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STABILITY OF TRIS-1,10-PHENANTROLINE IRON (II) COMPLEX IN BIOMINERAL PARTICLES PRODUCED BY *KLEBSIELLA OXYTOCA*

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Ангел Л. В. и др. E14-2011-75

Стабильность комплекса «железо (II) – фенантролин»
в биоминеральных частицах, продуцируемых бактериями *Klebsiella oxytoca*

Состав образцов, содержащих ионы железа, оказывает огромное влияние на стабильность комплекса «железо (II) – фенантролин» во время определения общего содержания железа. Предметом данной работы является определение стабильности комплекса «железо (II)–фенантролин» в биоминеральных частицах, продуцируемых бактериями *Klebsiella oxytoca*. Стабильность этого комплекса была проверена в период времени от 0 до 60 мин. Определена концентрация биогенных частиц ферригидрита в исследуемых образцах и временной интервал, в котором оптическая плотность комплекса является максимальной. Полученные результаты показывают, что более точное определение концентрации ферригидрита в исследуемых образцах происходит при измерении оптической плотности комплекса «железо (II) – фенантролин» по истечении 25 мин с момента добавления ортофенантролина.

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Stability of Tris-1,10-Phenantroline Iron (II) Complex
in Biomineral Particles Produced by *Klebsiella oxytoca*

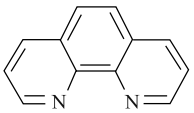
The composition of composites has a huge impact on the stability of tris-1,10-phenantroline iron (II) complex during the determination of total iron content. The subject of this work is the determination of the stability of tris-1,10-phenantroline iron (II) complex in samples of biominerals produced by bacteria *Klebsiella oxytoca*. The stability of this complex was monitored in the time period of 0–60 min. The aim of this work is to determine the concentration of the biogenic ferrihydrite in the samples and the time interval in which the absorbance of the complex is highest. The UV-Vis spectrophotometric method was used for the determination. Obtained results indicate that for more exact estimations of the concentration of biogenic ferrihydrite, absorbance of tris-1,10-phenantroline iron (II) complex should be measured within 25 min from the moment ortho-phenantroline was added.

The investigation has been performed at the Frank Laboratory of Neutron Physics, JINR.

Communication of the Joint Institute for Nuclear Research. Dubna, 2011

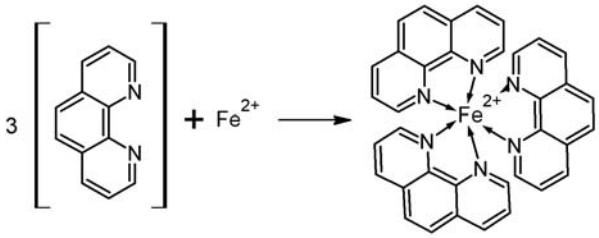
INTRODUCTION

According to Werner's coordination theory, the atoms or the ions, particularly those of transition elements can be bounded or coordinated in a certain number or they create spatial distribution of atoms, ions or molecules thus forming complex (coordination) compounds. Complex compounds consist of the central atom ion (builder of the complex) around which two or more ligands are coordinated. The number of ligands that are directly linked to the central ion in the complex is observed as the coordination number. It depends on the nature of the central atom, its electronic configuration and size, as well as a coordination ability of the ligand [1].



Ortho-phenantroline (1,10-phenantroline) is a heterocyclic organic compound ($M_r = 198.22$ g/mol). As a bidentate ligand in coordination chemistry, it forms strong complexes with most metal ions. Therefore it is used for photometric determination of the following elements: Cu (II) at wavelength of 272 nm; Cu (I) (435 nm) Co (226 and 270 nm); Cd (226 and 270 nm); Fe (II) (508 nm); Mn (226 and 268 nm); Ru (448 nm); Ni (228 and 270 nm) and Zn (226 and 270 nm) [2].

With orto-phenantroline, Fe (II) forms a stable complex of red color, which is called Ferrand. The color of the complex is stable in the interval of pH 2–9 [2].



Smaller cations and bivalent metals obstruct the process of determining iron using ortho-phenantroline complex, forming complexes or hardly soluble complexes with Fe (II), Co (II), Ni (II), Sn (II) and Cu (II).

Since ferrous iron is determined by the method, therefore ferric iron is reduced to ferrous using a hydroxylamine hydrochloride solution:

$$2\mathrm{Fe}^{3+} + 2\mathrm{NH_2OH} + 2\mathrm{OH}^- \rightarrow 2\mathrm{Fe}^{2+} + \mathrm{N_2} + 4\mathrm{H_2O}.$$

The determination of iron with 1,10-phenantroline depends on which form is iron bounded in the analyzed sample. In this paper biogenic ferrihydrite samples are used.

