

Iron(III) Complexation with Barbituric and 2-Thiobarbituric Acids in Aqueous Solution

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Abstract—Complexation in systems containing iron(III) chloride and barbituric (H₂BA) or 2-thiobarbituric (H₂TBA) acid has been studied by spectrophotometry and pH-metry in the pH range of 1.3–3.3 (*I* = 0.1 (NaCl), *t* = 20°C). The presence of 1 : 1 complexes with mono- and deprotonated forms of the ligand has been established, and their stability constants (in log units) have been determined: ¹[FeHBA]²⁺ (3.49 ± 0.15), [FeHTBA]²⁺ (2.69 ± 0.07), [FeBA]⁺ (12.22 ± 0.13), and [FeTBA]⁺ (11.05 ± 0.08). It has been shown that the higher thermodynamic stability of barbiturate complexes compared to 2-thiobarbiturate ones is due to the greater basicity of the barbiturate anion. Based on the stability constants obtained, it has been proposed to use orthophosphate, fluoride, and ethylenediaminetetraacetate ions to eliminate the interfering effect of iron(III) in the determination of malondialdehyde by the thiobarbiturate method. Orthophosphoric acid is the most convenient for practical applications, as it makes it possible to mask iron(III) and to create a strongly acidic medium necessary for the formation of a colored malondialdehyde–H₂TBA adduct.

Keywords: barbituric acid, thiobarbituric acid, ferric ions, complex formation, stability constants

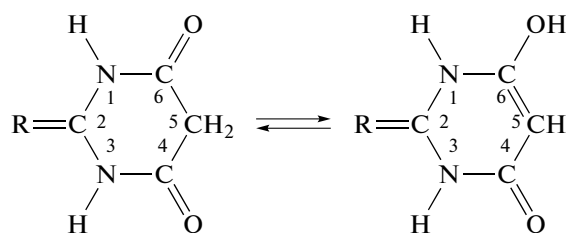
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INTRODUCTION

Iron is a vital trace element that regulates the processes of respiration, cell growth, metabolism, DNA synthesis and repair [1–3]. In particular, iron(III) in the form of heme is a component of cytochrome P450, whose isoenzymes are involved in the first phase of the metabolism of a large number of exogenous and endogenous compounds and in the biosynthesis of steroid hormones, cholesterol, bile acids, and prostanoids [4]. Obviously, iron(III) acts as an active binding site for various bioligands containing donor atoms of nitrogen, oxygen, and sulfur in their structure. Typical representatives of the latter are barbituric acids, belonging to the class of exogenous origin ligands.

Barbituric acid (C₄H₄N₂O₃, H₂BA) and 2-thiobarbituric acid (C₄H₄N₂O₂S, H₂TBA) are the parent molecules of a large group of drugs with anesthetic, antisclerotic, anticonvulsant, and sedative effects [5, 6]. Some members of the class demonstrate antibacterial, antifungal, anti-inflammatory, antiviral, and anticancer activity [5–10]. In analytical practice, H₂BA and H₂TBA are widely used in the colorimetric determina-

tion of cyanides (König reaction) [11] and malondialdehyde (MDA) [12], respectively. In aqueous solutions, acids (R = O for H₂BA and R = S for H₂TBA) exist predominantly in the keto (left) and enol (right) forms [7]:



The presence of electron-donating nitrogen, oxygen, and sulfur atoms in the imide >N–H, carbonyl >C=O, and thiocarbonyl >C=S groups determines the ability of H₂BA and H₂TBA to act as mono- or bidentate ligands. The acidic properties are due to the hydrogen atoms of the hydroxyl –OH (given the possible enolization of the >C=O group) and imide groups (both forms, positions 1 and 6).

There are data in the literature on the composition and properties of iron(III) compounds with H₂BA [13, 14] and H₂TBA [7, 15]. Due to the formation of intensely colored complexes, soluble iron(III) salts are

¹ For equilibrium of the formation of acidic complex formation: $\text{Fe}^{3+} + \text{HL}^- \xrightarrow{\beta_{11}} [\text{FeHL}]^{2+}$.

Table 1. Results of the stability constants determination for complex particles of iron(II, III) with barbituric and 2-thiobarbituric anions in an aqueous solution by spectrophotometry and pH-metry

