Chapter 2

Formation of Fe(III) Ternary Complexes with Related Bio-relevant Ligands

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Additional information is available at the end of the chapter

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Abstract

Ternary complexes of iron(III)-glycine(Gly)-nitrilotriacetate (NTA) system determined by electrochemical measurements of the dissolved iron(III)-Gly-NTA mixed ligand system in the 0.1 mol·dm^{-3} $NaClO_4$ aqueous solution at pH = 8.0 ± 0.1and 25 ± 1°C. The coordination number of Fe in Fe(EDTA)-L is seven in coordinate complex, where L can be a DNA constituent like uracil, uridine, thymine, thymidine, and inosine. The nonlinear least-squares program MINIQUAD-75 is used to deduce the hydrolysis constants of $[Fe(EDTA)(H_2O)]^-$ and its formation constant in solution. The antimicrobial activity of Fe(III) complexes of salicylhydroxamic acid (SHAM) and 1,10-phenanthroline (PHEN) studied against representative pathogenic bacteria and fungi.

Keywords: iron(III), MINIQUAD-75, speciation, stability constant, glycine, sulfamethoxazole, salicylhydroxamic acid

1. Introduction

Iron is consider as an essential element; its chemical and biological functions evolved from its oxidation and reduction processes and interactions with oxygen [1]. These are very important biogeochemical in natural aquatic systems [2, 3]. It is one of the most abundant metals in the Earth's crust [4]. However, very low concentrations (<10^{-9} mol·dm^{-3}) of dissolved, mostly iron (III) organic complexes are present in natural waters due to the low solubility of its thermodynamically stable 3+ ionic form [5, 6]. Iron is used to treat chlorosis (green disease), which often resulting from deficiency of the iron concentration [7]. However, 80 years ago, we did not have any information about the importance of inorganic iron in synthesis of hemoglobin [8]. Many years ago, the nutritional experts became interested in the role of iron in oxygen transport and hemoglobin formation [9]. Most anemia diseases in industrialized countries result from low

iron intake and bioavailability. On the other side, they are responsible for only about half of the anemia in developing countries [10]. There are other important causes [11] like infectious and inflammatory diseases (especially malaria), blood loss from parasitic infections, and other nutrient deficiencies (vitamin A, riboflavin, folic acid, and vitamin B12).

2. Analysis of aqueous solution complexes by different methods

1. The first and most common method is ion-selective electrode. It defines the position of dynamic equilibrium. The most important electrode is the glass one. There is also hydrogen gas electrode that can be used in hydrogen ion calculations.
2. Metal amalgam electrodes are a second choice. It can be used for some metal ions, but they are not as precise as the hydrogen ion electrode. We prefer the ion-selective electrode in-calculation because the results are collected from series of data taken through a titration procedure. A good method to check for this prerequisite is to make repeated high-resolution electrode readings at predetermined time intervals, since this will make sluggish attainments of equilibrium clearly visible.
3. Spectrophotometry can be used if the metal ion or the ligand is colored, so that the color will change (in intensity and/or frequency) upon complexation.

There are other experimental techniques that we are going to give some examples for them in the following lines. The specific method for diamagnetic metal ion is the nuclear magnetic resonance (NMR). It gives one separated signal for each unique chemical surrounding. In other words, it can inform us about concentration of the ligand, the free metal ion, the number of species, and their concentration for a given chemical composition. The important feature of this technique is that the positions of these selective signals are responsible for the protonation and deprotonation ones. In the case of fast reactions, we can use a stopped-flow technique.

2.1. The addition of water to iron(III)

It is observed that Fe^{3+} hydrolyses in water goes as follows:

$$Fe^{3+} + H_2O \leftrightharpoons FeOH^{2+} + H^+ \quad (1)$$

The addition of water to Fe^{3+} is carried out through a series of deprotonation reactions, resulting in formation of ferric hydroxides and oxyhydroxides [12, 13] as in Eq. (1), the equilibrium constant for this reaction was calculated to be 6.78×10^{-3} at 298 K (total iron(III) concentration of 0.5 mol·dm^{-3} and an ionic strength of 0.1 mol·dm^{-3}). **Figure 1** shows the speciation of the two iron-containing species of Eq. (1) as a function of pH, calculated at $T = 298$ K. It seen that $FeOH^{2+}$ will become the common species above pH = 2.17. At lower pH, there are small amount of it; at pH value 1.2, more than 9% of all iron (III) is present as the hydroxide. As a result of that, the calculations for the spectroscopic measurements were carried out at pH values up to 1. The equilibrium constant for the reaction shown in Eq. (1) has been studied at temperature range from

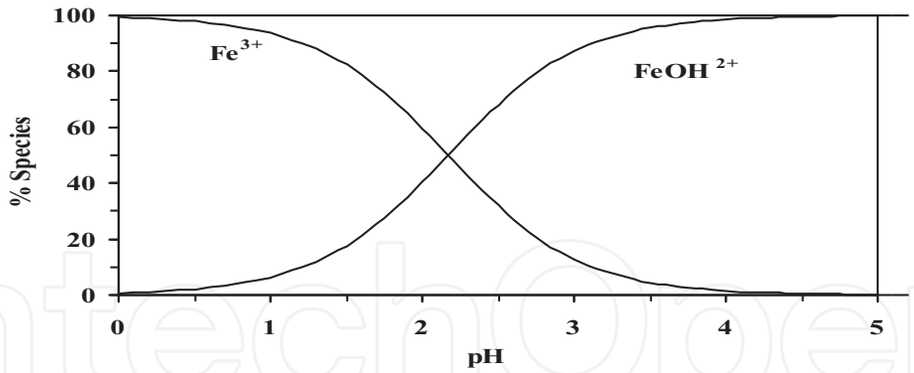

Figure 1. The speciation of the two species of iron as in Eq. (1) as a function of pH at $T = 298$ K for all the iron (III) concentration of 0.5 mol·dm^{-3} and an ionic strength of 0.1 mol·dm^{-3}.

$T = 298$ to 353 K and ionic strength range from $I = 0.1$ until 2.67 mol·dm^{-3} in perchlorate media [14].

For Eq. (1) to be done we must prevent the formation of hydrolyzed iron(III). This can be accomplished under the conditions of $T = 293$–323 K, and $I \sim 0.1$ mol·dm^{-3}.

