World Health Organization

GELATIN

(GELATINA)

Draft proposal for revision for *The International Pharmacopoeia*

(January 2021)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Claire Vogel (vogelc@who.int) by **31 March 2021**.

Our working documents are sent out electronically and they will also be placed on the WHO Medicines website (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/pharmaceuticals/current-projects) for comments under the "*Working documents in public consultation*" link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/21.874:

GELATIN

(GELATINA)

Description	**Date**
Monograph drafted based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group.	December 2020
Draft monograph sent out for public consultation.	February – March 2021
Discussion at the Consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.	May 2021
Presentation to the 56th WHO Expert Committee on Specifications for Pharmaceutical Preparations.	October 2021
Further follow-up action as required.	

*[**Note from the Secretariat**. It is proposed to revise the monograph on Gelatin in* The International Pharmacopoeia.

The monograph is based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group (PDG). Editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia.

Changes to the current chapter are indicated in the text by insert or ~~delete.~~]

GELATIN (GELATINA)

This monograph is based on the corresponding, internationally-harmonized text developed by the Pharmacopoeial Discussion Group (PDG). Editorial modifications have been made in order to be in line with the style used in The International Pharmacopoeia .

Chemical name. Gelatin; CAS Reg. No. 9000-70-8.

Description

Gelling grades: faintly yellow or light yellowish-brown solid, usually occurring as translucent sheets, shreds, granules or powder.

Non-gelling grades: faintly yellow or white granules or powder.

Solubility

Gelling grades: practically insoluble in common organic solvents; swell in cold water and give on heating a colloidal solution which on cooling forms a more or less firm gel.

Non-gelling grades: soluble in cold or warm water, practically insoluble in common organic solvents.

Category. Encapsulating agent; tablet binder; coating agent; suspending agent; viscosity-increasing agent.

Storage. Gelatin should be kept in a well-closed container.

Labelling. For gelling grades, the designation on the container should state the nominal gel strength of the gelatin.

Additional information. These specifications do not necessarily apply to gelatin for parenteral use or other particular application. Attention should be paid to the microbiological quality since gelatin is of natural origin.

Requirements

Definition. Gelatin is a purified protein obtained from collagen of animals by partial alkaline and/or acid hydrolysis and/or enzymatic hydrolysis, or by thermal hydrolysis. The hydrolysis leads to either gelling or non-gelling product grades. This monograph covers both gelling grades and non-gelling grades.

Solution S. Dissolve 1.00 g of the test substance in carbon-dioxide-free water R, heat to about 55 °C, and dilute to 100 mL with the same solvent. Keep the solution at this temperature to carry out the tests in which the use of solution S is indicated.

Identity tests

For gelling grades, carry out tests A and B. For non-gelling grades, carry out tests A, B and C.

A. To 2 mL of solution S add 0.05 mL of copper(II) sulfate (80 g/L) TS, mix, and add 0.5 mL of sodium hydroxide (~85 g/L) TS; a violet colour is produced.

B. Transfer 0.5 g of the test substance to a test-tube, add 10 mL of water, and allow to stand for 10 minutes. Heat at 60 °C for 15 minutes and keep the tube in a vertical position at 2-8 °C for 6 hours. Invert the tube; for gelling grades, the contents do not flow out immediately; for non-gelling grades, the contents flow out immediately.

C. To 0.5 g of the test substance in a 250 mL bottle, add 10 mL of water R and 5 mL of sulfuric acid (~1760 g/L) R. Place the bottle, partly but not completely closed (for example, using a watch glass), in an oven at 105 °C for 4 hours. Allow to cool and add 200 mL of water R. Adjust to pH 6.0-8.0 using sodium hydroxide (~200 g/L) TS. Place 2 mL of the solution in a test-tube and add 2 mL of a solution prepared immediately before use containing 14 g/L of tosylchloramide sodium R in phosphate buffer solution pH 6.8 R. Mix and allow to stand for 20 minutes. Add 2 mL of 4-dimethylaminobenzaldehyde TS8. Mix and place in a water-bath at 60 °C for 15 minutes; a red to violet colour develops.

pH value. pH of solution S, measured at 55 °C; 3.8-7.6.

Conductivity. Use a 10.0 g/L solution of the test substance at 30 ± 1.0 °C. Proceed with the test as described under *1.18 Conductivity [**Note from the Secretariat**. The chapter on conductivity is currently under elaboration]*.without the use of a temperature compensation device; the conductivity is not more than 1 $mS \cdot cm^{-1}$.

