.
**Chromium** Not more than 10 mg/kg.
**Fluoride** Not more than 0.005%.
**Heavy Metals** (as Pb) Not more than 0.002%.
**Lead** (as Pb) Not more than 1.5 mg/kg.
**Loss on Drying** Not more than 15.0%.
**Microbial Limits**:
  **E. coli** Negative in 25 g.
  **Salmonella** Negative in 25 g.
**Pentachlorophenol Limit** Not more than 0.3 mg/kg.
**Protein** The specification conforms to the representations of the vendor.
**Sulfur Dioxide** Not more than 0.005%.

## TESTS

**Ash** Proceed as directed under *Ash (Total)*, Appendix IIC.

**Chromium**

*Test Solution* Transfer 10 g of the sample, accurately weighed, to a 100-mL silica dish. Using a very low flame, heat the dish over a Bunsen burner, taking care that the sample does not swell over the lip of the dish or catch fire. Gradually increase the flame until the sample is completely charred, transfer to a muffle furnace at 550°, and ash overnight. Cool to room temperature, add 10 mL of hydrochloric acid and 10 mL of nitric acid, and heat on a steam bath for 10 min. Cool and transfer to a 25-mL volumetric flask, dilute to volume, and mix.

*Chromium Stock Solution* Transfer 192.3 mg of chromium trioxide, accurately weighed, to a 1000-mL volumetric flask, dissolve in 100 mL of water and 10 mL of nitric acid, dilute to volume, and mix. This solution contains 0.1 mg of chromium per mL. Transfer 100.0 mL to a 1000-mL volumetric flask, dilute to volume, and mix. This solution contains 10 µg of chromium per mL.

*Standard Preparations* To separate 100-mL volumetric flasks, transfer 10.0, 30.0, 50.0, and 70.0 mL, respectively, of *Chromium Stock Solution*, dilute to volume, and mix. The *Standard Preparations* contain, respectively, 1.0, 3.0, 5.0, and 7.0 µg of chromium per mL.

*Procedure* Concomitantly determine the absorbances of the *Standard Preparations* and the *Test Solution* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer equipped with a chromium hollow-cathode lamp and a slightly reducing air–acetylene flame, using water as the blank. Plot the absorbances of the *Standard Preparations* versus concentration, in µg/mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, $C_S$, in µg/mL, of chromium in the *Test Solution*. Calculate the concentration of chromium, in mg/kg, in the portion of Gelatin taken, by the formula

$$25C_S/W,$$

in which $W$ is the quantity, in g, of the sample taken.

**Fluoride** Fluoride is separated from the sample by distillation from a solution containing sulfuric acid. Depending on the amount of chloride in the sample, silver sulfate solution is added to the solution being distilled. To ascertain how much silver sulfate solution is necessary, the concentration of chloride in the sample must be determined, as described below, under *Procedure for Chloride*.

The temperature of the distillation must be carefully controlled to prevent excess sulfate (as sulfur trioxide) from carrying over into the fluoride-containing distillate. Since excess sulfate ($SO_4$) may interfere with the colorimetric determination of fluoride, it is necessary, after measuring the fluoride, to determine sulfate in the distillate, and if the result so indicates, to repeat the distillation, paying closer attention to controlling the temperature of the solution being distilled.

*Apparatus* The distillation apparatus consists of a 2-L round-bottom borosilicate flask, a connector with a thermometer opening, a thermometer reading to 200° and long enough to reach almost to the bottom of the flask, a condenser with an inner spiral tube, and an electric heating mantle for the flask. All glassware shall be fitted together with standard-taper joints.

*Standard Fluoride Solution* Accurately weigh 221 mg of sodium fluoride, previously dried at 200° for 4 h, dissolve in water in a 1000-mL volumetric flask, dilute to volume, and mix. Transfer a 10.0-mL aliquot into a separate 1000-mL volumetric flask, dilute to volume, and mix. This solution contains 1 µg of fluoride per mL.

*SPADNS Solution* Dissolve 958 mg of SPADNS (sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate) in water, dilute to 500 mL, and mix. Protect from direct sunlight.