Synthetic ferrihydrite is an iron oxyhydroxide with the molecular formula $\mathrm{Fe_5HO_8 \cdot 4H_2O}$, although $5\mathrm{Fe_2O_3 \cdot 9H_2O}$ and others have been accepted [3]. Structurally, it is believed to be based on simple chains of iron octahedral, although tetrahedrally coordinated iron has also been proposed. Ferrihydrite crystallographic modifications are commonly designated as «2-line» and «6-line» ones on the basis of the number of maxima observed in the X-ray diffraction (XRD) patterns. Typically, ferrihydrite nanoparticles are 2–6 nm in size [4]; their large surface area/volume ratio imparts to them high sorptive capabilities, especially for heavy and transition metals. This makes the nanodisperse ferrihydrite an issue of considerable environmental and industrial importance [3].

In the literature, several methods of synthesis and purification of ferrihydrite are described [5]. Typically, ferrihydrite emerges in the situations, where Fe^{2+} is oxidized rapidly and/or where crystallization inhibitors are present. The oxidation can proceed via an inorganic pathway, but may also be assisted by microorganisms. Earlier investigations have shown that bacterium *Klebsiella oxytoca* creates two types of ferrihydrite nanoparticles depending on the microorganism growth conditions [6, 7]. These two fractions are quite well identified by means of the Mossbauer spectroscopy [8, 9, 14], static magnetic measurement analysis [10, 11], scanning electron microscopy and small-angle X-ray scattering methods [12] on dry powder samples. SAXS studies on concentrated and nonultrasonicated samples of water dispersions of ferrihydrite have revealed a scattering object with the form factor of a cylinder of radius $R = 4.87 \pm 0.02$ nm and length $L = 2.12 \pm 0.04$ nm [13, 15] as obtained with the aid of FITTER program [16]. Structural investigations of ultrasonic assisted samples of different concentration of water-dispersed particles are performed. Model calculations and fitting procedures reveale scattering objects of an elongated shape with a radius of gyration 6.73 ± 0.16 nm [17]. It was shown also by HRTEM analysis, that nanoparticles or their clusters bear some adsorbed protein molecules on their surfaces [12]. The X-ray fluorescence analysis on the contents of chemical elements in bacterial ferrihydrite gave following chemical composition at $T = 20$ °C: 60.5343% O, 16.5057% C, 8.4520% P, 9.198% Fe, 2.9274% Ca, 1.6764% K, 0.3269% Cl and 0.3793% of other elements [9]. Hereby, we present a method of quantity analysis of ferrihydrite water dispersed samples.

EXPERIMENTAL

For the experimental part of the work, samples of biogenic ferrihydrite nanoparticles dispersed in light and heavy water were used. Aqueous samples of biogenic particles of ferrihydrite were provided by the Siberian Federal University, Krasnoyarsk, Russia.

Ferrihydrite particles were obtained from microorganism isolated from Borovoe Lake (Krasnoyarsk Region, Russia). Ferrihydrite solution was prepared by dissolving 5 g of biomineral particles in 400 ml of distilled water. This solution is the basic solution of ferrihydrite used in this paper for the analysis on the UV-Vis spectrophotometer.

Figure 1 presents the absorption spectrum of biogenic ferrihydrite nanoparticles. Obtained spectra reveal a strong absorption of the ultraviolet radiation in the region of 280–400 nm.

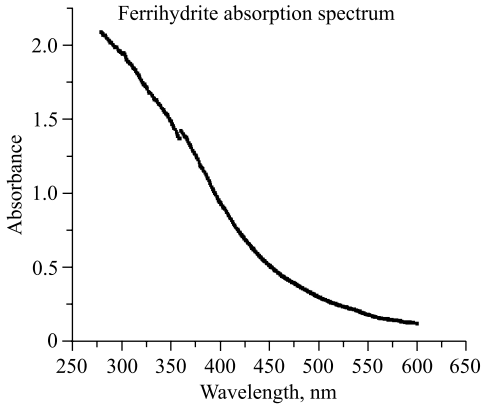


Fig. 1. Absorption spectrum of biogenic ferrihydrite

Reagents and Standard Solutions. All the high-quality (A.C.S.) chemicals used in the analysis were supplied by Sigma Aldrich Company (Germany). Stock solution of ferrous ammonium sulfate hexahydrate (used as standard solution of iron) was prepared by dissolving 0.07 g into 1000 cm^3. Stock of 1% orto-phenantroline solution was prepared by dissolving 10 g of $C_{12}H_8N_2$ in 100 cm^3 of distilled water. Buffer solution of 10% sodium acetate was prepared by dissolving 10 g of $CH_3COONa \cdot 3H_2O$ in 100 cm^3 of distilled water. The reducing agent solution of 10% hydroxylamine hydrochloride was prepared by dissolving 10 g of $NH_2OH \cdot HCl$ in 100 cm^3 of distilled water. All solutions were freshly prepared daily.

