



*Original Contribution*

**DETERMINATION OF TRACE IRON (III) BY CATALYTIC KINETIC SPECTROPHOTOMETRY WITH N,N-DIPHENYLAMINE**

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**ABSTRACT**

A catalytic kinetic spectrophotometric method for the determination of trace iron (III) is described based on the catalytic effect of iron (III) on the reaction between N,N-diphenylamine (DPA) and hydrogen peroxide in acidic medium. The reaction was monitored spectrophotometrically by measuring the absorbance of the reaction product at 580 nm. The variables that affected the reaction rate were investigated and the reaction conditions were established. Iron (III) can be determined in the range of 2 –100 ng/ml with detection limit of 0.94 ng/ml. The proposed method was successfully applied to the determination of iron (III) in industrial water.

**Key words:** iron, catalytic kinetic spectrophotometry

**INTRODUCTION**

Iron is the fourth most abundant element in the earth's crust. It occurs in a variety of rock and soil minerals in oxidation states 2 and 3; but it is only a trace element in biologic systems. Iron plays a central role in the biosphere. It is an essential component or cofactor of numerous metabolic reactions. Every living cell in both plants and animals contains iron. It is involved in oxygen transport and electron transfer and in enzymes including hydroxylases, peroxidases and dismutases [1]. Iron deficiency anaemia is one of the world's most common nutritional deficiency diseases. Evidence has been presented that at low levels iron is an essential element in the diet, whereas at higher concentrations it is toxic. The toxicity of iron, and in particular iron overload, has aroused considerable interest in recent years [2].

A number of sensitive analytical methods are available for the determination of iron.

At present some of the most commonly used methods include spectrophotometry [3-5], chemiluminescence [6], fluorescence analysis [7], polarographic and voltametric analysis [8, 9], flow-injection analysis [6, 10, 11], atomic emission and atomic absorption

spectrometry [12-14] and others [15, 16]. Atomic absorption spectrometry methods are disadvantageous in terms of cost and instruments used in routine analysis. Extractive methods are highly sensitive but are generally lacking in simplicity.

Catalytic kinetic methods are an attractive alternative for the determination of trace amounts of iron. Such methods have the general advantage of combining high sensitivity with relatively simple procedures and apparatus. Several kinetic methods based on the catalytic action of iron on the oxidation of various organic compounds have been reported in several reviews [17-19] and original papers [20-22]. The chief shortcoming of the kinetic catalytic determinations is that in most cases highly sensitive methods are poorly selective, or vice versa.

Therefore, more sensitive and selective methods that can determine the low concentrations of iron rapidly and conveniently are still needed.

This paper describes a simple and accurate visible catalytic spectrophotometric method and optimum conditions for the determination of iron (III). The method is based on the catalytic effect of the iron (III) on the reaction between N,N-diphenylamine (DPA) and hydrogen peroxide in acidic medium.

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## EXPERIMENTAL

### Reagents

All solutions were prepared from analytical grade chemicals and double distilled water. The laboratory glassware was treated with an alkaline solution, and then kept in HCl (1:1) overnight, followed by rinsing with distilled and double distilled water. A stock Fe (III) solution ( $\approx 0.1$  M) was prepared by dissolving  $\approx 7$  g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (Merck) in 250 ml of 2M HCl (Merck). Its exact concentration was determined gravimetrically. Working solutions were daily prepared by appropriate dilution with 0.01 M HCl. Diphenylamine (DPA) (Merck) stock standard solutions ( $1.0 \times 10^{-2}$  M) were prepared by dissolving the reagent in concentrated formic acid (Merck), thus ensuring acidic medium. Water solutions of hydrogen peroxide were prepared from 35% hydrogen peroxide (Merck).

### Apparatus

Kinetic measurements were made on a Specol 11 spectrophotometer fitted with 1-cm cells. The spectrophotometer cell compartments were thermostated by means of circulating water.

Absorption spectra were recorded on a Specord-UV-Vis spectrophotometer using 1-cm quartz cells.

