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

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Abstract: The reaction of iron (II) with 2, 6-diacetylpyridine dioxime (H$_2$dapd) 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 H$_2$dapd 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 H$_2$dapd 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)$_2$]$_{2+}$ complex with less selectivity and sensitivity than H$_2$dapm reagent. Calibration graph with [Fe(H$_2$dapd)$_2$]$_{2+}$ is linear over the range 0.28 µg mL$^{-1}$ with an apparent molar absorptivity of 8.48×10$^3$ L mol$^{-1}$cm$^{-1}$ at $\lambda_{m}$ 428 nm. Linear dynamic ranges are 0.01-11.0 and 0.07-11.0 µg mL$^{-1}$ iron (II) as [Fe(H$_2$dapd)$_2$]$_{2+}$ complex for direct and derivative modes, respectively. The analytical sensitivity is 4.44×10$^{-3}$ µg mL$^{-1}$ for direct and 9.15×10$^{-3}$ µg mL$^{-1}$ for derivative spectrophotometry with [Fe(H$_2$dapd)$_2$]$_{2+}$ complex. First derivative method enhances the sensitivity of the Fe(H$_2$dapd)$_2$$_{2+}$ more than direct one with two folds for Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and Cr$^{3+}$. A linear equation was derived from iron (II) determining as Fe(H$_2$dapd)$_2$$_{2+}$ in the presence of synthetic solution containing Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$ and Cr$^{3+}$. The use of H$_2$dapd 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$^{[3]}$. 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$^{[4]}$, 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-benzenoaxamic acid$^{[3]}$ in faintly acidic medium after extraction iron complex with trioctylamine in toluene and 2 [2 (6-methylbenzothiazolyl) azo] -5- diethylaminobenzoic acid$^{[4]}$ in acidic medium after extraction iron complex with trioxylamine in toluene and 2 [2 (6-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-cyclohexanetetrione 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 low and detection limit were 8.9×10\(^3\) L mol\(^{-1}\) cm\(^{-1}\), 0.7-5.0 µg mL\(^{-1}\) and 0.05 µg mL\(^{-1}\), respectively. Results also showed that Co\(^{2+}\), Ni\(^{2+}\), Cr\(^{3+}\) and Cu\(^{2+}\) 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 Co\(^{2+}\) and Cr\(^{3+}\) 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 (H\(_{2}\)dapd) 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 \(\alpha\)-nucleophilies\(^{[13]}\). Finally, H\(_{2}\)dapd possesses structural features in common with DCDT organic reagents. Based on the preliminary studies, H\(_{2}\)dapd 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 \textit{Vicia faba} bean is considered to be one of 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\(^{[13]}\). Most organic matter in the extracts was destroyed by this process.

