Spectroscopic and Structural Study of the Zinc(II)-hesperidin Complexes and Its Analysis Application

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Summary: The coordination mode of hesperidin with Zinc(II) was investigated by combined methods of Complete Neglect of Differential Overlap calculation (CNDO), continual variations of equimolar solutions, mole ratios, and IR spectra analysis. The results indicated that Zinc(II) ions and hesperidin form an ocher-yellow complex with an absorption maximum at 369 nm. Hesperidin can form a complex of 4:1 with Zinc(II) through its 4-carbonyl and 5-hydroxyl group. The relative stability constant of the complex, log β_1 ranged from 2.79 at pH=8.50 to 4.48 at pH=10.50. The conditions for the spectrophotometric determination of hesperidin, by means of the complex formation reaction, were investigated. It was found that hesperidin can be determined in the concentration range from 2.00×10^{-4} to 2.00×10^{-3} mol/L. The application of the coordination reaction for determining the concentration of hesperidin in orange juice is demonstrated. All investigations were carried out in 70% methanol solutions at room temperature (28ºC), constant values of pH (10.50), and ionic strength (0.01).

Key words: Complex; Hesperidin; Zinc chloride; CNDO calculation; Spectroscopic method

Introduction

Hesperidin belongs to the group of flavonoids of flavanone type, occurring mainly in the dried tangerine peel, fructus aurantii immaturus and citrus peel [1-3]. It displays a remarkable array of physiological and biological activities, such as anti-inflammatory, antimicrobial, anticancer, and reduction of capillary permeability [4-6]. In particular, it can significantly scavenge radicals, nitrogen species and reactive oxygen because of its strong antioxidant activity [7, 8].

Hesperidin($C_{28}H_{32}O_{15}$) is also called as hesperetin-7-rutinoside, or hesperetin 7-rhamnoglucoside and (*S*)-7-[[6-0-(6-deoxy-alpha-L-mannop-yranosyl)-beta -D-glucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one (Fig. 1). Hesperidin is a glycosidic flavonoid containing two hydroxyl groups in positions 5, 3′ and a carbonyl group in position 4. Besides, hesperidin obtains strong super delocalizability and a complete π conjugated system [9]. Owing to this kind of structure, hesperidin can coordinate with multiple trace metal ions and metal groups under certain conditions, such as Cu(Ⅱ) [10], VO(Ⅳ) [11], Al(Ⅲ) [12], Zr(Ⅳ) [13], etc.

The bioavailability of hesperidin is limited by its poor solubility in water. Complex formation of active constituent with trace metal elements can not only improve physic-chemical characters of hesperidin, promoting the absorption and utilization of the human body, but also can enhance its physiological functions and even produce some new biological activity through synergistic effects. In addition, the formation of complexes plays an important role in the absorption, transport and metabolism of trace metals [14]. S. B. Etcheverry et al [15] studied the antioxidation effect of the VO-hesperidin complex, and results showed that the ability of scavenging free radicals of the VO-hesperidin complex was higher than that of hesperidin. The hesperidin complex improves the superoxide dismutase (SOD)-like activity of the ligand, and H. M. Qing et al [16] found that the hesperidin coordinate with Cu(Ⅱ) increased the antimicrobial activity.

