

A New Spectrophotometric Method for Determination of Iron in Herbs, Spices and Beans with 2,6-Diacetylpyridine Dioxime and 2-Acetylpyridine Monoxime

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Abstract: The reaction of iron (II) with 2, 6-diacetylpyridine dioxime (H₂dapd) and 2-acetylpyridine monoxime (Hapm) in 10% and 60% v/v ethanol-water solution at pH 2.5 and 7.5, respectively, were studied using direct and first derivative spectrophotometry. Fe (III) is reduced by H₂dapd and gives iron (II) complex. A simple, rapid, selective and sensitive method for the determination of Fe (II), Fe (III) and a mixture of them with the H₂dapd reagent in acid water-ethanol medium after the solution attained to stand periods of 30 sec, 4 hr and 4 hr, respectively, are proposed. Hapm also reacts with Fe (II) to form [Fe(Hapm)₃]²⁺ complex with less selectivity and sensitivity than H₂dapm reagent. Calibration graph with [Fe(H₂dapd)₂]²⁺ is linear over the range 0.28 µg mL⁻¹ with an apparent molar absorptivity of 8.481×10³ L mol⁻¹cm⁻¹ at λ_m 428 nm. Linear dynamic ranges are 0.01-11.0 and 0.07-11.0 µg mL⁻¹ iron (II) as [Fe(H₂dapd)₂]²⁺ complex for direct and derivative modes, respectively. The analytical sensitivity is 4.44×10⁻³ µg mL⁻¹ for direct and 9.15×10⁻⁵ µg mL⁻¹ for derivative spectrophotometry with [Fe(H₂dapd)₂]²⁺ complex. First derivative method enhances the sensitivity of the Fe(H₂dapd)₂²⁺ more than direct one with two folds for Ni²⁺, Co²⁺, Cu²⁺ and Cr³⁺. A linear equation was derived from iron (II) determining as Fe (H₂dapd)₂²⁺ in the presence of synthetic solution containing Ni²⁺, Co²⁺, Cu²⁺ and Cr³⁺. The use of H₂dapd reagent for the determination of total iron spectrophotometrically in foodstuffs, herbs, spices (rocks) after wet ashing (wet digestion) in the absence of reducing agent is compared with that employing AAS.

Key words: Iron, spectrophotometry, iron(ii)-2, 6-diacetylpyridine dioxime complex, spices, herbs, beans, rocks

INTRODUCTION

Recently, there has been a growing concern about the role of iron (II) in biochemical and environmental systems, regarding man, animals and plants. Iron (II) lead to many diseases such as giddiness, diarrhea and cardiac collapse-conditions found amongst people, to damage of the gastrointestinal tract and liver^[1]. These diseases can lead to a problem in iron absorption and in turn iron toxicity.

Because of the ever-increasing interest in the determination of different oxidation state of iron in outdoor samples, there is a need for the development of more sensitive and selective analytical methods. The common availability and low cost of instrumentation, the simplicity of procedures, the accuracy and selectivity of the technique make spectrophotometric method advantage for the determination of iron. Flow Injection Analysis (FIA), intensively a developed over the past 20 years, offers several valuable advantages for speciation of iron^[2], where individual signals were obtained for Fe (II) and Fe (III). However, the determination of iron with the FIA is expensive and with complicated experimental conditions that makes it not an alternative for routine analysis.

Much attention was given for measuring iron (II) with organic reagents spectrophotometrically in an acidic medium and first derivative, which not only offers convenient solutions to a number of analytical problems such as avoiding extraction process, resolution of component systems and matrix background, but also enhances the selectivity and sensitivity of the method. A few of them used N-m-chlorophenyl-p-methoxy-benzohydroxamic acid^[3] in faintly acidic medium after extraction iron complex with trioctylamine in toluene and 2 [2 (6-methylbenzothiazolyl) azo] -5- diethylaminobenzoic acid^[4] after reduction iron (III) samples with ascorbic acid in acetate buffer (pH=5. 8). Whereas, 5-[(3,5-dibromo-2-pyridylazo)] -2,4-[diamino-toluene [3, -5-DBPADAT]^[5] or 2,2'-bipyridyl reagents were used in acetate buffer at pH = 4.7 or 4.5^[6]. Finally, sulfosalicylic acid^[7] in the presence of hexamine/HCl buffer solution (pH=5. 8) was used.

5,5-dimethyl-1,2,3-cyclohexanetrione 1,2-dioxime-3-thiosemicarbazone (DCDT) legend has been proposed as a spectrophotometric reagent for determining iron (II) in wines, food, minerals, commercial acids and fruit juices^[8-10]. DCDT reacts in a strong acidic medium with Fe (II) and Fe (III) to form a violet iron (II)-complex. The optimum acidity range in

HCl medium, complex formation is ranged from 0.1 to 7.5 mol L⁻¹.

The iron reaction with DCDT is unusual due to the high acidity of the medium. In addition, results showed that molar absorptivity, Beer's law and detection limit were 8.9×10³ L mol⁻¹ cm⁻¹, 0.7-5.0 µg mL⁻¹ and 0.05 µg mL⁻¹ respectively. Results also showed that Co²⁺, Ni²⁺, Cr³⁺ and Cu²⁺ foreign ions in synthetic iron (II) solutions could be tolerated to 1.0, 5.0, 25.0 and 50.0 folds, respectively. Whereas after extracting iron into amyl alcohol the tolerance limit of Co²⁺ and Cr³⁺ were 5.0×10⁻³ and 1.5×10⁻² µg mL⁻¹, respectively.

Considering the relative stabilities and specific character of the axioms (=N-OH) complex of iron (II), 2,6-diacetylpyridine dioxin (H₂dapd) and 2-acetylpyridine monoxide (Hapm) had much attention for the spectrophotometric kinetic studies^[11-12]. Previous studies indicated that oximate anions were characterized with strong α-nucleophilicities^[13]. Finally, H₂dapd possesses structural features in common with DCDT organic reagents. Based on the preliminary studies, H₂dapd and Hapm oxen are chosen as analytical reagents for iron (II) determination.

Recently, spices and herbs have gained an important role in agronomy production and pharmacy because of their increased needs as a raw material for flavorings beverage, food metabolites and medicinal components. A *Vicia faba* bean is considered to be one the most popular food in Egypt. Generally, direct determination of iron in herb, spice and bean samples is difficult due to high amounts of protein, amino acids, alkaloids, cellulose, starch, volatile and fixed oil and pigments in the extracts^[14], which caused a serious interference. Therefore, the iron determination is a requisite routine plant control analysis. Procedures usually used wet ashing in the preparation of the solutions for analysis^[15]. Most organic matter in the extracts was destroyed by this process.

In this work, the analytical properties of H₂dapd or Hapm with iron (II) in acid or faintly alkaline medium are examined Spectro photo metrically. Comparison between direct and first derivative spectral methods has been established. The ability to measure trace amounts of Fe (III) and a mixture of Fe (II) and Fe (III) with H₂dapd is also exploited with the aim of H₂dapd that could reduce the Fe (III) to Fe (II). This work is considered as an attempt to develop a new, rapid and sensitive method to determine two different oxidation states of iron individually and in a binary mixture. H₂dapd organic reagent is applied for the determination of total iron as Fe (II) -complex in foodstuffs, herbs, spices and rocks.