Instrumentation. For the determination of the iron in ferrihydrite samples a spectrometric method was used. Spectrophotometry is any technique that uses light to measure chemical concentration of analyzed samples.

In UV-Vis spectrophotometric analysis a source of radiation is used that extends into the ultraviolet region of the spectrum. The spectrophotometer possesses an optical system which forms the incident electromagnetic radiation beam, and measures the intensity of the transmitted radiation at selected wavelength of the spectral domain. Common analytical applications of spectrophotometry take advantage of the Beer–Lambert Law (often simply called Beer's Law) that relate the concentration of any analyzed substance to its absorbance of monochromatic radiation as shown below:

$$A = -\log T = \varepsilon\, bc,$$

where A is absorbance, T is transmittance, ε is the proportionality constant called the molar absorptivity, b is the path length through the sample, c is analyte concentration.

In our studies a UV-Vis spectrophotometer HITACHI DOUBLE BEAM (U-200) was used. All the experiments were performed in the Laboratory of Biochemistry of YuMO group, Condensed Matter Department of the Frank Laboratory of Neutron Physics, JINR (Russia).

Procedure. The spectrometric method of iron determination is based on the previous reduction of ferric iron using hydroxylamine hydrochloride, then on the formation of the red tris-1,10-phenantroline iron (II) complex under strictly defined pH (8.90–9.01) which was adjusted by addition of sodium acetate, and the photometric determination at wavelength of 508 nm [18].

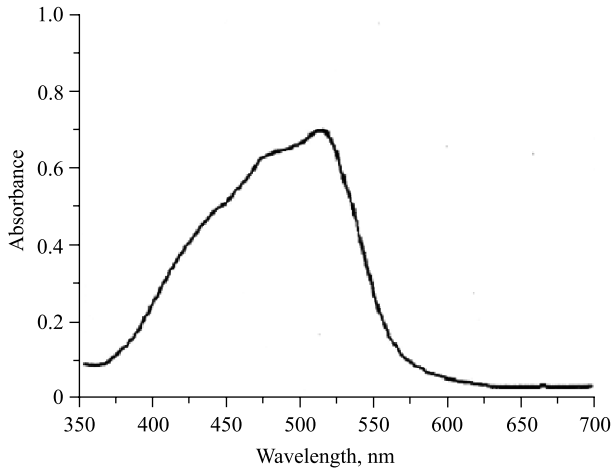


Fig. 2. Visible spectrum of $(phen)_3Fe(II)$ recorded in the range of 350–700 nm

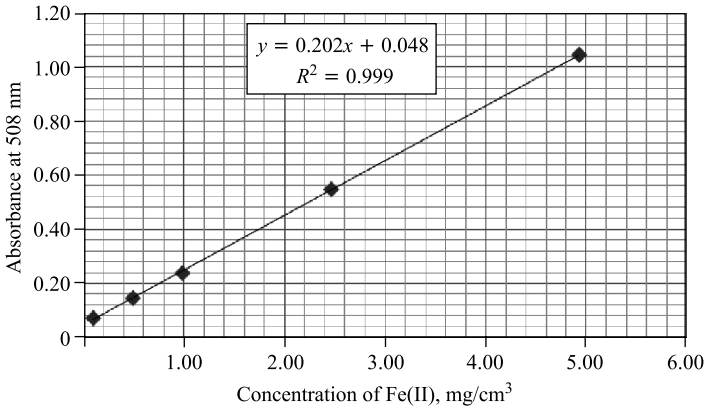


Fig. 3. Calibration curve showing the validity of Beer's law for the $(phen)_3Fe(II)$ complex used in iron determination

The selection of 508 nm was performed as follows: absorption spectra of the prepared standard solution of iron were recorded in the range of 350–700 nm. Resulted absorption spectrum of tris-1,10-phenantroline iron (II) complex is presented in Fig. 2.

After that, absorption maximum was determined, so for the wavelength of this maximum in accordance with Beer's law a linear dependence of absorbance on the concentration is presented and the steeper linear relation was selected (Fig. 3.).

Analysis of Biogenic Ferrihydrite Samples. The proposed method was applied to the analysis of iron content in biogenic ferrihydrite samples which will allow us to recalculate the ferrihydrite concentration in the analyzed samples. Therefore, to 250 μl from the basic solution of the ferrihydrite samples was dissolved with the addition of HCl (1 : 1) and transferred to 50 ml volumetric flask. Then the iron content was measured according to the recommended procedure.

