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Spectrophotometric investigation of the Cu(II)-hesperidin complex

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Hesperidin is a glycosidic flavonoid of the flavanone type with a broad spectrum of biological activities. This flavonoid manly occurs in the peel of *Citrus* fruits and consequently, in food and plant based beverages like fruit juices. Its chemical structure, including a hydroxyl group in position 5 and a carbonyl group in position 4, is suitable for the formation of complex compounds with metals. Therefore, hesperidin has been used a coloring reagent for the quantitative determination of many metals via complexing reactions [1]. Earlier, we investigated hesperidin complexes with Zr(IV), Al(III) and UO_2(II) ions [2–4]. Here, we report about a Cu(II)-hesperidin complex, its composition, stability constants and conditions for the spectrophotometric determination of hesperidin, 50% methanol.

$Cu(NO_3)_2$ and hesperidin form a complex of yellow color, having an absorption maximum at 382.4 nm at pH = 5.7. The composition of this complex was determined at pH 6.0 and 10.0, using two methods: the method of continual variations of equimolar solutions [5] and the molar ratios method [6].

According to the first method, mixed solutions of $Cu(NO_3)_2$ and hesperidin having the total concentration $c_0 = 1.25 \times 10^{-3}$ M were used. The curve obtained at both pH values had the same maximum at $x_{Cu} = 0.33$, denoting the formation of a Cu(II): hesperidin = 1 : 2 complex. According to the second method, solutions containing a constant $Cu(NO_3)_2$ concentration (2.5×10^{-4} M) and varied hesperidin concentrations (from 1.25×10^{-4} to 1×10^{-3} M) were used. A straight line $A = f(c_{Hesp}/c_{Cu})$ with interception at $c_{Hesp}/c_{Cu} = 2$ was obtained and this also proves a stoichiometric ratio of Cu(II) : hesperidin in the complex of 1 : 2 throughout the investigated pH range.

The complex formation takes place with liberation of H^+ ions, which is evident from the pH measurements. The pH of equimolar solutions of $Cu(NO_3)_2$ and hesperidin are 5.66 and 6.74, respectively. The pH of a mixture of $Cu(NO_3)_2$ and hesperidin (in which the concentrations are the same as in individual solutions), is however, only 4.25, indicating that H^+ ions are delivered. Therefore, the reaction can be presented as:

$$Cu^{2+} + 2\,C_{28}H_{31}O_{15}H \Leftrightarrow [Cu(C_{28}H_{31}O_{15})_2] + 2\,H^+$$

For the calculation of the relative stability constant, two solutions were prepared: a mixture containing 5.0×10^{-5} M of $Cu(NO_3)_2$ and 1.0×10^{-3} M hesperidin and a solution of 1.0×10^{-3} M hesperidin alone. The absorbance of these two solutions was measured at different pH values; two curves were obtained, of which the curve of the complex absorption $\Delta A = f(pH)$ was calculated, having the maximum at pH = 10.2. The relative stability constant K_2:

$$K_2 = \frac{[Cu(C_{28}H_{31}O_{15})_2]}{[Cu^{2+}][C_{28}H_{31}O_{15}^-]_0^2}$$

is calculated for four pH values (Table).

The concentration of the complex, as well as the stability constants, increase with pH, because a higher pH favorites the dissociation of hesperidin and hesperidin reacts in dissociated form with Cu(II) ions. The highest stability constant at pH 10.0 indicates the best conditions for complex formation.

Table: Relative stability constants of the Cu(II)-hesperidin complex at different pH values

pH	$[Cu^{2+}]$/M	[Complex]/M	K_2	log K_2
7.0	3.12×10^{-5}	1.87×10^{-5}	6.00×10^{5}	5.78
8.0	2.85×10^{-5}	2.12×10^{-5}	7.46×10^{5}	5.87
9.0	2.62×10^{-5}	2.37×10^{-5}	9.05×10^{5}	5.96
10.0	1.25×10^{-6}	4.85×10^{-5}	3.90×10^{7}	7.59

The complexing reaction between Cu(II) ions and hesperidin allows the determination of microquantities of hesperidin in 50% methanol. Series of standard solutions with different hesperidin concentrations and a constant $Cu(NO_3)_2$ concentration (3.75×10^{-3} M) were prepared. A linear dependence of the absorbence on the concentration of hesperidin was obtained for a concentration range of hesperidin from 2.50×10^{-5} to 4.37×10^{-4} M. The calibration curve, obtained at 410 nm and pH = 4.55, had the regression equation y = 1391.2x + 0.025 and a correlation coefficient of r = 0.9994.

Experimental

Spectrophotometric measurements were performed on a Beckman DU-650 spectrophotometer using a 1 cm quartz cell. For pH-metric measurements, a Radiometer pHM 28 pH-meter was used with saturated calomel-glass electrode. The reagents used were: $Cu(NO_3)_2$, CH_3OH, HNO_3, NaOH (all Merck); $NaNO_3$ (Mallinckrodt Chemica); hesperidin (Fluka AG), recrystalized several times from CH_3OH.

All investigations were carried out with 50% CH_3OH solutions at room temperature and constant ionic strength (0.01 M). The pH was adjusted by addition of HNO_3 and/or NaOH, and ionic strength by addition to $NaNO_3$.

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