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LUMINESCENT DETERMINATION OF ASCORBIC ACID IN DIETETIC ADDITIVES

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Annotation. The article presents the results of the ascorbic acid **(**AA) determination with using a complex of an Tb (III) ion with ciprofloxacin (CF) as a lanthanide luminescent marker. The luminescent properties of the Tb (III)-CF complex in the presence of AA were studied. The excitation and luminescence spectra, triplet level of ligand, the kinetics of the luminescence decay of the Tb(III)-CF complex in the presence of AA were analyzed. The excitation spectrum of the Tb (III)-CF complex has broad bands with maxima at 302 and 355 nm that corresponding to the n→π* electronic transition in the absorption spectrum of ligand. The luminesce spectra demonstrate the emission transitions arising from $^5D_4$ energy level to ^7Fj multiplet ground state. The luminescence of the Tb(III)-CF complex was found to be quenched by AA. It was established that the lifetime of the excited $^5D_4$ state of the Tb (III) ion decreases with AA concentration increasing up to 0,25 mg/cm^3. Luminescence quenching of the Tb (III)-CF complex by AA follows the Stern-Volmer relationship of AA. The Stern-Volmer constant K is 2478 dm^3/mol. The biomolecular quenching rate constant k_q is $1{,}25\cdot10^7$ dm^3/mol. The effect of luminescence quenching of the Tb (III)-CF complex was used to developing the procedure for determining of AA in the dietetic additives «Asvitol» and «Ascorbic acid». The linear calibration plot for AA was obtained over the concentration range of 0,02 to 0,25 mg/cm^3.

Keywords: luminescence, ion of terbium (III), ascorbic acid, dietetic additives.

ЛЮМІНЕСЦЕНТНЕ ВИЗНАЧЕННЯ АСКОРБІНОВОЇ КИСЛОТИ В ДІЄТИЧНИХ ДОБАВКАХ

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Анотація. У статті представлено результати визначення аскорбінової кислоти (АК) з використанням комплексу іона Tb (III) з ципрофлоксацином (CF) в якості лантанідного люмінесцентного маркера. Вивчено люмінесцентні властивості комплексу Tb (III)-CF в присутності АК. Проаналізовано спектри збудження люмінесценції, триплетний рівень ліганда, кінетика затухання люмінесценції комплексу Tb (III)-CF у присутності АК. Спектр збудження комплексу Tb (III)-CF має широкі смуги з максимумами при 302 і 355 нм, що відповідає електронному переходу n→π* в спектрі поглинання ліганду. В спектрах люмінесценції спостерігаються переходи з збудженого енергетичного рівня $^5D_4$ на рівні ^7F_j мультіплетного основного стану. Виявлено, що люмінесценція комплексу Tb (III)-CF гаситься АК. Встановлено, що час життя збудженого стану $^5D_4$ іона Tb (III) зменшується зі збільшенням концентрації АК до 0,25 мг/см^3. Гасіння люмінесценції комплексу Tb (III)-CF за допомогою АА підпорядковується співвідношенню Штерна-Фольмера. Константа Штерна-Фольмера К становить 2478 дм^3/моль. Константа швидкості біомолекулярного гасіння kq становить $1{,}25\cdot10^7$ дм^3/моль. Ефект гасіння люмінесценції комплексу Tb (III)-CF було використано для розробки методу визначення АК в дієтичних добавках «Асвітол» і «Аскорбінова кислота». Калібрувальний графік лінійний у діапазоні концентрацій від 0,02 до 0,25 мг/см^3 АК.

Ключові слова: люмінесценція, іон тербію (III), аскорбінова кислота, дієтичні добавки.

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Introduction

Ascorbic acid (AA) is found in many biological systems and food products and is important for many metabolic functions and oxidation-reduction reactions, plays a key role in the formation and maintenance of collagen, strengthens and protects the immune system, increases the bioavailability of iron. Biologically active is only one of the isomers of AA – L-ascorbic acid, which is called vitamin C.

