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Fe (III)-Morin complex: DNA interaction studies, speciation, flotation and spectrometric determination of iron (III) in media of diverse origin

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Abstract

A simple and rapid procedure was developed for the quantitative flotation of iron(III) from aqueous solutions. 3, 5, 7, 2', 4'-pentahydroxyflavone (Morin) has been used as a complexing agent and oleic acid as surfactant. The different parameters, affecting the flotation process, namely, ligand and surfactant concentrations, foreign ions (which are normally present in fresh and saline waters), pH and temperature are examined. Nearly 100% of iron(III) ions are floated at a metal: ligand ratio of 1:3, pH ~ 3.5 and at a temperature ~ 25ºC. The proposed preconcentration procedure was applied successfully to the determination of trace Fe (III) in certified reference samples, pharmaceutical and natural water samples. The flotation mechanism is suggested. The DNA-binding studies of the Fe (III)-Morin complex has been evaluated by examining its ability to bind to calf-thymus DNA (CTDNA) with UV- spectroscopy. The studies have shown that the Fe (III)-Morin complex can bind to CT-DNA by the electrostatic binding mode.

Key words: Fe (III); Morin; flotation; FAAS; UV-Vis spectrophotometry; Calf Thymus DNA.

1. Introduction

Iron is the fourth most abundant element in the earth's crust; it is present in a variety of rock and soil minerals both as iron (II) and iron (III) [1]. It plays an essential role in photosynthesis [2-5] and is a limiting growth nutrient for phytoplanktons in some parts of the open oceans [6-9]. Both iron (II) and iron (III) are important in the biosphere, serving as an active center of a wide range of proteins such as oxidases, reductases and dehydrases [10]. The observed concentrations of the total dissolved iron in natural water systems vary from 0.2 nmol L^{-1} in mid-ocean surface water [8] up to 400 mol L^{-1} in polluted urban cloud [11].

Metal speciation is important in a variety of environmental, biological, geological and medical applications. The chemical and physical properties of metal species depend very much on its oxidation state; hence an accurate determination of each species is important to evaluate the potential risk of some metals. Recently, speciation of iron(II) and iron(III) has been studied with different techniques [12-22]. A table containing the summary of analytical techniques for determination of iron (II) and iron (III) is shown in Pehkonen's review [14]. However, for routine analysis a simple conventional detection based on UV/ViS detection is more favourable. In this technique, the analysis often involves the converting of one or both metal species into stable derivatives prior to analysis using selective complexation [23-26]. In this context, two significant advantages for complexation can be identified: (i) more sensitive direct spectrophotometric detection can be performed and (ii) the preservation of the original oxidation states is often possible by choosing suitable complexing agents. Methods for separation and preconcentration of metals by flotation and solvent sublation, followed by their spectrophotometric and/or AAS determination, have increased in popularity in recent years [27-30].

Interaction between DNA and drug molecules is of current general interest and importance [31], especially for the designing of new DNA-targeted drugs and the screening of these *in vitro*.

Flavonoids have recently attracted a great interest as potential therapeutic agents against a large variety of diseases, such as anti-viral, anti-allergic, anti-platelet and anti-inflammatory, and possible protective effects against chronic disease.[32] In this context, it is generally considered that these flavonoids form

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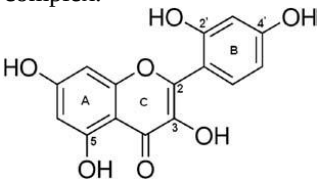
coordination complexes with some essential trace metals, and it is believed that this is the active form of the compound, which is medicinally beneficial. [33]

Morin (3,5,7,2',4'-pentahydroxyflavone), scheme 1, is a flavonol that has been identified in fruits, vegetables, tea, wine, and many Chinese herbs which exhibits a variety of pharmacological activities such as antioxidant activity, antinociceptive activity and anti-tumor activity.

Many techniques have been used to study the binding properties between metal complexes and DNA [34-40].

In the present study, a method is proposed for the determination of iron (III) and also for preconcentration and speciation of iron (III) using Morin as a complexig agent and HOL as a surfactant. A number of parameters have been evaluated viz.; pH, temperature, concentration of surfactant, analyte and ligand, etc.... In addition to measurements of Fe (III) in certified samples, other samples as tap, Nile, waste and sea water and pharmaceutical samples were also analyzed to assess the recommended procedure and to show the versatility of the reagent Morin. The feasibility of flotation and specificity of the reagent Morin enabled the ease of preconcentration of traces of Fe (III) with enhancement of the measurement. The objective of the present study is extended to through light on the anti-DNA effects of the prepared Fe (III) - Morin complex.



Scheme 1: 3, 5, 7, 2', 4'-pentahydroxyflavone (Morin)

2. Experimental

2.1. Reagents and apparatus

All the reagents and solvents were of analytical grade and were used without further treatment. Doubly distilled water (DDW) was used throughout this study.

Iron (III) stock solution (1000 ppm) was prepared by dissolving Fe metal powder in HCl and minimum amount of HNO_3 and boiling to dryness and then diluting in water and adjusting the pH at 1.0 by adding enough HCl.

Iron (II) stock solution (1000 ppm) was prepared by dissolving of Fe $(NH_4).(SO_4)_2.6H_2O$ in water and adjusting the pH at 1.0 by adding enough HCl.

A 1.0×10^{-3} mol L^{-1} Morin solution was prepared daily by dissolving 0.0302 g Morin in ethanol:H_2O (1:1) and diluted to 100 mL with water in a 100 mL volumetric flask. The solution was kept in a refrigerator at 4 ºC in dark. More diluted solutions were prepared by serial dilution with DDW.

