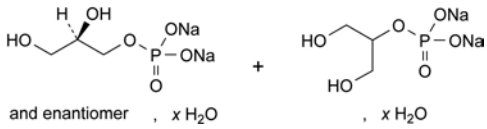
01/2009:1995
corrected 6.6

SODIUM GLYCEROPHOSPHATE, HYDRATED

Natrii glycerophosphas hydricus



$C_3H_7Na_2O_6P, xH_2O$ M_r 216.0 (anhydrous substance)

DEFINITION

Mixture of variable proportions of sodium (2*RS*)-2,3-dihydroxypropyl phosphate and sodium 2-hydroxy-1-(hydroxymethyl)ethyl phosphate. The mixture may contain various amounts of other glycerophosphate esters. The degree of hydration is 4 to 6.

Content: 98.0 per cent to 105.0 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white, crystalline powder or crystals.

Solubility: freely soluble in water, practically insoluble in acetone and in ethanol (96 per cent).

IDENTIFICATION

A. Solution S (see Tests) gives reaction (a) of sodium (*2.3.1*).

B. To 0.1 g add 5 mL of *dilute nitric acid R*. Heat to boiling and boil for 1 min. Cool. The solution gives reaction (b) of phosphates (*2.3.1*).

C. In a test-tube fitted with a glass tube, mix 0.1 g with 5 g of *potassium hydrogen sulfate R*. Heat strongly and direct the white vapour into 5 mL of *decolorised fuchsin solution R*. A violet-red colour develops which becomes violet upon heating for 30 min on a water-bath.

TESTS

Solution S. Dissolve 10.0 g in *carbon dioxide-free water R* prepared from *distilled water R* and dilute to 100 mL with the same solvent.

Appearance of solution. Solution S is not more opalescent than reference suspension II (*2.2.1*) and not more intensely coloured than reference solution Y_6 (*2.2.2, Method II*).

Alkalinity. To 10 mL of solution S add 0.2 mL of *phenolphthalein solution R*. Not more than 1.0 mL of *0.1 M hydrochloric acid* is required to change the colour of the indicator (n_2).

Glycerol and ethanol (96 per cent)-soluble substances: maximum 1.0 per cent.

Shake 1.000 g with 25 mL of *ethanol (96 per cent) R* for 10 min. Filter. Evaporate the filtrate on a water-bath and dry the residue at 70 °C for 1 h. The residue weighs not more than 10 mg.

Chlorides (*2.4.4*): maximum 200 ppm.

Dilute 2.5 mL of solution S to 15 mL with *water R*.

Phosphates (*2.4.11*): maximum 0.1 per cent.

Dilute 1 mL of solution S to 10 mL with *water R*. Dilute 1 mL of this solution to 100 mL with *water R*.

Sulfates (*2.4.13*): maximum 500 ppm.

Dilute 3 mL of solution S to 15 mL with *water R*.

Iron (*2.4.9*): maximum 20 ppm.

Dilute 5 mL of solution S to 10 mL with *water R*.

Heavy metals (*2.4.8*): maximum 20 ppm.

Dilute 10 mL of solution S to 20 mL with *water R*. 12 mL of the solution complies with test A. Prepare the reference solution using 10 mL of *lead standard solution (1 ppm Pb) R*.

Water (*2.5.12*): 25.0 per cent to 35.0 per cent, determined on 0.100 g.

ASSAY

Dissolve 0.250 g in 30 mL of *water R*. Titrate with *0.05 M sulfuric acid*, determining the end-point potentiometrically (*2.2.20*), (n_1).

Calculate the percentage content of sodium glycerophosphate (anhydrous substance) using the following expression:

$$\frac{216.0\left(n_1 - \frac{n_2}{4}\right)}{m\,(100 - a)}$$

a = percentage content of water;

n_1 = volume of *0.05 M sulfuric acid* used in the assay, in millilitres;

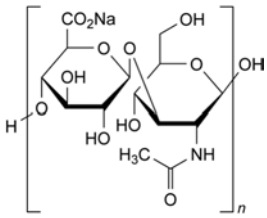
n_2 = volume of *0.1 M hydrochloric acid* used in the test for alkalinity, in millilitres;

m = mass of the substance to be examined, in grams.

01/2011:1472

SODIUM HYALURONATE

Natrii hyaluronas



$(C_{14}H_{20}NNaO_{11})_n$
[9067-32-7]

DEFINITION

Sodium salt of hyaluronic acid, a glycosaminoglycan consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine disaccharide units. It is extracted from cocks' combs or obtained by fermentation from *Streptococci*, Lancefield Groups A and C.