AA is a powerful antioxidant that reacts with the active forms of oxygen and free radicals and is therefore widely used as a food additive (antioxidant E300) in the food and beverage industry. Despite the necessity and importance of AA for the human body, excessive consumption of it can lead to urolithiasis, cardiac pathology, stomach cramps and diarrhea [1]. In addition to immediate toxic effects, the presence of large amounts of vitamin C may interfere with the correct in-

terpretation of the results of clinical blood and urine tests for the presence of various metabolites.

Therefore, the rapid, sensitive, and selective detection of AA level is significant for food and pharmaceutical analysis.

Formulation of the problem

Up to date, various types of approaches have been developed for AA detection. Most of these methods were intricate and time-consuming, and some of these usually required specialized and expensive instruments. Compared to other detection methods, luminescent methods have attracted more attention due to their good reproducibility and high sensitivity. The sensitized luminescence of lanthanide ions in complexes with organic ligands has been widely used for the determination of various inorganic and organic anions that are not the sensitizers of lanthanide luminescence, but can increase or quench its intensity. This paper presents the results of studies on the determination of AA in dietary supplements with using of a luminescent Tb (III) ion complex with ciprofloxacin (CF) as ligand.

Literature review

To date, various methods have been developed for the detectiion and quantitative determination of AA. The basis of most analytical techniques is the ability of AA to participate in the redox reactions. Some methods are based on the determination of the total amount of AA in both oxidized and reduced forms, which is more preferable, since many useful properties of AA are inherent in both AA itself and its oxidation products [2-8]. Liamas et al. [3] reported a flow-injection spectrophotometric determination with a photodegradation step to determine AA and total sugars. The flow-injection system included a simple ultraviolet photoreactor for the on-line photodegradation. The method was based on the determination of AA at 300 nm before the photodegradation step, followed by UV irradiation and measurement of total sugars at 268 nm. The proposed method was used to determine AA and total sugars in commercial and natural fruit juice samples. Kukoc-Modun et al. [4] proposed a flow-injection indirect spectrophotometric method for the determination of AA in pharmaceutical preparation. The method was based on the reduction of iron (III) to iron (II) by AA, and by the subsequent reaction of the produced iron (II) with 2,4,6-tripyridyl-s-triazine in buffered medium to form a colored complex. The linear range of the method is from 0.08 to 10 μmol/l of AA, with the detection limit 24 nmol/l of AA. The proposed method could be applied for the determination of AA in pharmaceutical preparations, down to picomolar quantity. Zhao et al. [7] fabricated a promising electrochemical biosensor for simultaneous detection of AA, dopamine (DA) and uric acid (UA) by electrochemical deposition of MgO nanobelts on a graphene-modified tantalum wire electrode. In the threefold co-existence system, the linear calibration plots for AA, DA and UA were obtained over the concentration range of 5.0 – 350 mmol/dm^3, 0.1 – 7 mmol/dm^3 and 1 – 70 mmol/dm^3 with detection limits of 0,03 mmol/dm^3, 0.15 mmol/dm^3 and 0.12 mmol/dm^3, respectively. The modified electrode showed excellent selectivity, good sensitivity and good stability, making it attractive as a sensor for simultaneous detection of AA, DA and UA in biological fluids.

Most of the known techniques for AA determination are complex, time-consuming, and for some of them expensive equipment is needed.

The ascorbic acid determination with using a complex of Tb (III) ion with ciprofloxacin

The aim of this research was to study the possibility of the ascorbic acid determination in biologically active additives with using the Tb(III)-ciprofloxacin complex as the luminescent marker.

A stock solution of AA ($1\cdot10^{-2}$ mol/dm^3) was prepared by dissolving a weighed quantity of AA in 100 cm^3 of distilled water. A stock solution of CF ($1\cdot10^{-2}$ mol/dm^3) was prepared by dissolving weighed quantities of CF in 100 cm^3 of ethanol. Terbium (III) chloride was prepared by dissolving high purity terbium (III) oxide (99.99 %) in hydrochloric acid (1:1) and excess hydrochloric acid was removed by evaporation. The concentration of Tb(III) was determined by complexometric titration with a standard solution of Complexone III (0.01 mol/dm^3) using arsenazo I as an indicator in a basic buffer solution of urotropine.