An oleic acid (HOL) stock solution, $6.3X10^{-2}$ mol L^{-1}, was prepared from the food grade product with sp. gr. 0.895 (from J.T. Baker chemical Co.) by dispersing 20 mL of the oleic acid in 1L of kerosene. Solutions of other foaming reagents (0.05 %) were prepared by dissolving appropriate amounts of surfactant.

Calf thymus DNA (CTDNA) was used as received without further purification. Its concentration was determined spectrophotometrically using the molar coefficient value, $\varepsilon 260 = 6600$ L mol^{-1} cm^{-1}. A 1.76×10^{-4} mol L^{-1} CTDNA was prepared by dissolving 0.010 g of CT DNA in 0.010 mol L^{-1} acetate buffer and 0.05 mol L^{-1} sodium chloride solution (pH 3.2) in a 25 mL volumetric flask.

Synthesis of the Fe (III)-Morin complexes

i) In aqueous solution

Stock solution (50 mL) of iron (III) ions (1.0×10^{-2} mol L^{-1}) was added into 150 mL of hot saturated solution of Morin in methanol (2×10^{-2} mol L^{-1}) and the result solution was diluted with water to a 1:1 $MeOH/H_2O$ ratio. The solution was stirred and the pH was fixed at 3.2 with 0.1 mol L^{-1}NaOH solution. Then the mixture was heated to 60 °C for 1 h and left at room temperature for 24 h. The precipitate, after being washed several times with methanol: water (1:1) solution, was centrifuged and dried in air at room temperature

ii) In oleic acid

The Fe (III)-Morin complex isolated from the scum was obtained by mixing molar amounts of both ligand and metal ion at the recommended ratio (M: L), pH and oleic acid concentration. The float was gathered by filtration in a sintered glass gooch (G_4), washed several times with DDW, ethanol and finally with diethylether. The precipitate was dried in an oven at 80 ºC and preserved in a desiccator.

Accuracy and precision of the proposed flotation procedures were checked by using certified reference material *viz*.: Phosphate 1 and Phosphate 2, kindly supplied by the Authority of Nuclear Materials (*Cairo*, Egypt).

Two types of flotation cells were used throughout [30]. The first type was a tube of 1.2 cm inner diameter and 29 cm length with a stopcock at the bottom. Such cell was used to study the factors affecting the efficiency of flotation. The second type was a cylindrical tube of 6 cm inner diameter and 45 cm length with a stopcock at the bottom and a quick-fit stopper at the top. This cell was used for the

separation of the investigated analytes from a relatively large volume.

A Perkin-Elmer Model 2380 atomic absorption spectrometer (USA) was used with Pye Unicam (England) hollow-cathode lamp and a conventional 10cm slit burner head for air acetylene flame. Absorbance values were taken after averaging over one second integration time. The instrumental conditions for Fe (III) were taken from the instrumental manual [41]. Spectrophotometric measurements were made at 500 nm with a Unicam UV2100/Vis spectrometer with 1- cm glass cells.

Adjustment of pH of the sample solutions was carried out in the range of 1-10 using 0.1 mol L^{-1}HCl and/or 0.5 mol L^{-1} NaOH. A Hanna Instruments 8519 digital pH meter was used.

2.2. Procedures

2.2.1. *DNA-binding experiments (Absorption spectrophotometric studies)*

Absorption spectra titrations were performed at room temperature in Tris–HCl/NaCl buffer (50 mM Tris–HCl/1 mM NaCl buffer, pH 7.5) to investigate the binding affinity between CT-DNA and Fe(III)-Morin complex. The absorption titration experiments were conducted by keeping the concentration of the CT-DNA constant (10 μM) while varying the complex concentration from 0 – 50 μM. The complex-DNA mixing solutions were incubated for 1 h before the absorption spectra were recorded.

2.2.2. Flotation- separation of iron (III)

In an Erlenmeyer flask, 2.0 ml of 2 x 10^{-4} mol l^{-1} Morin solution was added to an aliquot containing 2 x 10^{-6} mol L^{-1} Fe (III); the pH was adjusted to 2.0. The mixture was shaken well for few seconds to allow complete complexation of Morin with Fe (III) ions. A faint green color of Fe (III)-Morin complex developed instantaneously. All contents were quantitatively transferred into flotation cell (type a) and its volume was adjusted to 10 ml. Then, 3 mL of 4 x 10^{-4} mol L^{-1} HOL was added. The flotation cell was shaken upside down for 2 min by hand. Vigorous shaking of the flotation cell in the presence of a surfactant (HOL) creates bubbles in the solution which enhance the floatability of Fe (III)-Morin. At equilibrium, a scum layer was obtained and the aqueous solution in the cell became completely cleared of the colored complex. The processed aqueous phase was run off through the bottom of the cell. The scum layer, in which Fe (III) was concentrated, was taken into a small vial to determine Fe (III) by spectrophotometric and Flame AAS measurements.

To determine the concentration of Fe (III) spectrophotometrically, a suitable volume was transferred to a 1-cm glass cell and the absorbance was measured at 420 nm against a reagent blank.

To determine the concentration of iron by flame AAS, the scum layer was stripped with 2 mL of (1:1) HNO_3 and the resultant clear solution was aspirated directly to the flame to measure iron concentration at 248.3 nm.

The efficiency of flotation of the Fe (III) in the scum was determined from the relationship:

$$F_{Fe\ (III)} = (C_f/C_i) \times 100\ \% \quad (1)$$

Here C_f and C_i denote the concentration of Fe (III) in the scum and the initial concentration of Fe (III), respectively.