RESULTS AND DISCUSSION

The stability of the tris-1,10-phenantroline iron (II) complex was monitored in solutions of light and heavy water dispersed biogenic ferrihydrite nanoparticles in the time interval of 0–60 min. For the sample of light water dispersed nanoparticles containing 2.468 g/cm^3 of ferrihydrite, tris-1,10-phenantroline iron (II) complex is unstable in the first 10 min; in the interval of 10–25 min it reaches the absorbance maxima; after absorbance changes, it reaches at the end a value close to the initial one (Table 1). For the sample of heavy water dispersed nanoparticles containing 1.450 g/cm^3 of ferrihydrite, tris-1,10-phenantroline iron (II) complex presents the instability in the interval of 0–25 min reaching maximum value of

Table 1. Dependence between absorbance and concentration of iron in ferrihydrite samples measured at different time

Colour development, min	Solution 1, ferrihydrite in H_2O			Solution 2, ferrihydrite in D_2O		
	A	$C_{Fe(II)}$, mg/cm^3	$C_{ferrihydrite}$, mg/cm^3	A	$C_{Fe(II)}$, mg/cm^3	$C_{ferrihydrite}$, mg/cm^3
5	0.698	1.609	2.430	0.418	0.916	1.383
7	0.701	1.616	2.441	0.420	0.921	1.391
10	0.706	1.629	2.460	0.426	0.936	1.414
15	0.706	1.629	2.460	0.429	0.943	1.424
20	0.706	1.629	2.460	0.431	0.948	1.432
25	0.708	1.634	2.468	0.436	0.960	1.450
30	0.704	1.624	2.453	0.425	0.933	1.409
35	0.703	1.621	2.448	0.420	0.921	1.391
40	0.702	1.619	2.445	0.413	0.903	1.364
60	0.690	1.589	2.399	0.406	0.886	1.338

absorbance at 25 min, followed by a decrease of the absorbance at the end up to a lower value than the initial one (Table 1).

Based on the absorbance measurements, the stability of the solution in the examined time period is observed (Fig. 4.). From the obtained results, it can be concluded that the tris-1,10-phenantroline iron (II) complex reaches a maximum

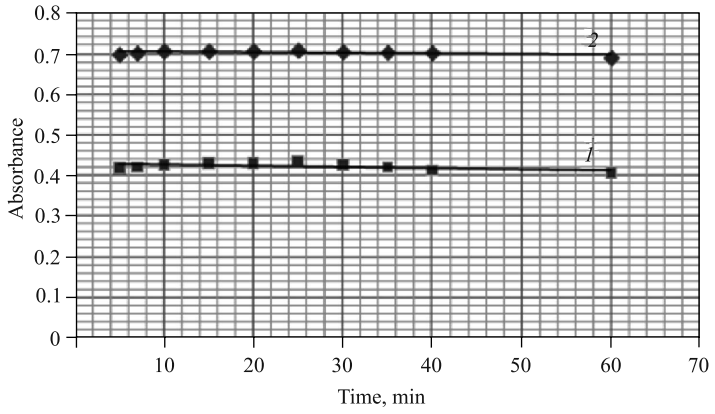


Fig. 4. Dependence of absorbance on the time for two different samples of biogenic ferrihydrite: *1*) ferrihydrite in D_2O; *2*) ferrihydrite in H_2O

of the absorbance value at 25 min from the moment that orto-phenantroline has been added, after which a decrease in the absorbance occurs.

This indicates that most probably the tris-1,10-phenantroline iron (II) complex disintegrates after a certain period of time. Figure 4 presents the dependence of the absorbance values obtained for samples of ferrihydrite nanoparticles dispersed in light and heavy water. Obtained plot does not reveal any oscillations of the absorbance depending on the time for the analyzed samples which indicates that the formation of the complex is not disturbed. This means that orto-phenantroline added to the analyzed sample strictly reacts only with the iron ions and there are no impurities that could influence the formation of the tris-1,10-phenantroline iron (II) complex. Therefore this method of determining iron in biogenic ferrihydrite samples can be the method of choice.

CONCLUSIONS

The tris-1,10-phenantroline iron (II) complex obeys Beer's law, i.e., presents a stability, for tested samples of ferrihydrite. For the concentration of biogenic ferrihydrite 2.68 g/cm^3, tris-1,10-phenantroline iron (II) complex of iron presents a maximum absorption at 25 min from the moment when ortho-phenantroline has been added. This indicates that for more exactly estimations of iron content in biogenic ferrihydrite samples, absorbance of tris-1,10-phenantroline iron (II) complex should be measured at 25 min from the moment when ortho-phenantroline has been added. During the spectrophotometric determination of the tris-1,10-phenantroline iron (II) complex the absorbance does not change significantly. Therefore this method of determining iron in biogenic ferrihydrite samples can be the method of choice.

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