MATERIALS AND METHODS

A Jasco V-530E Spectrophotometer with 1.0 cm quartz cells, a Buck Scientific Model 210 VGP Atomic Absorption Spectrophotometer (AAS), Shimadzu

FTIR-8101 Spectrophotometer, Perkin-Elmer 240C Elemental Analyzer and an Orion pH electrode fitted with a combined glass electrode were used. Millipore-water purification system was used.

Preparation of H₂dapd and Hapm Reagents: The H₂dapd or Hapm reagents were prepared from 2,6-diacetylpyridine or 2-diacetylpyridine via the modified procedure^[16]. Briefly, into a 250 mL flask 4.1 g of 2,6-diacetylpyridine or 20 mmol of 2-diacetylpyridine were mixed with 4.6 or 2.5 g of hydroxylamine hydrochloride and 4.8 or 2.4 g of barium carbonate, respectively, in 100 mL ethanol. The warmed mixture was stirred on a heating-stirrer and then refluxed for 5 hr. After filtration, the filtrate was left to stand overnight. The H₂dapd and Hapm oxime compound was re-crystallized from ethanol and dried.

Chemicals and Reagents: All entire reagents and solvents used were from BDH chemical grade except where indicated. 2,6-diacetylpyridine and 2-diacetylpyridine were obtained from Aldrich. HCl and HNO₃ were ultrapure acids (Merck). A stock solution of 1.0 (10⁻² M H₂dapd and Hapm reagents were prepared by dissolving reagent in ethanol. Stock solutions containing 1.0×10⁻³ M iron(II) and iron(III) (Merck) were prepared by dissolving an accurately weighed amounts of ferrous and ferric ammonium sulphate in 0.05 M H₂SO₄ and standardized titrimetrically with a standard K₂Cr₂O₇ solution (daily) and gravimetrically by weighing as F₂O₃, respectively. Working solutions were prepared by appropriate dilution with ultrapure water. Air must be removed from all solutions by purging with free oxygen-nitrogen prior to the experiments and all additions of reagents must be carried out. All glassware was cleaned as the procedure described elsewhere^[17].

Calibration Plot: Under the optimum conditions, a linear calibration graph was obtained up to 11.0 and 16.0 µg mL⁻¹ of iron (II) with H₂dapd and Hapm reagents, respectively. A plot showed that the optimum range was 0.28 to 11.0 (0.56 to 16.0) µg mL⁻¹ iron (II) with H₂dapd (Hapm) reagents using direct and derivative modes. The concentration of iron was calculated by the regression equations (Table 1).

Recommended Procedures for Direct and First Derivative Spectrophotometry for the Determination of Iron (II)

Procedure (I): A portion of ethanol solution containing 193.0 µg H₂dapd, 28.0 µg fresh iron(II) was mixed with 1.0 mL of 0.1 M HCl (to adjust pH to 2.5), 1.0 mL of 0.1M NaNO₃, and 6.0 mL ultrapure water in a 10.0 mL flask. The solution was shaken for a few seconds. The final ethanol concentration was 10.0% in the medium.

Table 1: Relationship Between Fe(II) Concentration (mol L⁻¹) and Maximum Absorbance (in Direct Mode) and Vertical Amplitude (in Derivative Mode)

| Regression equation | r | Y residual |
|--|--------|-----------------------|
| Direct mode | | |
| [Fe(H ₂ dapd) ₂] ²⁺ * | | |
| A ₄₂₈ = (8481 ± 1.82×10 ⁻³) [Fe ²⁺] - (6.2×10 ⁻³ ± 4.7×10 ⁻⁴) | 0.9998 | 1.42×10 ⁻³ |
| A ₅₀₂ = (7871 ± 3.50×10 ⁻³) [Fe ²⁺] - (4.0×10 ⁻³ ± 8.9×10 ⁻⁴) | 0.9994 | 2.68×10 ⁻³ |
| [Fe(Hapm) ₃] ²⁺ ** | | |
| A ₅₂₄ = (6905 ± 3.63×10 ⁻³) [Fe ²⁺] - (4.2×10 ⁻³ ± 1.20×10 ⁻⁴) | 0.9997 | 2.49×10 ⁻³ |
| First derivative mode | | |
| [Fe(H ₂ dapd) ₂] ²⁺ * | | |
| D ₄₁₂ = (169.6 ± 5.90×10 ⁻⁶) [Fe ²⁺] - (1.8×10 ⁻³ ± 1.5×10 ⁻⁶) | 0.9979 | 4.61×10 ⁻⁶ |
| D ₄₃₆ = (121.9 ± 8.80×10 ⁻⁷) [Fe ²⁺] - (5.3×10 ⁻⁴ ± 2.3×10 ⁻⁷) | 0.9994 | 6.89×10 ⁻⁷ |
| D ₄₈₁ = (63.90 ± 4.10×10 ⁻⁷) [Fe ²⁺] - (4.6×10 ⁻⁴ ± 1.0×10 ⁻⁷) | 0.9989 | 6.56×10 ⁻⁷ |
| D ₅₂₃ = (217.6 ± 8.40×10 ⁻⁷) [Fe ²⁺] - (1.3×10 ⁻⁴ ± 2.2×10 ⁻⁷) | 0.9998 | 3.19×10 ⁻⁷ |
| [Fe(Hapm) ₃] ²⁺ ** | | |
| D ₄₈₀ = (76.00 ± 5.80×10 ⁻⁷) [Fe ²⁺] - (7.0×10 ⁻⁴ ± 1.9×10 ⁻⁷) | 0.9996 | 9.19×10 ⁻⁷ |
| D ₅₅₅ = (134.8 ± 2.30×10 ⁻⁶) [Fe ²⁺] - (9.0×10 ⁻⁴ ± 4.3×10 ⁻⁷) | 0.9997 | 3.99×10 ⁻⁷ |

A=Absorbance; D= dA/dλ=Vertical Amplitude from Zeroth Line; r=Correlation Coefficient; *n =10 Measurements; ** n = 10 measurements

Table 2: Analytical Characteristics of Direct and First Derivative Modes

| Sensitivity S(b) s _b | Limit of detection (LOD) | | | | Dyn. Range | |
|---|--------------------------|-----------------------|-----------------------|-----------------------|----------------------|--|
| | S _A | y _B | s _B | y _L | C _L (k=3) | C _q (k=6) μg mL ⁻¹ |
| Direct Mode | | | | | | |
| [Fe(H ₂ dapd) ₂] ²⁺ * | | | | | | |
| λ ₄₂₈ 0.151 6.71×10 ⁻⁴ 4.44×10 ⁻³ | 2.13×10 ⁻³ | 1.48×10 ⁻⁴ | 2.20×10 ⁻² | 4.98×10 ⁻³ | 0.01 | 0.01-11 |
| [Fe(Hapm) ₃] ²⁺ ** | | | | | | |
| λ ₅₂₄ 0.123 1.43×10 ⁻³ 1.16×10 ⁻³ | 3.29×10 ⁻² | 2.59×10 ⁻³ | 3.53×10 ⁻² | 1.95×10 ⁻² | 0.04 | 0.04-16 |
| Derivative Mode | | | | | | |
| [Fe(H ₂ dapd) ₂] ²⁺ * | | | | | | |
| (d _A /dλ) ₅₂₃ 0.004 3.71×10 ⁻⁷ 9.15×10 ⁻⁵ | 3.80×10 ⁻⁴ | 4.47×10 ⁻⁵ | 5.14×10 ⁻⁴ | 3.40×10 ⁻² | 0.07 | 0.07-11 |
| [Fe(Hapm) ₃] ²⁺ ** | | | | | | |
| (d _A /dλ) ₅₅₅ 0.002 5.50×10 ⁻⁷ 2.29×10 ⁻⁴ | 4.60×10 ⁻⁴ | 5.48×10 ⁻⁵ | 6.24×10 ⁻⁴ | 6.80×10 ⁻² | 0.14 | 0.14-16 |