The steady-state luminescence spectra and the luminescence decay curves were recorded by using a Fluorolog FL 3-22 spectrofluorometr (Horiba Jobin Yvon).

We have previously shown [11,12] that the lanthanide trivalent cations form complexes with quinolone carboxylic acid derivatives, in particular CF, with intense luminescent properties, which have been chosen as a luminescent sensor for AA determination.

The excitation spectrum of the Tb (III)-CF complex has broad bands with maxima at 302 and 355 nm that corresponding to the n→π* electronic transition in the absorption spectrum of ligand (Fig. 1).

In order to avoid the direct excitation of the lanthanide ion, the excitation of the complex was performed into the ligand's lowest-energy centred absorption band. The luminescence spectra are given in Fig. 2.

The luminesce spectra demonstrate the emission transitions arising from $^5D_4$ energy level to ^7F_j multiplet ground state [10,13]. The major emission bands at 487, 545, 587, 618 nm have been ascribed to $^5D_4\rightarrow{}^7F_6$, $^5D_4\rightarrow{}^7F_5$, $^5D_4\rightarrow{}^7F_4$, $^5D_4\rightarrow{}^4F_3$ transitions respectively [10,13]. The band corresponding to the $^5D_4\rightarrow{}^7F_5$ transition with a maximum of luminescence at 545 nm has the highest intensity.

According to the literature data [10], the photoluminescent properties of the the Tb (III)-CF complex depend on the relative position of the energy levels of

the CF ligand relative to the emitting (resonance) level of the lanthanide ion. As shown previously [11-13], the lowest triplet energy level of CF (E_T = 21000 cm^{-1}) is larger than the resonance energy level of the Tb(III) ion (E = 20500 cm^{-1}) and in this case the intramolecular energy transfer from the triplet level of CF to the $^5D_4$ exited 4f-state of the terbium ion occurs. Thus, sensitization of lanthanide luminescence via its ligand takes place.

In the presence of AA, the excitation and luminescence spectra of the Tb(III)-CF complex do not change, but the intensity of the luminescence bands decreases when the concentration of AA is increases (Fig. 1,2). This indicates that the luminescence quenching process takes place. Luminescence quenching refers to any process that decreases the luminescence intensity of the Tb (III)-CF complex. A variety of molecular interactions can result in quenching. These include excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation (static quenching), and collisional quenching (dynamic quenching) resulting from collisional encounters between the exited molecule and quencher.

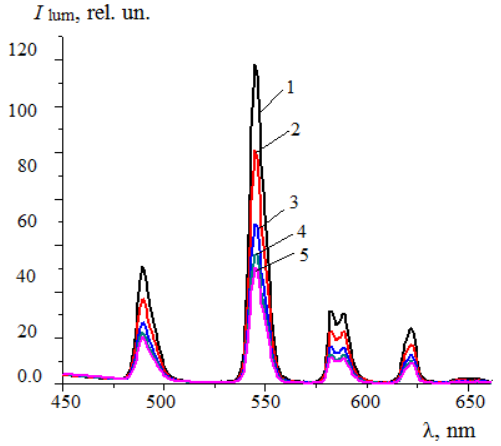

Fig.1. Excitation spectra of the luminescence of the Tb (III)-CF complex: in the absence (1) and in the presence of various concentrations of AA: 0.05 mg/sm^3 (2), 0.1 mg/sm^3 (3); λ_{lum}= 545 nm

