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PAPER

OPTIMAL CONDITIONS FOR SPECTROPHOTOMETRIC DETERMINATION OF IRON (III) IONS USING INDIGO

S.Majidov [1,*] and I. Otaqulov [2]

[1]Gulistan State University, PhD
[2]Gulistan State University, PhD student

* majidov@gmail.com

Abstract

Indigo is one of the most stable compounds found among heterocyclic compounds and is abundant in natural sources. Due to the presence of donor groups such as =CO and -NH_2, it is possible to form metal complexes. In our study, we investigated the optimal conditions for the spectrophotometric determination of iron (III) ions using indigo as a reagent. The maximum absorption region of the metal complex was found to be: λ max (FeR) = 370 nm, with an optimal solution pH (FeR) = 5.6. The range of adherence to the Beer-Lambert law was determined to be: $C25\mu g/ml(FeR) = 1 - 35\mu g/25ml$. Additionally, the reproducibility and accuracy of the method were assessed, and calibration curves were developed.

Key words: Indigo, metal complex, optimal conditions, reagent, buffer solution, order of addition, law of light absorption, Asmus method, Tolmachev graph, calibration curve, quantum chemical calculations, isomolar series method, thin-layer chromatography, and others.

Introduction

In the group of heavy metals, iron plays such a crucial role that without it, fundamental life processes like DNA synthesis, respiration, and photosynthesis cannot occur. This is precisely why it is considered an essential microelement for almost all living organisms [1; 3-b 2; 139-b]. Iron's participation in biochemical reactions and processes as a non-protein component of various enzymes, as well as its role in transporting protons and electrons through the transport chains of photosynthesis and respiration, is of great importance. Furthermore, it has been established that iron influences the structure and function of chloroplasts, as well as the synthesis of chlorophyll [3; 471-b]. The imbalance between the body's iron consumption and its requirement for processes and reactions is one of the primary molecular causes of chlorosis development, a very common condition [4; 10-b]. Moreover, such imbalance can occur due to both iron deficiency and excess iron in the rhizosphere. Excessive consumption of these metal ions leads to various diseases, among which the development of oxidative stress is frequently observed [5; 21-b].

The human body contains 3-5 g of iron. A large portion of it is concentrated in blood hemoglobin (70%). Iron is also a component of enzymes (catalase and peroxidase) and certain proteins that store and transport iron. One of the most important intracomplex compounds created by nature is hemoglobin. This is a complex protein that includes a non-protein (prosthetic) group called heme, which constitutes about 4% of hemoglobin. Heme is a bioinorganic chelate complex of iron (II) with a tetradentate ligand - porphyrin, possessing a planar structure. The fifth orbital of iron in heme binds to the protein part (globin), while the sixth orbital remains free and can bind various low molecular weight ligands: O2, H2O2, CO, CN- [6; p. 100].

There are about 50 types of iron-containing enzymes called cytochromes, which catalyze the process of electron transfer in the respiratory chain by changing the oxidation state of iron: $Fe^{3+} + e^- \rightarrow Fe^{2+}$; E_0 = +0.77V. Catalase and peroxidase enzymes contain iron in the +3 oxidation state. Catalase effectively accelerates the breakdown of hydrogen peroxide, which is formed during oxygen respiration and has a harmful effect on cellular components. Iron deficiency in the body disrupts the synthesis of hemoglobin and iron-containing enzymes, which can lead to the development of iron deficiency anemia. For treatment, preparations containing iron (II) sulfate or iron (II) chloride are used. In pharmacies, iron ammonium alum, iron (III) chloride, and iron (III) sulfate are

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used as reagents for detecting hydrolyzable tannins in medicinal plant raw materials. Qualitative determination of iron (II) and iron (III) ions in iron-containing preparations is carried out using pharmacopoeial reactions with potassium hexacyanoferrate (III), potassium hexacyanoferrate (II), and other reagents [7; pp. 26-41].

In the literature, a highly sensitive and selective spectrophotometric method for determining iron concentration has been developed based on extracting iron (III) from an aqueous solution with bis (1,9-2,5-trimethylpentyl) dithiophosphonic acid in toluene at pH 2.0-2.4 [8; p. 3071, 9; p. 108]. The green toluene extract exhibits maximum absorption at 613 nm and has a molar absorption coefficient of 5.048·103 mol-1/cm3:

1. Ber's law for the iron (III) ion is valid in the range of 0.2-4.8 ppm. The Sandell's sensitivity value is 0.102 μg/cm^{-2}.

2. The calibration graph at pH 2.2 gives a linear plot with a slope of 2.8 [10; p. 123, 11; p. 55].

An aliquot of the aqueous iron (III) solution is adjusted to pH 2.2 in a total volume of 25 ml. The solution is transferred to a separatory funnel, 10 ml of the reagent solution in toluene is added, and the mixture is shaken for 3 minutes. The two phases are then separated. The organic phase is dried over anhydrous sodium sulfate. The optical density of the sample should be measured at 613 nm. When applying the above method in the presence of various ions, it was found that magnesium ions have a greater potential for interference as foreign ions (with an error of 2%). Most ions were analyzed in the 1:10 range [12; p. 111, 13; p. 420, 14; p. 1959]. In most pharmaceutical, biological, and food samples, the copper content is usually lower than that of iron [15; p. 685]. Therefore, this method was chosen for the assessment of iron in these samples [16; p. 28].