2.2.3. Determination of Fe (III) and Fe (II) in each other's presence

Into a 50 ml measuring flask, 10 ml of a solution containing a definite amount of both Fe (II) and Fe (III) (each 2x10^{-5} mol L^{-1}) and 2 ml of conc.HNO_3 acid. This mixture was boiled well for 5 min to ensure complete oxidation of Fe (II) into Fe (III). Then, 2 ml of Morin (1x10^{-3} mol L^{-1}) were added and the mixture was again shaken well for 2 min to ensure complete complexation of Fe (III) with Morin. The contents are, then, quantitatively transferred to the flotation cell; the same steps of flotation and spectrophotometric determination of the floated Fe (III)-Morin complex, were followed as described before. The total concentration of iron, as Fe (III), was calculated from the calibration curve that was constructed by following the same flotation procedure, in which the concentration of the standard Fe (III) in the colored scum covers the range (1-10)x10^{-5} mol L^{-1} and measured spectrophotometrically or colorimetrically at 420 nm against a blank containing the same concentrations of the constituents as in the sample solution without the analyte. The concentration of iron(II) was calculated as the difference between the total iron concentration and Fe (III) concentration.

2.2.4. Sample analysis

Certified samples A 0.5 g of each certified reference sample was completely dissolved in a Teflon beaker with a mixture of acid (45 ml HF, 15 ml H_2SO_4 and 5 ml HNO_3). After complete dissolution, the solution was evaporated till dryness. The residue was dissolved in 20 ml HCl (1:1) and completed to 100 ml in a measuring flask using DDW. The previous steps of oxidation, flotation and spectrophotometric and/or FAAS determination were followed.

Natural water samples (recovery test) Water samples were collected from the city of Mansoura (Mansoura is an agro-industrial area located in the Nile delta of Egypt) and its neighborhood. Samples were filtered

using a 0.45 μm pore size membrane filter to remove any suspended particulate matter and immediately treated with few milliliters of conc. HNO_3 to prevent the possible hydrolytic precipitation of some mineral salts.

Different concentrations of Fe (III) were introduced into 20 ml aliquots of water samples; 2 ml of $1x10^{-3}$ mol l^{-1} Morin and 5 ml of $1x10^{-3}$ mol l^{-1} of HOL were added; the same previous steps of flotation and spectrophotometry were carried out and the recovery percentages were calculated.

To determine the total iron, 100 mL water sample was added to 2 mL of conc. HNO_3 and heated to boiling until volume 25 mL. Cool to room temperature. Fe (II) is oxidized to Fe (III) by boiling after adding conc. HNO_3. After cooling to room temperature and after adjustment of pH, the sample was analyzed by the general analytical procedure.

Pharmaceutical samples Three iron containing vitamin samples were selected for the analysis of iron. The samples were brought into solution by adopting the following procedure. Each sample was treated separately with concentrated nitric acid on a hot-plate, at a low temperature, to avoid violent spurting. The residue of each sample was cooled and again 1:1 nitric acid is added. The temperature of the hot-plate was gradually increased to 300°C. The residue obtained was dissolved in nitric acid (1:1) and was slowly heated for 2 h to procure a dry mass. Finally, the residue was dissolved in a minimum amount of DDW. The sample solution was quantitatively transferred into a 100-ml volumetric flask and then made to the mark with DDW and analyzed for iron by using the recommended general procedure.

To an aliquot of each dissolved drug sample 2 ml of conc. HNO_3 acid was added and the mixture was shaken well to allow complete oxidation of iron into Fe (III). The process of flotation and determination of iron was followed.

3. Results and discussion

3.1. Interaction between Morin and Fe (III)

3.1.1. Spectrophotometric studies

As it has been previously reported [42], Morin has two maxima bands; at 252 nm (benzoyl of ring A) and the other at 350 nm (cinnamoyl of ring B). [52] The absorption spectra of the Fe (III) - Morin complex in the aqueous solution acid has a maximum absorbance at 420 nm, Figure 1. At this wavelength, the ligand didn't show any absorbance. There are two possible groupings on Morin that can interact with Fe (III): the 2'-hydroxyl of ring B with the 1-oxo of ring C and 3 or 5-hydroxyls with the 4-carbonyl of ring C (Scheme 1). The new emerged absorption peak at 420 nm suggests that Fe (III) has bonded with the ring B. The stoichiometry of the complex was determined using mole ratio method. The formation of a 2:1 ligand-metal complex extends the conjugated system, with the inclusion of the C rings, which leads to further molecular stabilization.[43] Thus, the bonding of Fe (III) with ring C of Morin produced an electronic redistribution between Morin and Fe (III) ions, which resulted in an extended 4 bond system. On this basis, the n-π* electronic transition of Morin changed to a π-π* one, with a consequent decrease in energy as reflected by the appearance of a new peak at the longer wavelength (420 nm). This relationship is also in accord with previous literatures [44]