The acidity of the reaction mixture was controlled on a pH-meter "Consort R 400".

### Calibration Procedure

In a three-compartment reaction vessel (mixer) were placed, 6.5 ml of  $6.0 \times 10^{-5}$  M DPA solution in the first; 0.5 ml of 9.0 M hydrogen peroxide solution in the second and 0.5 ml of working Fe (III) solutions or sample in the third compartment.

The well-closed mixer with reagents was kept for 10 min at a temperature of 30°C and then the reagents were mixed by energetic shaking and transferred into a 1 cm constant temperature cell of a Specol 11 spectrophotometer. The absorbance at 580 nm was recorded as a function of time. The reaction rate was calculated from the absorbance-time kinetic curves by using the tangent method. When the "fixed time" method was used the absorbance was read 15 min after the mixing.

### Procedure for Analysis of Water Samples

Collected samples were analysed within 24 h after filtration.

In a test tube were placed: 6.5 ml of

$6.0 \times 10^{-5}$  M DPA solution, 0 - 0.5 ml of standard Fe (III) solution or water sample solution, or distilled water. The test tube was kept at 30°C in thermostated water bath for 10 min. Then 0.5 ml of 9.0 M hydrogen peroxide was added, the mixture was homogenised by shaking and transferred into a 1 cm constant temperature cell of the spectrophotometer. The absorbance was read at 580 nm 15 min after adding of hydrogen peroxide.

## RESULTS AND DISCUSSION

The oxidation of N,N-diphenylamine by hydrogen peroxide in acidic medium yields blue-violet product diquinonediimine [23], which has an absorbance maximum at  $\lambda = 583$  nm. This reaction is slow, but it is sharply increased by the addition of trace amounts of Fe (III). This process was monitored spectrophotometrically by measuring the increase in absorbance at 580 nm versus time.

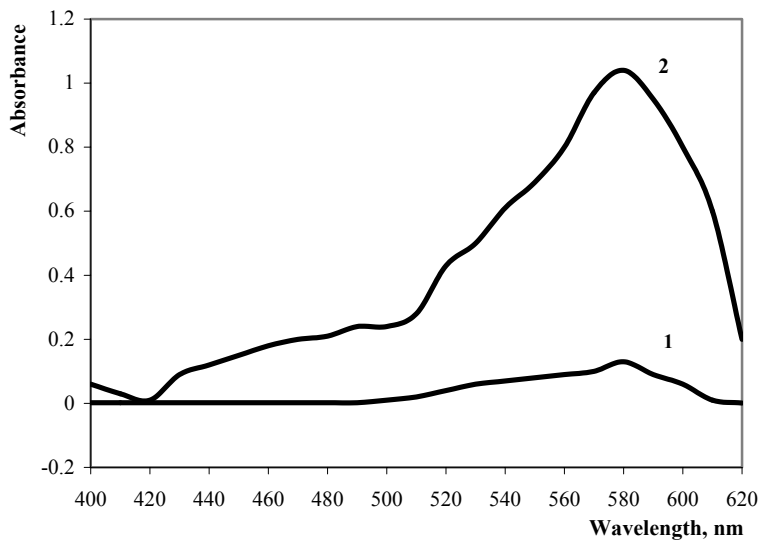
The influence of the reaction parameters (absorption spectra, acidity, temperature, reagent concentration) on the reaction rate was studied in the presence and absence of Fe (III) to establish the optimum reaction conditions. The system was optimised by altering each variable in turn, while the others were kept constant.

The absorption spectra of the reaction products are presented in **Figure 1**. As can be seen, the reaction products in the presence (**curve 2**) and in the absence of the catalyst (**curve 1**) have absorption maximum at 580 nm, which was chosen as the measurement wavelength. In the absence of the catalyst the reaction proceeded very slowly.