Content: 95.0 per cent to 105.0 per cent (dried substance).

Intrinsic viscosity: 90 per cent to 120 per cent of the value stated on the label.

PRODUCTION

Where applicable, the animals from which sodium hyaluronate is derived must fulfil the requirements for the health of animals suitable for human consumption.

When produced by fermentation of gram-positive bacteria, the process must be shown to reduce or eliminate pyrogenic or inflammatory components of the cell wall.

CHARACTERS

Appearance: white or almost white, very hygroscopic powder or fibrous aggregate.

Solubility: sparingly soluble or soluble in water, practically insoluble in acetone and in anhydrous ethanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *Ph. Eur. reference spectrum of sodium hyaluronate.*

B. It gives reaction (a) of sodium (*2.3.1*).

TESTS

Solution S. Weigh a quantity of the substance to be examined equivalent to 0.10 g of the dried substance and add 30.0 mL of a 9 g/L solution of *sodium chloride R*. Mix gently on a shaker until dissolved (about 12 h).

Appearance of solution. Solution S is clear (*2.2.1*) and its absorbance (*2.2.25*) at 600 nm is not greater than 0.01.

pH (*2.2.3*): 5.0 to 8.5.

Dissolve the substance to be examined in *carbon dioxide-free water R* to obtain a solution containing a quantity equivalent to 5 mg of the dried substance per millilitre.

Intrinsic viscosity. *Sodium hyaluronate is very hygroscopic and must be protected from moisture during weighing.*

Buffer solution (0.15 M sodium chloride in 0.01 M phosphate buffer solution pH 7.0). Dissolve 0.78 g of *sodium dihydrogen phosphate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 mL with the same solvent (solution A). Dissolve 1.79 g of *disodium hydrogen phosphate R* and 4.50 g of *sodium chloride R* in *water R* and dilute to 500.0 mL with the same solvent (solution B). Mix solutions A and B until a pH of 7.0 is reached. Filter through a sintered-glass filter (4) (*2.1.2*).

Test solution (a). Weigh 0.200 g (m_{0p}) *(NOTE: this value is only indicative and should be adjusted after an initial measurement of the viscosity of test solution (a))* of the substance to be examined and dilute with 50.0 g (m_{0s}) of buffer solution at 4 °C. Mix the solution by shaking at 4 °C during 24 h. Weigh 5.00 g (m_{1p}) of the solution and dilute with 100.0 g (m_{1s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*), and discard the first 10 mL.

Test solution (b). Weigh 30.0 g (m_{2p}) of test solution (a) and dilute with 10.0 g (m_{2s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Test solution (c). Weigh 20.0 g (m_{3p}) of test solution (a) and dilute with 20.0 g (m_{3s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Test solution (d). Weigh 10.0 g (m_{4p}) of test solution (a) and dilute with 30.0 g (m_{4s}) of buffer solution at 25 °C. Mix this solution by shaking for 20 min. Filter the solution through a sintered-glass filter (100) (*2.1.2*) and discard the first 10 mL.

Determine the flow-times (*2.2.9*) for the buffer solution (t_0) and for the 4 test solutions (t_1, t_2, t_3 and t_4), at 25.00 ± 0.03 °C. Use an appropriate suspended level viscometer (specifications: viscometer constant about 0.005 mm²/s², kinematic viscosity of 1-5 mm²/s, internal diameter of tube *R* 0.53 mm, volume of bulb *C* 5.6 mL, internal diameter of tube *N* 2.8-3.2 mm) with a funnel-shaped lower capillary end. Use the same viscometer for all measurements; measure all outflow times in triplicate. The test is not valid unless the results do not differ by more than 0.35 per cent from the mean and if the flow time t_1 is not less than 1.6 and not more than 1.8 times t_0. If this is not the case, adjust the value of m_{0p} and repeat the procedure.