In this work, the analytical properties of H\(_{2}\)dapd 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 H\(_{2}\)dapd is also exploited with the aim of H\(_{2}\)dapd 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. H\(_{2}\)dapd 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 H\(_{2}\)dapd and Hapm Reagents:** The H\(_{2}\)dapd 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 H\(_{2}\)dapd 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 HNO\(_3\) were ultrapur acids (Merck). A stock solution of 1.0 (10\(^{-2}\) M H\(_{2}\)dapd 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 H\(_2\)SO\(_4\) and standardized titrimetrically with a standard K\(_2\)Cr\(_2\)O\(_7\) solution (daily) and gravimetrically by weighing as F\(_2\)O\(_3\), 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 H\(_{2}\)dapd 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 H\(_{2}\)dapd (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 H\(_{2}\)dapd, 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 NaNO\(_3\), 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(Hdap)d₃]²⁺ [Fe(Hapm)]²⁻                                                       | 0.9998  | 1.42×10⁻³  |
| A₁₂₈ = (8481 ± 1.82×10⁻⁴) [Fe⁺] – (6.2×10⁻³ ± 4.7×10⁻⁵)                              |         |            |
| A₃₂₄ = (7871 ± 3.50×10⁻⁴) [Fe³⁺] – (4.0×10⁻³ ± 8.9×10⁻⁵)                           | 0.9994  | 2.68×10⁻³  |
| [Fe(Hapm)]³⁻                                                             **       |         |            |
| A₅₂₄ = (6905 ± 3.63×10⁻³) [Fe⁺] – (4.2×10⁻³ ± 1.20×10⁻⁴)                           | 0.9997  | 2.49×10⁻³  |
| First derivative mode                                                             |         |            |
| [Fe(Hdap)d₃]²⁺ [Fe(Hapm)]²⁻                                                       | 0.9979  | 4.61×10⁻⁶  |
| D₁₂₈ = (169.6 ± 5.90×10⁻⁵) [Fe²⁺] – (1.8×10⁻³ ± 1.5×10⁻⁵)                         |         |            |
| D₃₉₀ = (121.9 ± 8.80×10⁻⁷) [Fe²⁺] – (5.3×10⁻⁴ ± 2.3×10⁻⁴)                         | 0.9994  | 6.89×10⁻³  |
| D₅₸₁ = (63.90 ± 4.10×10⁻⁷) [Fe²⁺] – (4.6×10⁻⁴ ± 1.0×10⁻⁴)                         | 0.9989  | 6.56×10⁻⁵  |
| D₇₂₃ = (217.6 ± 8.40×10⁻⁷) [Fe²⁺] – (1.3×10⁻⁴ ± 2.2×10⁻⁵)                         | 0.9998  | 3.19×10⁻⁷  |
| (Fe(Hapm)]³⁻                                                             **       |         |            |
| D₅₸₁ = (76.00 ± 5.80×10⁻⁷) [Fe²⁺] – (7.0×10⁴ ± 1.9×10⁻⁵)                          | 0.9996  | 9.19×10⁻²  |
| D₅₸₅ = (134.₈ ± 2.₃0×1₀⁻⁷) [Fe²⁺] – (9.0×10⁴ ± 4.₃₀×1₀⁻⁵)                      | 0.9997  | 3.₉₀×1₀⁻²  |

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 | Limit of detection (LOD) | Dyn. Range |
|-------------|--------------------------|------------|
| S(b) s₀     | S₀                       | y₀          | Cₙ(k=3) Cₙ(k=6) µg mL⁻¹ |
| Direct Mode |                          |            |                         |
| [Fe(Hdap)d₃]²⁺ [Fe(Hapm)]²⁻ | λ₃₂₄ 0.151 6.71×10⁻⁴ 4.44×10⁻³ | 2.13×10⁻³  | 1.48×10⁻⁴ | 2.20×10⁻²  | 4.98×10⁻⁵ | 0.01 0.01-11 |
|            | λ₅₂₄ 0.123 1.₃4×1₀⁻³ 1.₁₆₀×1₀⁻³ | 3.₂₀×1₀⁻²  | 2.₅₀×1₀⁻³ | 3.₅₃×1₀⁻² | 1.₉₅×1₀⁻² | 0.₀₄ 0.₀₄-1₆ |
| Derivative Mode | [Fe(Hdap)d₃]²⁺ [Fe(Hapm)]³⁻ | λ₃₂₄ 0.004 3.₇₁×1₀⁻₅ 9.₁₅×1₀⁻₃ | 3.₈₀×1₀⁻⁴ | 4.₄₇×1₀⁻⁴ | 5.₁₄×1₀⁻⁴ | 3.₄₀×1₀⁻² | 0.₀₇ 0.₀₇-1₁ |
|            | λ₅₂₄ 0.002 5.₃₀×1₀⁻⁷ 2.₂₉₀×1₀⁻⁴ | 4.₆₀×1₀⁻⁴ | 5.₄₈×1₀⁻⁴ | 6.₂₄×1₀⁻⁴ | 6.₈₀×1₀⁻² | 0.₁₄ 0.¹₄-₁₆ |

