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Preparation of Some *as*-Triazines, Their Evaluation as Spectrophotometric Reagents and Determination of Trace Amount of Iron in Certain Food and Natural Samples

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Abstract

Spectrophotometric reagents 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) and 3-(2-pyridyl)-5,6-bis(p-methoxyphenyl)-1,2,4-triazine (PBMPT) were prepared from commercially available reactants simply by condensing pyridyl-2-hydrazidine with 1,2-dicarbonyl compounds. These organic reagents were used further to determine the trace amount of iron in various natural and food samples by spectrophotometric method. The absorptiometric features of iron (II) complexes of PDT and PBMPT were determined. The molar absorptivites of iron (II) complexes of PDT and PBMPT were found to be 24,181 L mol-1 cm-1 (at 556.0 nm) and 32,091 L mol-1 cm-1 at (561.8 nm) respectively. The reliability of these absorptivities were verified by standard addition technique and also by comparing the results of atomic absorption spectroscopy method. The complexes of both the reagents obey Beer's law within the experimental concentration range 0.032mg/L - 0.159 mg/L.

Keywords: as-triazine; spectrophotometric method; food samples; iron.

I. Introduction

Iron is an essential naturally occurring micronutrient involved in human immune function, cardiovascular health and a crucial structural element in many complex enzyme systems that perform significant metabolic actions[1-3]. Iron's most well-known job in human nutrition is in the synthesis of hemoglobin, a protein that delivers oxygen to all of the body's cells. Iron deficiency, anemia, lethargy, and other issues can result from low iron storage in the body. Hemochromatosis, a severe condition that damages the body's organs, can be caused by consuming significant amounts of iron on a regular basis.

Iron deficiency has become a foremost contributor to the global affliction of disease. Reported data shows that worldwide frequency of Fe deficiency to be about 30% [4]. The burden of Fe related diseases are common in low-income countries as well as resource rich countries. Inadequate intake of this micronutrient becomes a public health concern. The recommended intake of Fe is not always achieved even in USA, Great Britain and Germany as stated by the nutritional surveys conducted in those countries [5-6]. So, quantification of iron by using low cost but highly sensitive reagent can play a significant role in treating the diseases caused by iron deficiency or over intake of iron; especially in developing countries like ours.

Since food samples such as banana, prune, potato, carrot and other fruits, vegetables and various types of seeds contain iron, quantification of iron is extremely important in nutrition diagnosis and food management research. That's why, rapid and sensitive technologies for determining iron are in high demand.

Iron may be determined from natural samples using a variety of methods. AAS, ICP-MS, and colorimetric procedures are among the most popular [7-8]. Because of its availability, simplicity, and low cost, the colorimetric approach was used in this investigation [9-12]. When a suitable chromogenic reagent is employed, this approach can be used to determine trace levels of iron. Ferroin functional group is quite selective reagent for iron. 1,2,4-triazines containing the ferroin-producing chromogen are highly sensitive to the formation of colorful complexes with iron (II) ions [13-18]. 1,2,4-triazines can be easily made in the laboratory using readily accessible, relatively inexpensive chemicals [13,19]. PDT and PBMPT are extremely stable organic reagents with high stability constant with iron (II)[20]. Cu(II) complexes of these 1,2,4-triazines (PDT, PBMPT) were synthesized as well as reported to possess promising biological activities [21]. Amount of iron can be enumerated using a UV/Vis spectrophotometer in terms of iron (II) complexes of these triazines [15,22].

Recently, the determination of iron in iron solutions using the chromogenic reagents PDT, PBMPT, and PBBT has been carried out by Islam *et al.* [22]. Moreover, they investigated the stability constant of these reagents' complexes with iron. The Fe(II) complexes of PDT, PBMPT and PBBT were found to be exceptionally stable complex with the mean logK values 16.48, 17.48 and 15.54 respectively. These values are significantly similar to the iron (II) complexes of Ferene, 2,2'-bipyridine, Ferrozine and 1,10-phenanthroline that have been reported [20]. The possibility of using these compounds as promising chromogenic reagents for iron measurement is reflected by their higher sensitivity and stability constant values.