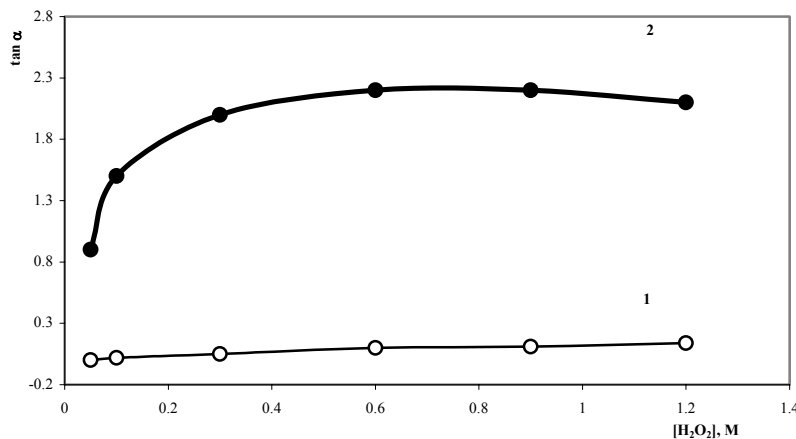
The influence of the acidity on the reaction rate was studied at different pH values, obtained by adding appropriate volumes of 0.2 M sodium hydroxide to 100 ml of three-component mixture of 0.04 M boric, phosphoric and acetic acid. The experiments led to the conclusion that at very low or higher (above pH 4) pH values, the observed reaction rate dramatically decreases. It was experimentally confirmed that the reaction proceeds faster without buffer solutions and high concentrations of formic acid ensure constancy of the pH in the reaction mixture (pH 3.8). That is why formic acid is used as the solvent throughout.

The influence of the temperature was studied between 20 and 60°C (data not shown). As the reaction temperature increased, there was an increase of the reaction rate. A temperature of 30°C was adopted as optimum since it provides good reaction rate, low background and can be easily maintained.

**Figure 1. Absorption spectra of the reaction products. (1) in the absence of the catalyst and (2) in the presence of the catalyst Fe(III)  $5 \times 10^{-7}$  M .**  
(temperature  $30^{\circ}\text{C}$ ;  $5.2 \times 10^{-5}$  M DPA; 0.6 M  $\text{H}_2\text{O}_2$  in a concentrated formic acid medium)



**Figure 2. Influence of hydrogen peroxide concentration.**  
Other conditions and symbols as in Figure 1.



**Figure 2** reflects the influence of the hydrogen peroxide concentration. The reaction rate increases with increasing hydrogen peroxide concentration up to 0.6 M  $\text{H}_2\text{O}_2$ . Higher concentrations do not increase the reaction rate, but instead a slight decrease is observed. Therefore the concentration of 0.6 M of hydrogen peroxide was chosen as the optimal.

The effect of the diphenylamine concentration on the catalysed and non-catalysed reaction was also investigated, in order to obtain a constant and maximum sensitivity. The results reveal that the rate of the catalysed reaction increases with increasing DPA concentration, attaining the maximum value, and then remains constant. A

concentration of  $5.2 \times 10^{-5}$  M was finally selected (**Figure 3**).

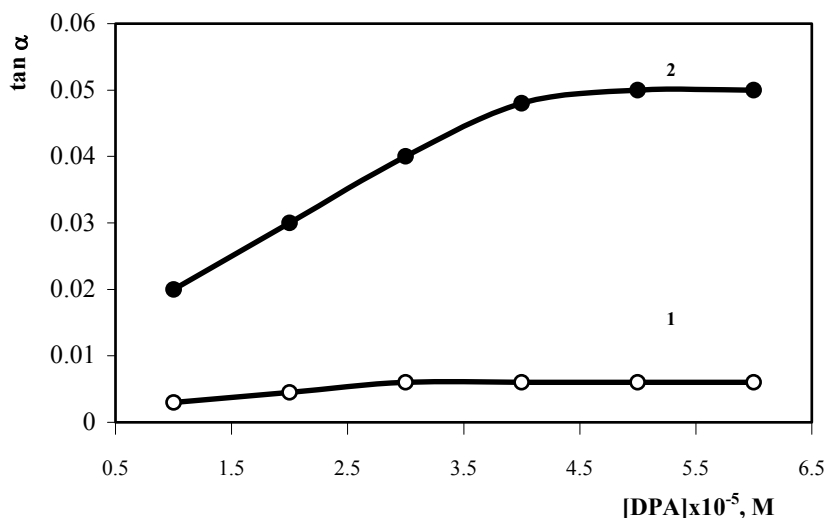
The most suitable reaction conditions proved to be:

$\lambda$  580 nm; temperature  $30^{\circ}\text{C}$ ; 0.6 M  $\text{H}_2\text{O}_2$  and  $5.2 \times 10^{-5}$  M DPA in a concentrated formic acid medium.

#### Calibration graph

Under the optimal conditions a linear calibration graph in the range of 2 –100 ng/ml was obtained, using “fixed time” method. This method was applied to the change in absorbance over an interval of 15 min from initiation of the reaction because it provided the best regression and sensitivity.

**Figure 3. Influence of DPA concentration.** Other conditions and symbols as in Figure 1.



The equation of the calibration graph was:  $A = 0.078 + 0.034 C$ , where  $C$  is the concentration of iron (III) expressed in ng/ml. The correlation coefficient of the graph was 0.9978. The relative standard deviation for ten

replicate determinations of 24 ng/ml ( $n=10$ ) was 2.7%, and the detection limit calculated from  $LOD = KS_b/b$  ( $K=3$ ,  $S_b$  is the standard deviation of the blank and  $b$  is the slope of calibration graph) was 0.94 ng/ml.

**Table 1.** Effect of foreign ions on the determination of iron(III) (24 ng/ml)

Foreign ion	Tolerated ratio
	[ion] : [Fe(III)]
Na(I), K(I), NH <sub>4</sub> (I), Mn(II), Fluoride, Chloride	1000
Al(III), Mg(II), Sn(IV), Cd(II), Pb(II), Zn(II)	200
Li(I), Ca(II), Ni(II), Cu(II), Co(II), Cr(III), Cr(VI), Sulphate	100
Ag(I), Bi(III), Mo(VI), Nitrate	50
Phosphate, Citrate	30
V(V)	20
Fe(II)	5

**Table 2.** Recovery test for iron(III) determination in water samples

Sample	Iron(III) concentration, ng/ml		Recovery, %	RSD, %
	Added	(mean ± standard deviation) (n=5)		
Industrial water	-	101.3 ± 2.6	-	2.6
	25.00	126.0 ± 4.4	98.8	3.5
	50.00	152.6 ± 3.2	102.6	2.1
	100.00	202.7 ± 5.9	101.4	2.9

### Interference study

To study the selectivity of the proposed method, the influence of foreign ions on the determination of 24 ng/ml Fe (III) was

investigated. The tolerance limit was defined as the concentration at which the species caused an error of less than ±5%. The interferences are listed in **Table 1**.

The results obtained indicate that almost all the common cations that are normally associated with iron in various types of sample, e.g. mineral, industrial and biological samples, do not interfere with the determination of iron (III).

### Application

The usefulness of the proposed method was tested by determining the iron (III) concentration in samples of circulating industrial water from a glass factory (Pleven, Bulgaria). This was previously determined by AAS to be 1.83  $\mu\text{M}$  (102.2 ng/ml). Iron (III) concentration was determined by applying the calibration curve and standard addition methods. Recovery tests (**Table 2**) were satisfactory (about 98.8–102.6%) with relative standard deviations of 2.1–3.5 %.

### CONCLUSIONS

The kinetic spectrophotometric method developed for the determination of iron (III) is simple and inexpensive, uses readily available reagents, allows rapid determination and shows adequate selectivity, low limit of detection and good precision. With this method it is possible to determine concentrations as low as 1 ng/ml without the need for any pre-concentration step. It was successfully applied to the determination of iron (III) in industrial water.

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