*Zirconyl–Acid Reagent* Dissolve 133 mg of zirconyl chloride octahydrate ($ZrOCl_2.8H_2O$) in 25 mL of water, add 350 mL of hydrochloric acid, dilute to 500 mL, and mix.

*Zirconyl–Acid/SPADNS Reagent* Mix equal volumes of *SPADNS Solution* and *Zirconyl–Acid Reagent*.

*Reference Solution* Add 10 mL of *SPADNS Solution* to 100 mL of water, add 10 mL of hydrochloric acid (7 in 10), and mix.

*Sodium Arsenite Solution* Dissolve 5.0 g of sodium arsenite in 1 L of water, and mix.

*Silver Sulfate Solution* Dissolve 500 mg of silver sulfate in 100 mL of water, and mix.

*Calibration Standards* Transfer the following volumes of the *Standard Fluoride Solution* into separate 50-mL volumetric flasks, dilute each to volume, and mix:

| Volume | Concentration |
|---|---|
| 0.0 mL Reagent Blank (water) | 0.0 μg/mL |
| 5.0 mL | 0.1 μg/mL |
| 25.0 mL | 0.5 μg/mL |
| 50.0 mL | 1.0 μg/mL |

*Procedure for Chloride* Transfer 5 g of the sample, accurately weighed, into a 400-mL beaker. Add 150 mL of water, stir to hydrate the sample, and warm to dissolve. Add 25.0 mL of 0.1 *N* silver nitrate and 15 mL of nitric acid, mix well, and carefully heat the contents of the beaker to boiling. Slowly add saturated potassium permanganate solution in 5-drop increments, boiling the sample solution after each addition, until it becomes colorless. Cool the solution to room temperature, add 3 mL of ferric ammonium sulfate TS, and while agitating the solution vigorously, titrate with 0.1 *N* ammonium thiocyanate to a pale rose-colored endpoint that persists for 30 s.

> **Note**: If the solution becomes pink upon the addition of the first few drops of 0.1 *N* ammonium thiocyanate, the determination must be repeated using a smaller sample.

Each mL of 0.1 *N* silver nitrate consumed is equivalent to 3.55 mg of chloride ion. Calculate the chloride, in mg/g, by the formula

$$C/S,$$

in which $C$ is the weight, in mg, of chloride found, and $S$ is the quantity, in g, of the sample taken.

*Procedure for Fluoride* Place 400 mL of water in the 2-L flask of the distillation apparatus, and cautiously add 200 mL of sulfuric acid. Swirl to mix, add 25 to 30 glass beads, and assemble the apparatus, making certain that all joints are tight. Distill into a receiver until the temperature of the solution reaches 180°, or until 250 mL of distillate have been collected in the receiver. Discontinue the distillation, and discard the distillate. Bring the contents of the distilling flask to 120°, or below. Then add 5 g of the sample, accurately weighed and dissolved in 300 mL of water, followed by 1 mL of silver sulfate per mg of chloride in the sample, as determined above. Again, assemble the apparatus and distill, collecting the distillate in a 250-mL volumetric flask. Continue the distillation until the temperature in the distilling flask reaches exactly 180° and no higher. If fewer than 250 mL have distilled, dilute the distillate to volume, and mix. If more than 250 mL have distilled, determine and record the volume, and mix.

Measure fluoride in the distillate as follows: Cool the distillate to a temperature of 25°. Transfer the standards, the reagent blank, and a 50-mL aliquot of the sample into separate beakers, being certain that the temperatures of all of the solutions fall within a range of 1°. Add 0.05 mL of *Sodium Arsenite Solution* to each beaker, followed by 10 mL of *Zirconyl–Acid/SPADNS Reagent*. Mix, allow to stand for 10 min, and measure the absorption of each solution against the reference solution at 570 nm in a suitable spectrophotometer.

Plot the absorbances of the *Standard Solutions* versus concentration, in μg/mL, of fluoride, and draw the straight line best fitting the data points. From the graph so obtained, determine the concentration, $F$, in µg/mL, of fluoride in the distillate, and calculate the percent fluoride by the formula

$$FV/10{,}000\ W,$$

in which $V$ is the volume, in mL, of the distillate, and $W$ is the quantity, in g, of the sample taken for the distillation.