2.2. How can you determine the stability constants of mixed ligand complexes

For inorganic chemistry, it is very important to determine the stability constant, or the equilibria constant or we can refer to it as the formation constant, for the reaction [15]. It is not easy to get the solution equilibria constants between the ligands and the metal ions. Proton ions and a range of metal ions fight for a range of donor sites. There are many factors that decide who will be the winner whether the proton ions or the metal ions. These factors are the concentration and pH. Potentiometry and spectrophotometry are used to determine the stability constants of metal complexes. Legget [16] and Meloum et al. [17] calculated the equilibrium constants from experimental data for the first time. Nowadays, many programs were published for these calculations using microcomputers. **Table 1** presents some of these programs [15, 19–30]. These programs are very helpful as they quickly present the best fit. They use the least-square method to reduce the differences between calculated and experimental data. The sum of square of residuals between calculated and experimental values is very small; it is nearly between 10^{-6} and 10^{-9}. Potentiometry is used to determine the stability constants of metal complexes. It is based on pH-metric titration of the ligand, and the availability of metal ions. Data obtained from potentiometry are analyzed by the least-square method to derive the formation constant. This later can describe the solution equilibria. For the measurements, there must be two conditions: the first one is a constant ionic strength of the solution and the second condition concerns the ionic strength that have to be higher than the concentration of metal ion. The reaction of all mixed complex:

$$\mathrm{L(M)} + p(\mathrm{L_1}) + q(L_2) + r(\mathrm{H}) \leftrightarrow \left[(\mathrm{M})_1(\mathrm{L_1})_p(\mathrm{L_2})_q(\mathrm{H})_\mathrm{r}\right] \tag{2}$$

System	Data type[a]	Reference
MINIQUAD	V	[19]
MINIQUAD75	V	[20]
TITAN	V	[25]
SCOGS2a	V	[21]
D SCOGS2ba	V	[26]
MINIQUAD	V, A	[22]
PSEQUAD	V, A	[23]
SPECFIT	A(E)	[18]
PKAS	V	[15]
HYDROUAD	V	[24]
STAR	A	[29]
HYPNMR	N	[30]

V, potentiometric experiments; A, spectrophotometric experiments; E, ESR; N, NMR.
[a]Additional data used in calculations are taken from different sources.

Table 1. The programs commonly used for calculating formation equilibrium constants.

The total stability constant, β_{lpqr}, can be calculated from the equation by:

$$\beta_1 pqr = [(\mathrm{M})l(\mathrm{L}_1)p(\mathrm{L}_2)\mathrm{q}(\mathrm{H})r]/[\mathrm{M}]^1[\mathrm{L}_1]p[\mathrm{L}_2]q[\mathrm{H}]_r(\text{for simplicity charges are omitted}) \quad (3)$$

where M, L_1, L_2, and H stand for metal ion, ligand (1), ligand (2), and proton, respectively. For OH^-, the coefficient (r) for $H = -1$.

2.3. Calculation of speciation

Pettit program computes speciation based on the concentrations of metal ions and the complexing species. This program specifies a certain pH range. Then the former calculates the species distribution of a certain series of complexes and plots it. We enter some data such as the total concentrations of metal and ligand ions and pH range. After that the best-fit set of β values will be used later to compute the equilibrium concentrations of those complex species over the pH range which we have specified before. We can use this program for all types of complexes: mixed complexes, protonated, hydroxo, and polynuclear species. The program produces a graphical recording of the most predominant complex species at any pH and the physiological pH range. In this chapter, we reviewed several iron complexes.

3. Different complexes between Fe(III) and biological active ligands

3.1. Studies of binary and ternary complexes of sulfamethoxazole (SMZ) and glycine with metal ions

Sulfamethoxazole (4-amino-N-(5-methyl-3-isoxazolyl)-benzenesulfonamide (SMZ)) is the most predominant sulfonamide in human medicine. Sulfonamides are synthetic antimicrobial agents

derived from sulfanilamide, whose antibacterial activity was discovered in the early 1930s by Domagk and Tréfouel [31–33].

3.1.1. Stability constants of ternary complexes (metal-SMZ-Gly)

Different metal ions Ti(II), Zr(IV), Sr(II), Al(III), Cr(III), Fe(III), Th(IV), Pb(II), La(III), and Co(II) were selected to make further investigation to elucidate the interaction of these metal ions with solution of SMZ and Gly (mixed ligand complexes). The potentiometric equilibrium measurements were made, at constant ionic strength $I = 0.1$ M $NaClO_4$ at 25 ± 0.1°C, for the interaction of SMZ and the selected 10 metal ions, with biologically important secondary ligand glycine (Gly) in a (1:1:1) molar ratio (1×10^{-3} M for each). The solutions were titrated pH-metrically against standard carbonate-free NaOH solution, as illustrated in **Figure 2**.

Figure 2 represents the titration curves for the metal-SMZ-Gly system studied. It is observed that the metal ion-SMZ titration curve (c) diverges from the SMZ curve (b) at variable pH values (pH ≈ 2.8 for Fe(III), pH ≈ 3.5 for La(III), pH ≈ 4.2 for Th(IV), pH ≈ 5.5 for Zr(IV), pH ≈ 4.5 for Al(III), and pH ≈ 6.06 for Co(II)) denoting the formation of metal ions-SMZ binary complexes. For the titration curves of the ternary systems studied, it can be observed that the curves (c) and (f), however, overlap with each other at lower pH values in the case of Fe(III) and La (III), whereas that for Sr(II), Pb(II), Cr(III), and Ti(II) are well separated. This indicates the formation of metal ions-SMZ-Gly ternary complexes at lower pH values, which can be considered as an evidence for the formation of protonated SMZ mixed ligand complex. The stability constants of the ternary metal ion complexes containing SMZ and Gly were calculated from Eqs. (4) and (5),

$$\mathrm{M} + \mathrm{SMZ} \leftrightarrow \mathrm{M}\,(\mathrm{SMZ}) \tag{4}$$

$$K^{\mathbf{M}(\mathbf{SMZ})}_{\mathbf{M}(\mathbf{SMZ})(\mathbf{Gly})} = \frac{[\mathrm{M(SMZ)(Gly)}]}{[\mathrm{M(SMZ)(Gly)}]} \tag{5}$$

using the data obtained from potentiometric titrations ($I = 0.1$M $NaClO_4$ at 25 ± 0.1°C).

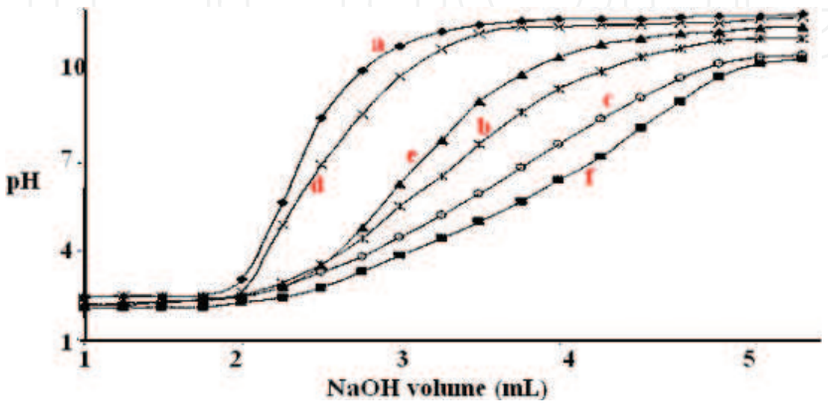

Figure 2. Potentiometric curves of SMZ in 0.1 M $NaClO_4$ at 25 ± 0.1°C: (a) 0.01 M $HClO_4$, (b) a + 0.001 M SMZ, (c) b + 0.001 M Sr (II), (d) b + 0.001 M Pb(II), (e) b+ 0.001 M Co(II), (f) b + 0.001 M Fe(III), and (g) b + 0.001 M Al (III).