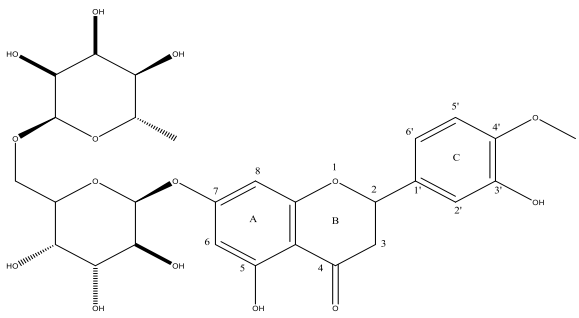

Fig. 1: Structure of hesperidin

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However, there is little detailed theoretical and experimental study on the interaction between Zinc(II) ions and hesperidin and on the application of the coordination reaction for determining the concentration of hesperidin in orange juice. Zinc(II) is a transition metal and necessary microelement for organic activities. As an important composition in many cellular enzymes, Zinc(II) can not only restrain the production of free radicals but also eliminate free radicals. The synthesis and biological activity of Zinc(II)-flavonoid complexes has been reported in some literature. The Zinc(II)-baicalin complex has better antioxidative and repairing abilities than single baicalin and trace element Zinc(II) [17]. The Zinc(II)-naringenin has stronger inhibition on CNV in the rat model than that of free ligind [18]. Hesperidin is difficult in vivo absorption and utilization due to its poor water-soluble ability, which greatly reduces its unique bioavailability. Complexation of hesperidin with Zinc(II) may therefore improve the biological activities of hesperidin. The present work aims to explore the coordination modes between Zinc(II) and hesperidin, to determine the complex stability constants, and the molecular structures and free charges of the Zinc(II)-hesperidin complex were optimized or calculated via semi-empirical calculation method using a Hyperchem software, to provide the theoretical reference for preparing this kind of complex in solution reaction system. Meanwhile, the work also aims to optimize the complexation reaction conditions, determine the solubility of the Zinc(II)-hesperidin complex and establish an easy spectrophotometric method for hesperidin determination in commercial juice.

Experimental

Materials and Chemicals

$ZnCl_2$, absolute methanol, NaOH and NaCl were acquired from Guangzhou Chemical Reagent Co. (Guangzhou, China). Orange juice was purchased from the local supermarket. Hesperidin was obtained from Connaught Li Co. (Zhengzhou, China), and was recrystallized out several times from methanol again. Since hesperidin can't be dissolved in water and $ZnCl_2$ can't be dissolved in pure methanol, 70% of methanol was selected as solvent with appropriate solubility [19].

Preparation of the complex

The complex was prepared via mixing of the 1.00×10^{-3} mol/L hesperidin with 1.00×10^{-3} mol/L $ZnCl_2$ according to the proportion of 4:1. After that, 500 mL blended liquid was placed in a round-button flask, then heated, stirred and refluxed for 3h at 70°C until it was completely dissolved, then the pH of the mixture was adjusted to 10.50 using 0.1 mol/L NaOH. After 5 min, this solution was concentrated to 50 mL using a rotary evaporator, then the residue was washed several times with absolute methanol to remove impurities. Finally, the complex was dried under vacuum for 12h, and used as a sample for infrared analysis.

Solubility analysis

Using 50 mL 70% (v/v) methanol as solvent, excess Zinc(II)-hesperidin complex was added into a flask. The mixture was refluxed for 2h at 70°C, then filtration, washing and drying processes after cooling to room temperature were done. Then the weight difference of complex before and after dissolving was done.

Preparation of the orange juice

The orange juice was diluted l00 times in methanol, 10 mL of orange juice was sampled and mixed with 8 mL of methanol, and then the blend was shaken thoroughly and filtered through a 0.45 μm microporous filter. After that the supernatant was transferred to a flask and the pH value was adjusted to 10.50, and 0.2 mL of the supernatant solution was blended with 2 mL of 1.00×10^{-3} mol/L $ZnCl_2$, l mL of water and 5 mL of methanol, then the absorbance of the blend was measured at 369 nm.

Theoretical Computation Method

The CNDO calculation was conducted using the Hyperchem (version 8.0) program to get the hesperidin structure information, and the free charges of all oxygen atoms and the bond lengths of hydroxyls in hesperidin were concluded.

UV-visible Spectroscopy

Ultraviolet absorption spectra were recorded on a double-beam spectrophotometer (TU-1810) at room temperature by using 1 cm quartz cell. The reaction of hesperidin with Zn^{2+} was determined at pH=10.50. The spectra were recorded from 200 nm to 800 nm.

Fourier Transform Infrared Spectroscopy (FT-IR)

The IR spectra of hesperidin and the Zinc(II)-hesperidin complex were recorded in the form of wave number ranging from 400 to 4000 cm^{-1} on a VERTEX 33 infrared spectrophotometer (Bruker, Germany) with a blank KBr disk as background.