After measuring the fluoride, determine sulfate in the distillate by the procedure that follows. If the concentration of sulfate is greater than 200 mg/L, repeat the distillation, being even more careful to control the temperature of the solution in the distillation flask so that it does not exceed 180°.

*Procedure for Sulfate in the Fluoride Distillate* Accurately determine the volume of distillate remaining from the fluoride distillation, and transfer it to a suitable beaker. Determine the pH with a pH meter, and if necessary, add dilute hydrochloric acid (1:3) to bring the pH to 4.5. Add an additional 2 mL of the hydrochloric acid, heat the solution to boiling, and while stirring, slowly add 30 mL of barium chloride TS to the hot solution. Boil the solution for 5 min, and allow it to stand until any precipitate settles out. If any precipitate has formed, filter the contents of the beaker through a tight, ashless filter paper, and quantitatively transfer the precipitate to the paper. Thoroughly wash the precipitate on the paper with hot water, and then transfer the paper to a tared crucible, previously ignited for 2 h at 1000° in a muffle furnace. Dry the paper in the crucible for 1 h at 105°, and then carefully char it at low heat. Gradually increase the heat to burn away the paper, and finally ignite the crucible and its contents for 2 h at 1000°. Cool and weigh, and calculate, in mg/L, the sulfate in the distillate by the formula

$$(B \times 0.4115 \times 1{,}000{,}000)/M,$$

in which $B$ is the weight, in g, of the barium sulfate residue, and $M$ is the mL of distillate taken.

**Heavy Metals** Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, Appendix IIIB, using 20 µg of lead ion (Pb) in the control (*Solution A*).

**Lead** A Sample Solution containing 2 g of the sample prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, Appendix IIIB, using 3 µg lead ion (Pb) in the control.

**Loss on Drying,** Appendix IIC Dry at 105° to constant weight.

**Microbial Limits:**

**E. coli** Proceed as directed in chapter 4 of the *FDA Bacteriological Analytical Manual*, Seventh Edition, 1992.

**Salmonella** Proceed as directed in chapter 5 of the *FDA Bacteriological Analytical Manual*, Seventh Edition, 1992.

**Pentachlorophenol**

*Pentachlorophenol (PCP) Stock Solution* Transfer about 4.0 mg of PCP Reference Standard (Standard No. 5260, Pesticide Reference Standards Section, Environmental Protection Agency, Research Triangle Park, NC 27711, or equivalent, available from Aldrich Chemical Co.), accurately weighed, to a 1000-mL volumetric flask, dissolve in pesticide-grade benzene, dilute with benzene to volume, and mix. Each mL of this solution contains 4.0 µg of PCP.

*PCP Standard Solutions* Prepare a series of *PCP Standard Solutions* by serially diluting the *PCP Stock Solution* in hexane. Into separate 1000-mL volumetric flasks, pipet 0.0, 1.0, 5.0, 10.0, 25.0, 50.0, and 100.0 mL, respectively, of *PCP Stock*

*Solution*, dilute to volume with hexane, and mix. The *PCP Standard Solutions* contain in each mL 0.0, 0.004, 0.020, 0.040, 0.100, 0.200, and 0.400 µg of PCP, respectively.

*Sample Preparation* Transfer about 2 g of the Gelatin sample, accurately weighed, and 2.0 mL of water (to serve as the blank) into separate 25- × 150-mm screw-cap test tubes equipped with Teflon-lined caps. Treat each in the following manner: Add 10 mL of 12 *N* sulfuric acid. Close the tube, tighten the cap, and heat for 1 h in a water bath maintained at 100° in a fume hood, removing the tube periodically and mixing the sample by shaking. Remove the tube from the bath and allow to cool to room temperature. Add 10 mL of hexane:isopropanol (4:1 v/v) to the tube, and shake vigorously. Centrifuge for 2 min at 1000 × *g* in a suitable centrifuge (International Equipment Co., Needham Heights, MA 02194) with a head equipped to accommodate 25- × 150-mm test tubes. Transfer the upper hexane layer to a second 25- × 150-mm test tube with a Pasteur pipet. Repeat the extraction and centrifugation two additional times, and combine the hexane extracts in the second test tube. To the combined extracts, add 5.0 mL of 1.0 *N* potassium hydroxide, tighten the cap, shake the test tube vigorously, and centrifuge for 2 min at 1000 × *g* as before. Remove the upper layer with a Pasteur pipet, and discard. Add 10 mL of hexane to the test tube, tighten the cap, shake the test tube vigorously, and centrifuge as before. Remove the upper layer with a Pasteur pipet, and discard. Add 5.0 mL of 12 *N* sulfuric acid to the test tube, tighten the cap, and mix by carefully swirling the tube. Add 5.0 mL of hexane, tighten the cap, shake the test tube vigorously, centrifuge as before, and transfer the upper layer to a 10-mL volumetric flask. Repeat twice, using 2.0 mL of hexane each time, and transfer the upper layer to the 10-mL volumetric flask. Dilute to volume with hexane.

