CHLOROPHYLLS

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 'not limited' was established at the 13th JECFA (1969)

SYNONYMS Magnesium chlorophyll, magnesium phaeophytin, CI Natural Green 3; C.I. (1975) No. 75810; INS No. 140

DEFINITION Obtained by solvent extraction of grass, lucerne, nettle and other plant material; during the subsequent removal of solvent, the naturally present co-ordinated magnesium may be wholly or partly removed from the chlorophylls to give the corresponding phaeophytins; the principal colouring matters are the phaeophytins and magnesium chlorophylls; the extracted product, from which the solvent has been removed, contains other pigments such as carotenoids as well as oils, fats and waxes derived from the source material.
Only the following solvents may be used for the extraction: acetone, dichloromethane, methanol, ethanol, propan-2-ol and hexane.

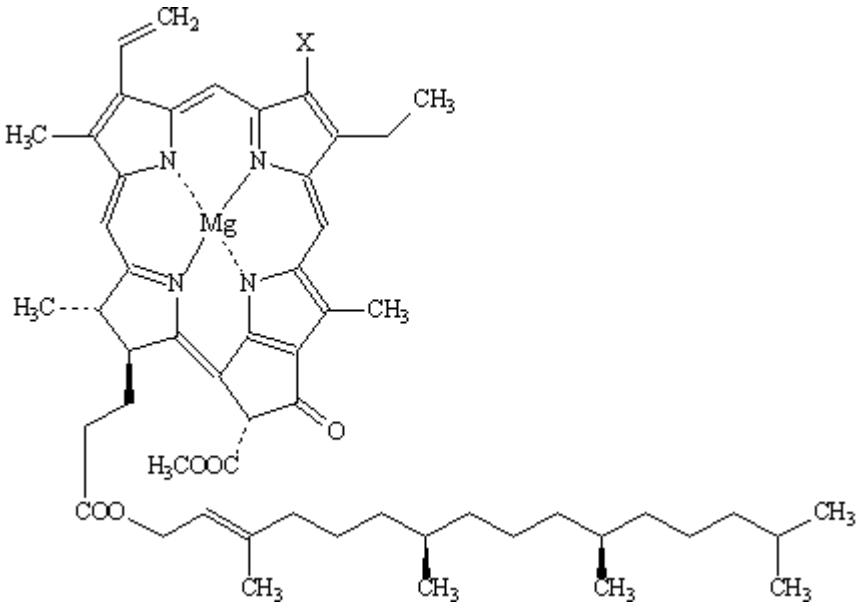
Chemical names The major colouring principles are:

Phytyl (13^2R,17S,18S)-3-(8-ethyl-13^2-methoxycarbonyl-2,7,12,18-tetramethyl-13'-oxo-3-vinyl-13^1-13^2-17,18-tetrahydrocyclopenta [at]-prophylrin-17-yl)propionate, (Pheophytin a), or as the magnesium complex (Chlorophyll a).
Phytyl (13^2R,17S,18S)-3-(8-ethyl-7-formyl-13^2-methoxycarbonyl-2,12,18-trimethyl-13'-oxo-3-vinyl-13^1-13^2-17,18-tetrahydro-cyclopenta [at]-prophylrin-17-yl)propionate, (Pheophytin b), or as the magnesium complex (Chlorophyll b).

C.A.S. number Phaeophytin a, Magnesium complex: 479-61-8
Phaeophytin b, Magnesium complex: 519-62-0

Chemical formula Phaeophytin a Magnesium complex (Chlorophyll a): $C_{55}H_{72}MgN_40_5$
Phaeophytin a: $C_{55}H_{74}N_40_5$
Phaeophytin b Magnesium complex (Chlorophyll b): $C_{55}H_{70}MgN_40_6$
Phaeophytin b: $C_{55}H_{72}N_40_6$

Structural formula



where
X = CH_3 for the "a" series
X = CHO for the "b" series
Loss of Mg converts chlorophyll into the corresponding phaeophytin.

Formula weight

Phaeophytin a magnesium complex (Chlorophyll a) 893.51
Phaeophytin a 871.22
Phaeophytin b magnesium complex (Chlorophyll b) 907.49
Phaeophytin b 885.20

Assay

Content of total combined phaeophytins and their magnesium complexes is not less than 10%.

DESCRIPTION

Waxy solid ranging in colour from olive green to dark green depending on the content of co-ordinated magnesium.

FUNCTIONAL USES

Colour

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4)

Insoluble in water; soluble in ethanol, diethyl ether, chloroalkanes, hydrocarbons and fixed oils

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 60 TLC 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 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:

Phaeophytin a: 0.77, grey/brown
Phaeophytin b: 0.75, yellow/brown
Chlorophyll a: 0.50, blue/green
Chlorophyll b: 0.63, yellow/green

In addition spots may be visible for ß-carotene at R_f 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 by *gas chromatographically* using either the method of entrainment distillation (*Determination of Residual Solvents*) or headspace analysis (*Limit Test for Solvent Residues*).

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 of the solution in a 1 cm cell against a diethyl ether blank at 660.4 nm, 642.0 nm, 667.2 nm and 654.4 nm. (These being the absorbance maxima in diethyl ether for chlorophyll a, chlorophyll b, phaeophytin a, and phaeophytin b, respectively). In addition measure at 649.8 nm and 628.2 nm. To the remaining diluted solution add one crystal of oxalic acid and after dissolution and mixing, remeasure the absorbances at the same wavelengths. "delta A" is the difference between the absorbances between the absorbance at the respective wavelengths, before and after addition of oxalic acid.

Calculate the concentration of the individual compounds in micromoles per litre from the following equations:

Chlorophyll a = 17.7 delta A (660.4 nm) + 7.15 delta A (642.0 nm)
Chlorophyll b = 19.4 delta A (642.0 nm) - 2.92 delta A (660.4 nm)
Phaeophytin a = -4.89 A (649.8 nm) + 0.0549 A (628.2 nm)
+18.7 A (667.2 nm) + 0.0575 A (654.4 nm) - chlorophyll a
Phaeophytin b = -71.0 A (649.8 nm) + 2.51 A (628.2 nm)
- 13.5 A (667.2 nm) + 84.3 A (654.4 nm) - chlorophyll b
Convert the figures in micro moles per litre to percentages using the following equations:

$$\%\ chlorophyll\ a\ =\ \frac{micromoles\ x\ 0.8935\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ chlorophyll\ b\ =\ \frac{micromoles\ x\ 0.9075\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ Phaephytin\ a\ =\ \frac{micromoles\ x\ 0.8712\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$

$$\%\ Phaeophytin\ b\ =\ \frac{micromoles\ x\ 0.8852\ x\ 12.5\ x\ 100}{mass\ of\ sample\ (mg)}$$