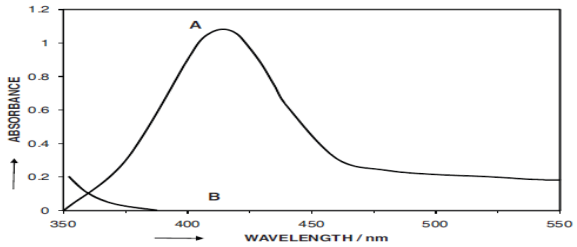


Figure 1 Absorption spectra of. a) The reagent Morin, b) Fe-Morin system

3.1.2. Infrared spectra of Fe (III) - Morin complex in aqueous and in HOL

Infrared spectra of Fe (III) - Morin complexes in the aqueous solution and have also been used to validate the proposed complex structure. Table 1 shows the position of the most important IR bands of Morin and its complex with Fe (III) in aqueous solution. The comparison of these two spectra with each other shows important spectral changes, notably in 1400-1700 cm^{-1}. The position of ν(C=O) is diagnostic for the involvement of 4C=O chromophore in coordination. The complexation should lengthen the C–O bond in the carbonyl group and lessen the force constant, which, in turn, may shift the IR band of the carbonyl group towards a smaller wavenumber.[45] On the complexation of Morin with Fe (III) ion, a shift of ν(C=O) is observed from 1662 cm^{-1} for Morin to 1653-1652 cm^{-1} for the complex. Hence, it may be assumed that the carbonyl group participates in binding the Fe (III) ion. Similar frequency values of this group are shown by the complexes of quercetin-5'-sulfonic acid with lanthanides, where the metal is bound via 3C–OH and 4C=O groups. [46, 47] In the spectra of the complex there also appear new bands due to the formation of the M–O bond. These are the bands of the frequencies 1550-1546, 811-805, 508-492 cm^{-1}. The 1550-1546 cm^{-1} band is related to the formation of the chelate ring > C=O---M–O–.48

In the FTIR spectra of the Fe-Morin complex isolated in the absence and presence of HOL, the positions of the function groups participated in the chelation (carbonyl group and

deprotonated hydroxyl group) are identical (Figure 2), reflecting the fact that there are no chemical bonds has been formed between HOL and the Fe-Morin chelate.

Table 1 Position of the most important spectra bands in IR spectra of Morin and its complex with iron (III)

Type of bonds	Morin/cm^{-1}	Fe (III)-Morin complex/cm^{-1}
Stretching vibrations OH	3300	3160
	2920	
Valence band C=O	1662	1650
Stretching vibrations C=C in aromatic ring, ring vibrations	1629	-
	1613	1603
	-	1549*
	1508	1507
	1459	1443
Deformation vibrations –C-OH	1379	1370
	1310	1321
Stretching vibrations –C-OH	1257	1257
	1227	1238
	1201	1198
	1173	1172
	1104	-
	1085	1095
	1011	1008
Deformation vibrations –C-H outside plane, related to substitution of aromatic rings of multiring compounds	970	975
	876	884
	-	805*
	796	-
	703	705
	635	646
	585	587
	565	566

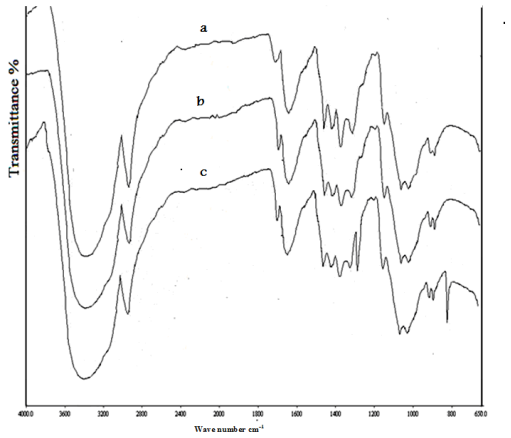


Figure 2 Infrared spectra of a) Morin in KBr, b) Fe (III)-Morin isolated in aqueous solution in KBr and c) Fe (III)-Morin-HOL isolated in oleic acid in KBr.

3.2. Fe (III)-Morin DNA interaction spectral studies

The interaction of different concentration (10-20-30-40-50μM) of Fe (III)-Morin complex with DNA (10μM) has been investigated using UV spectroscopic studies. The results obtained indicted that increasing the concentration of Fe (III)-Morin complex in the presence of DNA (10μM), resulted in an increase in the intensity (hyperchromatism) and red shift of the maxima of absorption spectra of Fe (III)-Morin, **Figure 3**. Hyperchromatism has been observed for the interaction of many drugs with DNA [48]. The hyperchromic effect might be ascribed to external contact (electrostatic binding [49]) or to partial uncoiling of the helix structure of DNA, exposing more bases of the DNA [50].

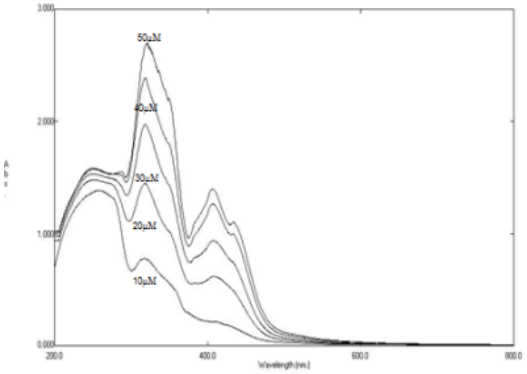


Figure 3 Absorption spectra of different concentrations of Fe (III)-Morin in the presence of 10 μM DNA.

3.3. Flotation of Fe (III)- Morin complex

3.3.1. Effect of pH

A series of experiments was carried out to float Fe (III) using HOL alone. A suitable concentration of the analyte (2x 10^{-5}mol L^{-1}) was taken in the flotation cell and a sufficient quantity of HOL surfactant (2 x 10^{-4}molL^{-1}), which is still less than the critical micelle concentration (CMC) was added to float Fe (III) at different pH values. The data in Figure 4 (curve a) prove that not more than 20% of the analyte was separated at any pH 3. Such a separation percent is not analytically satisfactory in which Fe (III) floats as Fe-oleate. Accordingly, many trials were carried out to separate (III) quantitatively and selectively using different organic collectors. Of these, Morin imposes itself as an excellent collector. The data in Figure 4 (curve b) show that complete separation (≈ 100%) of Fe (III) was obtained in the 3-4 pH range in the presence of 2 x 10^{-4}mol L^{-1} of Morin. In such a case, Fe (III) floats in the form of the Fe-Morin complex as a faint green color.