*Chromatographic System* Use a suitable gas chromatograph equipped with a $^{63}$Ni electron capture detector and a 1.8-m × 4-mm id glass column containing 1% SP-1240DA on 100- to 120-mesh Supelcoport (Supelco Inc.) or equivalent. Place a small plug (2 to 3 mm) of phosphoric acid-washed glass wool in the detector end of the column. The carrier gas is 5% methane in argon at a flow rate of 60 mL/min. Condition the column by purging with carrier gas at ambient temperature for 10 to 15 min; program the column oven to increase from 70° to 190° at 1°/min, and hold the temperature at 190° for 8 h while continuing to purge with carrier gas.

> **Caution**: Use only recently prepared and thoroughly conditioned columns; the appearance of ghost PCP peaks may be noted following the injection of samples containing high levels of PCP; repeated injections of solvent may be necessary until ghost PCP peaks disappear.

For sample analyses, the temperature of the column oven, injector port, and detector are maintained at 180°, 250°, and 350°, respectively. The electrometer should be adjusted to provide about half of the full-scale deflection when 0.1 ng of PCP is injected.

*Procedure* Inject 5-µL portions of each of the *PCP Standard Solutions* (0.0, 0.02, 0.10, 0.20, 1.0, and 2.0 ng, respectively) and the reagent blank into the gas chromatograph sequentially, and record the chromatograms. Measure the areas under the PCP peaks and the peak heights for each of the *PCP Standard*

*Solutions* (retention time for PCP should be about 10 min), corrected for the reagent blank. The maximum acceptable reagent blank for satisfactory performance of the method is 0.01 µg/g. Similarly, inject 5 µL of the *Sample Preparation* into the gas chromatograph, and record the chromatogram. Measure the area under the PCP peak and the peak height, corrected for the reagent blank. Determine the amount of PCP in the *Sample Preparation* by comparing the peak area and height to the peak area and height obtained from injection of known amounts of *PCP Standard Solutions*; to ensure valid measurement of PCP in the *Sample Preparation*, the size of the PCP peak from the *Sample Preparation* and the standards should be within ±10%. The *Sample Preparation* may require further dilution. Designate as $A_S$ the amount of PCP, expressed in ng, in the aliquot of the *Sample Preparation*. Each *PCP Standard Solution* and *Sample Preparation* should be injected twice to ensure that consistent responses are obtained. Following each injection of *PCP Standard Solutions* or *Sample Preparation*, rinse the syringe 10 times with hexane. After each injection of *PCP Standard Solutions* or *Sample Preparation*, inject 5 µL of hexane onto the gas chromatograph, and record the chromatogram. If peaks are observed at the retention time for PCP, repeat the hexane injection until such peaks are no longer encountered. Calculate the concentration of PCP, in µg/g, in the sample by the formula

$$5A_S.$$

**Protein** Transfer about 1 g, accurately weighed, to a 500-mL Kjeldahl flask, and proceed as directed under *Nitrogen Determination*, Appendix IIIC. Percent protein equals percent $N \times 5.55$.

**Sulfur Dioxide** Determine as directed in the general method, Appendix X. Instead of using a 50-g sample, dissolve 20.0 g in 100 mL of a 5% alcohol in water mixture, and proceed as directed under *Sample Introduction and Distillation*.

**Packaging and Storage** Store in tight containers.