3.2. Ternary complexes of iron(III)-glycine(Gly)-nitrilotriacetate (NTA) system

Electrochemical measurements of the dissolved iron(III)-Gly-NTA mixed ligand system in the 0.1 mol·dm^{-3} $NaClO_4$ aqueous solution were performed at pH = 8.0 ± 0.1and 25 ± 1°C, using differential pulse cathodic voltammetry (DPCV), cyclic voltammetry (CV), and direct current (d. c.) polarography. Iron(III) concentrations were varied from 2.5 × 10^{-5} to 6 × 10^{-4} mol·dm^{-3}, NTA total concentrations varied from 2 × 10^{-5} to 1 × 10^{-3} mol·dm^{-3} and glycine total concentrations were 0.2, 0.02, and 0.002 mol·dm^{-3}. **Figure 3** shows the differential pulse voltammograms of iron (III) in a mixture of glycine (0.2 mol·dm^{-3}) and NTA (5 × 10^{-4} mol·dm^{-3}). Reduction peak currents of mixed ligand complex depend on iron(III) concentrations, as shown in **Figure 3**. Basic line (voltammogram) represents the solution with both ligands present, without iron(III). It does not contain any reduction peak. This later implies electrochemical inactivity of these two ligands under the applied experimental conditions. When iron(III) is added, the reduction peak potentials remain constant at –0.112 V, indicating stability of the formed species. These peaks are the response to iron(III) reduction in mixed ligand complexes.

3.3. Determination of formation equilibria of seven-coordinate Fe(EDTA) complexes with DNA and related biorelevant ligands

Fe(EDTA)-L is a seven-coordinate complex as the coordination number of Fe is seven. L can be a DNA constituent like uracil, uridine, thymine, thymidine, and inosine. To understand the chemistry of this seven-coordinate complex, we did some investigations using methylamine, ammonium chloride, or imidazole. The complexes produced are 1:1 with DNA constituents and other ligands. This complex indicates that the total coordination number of Fe(III) ion is seven. Potentiometric titration is carried out at 25°C and ionic strength 0.1 mol·L^{-1} using $NaNO_3$ to measure the stability constant. Besides, the nonlinear least-squares program MINIQUAD-75 is used to deduce the hydrolysis constants of $[Fe(EDTA)(H_2O)]^-$ and its formation constant in solution. The concentration distributions of the different species formed in solution were evaluated as a pH dependent.

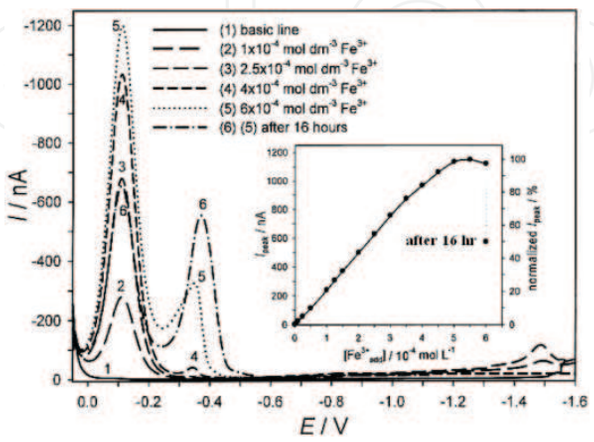

Figure 3. DPC voltammograms; iron(III)-Gly-NTA peak currents on added iron(III). 0.2 mol·dm^{-3} glycine, 5 ± 10^{-4} mol·dm^{-3} NTA, 0.1 mol·dm^{-3} $NaClO_4$; pH = 8.0 ± 0.1, E_{inc} = 2 mV, a = 25 mV, t_p = 0.05 s, t_{int} = 0.2 s.

3.3.1. Calculated equilibria of the [Fe(EDTA)(H_2O)]$^-$ ion

Different equilibrium models were tested [34] to fit the experimental potentiometric data for the hydrolysis of [Fe(EDTA)(H_2O)]$^-$ ion. The best-selected model consists of the formation of the 10^{-1} species, as given in Eq. (6). This supports the presence of one water molecule coordinated in the [Fe(EDTA)(H_2O)]$^-$ ion:

$$\underset{(100)}{[\mathrm{Fe(EDTA)(H_2O)}]^-} \overset{K_{a1}}{\rightleftharpoons} \underset{(10-1)}{[\mathrm{Fe(EDTA)(OH)}]^{2-}} + \mathrm{H^+} \tag{6}$$

The pH-meter readings (B) recorded in dioxane-water mixtures were converted to hydrogen ion concentrations [H^+] with the widely used relation given by the Van Uitert and Haas equation [35]:

$$-\log_{10}[\mathrm{H^+}] = B + \log_{10} U_{\mathrm{H}} \tag{7}$$

where $\log_{10} U_{\mathrm{H}}$ is the correction factor for the solvent composition and ionic strength at which β was determined. Values of pK_w in dioxane-water mixtures were determined as described previously [36, 37]. Different amounts from NaOH of the known concentration were added to a solution of ionic strength 0.1. The amount of base added determines the [OH^-], unlike [H^+], which is calculated from the pH value. The product of ([OH^-]. [H^+]) is used to calculate the mean values (p$K_{w)}$ which is $-\log_{10}$ [H^+][OH^-]. The mean values at 25°C are 14.17, 14.37, 14.50, and 15.44 for 12.5, 25, 37.5, and 50% dioxane, respectively. These percentages are the mass percentage of dioxane in water solution. The equilibrium constants obtained from the titration data (summarized in **Table 2**) are defined by Eqs. (8) and (9), where M, L, and H stand for [Fe(EDTA)(H_2O)]$^-$, ligand, and proton, respectively

$$p\mathrm{M} + q\mathrm{L} + r\mathrm{H}\ \mathrm{M}_p\mathrm{L}_q\mathrm{H}_r \tag{8}$$

$$\beta_{pqr} = \left[\mathrm{M}_p\mathrm{L}_q\mathrm{H}_r\right] / [\mathrm{M}]^p[\mathrm{L}]^q[\mathrm{H}]^r \tag{9}$$

The speciation distribution diagram for the hydrolysis of [Fe(EDTA)(H_2O)]$^-$is given in **Figure 4**. The fraction of the monohydroxo species increases with increasing pH, attaining a maximum of 99.9% at a pH = 10.6.

3.3.2. Complex formation equilibria of the [Fe(EDTA)(H_2O)]$^-$ion

The potentiometric titration curve, given in **Figure 5**, illustrates the result where imidazole is taken as an example. This curve has two plots one for the [Fe(EDTA)(H_2O)]$^-$_imidazole system and the other for imidazole. The complex formation curve for the [Fe(EDTA)(H_2O)]$^{--}$_imidazole system is lower than the imidazole's one. This is because of the hydrogen ion evolved during the formation of a complex species. This potentiometric data are products for an experiment composed of the species 110.