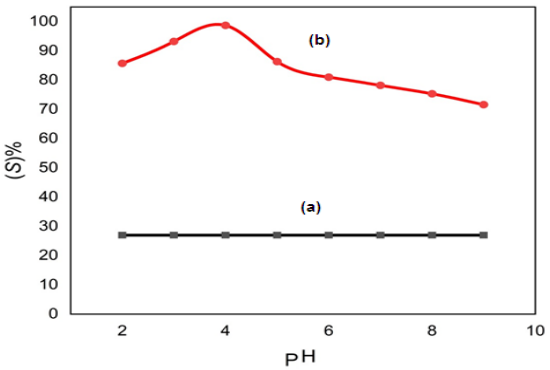


Figure 4.Effect of the pH on the separation efficiency of 2x 10^{-5} mol L^{-1} Fe (III) a) In the absence of Morin, b) In the presence of 2 x 10^{-4} mol L^{-1} Morin, using 2x 10^{-4} mol L^{-1} HOL

Iron (II) flotation with Morin, on the other hand, was extremely poor and no quantitative flotation of iron (II) was attained in the pH range 1-10. Iron (III) was floated at pH 3 at which iron (II) was not separated at all. In order to prevent the oxidation of iron (II) to iron (III), 2 mL of 2 mol of ascorbic acid for 1 mole of iron (II) was added into the aqueous solutions before addition of the ligand. Moreover, solutions containing 10^{-5} mol L^{-1} Fe (III) and various concentrations of Fe (II) were floated in the presence of 2.5x10^{-3} mol L^{-1} Morin and 5 mL 2x 10^{-4} mol /L HOL at pH 3. The results obtained (Table 2) showed that the flotation efficiency of Fe (III) did not vary with the concentrations of Fe (II).

Table 2: Influence of Fe (II) concentration on the flotation of Fe (III) (Conditions: Morin, 2.5x10^{-3} mol L^{-1}; HOL, 4 x 10^{-3} mol L^{-1}; pH 3.0; Temp. 25C)

Fe (III) added (μg l^{-1})	Fe (II) added (μg l^{-1})	*F*, %
10.0	-	100.0 ± 0.2
10.0	10.00	100.0 ± 0.5
10.0	100.0	100.0 ± 0.1
-	100.0	0.005

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3.3.2. Effect of ligand and metal concentration

At pH 3, a conductive series of experiments was carried out to investigate the type of interaction between Fe (III) and Morin in solution. It was found that Fe (III) forms a 1:3 complex with Morin, and complete flotation-separation was achieved whatever amount of Morin was added (Figure 5). This simplifies the procedure for the analytical separation and determination of Fe (III), especially in samples containing unknown amounts of the analyte. To confirm the previous data, another series of experimental work was carried out by changing the metal concentration. The results obtained indicated that complete separation occurred up to the previous ratio of 1:3, Fe(III):Morin. Above such a ratio of increasing Fe (III), a small degradation to a lower separation percentage was noticed. This reflects that insufficient ligand is present for complete complexation and indirect separation.

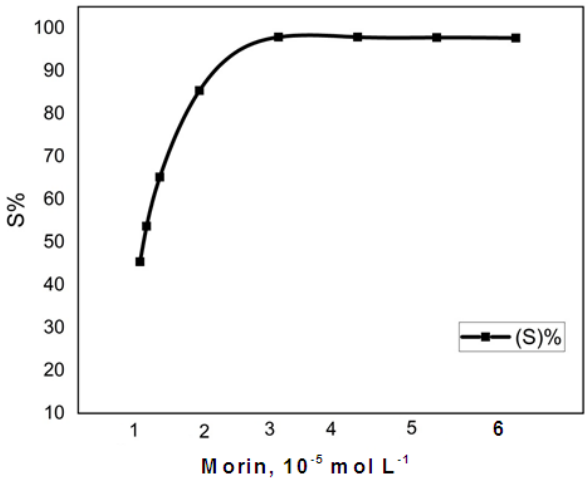


Figure 5 Effect of concentration of morin on the Flotation efficiency of 1 x 10^{-5} mol L^{-1} of Fe (III) at pH 3 using 2 x 10^{-4} mol L^{-1} HOL at pH 3.

3.3.4. Effect of HOL concentration

In general, a surfactant is added to float some materials in an aqueous solution by making them hydrophobic. Hydrophobic materials can be more effectively seeded from aqueous solutions than hydrophilic materials. The material can be a precipitate, complex ions or ion associate (ion pairs) species. The concentration of HOL is an important parameter; up to a limit the separation percentage increases as the concentration of the surfactant increases. The effect of HOL concentration on the flotation efficiency of Fe(III)-Morin complex was investigated. The results obtained indicated that the flotation efficiency of Fe(III)-Morin complex reaches its maximum (100%) over a wide range of HOL concentrations ($1X10^{-3}$$1X10^{-4}$ mol L^{-1}) until its critical micelle concentration (CMC) is reached. At a higher HOL concentration, there will be a concentration, at which the surfactant molecules gather together to form a microball, called *micelle*. Micelles compete for the ion pairs and since they stay in solution, they reduce the effectiveness of separation. The concentration of the surfactant also changes the bubble size with the size getting smaller as the surfactant increases. This makes creamier foam. A suitable concentration ($2X10^{-4}$ mol L^{-1}) of HOL was selected throughout this work.

3.3.5. Effect of temperature

A series of experiments was conducted in aide temperature range (20-80°C), to find out the proper temperature required for maximum flotation of Fe-Morin complex. It was found that the flotation efficiency was not markedly affected in the 20-60°C range, Fig.6. Therefore, subsequent measurements were carried out at room temperature, i.e., ~25°C.