When determining iron, the main focus is on the formation of one of its colored compounds, $Fe(SCN)_3$ [17; p. 190, 18; p. 448].

A specially purified sample of Mohr's salt is used as the standard solution. Standard solutions with a concentration of 0.1 g/L typically yield good results [19; p. 544].

863.4 mg of Mohr's salt, measured on analytical scales, is dissolved in 5.0 mL of sulfuric acid with a density of 1.84 g/cm3, and the solution is prepared and mixed in a 1 L volumetric flask using distilled water [20; p. 65].

Iron (III) ions can form colored complexes with sulfosalicylic acid at various wavelengths. The wavelength range is defined as 360-570 nm. It was determined that this complex usually absorbs more light at a wavelength of 490 nm, and calibration graphs were compiled accordingly [21; p. 5598].

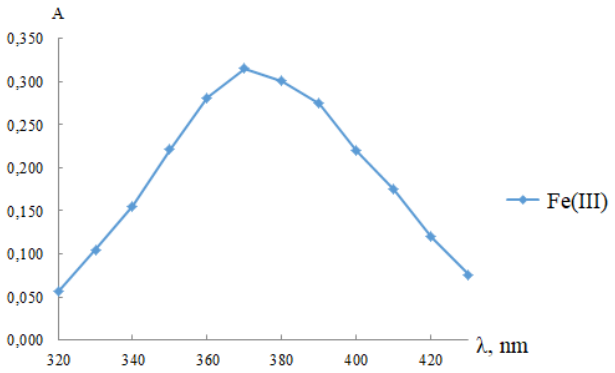
Determination procedure: Standard solution is added to 100 ml volumetric flasks in sequential amounts (5, 10, 15, 20 ml). Then, 10 ml of acetic acid buffer solution with pH=4 and 2.0 ml of 10% alcoholic solution of salicylic acid are added. A 1 molar solution of sodium hydroxide is poured into the flasks and mixed, after which distilled water is added up to the mark. The samples are examined at wavelengths between 360-570 nm, and their optical densities are recorded. Using the resulting graph, the concentration of iron (III) ions is calculated [22; p.426, 23; p.225].

Experimental part

To select the optimal light filter for the complex compound of iron (III) ions with the indigo reagent, 1.0 ml of a 0.01% indigo solution and 1.0 ml of a 20 μg/ml metal ion solution were added to a 25 ml volumetric flask, and the solvent was added up to the mark on the flask. The optical density of the resulting complex compound was measured using a SP-UV 1100 spectrophotometer in a cuvette with a light absorption path length of l=1.0 cm using various light filters. A solution of indigo in DMFA was used as a reference solution (see Table 1 and Figure 1).

As can be seen from the obtained results, the iron complex compound exhibited high optical density at λmax=370 nm.

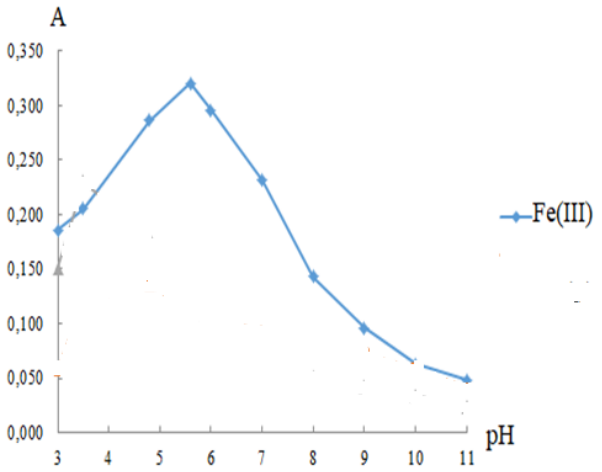
Figure 1. Graph showing the relationship between optical density and wavelength for the complex formed by indigo with iron (III) ions



Subsequent measurements were conducted at λmax=370 nm.

When studying the dependence of complex formation on the solution medium, the following results were obtained (refer to Figure 2 and Table 2).

Figure 2. Dependence of the optical density of metal ion-indigo reagent complex on solution pH



It was determined that the optical density of the formed complexes has a high value in an acidic medium, and solutions with a pH (Fe (III)) of 5.6 were selected. These solutions were used in subsequent analyses.

To study the dependence of the iron (III) ion complex with indigo on the amount of reagent added, varying quantities (0.2-1.4 ml) of 0.01% indigo solution, 1.0 ml of 20 μg/ml iron (III) ion solution, and 5 ml of acetate buffer solution with a pH of 5.6 were added to a 25 ml volumetric flask, mixed, and then DMFA was added up to the mark on the flask.