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Besides, the trace amount of iron in water, food, and pharmaceutical samples has also been studied by using PDT and PBMPT spectrophotometric reagents by Hossain *et. al*[7].

Based on these references, two *as*-triazine reagents have been used spectrophotometrically in this research work, to determine the iron contents of some food samples collected from Dhaka. The key objective of our current work is to establish a simple and robust method to quantify Fe (II) content in food samples and verify its reliability by comparing the results with that of AAS method.

II. Experimental

Preparation of Spectrophotometric Reagents

3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT), and 3-(2-pyridyl)-5,6-bis (*p*-methoxyphenyl)-1,2,4-triazine (PBMPT) were prepared according to the method described by Stephen and Islam [13]. In the first step 1,2-diketones were prepared by oxidation of their corresponding benzoin which were previously prepared by benzoin condensation of benzaldehyde. Oxidation of benzoin was done under mild refluxing condition for 90 minutes in presence of ammonium nitrate, copper acetate and 80% acetic acid. Bright yellow needle shaped crystal was collected by suction filtration, washed with water and air dried. Secondly Pyridyl-2-hydrazidine was prepared from 2-cyano pyridine and hydrazine hydrate. A mixture of 2-cyanopyridine, (99%) hydrazine hydrate in ethanol was stirred on a magnetic stirrer at room temperature for about two hours.

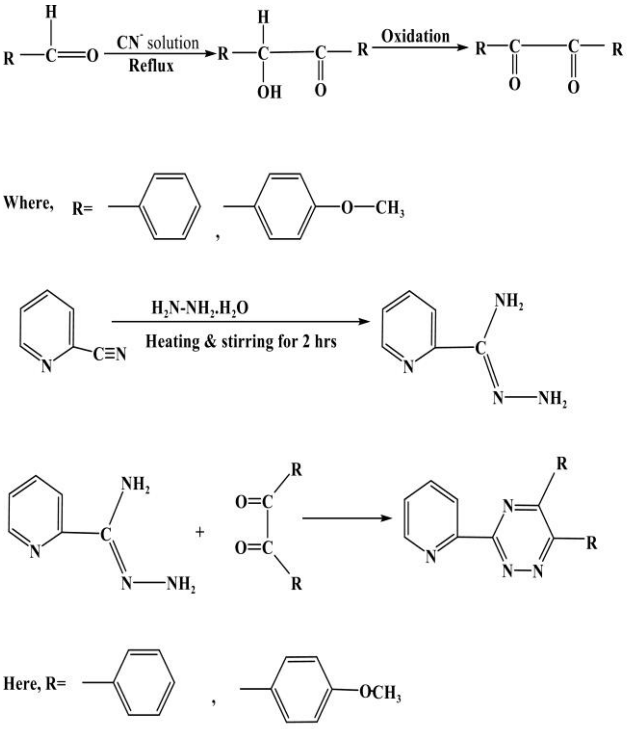
Table 1. Comparison of Melting Points of Spectrophotometric Reagents (PDT & PBMPT)

Organic Reagents	Literature Value (°C)	Experimental Value (°C)
PDT	188-190 [22]	186-188
PBMPT	153-155 [22]	153-155

Table 2. Percentage of Carbon, Hydrogen and Nitrogen in the organic reagents

Reagents	%C found (calcd.)	%H found (calcd.)	%N found (calcd.)
PDT	77.05 (77.39)	4.12 (4.55)	18.11 (18.06)
PBMPT	72.01 (71.34)	4.54 (4.89)	15.10 (15.12)

Dichloromethane was used for the extraction of the product in organic layer and then dried over anhydrous sodium sulphate. Recrystallization of crude product was done from hot toluene after evaporation of solvent completely. Needle-shaped white crystalline product obtained were collected by filtration. Finally equimolar mixture of 1,2-diketone and pyridyl-2-hydrazidine was refluxed and kept overnight. The yellow crystals were collected and air dried after washing with hexane. The reaction scheme is:



Melting points and elemental analysis data affirmed the purity of the prepared triazines.