Heavy metals. Use 1.0 g of the test substance for the preparation of the test solution as described under *2.2.3 Limit test for heavy metals*, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.

Loss on drying. Dry 5 g of the test substance at 105 °C for 16 hours; it loses not more than 150 mg/g.

Sulfur dioxide. Proceed with the test described under *2.11 Determination of Sulphur Dioxide [**Note from the Secretariat**. This chapter is currently under elaboration.]*; the sulfur dioxide concentration is not more than 50 µg/g.

Peroxides. Carry out the test described below using peroxide test strips R that contain peroxidase and that comply with the suitability test described below. The peroxidase present in the test strips transfers oxygen from peroxides to an organic redox indicator which is converted to a blue oxidation product. The intensity of the colour obtained is proportional to the quantity of peroxide and can be compared with a colour scale provided with the test strips, to determine the peroxide concentration.

Suitability test. Dip a test strip for 1 second into hydrogen peroxide standard (2 µg H_2O_2/mL) TS, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and, after 15 seconds, compare the reaction zone with the colour scale provided. The test strips are suitable if the colour matches that of the 2 ppm concentration.

Test. Weigh 20.0 ± 0.1 g of the test substance in a beaker and add 80.0 ± 0.2 mL of water R. Stir to moisten all the gelatin and allow the sample to stand at room temperature for 1-3 hours. Cover the beaker with a watch-glass. If dissolution is not complete, place the beaker for 20 ± 5 minutes in a water-bath at 65 ± 2 °C to dissolve the sample. Stir the contents of the beaker with a glass rod to achieve a homogeneous solution. Dip a test strip for 1 second into the test solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and, after 15 seconds, compare the reaction zone with the colour scale provided.

Multiply the concentration read from the colour scale by a factor of 5 to calculate the concentration in µg/g of peroxide in the test substance; the peroxide concentration is not more than 10 µg/g.

Gel strength (Bloom value). For gelling grades, carry out the following test:

The gel strength is expressed as the mass in grams necessary to produce the force which, applied to a plunger 12.7 mm in diameter, makes a depression 4 mm deep in a gel having a concentration of 6.67 per cent (*m/m*) and matured at 10 °C.

The apparatus consists of a texture analyser or gelometer with a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane pressure surface with a sharp bottom edge; and a bottle 59 ± 1 mm in internal diameter and 85 mm high.

Adjust the apparatus according to the manufacturer's manual. Settings are: distance 4 mm, test speed 0.5 mm/s.

Prepare a gel as follows: Place 7.5 g of the test substance in the bottle. Add 105 mL of water R, close the bottle and allow to stand for 1-4 hours. Heat in a water-bath at 65 ± 2 °C for 15 minutes. While heating, stir gently with a glass rod. Ensure that the solution is uniform and that any condensed water on the inner walls of the bottle is incorporated. Allow to cool at room temperature for 15 minutes and transfer the bottle to a thermostatically controlled bath at 10.0 ± 0.1 °C, fitted with a device to ensure that the platform on which the bottle stands is perfectly horizontal. Close the bottle with a rubber stopper and allow to stand for 17 ± 1 hours.

Remove the bottle from the bath and quickly wipe the water from the exterior of the bottle. Centre the bottle on the platform of the apparatus so that the plunger contacts the sample as near to its midpoint as possible and start the measurement.

The gel strength is not less than 80 per cent and not more than 120 per cent of the nominal value stated on the labelling.

Iron. Determine by atomic absorption spectrophotometry as described under *1.8 Atomic spectrometry: emission and absorption*, Method 2 *[**Note from the Secretariat.** Chapter 1.8 Atomic spectrometry: emission and adsorption is currently under revision.]*, at a wavelength

of 248.3 nm. Prepare the test solution as follows: To 5.00 g of the test substance to be examined, in a conical flask, add 10 mL of hydrochloric acid (~420 g/L) TS. Close the flask and place in a water-bath at 75-80 °C for 2 hours (if necessary for proper solubilisation, the gelatin may be allowed to swell after addition of the acid and before heating, the heating time may be prolonged, and a higher temperature may be used). Allow to cool and adjust the contents of the flask to 100.0 g with water R. Use iron standard (8 µg Fe/mL) TS to prepare the reference solutions, diluting with water R; the iron content is not more than 30 µg per g.

