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Catalytic Kinetic Spectrophotometric Method for the Determination of Traces of Iron

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A novel kinetic spectrophotometric method for the determination of trace iron is developed based on the catalytic effect of Fe(III) on the oxidation reaction of dibromo-*p*-chloro-chlorophosphonazo (DBC-CPA) by potassium periodate. In the medium of 1.0×10^{-2} mol/L of sulfuric acid, the maximum absorption peak of Fe(III)-(DBC-CPA)-KIO_4 system locates at 548 nm. The absorbance difference (ΔA) is linearly related with the concentration of iron(III) over the range of 0.010-0.15 μg/mL and fitted the equation: $\Delta A = 5.810C$ (C: μg/mL) + 0.1592, with a regression coefficient of 0.9936 at the wavelength. The detection limit of the method is 5.06 ng/mL. The method was used to determine trace iron in the blood sample of human. The relative standard deviation was 0.87-1.42 % for 11 replicate determinations. The recovery of the standard addition was 99.98-100.7 %. The analytical results were in good agreement with those of atomic absorption spectrometry.

Key Words: Iron, Catalytic kinetic spectrophotometry, Dibromo-*p*-chloro-chlorophosphonazo, Potassium periodate, Blood sample.

INTRODUCTION

Iron is not only the fourth element from abundance in the earth's crust, but also it is one of the most abundant elements in a human body. It is an important component of human tissues. Iron combines with original porphyrin to form haemachrome, with which globin combines to form many important functional enzymes in ferrohemoglobin, muscle proteins and cells such as cell colouring matters, cell colouring matter oxidation enzymes, reductive type niacinamide adenine dinucleotide dehydrogenase, *etc*. These contain the iron that combines with the proteins. Iron element has a function of making blood, participates in the syntheses of blood protein, cell pigment and various enzymes and accelerates growth. Iron takes an action of transporting oxygen and nutrient substances in blood. Human body lacks iron results in small cell cellularity anaemia, the reduction drop of immunity function and metabolism turbulence. If iron matter is not enough, it results in anaemia lacking iron property and makes a person's colour fading-yellow. Thus, developing highly sensitive method for the determination of iron and applying some practical biological sample analysis such as the determination of blood sample has a very important applied value.

The methods for the determination of iron proposed have atomic absorption spectrometry[1,2], polarography[3], stripping voltammetry[4], fluorescence method[5], which have the disadvantages that the instruments are expensive and operations are complex, *etc*. Photometry is more suitable to the determination of iron due to the advantages that operation is simple and the instrumentation is cheap, *etc*. Although some determination systems have been proposed using kinetic photometry of the determination of iron[6-8], the selectivity of the methods is very poor to common elemental ions Cu^{2+}, Ti^{4+}, *etc*. Thus, development of a new kinetic spectrophotometric method for the determination of iron still has an important sense.

Dibromo-*p*-chloro-chlorophosphonazo[9] (simplified as DBC-CPA, $C_{22}H_{13}N_4O_{11}PS_2Br_2Cl_2$), is a chromogenic agent, dark-perple powder, easily soluble in water and was used in the determination of copper[10]. Its structure is as follows:

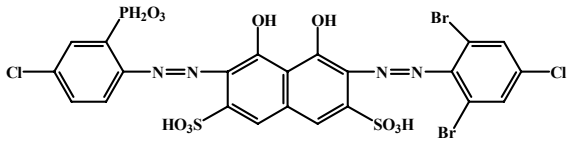
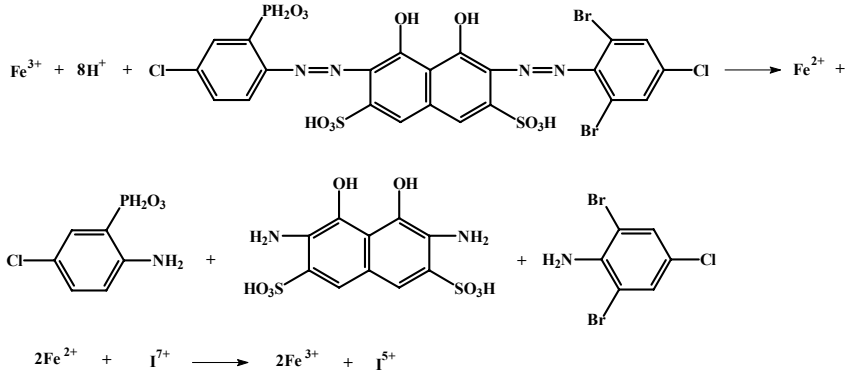