λ_m = Maximum Wavelength; S = Sensitivity=Slope of Calibration Curve; s_b = Standard Deviation of Sensitivity; S_A = Analytical Sensitivity; y_B = Blank Signal; (Average of Five Measurements); s_B = Standard Deviation of Blank; y_L = Lowest Detectable Instrument Signal; C_L = Limit of Detection; C_q = Limit of Quantification; dyn= Dynamic Range; * (n=10 measurements); ** (n=8 measurements)

Procedure (II): A portion of ethanol solution containing 408.0 μg Hapm, 56.0 μg of iron (II) was mixed with 4.0 mL ethanol, 1.0 mL 0.1M NaNO₃ and 2.0 mL ultrapure water into 10 mL beaker, stir the solution and the pH was adjusted to 7.5 with NaOH. The final ethanol concentration was 60% in the medium.

Transfer both solutions to a 10.0 ml flask and make up to the mark with ultrapure water. In procedures (I) and (II), the absorption (A) and amplitude signals (dA/dλ) against a reagent blank were recorded. Then the concentration of ferrous ion as Fe (II) - and Fe (II) -Hapm complexes were measured at 428 (523) or 524 (555) NM with direct (first derivative) mode, (Table 1).

Determination the Fe(II) and Fe(III) in a Synthetic Solution: X mL solutions containing 0.1675-1.117 μg mL⁻¹ Fe(III) and 1.0 mL of 1.0×10⁻² M (0.5585 μg mL⁻¹) Fe(II) were placed in a flask and flow the nitrogen in the mixture. After duration for 15 sec, y mL of 1.0×10⁻⁴ M H₂dapd, 1.0 mL 0.1 M HCl and 1.0 mL of 0.1 M NaNO₃ were added. Dilute the mixture with water to the mark. The absorbance of total iron as Fe(II)-H₂dapd complex was measured

after standing 4 hr. The concentration of iron was calculated at λ_m 428 nm using regression equations (Table 1).

Determination of Total Iron in Herb, Spice, Bean and Rock Samples: The recommended procedure (I) was applied to determine iron in four herbs and spices (kakade, anise, cumin and black pepper), one *Vicia faba* bean (testa, cptyledons and testa+ cptyledons) and six rock samples. Four herbs and spice or six rock samples were purchased from a supermarket in Sohag area are collected from the red sea area, Upper Egypt. To prepare dry weight of herb, spice and bean, the samples washed with water to avoid any contamination on the surface^[15]. After drying, the samples were ground with titanium blade (food blender) to make the homogeneous and sieved to separate particle size fraction (<150 μm). Whereas rock samples were ground with ceramic mortar and sieved to separate particle size fraction (<150 μm). A certain amount of ground sample (herb, spice and bean) was heated at 500°C for 2 hr in a muffle furnace. A 1.0 g of resultant or rock sample was mixed with 10 mL concentrated HCl and HNO₃ (1:1). Evaporate till appearance of white residue, then

dissolve in 10 mL water and evaporated again. After cooling, 50.0 mL of water was added and the solution was filtered into a 100 mL flask, brought to volume. Aliquots of 1.0-3.0 or 5.0 ml from an herb, spice and bean or rock solutions were used in spectrophotometric determination of iron by procedure (I). The absorbance of iron as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex was measured at 428 (523) nm using direct (first derivative) mode after standing 4 hr. The iron concentration was calculated using regression equations (Table 1).

RESULTS AND DISCUSSION

Effect of pH: The influence of pH on the formation of $\text{Fe}(\text{H}_2\text{dapd})_2^{2+}$ ($[\text{Fe}(\text{Hapm})_3]^{2+}$) complex in 10% (60%) v/v water-ethanol mixture was studied in the pH range from 0.5 to 12.0, Fig. 1 (a, b). Obviously, observance is constant and maximal at pH 2.0-3.0 (7.0-10.0) for Fe (II) - H_2dapd (Fe (II) - Hapm) complex. So, pH 2.5 or 7.5 were selected for the iron (II) determination with H_2dapd or Hapm reagents.

Effect of Time on H_2dapd Reagent: Figure 2 (a) shows the influence of varying period times (30.0 sec to 12 hr) on the stability of 1.0×10^{-3} M H_2dapd at pH 2.5. Obviously, the absorbance at λ_m 297 nm attained constant value from 30 sec to 4.0 hr with relative error less than 1.8%. On further increasing period times in 12 hr, the absorbance decreases slowly with relative error range 2.5-11.2%. After three days, the absorbance decreases rapidly with relative error 33%. The same result is also found in λ_m 258 nm, suggesting that H_2dapd reagent is stable within 4.0 hr. For this fact, it is advisable to prepare this reagent daily.

Oxidation-reduction Character of H_2dapd : The presence of ascorbic acid does not affect the Fe (II) - H_2dapd complex, whereas changes occur in the presence of H_2O_2 . This observation revealed that H_2dapd reagent behaves as a reducing agent. To assess the ability and power of H_2dapd as a reducing agent, a study was carried out with four standard solutions: Two solutions contain 2.8 and $5.6 \mu\text{g mL}^{-1}$ Fe (II) with $18.3 \mu\text{g mL}^{-1}$ H_2dapd ; Fig 2 (d, e), While other two solutions contain 2.5 and $5.6 \mu\text{g mL}^{-1}$ Fe(III) with $38.6 \mu\text{g mL}^{-1}$ H_2dapd ; Fig 2 (b,d), following the optimum condition of spectrophotometric procedure (I). The ratio between iron: reagent is about 1:10. The absorbance of Fe(II) or Fe(III) complexes at λ_m 428 nm after allowed periods range 30 sec -12.0 hr were measured. Figure 2 (b,d) shows that the observance of Fe (III) system increases gradually with increasing time from 30 sec to 4.0 hr. With increasing time from 4 to 6.5 hr, the observance remains constant. At still increasing time, the absorbance decreases slowly. Figure 2(c,e) shows the rapid formation of the Fe(II)-complex indicating that Fe(II)-complex is formed and it's stable for at least 8 hr but with increasing time to 12 hr, absorbance decreases slowly.