Calculation of the relative viscosities

Since the densities of the sodium hyaluronate solutions and of the solvent are almost equal, the relative viscosities η_{ri} (being η_{r1}, η_{r2}, η_{r3} and η_{r4}) can be calculated from the ratio of the flow times for the respective solutions t_i (being t_1, t_2, $t_3$and t_4) to the flow time of the solvent t_0, but taking into account the kinetic energy correction factor for the capillary (B = 30 800 s²), using the following expression:

$$\frac{t_i - \frac{B}{t_i^2}}{t_0 - \frac{B}{t_0^2}}$$

Calculation of the concentrations

Calculate the concentration c_1 (expressed in kg/m³) of sodium hyaluronate in test solution (a) using the following expression:

$$\frac{m_{0p} \times x \times (100 - h) \times m_{1p} \times \rho_{25}}{100 \times 100 \times (m_{0p} + m_{0s}) \times (m_{1p} + m_{1s})}$$

x = percentage content of sodium hyaluronate as determined under Assay;

h = percentage loss on drying;

ρ_{25} = 1005 kg/m³ (density of the test solution at 25 °C).

Calculate the concentration c_2 (expressed in kg/m³) of sodium hyaluronate in test solution (b) using the following expression:

$$c_1 \times \frac{m_{2p}}{m_{2s} + m_{2p}}$$

Calculate the concentration c_3 (expressed in kg/m³) of sodium hyaluronate in test solution (c) using the following expression:

$$c_1 \times \frac{m_{3p}}{m_{3s} + m_{3p}}$$

Calculate the concentration c_4 (expressed in kg/m³) of sodium hyaluronate in test solution (d) using the following expression:

$$c_1 \times \frac{m_{4p}}{m_{4s} + m_{4p}}$$

Calculation of the intrinsic viscosity

Calculate the intrinsic viscosity $[\eta]$ by linear least-squares regression analysis using the Martin equation:

$$\log\left(\frac{\eta_r - 1}{c}\right) = \log[\eta] + k[\eta]c$$

The decimal antilogarithm of the intercept is the intrinsic viscosity expressed in m³/kg.

Sulfated glycosaminoglycans: maximum 1 per cent, if the product is extracted from cocks' combs.

Appropriate safety precautions are to be taken when handling perchloric acid at elevated temperature.

Test solution. Introduce a quantity of the substance to be examined equivalent to 50.0 mg of the dried substance into a test-tube 150 mm long and 16 mm in internal diameter and dissolve in 1.0 mL of *perchloric acid R*.

Reference solution. Dissolve 0.149 g of *anhydrous sodium sulfate R* in *water R* and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with *water R*. Evaporate 1.0 mL in a test-tube 150 mm long and 16 mm in internal diameter in a heating block at 90-95 °C, and dissolve the residue in 1.0 mL of *perchloric acid R*.

Plug each test-tube with a piece of glass wool. Place the test-tubes in a heating block or a silicone oil bath maintained at 180 °C and heat until clear, colourless solutions are obtained (about 12 h). Remove the test-tubes and cool to room temperature. Add to each test-tube 3.0 mL of a 33.3 g/L solution of *barium chloride R*, cap and shake vigorously. Allow the test-tubes to stand for 30 min. Shake each test-tube once again, and determine the absorbance (*2.2.25*) at 660 nm, using *water R* as a blank.

The absorbance obtained with the test solution is not greater than the absorbance obtained with the reference solution.

Nucleic acids. The absorbance (*2.2.25*) of solution S at 260 nm is maximum 0.5.

Protein: maximum 0.3 per cent; maximum 0.1 per cent, if intended for use in the manufacture of parenteral preparations.

Test solution (a). Dissolve the substance to be examined in *water R* to obtain a solution containing a quantity equivalent to about 10 mg of the dried substance per millilitre.

Test solution (b). Mix equal volumes of test solution (a) and *water R*.

Reference solutions. Prepare a 0.5 mg/mL stock solution of *bovine albumin R* in *water R*. Prepare 5 dilutions of the stock solution containing between 5 µg/mL and 50 µg/mL of *bovine albumin R*.

Add 2.5 mL of freshly prepared *cupri-tartaric solution R3* to test-tubes containing 2.5 mL of *water R* (blank), 2.5 mL of the test solutions (a) or (b) or 2.5 mL of the reference solutions. Mix after each addition. After about 10 min, add to each test-tube 0.50 mL of a mixture of equal volumes of *phosphomolybdotungstic reagent R* and *water R* prepared immediately before use. Mix after each addition. After 30 min, measure the absorbance (*2.2.25*) of each solution at 750 nm against the blank. From the calibration curve obtained with the 5 reference solutions determine the content of protein in the test solutions.

Chlorides (*2.4.4*): maximum 0.5 per cent.

Dissolve 67 mg in 100 mL of *water R*.

Iron: maximum 80 ppm.