Table-1: Electron of the oxygen atoms and hydroxyl bond length determined by the CNDO method.

labels	O1	O8	O10	O18	O20	O26	O32	O33	O34
Charge	-0.401*	-0.268	−0.357	-0.241	-0.210	-0.215	-0.246	-0.197	-0.290
Bond length/nm	1.299	1.384	1.089	1.033	1.392	1.399	1.383	1.032	1.104
labels	O35	O36	O37	O38	O40	O42			
Charge	-0.236	-0.239	-0.255	-0.252	-0.279	-0.257			
Bond length/nm	1.0323	1.036	1.033	1.037	1.087	1.388			

- It indicates that the charge of atom O1 is 0.401 times that of an electron.

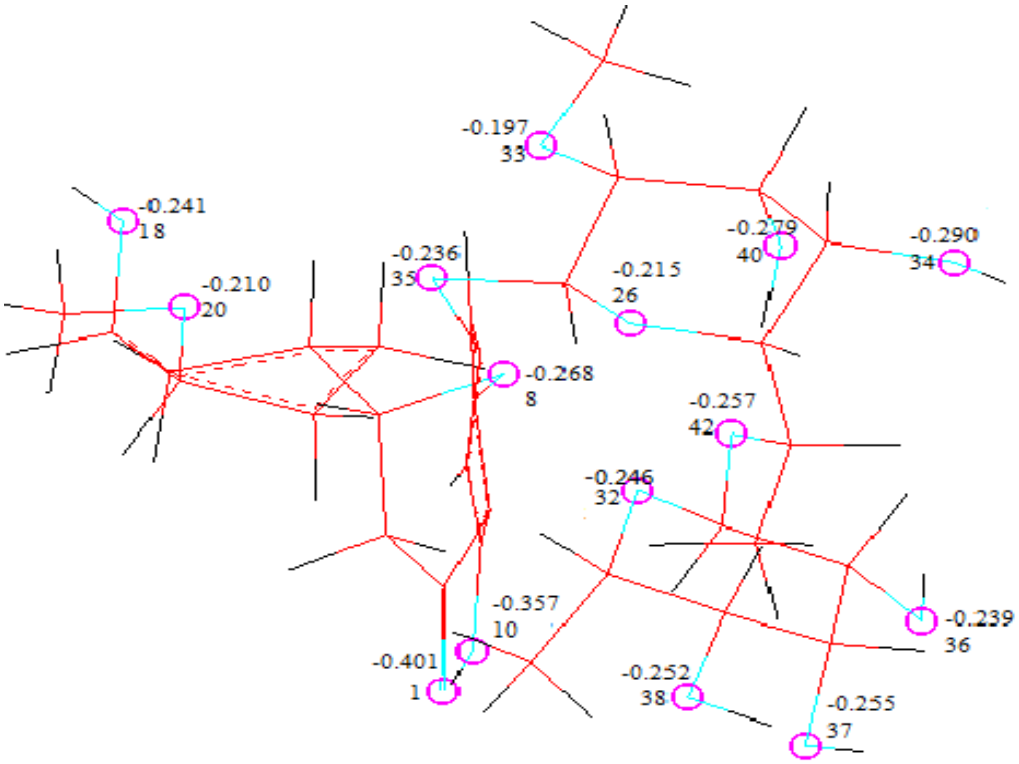

Fig. 2: Spatial structural of hesperidin optimized by the CNDO method.

Results and Discussion

Calculation of hesperidin by the CNDO method

Semi-empirical calculation has been widely used in the study of theoretical research of various organic molecules [20, 21], the electron density of hesperidin can be accurately calculated by the CNDO method, and results showed that the single hesperidin molecule presented coil and non-planar structure. The coordination pattern of hesperidin can be speculated according first to the relative charges of oxygen atoms and the bond lengths of hydroxyl groups (Table-1), and second to the maximum electric charge carried by the O1 and the O10, both obviously larger than that of other O atoms in this molecule. If groups or atoms get more net charges, they will give priority to gain or loss charges [22], so the most likely metal-chelating sites reacting with transition metal ions were O1 and O10.

