E55A GELATIN, GELLING GRADE

Gelatina

DEFINITION

Purified protein obtained from collagen of animals (including fish and poultry) by partial alkaline and/or acid hydrolysis, by enzymatic hydrolysis or by thermal hydrolysis.

The hydrolysis leads to gelling or non-gelling grades. This monograph covers the gelling grades

CHARACTERS

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

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

The isoelectric point is a relevant quality parameter for use of gelatin in different applications: different gelatins have an isoelectric point in the range pH 4.7-9.5 and for specific applications a narrower tolerance is usually applied.

Gelatin forms aqueous solutions that vary in clarity and colour. For a particular application, a suitable specification for clarity and colour may be required.

IDENTIFICATION

A. To 2 ml of solution S (see Tests) add 0.05 ml of a 125 g/l solution of copper sulphate pentahydrate. Mix and add 0.5 ml of an 85 g/l solution of sodium hydroxide. A violet colour is produced.

B. To 0.5 g in a test-tube add 10 ml of water. Allow to stand for 10 min, heat at 60 °C for 15 min and keep the tube upright at 0 °C for 6 h. Invert the tube; the contents do not flow out immediately for gelling grades.

C. Place 0.5 g in a 250 ml bottle. Add 10 ml of water and 5 ml of sulphuric acid. Place the bottle, partly but not completely closed (for example, using a watch glass), in an oven at 105 °C for 4 h. Allow to cool and add 200 ml of water. Adjust to pH 6.0-8.0 using a 200 g/l solution of sodium hydroxide. Place 2 ml of the solution in a test-tube and add 2 ml of oxidising reagent [14 g/l solution of chloramine in phosphate buffer solution pH 6.8; prepare immediately before use]. Mix and allow to stand for 20 min. Add 2 ml of colour reagent [prepared immediately before use by dissolving 1.0 g of dimethylaminobenzaldehyde in 3.5 ml of perchloric acid (600 g/l $HClO_4$) and slowly adding 6.5 ml of 2-propanol]. Mix and place in a water-bath at 60 °C for about 15 min. A red colour develops.

TESTS

Solution S. Dissolve 1.00 g in carbon dioxide-free water at about 55 °C, dilute to 100 ml with the same solvent and keep the solution at this temperature to carry out the tests.

pH: 3.8 to 7.6 for solution S, measured at 55 °C.

Conductivity: maximum 1 $mS \cdot cm^{-1}$, determined on a 1.0 per cent solution at 30 ± 1.0 °C (without the use of the temperature compensation).

Sulphur dioxide: maximum 50 ppm.

Introduce 150 ml of water into the flask (*A*) (see Figure 1) and pass carbon dioxide through the whole system for 15 min at a rate of 100 ml/min. To 10 ml of hydrogen peroxide solution (30 g/l H_2O_2) add 0.15 ml of a 1 g/l solution of bromophenol blue in alcohol (20 per cent *V/V*). Add 0.1 M sodium hydroxide until a violet-blue colour is obtained, without exceeding the end-point. Place the solution in the test-tube (*D*). Without interrupting the stream of carbon dioxide, remove the funnel (*B*) and introduce through the opening into the flask (*A*) 25.0 g (*m* g) of the substance to be examined with the aid of 100 ml of water. Add through the funnel 80 ml of dilute hydrochloric acid (73 g/l HCl) and boil for 1 h. 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. Add 0.1 ml of 1 g/l solution of bromophenol blue in alcohol (20 per cent *V/V*) and titrate with 0.1 M sodium hydroxide until the colour changes from yellow to violet-blue (V_1 ml). Carry out a blank titration (V_2 ml). Calculate the content of sulphur dioxide in parts per million from the expression:

$$32030 \times (V_1 - V_2) \times n/m$$

n = molarity of the sodium hydroxide solution used as titrant

Peroxides: maximum 10 ppm, determined using peroxide test strips.