Atomic absorption spectrometry (*2.2.23, Method II*).

Test solution. Dissolve a quantity of the substance to be examined equivalent to 0.25 g of the dried substance in 1 mL of *nitric acid R* by heating on a water-bath. Cool and dilute to 10.0 mL with *water R*.

Reference solutions. Prepare 2 reference solutions in the same manner as the test solution, adding 1.0 mL and 2.0 mL respectively of *iron standard solution (10 ppm Fe) R* to the dissolved substance to be examined.

Source: iron hollow-cathode lamp using a transmission band of 0.2 nm.

Wavelength: 248.3 nm.

Atomisation device: air-acetylene flame.

Heavy metals (*2.4.8*): maximum 20 ppm; maximum 10 ppm if intended for use in the manufacture of parenteral preparations.

1.0 g complies with test F. Prepare the reference solution using 2.0 mL of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*): maximum 20.0 per cent, determined on 0.500 g by drying at 100-110 °C over *diphosphorus pentoxide R* for 6 h.

Microbial contamination

TAMC: acceptance criterion 10^2 CFU/g (*2.6.12*). Use 1 g of the substance to be examined.

Bacterial endotoxins (*2.6.14*): less than 0.5 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins; less than 0.05 IU/mg, if intended for use in the manufacture of intra-ocular preparations or intra-articular preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Determine the glucuronic acid content by reaction with carbazole as described below.

Reagent A. Dissolve 0.95 g of *disodium tetraborate R* in 100.0 mL of *sulfuric acid R*.

Reagent B. Dissolve 0.125 g of *carbazole R* in 100.0 mL of *anhydrous ethanol R*.

Test solution. Prepare this solution in triplicate. Dissolve 0.170 g of the substance to be examined in *water R* and dilute to 100.0 g with the same solvent. Dilute 10.0 g of this solution to 200.0 g with *water R*.

Reference stock solution. Dissolve 0.100 g of *D-glucuronic acid R*, previously dried to constant mass in vacuum over *diphosphorus pentoxide R* (*2.2.32*), in *water R* and dilute to 100.0 g with the same solvent.

Reference solutions. Prepare 5 dilutions of the reference stock solution containing between 6.5 µg/g and 65 µg/g of *D-glucuronic acid R*.

Place 25 test-tubes, numbered 1 to 25, in iced water. Add 1.0 mL of the 5 reference solutions in triplicate to the test-tubes 1 to 15 (reference tubes), 1.0 mL of the 3 test solutions in triplicate to the test-tubes 16 to 24 (sample tubes), and 1.0 mL of *water R* to test-tube 25 (blank). Add to each test-tube 5.0 mL of freshly prepared reagent A, previously cooled in iced water. Tightly close the test-tubes with plastic caps, shake the contents, and place on a water bath for exactly 15 min. Cool in iced water, and add to each test tube 0.20 mL of reagent B. Recap the tubes, shake, and put them again on a water-bath for exactly 15 min. Cool to room temperature and measure the absorbance (*2.2.25*) of the solutions at 530 nm, against the blank.

From the calibration curve obtained with the mean absorbances read for each reference solution, determine the mean concentrations of D-glucuronic acid in the test solutions.

Calculate the percentage content of sodium hyaluronate using the following expression:

$$\frac{c_g}{c_s} \times Z \times \frac{100}{100 - h} \times \frac{401.3}{194.1}$$

c_g = mean of concentrations of D-glucuronic acid in the test solutions, in milligrams per gram;

c_s = mean of concentrations of the substance to be examined in the test solutions, in milligrams per gram;

Z = determined percentage content of $C_6H_{10}O_7$ in *D-glucuronic acid R*;

h = percentage loss on drying;

401.3 = relative molecular mass of the disaccharide fragment;

194.1 = relative molecular mass of glucuronic acid.

STORAGE

In an airtight container, protected from light and humidity. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

LABELLING

The label states:

- the intrinsic viscosity;
- the origin of the substance;
- the intended use of the substance;
- where applicable, that the substance is suitable for parenteral administration other than intra-articular administration;
- where applicable, that the substance is suitable for parenteral administration, including intra-articular administration;
- where applicable that the material is suitable for intra-ocular use.

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SODIUM HYDROGEN CARBONATE

Natrii hydrogenocarbonas

$NaHCO_3$ M_r 84.0
[144-55-8]

DEFINITION

Content: 99.0 per cent to 101.0 per cent.