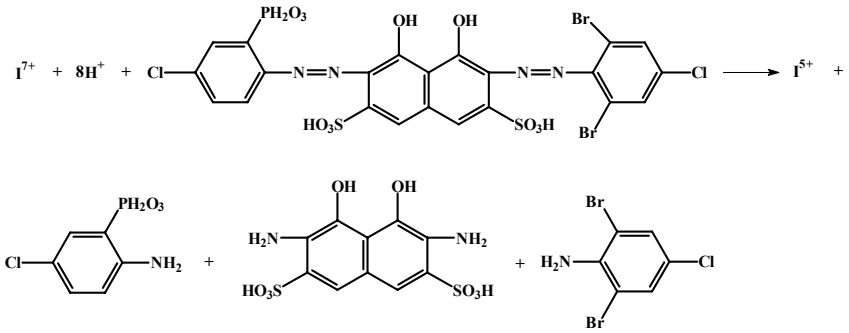
Fig. 1. Molecular structure of DBC-CPA

The reagent not only has strong complex ability and form various water-soluble complexes with metal ions, but also DBC-CPA contains -N=N- group and itself can produce colour. When it is oxidized or deoxidized, the -N=N- group is destroyed, which results in the colour of solution become shallow even achromaticity.

It is found in present studies that in presence of sulfuric acid of concentration 1.0×10^{-2} mol/L, iron(III) catalyzes the decolouring reaction of DBC-CPA oxidized by KIO_4 and a new method for the determination of trace iron was developed. The sensitivity of the present procedure is high, operation is simple and analytical cost is low. It has been successfully applied to the determination of trace iron in the human blood samples with satisfactory results. Based on literature[11] we propose the mechanism of the reaction for the catalytic kinetic spectrophotometric determination of trace iron as follow:



Catalytic reaction



Non-catalytic reaction

EXPERIMENTAL

A 722S spectrophotometer (Shanghai Lingguang Technique Co. Ltd., China) equipped with 1 cm cells was employed for all absorbance measurements. A HH-2 digital display constant temperature water-bath boiler (Jiangsu Jintan Ronghua Apparatus Manufacture Co., Ltd, China) was used to control the reaction temperature. A stop watch was used to record the reaction time.

Iron(III) standard solution: 2.7×10^{-7} mol/L (0.0863 g) of $FeSO_4NH_4(SO_4)_2 \cdot 12H_2O$ (Shenyang First Plant for Regent) was accurately weighed and was diluted with 0.1 mol/L of sulfuric acid solution. The volume of the solution was made to 100 mL by the dilution of water. A concentration of 2.7×10^{-5} mol/L stock solution was obtained. The stock solution was appropriately diluted by water to obtain 2.7×10^{-7} mol/L (1.0 µg/mL) working solution of Fe(III); DBC-CPA (Shanghai Changke Research Institute for Reagent) solution: 0.05 % (w/v) of water solution. Its molar concentration was 5.9×10^{-4} mol/L. 0.0050 g DBC-CPA was weighed and diluted with a definite amount of water. Then, the solution was made to the constant volume of 100 mL with water; KIO_4 (The Chemical Reagent Limited Company of National Medical Group) solution: 0.2300 g of KIO_4 was accurately weighed and dissolved in a definite amount of water. The solution was further transferred to a 100 mL volumetric flask and diluted to the constant volume by water. A 0.010 mol/L KIO_4 solution was obtained; H_2SO_4 (Beijing Chemical Plant, GR) aqueous solution: 0.10 mol/L. All the above reagents were of analytical grade unless specially stated. Deionized water obtained from the ion-exchange resin processed distilled water.