Chromium. Determine by atomic absorption spectrophotometry as described under *1.8 Atomic spectrometry: emission and absorption*, Method 2, at a wavelength of 357.9 nm. Prepare the test solution as described in the test for Iron. Use chromium standard (100 µg Cr/mL) TS to prepare the reference solutions, diluting with water R; the chromium content is not more than 10 µg per g.

Zinc. Determine by atomic absorption spectrophotometry as described under *1.8 Atomic spectrometry: emission and absorption*, Method 2, at a wavelength of 213.9 nm. Prepare the test solution as described in the test for Iron. Use zinc standard (10 µg Zn/mL) TS to prepare the reference solutions, diluting with water R; the zinc content is not more than 30 µg per g.

Microbial contamination. Determine as described under *3.3. Microbiological examination of non-sterile products*. The acceptance criteria are: TAMC 103 CFU/g (3.3.1), TYMC 102 CFU/g (3.3.1), Absence of *Escherichia coli* (3.3.2) and Absence of *Salmonella* (3.3.2).

REAGENTS to be amended

Tosylchloramide sodium R

Amend the entry to include the synonym '*chloramine T*'.

Hydrogen peroxide (~30 g/L) TS

Change the content statement from *' about 30 g of H_2O_2 per litre'* to *'…….not less than 25 g and nt more than 35 g of H_2O_2 per litre."*

REAGENTS to be added

Phosphate buffer solution pH 6.8 R

Procedure. Mix 77.3 mL of a 71.5 g/L solution of disodium hydrogen phosphate R with 22.7 mL of a 21 g/L solution of citric acid R.

4-Dimethylaminobenzaldehyde TS8

Procedure. Dissolve 1.0 g of 4-dimethylaminobenzaldehyde R in 3.5 mL of perchloric acid (~600 g/L) TS and slowly add 6.5 mL of 2-propanol R.

Note: 4-Dimethylaminobenzaldehyde TS8 should be prepared immediately before use.

Perchloric acid (~600 g/L) TS

Perchloric acid (~1170 g/L) TS, diluted with water to contain 600 g/L of $HClO_4$.

Peroxide test strips R

Use commercial test strips with a suitable scale in the range from 0 ppm to 25 ppm peroxide.

Hydrogen peroxide standard (2 µg H_2O_2/mL) TS

Procedure. Dilute 10.0 mL of hydrogen peroxide (~30 g/L) TS to 300.0 mL with water R. Dilute 2.0 mL of this solution to 1000.0 mL with water R.

Note: Hydrogen peroxide standard (2 µg H_2O_2/mL) TS should be prepared immediately before use.

Iron standard (8 µg Fe/mL) TS

Procedure. Dissolve 80 mg of reduced iron R in 50 mL of hydrochloric acid (~220 g/L) TS and dilute to 1000.0 mL with water R. Immediately before use, dilute this solution to 10 times its volume using water R. Each mL of the resultant solution contains 8 µg of iron.

Hydrochloric acid (~220 g/L) TS

A solution of hydrochloric acid (~420 g/L) TS in water containing approximately 220 g of HCl per litre (about 6 mol/L).

Chromium standard (100 µg Cr/mL) TS

Procedure. Dissolve potassium dichromate R equivalent to 0.283 mg of $K_2Cr_2O_7$ in water R and dilute to 1000.0 mL with the same solvent. Each mL of this solution contains 100 µg of chromium.

Zinc standard (10 µg Zn/mL) TS

Procedure. Dissolve 0.440 g of zinc sulfate R and 1 mL of acetic acid (~300 g/L) TS in water R and dilute to 100.0 mL with the same solvent. Immediately before use, dilute this solution to 100 times its volume using water R. Each mL of the resultant solution contains 10 µg of zinc.

