

PYRIDINE-DERIVED SCHIFF BASE-ANALYTICAL REAGENT FOR IRON (II) IONS

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PYRIDINE-DERIVED SCHIFF BASE-ANALYTICAL REAGENT FOR IRON (II) IONS

(Abstract): **Aim:** Development of a new spectrophotometric method for quantitative determination of Fe (II) ions in pharmaceutical products based on the complexation reaction of the cations with an original Schiff base-2-salicylidene-aminopyridine, when a red complex, extractable in chloroform, with a maximum absorption at 525 nm has formed. **Material and methods:** A UV-VIS Hewlett-Packard 8453 Spectrophotometer has been used for all determinations. The optimum working conditions of the complexation reaction have been established by studying the influence of pH, ionic strength, and combining ratio on the complexation reaction. **Results:** The calibration curve had been plotted, and the detection limit was determined ($L_D=1 \mu\text{g}$). The proposed method has a good linearity in the 5-50 $\mu\text{g/mL}$ range with a correlation coefficient $r = 0.9946$. The stability constant and the molar extinction coefficient were calculated ($\epsilon = 4.16 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$). The cation-ligand ratio was 1:2. The complexation reaction of Fe (II) ions with the Schiff base had no cation interferers, not even Fe (III) ions. **Conclusions:** The proposed method has been applied for the determination of Fe (II) in pharmaceutical products with very good results. **Keywords:** SCHIFF BASE, IRON (II) IONS, SPECTROPHOTOMETRY.

Iron is an essential element for living organisms, as it is involved in the transport of oxygen and in cellular oxidative processes. It is found in amounts of 5 mg/kg bodyweight for men and 30 mg/kg bodyweight for women. Approximately 2/3 of all iron ions are found in circulating red blood cells in the composition of hemoglobin, while smaller percentage may be found in myoglobin (4%), hemic enzymes (0.2%), transferrin (0.12%) and about 25% is deposited as ferritin and hemosiderin. Depending on the way iron they are bound, iron-protein complexes can be classified

into three groups: hemoproteins, iron-sulfur proteins and iron-proteins. In hemoproteins, iron is incorporated into a system of four tetrapyrrole rings linked to proteins, such as myoglobin, hemoglobin, cytochrome c and enzymes such as cytochrome oxidase, catalase and peroxidases. The binding of Fe to proteins occurs at certain sites of amino acids (1-4).

Schiff bases are a sub-class of imines. They are common enzymatic intermediates. During physiological processes involving pyridoxal-5'-phosphate, intermediate Schiff bases form between the $\alpha\text{-NH}_2$ group of L-

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valine, the ϵ -NH₂ group of lysine and the protein chains (5). In aqueous medium at pH = 4.5-8.5, fluorescent Schiff bases of 5'-dioxypyridoxal with n-hexylamines, are formed (6). In the synthesis of temperature-dependent tryptophan and the pH of the medium, an intermediate ligand is formed which is a Schiff base, pyridoxal-5'-phosphate-L-serine (7). There are also Schiff bases that exhibit electrical and magnetic properties (8).

The Schiff bases can be used as analytical reagents for the quantitative determination of important cations in the body (9). We have performed an analytical study on a Schiff base: 2-salicylidene-amino pyridine (fig. 1). It is an organic ligand obtained through the reaction of salicylaldehyde with 2-aminopyridine (10, 11).

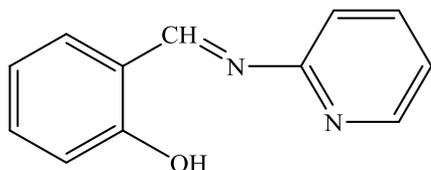


Fig. 1. 2-salicylidene-aminopyridine

The paper presents the development and validation of a new spectrophotometric method for the quantitative determination of Fe (II) ions and its application for the analysis of pharmaceutical products.

MATERIAL AND METHODS

A UV-VIS Hewlett-Packard 8453 spectrophotometer has been used for all determinations. All reagents and solvents were analytical grade.

A 0.1 mg/mL Fe (II) stock solution was prepared in distilled water, and then by suitable dilutions a 5-50 μ g/mL Fe (II) standard solution was obtained. Reagent solution 0.1% (w/v) was prepared by dis-

solving 2-salicylidene-amino pyridine (SB) in methanol. A 0.2M sodium acetate and acetic acid buffer solution with pH = 5.6 has been used.

