CHLOROPHYLLS, COPPER COMPLEXES

Prepared at the 31st JECFA (1987), published in FNP 38 (1988) and in FNP 52 (1992). Metals and arsenic specifications revised at the 59th JECFA (2002). An ADI of 0-15 mg/kg bw was established at the 13th JECFA (1969)

SYNONYMS Copper chlorophyll, copper phaeophytin, CI Natural Green 3; C.I. (1975) No. 75810, INS No. 141(i)

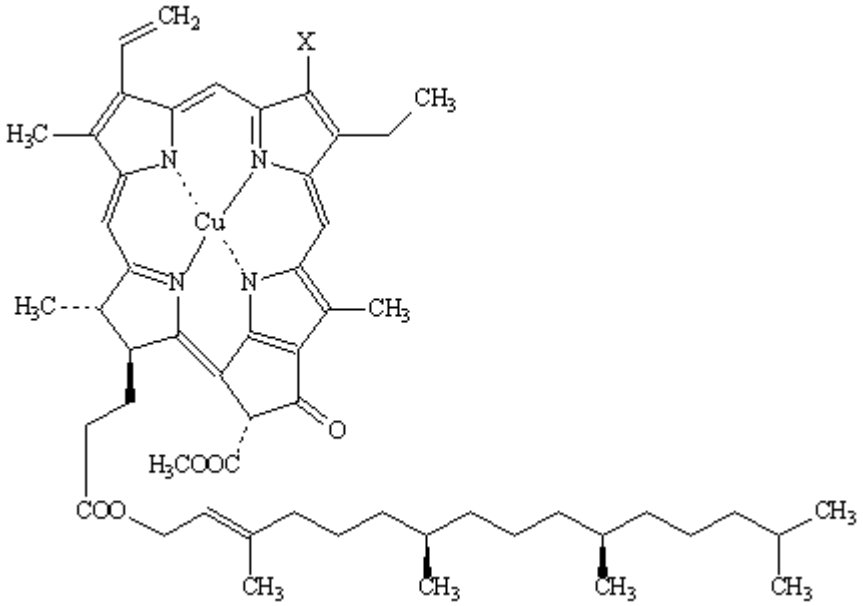
DEFINITION Obtained by addition of an organic salt of copper to the substance obtained by solvent extraction of grass, lucerne, nettle and other plant material; the product, from which the solvent has been removed, contains other pigments such as carotenoids as well as fats and waxes derived from the source material; the principal colouring matters are the copper phaeophytins.
Only the following solvents may be used for the extraction: Acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane.

Chemical names [Phytyl (13^2R,17S,18S)-3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13^1-oxo-3-vinyl-13^1,13^2,17,18-tetra-hydrocyclopenta [at]-prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll a)
[Phytyl (13^2R,17S,18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2,12,18-trimethyl-13^1-oxo-3-vinyl-13^1,13^2,17,18-tetrahydro-cyclopenta [at]prophyrin-17-yl)propionate] copper (II) (Copper chlorophyll b)

C.A.S. number 65963-40-8

Chemical formula Copper phaeophytin a: C_{55} H_{72}Cu $N_4 0_5$
Copper phaeophytin b: C_{55} H_{70}Cu $N_4 0_6$

Structural formula



where
X = CH_3 for the "a" compound
X = CHO for the "b" compound

Formula weight: Copper phaeophytin a: 932.75
Copper phaeophytin b: 946.73

Assay: Not less than 10% of total copper phaeophytins

DESCRIPTION Waxy solid ranging in colour from blue green to dark green depending on the source material.

FUNCTIONAL USES Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4): Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils

Spectrophotometry (Vol. 4): A (1%, 1 cm) at 422 nm in chloroform is not less than 54.

Thin-layer chromatography: Apply a 1 in 20 solution of the sample in chloroform as a band of the length of 2 cm to a Silica 60C plate. After drying, develop the plate by a mixture of 50% hexane, 45% chloroform and 5% ethanol (general purpose reagent grade chloroform is supplied with 2% of added ethanol as a stabilizer. The 5% ethanol in the solvent mixture is in addition to this), until the solvent ascends to a point 15 cm above the initial spots. Allow the solvent to evaporate, then visually chromatography examine the separated spots and identify the components of interests by their R_f values and colours. Approximate R_f values and colour of the spots are as follows:

Copper phaeophytin a: 0.5, green
Copper phaeophytin b: 0.73, yellow/green

In addition spots may be visible for ß-carotene at Rf 0.81 and xanthophyll at R_f 0.47 and 0.23.

PURITY

Residual solvents (Vol. 4): Acetone, methanol, ethanol, propan-2-ol, hexane: Not more than 50 mg/kg, singly or in combination
Dichloromethane: Not more than 10 mg/kg
Determine *gas chromatographically* using either the method of entrainment distillation (*Determination of Residual Solvents*) or headspace analysis (*Limit Test for Solvent Residues*).

Free ionizable copper: Not more than 200 mg/kg
Accurately weigh about 1 g of the sample and dissolve in 20 ml of arachid oil, with the aid of gentle heat. Add exactly 200 ml of water, stir mechanically, and adjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid (avoid over-shooting). Allow the mixture to stand for 10 min. If necessary readjust to pH 3.0 by careful addition of 0.5 N hydrochloric acid. Transfer to a separating

funnel and allow to stand for about 20 min. Filter the aqueous phase through a No. 50 Whatman filter paper, rejecting the first 10 ml. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Total copper

Not more than 8% of the total copper phaeophytins
Ignite about 0.1 g, accurately weighed, of the sample contained in a silica dish, at a temperature not exceeding 500º, until all carbon is removed; moisten with one or two drops of concentrated sulphuric acid and re-ash. Dissolve the ash by boiling with 3 portions (each of 5 ml) of 10% (w/w) hydrochloric acid, filtering each addition through the same small filter paper into a 100 ml volumetric flask. Cool, and make up to volume with purified water. Subject this solution to analysis for copper by *atomic absorption spectrometry* (see Volume 4).

Arsenic (Vol. 4)

Not more than 3 mg/kg (Method II)

Lead (Vol. 4)

Not more than 5 mg/kg
Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, “Instrumental Methods.”

METHOD OF ASSAY

Accurately weigh about 100 mg of the sample and dissolve in diethyl ether, making the volume to 100 ml. Dilute 2 ml of this solution to 25 ml with diethyl ether. The concentration of the sample should not give an absorbance at 660.4 nm that is in excess of the working range for Absorbance measurements, i.e., not in excess of 0.7.

Measure the absorbances (A) of the solution in a 1 cm cell against a diethyl ether blank at 667.2 nm, 654.4 nm, 649.8 nm and 628.2 nm. (The latter two wavelengths being the absorbance maxima in diethyl ether for copper phaeophytin a and copper phaeophytin b respectively).

Calculate the concentration of the individual compounds in micromoles per liter from the following equations:
Copper phaeophytin a = 45.6 A (649.8nm) - 2.75 A (628.2nm) + 3.10 A (667.2nm)- 35.4 A (654.4nm)
Copper phaeophytin b = -8.46 A (649.8nm)+ 20.7 A (628.2nm) - 1.69 A (667.2nm)+ 5.13 A (654.4nm)
Convert the figures in micromoles per liter to percentages using the following equations:

$$\%\ copper\ phaephytin\ a\ =\ \frac{micromoles\ x\ 0.9327\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ copper\ phaeophytin\ b\ =\ \frac{micromoles\ x\ 0.9467\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$