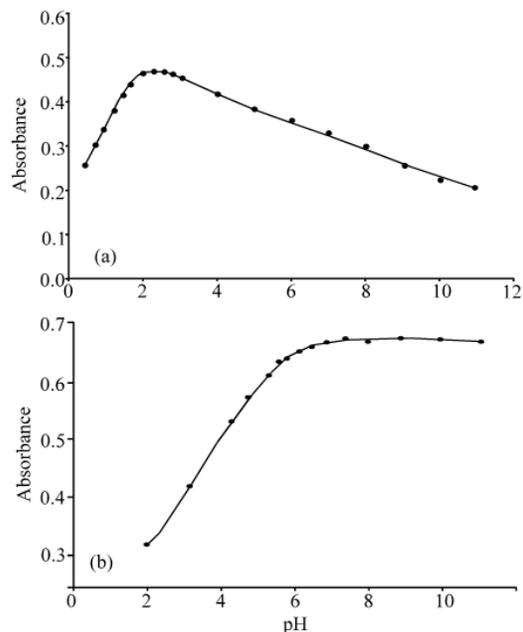


Fig. 1: Effect of pH on the Fe(II)- H_2dapd and Fe(II)- Hapm systems: a)- $2.8 \mu\text{g mL}^{-1}$ Fe(II) and $19.3 \mu\text{g mL}^{-1}$ H_2dapd ; b)- $5.6 \mu\text{g mL}^{-1}$ Fe(II) and $40.8 \mu\text{g mL}^{-1}$ Hapm

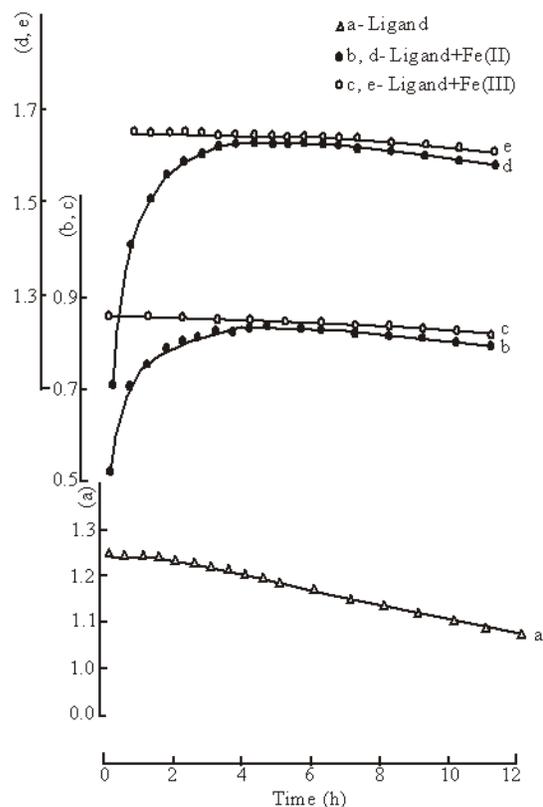


Fig. 2: Stability and radix character: (a) 19.3 mg L^{-1} H_2dapd ; b or c) a + 2.8 or $5.6 \mu\text{g L}^{-1}$ Fe (II) or Fe (III); d and e) 2a + 2.8 or $5.6 \mu\text{g L}^{-1}$ Fe (II) or Fe (III) using a procedure (I).

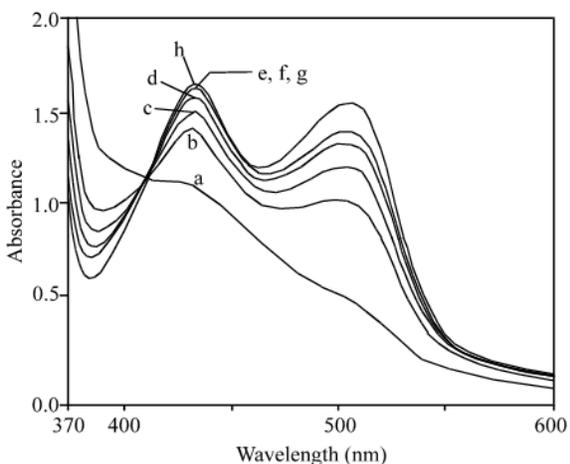


Fig. 3: Direct spectra obtained from a mixture of iron and Fe(II) in a solution containing $38.6 \mu\text{g L}^{-1}$ H_2dapd : a) $2.8 \mu\text{g mL}^{-1}$ Fe(III) and $2.8 \mu\text{g mL}^{-1}$ Fe(II) at 30 sec; b) a at 0.5 hr; c) a at 1.0 hr; d) a at 2.0 hr; e) a at 3.5 hr; f) a at 4.0 hr; g) a at 6.0 hr; h) $5.6 \mu\text{g mL}^{-1}$ Fe(II) at 30 sec.

Figure 2 (b, c, d, e), also shows that the maximum absorbance of Fe(II) or Fe(III) systems are nearly identical at time periods range 4.0-6.0 hr. This finding suggests that Fe(II) reacts with the H_2dapd reagent to form Fe(II)- H_2dapd complex at 30 sec (instantaneously) whereas the reaction in Fe(III) system needs standing time within 4.0-6.5 hr forming Fe(II)- H_2dapd complex. To confirm above results two solutions were prepared, one of them contains an iron mixture (2.5 ferric and $2.5 \mu\text{g L}^{-1}$ ferrous) while the other contains only ferrous ($5.6 \mu\text{g L}^{-1}$). Under the optimum condition of procedure I, the absorbance of iron in iron mixture and Fe(II) systems at λ_m 428 nm were measured at seven different period time (30 sec-6 hr) and at 30 sec, respectively. The results are illustrated in Fig. 3. Obviously, the absorbance values in Fe (III) system increase gradually with increasing time of reaction. In addition, the absorbance value of Fe (III) system will be fixed at time ranges from 4 to 6 hr. Results also show that the recovery of ferric reduction to ferrous ion with H_2dapd are 73.6, 85.6, 91.6, 96.2, 99.1, 99.5 and 99.7 % at 30 Sec, 0.5, 1.0, 2.0, 3.5, 4.0 and 6.0 hr, respectively, assuming the standard value of absorbance for ferrous at 30 Sec. On the other hand, observance of iron in the ferrous system (curve h) at 30 Sec is analogous with absorbance in iron mixture at 4.0 and 6.0 hr (curves f and g). These observations reveal that H_2dapd reagent reduced ferric ion completely to ferrous within period at least 4 hr without shift in λ_m (428 nm). Also, ferrous ion in a binary mixture of ferric and ferrous ions could not be measured at 30 Sec, i.e. The reaction is instantaneous, resulting incomplete reduction process of ferric system.