λ₃₂₄ = Maximum Wavelength; S = Sensitivity=Slope of Calibration Curve; s₀ = Standard Deviation of Sensitivity; S₀ = Analytical Sensitivity; y₀ = Blank Signal; (Average of Five Measurements); s₀ = Standard Deviation of Blank; y₀ = Lowest Detectable Instrument Signal; Cₙ = Limit of Detection; Cₙ = 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(II) 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 H₃dap, 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)-H₃dap complex was measured after standing 4 hr. The concentration of iron was calculated at λ₃₂₄ 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. 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 tell 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 \([Fe(H_2dapd)_2]^{2+}\) 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 \(Fe(H_2dapd)_2\)\((Fe(Hapm)_3)2^+\) 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) -H2dapd (Fe(II) -Hapm) complex. So, pH 2.5 or 7.5 were selected for the iron (II) determination with H2dapd or Hapm reagents.

**Effect of Time on H2dapd Reagent:** Figure 2 (a) shows the influence of varying period times (30.0 sec to 12 hr) on the stability of 1.0x10^{-3} M H2dapd at pH 2.5. Obviously, the absorbance at \(\lambda_m\) 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 \(\lambda_m\) 258 nm, suggesting that H2dapd reagent is stable within 4.0 hr. For this fact, it is advisable to prepare this reagent daily.

**Oxidation-reduction Character of H2dapd:** The presence of ascorbic acid does not affect the Fe(II) - H2dapd complex, whereas changes occur in the presence of H2O2. This observation revealed that H2dapd reagent behaves as a reducing agent. To assess the ability and power of H2dapd as a reducing agent, a study was carried out with four standard solutions: Two solutions contain 2.8 and 5.6 \(\mu\)g mL⁻¹ Fe (II) with 18.3 \(\mu\)g mL⁻¹ H2dapd; Fig 2 (d, e), While other two solutions contain 2.5 and 5.6 \(\mu\)g mL⁻¹ Fe(III) with 38.6 \(\mu\)g mL⁻¹ H2dapd; 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 \(\lambda_m\) 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. 3: Direct spectra obtained from a mixture of iron and Fe(II) in a solution containing 38.6 μg L⁻¹ H₂dapd: a) 2.8 μg mL⁻¹ Fe(III) and 2.8 μg 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 μg 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 H₂dapd reagent to form Fe(II)-H₂dapd complex at 30 sec (instantaneously) whereas the reaction in Fe(III) system needs standing time within 4.0-6.5 hr forming Fe(II)-H₂dapd complex. To confirm above results two solutions were prepared, one of them contains an iron mixture (2.5 ferric and 2.5 μg L⁻¹ ferrous) while the other contains only ferrous (5.6 μg L⁻¹). Under the optimum condition of procedure I, the absorbance of iron in iron mixture and Fe(II) systems at λₘ 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 H₂dapd 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 H₂dapd reagent reduced ferric ion completely to ferrous within period at least 4 hr without shift in λₘ (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 H₂dapd 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) -H₂dapd (curves a, b) and Fe(II) -Hapm (curves b, b) complexes and their reagent blanks (curve c, c, d, d). The absorption bands in direct mode of Fe(II)-H₂dapd 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 [Fe(H₂dapd)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 ⁵A₁ and ⁵B₁ 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) -H₂dapd or Fe (II) -Hapm complex at pH 2.5 or 7.5. A 1:2 and 1:3 of the Fe (II)-Hapm 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)-H₂dapd (Fe(II)-Hapm) complex, calibration graphs by direct or derivative modes were plotted at λₘ 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)-H₂dapd (Fe(II)-Hapm) complexes were 8.481×10⁵ (6.905×10⁵) L mol⁻¹ cm⁻¹ at 428 and 524 nm and 217.6 (134) L mol⁻¹ cm⁻¹ at 523 (555) nm, respectively, suggesting that H₂dapd 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, ŷ and y-ŷ residuals are introduced. Y-ŷ represents the difference between the experimental y values and fitted ŷ values. By comparing ŷ-residual in direct and derivative modes for both irons-complexes, results show that low value in ŷ-residual appeared at λₘ 428 and D at 523 nm for [Fe(H₂dapd)₂]²⁺ complex.
Fig. 4: Direct (a, b, c, d) and first derivative (a’, b’, c’, d’) spectra of \([\text{Fe (H}_{2}\text{dapd)}^{2+}, [\text{Fe (Hapm)}]^{3+}, \text{H}_{2}\text{dapd} and \text{Hapm. Conditions: (a,a’) 6.72 \mu g mL}^{-1} \text{Fe(II)+46.32 \mu g mL}^{-1} \text{H}_{2}\text{dapd; (b,b’) 5.6 \mu g mL}^{-1} \text{Fe(II)+40.8 \mu g mL}^{-1} \text{Hapm, (c,c’) 46.32 \mu g mL}^{-1} \text{H}_{2}\text{dapd; (d,d’) 40.8 \mu g mL}^{-1} \text{Hapm.}