Reagents

Preparation of Standard Iron (II) solution

AnalaR grade Mohr's salt and concentrated H_2SO_4 were used for the preparation of standard stock iron (II) solution. 3.5100 g of Mohr's salt was dissolved in little amount of distilled water followed by the addition of a small volume (5mL) of H_2SO_4. Then the solution was diluted to 500mL with distilled water. Suitable aliquots were pipetted for the preparation of solution with required concentration.

Preparation of Organic Reagent (PDT & PBMPT) Solution

A stock solution of concentration 0.005 M of the organic reagent was prepared by dissolving 0.3881 g PDT and 0.4625 g PBMPT in small volume of ethanol into two different 250 ml volumetric flask respectively. To dissolve PDT a few drops of concentrated hydrochloric acid was added and diluted up to the mark with ethanol. Further dilution was made to prepare the required concentration of the solution for spectrophotometric examination.

Preparation of Buffer Solution

Acetic acid-sodium acetate buffer with pH 4.76 was prerequisite for the preparation of Iron (II) complex solution. 250 mL 2M CH_3COOH solution and 250 mL 2M CH_3COONa solution were mixed in a 500 mL volumetric flask to prepare required acidic buffer solution.

Preparation of reducing agent solution

10% (w/v) solution of reducing agent was prepared in 250 mL volumetric flask by dissolving 25 g of the pure hydroxylamine hydrochloride in distilled water.

Apparatus

A double beam UV-visible Spectrophotometer (Model: UV-1800, SHIMADZU, Japan), rectangular glass cells of path length 1 cm, a rotary evaporator (Model: BUCHI Labortechnik AG, Switzerland), a melting point meter (Model: KRUSS, A. KRUSS OPTRONIC, Germany) were used in this research work. Elemental analysis were performed using CHNS elemental analyzer in the INARS laboratory of BCSIR by ASTM method.

Samples Collection and Preservation

Food samples (Potato, Carrot, Honey, Horlicks, Black Seed, Milk, Prune, Banana, White Bread, Aloe Vera) were collected from the local markets of Dhaka. For further analysis preservation of the samples were ensured in suitable dry condition.

Analytical procedure

General Procedure of Iron (II) Complex solutions of Organic Reagents

Preparation of the iron (II) complexes of PDT and PBMPT for spectrophotometric determination were done by the following general procedure: 5.0 mL of standard iron solution was transferred to a 25 mL volumetric flask and 2 mL of 10% (w/v) hydroxylamine hydrochloride and 5 mL of the organic reagent solution were added, followed by 5 mL buffer solution of pH 4.76. The content of the flask was diluted to up to the mark by the addition of ethanol. Spectra of all solutions were recorded against reagent blanks in the aqueous-ethanol medium as the buffer solution and reducing agents were dissolved in water and organic reagents PDT and PBMPT were dissolved in ethanol. The wavelengths of maximum absorbance, (λ_{max}) of PDT iron (II) and PBMPT-iron (II) complexes were recorded. Furthermore calibration curve of both the iron (II)-PDT and iron (II)-PBMPT were plotted. The linear regression coefficient of the graph was used for further calculation of specific absorptivity (a) and molar absorptivity (ε) of different iron complexes with different Fe(II) at the wavelength of maximum absorbance.