Equilibrium	log <i>K</i>	Method of determination	Conditions	Ref.
$\text{Fe}^{3+} + \text{HBA}^- \rightleftharpoons [\text{FeHBA}]^{2+*}$	3.89 ± 0.11	pH-metry	$T = 25^\circ\text{C}$, $I = 0.1$ (NaCl), pH 1.5–2.0	[13]
	3.56 ± 0.15	B–H**		
	3.29 ± 0.08	Isomolar series	$T = 20^\circ\text{C}$, $I = 0.1$ (NaCl), pH 1.3–3.0	This study
	3.71 ± 0.19	B–H		
	3.65 ± 0.11	<i>D</i> –pH		
3.30 ± 0.20	pH-metry			
$\text{Fe}^{3+} + \text{BA}^{2-} \rightleftharpoons [\text{FeBA}]^+$	12.13 ± 0.23	B–H	$T = 20^\circ\text{C}$, $I = 0.1$ (NaCl), pH 2.6–3.3	
	12.30 ± 0.03	pH-metry		
$\text{Fe}^{3+} + \text{HTBA}^- \rightleftharpoons [\text{FeHTBA}]^{2+*}$	2.45 ± 0.07	<i>D</i> –pH	$T = 20^\circ\text{C}$, $I = 0.1$ (NaCl), pH 1.6–3.1	
	2.92 ± 0.07	pH-metry		
$\text{Fe}^{3+} + \text{TBA}^{2-} \rightleftharpoons [\text{FeTBA}]^+$	10.83 ± 0.12	Isomolar series	$T = 20^\circ\text{C}$, $I = 0.1$ (NaCl), pH 2.5–3.1	
	11.27 ± 0.03	pH-metry		
$\text{Fe}^{2+} + \text{TBA}^{2-} \rightleftharpoons [\text{FeTBA}]^0$ $[\text{FeTBA}]^0 + \text{TBA}^{2-} \rightleftharpoons [\text{Fe}(\text{TBA})_2]^{2-}$	6.56 ± 0.10	pH-metry	$T = 18^\circ\text{C}$, $I = 0.1$ (NaClO ₄)	[19]
	4.72 ± 0.10			

* Acid complex formation equilibrium [27, 28]. ** Benesi–Hildebrand method.

used both in the quantitative assessment of the content of these barbituric acids (pH 2–4) [13, 16] and in determining the authenticity of their derivatives in pharmaceutical chemistry [17]. Morelli [18] suggested using H₂TBA as an analytical reagent for Cu²⁺ and Fe³⁺ ions in their joint determination without preliminary separation. Using the method of molar ratios at various pH values, the 1 : 2 stoichiometric composition of the dominant complexes in the FeCl₃–H₂TBA system ($\lambda_{\text{max}} = 375$ nm) was established. Nevertheless, complexation between the studied components in an aqueous solution are practically not studied to date, especially this concerns the quantitative characteristics of equilibria (Table 1).

In 1964, Wills [20] noticed that the spectrophotometric determination of MDA by the thiobarbiturate method in the presence of iron(III) leads to erroneous results because of a significant increase in absorption ($\lambda = 535$ nm). The author suggested that the reason was the formation of iron(III) 2-thiobarbiturate complexes. Later, Zhou and colleague [21] showed that the previously described interfering effect of Fe³⁺ ions is indeed due to the formation of complex particles between iron(III) and H₂TBA, which have pronounced absorption at 532 nm or fluorescence at 553 nm (characteristic maxima for the MDA–H₂TBA adduct). The authors give the probable stoichiometric composition

of the complex as 1 : 3 (precipitation was observed). Thus, the presence of iron, given its biogenic origin, can lead to false positive results of biochemical tests when quantifying MDA, which is a marker of oxidative stress, by the thiobarbiturate method. According to the described spectrophotometric procedures [12, 22–24], the determination of MDA in the form of an adduct with H₂TBA is carried out in a strongly acid medium using orthophosphoric and glacial acetic acids, as well as its chlorine-substituted derivatives.

The aim of this work is to study complexation in the iron(III)–barbituric (2-thiobarbituric) acid system, including determination of the composition and thermodynamic stability of complex particles in an aqueous solution in an acidic medium.

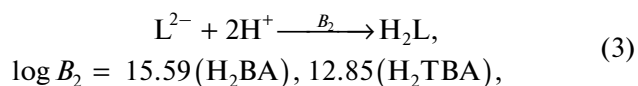
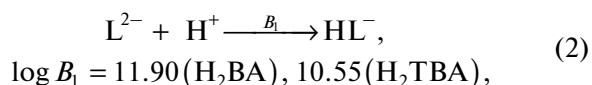
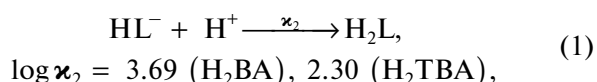
EXPERIMENTAL

Reagents and equipment. Barbituric (Reakhim, Russia) and 2-thiobarbituric (Diam, Russia) acids (pure for analysis grade, Russian State Standard), iron(III) chloride hexahydrate (Reakhim, Russia, chemically pure grade), sodium hydroxide (Khimreaktiv, Russia, pure for analysis grade), sodium chloride (Base No. 1 Khimreaktivov, Russia, chemically pure grade), potassium dihydroorthophosphate (Base No. 1 Khimreaktivov, Russia, chemically pure grade), potassium fluoride dihydrate (Reakhim, Russia, pure

for analysis grade), orthophosphoric (Khimprom, Russia, chemically pure grade) and hydrochloric (Sigma Tech, Russia, special quality grade) acids, sulfosalicylic acid dihydrate (Base No. 1 Khimreaktivov, Russia, chemically pure grade), Trilon B (standard titer, Reakhim, Russia) were used as starting reagents. The working solutions were maintained at a constant ionic strength $I = 0.1$ (NaCl) and temperature $t = 20^\circ\text{C}$. The concentration of H_2L ligands was preliminarily refined by pH-metric titration with an alkali solution; iron(III) was refined by direct complexometric titration with a Trilon B solution using sulfosalicylic acid as an indicator according to a well-known method [25]. To prevent the hydrolysis process, the iron(III) chloride solution was acidified with hydrochloric acid. In the experiments, we utilized ultrapure water (type I) obtained using a specialized Millipore Direct-Q 5 UV water purification system (Merck, USA).