There are many examples for the pyrimidinic species like uridine, uracil, thymine, and thymidine. The dissociable proton of the pyrimidinic species lies in the N3–C4O group. The acid dissociation constants for pyrimidinic species and the N1 proton of inosine are compared. The

System	*p*	*Q*	*r*[a]	$\log_{10}\beta$[b]	*S*[c]
$[Fe(EDTA)(H_2O)]^-$	1	0	−1	−7.60(0.008)	4.7E–8
Uracil	0	1	1	9.35(0,002)	4.5E–7
	1	1	0	5.12(0.1)	8.8E–6
Thymine	0	1	1	9.50(0.01)	8.1E–8
	1	1	0	5.98(0.1)	2.5E–6
Thymidine	0	1	1	9.06(0.01)	8.7E–8
	1	1	0	5.89(0.1)	3.0E–5
Uridine	0	1	1	9.01(0.02)	1.1E–7
	1	1	0	4.93(0.05)	2.0E–5
Methylamine·HCL	0	1	1	10.03 (0.04)	4.4E–7
	1	1	0	6.13(0.1)	2.2E–5
Ammonium chloride	0	1	1	9.32(0.01)	7.2E–5
	1	1	0	3.91(0.03)	1.2E–6
Imidazole	0	1	1	7.04(0.01)	2.6E–9
	1	1	0	2.23(0.04)	5.6E–7
Inosine	0	1	1	8.43(0.01)	5.0E–9
	1	1	0	5.96(0.02)	2.9E–5
	1	1	1	13.14(0.06)	

[a]*p*, *q*, and *r* are the stoichiometric coefficients corresponding to $[Fe(EDTA)(H_2O)]^-$, L, and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the squares of residuals.

Table 2. Stability constant of mixed complexes in water at 25 ± 0.1 °C and 0.1 ionic strength.

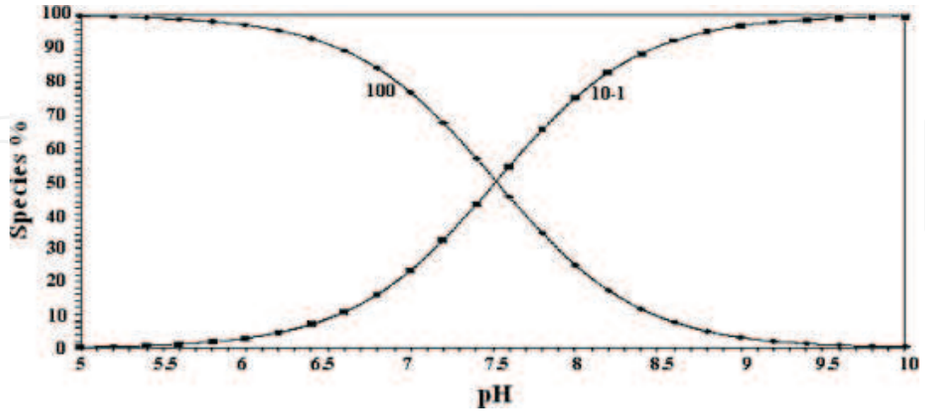

Figure 4. Speciation distribution of different species as pH dependence in the Fe(EDTA)-OH system at 1.25 mmol·L^{-1} $[Fe(EDTA)(H_2O)]^-$, in aqueous solution at 25°C and ionic strength $I = 0.1$.

latter is slightly more acidic. The anionic form of purinic derivatives is the reason for that as it occurred in a large number of resonance forms. These resonance forms are created by the two condensed rings in the inosine ligand, as shown in **Scheme 1**. We can conclude that uracil,

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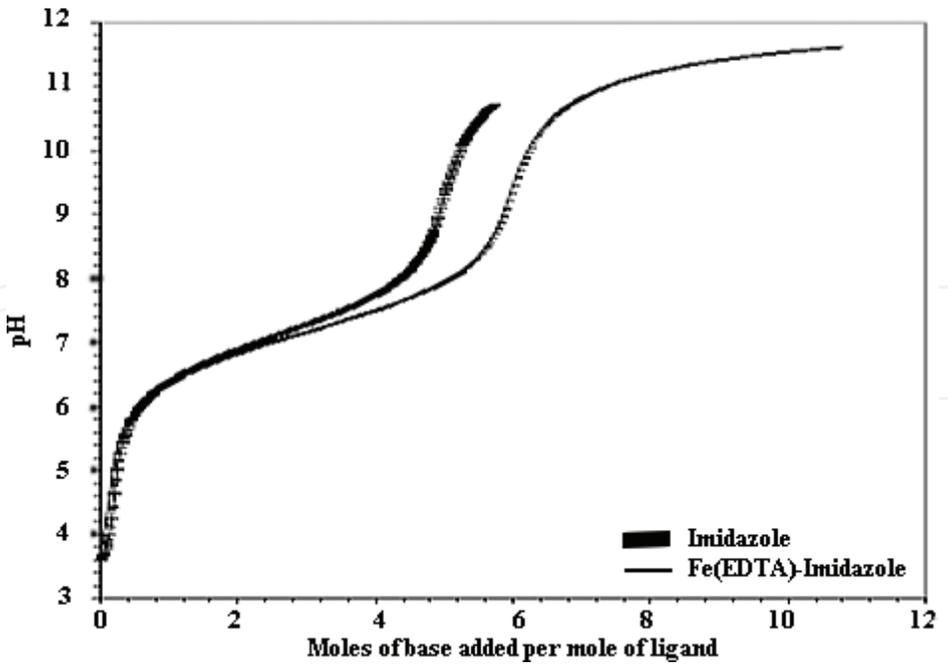
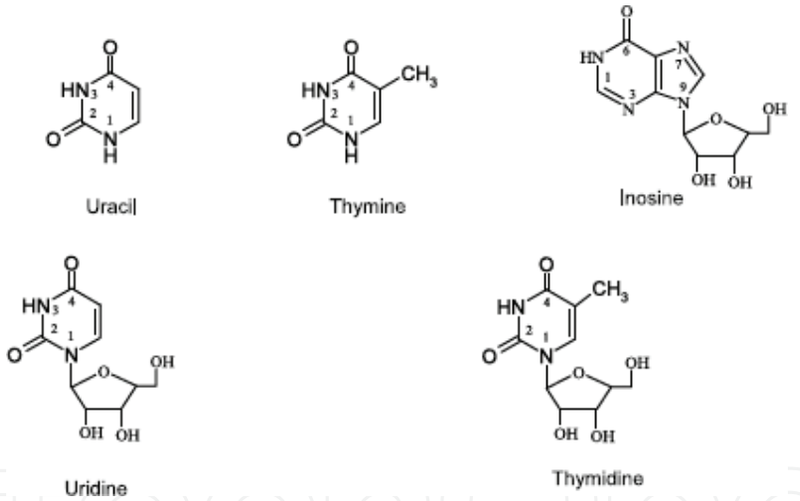


Figure 5. Titration curves of the Fe(EDTA)-imidazole system in aqueous medium.



Scheme 1. Structural formulae of the DNA used.

uridine, thymine, and thymidine do not form protonated complexes. They coordinates through N_3 in their deprotonated form; the monoanion one.

Thymine and thymidine have a methyl group that donates an extra electron of an inductive effect. This increases the basicity of the N3 site of thymine and thymidine complexes and stabilizes them more than uracil and uridine ones. Pyrimidines are monodentate ligands of p*K*a ≈ 9, so their complexes are absent below pH = 6. This indicates that in the neutral or nearly basic pH media, the negatively charged nitrogen donors of pyrimidines bases are vital binding sites.