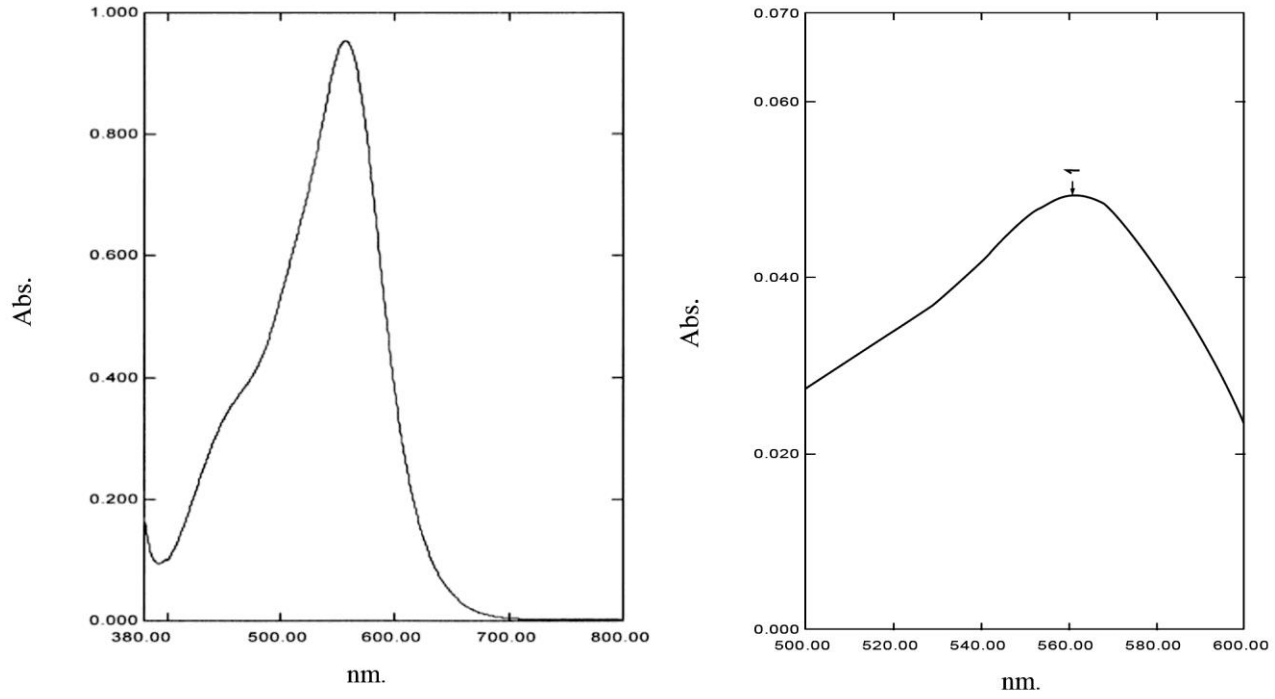


Fig. 1. UV-Vis spectra of Fe (II) complex of PDT (left side) and PBMPT (right side) in aqueous ethanol medium recorded against reagent blank

Table 3. Data for Sensitivity Determination of PDT-Iron (II) Complex in Aqueous-Ethanol Medium

Concentration of iron (II) in mg/L	Absorbance at 556 nm (λ_{max})	Sensitivity at 556 nm (λ_{max})
0.0	0.000	
0.8	0.3464	
1.6	0.6927	a = 0.078 ε = 24,181
2.4	1.0391	
3.2	1.3855	
4.0	1.7318	
4.8	2.078	
5.6	2.4246	

Table 4. Data for Sensitivity Determination of PBMPT-Iron (II) Complex in Aqueous-Ethanol Medium

Concentration of iron (II) in mg/L	Absorbance at 556 nm (λ_{max})	Sensitivity at 556 nm (λ_{max})
0.0	0.000	
0.9	0.5171	
1.8	1.0343	
2.7	1.5514	a = 0.086 ε = 32,091
3.6	2.0685	
4.5	2.5857	
5.4	3.1028	
6.3	3.6199	