Spectrophotometric measurements in the UV and visible spectrum regions were carried out on a Cary-50 spectrophotometer (Varian, USA) in quartz cuvettes ($l = 10$ mm, against water). pH was measured using a 673 pH meter (Analitpribor, Georgia) on a concentration scale. For that, glass electrode was preliminarily calibrated against (H, Na)Cl solutions with different HCl concentrations ($I = 0.1$). pH-metric titration in determining the protonation constants of ligand anions and the stability constants of complex particles was carried out with a solution of preliminarily standardized carbonate-free alkali ($I = 0.1$) with continuous stirring and passage of purified nitrogen through the titrated solution.

Choice of ligand protonation constants. The protonation constants of singly (HL^-) and doubly charged (L^{2-}) anions of the studied ligands were used in the calculations, characterizing the following equilibria:



where κ_2 is the stepwise protonation constant; B_1 and B_2 are the overall protonation constants of the L^{2-} ion.

The stepwise protonation constants κ_2 (in log units) of the ligands were preliminarily determined by pH-metry ($I = 0.1$, $T = 20^\circ\text{C}$): 3.69 ± 0.02 (H_2BA), 2.30 ± 0.01 (H_2TBA). The overall protonation constants for the first step B_1 (in log units) were taken from [19, 26]: 11.90 ± 0.04 for H_2BA ($I = 0.1$ (KNO_3), $T = 25^\circ\text{C}$) and 10.55 ± 0.10 for H_2TBA ($I = 0.1$

(NaClO_4), $T = 18^\circ\text{C}$). The overall protonation constants for the second step B_2 are obtained from the equation: $\log B_2 = \log B_1 + \log \kappa_2$. The temperature difference was not taken into account, since it was insignificant (according to [19], an increase in temperature by 13°C led to a change in the value of $\log B_1$ by only 0.11 units).

Determination of the composition and stability of complex particles. The composition and stability of the complex particles formed in the studied systems under specified conditions were determined from the data of spectrophotometric (isomolar series, Benesi–Hildebrand, D -pH methods [27, 28]) and pH metric measurements in the pH range of 1.3–3.3. The effective wavelength, where the observed absorption is maximum for the complex and minimum for the free metal and ligand ($D_L = 0$) was taken to be 440 or 540 nm for the FeCl_3 - H_2BA system and 490 or 540 nm for the FeCl_3 - H_2TBA system. Before absorbance measurements, the mixtures were kept for 1 min until a nearly constant D_i value was established.

Processing of measurement and calculation results. Calculation of protonation constants (“Acid 1”) and stability constants of complex particles according to spectrophotometry (“ D -pH”) and pH-metry (“Bjerrum 1”), construction of species distribution diagrams of the studied ligands (“Acid Yield”), as well as statistical processing of the obtained results (“Delta”) was performed using the programs indicated in brackets [28]. When calculating the overall stability constants of complex particles, we took into account the possible protonation of the ligand (Eqs. (1)–(3)) and hydrolysis of the metal ion in the first step ($\log K_{h1} = -2.84$ [28]). In the case of the D -pH series, the complexation of Fe^{3+} ions with supporting electrolyte anions (Cl^-) was also given. For each studied system, at least two parallel experiments were performed.

Iron(III) masking in the presence of 2-thiobarbiturate anion. When assessing the possibility of iron(III) masking in the presence of 2-thiobarbiturate anion, a number of well-known masking agents were used: orthophosphate, fluoride, and ethylenediaminetetraacetate anions. The reference samples were aqueous solutions of 1.68×10^{-3} M FeCl_3 (solution 1, pH 1.35) and FeCl_3 - H_2TBA (1 : 1, solution 2, $C_{\text{Fe}} = C_{\text{L}} = 1.68 \times 10^{-3}$ M, pH 1.36). Next, a stoichiometric amount of an aqueous solution of Trilon B (solution 3, pH 1.53) was added to the resulting mixture; solid KF (solution 4, pH 1.89) and KH_2PO_4 (solution 5, pH 1.77) were also added at the tip of a spatula. Then the solutions were mixed, left for 10 min, and their color was fixed (Fig. 1). In all cases, the constant ionic strength $I = 0.1$ was maintained by adding a mixture of (H, Na)Cl at $T = 20^\circ\text{C}$. Instead of KH_2PO_4 , 10 μL of concentrated H_3PO_4 ($V_{\text{total}} = 6$ mL) was also added to solution 5. All test solutions were stable for at least 24 h.