Inosine complex chelates as a monodentate has two sites of chelation N(1) and N(7). In the acidic medium, N1 is protonated and N7 is attached to the metal ion. When pH increases, the

metal ion moves from N7 to N1. This motion was recorded by nuclear magnetic resonance (NMR) spectroscopy [38, 39]. Chelation depends on the pH range; in basic medium N(1) is a coordination site in the complex formation [40]. The data show the formation of the ternary complexes with stoichiometric coefficients 110 and 111. To know the main features observed in the species distribution in these systems, the speciation diagram obtained for the Fe(EDTA)-uracil and Fe(EDTA)-inosine complexes, as shown in **Figure 6**, as examples of DNA constituents. The pK_a value of the N1H group of the protonated complex (log10 β111 − log10 β110) amounts to 7.18. This indicates the acidification of the N1H site by 1.25 log units through coordination with the $[Fe(EDTA)(H_2O)]^-$ complex, which is in agreement with previous results for similar systems [41]. Detection of the concentration distribution of the various species in solution provides a useful picture of metal ion binding. At pH 4.0, the mixed complex of Fe(EDTA) with uracil, species 110, occurs. This occurrence increases with increasing the pH of the medium, up to it reaches 84% at pH 8.5. After 8.5 the concentration of Fe(EDTA)-uracil system

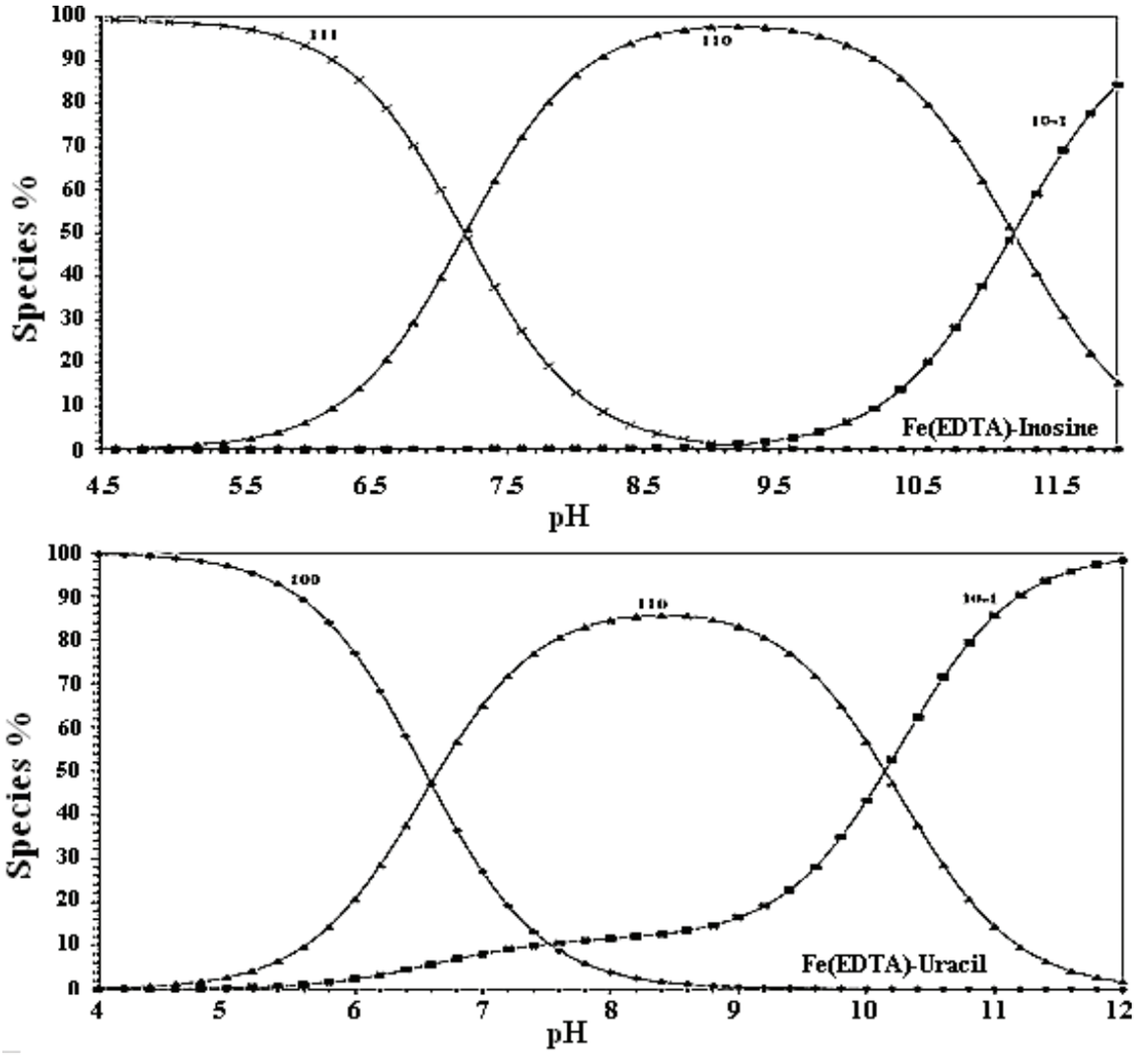


Figure 6. Speciation distribution curve of different species as pH dependence in the Fe(EDTA)-inosine and Fe(EDTA)-uracil systems (at 1.25 mmol·L^{-1} for Fe(EDTA) and 6.25 mmol·L^{-1} for inosine and uracil), in water at room temperature and 0.1 ionic strength.

drops and $[Fe(EDTA)(OH)]^{2-}$ develop. This result concludes that the Fe(EDTA) complex can interact with bioligands as DNA units. Not only does the pH affects the appearance of Fe (EDTA)-uracil system, it also affects the Fe(EDTA)-inosine system. At low pH mediums, species 111 is present; N7 coordinates to the complex and the N1 nitrogen is protonated. Whereas at high pH like 8.8, species 110 occurs where the concentration of N1 coordinated is 98%. This is the maximum concentration obtained for N1 coordinated. As the pH plays an important role before in the absence and presence of some systems, it has its role with cytosine and cytidine chelates. At low pH, they have their N3 protonated. It was recorded by the NMR spectroscopy in its solution state and with X-ray crystallography in its solid state.

3.3.3. The influence of thermodynamic parameters

Thermodynamic parameters are useful tools for studying the interactions with DNA constituents and understanding the relative stability of the complexes formed. The thermodynamic parameters ΔG^0, ΔH^0, and ΔS^0 were easily determined using Van't Hoff relation (Eq. (10)). If we take the protonation of uracil and its complex formation with [Fe(EDTA)], as representative example. Since, we have a known value for protonation constant, the stability formation constant (K) and gas constant (R) in this reaction. So, we can apply the Van't Hoff equation to obtain the value of those parameters at the required temperature (T) in Kelvin. Then we can use the results to draw a graph of ln K versus $1/T$ and the intercept will be $\Delta S^0/R$.

$$lnK = -\Delta H^0/RT + \Delta S^0/R \tag{10}$$

and a slope parameters ΔH^0. The formation constants and the thermodynamic parameters values are presented in **Tables 3** and **4** and can be interpreted as follows:

1. The protonation reaction of uracil can be represented as:

$$\mathbf{L^+ + H^+ \ LH} \tag{11}$$

The thermodynamic processes accompanying the protonation reactions are as follows:

i. The neutralization reaction is considered as an exothermic equation

ii. Desolvation of ions is considered as an endothermic process; and

iii. Changes in the configurations and arrangements of the hydrogen bonds around the free and protonated ligands.