Peroxidase 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 s into hydrogen peroxide standard solution (10 ppm H_2O_2) [prepared by dilution of hydrogen peroxide solution (30 g/l H_2O_2)], such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and after 15 s compare the reaction zone with the colour scale provided. The test strips are suitable if the colour matches that of the 10 ppm concentration.

Test. Weigh 20.0 ± 0.1 g of the substance to be tested in a beaker and add 80.0 ± 0.2 ml of water. Stir to moisten all the gelatin and allow the sample to stand at room temperature for 1-3 h. Cover the beaker with a watch-glass. Place the beaker for 20 ± 5 min 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 s into the test solution, such that the reaction zone is properly wetted. Remove the test strip, shake off excess liquid and compare the reaction zone after 15 s with the colour scale provided. Multiply the concentration read from the colour scale by a factor of 5 to calculate the concentration in parts per million of peroxide in the test substance.

Gel strength (Bloom value):, 80 to 120 per cent of the labelled nominal value.

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.

Apparatus. Texture analyser or gelometer with:

— a cylindrical piston 12.7 ± 0.1 mm in diameter with a plane pressure surface and a sharp bottom edge,

— 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.

Method. Place 7.5 g of the substance to be tested in a bottle. Add 105 ml of water, place a watch-glass over the bottle and allow to stand for 1-4 h. Heat in a water-bath at 65 ± 2 °C for 15 min. 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 min and transfer the bottle to a thermostatically controlled bath at 10.0 ± 0.1 °C, and 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 h. Remove the sample 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 nearly at its midpoint as possible and start the measurement.

Iron: maximum 30 ppm.

Atomic absorption spectrometry, standard additions method

Test solution. To 5.00 g of the substance to be examined, in a conical flask, add 10 ml of hydrochloric acid (37 per cent *m/m* HCl). Close the flask and place in a water-bath at 75-80 °C for 2 h. (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 content of the flask to 100.0 g with water.

Reference solutions. Prepare the reference solutions using *iron standard solution (8 ppm Fe)*, diluted as necessary with water.

Wavelength: 248.3 nm.

Chromium: maximum 10 ppm.

Atomic absorption spectrometry, standard additions method

Test solution. Test solution described in the test for iron.

Reference solutions. Prepare the reference solutions using *chromium standard solution (100 ppm Cr)*, diluted if necessary with water.

Wavelength: 357.9 nm.

Zinc: maximum 30 ppm.

Atomic absorption spectrometry, standard additions method

Test solution. Test solution described in the test for iron.

Reference solutions. Prepare the reference solutions using *zinc standard solution (10 ppm Zn)*, diluted if necessary with water.

Wavelength: 213.9 nm.

Loss on drying: maximum 15.0 per cent, determined on 5.000 g, by drying in an oven at 105 °C for 16 h.

Microbial contamination.

TAMC: acceptance criterion 10^3 CFU/g.
TYMC: acceptance criterion 10^2 CFU/g.
Absence of *Escherichia coli*.
Absence of *Salmonella*.

STORAGE

Protect from heat and moisture.

LABELLING

The label states the gel strength (Bloom value)

REAGENTS

Chromium standard solution (100 ppm Cr): solution of potassium dichromate in water.

Iron standard solution (8 ppm): dissolve 80 mg of iron in 50 ml of hydrochloric acid (220 g/l of HCl) and dilute to 1000.0 ml with water. Immediately before use, dilute a portion of the solution to 10 times its volume with water.

Zinc standard solution (10 ppm): dissolve 0.440 g of zinc sulphate heptahydrate and 1 ml of acetic acid (300 g/l of $C_2H_4O_2$) in water and dilute to 100.0 ml. Immediately before use, dilute a portion of the solution to 100 times its volume with water.

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

Phosphate buffer solution pH 6.8. Mix 77.3 ml of a 71.5 g/l solution of disodium hydrogen phosphate dodecahydrate with 22.7 ml of a 21 g/l solution of citric acid.

Fig 1. Apparatus for determination of sulphur dioxide