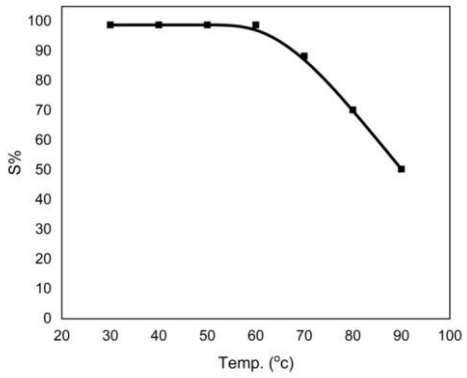


Fig.6 Effect of temperature on the separation efficiency of 2x 10^{-5} mol L^{-1} Fe (III) at pH 3.0 in the presence of 2x 10^{-4} mol L^{-1} Morin using 2 x 10^{-4} mol L^{-1} HOL.

3.3.6. Effect of shaking time

The influence of shaking time of the flotation cell on separation of 2x 10^{-5} mol L^{-1} Fe (III) using Morin (4 × 10^{-4}mol L^{-1}) and 2 ×10^{-4}mol L^{-1} HOL was investigated. It was found that the flotation efficiency reaches its maximum (~100%) after about 2 minutes and remains constant up to 30 min, Fig.7. Therefore, a shaking time of 3 minutes was used in the subsequent experiments to ensure complete metal separation. This means that the flotation separation procedure is not time consuming.

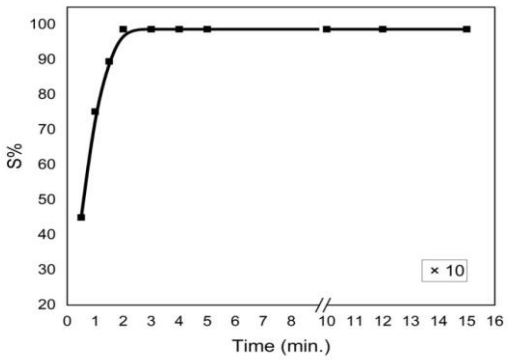


Figure 8.Effect of time on the separation efficiency of 2x 10^{-5} mol L^{-1} Fe (III) at pH 3.0 in the presence of 2x 10^{-4} mol L^{-1} Morin using 2 x 10^{-4} mol L^{-1} HOL

3.3.7. Effect of ionic strength

The effect of changing the ionic strength of different salts on the flotation efficiency of 2.0 x10^{-5}M Fe (III) ions with 2.0 x 10^{-4}M HOL in the presence of 2.0 x 10^{-4} mol L^{-1} Morin at pH 3 is shown in Table 3. The salts used in modifying the ionic strength usually look like individual salts present in

natural water samples. As it can be noticed, the ionic strength of the medium has not evidently affected the flotation process or the determination of iron.

Table 3 Effect of Ionic Strength on the Floatability of 2x 10^{-5} mol/L of Fe (III) using 2 x 10^{-4} mol L^{-1} of Morin, 2.0 x 10^{-4} mol L^{-1} HOL at pH 3.

Ionic strength (mol L^{-1})	Adjuster	Recovery %
0.001	NaCl	99.8
	Na_2SO_4	98.8
0.01	NaCl	98.6
	Na_2SO_4	99.4
0.05	NaCl	98.4
	Na_2SO_4	98.3
0.001	$Na_2C_2O_4$	99.5
	CH_3COONa	99.3
0.01	$Na_2C_2O_4$	99.1
	CH_3COONa	99.7
0.05	$Na_2C_2O_4$	96.2
	CH_3COONa	99.3
0.001	$NaNO_2$	98.7
	Na_2CO_3	98.6
0.01	$NaNO_2$	98.4
	Na_2CO_3	96.5
0.05	$NaNO_2$	95.7
	Na_2CO_3	95.2
0.01	EDTA	41.9
0.001	EDTA	80.7
0.0001	EDTA	95.6
0.00001	EDTA	97.8

3.3.8. Effect of interfering ions

The effect of interfering ions on the floatability of Fe (III) with Morin has been studied in detail, Table 4. The obtained results, revealed that in spite of the high tendency of Morin to form complexes with different transition metal ions. Fortunately, most of these complexes are not floated with the Fe (III)-Morin complex at pH 3. The experimental data showed that Na(I), K(I), Ag(I),Ca(II), Mg(II), Sr(II), Pb(II), Cd(II) and Ni(II) as chlorides, sulphates or nitrates have no effect, whereas Al(III), Hg(II), U(IV),V(V) and Os(V) have little interfering effects (~1%). All of these interferences were completely controlled by adding excess Morin (2x10^{-3} mol /L). Hence, one can predict that the interfering effects may be due to complex formation which is accompanied by a decrease in the ligand concentration. Consequently, masking of the interfering effects by adding excess Morin offers a highly selective procedure for the separation and determination of micro amounts of Fe (III) in various complex materials, like environmental, drug and biological samples.

Table 4 Influence of foreign ions on the flotation efficiency of Fe (III) (Conditions: Fe (III): 10 μgl^{-1}, Morin: 2x10^{-3} mol l^{-1}; HOL. 4 x 10^{-3} mol l^{-1}, pH 3.0 at ~25ºC).