2. The $\log_{10} K^{H}$ values decrease with increasing temperature, revealing that their acidity increases with increasing temperature, as shown in **Figure 7**.

3. The protonation reaction of uracil has a positive entropy change, which may be due to increased disorder as a result of desolvation processes and the breaking of hydrogen bonds.

4. The negative value of ΔH^0 for the protonation process of uracil ligand indicates that its association process is accompanied by a release of energy and the process is exothermic.

System	T (°C)	p	q	r[a]	$\log_{10}\beta$[b]	S[c]
Uracil	15	0	1	1	9.55(0.002)	1.6E–8
[Fe(EDTA)]		1	0	−1	−7.78(0.009)	1.6E–7
[Fe(EDTA)-uracil]		1	1	0	5.20(0.08)	1.3E–5
Uracil	20	0	1	1	9.46(0.002)	1.1E–8
[Fe(EDTA)]		1	0	−1	−7.69 (007)	1.0E–7
[Fe(EDTA)-uracil]		1	1	0	5.07(0.06)	9.7E–6
Uracil	25	0	1	1	9.35(0.002)	4.5E–7
[Fe(EDTA)]		1	0	−1	−7.60(0.008)	3.7E–8
[Fe(EDTA)-uracil]		1	1	0	4.93(0.05)	8.8E–6
Uracil	30	0	1	1	9.25(0.003)	8.6E–9
[Fe(EDTA)]		1	0	−1	−7.53(0.008)	1.2E–7
[Fe(EDTA)-uracil]		1	1	0	4.80(0.09)	1.0E–5
Uracil	35	0	1	1	9.15(0.003)	3.1E–7
[Fe(EDTA)]		1	0	−1	−7.46(0.008)	1.4E–7
[Fe(EDTA)-uracil]		1	1	0	4.69(0.14)	1.0E–5

[a]p, q, and r are the stoichiometric coefficient corresponding to $[Fe(EDTA)(H_2O)]^-$, uracil, and H^+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the squares of residuals.

Table 3. Protonation constants of uracil and the formation constants of the Fe(EDTA)-uracil complex in aqueous solution and different temperatures and 0.1 ionic strength.

Equilibrium	ΔH^0 (kJ·mol^{-1})	ΔS^0 (J·k1^{-1}·mol^{-1})	ΔG^0 (kJ·mol^{-1})
Fe-EDTA hydrolysis			
(1) $[Fe(EDTA)(H_2O)]$ ($[Fe(EDTA)(OH)]^- + H^+$	−54.40 ± 0.75	27.20 ± 2.5	43.4 ± 1.5
Uracil			
(2) $L^- + H^+$ (LH	−34.29 ± 0.75	63.91 ± 2.5	−53.39 ± 1.5
Fe-EDTA-uracil			
(3) $[Fe(EDTA)(H_2O)] + L$ ([Fe-EDTA-L] + H_2O	−43.84 ± 0.68	−52.64 ± 2.3	−28.15 ± 1.4

L denotes uracil.

Table 4. Thermodynamic parameters (ΔH^0, ΔS^0 and ΔG^0) for the interaction n of Fe–EDTA with uracil in aqueous solution.

The values of formation constants of the complexes are plotted in **Figure 8** at different temperatures. The plotted line shows that the formation constants of the complexes are inversely proportion to increasing temperature. Therefore, the complexation process requires low temperatures. At the end, we can say that

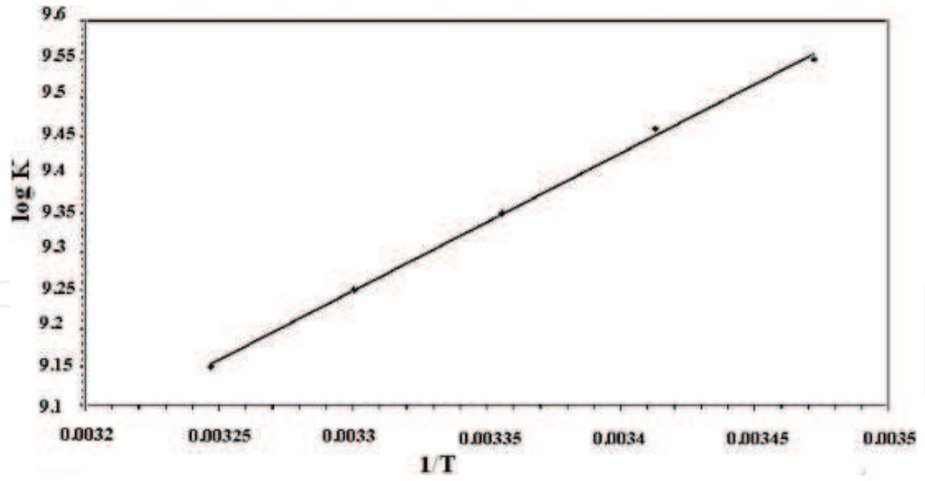


Figure 7. Effect of temperature on the protonation constant of uracil.

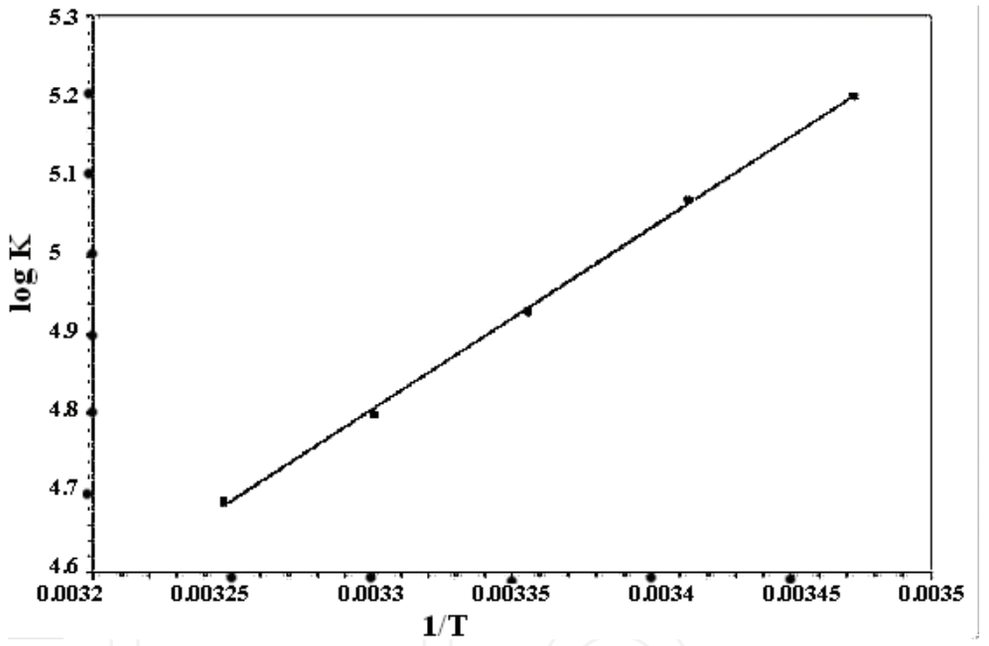


Figure 8. Effect of temperature on the stability constant of $[Fe(EDTA)(uracil)]^{2-}$.