~~**Gelatin (Gelatina)**~~

~~**Chemical name**. Gelatin; CAS Reg. No. 9000-70-8.~~

~~**Description**. Faintly yellow to amber-coloured sheets, flakes, granules, or powder; practically odourless; in solution it has a slight, characteristic, bouillon-like odour.~~

~~**Solubility**. Practically insoluble in most organic solvents. In cold water it swells and softens, absorbing 5–10 times its own mass of water. After swelling, soluble in hot water, in acetic acid (~300 g/l) TS, and in a hot mixture of glycerol R and water.~~

~~**Category**. Encapsulating agent; tablet binder; coating agent; suspending agent; viscosity-increasing agent.~~

~~**Storage**. Gelatin should be kept in a well-closed container.~~

~~**Additional information**. These specifications do not necessarily apply to gelatin for parenteral use or other particular application. Attention should be paid to the microbiological quality since gelatin is of natural origin.~~

~~The type of gelatin may be distinguished by the following test:~~

~~Dissolve 1 g in 100 mL of hot water. Place aliquots of 5 mL into six separate test-tubes and add 5 mL of a buffer to each tube, using buffers of pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 (citrate buffer, pH 4.0, TS, phosphate buffer, pH 4.0, TS, or phthalate buffer, pH 4.0, TS; acetate buffer, pH 5.0, TS; phosphate/citrate buffer, pH 6.0, TS or acetate buffer, pH 6.0, TS; phosphate buffer, pH 7.0, TS; phosphate buffer, pH 8.0, TS or buffer borate, pH 8.0, TS; buffer borate, pH 9.0, TS). Cool the test-tubes and allow them to stand at 4 °C for 24 hours; the type of gelatin is recognized by the resulting opalescence – a maximum opalescence appearing at pH 5.0 indicates gelatin type B, while a maximum opalescence between pH 7.0 and pH 9.0 indicates gelatin type A.~~

~~Requirements~~

~~**Definition.** Gelatin is a purified protein obtained either by the partial acid hydrolysis (type A) or by the partial alkali hydrolysis (type B) of animal collagen. It can exist as a mixture of both types.~~

~~Identity tests~~

~~A. Dissolve 1 g in carbon-dioxide-free water R, heat to about 55 °C, and dilute to 100 mL with the same solvent. Keep the solution at this temperature throughout the following test (retain the solution for test C): to 2 mL add 0.05 mL of copper(II) sulfate (160 g/l) TS, mix, and add 0.5 mL of sodium hydroxide (~80 g/l) TS; a violet colour is produced.~~

~~B. Transfer 0.5 g to a test-tube, add 10 mL of water, and allow to stand for 10 minutes. Heat at 60 °C for 15 minutes and keep the tube in a vertical position at 0 °C for 6 hours. Invert the tube; the content does not immediately flow out.~~

~~C. Acidify 2 mL of the solution prepared for test A and add 0.5 mL of potassium dichromate (100 g/l) TS; a yellow precipitate is formed.~~

~~**Heavy metals**. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 3; determine the heavy metals content according to Method A; not more than 10 µg/g.~~

Arsenic. Use a solution of 1.0 g in a mixture of 2.5 mL of sulfuric acid (~1760 g/l) TS, 2.5 mL of nitric acid (~1000 g/l) TS, and a slight excess of bromine TS1, allow to stand for 30 minutes, and boil under a reflux condenser for 1 hour. Proceed with the test as described under 2.2.5 Limit test for arsenic; the arsenic content is not more than 1 µg/g.

Odour and water-insoluble substances. Dissolve 1 g in 40 mL of hot water; no disagreeable odour is perceptible. Observe the solution through a layer of 2 cm; only a slight opalescence appears.

Sulfated ash. Use 2.0 g; not more than 30 mg/g.

Loss on drying. Weigh 10 g and dry to constant mass at 105 °C; it loses not more than 150 mg/g.

Sulfur dioxide. Dissolve 20 g in 150 mL of hot water using a round-bottom flask with a long neck. Add 5 mL of phosphoric acid (~1440 g/l) TS and 1 g of sodium hydrogen carbonate R, and without delay connect the flask to a condenser. (*Note*. Excessive foaming can be reduced by adding a few drops of an antifoaming agent.) Distil 50 mL, allowing the distillate to be collected under a 50 mL surface of iodine (0.05 mol/l) VS. Acidify the distillate with a few drops of hydrochloric acid (~70 g/l) TS, add 2 mL of barium chloride (50 g/l) TS, and heat on a water-bath until the liquid is nearly colourless. If any, filter the precipitated barium sulfate, wash, ignite, and weigh. Repeat the procedure without the Gelatin being examined and make any necessary corrections. The content of barium sulfate is not more than 109.3 mg, which corresponds to not more than 1.5 mg/g of sulfur dioxide.

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