Considering from the perspective of bond energy, the coordination mode of hesperidin with Zinc(Ⅱ) can be further explained by the following facts: the bond length of O1-H was longer than O10-H (Table-1), because of the force constants of O10-H was weakened by carbonyl, the O10-H was point at O1 after the molecule structure had been optimized by the HyperChem software (Fig. 2), suggesting that it was the hydrogen bond between O1 and O10-H which weaken the force constants of O10-H. In theory, according to these facts H ions were released into the medium during the coordination of hesperidin with Zinc (Ⅱ) due to the fact that the O10-H group breaks easier than other hydroxyl groups.

The Absorption Spectra of Hesperidin and the Complex

$ZnCl_2$ and hesperidin forms a complex of distinctive ocher-yellow color with an absorption

maximum at 369 nm (Fig. 3, curve 1), while the maximum absorbance wavelength of hesperidin at pH=10.50 was 332 nm (Fig. 3, curve 2). Since the absorption of hesperidin is negligible at wavelengths beyond 369 nm, all following measurements were performed at 369 nm against 70% methanol.

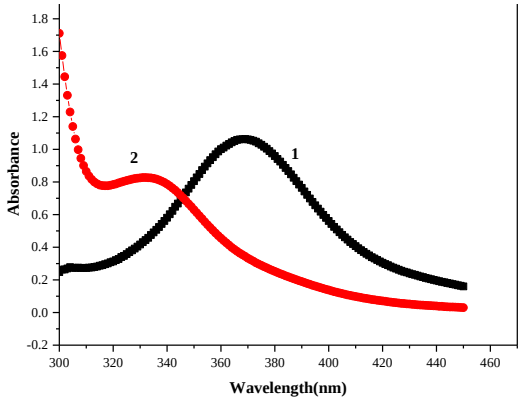

Fig. 3: UV spectra. Curve 1: mixture of 1.00×10^{-5}mol/L $ZnCl_2$ and 4.00×10^{-5}mol/L hesperidin, blank is 4.00×10^{-5}mol/L hesperidin; Curve 2: 4.00×10^{-5}mol/L hesperidin, blank is 70% (v/v) methanol.

The Composition of the Complex

The composition of the complex was determined by the molar ratios method and the method of continual variations of equimolar solutions. In the first method, solutions containing a constant concentration of $ZnCl_2$ (1.00×10^{-4} mol/L) and varying concentrations of hesperidin (ranges from 1.00×10^{-4} to 7.00×10^{-4} mol/L) were used, and adjusting the pH value to 10.50 after having mixed the two solutions. Two tangent lines were obtained which intercept at $C_{hesp}/C_{Zn}^{2+}=4$. This demonstrates that the stoichiometric ratio of hesperidin to Zn^{2+} in the complex was 4:1 (Fig. 4).

The second method involved the use of solutions obtained by mixing hesperidin and $ZnCl_2$ solutions, and the total concentration of C_{Hesp} plus C_{Zn}^{2+} was 1.00×10^{-3}mol/L. The curve had a maximum absorbance at 369 nm while the representative partition of Zn^{2+} was $X_{Zn}^{2+}=0.2$, denoting the formation of the hesperidin: Zn^{2+} = 4:1 complex (Fig. 5).

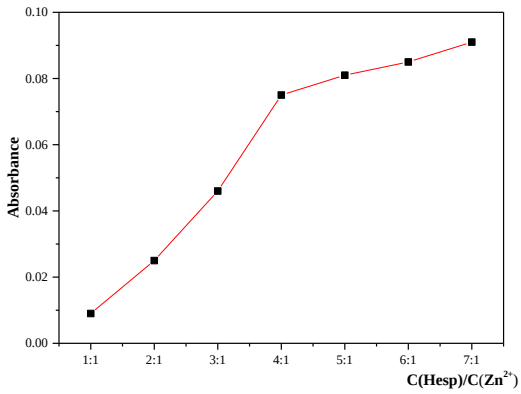

Fig. 4: Method of molar ratios: mixture of 1.00×10^{-4}mol/L $ZnCl_2$ and hesperidin (ranging from 1.00×10^{-4} to 7.00×10^{-4}mol/L), blank is hesperidin, as in mixtures. λ=369 nm.