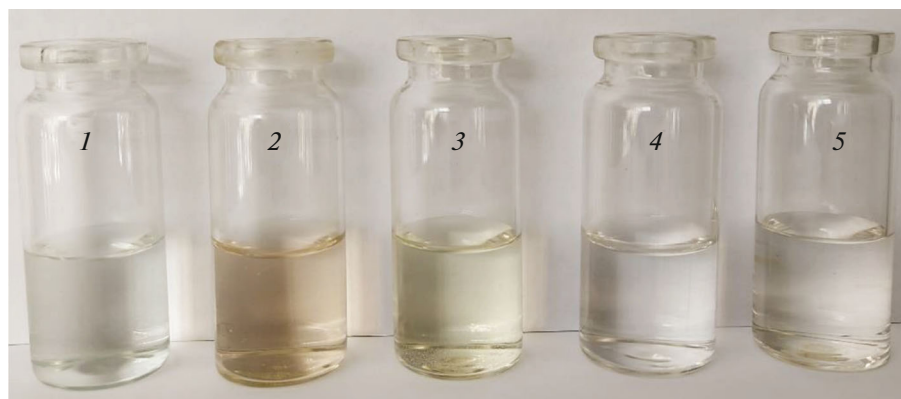


Fig. 1. Visual representation of solutions used in assessing the possibility of iron(III) masking in the presence of H₂TBA ($C_L = C_{Fe} = C_{mixture} = 1.68 \times 10^{-3}$ M, $V_{total} = 6$ mL, $I \sim 0.1$, $t = 20^\circ\text{C}$): (1) FeCl₃ (pH 1.35), (2) FeCl₃–H₂TBA (pH 1.36), (3) FeCl₃–H₂TBA + Na₂H₂Edta ($C_{additive} = 1.68 \times 10^{-3}$ M, pH 1.53), (4) FeCl₃–H₂TBA + KF(solid) (pH 1.89), (5) FeCl₃–H₂TBA + KH₂PO₄(solid) (pH 1.77).

RESULTS AND DISCUSSION

Composition and Stability of Complex Particles

When aqueous solutions of FeCl₃ and H₂L were mixed, the resulting solution turned red-brown accompanied by a significant increase in absorption in the UV and visible ($D_L = 0$) spectrum regions (Figs. 2a and 2b). The electronic absorption spectra of H₂L solutions in the UV range (Figs. 2a and 2b) contain pronounced maxima (Table 2) due to intraligand transitions $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ in the heterocyclic fragments of the considered acids and their functional groups [14], as well as those undergoing a hypsochromic shift in the absorption spectra of the complexes. The absorption band of the FeCl₃–H₂BA and FeCl₃–H₂TBA systems in the visible range (Figs. 2a and 2b) forms a pronounced shoulder emanating from the UV region and may belong to a charge transfer complex. According to Ref. [13], the spectra of the FeCl₃–H₂BA system also contained a charge transfer band in the region of 430–

480 nm. The above facts unambiguously indicate the occurrence of complexation processes in the studied systems. This is also indirectly confirmed by the fact that the titration curves of the FeCl₃–H₂L systems are located significantly lower than the titration curves of the acids themselves, since the Fe³⁺ ion promotes the displacement of the ligand proton [13].

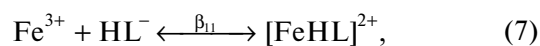
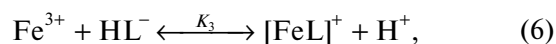
The determination of the composition and thermodynamic stability of iron(III) complex particles with H₂BA and H₂TBA anions was preceded by a study of the acid–base properties of the ligands by pH-metry. The obtained values of the logarithms of the stepwise protonation constants of the anions BA²⁻ (3.69 ± 0.02) and TBA²⁻ (2.30 ± 0.01) at $I = 0.1$ (NaCl) and $T = 20^\circ\text{C}$ satisfactorily agree with the currently available literature data [7, 13, 19, 26].

The study of the FeCl₃–H₂L systems using the isomolar series (Fig. 3) and D –pH ($M : L \sim 1 : 1$) showed dominance of the 1 : 1 complex particles containing the protonated form of the ligand under experimental conditions (pH 1.3–2.4, $I = 0.1$, $T = 20^\circ\text{C}$). At the same time, according to the results of pH-metry (pH 2.6–3.1), the simultaneous presence of protonated [FeHL]²⁺ and deprotonated [FeL]⁺ complexes was established. Thus, given the species distribution diagrams of H₂L, the formation of barbiturate and 2-thio-barbiturate iron(III) complexes in an aqueous solution can be represented by the following equilibria:

Table 2. Results of UV spectrophotometric study of FeCl₃–H₂L systems and free ligands in aqueous solution ($C_{Fe} = C_L = 3.0 \times 10^{-5}$ M, $I = 0.1$, pH ~ 3.0 , $T = 20^\circ\text{C}$, $l = 10$ mm)

System	λ_{max} , nm	$\Delta\lambda$, nm*	D_{max}
H ₂ BA	260	–	0.313
FeCl ₃ –H ₂ BA	257	–3	0.617
H ₂ TBA	242	–	0.176
	267	–	0.300
	276	–	0.289
FeCl ₃ –H ₂ TBA	242	0	0.703
	263	–4	1.222
	271	–5	1.164

* For $\Delta\lambda$ values, the hypsochromic shift is indicated by a minus sign.



