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Chlorophyllin copper complex: Quality control

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Synopsis

The currently employed spectrophotometric method in its present version is not reliable to control the quality of commercially available chlorophyllin copper complex (CCC). This quantitative assay may significantly overestimate the purity of CCC compared to that obtained on the basis of elemental analysis (copper and nitrogen content). The presence of uncoppered chlorophyll derivatives and carotenoids could account for the erroneous data. These results are discussed in the context of approaches for the improvement of CCC quality control.

Chlorophyllin copper complex (CCC) is a modified plant product known for decades to be used as a natural colorant (1) and as an internal deodorant and wound healing agent (2). It is obtained from chlorophyll by saponification and replacement of chelated magnesium with copper (3). Commercially available CCC is not a chemically pure compound but a mixture of several water-soluble chlorophyll derivatives, the composition of which varies depending upon the manufacturing conditions employed (4). A spectrophotometric assay was suggested for the quantitative analysis of CCC (5). This method, however, has been shown to be unreliable. Voigtlander and Henning (6), following the established procedure, found in some samples a purity reaching up to 160%. As discussed later, even in studies where the calculated amount of chlorophyll derivatives in CCC did not exceed 100%, the observations made by spectrophotometry contradict the data obtained from elemental analysis.

The following explanation can be given for the erroneous data resulting from the spectrophotometric assay of CCC. This method is based on the optical properties of CCC in the blue region of the spectrum. The absorptivity of CCC in this area increases significantly if other coloring compounds are present with the coppered chlorophyll derivatives. Here we will analyze results obtained elsewhere to demonstrate that metal-free chlorophyll analogs and carotenoids may account for the errors in the spectrophotometric quantitative assay of CCC.

Trisodium copper chlorin e_6 ($C_{31}H_{31}N_4Cu$ $(COONa)_3$) and disodium cooper isochlorin e_4 ($C_{31}H_{32}N_4Cu$ $(COONa)_2$) are the main chlorophyll derivatives reported in CCC (7–9). Based on these observations, we calculated that the theoretical content of chelated copper and nitrogen in a 100% pure CCC is 9.2% and 8.1%, respectively. The data have been compared with the values of these elements (3.9–4.3%) found in CCC

characterized by the spectrophotometric assay to be a good quality product (7). While the purity of CCC, as calculated from spectrophotometric analysis, was in a range of 78.0–94.4%, the amount of the CCC estimated by the content of copper and nitrogen was only between 42.4–46.7% and 48.2–53.1%, respectively. The poor quality of the CCC is confirmed by fractionation analysis, which reveals that the main bulk of the chlorophyll-derived constituents represents only 50% of the total product (9).

No substantial analytical data on CCC have been reported in recent years. The most intense studies on the quality of CCC have been carried out by Voigtlander and Henning (6). These authors investigated commercially available CCC, samples prepared in their laboratory, and purified individual chlorophyll derivatives. The following discussion is based on these data, some of which have been modified and recalculated.

It is implicit that the copper content in CCC reflects the level of chlorophyll derivatives complexed with metal. We attempted to correlate the amount of copper determined in CCC with the purity of the samples established by spectrophotometric assay (Table I). The comparison did not reveal any relationship between these two parameters. An alteration in the copper content of the samples was not accompanied by an appropriate change in their purity. While for some samples the increase in the amount of copper led to a higher quality of CCC, others were characterized by significant reduction of the purity values.

The ratio between copper and nitrogen content (Cu/N index) can also be a reliable indication of the quality of CCC. Our calculations indicate that the Cu/N index for 100% coppered chlorophyll derivatives is 1.1. We estimated the Cu/N index for different commercial samples of CCC (Table II). The data presented show that for the majority of these samples, the Cu/N index is far from the standard value of 1.1. Only four of the nine samples could be considered good quality products, since their Cu/N indices were found to be between 0.9 and 1.1. These values correspond to purities of CCC not less than 81.8%.

From the lack of correlation between copper content and purity determined by spectrophotometric assay, and from the inconsistent results relative to the Cu/N index, it can be concluded that in most samples of CCC studied, copper-containing chlorophyll derivatives are accompanied by their metal-free analogs. Spectrophotometric evaluation

Table I
Comparative Data on the Copper Content and Spectrophotometrically Determined Purity of Commercial CCC*

Sample number	Cu (%)	Purity (%)
1	0.80	42
2	1.27	23
3	2.90	30
4	3.05	48
5	3.10	43
6	3.33	102
7	3.68	160
8	3.86	89
9	4.70	82

* From reference 6, Table 1, section a.

Table II
Copper/Nitrogen Index and Content of Metal-Free Chlorophyll Derivatives in Commercial CCC*

Sample number	Cu/N	Metal-free derivatives as % of the total
1	0.3	72.7
2–4	0.6	45.5
5	0.7	36.4
6–7	0.9	18.2
8	1.0	9.1
9	1.1	0

* Based on data from reference 6, Table 1, section a.

of purified trisodium copper chlorin e_6 (one of the main components of CCC) and its metal-free analog, following the procedure currently used for CCC (5), revealed the purity as 106% for the former compound and 380% for the latter (6). By comparison of these data, it may be estimated that 100% copper-free trisodium chlorin e_6 exhibits a value of specific absorbance 3.6 times greater than that of its coppered counterpart. Investigations on the spectral properties of various metal-containing chlorophyll derivatives corroborate the conclusion of higher absorptivity if the metal is eliminated from these compounds (10). Hence, specific extinction coefficients determined at the absorption maxima between 400–450 nm for pheophytin a and its coppered analog were 126 and 67.8, respectively. Similarly, values of 182.2 and 93.9 were obtained for pheophorbide a and its coppered derivative. Therefore, the presence of even small quantities of metal-free chlorophyll-derived compounds in CCC can result in increased values of apparent purity, determined by spectrophotometric assay.

Valuable conclusions can be made from the study of chlorophyll-derived compounds intended to mimic commercially available CCC (Table III). Although the Cu/N indices of such samples are close to 1.1 (as required for completely coppered chlorophyll derivatives), the purity determined spectrophotometrically and on the basis of copper content is different. The values of purity exceeding 100% may be due to the presence of carotenoids, which have an intense absorptivity at 350–500 nm (11). On the other hand, those values below this level may come about because of samples containing colorless impurities. These two groups of compounds have been already found in CCC (7,9).

Table III
Percent Purity of Laboratory Preparations of CCC*

Sample number	Determined by	
	Copper content	Spectrophotometry
1	52	62
2	62	89
3	78	107
4	88	115
5	92	147
6	96	163

* Based on data from reference 6, Table 1, section b.

The present data clearly indicate that the currently used spectrophotometric assay erroneously estimates the purity of CCC as a result of metal-free chlorophyll derivatives having even higher extinction coefficients than their coppered counterparts and/or carotenoids. In order to control the quality of CCC, the Cu/N index should be employed in conjunction with the absolute percentage of copper and nitrogen content. These values have to be in range of those calculated for a 100% pure CCC. Interference by carotenoids can be avoided by obtaining the total absorptivity of the red peak for the preparation, rather than spectrophotometric measurements in the blue region of the spectrum, as presently employed.

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