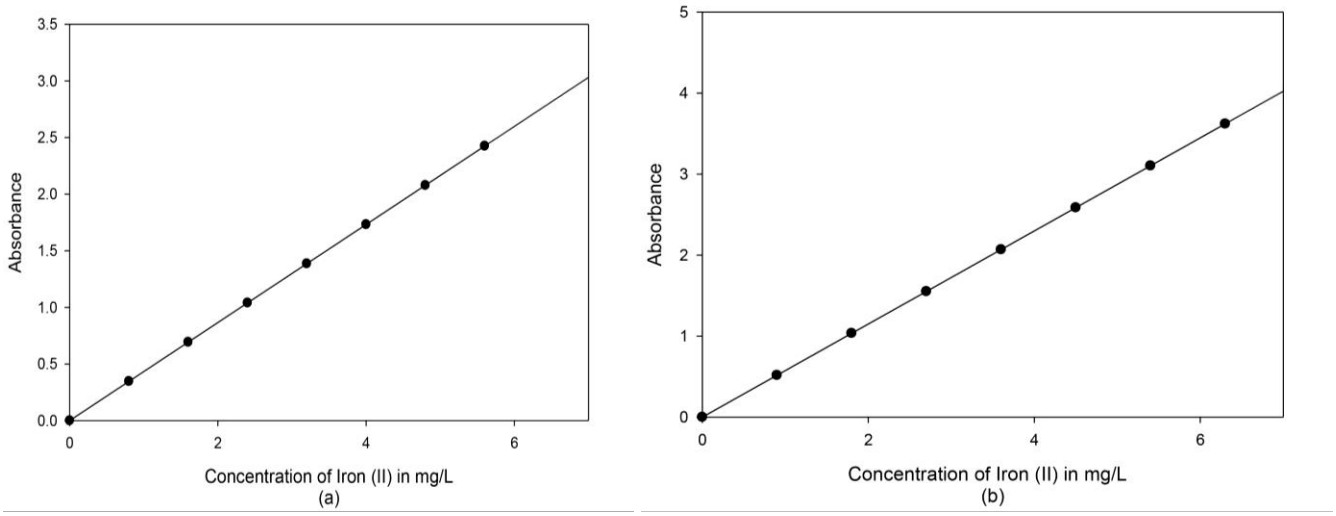


Fig. 2. Calibration curves of (a) Fe(II)-PDT complex and and (b) Fe(II)-PBMPT complex in aqueous-ethanol medium

Preparation of Food Samples

Solid foodstuffs were prepared following the scheme described by Dubey *et al* [23]. About 10 g of the homogenized sample was dried for 3 hours at 105°C temperature in oven. The dried samples were charred by heating in a porcelain crucible. In a muffle furnace the previously charred samples were heated till the formation of a whitish or greyish ash. Dilute H_2SO_4 was added to that ash with careful stirring. Then the solution was transferred to a volumetric flask followed by the filtration and dilution to 50 mL. The solution of the iron (II) complex was prepared following the procedure described by Islam *et al* [20].

Liquid samples were processed by the method of Sharma *et. al* [24]. 100mL of sample was evaporated without frothing by heating carefully in porcelain crucible. After complete removal of moisture, greyish ash was obtained on heating strongly in muffle furnace. Utmost precaution was taken to avoid loss by sputtering. The ash was treated with sulfuric acid and volume was made up to 50mL with water.

Determination of Iron Content in Food Samples

At λ_{max}, the absorbance of the prepared solutions was measured against similarly prepared reagent blank solution, and the iron content of the samples was determined using the prepared calibration curve. To minimize the experimental error at least two trials were carried out with each sample. Furthermore validity of the experiment was confirmed by the standard addition method and Atomic Absorption Spectroscopy Method (AAS). In standard addition method known amount of iron is added with the sample solution and repeated measurement is performed.

III. Results and Discussion

Organic reagents: PDT and PBMPT were prepared from pyridyl-2-hydrazidine and corresponding 1,2-diketones. Purity of the reagents were confirmed from the elemental analysis and melting point data. Results obtained from elemental analysis were in good agreement with the calculated data (Table 2.) indicating high purity of the prepared organic reagents.

The UV-Vis spectra exhibited a single sharp peak in the visible region with λ_{max} values of 556 and 561.8 nm for Fe(II)-PDT and Fe(II)-PBMPT complexes respectively. The molar absorptivities, (ε) of the Fe(II)-PDT and Fe(II)-PBMPT at λ_{max} in aqueous ethanol medium are 24,121 $Lcm^{-1}mol^{-1}$ and 32,091 $Lcm^{-1}mol^{-1}$ respectively. The stoichiometry of the Fe(II)-PDT/PBMPT complexes were found to be 1:3 ratio, λ_{max} and molar absorptivity (ε) values are in good agreement with that of reported by Islam *et. al* [13]. The results of that study demonstrated that these ligands (PDT/ PBMPT) form tris-Fe (II) complex revealing their bidentate property.