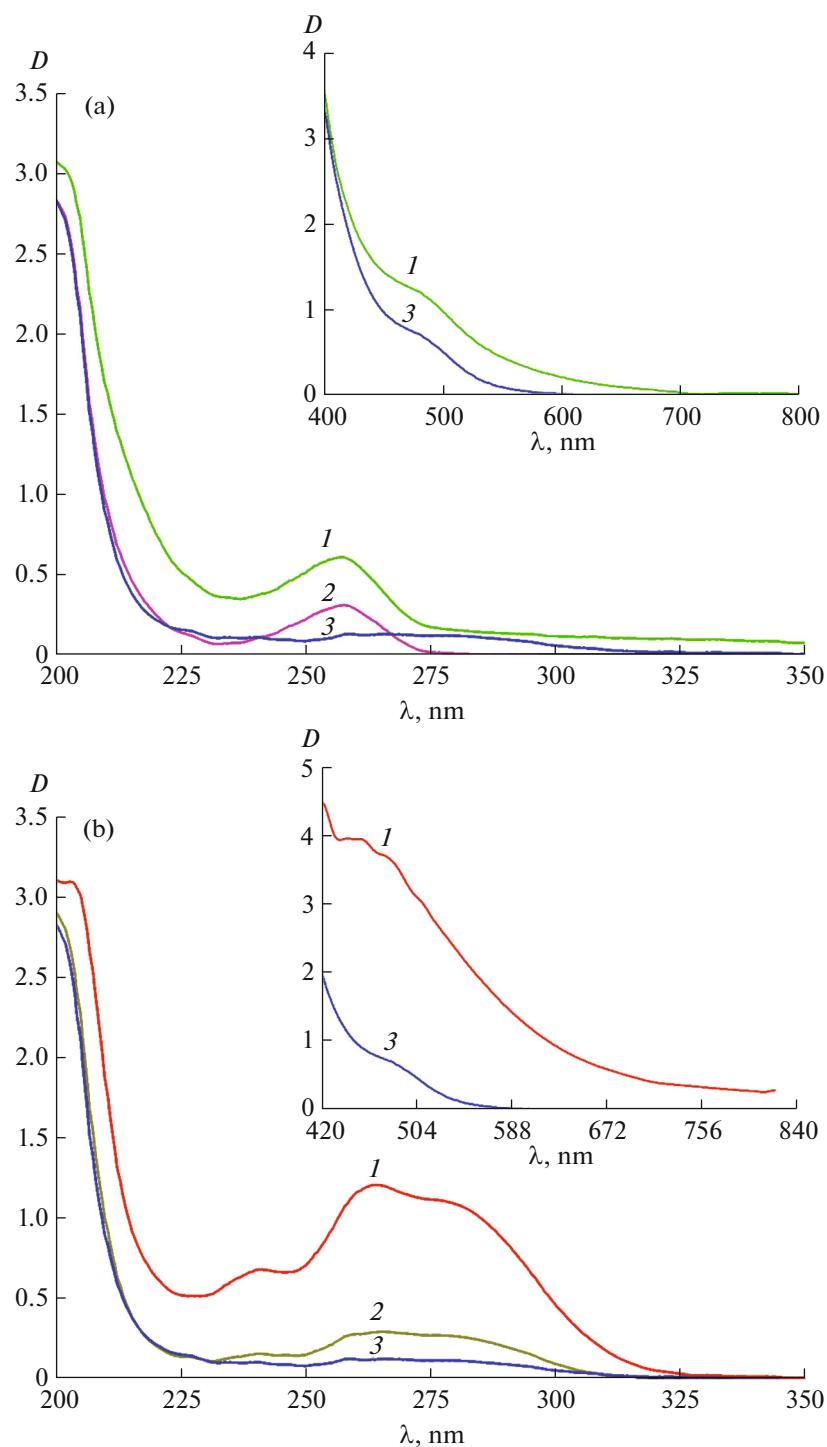
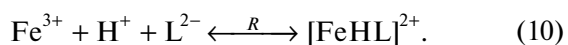
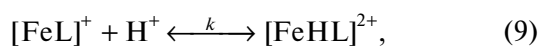


Fig. 2. Electronic absorption spectra ($I \sim 0.1$, $\text{pH} \sim 3.0$, $T = 20^\circ\text{C}$) of $\text{FeCl}_3\text{-H}_2\text{L}$ (1), H_2L (2) and FeCl_3 (3) aqueous solutions in UV ($C_L = C_{\text{Fe}} = C_{\text{mixture}} = 3.0 \times 10^{-5}$ M) and visible region ($C_L = C_{\text{Fe}} = C_{\text{mixture}} = 2.5 \times 10^{-2}$ M), where $\text{H}_2\text{L} =$ (a) H_2BA or (b) H_2TBA .