The Fe (II) ions react with 2-salicylidene-amino pyridine (SB) at pH = 6.0, and a complex combination is formed. It is extracted in chloroform and its absorbance measured at 525 nm is proportional to the concentration of the cations.

The optimum wavelength for detection was selected and the optimum working conditions were established by studying the influence of pH, formation time, and the stability of the complex (12, 13).

The cation/ligand combination ratio, the stability conditional constant (β_n), and the limit of detection were calculated, and the potential interferers have been evaluated.

The complexation reaction was influenced by the ionic strength of the solution. Thus, the concentration and volume of the KCl solution that provided the ionic strength that maximized the absorption must be established. 1 mL of 30 μ g/mL Fe(II) standard solution was treated at pH = 5.6 (0.2M sodium acetate and acetic acid buffer solution) with 0.5 mL solution with various concentration levels of KCl, 1 mL 1% (w/v) SB reagent solution. After extraction in 5 mL CHCl₃ and separation, anhydrous sodium sulfate was used to remove any traces of water, and the absorbance of the organic layer was measured at $\lambda = 525$ nm against a blank sample.

The complexation reaction was influenced also by the pH of the solution. So 1 mL of 30 μ g/mL Fe(II) standard solution was mixed with 1 mL sodium acetate and acetic acid buffer solutions with a pH that varied in the range 3.5-7.0. 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1 mL 1% (w/v) SB reagent solution. After the extraction of the

red complex in 5 mL CHCl₃ and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured at $\lambda = 525$ nm against a blank sample.

The influence of the number of extractions in chloroform of the complex combination on its absorbance was evaluated by comparing the results obtained when using 5 mL of organic solvent once versus using 2.5 mL of chloroform twice.

The metal-ligand combination ratio was determined using various volumes of Fe (II) standard solution and mixing then with SB reagent solution with various concentration levels ($7 \cdot 10^{-3}$ M; $5 \cdot 10^{-3}$ M; 10^{-2} M), thus achieving the following combination ratios: 0.2, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, and 3.0. The mixtures were processed as previously described.

The stability constant (K_s) - expressed as $L \cdot cm^{-1} \cdot mol^{-1}$ - of the complex combination was determined using Harvey-Manning dissociation method based on the instability constant (K_i), according to the following equations (14):

$$K_s = 1/K_i$$

$$K_i = \frac{\alpha^2 C}{1 - \alpha} \quad \text{and} \quad \alpha = \frac{A_m - A}{A_m}$$

where: α = degree of dissociation, A_m = maximum absorbance, A = equilibrium absorbance, c = molar concentration of Fe (II).

A second method for calculating the stability constant (β_n) was based on the following equation (15):

$$\beta_n = \frac{A/A_m}{(1 - A/A_m)n^2 C_L^n}$$

where: A_m = maximum absorbance, A = equilibrium absorbance,

n = the coordination number of the ligand, and C_L = molar concentration of the ligand.

The new spectrophotometric method was validated and applied for the quantitative determination of Fe (II) (16-21).

RESULTS AND DISCUSSION

The optimum pH value for the complexation was established to be 5.6 (fig. 2). It was achieved using a 0.2 M acetic acid-sodium acetate buffer solution.

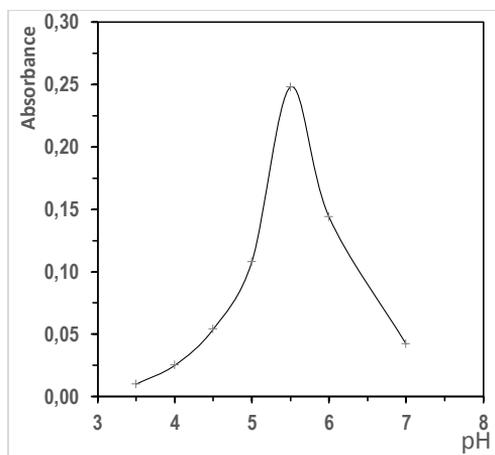


Fig. 2. Influence of pH on the absorbance of the SB-Fe (II) complex

The concentration and volume of the KCl solution providing the ionic strength for maximum absorbance of the complex was established based on the results shown in table I. It was established that a volume of 0.5 mL of KCl $2.5 \cdot 10^{-2}$ M solution provided a $\mu = 0.005526$ ionic strength and maximum absorbance.