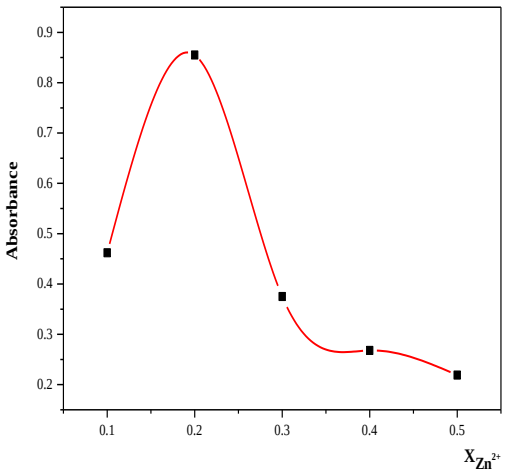

Fig. 5: Method of continual variations of equimolar solutions: C_{Zn}^{2+} plus C_{hesp} makes 1.00×10^{-3}mol/L, Blank is hesperidin as in mixtures, λ=369 nm.

The Infrared Spectra of Hesperidin and of the Complex

To disclose the nature of the linkage between Zinc(II) ion and hesperidin, the IR spectra of hesperidin and the Zinc(II)-Hesperidin complex as shown in the figure (Fig. 6) illustrated that the characteristic absorption spectrum of 4-carbonyl in ligand of hesperidin moved to low wave number after the complex was formed because the corresponding peak wavelength red shifts from 1648 cm^{-1} to 1603 cm^{-1}. This is due to a coordination bond formed between the carbonyl oxygen and Zn^{2+}, thus

the electron cloud density of C-O decreased, testifying the involvement of 4-carbonyl in the coordination process. Hesperidin has two hydroxyl groups in position 5 and 3′, and exhibits a strong broad band in the range of 3000 cm^{-1} to 3600 cm^{-1} due to partially overlapped of the two groups [23], peak wavelength of 5-OH and 3′-OH absorption spectrum is 3429 nm and 3479 nm respectively. In the spectrum of the Zinc(II)-hesperidin complex, the peak at 3429 cm^{-1} disappeared and one new strong peak at 3291 cm^{-1} was observed, indicating that the ligand was attached to the Zinc(II) ion by carbonyl and its ortho-hydroxyl group.

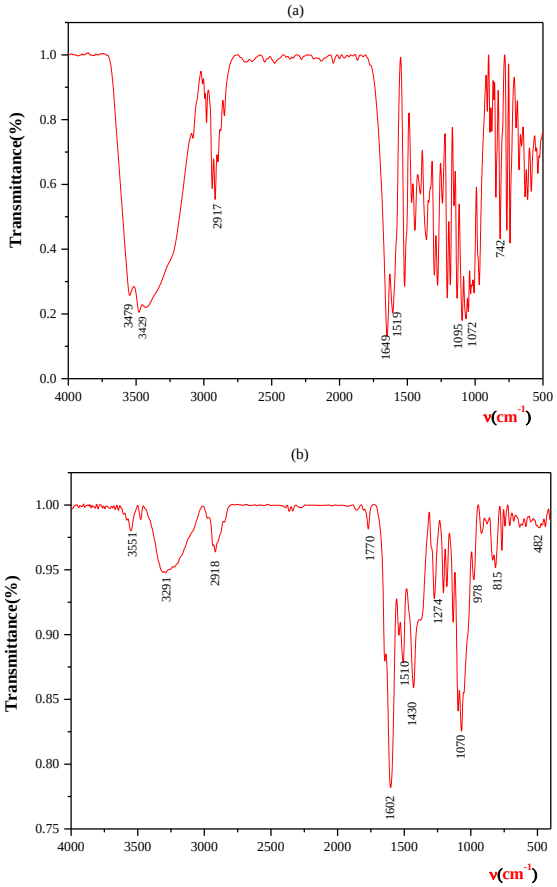

Fig. 6: IR spectra of hesperidin (a) and the Zinc(Ⅱ)-hesperidin complex (b).