The optical density of the formed complex compounds was measured using an SP-UV 1100 spectrophotometer in a cuvette with a light path length of l=1.0 cm using corresponding light filters. A solution of indigo in DMFA was used as a reference solution. (See Table 3 and Figure 3).

The obtained results show that 0.8 ml of a 0.01% solution of the indigo reagent is required for the complete binding of 20 μg of Fe (III) ion.

To study the dependence of the complex formed by the iron (III) ion with indigo on the amount of the element, the following procedure was carried out: In a 25 ml volumetric flask, 0.8 ml of a

Table 1. Relationship between wavelength and optical density of the complex formed by indigo and iron (III) ions

Wave length, nm	320	330	340	350	360	370	380	390	400	410	420	430
Optical density	0.056	0.105	0.155	0.221	0.281	0.315	0.301	0.275	0.220	0.175	0.120	0.076

Table 2. Dependence of the optical density of metal ion-indigo reagent complex compound on solution pH

pH	3.5	4.8	5.6	6.0	7.0	8.0	9.0	10.0	11.0
A	0.186	0.205	0.320	0.296	0.231	0.143	0.096	0.063	0.048

Table 3. Dependence of the optical density of the complex compound on the amount of reagent added

V, ml	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Fe(III)	0.068	0.132	0.196	0.260	0.324	0.324	0.324

Figure 3. Graph showing the relationship between the optical density of Fe (III) - indigo complexes and the amount of reagent added

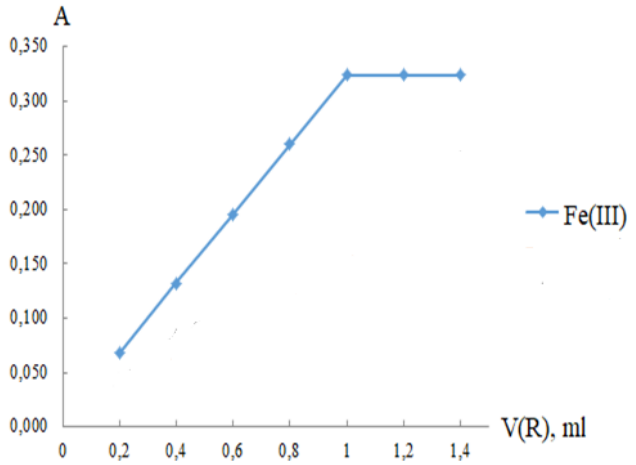
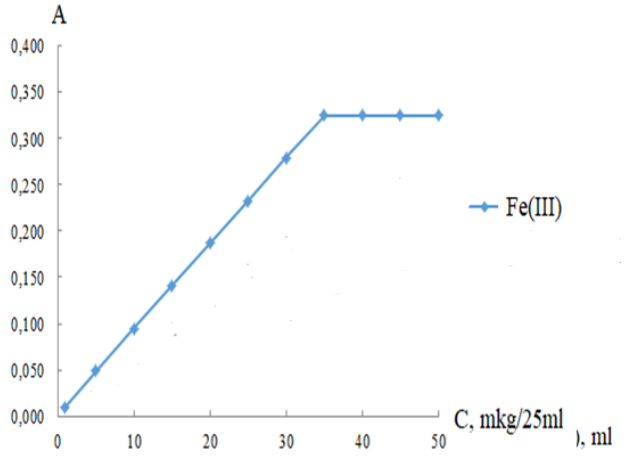


Figure 4. Graph showing the conformity of complexes to the Bouguer-Lambert-Beer law



0.01% indigo solution and varying amounts of a 50 µg/ml iron (III) solution were added. Then, 5 ml of an acetate buffer solution with a pH of 5.6 was added and mixed. Finally, DMFA was added to the mark of the flask (refer to Figure 4 and Table 4).

Table 4. Adherence of complexes to the law of dependence on the amount of elements (Bouguer-Lambert-Beer law)

№	Solution volume, ml	Metal content, µg	Optical density
1	0.1	1	0.021
2	0.5	5	0.062
3	1.0	10	0.11
4	1.5	15	0.16
5	2.0	20	0.21
6	2.5	25	0.258
7	3.0	30	0.306
8	3.5	35	0.325
9	4.0	40	0.352
10	4.5	45	-
11	5.0	50	-

From the obtained results, it can be seen that the complex formed by iron (III) ions with indigo obeys the Beer-Lambert law in the range of 1-35 $\mu g/ml$ in 25 ml of solution. At higher concentrations, a deviation from the linear relationship was observed[24].

In conclusion, the results of our research demonstrate that the optimal conditions studied for the spectrophotometric determination of iron (III) ions using indigo are suitable for conducting analytical analyses. Specifically, the maximum light absorption region of the resulting complex, the solution medium, the amount of reagent added, and the quantity of the element serve as examples of this and confirm the potential for using this method in the future.

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