The results of the study on the variation of absorbance according to the number of extractions are shown in table II, and they prove that one extraction was found to be effective.

TABLE I
Influence of ionic strength on absorbance

Fe(II) (µg/mL)	KCl concentration	1% SB reagent solution (mL)	CHCl ₃ (mL)	A _{525 nm}
30	7.5·10 ⁻¹ M	1	5	0.140
30	5·10 ⁻¹ M	1	5	0.147
30	2.5·10 ⁻¹ M	1	5	0.155
30	10 ⁻¹ M	1	5	0.167
30	2.5·10 ⁻² M	1	5	0.248

TABLE II
Absorbance variation depending on the number of extractions

Fe(II) (µg/mL)	Buffer solution pH = 5.6 (mL)	2.5·10 ⁻² M KCl (mL)	1% SB reagent solution (mL)	CHCl ₃ (mL)	A _{525 nm}
30	2.5	0.5	1	5.0	0.250
30	2.5	0.5	1	2.5 × 2	0.248

TABLE III
Absorbance variation depending on the number of extractions

Fe (II):SB Combination ratio	A _{525 nm}		
	5·10 ⁻³ M Fe (II)	7.5·10 ⁻³ M Fe (II)	5·10 ⁻² M Fe (II)
1:5	0.169	0.294	0.578
1:3	0.200	0.323	0.702
1:2	0.212	0.391	0.869
1:1.4	0.177	0.315	0.777
1:1	0.092	0.285	0.653
1:0.66	0.069	0.219	0.546

Based on the data obtained (tab. III) the ion: ligand combination ratio was plotted, and its value was 1:2.

Determination of complex stability constant was done using the graph method in order to determine the values for the maximum absorbance ($A_m = 0.890$) and the equilibrium absorbance ($A = 0.292$), and then to calculate the instability constant of the complex $K_i = 7.3 \cdot 10^{-5}$ and the stability constants $K_s = 1.4 \cdot 10^4$, considering that $n = 2$, and the concentration of the Fe (II) was

10^{-2} M. The calculated value of the extinction molar coefficient was $4.16 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$. The second method of calculating the stability constant produced results ($\beta_n = 1.5 \cdot 10^4$ and $1.45 \cdot 10^4$) which were very close to those obtained using the Harvey-Manning dissociation method.

The optimized procedure was: 1 mL Fe(II) standard solutions (5-50 µg/mL) were mixed with 2.5 mL sodium acetate and acetic acid 0.2M pH = 5.6 buffer solution, 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1

mL 1% (w/v) SB reagent solution. After one extraction of the red complex in 5 mL CHCl_3 and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured after 10 minutes at 525 nm against a blank sample in a 1 cm cuvette.

During the validation of the method, it was established that the absorbance was proportional to the concentration in Fe (II) and the Lambert-Beer Law was obeyed in the 5-50 $\mu\text{g/mL}$ range, with a correlation coefficient of 0.9946. The calibration curve is presented in fig. 3 and the linear equation was: $A = 0.008877 \cdot c - 0.02547$ (correlation coefficient = 0.9946, intercept = 0.008877, slope = 0.02547).

Table IV includes the results of the sta-

tistical data processed during the validation of the VIS spectrophotometric method for the quantitative determination of Fe (II).

