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Analytical Properties of Pyridine-2,4,6-tricarboxylic Acid: Determination of Iron in Food Samples and Quantification of Tetracyclines

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Pyridine-2,4,6-tricarboxylic acid is proposed as a new and sensitive reagent for the determination of Fe(II). The tridentate ligand combines with iron(II) in the acidic media at the pH of 2.2 to readily form an intense coloured red-purple iron(II) complex with an absorbance maximum at 488 nm in the first order derivative spectrum. The complex is stable for 3-4 h with no increase in the absorbance value. Beer's law is obeyed for iron(II) concentrations from 3.0-425.0 μg mL^{-1}. The molar absorptivity was found in the range of 0.186×10^3 L mol^{-1} cm^{-1}. The method has been successfully applied for the determination of iron(II) in food samples as well as indirect quantification of tetracycline class of antibiotics. Use of derivative spectroscopy eliminates commonly encountered spectral interferences in this determination due to impurities, excepients and additives in food and pharmaceutical samples.

Key Words: Pyridine-2,4,6-tricarboxylic acid, Ligand, Spectrometry, Tetracyclines.

INTRODUCTION

Pyridine-2,4,6-tricarboxylic acid ($ptCH_3$) has been recently picked ligand in coordination chemistry and can be easily synthesized by the oxidation of 2,4,6-trimethyl pyridine using $KMnO_4$[1]. It is a potential ligand with O and N as electron donor atoms and is water-soluble. In solid state, its complexes are now reported with zinc, cobalt, copper and Iron[2,3]. Although complexation of other pyridine carboxylic acids with metal ions in their solution state has been topic of special interest for analytical chemists, no attention has been yet paid to explore the potential use of $ptCH_3$ in chemical analysis. For example, dipicolinic acids has been demonstrated to be quite useful ligand for its ability to stabilize unusual oxidation states, for their use as electron carriers in model biological systems, in iron-induced activation of dioxygen and hydrogen peroxide and in chemical

analysis of iron at low concentration (down to 4 ppm)[4,5]. Determination of iron in particular, using organic complexing agents is of special significance in analytical chemistry because of its essential presence in food, pharmaceuticals and cement, *etc*. A variety of reagents have been proposed for the spectrophotometric determination of iron. Few reagents can be used in aqueous media[6-8] whereas most are based on solvent extraction[9-13], *e.g.*, 1,10-phenanthroline has been most widely used colourimetric reagent for Fe(II) in aqueous media, but errors can arise in the presence of Fe(III), which forms a yellow complex with 1,10-phenanthroline with slight absorbance contribution at λ_{max} for the Fe(II) complex. Another problem is increase in the absorbance of the Fe(II) complex with time because the Fe(III) complex is slowly reduced to Fe(II) complex at the pH range the procedure is applicable for analysis[14,15].

Herein, a selective determination of Fe(II) is proposed without facing the interference from Fe(III) using $ptCH_3$. Application of this reaction is described in the quantification of iron in food supplements, such as baby's milk and in the quantification of tetracycline class of antibiotics in their pharmaceutical formulations. In comparison to reported procedures for the analysis of tetracyclines such as HPLC[16-31], capillary electrophoresis[32] and spectrophotometric methods based upon UV-Visible techniques[33-35], the proposed procedure is found to provide even a more simple and accurate basis for their analysis.

EXPERIMENTAL

UV-Visible spectrophotometer Cecil-7200 with 1.0 cm matched cell was employed for measuring the absorbance values. Hanna pH meter was used to measure the pH.

Pyridine-2,4,6-tricarboxylic acid was synthesized following a literature method[1], by oxidation of 2,4,6-trimethyl-pyridine with an aqueous solution of $KMnO_4$ in 50 % yield. 2,4,6-Trimethyl-pyridine and $KMnO_4$ were purchased from Merck. Pure raw material of doxycycline, oxytetracycline and minocycline were kindly donated by Irza pharmaceuticals Lahore (Pakistan), while baby's milk sample and pharmaceuticals containing doxycycline, oxytetracycline and minocycline were purchased from the local market. All reagents used were of analytical grades and double distilled water was used throughout the experiments.