Spectral Characteristics: The iron (II) reacts rapidly within a minute with H_2dapd and Hapm reagents forming. Stable red and orange complexes at pH 2.5 and 7.5, respectively. Figure 4 shows the direct and first derivative spectra of Fe (II) - H_2dapd (curves a, \hat{a}) and Fe-(II) - Hapm (curves b, \hat{b}) complexes and their reagent blanks (curve c, \hat{c} , d, \hat{d}). The absorption bands in direct mode of Fe(II)- H_2dapd complex showed doubly absorption maximum at 428 and 502 nm while Fe(II) Hapm complex showed a singly absorption band at 524 nm. The splitting band of $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex indirect spectra was explained by a pronounced reduction in the ligand field caused by the greater stress requirements of the two legends molecules. Two absorption bands were assigned to the $^5\text{A}_1$ and $^5\text{B}_1$ states derived from the splitting of ^5E spectroscopic term in the tetragonal ligand^[18]. Maximum amplitude in the first derivative mode for di-(mono) complex appeared in 412, 439, 481 and 523 (480 and 555) NM.

Composition of the Complexes: The continuous variations and molar ratio methods were applied to ascertain the stoichiometric composition of Fe (II) - H_2dapd or Fe (II) - Hapm complex at pH 2.5 or 7.5. A 1:2 and 1:3 of the Fe (II) - H_2dapd and Fe (II) - Hapm complexes were indicated, respectively. The ratios are in agreement with that found by analysis of solid complexes with the CHN elemental analyzer and IR.

Selection of Wavelength: In order to select the best analytical signal for Fe(II)- H_2dapd (Fe(II)- Hapm) complex, calibration graphs by direct or derivative modes were plotted at λ_m 428 and 502 (524) nm or 412, 439, 481 and 523 (480 and 555), respectively. Linear equations ($y = bx + a$) of A or D signal against Fe (II) concentration for both complexes were listed in Table 1. Molar absorptivity in direct and derivative modes of Fe(II)- H_2dapd (Fe(II)- Hapm) complexes were 8.481×10^3 (6.905×10^3) $\text{L mol}^{-1} \text{cm}^{-1}$ at 428 and 524 nm and 217.6 (134) $\text{L mol}^{-1} \text{cm}^{-1}$ at 523 (555) nm, respectively, suggesting that H_2dapd ligand is the best one for determining iron(II). Miller^[19] has indicated that if the r closes 1 or -1, the gentle calibration graph is obtained. Table 1 shows a slight deviation from ideal linear. This deviation is attributed to random experimental errors, which make difficult to select the best wavelength or pulse amplitude. Accordingly, \hat{y} and $y - \hat{y}$ residuals are introduced. $Y - \hat{y}$ represents the difference between the experimental y values and fitted \hat{y} values. By comparing \hat{y} -residual in direct and derivative modes for both irons-complexes, results show that low value in \hat{y} -residual appeared at λ_m 428 and D at 523 nm for $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex.

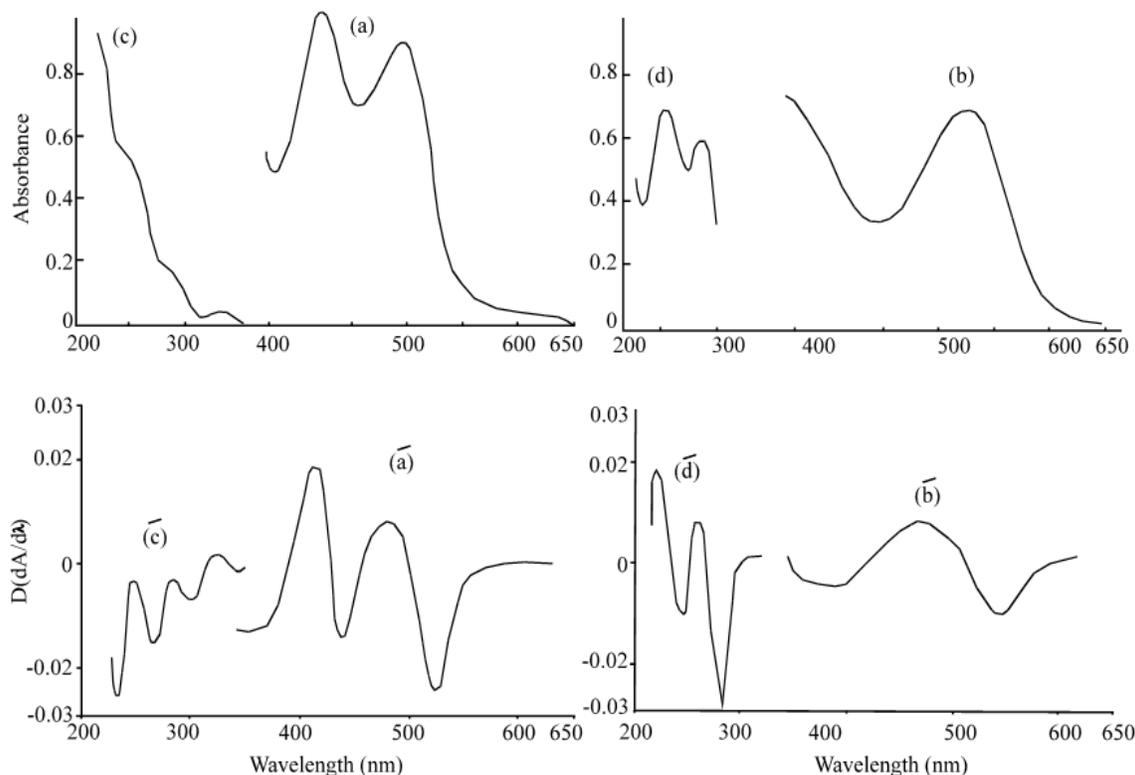


Fig. 4: Direct (a, b, c, d) and first derivative (a', b', c', d') spectra of $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$, $[\text{Fe}(\text{Hapm})_3]^{2+}$, H_2dapd and Hapm . Conditions: (a,a') $6.72 \mu\text{g mL}^{-1} \text{Fe(II)} + 46.32 \mu\text{g mL}^{-1} \text{H}_2\text{dapd}$; (b,b') $5.6 \mu\text{g mL}^{-1} \text{Fe(II)} + 40.8 \mu\text{g mL}^{-1} \text{Hapm}$; (c,c') $46.32 \mu\text{g mL}^{-1} \text{H}_2\text{dapd}$; (d,d') $40.8 \mu\text{g mL}^{-1} \text{Hapm}$

This is confirmed graphically, on plotting individual $y - \hat{y}$ -residuals vs. y signals (Fig. 5). Clearly, Fig. 5 (a, a') shows that the residuals should thus take if the unweighted regression line is a good model for the experimental spectra. There is a clear sequence of the positive residual, followed by sequence negative ones followed by a second positive sequence. The three numbers of runs give a significant data. While Fig. 5 (b, b') shows that, the regression line is inappropriate. Because the sign (+) or (-) of the residual is in random. Therefore, results indicate that H_2dapd reagent is considered the best reagent for iron(II) determination at 428 and 523 nm with direct and first derivative, respectively, while iron(II) measurement with Hapm reagent at 524 (555) nm with direct (first derivative) is inappropriate.

Sensitivity, Detection Limits, Dynamic Range, Accuracy and Precision: Analytical sensitivity (S_A)^[20], detection limit (y_L) and dynamic range^[20] of Iron (II) determination as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ and $[\text{Fe}(\text{Hapm})_3]^{2+}$ are summarized in Table 2. It is found that, direct and first order methods with H_2dapd reagent were sensitive with low detection limits than that obtained with Hapm suggesting, iron(II) can be successfully measured with procedure I.