Ion	Concentration	*F*, %
None	-	100 ± 0.5
Co(II)	0.10 mg l^{-1}	101.0 ± 0.6
Ni(II)	0.10 mg l^{-1}	100.8 ± 0.1
Ca(II)	0.5 mg l^{-1}	100.1 ± 0.9
NO_3^-	0.1 mmol l^{-1}	99.9 ± 0.4
NO_2^-	0.1 mmol l^{-1}	99.8 ± 0.9
PO_4^{3-}	0.1 mmol l^{-1}	100.0 ± 0.7
CN^-	0.1 mmol l^{-1}	101.0 ± 0.6
Tartarate	0.1 mmol l^{-1}	99.8 ± 0.3
Citrate	0.1 mmol l^{-1}	101.0 ± 0.4

3.3.9. Effect of volume

A series of experiments was achieved to float different concentrations of Fe^{3+}solution from different aqueous volumes using suitable large flotation cells under the recommended conditions. The results obtained revealed that, up to 100 μg of Fe^{3+}could be quantitatively separated from one liter into 10 mL of HOL with a preconcentration factor of 100. The high preconcentration factor (100) enhanced extremely the sensitivity of the proposed preconcentration procedure and permits the determination of Fe (III) in nanogram scale.

3.3.10. Analytical figures of merits

The applicability of Morin as a complexing agent for the spectrometric determination of Fe (III) was studied in the concentration range 5x10^{-6}- 5x10^{-4} mol l^{-1} Fe (III) solution at pH 3.0 using 2.5x10^{-3} mol l^{-1} Morin, and 3 ml of 2x10^{-4} mol l^{-1} HOL.

The effective molar absorption was calculated from the data obtained by the measurements of the organic phase absorbance as the condition of flotation was completed. The calibration graph obtained was a straight line passing through the origin over the concentration range mentioned above.

The effective molar absorption coefficients (ε) at λ_{max} 420 are 0.2 $x10^6$ and $2.81x10^4$ l mol^{-1} cm^{-1} in the organic layer and in the aqueous solution, respectively. The higher molar absorpitivity in the presence of HOL could be attributed to the selective separation of Fe (III)-Morin using the HOL surfactant. Beer's law is obeyed up to 1 mg ml^{-1} with an optimum range. The precision of the method was determined for ten different samples each containing $2x10^{-5}$ mol l^{-1} of solution. The mean value of Fe (III) was $2.03x10^{-5}$ mol l^{-1} Fe (III) with relative standard deviation of 2.2%.

The limit of detection, which was calculated as the concentration that gives a reading equal to three times the standard deviation of a series of ten determinations taken with solutions of concentrations, which are close to the level of the blank, was found to be $0.18x10^{-6}$ mol L^{-1}.

In order to validate the proposed flotation methodology, it was applied to two certified reference materials *viz.*: phosphate 1 and phosphate 2, kindly supplied by the Egyptian Authority of Nuclear Materials (Cairo, Egypt). The average contents of iron (n = 5) in the certified reference materials are shown in Table 5 with a precision as relative standard deviation (R.S.D.) up to 5 %.

Table 5, also, shows a comparison of the experimental mean ($\overline{X}$) and the true value (μ) by the $|t|_1$ test. From Table 5 it can be noticed that $|t|_1$= 0.56 and 2.240 for iron. This means that the null hypothesis of $|t|_1$ for P = 0.05 and n = 5 is retained where the calculated experimental values of $|t|_1$ are less than the tabulated value ($|t|_1$= 2.78). This means that all preconcentrated samples are not subject to any systematic error i.e. accurate.

Table 5 Statistical evaluation for analysis of some certified reference samples after preconcentration using the proposed flotation procedure Comparison of experimental mean ($\overline{X}$) with true value (μ) by $|t|_1$ test

Ore sample	Iron, %		S	$\|t\|_1$
	($\overline{X}$)	(μ)		RSD,%
Phosphate 1	1.57	1.56	0.08	0.56 1.60
Phosphate 2	1.36	1.34	0.01	2.23 2.70

($\overline{X}$): experimental value, (μ) true value.

$|t|_1$: For P = 0.05 and n = 5 (4 degree of freedom) = 2.78

R.S.D.%: Relative standard deviation.

3.3.11. Application

Recovery yields of iron in natural water samples

The developed procedure for the determination of Fe (III) using a flotation methodology and spectrophotometry was examined for several natural-water samples, Table 6. The recoveries of spiked known additions to different water samples lay within the range 96-104%.

Table 6 Recovery (R, %) of known Fe (III) concentrations added to different water sample (Conditions: 2 ml conc. HNO_3; Morin $2x10^{-3}$ mol l^{-1}, HOL $4x10^{-3}$ mol l^{-1}, pH 3.0 at ~25°C)

Water sample (source)	Fe (III) / μg ml^{-1}		Recovery (%)	RSD,%
	Added	Found[a]		
Tap water (Mansoura City)	0.5	0.51	102	2.9
	1.0	1.03	103	3.2
Nile River (Mansoura City)	0.5	0.48	96	4.3
	1.0	0.98	98	2.4
Sea Water (Ras El-Bar)	0.5	0.52	104	3.2
	1.0	1.02	102	1.3

a. Mean values were obtained by spectrophotometry using five known samples

Analysis of pharmaceutical samples

The proposed flotation procedure was successfully applied to the determination of iron in some iron-containing pharmaceutical samples with satisfactory results, Table 7.

Table 7 Determination of iron in some pharmaceutical samples by the proposed flotation technique (Conditions: 2 ml conc. HNO_3; Morin $2x10^{-3}$ mol l^{-1}, HOL $4x10^{-3}$ mol l^{-1}, pH 3.0) at ~25°C).

Sample (source)	Spectrophotometry		FAAS	
	Observed value[b]	RSD[c] (%)	Observed value	RSD (%)
Theregran Haematinic (Squib, Egypt)	66.60 (66.70)[a]	2.00	66.90	3.80
Totavit (Egyphar, Egypt)	18.20 (18.00)[a]	3.7	17.80	3.00
Haemacaps (Amoun Pharm. Co., Egypt)	349.8 (350.00)[a]	2.7	350.3	4.60

a. Calculated values (mg/capsule),
b. Mean (n=3),
c. Relative Standard Deviation.