Preparations of solutions: 5.0 mg mL^{-1} of iron(II) solution was prepared by dissolving 0.500 g of ammonium iron(II) sulphate hexahydrate in a small quantity of distilled water, to which few drops of dilute sulphuric acid were added to completely change any Fe(III) into Fe(II). The volume was made up to 100 mL to obtain 5.0 mg mL^{-1} of stock solution. All working solutions were prepared from this stock solution by dilution.

Samples of drugs (100 mg) were dissolved in distilled water and the volume was made up to 100 mL. This solution was diluted further as required. A solution of 2.07×10^{-3} M ammonium ferrous sulphate containing a few drops of dilute sulfuric acid was prepared in distilled water. An aqueous solution of $ptCH_3$ (2.37×10^{-2} M) was prepared by dissolving 0.5 g of $ptCH_3$ in distilled water on boiling and then diluted with distilled water up to 100 mL. Glycine-HCl buffer of pH 2.2 was used in the experiment.

Procedure for the determination of Fe(II) with $ptCH_3$: To an aliquot of 2.0 mL of 1.0 mg mL^{-1} $ptCH_3$, 2.0 mL of 1.0 mg mL^{-1} solution of Fe(II) and 6 mL of buffer solution of pH 2.2 was added. The contents of the test tube were shaken and red purple complex was obtained at room temperature. The absorption of the complex was then noted in the visible range of 380-750 nm.

Procedure for the determination of Fe(II) in food sample: For the determination of iron, MaMa Sustagen-a nutritional milk powder was chosen which contained 150 mg of iron in a net weight of 400 g packing. 10 g of dry powdered mass (containing 3.75 mg Fe) was moistened with 5.0 mL of concentrated hydrochloric acid, warmed on a steam bath for 10 min and then 20 mL of hot water was added. Any residue was filtered and washed. The filtrate and washings were collected in a 100 mL standard flask. To an aliquot of 2 mL of the above prepared sample of milk was added 2 mL of 2 mg mL^{-1} solution of $ptCH_3$. The contents of the test tubes were shaken and the absorbance value was noted. The concentration of Fe(II) present in the milk powder was then calculated by referring to the calibration graph/regression equation.

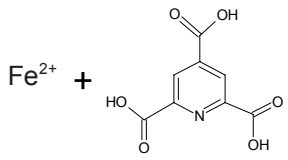
Procedure for assay of tetracyclines: 1 mL aliquots of standard solutions (10-220 μg mL^{-1}) of doxycycline, oxytetracycline and minocycline were transferred into 10 mL separate calibrated flasks containing 2 mL of ammonium ferrous sulphate and 5 mL of buffer solution of pH 2.2. The flasks were kept in a boiling water bath for 0.5 h and cooled to room temperature. 2 mL of $ptCH_3$ solution was then added and the volume was completed up to the mark with buffer solution. The absorbance of the resulting solution was measured at 488 nm against the corresponding reagent blank and calibration graph was constructed.

20 Tablets or capsules were weighed to get the average weight. An amount of powder equivalent to 100 mg each of doxycycline, oxytetracycline and minocycline were transferred into separate 100 mL volumetric flasks with 70 mL water and shaken for 5 min followed by making up to volume with water to provide a solution containing 1.0 mg mL^{-1}. An aliquot of this solution was subjected to analysis as described.