Based on the results of isomolar series, using regions with a molar ratio of components close to 1 : 1,

we calculated the stability constants of complex particles $[\text{FeHBA}]^{2+}$ ($\log \beta_{11} = 3.29 \pm 0.08$, $\text{pH} \sim 1.9$) and $[\text{FeTBA}]^+$ ($\log \beta_1 = 10.83 \pm 0.12$, $\text{pH} \sim 2.5$). The stability constants of acidic $[\text{FeHBA}]^{2+}$ ($\log \beta_{11} = 3.71 \pm 0.19$, $\text{pH} \sim 2.1$) and deprotonated $[\text{FeBA}]^+$ ($\log \beta_1 = 12.13 \pm$

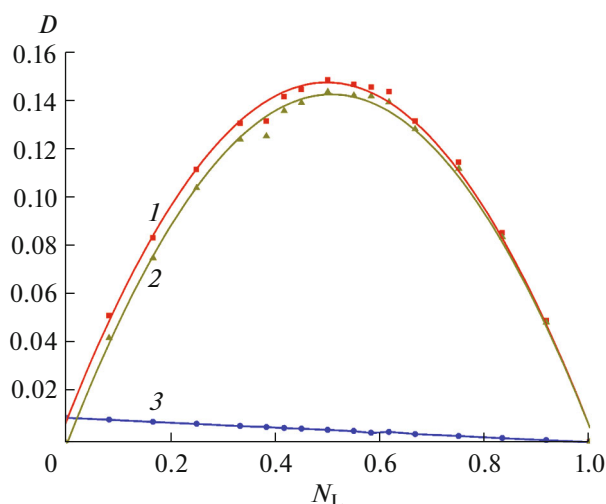


Fig. 3. Dependence of the absorption D on the molar content of the ligand N_L in a series of isomolar solutions of the system $\text{FeCl}_3\text{--H}_2\text{TBA}$: D_i (1), ΔD_i (2), $D_{\text{Fe}, i}$ (3) (i is the experimental point number, $\lambda_{\text{ef}} = 540$ nm, $l = 10$ mm, $C_L^\circ = C_M^\circ = 6.00 \times 10^{-3}$ M, $\Delta D_{\text{max}} = 0.144$, $V_{\text{total}} = 6$ mL, $\text{pH} \sim 2.5$, $I = 0.1$, $T = 20^\circ\text{C}$).

0.23, $\text{pH} \sim 3.3$) complexes were also determined by Benesi-Hildebrand method at $C_{\text{Fe}} \gg C_L$ and $C_L \gg C_{\text{Fe}}$. In addition, processing of the experimental data obtained by the D – pH method for the $\text{FeCl}_3\text{--H}_2\text{BA}$ system ($\text{pH} 1.3\text{--}2.4$) led to the following results: $n \approx 1$ and $\log R = 15.55 \pm 0.20$, where n is the number of protons in the complex, R is equilibrium constant (10). It follows from Eq. (2), (7), and (10) that $\log \beta_{11} = \log R - \log B_1 = 15.55 - 11.90 = 3.65$ ($s^2 = 3.02 \times 10^{-3}$). For the $\text{FeCl}_3\text{--H}_2\text{TBA}$ system ($\text{pH} 1.6\text{--}2.4$), we have $n \approx 1$ and $\log R = 13.00 \pm 0.90$; thus, $\log \beta_{11} = 13.00 - 10.55 = 2.45$ ($s^2 = 4.23 \times 10^{-3}$).

These methods assume the predominance of only a single complex in the equilibrium mixture. However, the expediency of these assumptions (taking into account only complex particles of the same type) is confirmed by pH -metry data, which makes it possible to take into account the simultaneous presence of several complex particles of different composition. Thus, the processing of pH -metric data for the $\text{FeCl}_3\text{--H}_2\text{BA}$ system ($\text{pH} 2.6\text{--}3.0$; Table 3) led to the following results: $\log \beta_1 = 12.30 \pm 0.03$, $\log \beta_{11} = \log R_{\text{opt}} - \log B_1 = 15.20 - 11.90 = 3.30$ ($s^2 = 1.03 \times 10^{-2}$). For the system $\text{FeCl}_3\text{--H}_2\text{TBA}$ ($\text{pH} 2.6\text{--}3.1$) we have the following: $\log \beta_1 = 11.27 \pm 0.03$, $\log \beta_{11} = \log R_{\text{opt}} - \log B_1 = 13.47 - 10.55 = 2.92$ ($s^2 = 4.23 \times 10^{-3}$). The values of the stability constants of $[\text{FeHL}]^{2+}$ and $[\text{FeL}]^+$ complex particles calculated by different methods are in satisfactory agreement both with each other and with the currently known literature data [13] (Table 1), which indicates the correctness of the chosen calculation model and the processes embedded in it.

When comparing the values of β_{11} and β_1 (Table 1), it can be seen that $[\text{FeHL}]^{2+}$ are less stable than $[\text{FeL}]^+$. This is due to the lower denticity of the HL^- ion compared to L^{2-} one, since in the first case the nitrogen atom of the imide group is blocked by a proton and is not able to participate in the coordination by the metal ion. According to the Pearson HSAB concept and the Ahrlund–Chatt–Davies classification [29], the Fe^{3+} cation, which has a half-filled d -sublevel and has a charge distribution close to spherical, is a typical hard acid (class “A” cation) and forms the most stable complexes with hard bases, which are ligands containing donor oxygen and nitrogen atoms. It is logical that the HL^- anion acts as a monodentate ligand, being coordinated by the Fe^{3+} ion via the deprotonated oxygen atom of the hydroxo group (see earlier keto-enol equilibrium), the proton of which is displaced first. The