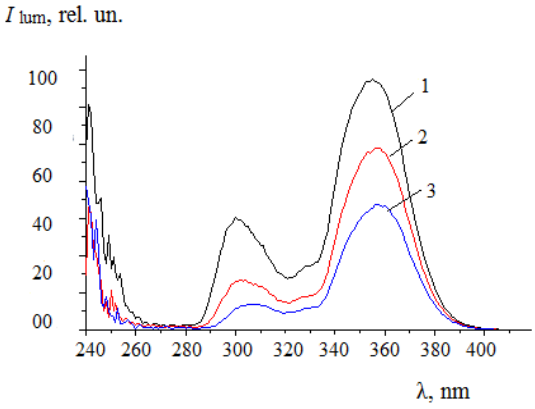

Fig. 2. Luminescence spectra of the Tb (III)-CF complex: in the absence of AA (1) and in the presence of various concentrations of AA: 0.02 mg/sm^3 (2), 0.05 mg/ sm^3 (3), 0.1 mg/ sm^3 (4), 0.15 mg/ sm^3 (5); λ_{ex} = 355 nm

An important characteristic of collisional quenching, which is an equivalent decrease in luminescence intensity and lifetime. The decrease in lifetime occurs because quenching is an additional rate process that depopulates the excited state. The decrease in yield occurs because quenching depopulates the excited state without emission. Static quenching does not decrease the lifetime because only the luminescence molecules are observed, and the uncomplex fluorophores have the unquenched lifetime τ_0 [14]. So the kinetics of the luminescence decay of the Tb(III)-CF complex in the presence of AA was studied. The results are shown in Table 1. Table 1 shows that the lifetime of the excited $^5D_4$ state of the Tb (III) ion decreases with the concentration of AA increasing.

Table 1 – The lifetime (τ) of the excited $^5D_4$ state of the Tb(III) ion in the complex with CF in the presence of AA

c(AA), mg/cm^3	0,0	0,02	0,05	0,10	0,15	0,25
τ, μs	198	135	112	97	85	78

The luminescence quenching efficiency is described by Stern-Volmer equation [14]:

$$\frac{I_0}{I} = 1 + k_q \cdot \tau_0 \cdot C = 1 + K \cdot C \qquad (1),$$

where I_0 and I are the luminescence intensity in the absence and presence of the quencher, respectively;

k_q is the biomolecule quenching rate constant, l/mol;

τ_0 is the lifetime of the fluorophore in the absence of quencher, s;

K is the Stern-Volmer constant, l/mol;

C is the concentration of quencher, mol/l.

Luminescence quenching of the Tb (III)-CF complex by AA follows the Stern-Volmer relationship. The Stern-Volmer constant K, calculated from equation (1) was 2478 dm^3/mol. The biomolecular quenching rate constant k_q was 1, $25 \cdot 10^7$ dm^3/mol c. Value of k_q smaller than the diffusion-controlled value can result from steric shielding of the fluorophore or a low quenching efficiency [14].

We used the effect of luminescence quenching of the Tb(III)-CF complex to developing the procedure for determining of AA in the dietetic additives «Asvitol» and «Ascorbic acid». Luminescence quenching of the Tb(III)-CF complex in the presence of AA is ob-

served in the concentration range of 0.02 to 0.25 mg/cm^3.

The determination was made by a calibration curve method. When plotting the calibration curve for the determination of AA, the following procedure was used: in 5 ml measuring tubes we placed 0.05; 0.1; 0.2; 0.3; 0.4; 0.5 cm^3 of a standard solution of AA (2 mg/dm^3). To each tube we added 0,2 cm^3 of terbium(III) chloride solution ($1\cdot10^{-2}$ mol/dm^3), 0,4 cm^3 of CF solution ($1\cdot10^{-2}$ mol/dm^3), 0.2 cm^3 of urotropine solution with mass fraction of 40 % and distilled water to 5 cm^3. The luminescence intensity of the Tb (III)-CF complex was measured at λ_{lum} = 545 nm upon excitation at 355 nm.

Based on the obtained data, a calibration curve was plotted. The calibration graph for AA is linear in the range of 0.02 to 0.25 mg/dm^3 (Fig. 3).