3.3.12. Mechanism of flotation of Fe (III)-Morin

According to Sebba [51], the separation mechanism in the flotation technique is very simple. As the gas bubbles pass through the liquid mass they collect the colligend-collector species, which is then transferred to the organic phase on the upper surface of the liquid mass. In studies concerning separation via flotation, the role of surfactant is very important. The nature of the interaction between oleic acid surfactant and the formed Fe (III)-Morin must be studied to approach the actual mechanism of flotation. The proposed mechanism may proceed through: i) a physical interaction through Van der Waal ; or ii) by forming a hydrogen bond between the hydrophilic part of the surfactant and the active sites in the ligand complex. In all cases, the hydrophobic part of the surfactant attaches to air bubbles and floats separating the analyte-containing species.

The flotation-separation mechanism is suggested as follows:
Fe^{3+} ions could react with Morin in an M:L ratio of (1:3) giving a green colored complex according to the following equation:

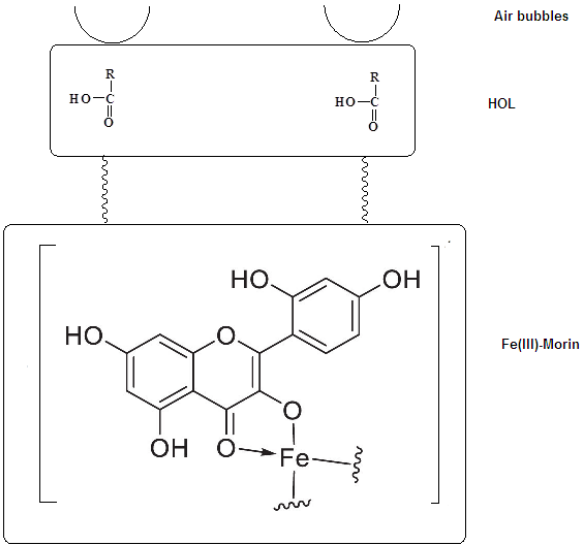
$$\mathbf{Fe^{3+} + 3HL \rightleftharpoons [Fe\ (L)_3] + 3H^+} \quad \mathbf{(2)}$$

The chemical species of HOL is pH-dependent in the solution [52], where it begins to dissociate at pH>5.2 [53] and the percentage of its forms was determined by IR analysis, and the data were reported in the literature. The IR spectra of HOL are associated with bands at 1300-1800 cm^{-1} which are characteristic for (-COOH, and -COO-Na^+) groups. Also, as reported [54], HOL has a C=O stretching band at 1705 cm^{-1} which is shifted on ionization to bands in the range of 1520-1540 cm^{-1} which is characteristic of sodium oleate. So, the suggested mechanism could take place by physical force formation as Van Der Waals forces or a hydrogen bond formation between the hydrophilic part of oleic acid and the active sites of Morin in the formed complex or by an interaction between HOL and the formed complex in solution through a coordinate bond which leads to the formation of a self-floatable species (Fe (III)-Morin-HOL). Hence, oleic acid could interact with formed complex either in its un-dissociated (R-COOH) or dissociated (R-COO-) form depending upon the solution pH as follows:

$$\mathbf{R\text{-}COOH + [Fe\ (L)_3] \rightleftharpoons R\text{-}COOH\ldots.\ [Fe\ (L)_3]} \quad \mathbf{(3)}$$

$$\mathbf{R\text{-}COO^- + [Fe\ (L)_3] \rightleftharpoons R\text{-}COO\text{-}\ldots.\ [Fe\ (L)_3]} \quad \mathbf{(4)}$$

This suggestion was tested by proceeding IR analyses, where the IR spectrum of the removed complex from the float layers (after good washing) has no bands concerning HOL which means that HOL can combine with metal-Morin chelate through weak bonds depending upon the pH of the solution [55]. The combination of HOL with the formed chelates gives aquaphobic aggregates that float with the help of air bubbles (created inside the cell of flotation by shaking) to the solution surface [56]. So, one can conclude that the proposed flotation/separation mechanism proceeded via physisorption with the help of air bubbles. The mechanism of flotation of Fe(III)-Morin complex is represented in scheme 2.



Scheme 2 Mechanism of flotation of Fe (III)-Morin complex

4. Conclusion

The proposed method is simple, rapid and selective. The performance of the method described here allows the determination of iron(III) species in most of water samples. The proposed method has the advantage that it determines directly the iron (III) concentration, which is contained at higher concentration in water samples. Therefore the Fe (II) concentration in water samples can be calculated from the difference between Fe (III) concentration before and after oxidizing the sample. Thus, it is possible to state that the speciation of Fe (III) and Fe (II) in aqueous solution can be carried out by the proposed procedure. It is possible to determine the concentration of Fe (II) even if it is below 10 µg L^{-1} after oxidizing to Fe (III). As a result it can be concluded that the proposed method enhances sensitivity, improve the detection limit and precision in terms of Fe (III). Also, in the proposed method only a limited number of ions have been found to interfere with the determination of Fe (III). In view of the above-mentioned advantages of the present proposed method, selective preconcentration and speciation of iron in different samples may be used as an alternative application. The mechanism is

suggested depending on the formation of physical bonding between oleic acid surfactant and Fe(III)-Morin complex. The DNA-binding studies of the Fe (III)-Morin complex has been evaluated by examining their ability to bind to calf-thymus DNA (CT DNA) with UV spectroscopy. The studies have shown that the complexes can bind to CT-DNA by the electrostatic binding mode.

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