In addition, the characteristic peaks of benzene skeletons of Ring C (Fig. 1) for hesperidin and its metal complexes basically had no change between 1600 cm^{-1} to 1450 cm^{-1}, showing the whole molecule retains the benzene ring structure of natural hesperidin. No major changes were observed in the frequency of (νC–O–C) that appear at 1072 cm^{-1} in hesperidin and at 1070 cm^{-1} in the complex, suggesting that the cyclic ether bond did not open loop under alkaline conditions. Besides, a new peak emerges at a rather low frequency value of 482 cm^{-1} on account of the formation of the complex, demonstrating that the Zinc(II) ion became one part of hesperidin molecule, since this peak did not appear in the IR spectrum of hesperidin molecule.

The Stability Constant of the Complex

The relative stability constants β_1 of the complex at different ionic strengths were determined by a modified version of Bjerrum's method [24]. For various pH values, the absorbance of a solution containing 1.00×10^{-3} mol/L hesperidin alone (Fig. 7, curve 1) and of a blend one containing 5.00×10^{-5} mol/L $ZnCl_2$ and 1.00×10^{-3} mol/L hesperidin (Fig. 7, curve 2) were measured. Two curves were given and the curve of the absorbance difference $\Delta A = f$ (pH) between two curves of hesperidin ant its complex was also measured (Fig. 7, curve 3). The maximum absorbance difference was found at pH 10.50, and it could be supposed that at that pH value the concentration of the complex is roughly equivalent to the total $ZnCl_2$ concentration, $[complex]\approx[Zn^{2+}]_0$, because all the Zn^{2+} were almost completely coordinated with hesperidin which was excessive in the blend. The given concentration of hesperidin in the solution was 20 times more than the $ZnCl_2$ concentration. Accordingly, the molar absorptivity of the complex was calculated from the expression:

$$a = A_{max} / [Zn^{2+}]_0 \quad (1)$$

And the complex and Zn^{2+} concentration were calculated from following equations:

$$[complex] = A/a \quad (2)$$

$$[Zn^{2+}]_0 = [Zn^{2+}] + [complex] \quad (3)$$

The relative stability constant β_1 was calculated from the following Equation:

$$\beta_1 = \frac{[Complex]}{[Zn^{2+}][Hesp]_0} \quad (4)$$

β_1 was calculated for four different pH values, the results are given in Table-2.

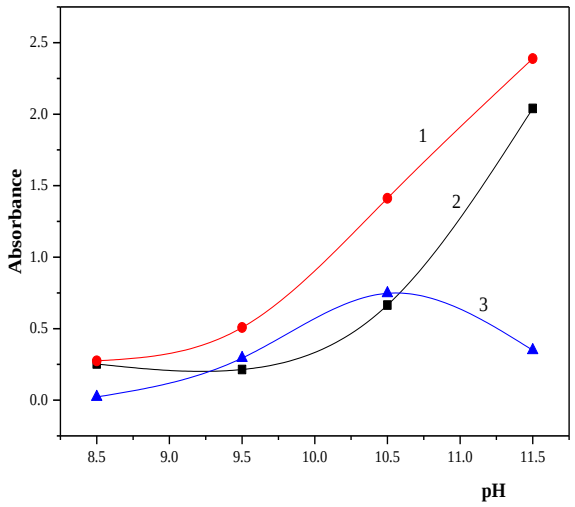

Fig. 7: Dependence of absorbance on pH at λ = 369 nm: (curve 1) 1.00×10^{-3}mol/L hesperidin, blank is methanol; (curve 2) mixture of 5.00×10^{-5}mol/L $ZnCl_2$ and 1.00×10^{-3} mol/L hesperidin, blank is 70% (v/v) methanol; (curve 3) ΔA= *f*(pH).