1. The values of ΔG^0 for the coordination process are negative, which indicates that the reaction is spontaneous.

2. The positive value of ΔS^0 upon complexation is because of the increase in entropy. Within complexation a bounded solvent molecule was released. The release of the molecule produces increased the entropy, however, the complexation process causes a small decreased in the entropy. Because the solvent molecules are arranged around the ligand in a certain way. Unlike, the metal ions are configured randomly upon complexation.

3. The negative values of ΔH^0 indicate that the coordination processes are exothermic, so that the complexation reactions are favored at low temperatures.

3.3.4. How can you expect the effect of solvent composition

It is well known that the "effective" or "equivalent solution" dielectric constants in a protein [42, 43], or active site cavities of enzymes [44] are small compared to that in bulk water. The dielectric constants detecting in such locations range from 30 to 70 [43, 44]. Therefore, by using aqueous solutions containing ~10–50% dioxane, one may expect to simulate to some degree the situation in active site cavities [45], and hence to extrapolate the data to physiological conditions. We asked what the relation between the solvent occurs in media and formation constant of complexes on the equilibrium constants (**Table 4**) reveals the following points:

1. The value of pK_a of uracil (N_3-site) increases directly with increasing dioxane in the medium as shown in **Figure 9**. Dioxane has a low-dielectric constant, which increased the electrostatic forces between the proton and the ligand. Finally, the pK_a increases.

2. The stability constant ($\log_{10} K_1$) of the Fe(EDTA)-uracil complex increases with increase of the dioxane concentration (**Figure 10**). This is due to that lowering the dielectric constant of

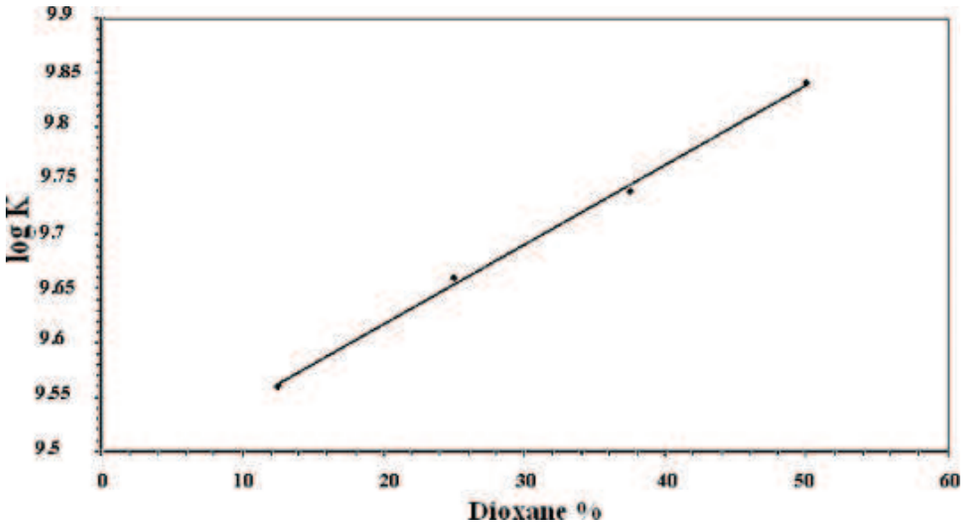


Figure 9. Effect of dioxane on the protonation constant of uracil.

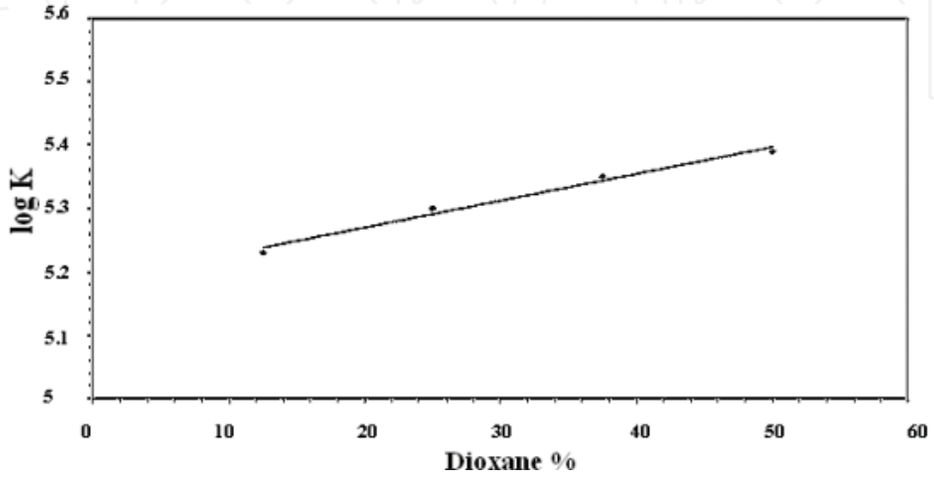


Figure 10. Effect of dioxane on the stability constant of the $[Fe(EDTA)uracil)]^{2-}$ species.

System	% dioxane	*p*	*q*	*r*[a]	$\log_{10}\beta$[b]	*S*[c]
[Fe(EDTA)]	12.5%	1	0	−1	−7.68(0.002)	2.5E–9
Uracil		0	1	1	9.56(0.004)	2.3E–8
[Fe(EDTA)-uracil]		1	1	0	5.23(0.16)	1.0E–5
[Fe(EDTA)]	25%	1	0	−1	−7.83(0.002)	4.1E–9
Uracil		0	1	1	9.66(0.009)	2.3E–7
[Fe(EDTA)-uracil]		1	1	0	5.30(0.06)	3.1E–6
[Fe(EDTA)]	35%	1	0	−1	−7.98(0.003)	6.9E–9
Uracil		0	1	1	9.74(0.01)	2.6E–7
[Fe(EDTA)-uracil]		1	1	0	5.35(0.19)	1.2E–5
[Fe(EDTA)]	50%	1	0	−1	−8.14(0.006)	2.0E–8
Uracil		0	1	1	9.84(0.01)	3.0E–7
[Fe(EDTA)-uracil]		1	1	0	5.39(0.13)	1.2E–5

[a]*p*, *q*, and *r* are the stoichiometric coefficient corresponding to [Fe(EDTA)(H_2O)]−, uracil, and H+, respectively.
[b]Standard deviations are given in parentheses.
[c]Sum of the squares of residuals.

Table 5. Effect of solvent (dioxane) on the stability constant of [Fe(EDTA)(uracil)] at 25°C.

the medium (by increasing the dioxane content) favors the interaction between Fe(EDTA) and uracil, and consequently the stability constant of the complex increases. These finding, shown in **Table 5**, is in agreement with literature data [46].