This is confirmed graphically, on plotting individual y-\(y\)-residuals vs. \(y\) signals (Fig. 5). Clearly, Fig. 5 (a, á) 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 \(\text{H}_{2}\text{dapd 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 [Fe (H\(_2\)dapd) ]\(^{2+}\) and [Fe (Hapm) ]\(^{3+}\) are summarized in Table 2. It is found that, direct and first order methods with \(\text{H}_{2}\text{dapd 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 \(\mu g mL^{-1}\) iron (II). Under the optimum conditions of a procedure I and II, ferrous ion as [Fe (H\(_2\)dapd) ]\(^{2+}\) ([Fe (Hapm) ]\(^{3+}\) ) was measured at \(\lambda_m\) 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 \(\mu g mL^{-1}\) 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, Fe (H\(_2\)dapd) ]\(^{2+}\) complex has the advantages of reasonable selectivity, good precision and low detection limit than Fe (Hapm) ]\(^{3+}\) complex.

**Effect of Foreign Ions:** The effect of 24 foreign ions on the determination of 2.8 or 5.6 \(\mu g mL^{-1}\) iron (II) with \(\text{H}_{2}\text{dapd 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 ŷ-residual of calibration and y signal for direct (a,b) and first derivative (a’,b’): (a) \([\text{Fe(H}_{2}\text{dapd})_{2}]^{2+}\) at 428 nm, (a’) \([\text{Fe(H}_{2}\text{dapd})_{2}]^{2+}\) at 523 nm, (b) \([\text{Fe(Hapm)}_{3}]^{2+}\) at 524 nm and (b’) \([\text{Fe(Hapm)}_{3}]^{2+}\) at 555 nm.

Tolerance limit of iron (II) as \([\text{Fe(H}_{2}\text{dapd})_{2}]^{2+}\) in presence of \(\text{Cu}^{2+}, \text{Cr}^{3+}, \text{Ni}^{2+}\) and \(\text{Co}^{2+}\), with direct and derivative modes is higher than that obtained with \([\text{Fe(Hapm)}_{3}]^{2+}\) 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\(^{-1}\) \(\text{Cu}^{2+}\), 0.155 (0.052) mg mL\(^{-1}\) \(\text{Cr}^{3+}\), 0.017 (0.007) mg mL\(^{-1}\) \(\text{Ni}^{2+}\) and 0.017 (0.009) mg mL\(^{-1}\) \(\text{Co}^{2+}\), an iron - (II) as \([\text{Fe(H}_{2}\text{dapd})_{2}]^{2+}\) complex was not affected with no significant difference in the presence of \(\text{Cu}^{2+}, \text{Cr}^{3+}, \text{Ni}^{2+}\) and \(\text{Co}^{2+}\) ions (Fig. 6a), while indirect mode, the observance of iron (II) -complex affected seriously with them ions (Fig. 6a), suggesting that \(\text{Cu}^{2+}, \text{Cr}^{3+}, \text{Ni}^{2+}\) and \(\text{Co}^{2+}\), interfering ions are completely masked with derivative mode. In contrast, direct and derivative spectra of iron (II) as \([\text{Fe(Hapm)}_{3}]^{2+}\) 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 \(\text{H}_{2}\text{dapd}\) reagent in presence \(\text{Cu}^{2+}, \text{Cr}^{3+}\), \(\text{Ni}^{2+}\) and \(\text{Co}^{2+}\), ions. Although \(\text{H}_{2}\text{dapd}\) reagent improved tolerance limits two folds with first derivative spectra, values of \(\text{Ni}^{2+}\) and \(\text{Co}^{2+}\) ions are still low (Table 3). This is possibly attributed to the formation of \(\text{Ni}^{2+}\) dimmer complexes \([\text{Ni(H}_{2}\text{dapd})_{2}]^{2+}\) and \([\text{Ni(dapd)}_{2}]^{2+}\) and quite oxidation of \(\text{Co}^{2+}\) to \(\text{Co}^{3+}\) in presence of \(\text{H}_{2}\text{dapd}\) ligand\(^{[21,12]}\).