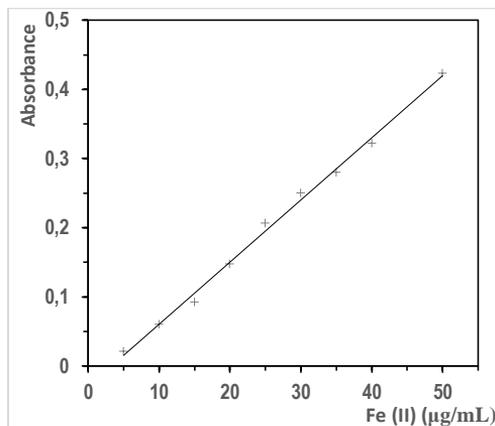


Fig. 3. Calibration curve

TABLE IV
Validation parameters

Fe (II) ($\mu\text{g/mL}$)	Analyzed Fe (II) ($\mu\text{g/mL}$)	Recovery (%)	Statistical parameters
5	5.18	98.95	Linearity range: 5.18-50.74 $\mu\text{g/mL}$ Fe(II) Intercept = 0.008877 and slope = 0.02547 $r = 0.9972$; $SD = 0.91$ Accuracy: 98.94 ± 1.009 $n = 18$; $t_{\alpha} = 2.11$; $\alpha = 0.95$ Repeatability: $CV_r = 0.92$ ($n = 9$) Reproducibility: $CV_R = 2.05$ ($n = 18$)
10	9.62		
15	13.23		
20	19.42		
25	26.00		
30	31.19		
35	34.21		
40	39.14		
50	50.74		

There were studies conducted that proved that the complexation reaction of Fe (II) using the Schiff base was not interfered by any other cation. The interference of Fe (III) on the complexation reaction at pH = 5.6 in the presence of KCl ($2.5 \cdot 10^{-2}$ M) has also been ruled out. The method could still be used for the indirect quantitative determination of Fe (III) after the reduction reaction to Fe (II) using reduction agents such as hydroxylamine or ascorbic acid.

The spectrophotometric determination method for Fe (II) using the Schiff base as reagent has been applied with very good results for the analysis of an antianemic pharmaceutical product formulated as syrup (tab. V).

The following procedure had been used: 2.5 mL syrup sample (7.2 mg/mL Fe (II)) was diluted with distilled water into a 100 mL graduated flask. 1 mL diluted sample solution was diluted even further with dis-

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tilled water using a 10 mL graduated flask. 1 mL of solution was mixed with 0.5 mL sodium acetate and acetic acid 0.2M pH = 5.6 buffer solution, 0.5 mL KCl solution $2.5 \cdot 10^{-2}$ M and 1 mL 1% (w/v) SB reagent solution. After one extraction of the red

complex in 5 mL CHCl_3 and separation, anhydrous sodium sulfate was used as desiccant, and the absorbance of the organic layer was measured after 10 minutes at 525 nm against a blank sample in a 1 cm cuvette.

TABLE V
Applications of the spectrophotometric method for Fe (II)

Labelled Fe(II) (mg/5mL)	Analyzed Fe(II) (mg/5mL)	Recovery (%)	Statistical parameters	
			Repeatability	Reproducibility
36	36.3794	101.05	n = 5 M = 36.3342 S = 0.1883 S _x = 0.0842 A = 36.3342 ± 0.2341 CV _r = 0.5184%	n = 15 t _α = 2.15 α = 0.95 M = 36.31 S = 0.34 A = 36.31 ± 0.188 CV _R = 0.93
36	36.6046	101.67		
36	36.1541	100.42		
36	36.3794	101.05		
36	36.1541	100.42		
FRX Criteria: 34.92-37.12 mg/5mL				

The method proved to be as accurate if not more as other spectrophotometric methods, while using a simple procedure (22-23).

CONCLUSIONS

We proposed a new spectrophotometric method for the determination of Fe (II) in antianemia pharmaceutical products, based on our study on the complexation reaction of those cations with 2-salicylidene aminopyridine. The Schiff base that was used as reagent, formed with Fe (II) at pH = 5.6 a red complex extractible in chloroform with a maximum absorption at $\lambda = 525$ nm. The Lambert-Beer Law was obeyed in the concentration range 5-50 $\mu\text{g/mL}$ Fe (II), and the linearity coefficient was $r = 0.9946$.

The statistical parameters highlighted the good precision of the spectrophotometric method. The optimization of the method included: a study of pH and ionic strength influence on the complexation reaction, calculus of the combination ratio, stability constant and molar extinction coefficient, plotting the calibration curve, establishing the detection limit, and the evaluation of interferers. The statistical parameters highlighted the accuracy of the method, with relative errors well within the limit of criteria for VIS spectrophotometric methods. The method has been applied successfully for the analysis of iron (II) ions in antianemia pharmaceutical products, and it could be also used for the indirect analysis of iron (III) ions.

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