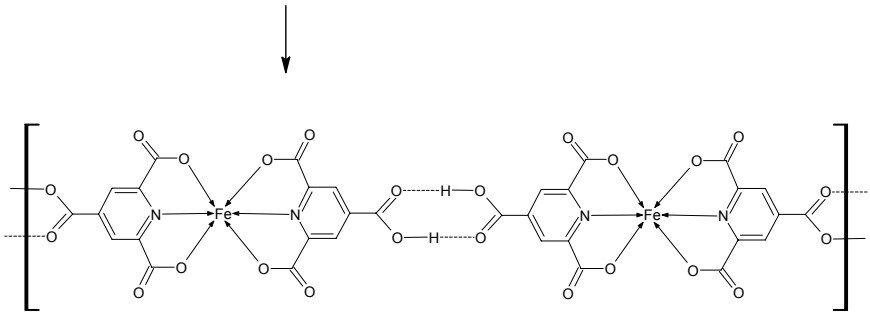
RESULTS AND DISCUSSION

In the present study, a new method was developed for the quantitation of Fe(II) in its complex matrices. A coloured complex of iron(II) with pyridine tricarboxylic acid was prepared and subsequently scanned in the visible range 380-780 nm. The spectrum showed λ_{max} at 488 nm. First order derivative spectra of the normal spectra was taken in order to eliminate spectral interferences from interfering cations and enhance sharp features of the conventional analytical band from which λ_{max} found to be 487.6 nm. The conditions like temperature, time, pH and concentration of $ptCH_3$, for chromogenic reaction were optimized. Preliminary tests revealed that temperature affects Fe(II)-$ptCH_3$ complex such that in the first 4 h there was a very slight change in the intensity on increase of temperature, beyond which the colour faded rapidly. Most stable and intensified complex was obtained at pH 2.2. It was found that any change in the pH by ± 0.1 units could affect the stability of the complex severely. From these findings, it can be inferred that Fe(II)-$ptCH_3$ complex neither requires any addition of acid or base, nor any stringent conditions of temperature. The calibration curve was drawn by measuring the absorbance for a series of concentrations of Fe(II) and the regression equation calculated is as follows:

$$Y = 0.251\ X + 0.664$$



Pyridine-2,4,6-tricarboxylic acid



Fe-$ptCH_3$ complex

The reaction of $ptCH_3$ with Fe(II) obeyed Beer's law in the concentration range of 0.003-0.425 mg × 10 mL^{-1} with molar absorptivity as 0.186 × 104 L mol^{-1} cm^{-1}. Recovery studies (Table-1) showed that iron content of

nutritional supplement-milk powder determined by this method was in good agreement with the manufacturer's claim on packing labels. A comparative data of the sensitivities of different spectrophotometric methods used for the determination of iron has been presented in Table-2. It is clear that methods based on solvent extraction are usually more sensitive than methods employing aqueous media. The presented method has the advantage of determining iron(II) in aqueous medium with comparable sensitivity. It has been found that the procedure is specific for the determination of Fe(II) because when $ptCH_3$ was reacted with iron in its trivalent state, no coloured complex was showing. This clearly depicts the selectivity and specificity of determination of Fe(II) even in the presence of matrices containing Fe(III) ions. In addition, this method is simple, rapid, stable, non-extractive and does not require additional reducing or oxidizing agent.

TABLE-1
RECOVERY OBTAINED IN THE DETERMINATION IRON(II) IN MaMa SUSTAGEN (MILK POWDER-NUTRITIONAL SUPPLEMENT) BY THE PROPOSED METHOD

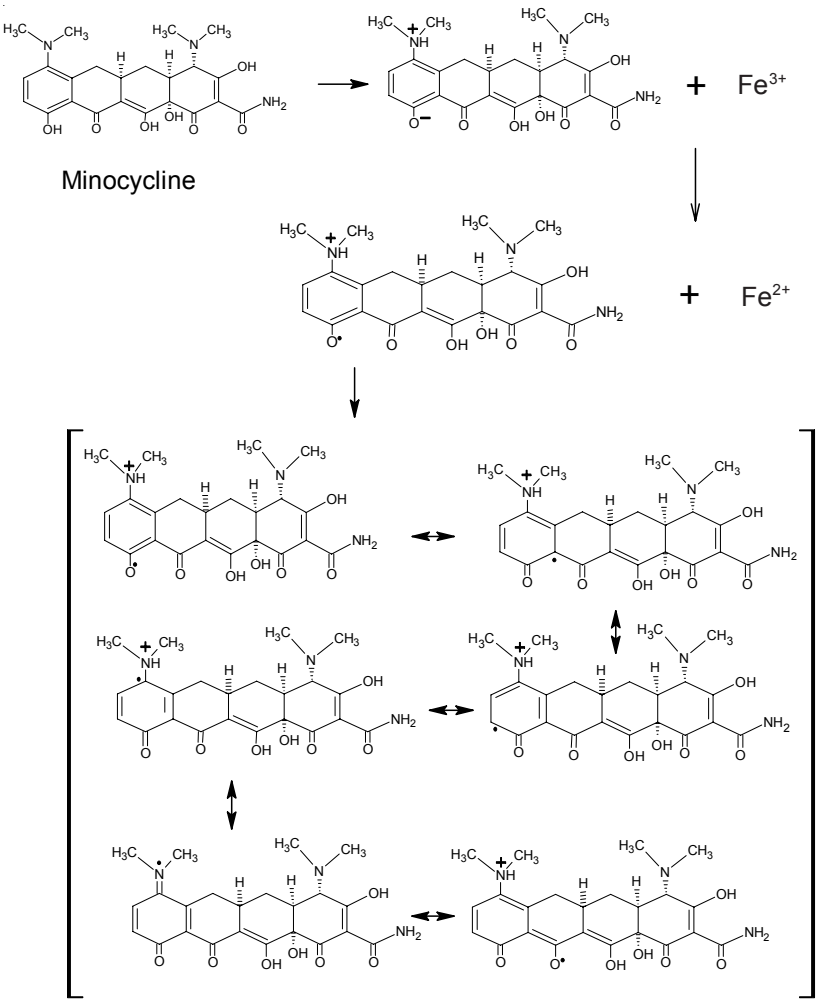
Sample no.	Amount labeled (mg/10 g)	Amount found (mg/10 g)			Mean	SD	RSD (%)
		1	2	3			
1		3.74	3.79	3.70	3.74	0.04	1.20
2	3.75						
3							