The accuracy and precision of I and II procedures were checked by analyzing two sets of 2.8 and 5.6 $\mu\text{g mL}^{-1}$ iron (II). Under the optimum conditions of a procedure I and II, ferrous ion as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ ($[\text{Fe}(\text{Hapm})_3]^{2+}$) was measured at λ_m 428 and 523 nm is using direct and D at 524 and 555 using first derivative, respectively. The mean value and standard deviation for a procedure I or II were 2.85 (0.018 or 5.55 (0.021 using direct and 2.81 (0.021 or 5.72 (0.018 $\mu\text{g mL}^{-1}$ iron (II) using first derivative, while relative errors were 0.71 and 0.35 % for direct and 5.0 and 2.8% for the first derivative, respectively. Thus, $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex has the advantages of reasonable selectivity, good precision and low detection limit than $[\text{Fe}(\text{Hapm})_3]^{2+}$ complex.

Effect of Foreign Ions: The effect of 24 foreign ions on the determination of 2.8 or 5.6 $\mu\text{g mL}^{-1}$ iron (II) with H_2dapd or Hapm reagent were examined individually by measuring the absorbance and vertical distance under the recommended procedures I and II. Metal ions were added as Na^+ or K^+ while the anions was added as NO_3^- ions. The tolerance limit was taken as the amount that caused 2.5% error. The results are given in Table 3.

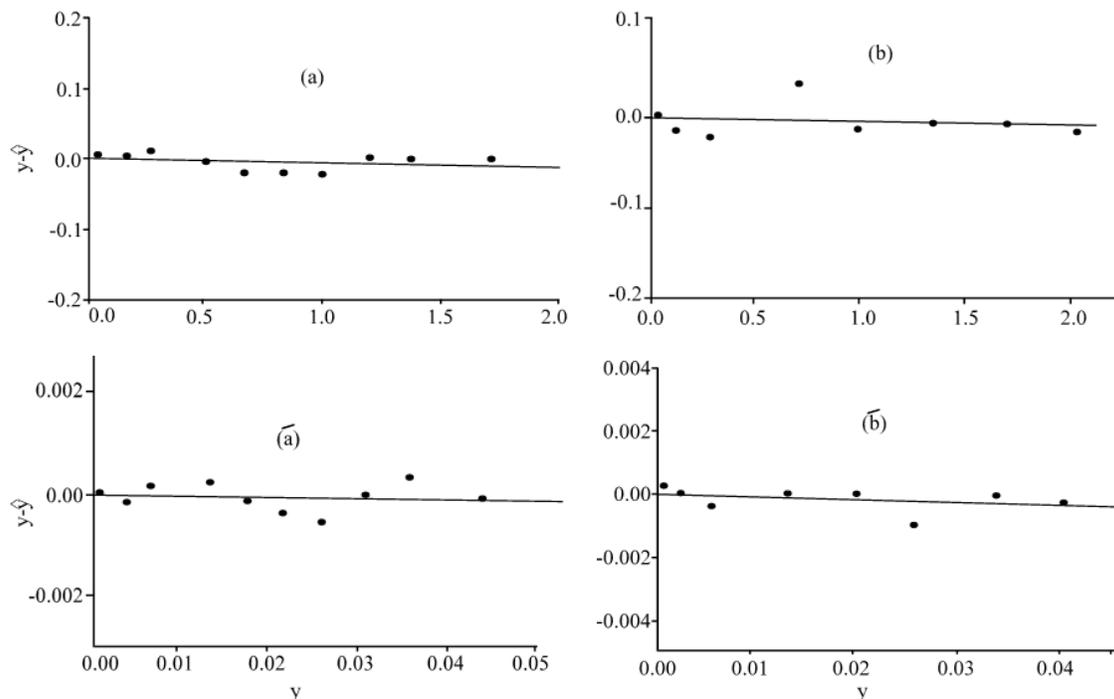


Fig. 5: Relation between individual \hat{y} -residual of calibration and y signal for direct (a,b) and first derivative (a',b'): (a) $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ at 428 nm, (a') $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ at 523 nm, (b) $[\text{Fe}(\text{Hapm})_3]^{2+}$ at 524 nm and (b') $[\text{Fe}(\text{Hapm})_3]^{2+}$ at 555 nm

Tolerance limit of iron (II) as $\text{Fe}(\text{H}_2\text{dapd})_2^{2+}$ in presence of Cu^{2+} , Cr^{3+} , Ni^{2+} and Co^{2+} , with direct and derivative modes is higher than that obtained with $[\text{Fe}(\text{Hapm})_3]^{2+}$ complex. Derivative spectra improved the tolerance limit of iron (II) fold twice than the direct spectra.

Figure 6 shows the influence of 0.152 (0.044) mg mL^{-1} Cu^{2+} , 0.155 (0.052) mg mL^{-1} Cr^{3+} , 0.017 (0.007) mg mL^{-1} Ni^{2+} and 0.017 (0.009) mg mL^{-1} Co^{2+} , an iron (II) as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ ($[\text{Fe}(\text{Hapm})_3]^{2+}$). Obviously, the amplitude signal in the first derivative (D) of iron (II) as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex was not affected with no significant difference in the presence of Cu^{2+} , Cr^{3+} , Ni^{2+} and Co^{2+} ions (Fig. 6a), while indirect mode, the observance of iron (II) -complex affected seriously with them ions (Fig. 6a), suggesting that Cu^{2+} , Cr^{3+} , Ni^{2+} and Co^{2+} , interfering ions are completely masked with derivative mode. In contrast, direct and derivative spectra of iron (II) as $[\text{Fe}(\text{Hapm})_3]^{2+}$ could not prevent the deviation in A and D, respectively, as shown in (Fig. 6 b, b'). Thus, the first derivative mode is used successfully for the determination of iron (II) with the H_2dapd reagent in presence Cu^{2+} , Cr^{3+} , Ni^{2+} and Co^{2+} , ions. Although H_2dapd reagent improved tolerance limits two folds with first derivative spectra, values of Ni^{2+} and Co^{2+} ions are still low (Table 3). This is possibly attributed to the formation of Ni^{2+} dimmer complexes $[\text{Ni}(\text{H}_2\text{dapd})_2]^{2+}$ and $[\text{Ni}(\text{dapd})_2]^{2+}$ and quite oxidation of Co^{2+} to Co^{3+} in presence of H_2dapd ligand^[21,12].