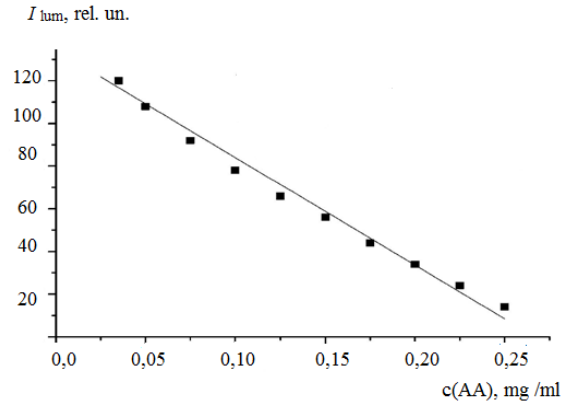

Fig. 3. Calibration curve for the AA determination

Method of determination: for analysis, 4 tablets of the sample were ground, 200 mg of sample was taken, transferred to a 50 cm^3 volumetric flask and dissolved in a small amount of distilled water. After completely dissolving the sample, the flask was diluted to scale with water. In three 5 cm^3 tubes 0.5 cm^3 of the assay solution were sampled and all the reagents were added, as in the plotting of the calibration curve. The luminescence intensities of these solutions at 545 nm were measured upon excitation at 355 nm.

Accuracy of the results of the analysis was checked using spike sample analysis. The value of the relative standard deviation does not exceed 6,5 % (Table 2).

Table 2 – Results of ascorbic acid determination in biologically active additives by spike sample analysis

(n = 5, P = 0,95)

Object of analysis	Added, mg/cm^3	Found, mg/cm^3	S_r, %
«Asvitol»	0.10	0.105±0,006	6.0
-	0.20	0.210±0,014	6.5
«Ascorbic acid»	0.10	0.095±0,005	5.7
-	0.20	0.193±0,011	5.8

Conclusion

The luminescent properties of the Tb (III)-CF complex in the presence of AA were studied. The excitation and luminescence spectra, the decay curves were analyzed. The luminescence of the Tb (III)-CF complex was found to be quenched by AA. A method for the detection of AA based on the luminescence quenching of the Tb (III)-CF complex in biologically active additives was developed.

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ЛЮМИНЕСЦЕНТНОЕ ОПРЕДЕЛЕНИЕ АСКОРБИНОВОЙ КИСЛОТЫ В ДИЕТИЧЕСКИХ ДОБАВКАХ

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Аннотация. В статье представлены результаты определения аскорбиновой кислоты (АК) с использованием комплекса иона Tb (III) с ципрофлоксацином (CF) в качестве лантанидного люминесцентного маркера. Изучены люминесцентные свойства комплекса Tb(III)-CF в присутствии АК. Проанализированы спектры возбуждения и люминесценции, триплетный уровень лиганда, кинетика затухания люминесценции комплекса Tb(III)-CF в присутствии АК. Спектр возбуждения комплекса Tb (III)-CF имеет широкие полосы с максимумами при 302 и 355 нм, что соответствует электронному переходу $n \rightarrow \pi^*$ в спектре поглощения лиганда. Спектры люминесценции демонстрируют переходы эмиссии, возникающие от возбужденного уровня энергии $^5D_4$ АК. Установлено, что время жизни возбужденного состояния $^5D_4$, иона Tb (III) уменьшается с увеличением концентрации АА до 0,25 мг/см^3. Люминесцентное тушение комплекса Tb (III)-CF с помощью АК следует соотношению Штерна-Фольмера АК. Константа Штерна-Фольмера К составляет 2478 дм^3/моль. Константа скорости биомолекулярного тушения k_q составляет $1{,}25 \cdot 10^7$ дм^3/моль. Эффект тушения люминесценции комплекса Tb (III)-CF был использован для разработки методики определения АК в биологически активных добавках «Асвитол» и «Аскорбиновая кислота». Калибровочный график линеен в диапазоне концентраций от 0,02 до 0,25 мг/см^3 АК.

Ключевые слова: люминесценция, ион тербия (III), аскорбиновая кислота, диетические добавки.

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