TABLE-2
COMPARISON OF SPECTROPHOTOMETRIC REAGENTS FOR ANALYSIS OF IRON(II) AND IRON(III)

Reagent	Molar absorptivity ($L\ mol^{-1}\ cm^{-1} \times 10^4$)	Reference
5,5-Dimethyl-1,2,3-cyclohexanetrione-1,2-dioxime-3-thiosemicarbazone	0.89	[6]
2-chloroquinoline-3-carbaldehydethiosemicarbazone	0.35	[7]
Diformylhydrazine	0.33	[8]
Pyridine-2-carbaldehyde-2-hydroxybenzoyl hydrazone	0.364	[9]
2,2´-Dipyridyltetraphenylborate	0.889	[10]
1,10-phenanthroline picrate	13	[11]
2,2´-Dipyridyl ketone picolinohydrazone	0.664	[12]
Cyclohexylthioglycolate	0.7	[13]
2,4,6-Pyridine tricarboxylic acid	0.186	This work

Application in the quantification of tetracyclines

Besides their antibiotic action in biological systems, tetracyclines have also been reported to exhibit antioxidant properties in recent years[36]. The reducing properties of tetracyclines could be exploited to reduce iron(III) to iron(II). The reaction proceeds in a quantitative manner and the Fe(II) ions thus formed could subsequently be complexed with $ptCH_3$ in buffer medium of pH 2.2. The proposed mechanism is shown in **Scheme-I**. Different parameters of the reactions like sensitivity, reproducibility and adherence to Beer's law were investigated using minocycline as the model representative compound (since other tetracyclines showed similar reaction). A red purple coloured product with maximum absorbance at 488 nm



Scheme-I. Proposed Mechanism of the reduction of Fe (III) and subsequent complexation with pyridine tricarboxylic acid

was formed when minocycline was allowed to react with ammonium ferric sulphate on boiling. The subsequent addition of $ptCH_3$ after cooling the reaction mixture to room temperature produced the characteristic colour of Fe(II)-$ptCH_3$ complex.

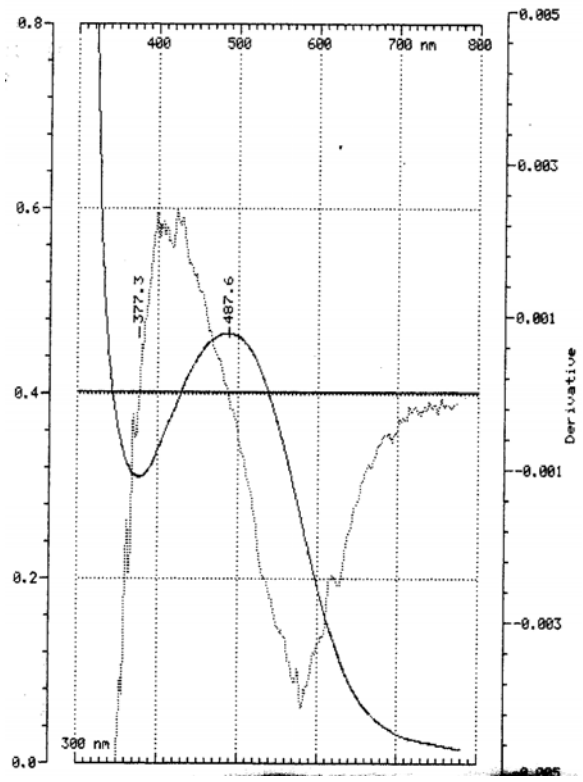


Fig. 1. First derivative spectra of reaction of minocycline with Fe(III) and then subsequent complexation with pyridine tricarboxylic acid

