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Chlorophyllin Copper Complex Sodium

DEFINITION

Chlorophyllin Copper Complex Sodium contains sodium salts of copper-chelated chlorophyll derivatives. It contains no artificial coloring.

IDENTIFICATION

• A. <u>Ultraviolet-Visible Spectroscopy (857)</u> (in the visible region)

Sample solution: 10 µg/mL

Medium: pH 7.5 phosphate buffer, prepared by mixing 0.15 M dibasic sodium phosphate and 0.15 M monobasic potassium phosphate (21:4)

Acceptance criteria: The ratio of $\rm A_{405}/A_{630}$ is 3.0-3.9.

OTHER COMPONENTS

CONTENT OF TOTAL COPPER

Stock solution 1: 1000 μg/mL of copper. Transfer 1.000 g of copper to a 1000-mL volumetric flask, dissolve in 20 mL of nitric acid, and dilute with 0.2 N nitric acid to volume. [Note—Store in a polyethylene bottle.]

Stock solution 2: 10 μg/mL of copper. Transfer 5.0 mL of *Stock solution 1* into a 500-mL volumetric flask, and dilute with water to volume. **Standard solutions:** Transfer 5.0, 10.0, 15.0, and 20.0 mL, respectively, of *Stock solution 2* to separate 100-mL volumetric flasks, and dilute the contents of each flask with water to volume. These *Standard solutions* contain 0.5, 1.0, 1.5, and 2.0 μg/mL of copper, respectively.

Sample solution: Transfer 100 mg of previously dried Chlorophyllin Copper Complex Sodium to a Kjeldahl flask. Add 2.0 mL of sulfuric acid, 1.0 mL of nitric acid, and 1.0 mL of hydrogen peroxide, and carefully heat under a fume hood until a light green color is obtained. [Note—If the solution has any hint of a brown tint, continue to add 0.5-mL portions of nitric acid until a green color is obtained.] Cool, transfer the contents quantitatively to a 1000-mL volumetric flask with several portions of water, dilute the contents of the flask with water to volume, and mix. Transfer 10.0 mL of this solution to a 50-mL volumetric flask, and dilute with water to volume.

Instrumental conditions

(See Atomic Absorption Spectroscopy (852).)

Mode: Atomic absorption spectrophotometry

Lamp: Copper hollow-cathode

Flame: Air-acetylene

Analytical wavelength: Copper emission line of 324.8 nm

Blank: Water Analysis

Samples: Standard solutions and Sample solution

Determine the absorbances of the *Standard solutions* and the *Sample solution*. Plot the absorbances of the *Standard solutions* versus the concentration, in μ g/mL, of copper, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, *C*, in μ g/mL, of copper in the *Sample solution*.

Calculate the percentage of copper in the portion of Chlorophyllin Copper Complex Sodium taken:

Result =
$$(C/W) \times (V/F) \times 100$$

C = concentration of the Sample solution determined from the graph (μ g/mL)

 $W \quad = \text{weight of Chlorophyllin Copper Complex Sodium taken to prepare the } \textit{Sample solution (mg)}$

V = final volume of Sample solution, 5000 mL

 $F = \text{conversion factor, } 1000 \, \mu\text{g/mg}$

Acceptance criteria: NLT 4.25% on the dried basis

• CONTENT OF CHELATED COPPER

Analysis: Calculate the percentage of chelated copper in the portion of Chlorophyllin Copper Complex Sodium taken by subtracting the percentage of ionic copper found in the test for *Limit of Ionic Copper* from the percentage of total copper found in the test for *Content of Total Copper*.

Acceptance criteria: NLT 4.0% on the dried basis

Content of Sodium

Standard stock solution: 100 μ g/mL of sodium. Dissolve 254.2 mg of sodium chloride, previously dried at 105° for 2 h, in 50 mL of water. Transfer to a 1000-mL volumetric flask, and dilute with water to volume.