To drive a linear regression equation for iron (II) as \([\text{Fe(H}_{2}\text{dapd})_{2}]^{2+}\) complex in the presence of four serious interfering ions, a series of a synthetic solution containing a varied concentration range 1.2-7.84 \(\mu\)g mL\(^{-1}\) Fe (II) and fixed concentration of \(\text{H}_{2}\text{dapd}\) (70 \(\mu\)g mL\(^{-1}\)) reagent in the presence of 0.152 mg mL\(^{-1}\) \(\text{Cu}^{2+}\), 0.155 mg mL\(^{-1}\) \(\text{Cr}^{3+}\) and 0.017 mg mL\(^{-1}\) \(\text{Ni}^{2+}\) and \(\text{Co}^{2+}\), 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:

\[
D_{523}=(3.92\times10^{-3}\pm1.18\times10^{-5})[\text{Fe}^{2+}]-3.60\times10^{-4}\pm1.66\times10^{-6}
\]

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) and varied concentration of Fe (III) ranges 0.1675-1.1170 \(\mu\)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 \(\lambda_m\) 428 nm.
Fig. 6: Direct (a, b) and first derivative (á, b/combiningacuteaccent) spectra of solution containing 19.3 (40.8) µg mL⁻¹ H₂dapd (Hapm) reagent, 15 (60) % ethanol, 0.1 M NaNO₃ at pH 2.5 (7.5):
1: (a, á, b, b/combiningacuteaccent): 2.8 x 10⁻³ and 5.6x10⁻³ mg mL⁻¹ Fe (II) as [Fe (H₂dapd)₂]²⁺ and [Fe (Hapm)₃]²⁺,
2: (a, á, b, b/combiningacuteaccent): 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/combiningacuteaccent): 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/combiningacuteaccent): 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/combiningacuteaccent): 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) µg mL⁻¹ with H₂dapd (Hapm) Reagent

| Diverse ions | Tolerance limits (w/w) | Diverse ions | Tolerance limit (w/w) |
|--------------|------------------------|--------------|-----------------------|
| [Fe(H₂dapd)₃]²⁺ |                         | [Fe(Hapm)₃]²⁺ |                       |
| Direct mode  |                         |              |                       |
| K⁺, Na⁺, Ca²⁺ | <1000                  | K⁺, Na⁺, Ca²⁺ | <1000                 |
| NO₃⁻, Cr, C₂O₄²⁻, SO₄²⁻, Ce⁴⁺, La³⁺, Cd²⁺, Zn²⁺ |              |              |                       |
| Mg²⁺, Ba²⁺, Th⁴⁺ | <500                   | Mg²⁺, Th⁴⁺, Ce⁴⁺ | <207                 |
| SCN⁻, CN⁻, U⁶⁺ | <253                   | U⁶⁺          | <84                   |
| Pb²⁺, Mn²⁺ | <118                   | Pb²⁺, Mn²⁺ | <29                   |
| Cu²⁺ | <36                    | Cu²⁺ | <7.9                  |
| Cr³⁺ | <28                    | Cr³⁺ | <9.3                  |
| Ni²⁺ | <2.5                   | Ni²⁺ | <1.25                 |
| Co³⁺ | <3.0                   | Co³⁺ | <1.60                 |
| First Derivative Mode |             |              |                       |
| Cu²⁺ | <54                    | Cu²⁺ | <7.9                  |
| Cr³⁺ | <55                    | Cr³⁺ | <9.3                  |
| Ni²⁺ | <6.0                   | Ni²⁺ | <1.25                 |
| Co³⁺ | <6.0                   | Co³⁺ | <1.60                 |