Maximum values of absorbance were obtained when the standard reaction flasks were kept on boiling water bath for 0.5 h after the addition of Fe(III) and buffer solution to the drug solution. The $ptCH_3$ solution was added after allowing the reaction mixture to cool down to room temperature. It was found that 2.07×10^{-3} M ammonium ferric sulphate solution in the range of 1-3 mL and 2.37×10^{-2} M $ptCH_3$ solution in the range of 2-5 mL resulted into maximum colour intensity and stability. Hence 2 mL of ammonium ferric sulphate solution, 2 mL of $ptCH_3$ solution and 5 mL of buffer solution were found to be optimum. Table-3 shows linear calibration range and equation parameters for this procedure. Separate determinations at different concentrations of each drug gave a coefficient of variation not exceeding to 2 %. The absorbance values were maximum and

remained constant in the temperature range of 80-100°C which otherwise were slow at room temperature. The complex was found to be stable for 3-4 h.

TABLE-3
OPTICAL AND REGRESSION CHARACTERISTICS OF TETRACYCLINE BY PROPOSED METHOD

Parameters	Doxycycline	Oxytetracycline	Minocycline
Colour	Red purple	Red purple	Red purple
λ_{max} (nm)	488	488	488
Stability	3 h	3 h	4 h
Beer's law limit (μg mL^{-1})	10-220	10-220	10-220
Molar absorptivity (mol^{-1} cm^{-1})	5.33×10^2	1.78×10^2	6.44×10^2
Regression equation[a]			
Slope	1.195	0.355	1.685
Intercept	0.00235	0.0004	-0.1985
Correlation coefficient	0.9990	0.9965	0.9984

[a]y=ax+ b where x is the concentration of doxycycline, oxytetracycline and minocycline in μg mL^{-1}

The interferences by various substances that often accompany tetracycline drugs in pharmaceutical formulations were studied by taking minocycline as representative drug. It was found that the commonly encountered pharmaceutical additives and excepients such as starch, lactose, talcum, glucose and aerosil do not interfere with the proposed procedure (Table-4).

TABLE-4
RECOVERY OF MINOCYCLINE HYDROCHLORIDE IN THE PRESENCE OF EXCEPIENTS AND OTHER SUBSTANCES

Material	Amount (mg)	Recovery (%) of Minocycline[a] ± SD[b]
Starch	50	100.59 ± 0.64
Lactose	50	98.83 ± 1.19
Talcum	50	98.56 ± 0.86
Glucose	50	100.10 ± 1.38
Aerosil	20	100.38 ± 1.12

[a]50 μg mL^{-1} of Minocycline taken; [b]Number of independent analytes n = 5

The applicability of this novel method to assay pharmaceutical preparations was tested. Commercial tablets and capsules containing tetracycline can be successfully analyzed by the proposed method and the results obtained are quite comparable with those already reported in United States Pharmacoepia [USP] (Table-5).

TABLE-5
COMPARISON OF THE PROPOSED METHOD WITH THE REFERENCE METHOD FOR THE DETERMINATION OF TETRACYCLINES

Drug	Label claim (mg per capsule/tablet)	Proposed method Recovery[a] %	Reference method[37] found (%)
Doxycycline	100	100.68 ± 0.73	100.32 ± 0.46
Oxytetracycline	100	100.36 ± 1.01	99.36 ± 0.73
Minocycline	100	99.63 ± 1.13	99.85 ± 0.38

[a]Proposed method-average ± standard deviation of 5 determinations.

Conclusion

Pyridine-2,4,6-tricarboxylic acid ($ptCH_3$) is demonstrated to be a versatile analytical reagent which can find variety of applications in quantitative analysis. Its complex with iron provides opportunity of its detection at lowest reported level. In addition, indirect quantification of tetracyclines is also accomplished. Thus this new method could be well suited for the routine assay and evaluation of the iron in food samples as wells as quantification of drugs in preformulation and dosage forms to assure high standard of quality control.

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