To drive a linear regression equation for iron (II) as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ complex in the presence of four serious interfering ions, a series of a synthetic solution containing a varied concentration range 1.2-7.84 $\mu\text{g mL}^{-1}$ Fe (II) and fixed concentration of H_2dapd (70 $\mu\text{g mL}^{-1}$) reagent in the presence of 0.152 mg mL^{-1} Cu^{2+} , 0.155 mg mL^{-1} Cr^{3+} and 0.017 mg mL^{-1} Ni^{2+} and Co^{2+} , were prepared. The optimum condition of the procedure I was applying. The vertical distance of amplitude signal to zero line (D) against blank was recorded at derivative amplitude (D) 523 nm. A linear calibration graphs passing through the origin is obtained by plotting DA/d (at 523 NM versus iron (II) concentration. The regression equation in derivative mode (95% confidence interval, n=8) is:

$$D_{523} = (3.92 \times 10^{-3} \pm 1.18 \times 10^{-6})[\text{Fe}^{2+}] - (3.60 \times 10^{-4} \pm 1.66 \times 10^{-6})$$

Determination of Iron in a Mixture Containing Fe (II) and Fe (III) Synthetic Solution: Table 4 shows the concentration of iron mixture in synthetic solution containing 0.5585 mg mL^{-1} Fe (II), varied concentration of Fe (III) ranges 0.1675-1.1170 $\mu\text{g mL}^{-1}$ in the presence of 1.0 ml 0.1 M HCl and 1.0 ml 0.1 M NaNO_3 .

The absorbance of iron mixture was recorded under the optimum condition of procedure I (0.1M NaNO_3 , pH = 2.5 and 10% ethanol) after standing 4 hr at λ_m 428 nm.

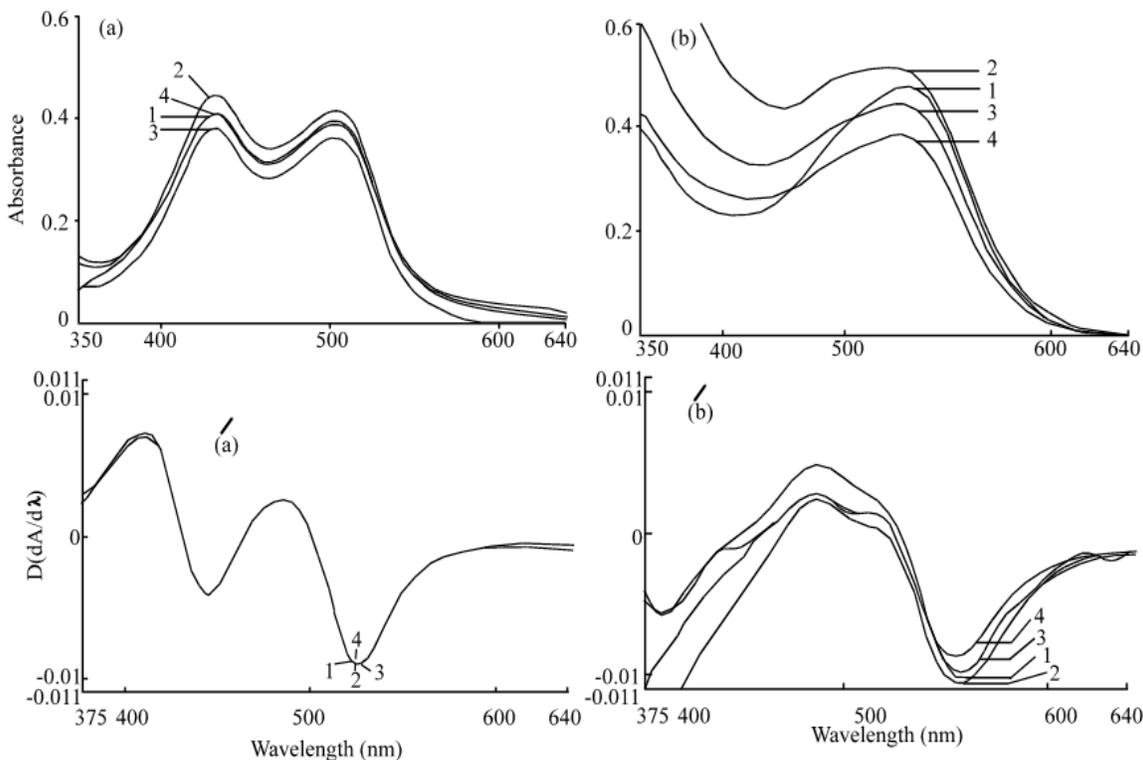


Fig. 6: Direct (a, b) and first derivative (a', b') spectra of solution containing 19.3 (40.8) $\mu\text{g mL}^{-1}$ H₂dapd (Hapm) reagent, 15 (60) % ethanol, 0.1 M NaNO₃ at pH 2.5 (7.5):

- 1: (a, a', b, b'): 2.8×10^{-3} and 5.6×10^{-3} mg mL⁻¹ Fe (II) as [Fe (H₂dapd)₂]²⁺ and [Fe (Hapm)₃]²⁺,
- 2: (a, a', b, b'): 1 (a) +0.017 and 0.007 mg mL⁻¹ Ni²⁺ with [Fe (H₂dapd)₂]²⁺ and [Fe (Hapm)₃]²⁺,
- 3: (a, a', b, b'): 1 (a) +0.017 and 0.009 mg mL⁻¹ Co²⁺ for [Fe (H₂dapd)₂]²⁺ and [Fe (Hapm)₃]²⁺,
- 4: (a, a', b, b'): 1 (a) +0.152 and 0.044 mg mL⁻¹ Cu²⁺ for [Fe (H₂dapd)₂]²⁺ and [Fe (Hapm)₃]²⁺,
- 5: (a, a', b, b'): 1 (b) +0.155 and 0.052 mg mL⁻¹ Cr³⁺ for [Fe (Hapm)₃]²⁺ and [Fe (Hapm)₃]²⁺

Table 3: Effect of Diverse Ions Amounts of Fe (II) Taken 2.8 (5.6) $\mu\text{g mL}^{-1}$ with H₂dapd (Hapm) Reagent

| [Fe(H ₂ dapd) ₂] ²⁺ | Tolerance limits | [Fe(Hapm) ₃] ²⁺ | Tolerance limit |
|--|------------------|--|-----------------|
| Diverse ions | (w/w) | Diverse ions | (w/w) |
| Direct mode | | | |
| K ⁺ , Na ⁺ , Ca ²⁺ | <1000 | K ⁺ , Na ⁺ , Ca ²⁺ | <1000 |
| NO ₃ ⁻ , Cl ⁻ , C ₂ O ₄ ²⁻ , SO ₄ ²⁻ , Ce ⁴⁺ , La ³⁺ , Cd ²⁺ , Zn ²⁺ | | La ³⁺ , Cd ²⁺ | |
| | | SO ₄ ²⁻ | <900 |
| | | Zn ²⁺ | <590 |
| Mg ²⁺ , Ba ²⁺ , Th ⁴⁺ | <500 | Mg ²⁺ , Th ⁴⁺ , Ce ⁴⁺ , | <207 |
| SCN ⁻ , CN ⁻ | | NO ₃ ⁻ , Cl ⁻ , C ₂ O ₄ ²⁻ | |
| U ⁶⁺ | <253 | U ⁶⁺ | <84 |
| Pb ²⁺ , Mn ²⁺ | <118 | Pb ²⁺ , Mn ²⁺ | <29 |
| | | SCN ⁻ , CN ⁻ | |
| Cu ²⁺ * | <36 | Cu ²⁺ * | <7.9 |
| Cr ³⁺ * | <28 | Cr ³⁺ * | <9.3 |
| Ni ²⁺ * | <2.5 | Ni ²⁺ * | <1.25 |
| Co ²⁺ * | <3.0 | Co ²⁺ * | <1.60 |
| First Derivative Mode | | | |
| Cu ²⁺ * | <54 | Cu ²⁺ * | <7.9 |
| Cr ³⁺ * | <55 | Cr ³⁺ * | <9.3 |
| Ni ²⁺ * | <6.0 | Ni ²⁺ * | <1.25 |
| Co ²⁺ * | <6.0 | Co ²⁺ * | <1.60 |