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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, H$_2$dapd reagent can be recommended for the determination of total iron in a mixture containing ferrous and ferric mixture as [Fe(H$_2$dapd)$_2$]$^{2+}$ complex after an allowed period of 4 hr for complete reduction Fe(III) to Fe(II).

### Determination of Total Iron in Outdoor Samples

#### Using H$_2$dapd 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(II)-complex to Fe(II)-complex with H$_2$dapd 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, H$_2$dapd reagent can be used successfully to determine total iron as [Fe(H$_2$dapd)$_2$]$^{2+}$ 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(H$_2$dapd)$_2$]$^{2+}$ complex in acidic water-ethanol medium (pH 2.5). H$_2$dapd 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 Cu$^{2+}$, Cr$^{3+}$, Ni$^{2+}$ and Co$^{2+}$ indicated that H$_2$dapd is sensitive and selective (except for Cu$^{2+}$). On comparing one method with existing flow

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Table 4: Results the Determination of a Mixture Containing Fixed or Variable Concentration of Fe (II) or Fe (III) with 19.3 µg mL$^{-1}$ H$_2$dapd Reagent (I = 0.1 M NaNO$_3$, 10% Ethanol at pH 2.5).

| No. of samples | Direct Mode Absorbance µg mL$^{-1}$ | Derivative Mode Absorbance µg mL$^{-1}$ | AAS Absorbance µg mL$^{-1}$ | True value mg g$^{-1}$ | Found value mg g$^{-1}$ | Relative Error % |
|----------------|-----------------------------------|--------------------------------------|----------------------------|-------------------------|-------------------------|-----------------|
| 1              | 10.19 (±0.04)                     | 11.09 (±0.05)                       | 10.58 (±0.07)              | 1.1170                  | 1.6755                  | 2.5002±2.6x10$^{-3}$ | 1.6502         |
| 2              | 7.45 (±0.02)                      | 8.07 (±0.03)                        | 7.74 (±0.15)               | 0.7253                  | 0.7260                  | 1.0956±2.1x10$^{-3}$ | 99.9           |
| 3              | 7.95 (±0.03)                      | 8.69 (±0.06)                        | 8.33 (±0.02)               | 0.8905                  | 0.8936                  | 1.3465±1.7x10$^{-2}$ | 99.6           |
| 4              | 5.12 (±0.07)                      | 5.49 (±0.07)                        | 5.32 (±0.11)               | 5.32 (±0.11)            | 5.35 (±0.07)            | 8.69 (±0.06)      | 99.3           |
| 5              | 4.09 (±0.05)                      | 4.45 (±0.06)                        | 4.29 (±0.14)               | 4.29 (±0.14)            | 4.33 (±0.05)            | 8.07 (±0.03)      | 98.7           |
| 6              | 8.88 (±0.04)                      | 9.14 (±0.05)                        | 8.98 (±0.04)               | 8.98 (±0.04)            | 9.00 (±0.05)            | 7.74 (±0.15)      | 98.5           |

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, H$_2$dapd reagent can be recommended for the determination of total iron in herb, spice, bean (rock) after wet ashing (wet digestion) of the samples. The results are listed in Table 4. The method based on the reduction of Fe(III)-complex to F(III)-complex with H$_2$dapd 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, H$_2$dapd reagent can be used successfully to determine total iron as [Fe(H$_2$dapd)$_2$]$^{2+}$ 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

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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 [Fe(H$_2$dapd)$_2$]$_2^{2+}$ in the absence of reducing agents.