4. Biological activity of mixed ligand Fe(III) complexes with bioactive ligands

4.1. The study of antibacterial activity of Fe Complex

4.1.1. The effect of microorganisms and media

Salicylhydroxamic acid **(**SHAM) and its binary and ternary complexes (I–VI) were screened [47] to do an experiment to test its antibacterial activity on six bacterial strains: *Escherichia coli* (E.c.), *Staphylococcus aureus* (S. a.), *Enterobacter cloacae* (E. c.), *Salmonella gallinarum* (S. g.), *Bacillus subtilis* (B. s.), and *Pseudomonas aeruginosa* (P. a.). This experiment is illustrated in **Table 6**. These strains were obtained from the Microbial Centre of Ain Shams University, Egypt. Some other microorganisms like (*Aspergillus fumigatus* (A. f.), *Candida albicans* (C. a.), *Alternaria alternata* (A. a.), *Penicillium italicum* (P. i.), *Saccharomyces cerevisiae* (S. c.), and *Microsporum canis* (M. c.) were used for the fungistatic evaluation. These were later provided by the National Research Centre and the Microbial Centre of A in Shams University, Egypt. The media used were Mueller Hinton agar medium, tryptic soy broth (TSB), (ICN, biochemical Co., USA).

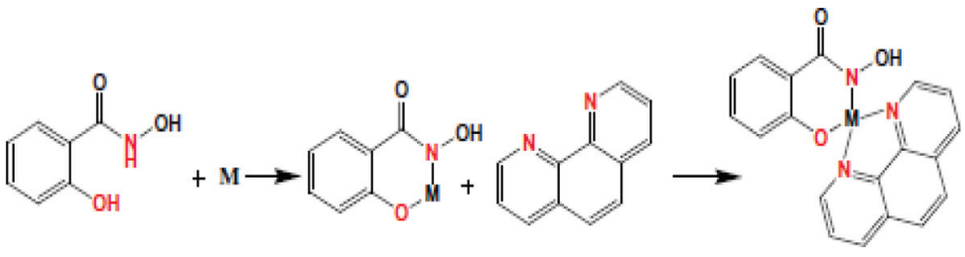
	Antibacterial bioassay						Antifungal bioassay					
	Bacterial strain						Fungi strain					
Compd.	***S.a.***	***S.g.***	***B.s.***	***P.a.***	***E.c.***	***E.c.***	***A.f.***	***C.a.***	***A.a.***	***P.i.***	***S.c.***	***M.c.***
SHAM	++++	++++	++++	++++	+++	++++	++++	+++	++++	++++	++++	++++
I	+++	+++	+++	+++	++	++	++++	+++	+++	+++	++++	+++
II	++	++	++	++	+	+	++	+++	++	++	+	+
III	+++	+++	+++	+++	++	++	+++	++	+++	+++	++	+++
IV	++++	++++	++++	++++	+++	++++	++++	++++	+++	++	++++	+++
V	+++	+++	+++	+++	++	+++	++++	++	+++	++++	++	++
VI	++++	+++	++++	++++	++++	+++	++++	+++	+++	+++	++	+++
A	+++	+++	++	++	+++	++++	+++	++	++++	++	++	++

I, II, III, IV, V, VI are complexes of SHAM (I-VI) $Cu(SHAM)_2{\cdot}2H_2O$ (I), $Ni(SHAM)_2{\cdot}2H_2O$ (II), $Fe(SHAM)_2{\cdot}2H_2O$ (III), [Cu(Phen)(SHAM)] (IV), [Ni(Phen)(SHAM)] (V), [Fe(Phen)(SHAM)] (VI), and the reference drug (A) against six bacterial species and six fungi species in the agar disc diffusion method measured by diameter of inhibition zones (DIZ, mm).

Table 6. Antimicrobial activities of SHAM and its complexes.

4.1.2. The calculation of minimum inhibitory concentration (MIC)

The apparatus used for the experiments are 0.5 mL volume 96-well microplates (ICN Biochem. Co., USA). Fill the first row of wells in each of 96-well microdilution plates with 100 μL of double strength TSB and fill the others with single strength TSB. Dilute the test compound and pour 100 μL in the first row. Every well filled must have its content mixed. The mixing technique based on suctioning and dispensing its content back five times. Take 100 μL from the first row and fill the second one. Then take from the second and fill the third. Repeat this procedure until the 23 rows. Each organism was experimented twice and the experiment as a whole was repeated three times. Before inoculating the 96-well plates with 96-prong inoculator, this later was in the inoculums. The microdilution plates were sealed with tape to prevent the drying of samples. At the end, plates were incubated at suitable temperatures to allow the growth to start. The growth was observed after 1 day.



Metal complexes of salicylhydroxamic acid (SHAM) and 1,10-phenanthroline **(**PHEN)

This stable can be summarized in the following points:

- DIZ 7–9 mm get + sign which indicates a weak activity.
- DIZ 10–14 mm get ++ sign which indicates a moderate activity.
- DIZ 15–18 mm get +++ sign which indicates a good activity.
- DIZ >18 mm get +++ sign which indicates a significant activity.

The bacteria used were (*S.a.*), (*S.g.*), (*B.s.*), (*P.a.*), (*E.c.*), and (*E.c.*). While the fungi used were (*A.f.*), (*C.a.*), (*A.a.*), (*P.i.*), (*S.c.*), and (*M.c.*). The reference drugs: (A) Ampicillin (H_2O).

5. Conclusion

In this chapter, a detailed survey of the formation equilibria of Fe^{3+} with ligands of biological significance is presented. The main conclusions may be summarized as follows:

- Stability constant of the ternary complexes formed between glycine combines with the binary complex (metal:SMZ) (1:1) in similar manner was calculated with respect to the binary complexes.
- Mixed ligand complex formed between iron(III) and glycine(Gly) as the first ligand and nitroacetic acid as the second one have been characterized using differential pulse cathodic voltammetry (DPCV), cyclic voltammetry (CV), and direct current (d.c.) polarography, where iron(III) concentrations varied from 5×10^{-6} to 6×10^{-4} mol·dm^{-3}, (nitroacetic acid), its concentrations varied from 2×10^{-5} to 1×10^{-3} mol·dm^{-3} and glycine's concentrations were 0.2, 0.02, and 0.002 mol·dm^{-3}, in a 0.1 mol·dm^{-3} $NaClO_4$ at pH = 8.0 ± 0.1 and 289 K.
- The concentration distribution curves of the complexes are plotted against the pH. The concentration of $[Fe(EDTA)(uracil)]^{2-}$ complex starts to increase from pH = 4.0 and continues up to pH 8.5, where it reaches 84% which represents its highest value. After that the concentration decreases and the concentration of the hydrolyzed species $[Fe(EDTA)(OH)]^{2-}$ develops.
- The antimicrobial activities of SHAM and PHEN as bioligands and their synthesized metal complexes (I–VI) against representative pathogenic bacteria and fungi. The minimum inhibitory concentration (MIC) value was defined as the lowest concentration of the antibacterial, antifungal agents at which there showed optically clear. Quality control was performed using ampicillin as a standard antibiotic.

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Abbreviations

A. a	*Alternaria alternata*
A. f	*Aspergillus fumigatus*
B. s	*Bacillus subtilis*
C. a	*Candida albicans*
E. c	*Enterobacter cloacae*
M. c	*Microsporum canis*
P. i	*Penicillium italicum*
P. a	*Pseudomonas aeruginosa*
S. c	*Saccharomyces cerevisiae*
S. g	*Salmonella gallinarum*
S. a	*Staphylococcus aureus*
Gly	glycine
NTA	nitrilotriacetate
PHEN	1,10-phenanthroline
SHAM	salicylhydroxamic
SMZ	sulfamethoxazole (4-amino-N-(5-methyl-3-isoxazolyl)-benzenesulfonamide

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