Table 4: Results the Determination of a Mixture Containing Fixed or Variable Concentration of Fe (II) or Fe (III) with 19.3 µg mL⁻¹ H₂dapd Reagent (I = 0.1 M NaNO₃, 10 % Ethanol at pH 2.5)

| True value Fe(II) µg mL ⁻¹ | Fe(III) µg mL ⁻¹⁺ | Found value Total iron µg mL ⁻¹ | Absorbance | Total iron µg mL ⁻¹ | Recovery % |
|---|---------------------------------|--|------------------------------|-----------------------------------|---------------|
| 0.5585 | 0.1675 | 0.7260 | 1.0956 ±2.1x10 ⁻³ | 0.7253 | 99.9 |
| | 0.3351 | 0.8936 | 1.3465±1.7x10 ⁻² | 0.8905 | 99.6 |
| | 0.5585 | 1.1170 | 1.6789±1.9x10 ⁻³ | 1.1095 | 99.3 |
| | 0.8377 | 1.3962 | 2.0879±2.3x10 ⁻³ | 1.3787 | 98.7 |
| | 1.1170 | 1.6755 | 2.5002±2.6x10 ⁻³ | 1.6502 | 98.5 |

Table 5: Direct and First Derivative Measurements of Iron (II) as [Fe (H₂dapd) ₂]²⁺ Complex in Beans, Herbs Species and Rocks

| No. of samples | Direct Mode | Derivative Mode | AAS | Relative Errors | |
|-------------------------|--------------------------|--------------------------|--------------------------|-----------------|-------|
| | mg g ⁻¹ (±sd) | mg g ⁻¹ (±sd) | mg g ⁻¹ (±sd) | direct-AAS | D-AAS |
| Herbs, Spices and beans | | | | | |
| 1 | 10.19 (±0.04) | 11.09(±0.05) | 10.58(±0.07) | 3.69 | 4.82 |
| 2 | 3.75 (±0.05) | 4.14(±0.06) | 3.95(±0.05) | 5.06 | 4.81 |
| 3 | 7.45 (±0.02) | 8.07(±0.03) | 7.74(±0.15) | 3.75 | 4.26 |
| 4 | 7.95 (±0.03) | 8.69(±0.06) | 8.33(±0.02) | 4.56 | 4.32 |
| 5 | 5.12 (±0.07) | 5.49(±0.07) | 5.32(±0.11) | 3.76 | 3.19 |
| 6 | 4.09 (±0.05) | 4.45(±0.06) | 4.29(±0.14) | 4.66 | 3.73 |
| 7 | 8.88 (±0.04) | 9.14(±0.05) | 8.98(±0.04) | 1.11 | 1.78 |
| Rocks | | | | | |
| 8 | 72.87 (±1.72) | 76.04(±1.09) | 75.0(±3.22) | 2.84 | 1.39 |
| 9 | 85.85 (±1.27) | 89.15(±1.26) | 88.15(±2.44) | 2.61 | 1.13 |
| 10 | 75.77 (±1.53) | 77.50(±1.04) | 75.96(±3.09) | 0.25 | 2.03 |
| 11 | 34.97 (±1.06) | 37.50(±1.27) | 36.30(±1.76) | 3.66 | 3.30 |
| 12 | 7.56 (±0.04) | 8.12(±0.05) | 7.88(±0.19) | 4.06 | 3.04 |
| 13 | 72.87 (±1.72) | 76.04(±1.09) | 100.8(±2.08) | 3.12 | 2.18 |

1-kakade; 2- anise; 3- cumin; 4- black pepper; 5- testa of *Vicia faba*; 6- cotyledons of *Vicia faba*; 7- testa and cotyledons; (8-10)- amphibolites; (11, 12)- migmatite leosome; 13- serpentine; D Derivative Spectra; AAS = Atomic Absorption Spectrophotometric

There is a good agreement between true and found concentration of iron. The recovery of measurements was found to be higher than 99.3%. Thus, H₂dapd reagent can be recommended for the determination of total iron in a mixture containing ferrous and ferric mixture as [Fe(H₂dapd)₂]²⁺ complex after an allowed period of 4 hr for complete reduction Fe(III) to Fe(II).

Determination of Total Iron in Outdoor Samples Using H₂dapd Reagent: The proposed procedure I was applied to determine iron in herb, spice, bean (rock) after wet ashing (wet digestion) of the samples. The results are listed in Table 5. The method based on the reduction of Fe(III)-complex to F(II)-complex with H₂dapd reagent after standing for 4 hr. The A or D signals were measured at 428 or 523 nm, respectively against the blank solution. The results obtained were compared with that measured by AAS. There are agreements between the results obtained by direct and derivative modes and AAS. No significant differences are observed (F=1.048 in direct mode-AAS and F=1.032 in derivative mode-AAS). The relative error was found to be less than 5%. Thus, H₂dapd reagent can be used successfully to determine total iron as [Fe (H₂dapd) ₂]²⁺ complex in the majority of wet ashing herb, spice and bean or wet digested rock samples

without reducing agent. A comparison between present (average of three values) and published results^[22]; that investigated the amount of iron in kakade (10.62 vs. 6.57 mg g⁻¹) and black pepper (8.32 vs. 3.9 mg g⁻¹) is higher whereas anise (3.95 vs.7.67 mg g⁻¹) and cumin (7.75 vs.8.28 mg g⁻¹) is lower than in the published results. Higher concentration may be possibly attributed to contaminated samples, which comes from the irrigation with contaminated water and addition of fertilizer and/or herbicides to soil.

CONCLUSION

One of the most important aspects of the present work is the simple, rapid method for spectrophotometric determination of ferrous and ferric individually or a mixture of ferrous and ferric as [Fe (H₂dapd) ₂]²⁺ complex in acidic water-ethanol medium (pH 2.5). H₂dapd reagent has the ability to react instantaneously with ferrous whereas ferric within a period of 4.0 hr. The sensitivity and selectivity of the method were improved with first derivative. Comparing the present procedure with the DCDT reagent before or after extraction into amyl alcohol concerning detection and tolerance limits for Cu²⁺, Cr³⁺, Ni²⁺ and Co²⁺ indicated that H₂dapd is sensitive and selective (except for Cu²⁺). On comparing one method with existing flow

injection analysis methods, indicates that the use is readily available, relatively inexpensive apparatus and simplicity of the experimental conditions make the proposed method a good alternative for routine analysis. Recommended method is economical since it is cheaper than AAS and does not need any gas maintenance. The method has been successfully used for determining total iron directly in digesting foodstuffs, herbs, spices and rock solution as $[\text{Fe}(\text{H}_2\text{dapd})_2]^{2+}$ in the absence of reducing agents.

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