Table 3. pH -metry data and calculation results of the $[\text{FeBA}]^+$ stability constant with the simultaneous presence of $[\text{FeHBA}]^{2+}$ ($V_M^\circ = V_L^\circ = 5.00$ mL, $C_L^\circ = 1.04 \times 10^{-2}$ M, $C_M^\circ = 1.00 \times 10^{-2}$ M, $C_{\text{HCl}} = 1.142 \times 10^{-3}$ M, $C_{\text{NaOH}} = 1.68 \times 10^{-2}$ M, $\log K_{\text{H}_1} = -2.84$, $\log B_1 = 11.90$, $\log B_2 = 15.59$, $I = 0.1$, $T = 20^\circ\text{C}$)

V_{alk} , mL	pH	$\log \beta_1$	V_{alk} , mL	pH	$\log \beta_1$	V_{alk} , mL	pH	$\log \beta_1$
0.20	2.60	12.25	1.10	2.71	12.38	2.00	2.90	12.25
0.40	2.60	12.38	1.20	2.73	12.37	2.10	2.91	12.27
0.50	2.61	12.40	1.40	2.78	12.31	2.20	2.92	12.29
0.60	2.63	12.38	1.50	2.80	12.30	2.40	2.95	12.30
0.80	2.66	12.39	1.60	2.83	12.25	2.50	2.96	12.33
0.90	2.68	12.37	1.80	2.87	12.23	2.60	2.97	12.35
1.00	2.69	12.40	1.90	2.89	12.23	2.80	2.98	12.42

$\log \beta_1 = 12.30 \pm 0.03$, $\log \beta_{11} = 3.30$ ($s^2 = 1.03 \times 10^{-2}$).

L^{2-} anion is probably coordinated via donor oxygen and nitrogen atoms.

The obtained values of β_{11} for $[\text{FeHTBA}]^{2+}$ and $[\text{FeHBA}]^{2+}$, β_1 for $[\text{FeTBA}]^+$ and $[\text{FeBA}]^+$ correlate with each other, as well as with the value of β_1 of iron(II) 2-thiobarbiturate complex $[\text{FeTBA}]^0$ [19] (Table 1). From simple electrostatic concepts for cations with a spherical charge distribution (or close to it), it follows that the complex is more stable when it includes ions with a larger charge and a smaller size. A convenient criterion for assessing the complexing ability of ions is the value of their ionic potential φ ($\varphi = z_i/r_i$) [30, 31]: the complex with the higher φ value (with the same composition of complexes) is more stable. Indeed, the higher stability of the iron(III) 2-thiobarbiturate complex $[\text{FeTBA}]^+$ compared to that of iron(II) $[\text{FeTBA}]^0$ (Table 1) is associated with a change in the electrostatic characteristics of the cations when passing from Fe^{2+} (d^6 , $r_u = 0.92 \text{ \AA}$, $z_u = +2$, $\varphi = 2.17$) to Fe^{3+} (d^5 , $r_u = 0.79 \text{ \AA}$, $z_u = +3$, $\varphi = 3.80$). As a consequence, a stronger electrostatic M–L interaction occurs in the $[\text{FeTBA}]^+$ complex than in the $[\text{FeTBA}]^0$ particle. In addition, a ligand with a higher basicity forms more stable complexes: the Fe^{3+} ion, like the proton, preferentially binds to the more basic ligand. Thus, when comparing the stability of 2-thiobarbiturate ($\log B_1 = 10.55$, $\log B_2 = 12.85$, $\log \kappa_2 = 2.30$) and barbiturate ($\log B_1 = 11.90$, $\log B_2 = 15.59$, $\log \kappa_2 = 3.69$) complexes (Table 1), it can be seen that the anions HBA^- and BA^{2-} are more basic than HTBA^- and TBA^{2-} ones. As a result, barbiturate complexes are an order of magnitude more stable than 2-thiobarbiturate analogs.

Iron(III) Masking in the Presence of 2-Thiobarbiturate Anion

It is known that biogenic metal ions present in the biological fluids of animals and humans can have a significant interfering effect on the results of various biochemical studies. In particular, the interfering effect of Fe^{3+} ions in studying the lipid peroxidation by the thiobarbiturate method is due to their interaction with H_2TBA and the formation of an intensely colored complex [20, 21].

It was established in Ref. [20] that the addition of various chelating agents (trilon B, gelatin, ovalbumin, diluted horse serum) to a mixture of MDA and H_2TBA in the presence of iron(III) leads to a noticeable decrease in the absorption of the solution. The observed phenomenon can be explained by the binding of iron(III) into a more stable and less colored complex compared to the 2-thiobarbiturate one. Thus, for $[\text{FeEdta}]^-$ (Edta^{4-} is the anion of ethylenediaminetetraacetic acid H_4Edta), $\log \beta_1 = 25.10$ ($I = 0.1$ (NaClO_4), $T = 20^\circ\text{C}$) [32], which is much higher