Determination of Iron

The absorption of the prepared solutions of food samples were measured by transferring the samples directly to the cuvette from the 25 mL volumetric flask following the method proposed by Dubey *et al* [24]. Experimental samples were analyzed multiple times using both PDT and PBMPT and the results are given in Table 5.

Quantitative data obtained were validated using the standard addition method. Results with a very small standard deviation proved this spectrophotometric determination of a trace amount of Fe(II) contents to be successful.

Table 5. Fe(II) content of Food Samples Determined by Both PDT and PBMPT Following Three Different Methods

Sample	Spectrophotometric Method (mg/L)		Standard Addition Method (mg/L)		AAS (mg/L)
	By PDT	By PBMPT	By PDT	By PBMPT	
Potato	1.590 ± 0.110	1.610 ± 0.110	1.670 ± 0.080	1.640 ± 0.110	1.620
Carrot	0.600 ± 0.170	0.620 ± 0.180	0.690 ± 0.050	0.660 ± 0.110	0.640
Honey	0.750 ± 0.180	0.770 ± 0.170	0.820 ± 0.070	0.790 ± 0.090	0.780
Horlicks	5.200 ± 0.270	5.230 ± 0.310	5.270 ± 0.080	5.250 ± 0.190	5.240
Black Seed	2.040 ± 0.780	2.070 ± 0.760	2.090 ± 0.140	2.110 ± 0.350	2.080
Milk	6.150 ± 0.001	6.339 ± 0.011	6.030 ± 0.035	6.305 ± 0.134	6.490
Prune	2.789 ± 0.002	2.981 ± 0.002	2.790 ± 0.025	3.185 ± 0.078	3.210
Banana	1.895 ± 0.008	1.963 ± 0.001	1.905 ± 0.001	2.010 ± 0.042	2.180
White Bread	2.927 ± 0.008	3.012 ± 0.002	2.940 ± 0.028	2.995 ± 0.078	3.010
Aloe Vera	0.979 ± 0.003	1.019 ± 0.029	0.960 ± 0.021	1.040 ± 0.071	1.080

IV. Conclusion

Preparations of *as*-triazine containing ferroin functional group 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT), and 3-(2-pyridyl)-5,6-bis(*p*-methoxyphenyl)-1,2,4- triazine (PBMPT) from commercially available relatively cheaper reactants have been described. These compounds were characterized by recording their melting points and elemental analysis.

The absorptiometric characteristics, sensitivity of iron (II) complexes of these compounds (PDT, PBMPT) has been studied. High values of molar extinction coefficients (ϵ) for these complexes reflects high sensitivity.

Estimation of trace amount of Fe(II) spectrophotometrically in food samples both PDT and PBMPT proved to be successful since the results are in well agreement with the literature value as well as with the AAS result with a small deviation [23, 24]. The higher sensitivity and easy preparative route of the compound PBMPT make this a particularly more useful chromogenic reagent for the determination of trace amount of iron in food samples over PDT. The spectrophotometric approach with sensitive reagent is more convenient over AAS because it is difficult to transport the AAS machine and maintain for continuous monitoring in agricultural or industrial settings.

In the present study, it is established that determination of trace amount of iron from natural and food samples using *as*-triazines is highly effective and convenient. In addition, the results obtained from this study indicate that the quantity of iron in food samples is of varying range. So, the people having the symptoms of iron deficiency should include iron-rich food like milk, prune, potato, black seed and Horlicks (in case of unavailability of natural sources) in their daily diets by the consultation of doctors. In contrast, people having iron toxicity and have the symptoms of hemochromatosis should avoid these iron rich foods and can consume low iron containing foods like honey, carrot etc. To maintain sound health, we should have proper daily diets with suggested amount of iron.

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