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Standard solutions: Transfer to each of four 100-mL volumetric flasks 10 mL of a nonionic wetting agent solution (1 in 500). To each flask add, respectively, 2.5, 5.0, 10.0, and 15.0 mL of the *Standard stock solution*, and dilute with water to volume. These *Standard solutions* contain 2.5, 5.0, 10.0, and 15.0 μg/mL of sodium, respectively.

Blank: Transfer 10 mL of a nonionic wetting agent solution (1 in 500) into a 100-mL volumetric flask, and dilute with water to volume. **Sample solution:** Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 1000-mL volumetric flask. Add 100 mL of a solution of nonionic wetting agent (1 in 500) and 400 mL of water, and shake by mechanical means for 5 min. Dilute with water to volume.

Analysis

Samples: Standard solutions and Sample solution

Set the flame photometer for maximum transmission at a wavelength of 589 nm. Adjust the instrument to zero transmittance with the *Blank*. Adjust the instrument to 100% transmittance with the most concentrated of the *Standard solutions*. Read the percentage of transmittance of the other *Standard solutions*, and plot the percentage of transmittance versus the concentration, in µg/mL, of sodium. Read the percentage of transmittance of the *Sample solution*, and from the graph read the concentration, *C*, in µg/mL, of sodium in the *Sample solution*.

Calculate the percentage of sodium in the portion of Chlorophyllin Copper Complex Sodium taken:

Result =
$$(C/W) \times (V/F) \times 100$$

 $C = \text{concentration of the } Sample \text{ } solution \text{ } determined \text{ } from \text{ } the \text{ } graph \text{ } (\mu g/mL)$

W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the Sample solution (mg)

V = volume of Sample solution, 1000 mL

 $F = \text{conversion factor, } 1000 \, \mu\text{g/mg}$

Acceptance criteria: 5%-7% on the dried basis
• <u>Nitrogen Determination (461)</u>, <u>Method I</u>: NLT 4.0%

IMPURITIES

• LIMIT OF IONIC COPPER

Standard solutions: Prepare as directed in the test for *Content of Total Copper*.

Sample solution: Transfer 100 mg of Chlorophyllin Copper Complex Sodium to a 150-mL conical flask. Add 75 mL of water, and shake by mechanical means for 3 min. Adjust with 1 N hydrochloric acid to a pH of 3.0, transfer the suspension thus obtained to a 100-mL volumetric flask, and dilute with water to volume. Filter this suspension, discarding the first 10 mL of the filtrate. Use the clear filtrate for analysis.

Analysis

Samples: Standard solutions and Sample solution

Proceed as directed in the test for Content of Total Copper.

Calculate the percentage of ionic copper in the portion of Chlorophyllin Copper Complex Sodium taken:

Result =
$$(C/W) \times (V/F) \times 100$$

C = concentration of the Sample solution determined from the graph (μ g/mL)

W = weight of Chlorophyllin Copper Complex Sodium taken to prepare the Sample solution (in mg on the dried basis)

V = volume of Sample solution, 100 mL

 $F = \text{conversion factor}, 1000 \,\mu\text{g/mg}$

Acceptance criteria: NMT 0.25% on the dried basis

• RESIDUE ON IGNITION (281): NMT 30% on the dried basis

Change to read:

• **▲**<u>Iron (241), Procedures, Procedure 1</u> (CN 1-Jun-2023): NMT 0.50%

SPECIFIC TESTS

• MICROBIAL ENUMERATION TESTS (61) and Tests for Specified MICROORGANISMS (62): It meets the requirements of the tests for absence of Escherichia coli and Salmonella species.

• PH (791): 9.5-10.7, in a solution (1 in 100)

• Loss on Drying (731)

Analysis: Dry at 105° for 2 h. **Acceptance criteria:** NMT 5%

• TEST FOR FLUORESCENCE

Sample solution: 10 mg/mL

Analysis: Apply 10 µL of *Sample solution* on filter paper, allow to dry, and examine the area of application under long-wavelength UV light through a red optical filter.

Acceptance criteria: No fluorescence is visible.

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• Packaging and Storage: Preserve in tight, light-resistant containers.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
CHLOROPHYLLIN COPPER COMPLEX SODIUM	<u>Documentary Standards Support</u>	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:

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