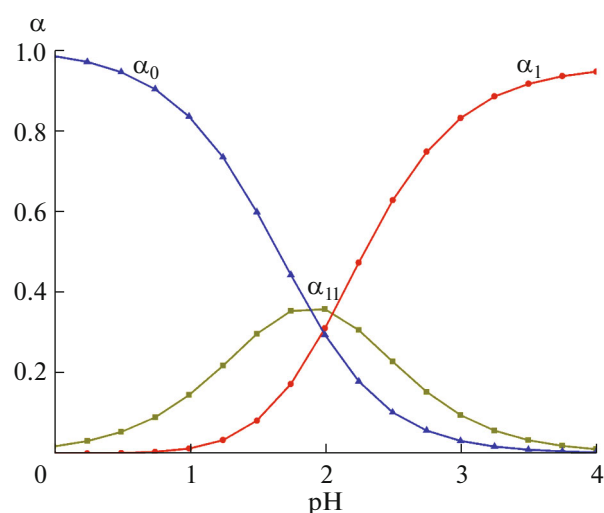


Fig. 4. Species distribution diagram of iron(III) 2-thiobarbiturate complexes in an aqueous solution ($C_L = 0.01 \text{ M}$, $\log B_1 = 10.55$, $\log B_2 = 12.85$, $\log K_{h1} = -2.84$, $\log \beta_{11} = 2.69$, $\log \beta_1 = 11.05$): $[\text{Fe}^{3+}]$ (α_0), $[\text{FeHTBA}^{2+}]$ (α_{11}), $[\text{FeTBA}^+]$ (α_1).

than the corresponding value for $[\text{FeTBA}]^+$ (Table 1). Proteins contain a large number of N- and O-donor functional groups and are able to bind the Fe^{3+} ion into strong chelate complexes. On the other hand, the addition of sodium citrate (Na_3Cit), glycine (HGly), alanine (HAla), phenylalanine (HPhe) or glutamic acid (H_2Glu) does not affect the absorption of the mixture. Apparently, this is due to the fact that the forming complex particles have less or close stability compared to $[\text{FeTBA}]^+$. For instance, for $[\text{FeGly}]^{2+}$ $\log \beta_1 = 8.57$ ($I = 0.5$ (NaNO_3), $T = 25^\circ\text{C}$) [33]; for $[\text{FeAla}]^{2+}$ $\log \beta_1 = 8.96$ ($I = 0.5$ (NaNO_3), $T = 25^\circ\text{C}$) [34]; for $[\text{FeGlu}]^+$ $\log \beta_1 = 11.81$ ($I = 0.5$ (NaNO_3), $T = 25^\circ\text{C}$) [35]; for $[\text{FeCit}]^0$ $\log \beta_1 = 11.21$ ($I = 0.1$ (KNO_3), $T = 25^\circ\text{C}$) [36].

It follows from the above that it is logical to use Trilon B to eliminate the interfering effect of iron(III) in the determination of MDA by the thiobarbiturate method in a strongly acidic medium. The forming $[\text{FeEdta}]^-$ complex is more stable than $[\text{FeTBA}]^+$ one and does not significantly absorb in the region of 530–540 nm (the interval of the characteristic maximum for the MDA– H_2TBA adduct) [37], which makes it possible to avoid possible spectral noise. Fluoride and orthophosphate ions are often used in analytical practice to mask Fe^{3+} ones. Since the stability of iron(III) fluoride complexes ($\log \beta_3 = 12.53$; $I = 0.1$ (KNO_3), $T = 25^\circ\text{C}$) [38] is higher than that of $[\text{FeTBA}]^+$, fluoride ions can also be recommended for masking Fe^{3+} ones. Iron(III) orthophosphate protonated complexes, e.g., $[\text{FeHPO}_4]^+$ ($\log \beta_{11} = 8.59$; $I = 0.15$ (NaCl),

$T = 25^\circ\text{C}$ [39]), are less resistant to $[\text{FeTBA}]^+$. In this regard, orthophosphate ions can be used as a masking agent in the region of dominance of the $[\text{FeHTBA}]^{2+}$ complex at $\text{pH} < 2$ (Fig. 4).

The assumptions made above based on a comparison of the stability constants of iron(III) complexes agree with the experimental data. As can be seen from Fig. 1, solution 4 with KF and solution 5 with KH_2PO_4 were colorless compared to the colored reference solution 2. Adding 10 μl of concentrated H_3PO_4 instead of KH_2PO_4 also causes the solution to become colorless. Thus, H_3PO_4 can be used to create an acidic medium ($\text{pH} \sim 2\text{--}3$ [23]), which is necessary for the reaction between MDA and H_2TBA , and to mask Fe^{3+} ions. When Trilon B solution (solution 3) is added, a yellow color appears (wavelength range 565–590 nm), which, as expected, should not affect the absorption of the MDA– H_2TBA adduct [37].

CONCLUSIONS

Using spectrophotometry and pH-metry in $\text{FeCl}_3\text{--H}_2\text{L--H}_2\text{O}$ systems ($\text{pH} 1.3\text{--}3.3$, $I = 0.1$, $T = 20^\circ\text{C}$), where H_2L is barbituric or 2-thioarbituric acid, the formation of complex particles was shown, the metal : ligand ratio was established, the forms of the ligand in the formed complexes ($[\text{FeHL}]^+$ and $[\text{FeL}]^+$) and their stability constants were determined. It was shown that when studying the state of lipid peroxidation, iron(III) as an interfering endogenous component can be masked by the addition of Trilon B, KF, and KH_2PO_4 (or H_3PO_4).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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