Table-2: IR Data(cm^{-1}) for hesperidin and Zinc(Ⅱ)-hesperidin complex

Compound	ν(C=O)	ν(O-H)	ν(Benzeneskeleton)	ν(C-O-C)	ν(M-O)
Hesperidin	1648	3429	1510	1072	—
Complex	1602	3291	1519	1070	482

Quantitative Determination of Hesperidin

The formation of the complex between Zn^{2+} and hesperidin permits the determination of micro-amounts of hesperidin in liquid commercial goods containing hesperidin. To get a calibration curve, solutions with a constant $ZnCl_2$ concentration (2.00×10^{-3} mol/L) and varying amounts of hesperidin were prepared. At pH 10.50, a linear dependence of the absorbance versus hesperidin concentration was obtained for a concentration range of hesperidin from 2.00×10^{-4} to 2.00×10^{-3} mol/L (Fig. 8). The regression equation is Y=0.0171x + 0.0357 and the square of correlation coefficient, r^2, is 0.9921. The accuracy of the method was tested for three different hesperidin concentrations.

Solubility Determination of Hesperidin and its Zinc(II) complex

After hesperidin coordinated with Zinc(II), the color of solution changed from white to ocher-yellow. The results showed that the solubility of Zinc(II)-hesperidin complex in 70% (v/v) methanol was 1.29 g/L, while the solubility of hesperidin in 70% (v/v) methanol was 0.02 g/L under the same operating conditions. The solubility of hesperidin increased a lot after forming a complex with Zinc(II), which might be one of the causes of the physiological activity of the Zinc(II)-hesperidin complex is better than that of hesperidin.

Determination of Hesperidin in Orange Juice.

The stability of the Zinc(II)–hesperidin complex provided the possibility for determining the hesperidin content in orange juice using UV spectrophotometry method. At 369 nm, the Zinc(II)-hesperidin complex were strongly absorbed, whereas the absorbance of hesperidin can be neglected. In addition, after filtration of the methanol extract of the juice at room temperature, its hesperidin concentration was stable. Two different kinds of orange juices were studied and the results were shown in Table-3.

Table-3: Stability constants of the Zinc(Ⅱ)-hesperidin complex.

pH	$[Zn^{2+}]$	[complex]	K_1	$\log K_1$
8.5	3.098×10^{-5}	1.902×10^{-5}	613.9	2.79
9.5	3.391×10^{-5}	1.609×10^{-5}	474.5	2.67
10.5	1.580×10^{-6}	4.842×10^{-5}	30645.6	4.48

a=13300 $cm^{-1}mol^{-1}$

Table-4: Spectrophotometric determination of hesperidin.

Taken(*M*)	Found(*M*)	SD	CV(%)
4.0×10^{-4}	4.10×10^{-4}	1.51×10^{-5}	3.68
1.2×10^{-3}	1.22×10^{-3}	1.72×10^{-5}	1.40
2.0×10^{-3}	1.98×10^{-3}	8.16×10^{-5}	4.11

n=5

Table-5: The content of hesperidin presented in orange juice.

Brand of orange juice	Hesperidin present(mg/L)	SD
HUIYUAN juice concentrate	147	8.56
Minute Maid	62	6.59

Conclusions

Hesperidin and Zinc (Ⅱ) ions form a 4:1 complex in 70% methanol under the alkaline condition through the 4-carboxyl and 5-hydroxyl of hesperidin. Optimal conditions for hesperidin determination by this coordination method are at wavelength λ=369 nm and pH=10.50. Since the complex was stable enough and has an intensive ocher-yellow color, the suggested method can be applied in the visible region and recommended for

quantitative determination the concentration of hesperidin in orange juice. Interference with other substances which may have absorbance in the UV region is avoided. The solubility of Zinc(II)-hesperidin complex increased relative to hesperidin. As for the anti-oxidation activity and mechanism, and whether the Zinc-hesperidin complex influences the electrochemical properties of hesperidin, need further research.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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