REFERENCES

1. Hartley, F.R., C. Burgess and R.M. Alcock, 1980. Excerpted from the Book Solution Equilibria. Published by John Wiley and Sons, pp: 1-357.
2. Campanella, L., K. Pyrzynska and M. Trojanowicz, 1996. Chemical speciation by flow-injection analysis. A Review, Talanta, 43: 825-838.
3. Agrawal, Y.K., 1997. Extraction and determination of iron (II) by spectrophotometry and inductively coupled plasma, atomic-emission spectrophotometry, in pharmaceutical and environmental samples. Indian Drugs, 34: 269-274.
4. Fan, X.Z., C.H. Zhu and H.C. Liu, 1997. A study of the color reaction of a new thiazolyalo reagent $2\{6$-methylbenzothiazolyl azo]-5-diethylaminobenzoic acid with iron (II) and its application. Fenxi Shiyanshi, 16: 23-26.
5. He, Q.H., Y.Y. Zhu and X.Q. Mao, 1997. Study on the color reaction of iron (II) with 5-(3,5-dibromo-2-pyridyldazo)-2,4-diaminotoluene. Lihua Jianyan Huaxue Fence, 33: 268-270.
6. Oliveira, P.C.C. and J.C. Masini, 2001. Sequential injection determination of iron (II) in an-anemic pharmaceutical formulations with spectrophotometric detection. Anal. Lett., 34: 389-397.
7. Liang, W.A., Z.Y. Zhang and S.F. Zou, 1997. Ultraviolet derivative spectro-photometric determination of trace iron with sulfosalicylic acid. Fenxi Kexue Xuebao, 13: 145-147.
8. Salinas, F., J.C.J. Sanchez and T.G. Diaz, 1986. Spectro-photometric determination of iron in wines, foods and mineral with 5,5-dimethyl-1,2,3-cyclo-hexanetione 1,2-dioxide 3-thiosemicarbazone complex. Talanta, 34: 655-656.
9. Hanania, G.I.H. and D.H. Irvine and F. Shurayh, 1965. The effect of Coordination on ionization. Part III. Pyridine-2, 6-dialdoxime and its iron (II) complex. J. Chem. Soc., pp: 1149-1154.
10. Yatsimirsky, A.K., P. Gomez-Tagle, S. Escalante-Tovar and L.R.R. Ramirez, 1998. Kinetics and mechanism of ester hydrolysis by metal complexes of 2,6-diacetylpyridine dioxin. Inorg. Chim. Acta, 273: 167-174.
11. Jencks, W.P., 1989. Excerpted from the Book Catalysis in chemistry and Enzymology Published by McGraw Hill New York.
12. Pruthi, J.S., 1980. Excerpted from the Book Spices and Condiments: Chemistry, Microbiology, Technology. Published by Academic Press Inc.
13. Allen, S.E., 1989. Excerpted from the Book Chemical Analysis of Ecological Material. Published by Black-Well Scientific, pp: 1-368.
14. Satake, M., N. Sugita and M. Katyal, 1990. Annali Di Chimica, 80: 385.
15. Komy, Z., E. Roekens and R. Van Grieken, 1988. Analysis of rain water by differential pulse stripping Voltammetry in nitric acid medium. Anal. Chim. Acta, 204: 179-187.
16. Mohan, M. and M. Kumar, 1985. Transition-metal chemistry of oxide-containing ligands-xxvii. Five- and six-coordinate complexes of iron (II) and iron (III) with 2,6-diacetylpyridine dioxin. Tetrahedron, 4: 1929-1939.
17. Miller, J.N., 1991. Basic statistical methods for analytical chemistry part 2. Calibration and regression methods. Analyst, 116: 3-14.
18. Agnihotri, N.K., V.K. Singh and H.B. Singh, 1997. Derivative spectrophotometric determination of copper (II) in non- Ionic micellar medium. Talanta, 45: 331-341.
19. Baucom, E.I. and R.S. Drago, 1971. J. Amer. Chem. Soc., 39: 469.
20. Abou-Arab, A.A.K. and M.A. Abou-Donia, 2002. Heavy metals in Egyptian spices, medicinal plants and the effect of processing on their levels. J. Agric. Food Chem., 48: 2300.