Recommended procedure for iron: In the following order, 1.0 mL of 0.10 mol/L sulfuric acid solution, 1.0 mL of 5.9×10^{-4} mol/L DBC-CPA solution and 1.0 mL of 0.010 mol/L potassium periodate solution were subsequently placed into two 10 mL calibrated flasks, respectively. In the one calibrated flask, an appropriate amount of the Fe(III) standard solution (for the conditional experiments, 4.0 µg) was put in the mixed solution (catalytic reaction, the absorbance was designed as A), while in the another one, the Fe(III) was not put in the mixture (non-catalytic reaction, the absorbance was designed as A_0). The mixtures were diluted up to the mark with water, shaked up, heated at a 100 ºC of water bath for 2 min. Then, they were

rapidly taken out and cooled by running water for 10 min. The absorbance (A_0) of non-catalyzed reaction solution and the absorbance (A) of catalytic reaction solution were measured at 550 nm in 1 cm cells against water and $\Delta A = A_0 - A$ and the $\log \frac{Ao}{A}$ values were calculated.

Determination of iron in blood sample: 1.00 mL of a human blood sample was taken in a 20 mL small beaker. 3 mL aqua regia (HNO_3:HCl = 1:3, v/v) was added to it and kept for 5-10 min. Then, the beaker was placed on an electronic oven to be heated at a low temperature. The contents were evaporated to near dryness. 1 mL of 0.2 mol/L HNO_3 solution was added to it and the contents were evaporated to near dryness and cooled down to a room temperature. The water was added to it and the obtained solution was diluted to the constant volume of 100 mL. From the solution 10 mL was taken out and diluted to 100 mL with water and the solution to be tested was obtained. 1.00 mL of the test solution was taken out and determined according to the standard procedure. At the same time a recovery of the method was determined according to the standard addition method. The determined results were contrasted with those of atomic absorption spectrometry.

RESULTS AND DISCUSSION

Absorption spectra: Fig. 2 shows the absorption spectra of different reaction systems. The curves a and b in the figure are the absorption curve of DBC-CPA system against water and (DBC-CPA) + KIO_4 system against water. From curve a and b it can be seen that the addition of KIO_4 can make the absorbance of the DBC-CPA present some degree of decrease,showing that KIO_4 can slowly oxidize the DBC-CPA to fade under the experimental condition but the change of value of the absorption peak is not large and the rate of fading reaction is slower. The system corresponding to curve c was DBC-CPA + KIO_4 + Fe(III) system with 0.50 µg of Fe(III) against water. Comparison between curve b and c shows that Fe(III) has a catalytic effect on the oxidation of DBC-CPA oxidized by KIO_4. The reactive system corresponding to curve d was the same as that of curve c except the Fe(III) amount of 1.0 µg. From the figure it can be seen that the peak values of both curves c and d decreased. Especially the decrease for curve d is more obvious, indicating as the addition amount of Fe(III) increased, the change of the fading reaction was bigger. Over a definite range of concentration,an amount of the catalyst Fe(III) and a fading extent present a linear relationship. This is a quantitative base of the determination of Fe(III).

Absorbance was determined for the solution of catalytic and non-catalytic reaction according to the standard procedure and the results are shown in Fig. 3. It shows that under the test conditions maximum absorption wavelength of non-catalytic reaction was 548 nm and maximum absorption wavelength of catalytic reaction was 362 nm. At 548 nm, absorbance difference ΔA was maximum. Thus, in the present study 548 nm was selected as measurement wavelength.

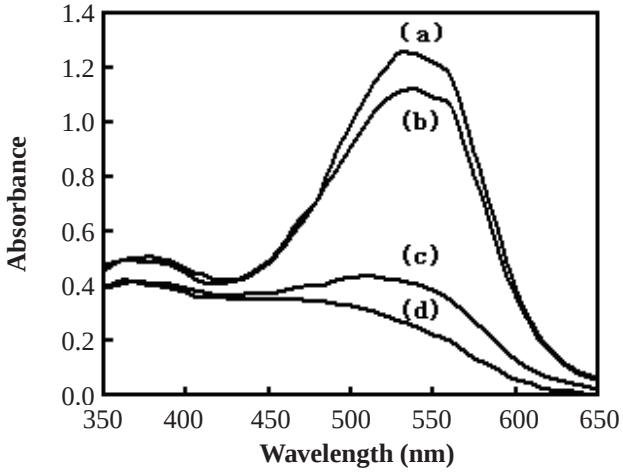


Fig. 2. Absorption spectra: (a) DBC-CPA (against water); (b) DBC-CPA + KIO_4 (against water); (c) DBC-CPA + KIO_4 + 0.50 µg Fe(III) (against water); (d) DBC-CPA + KIO_4 + 1.0 µg Fe(III) (against water). [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating temperature T = 100 ºC; Heating time t = 2 min

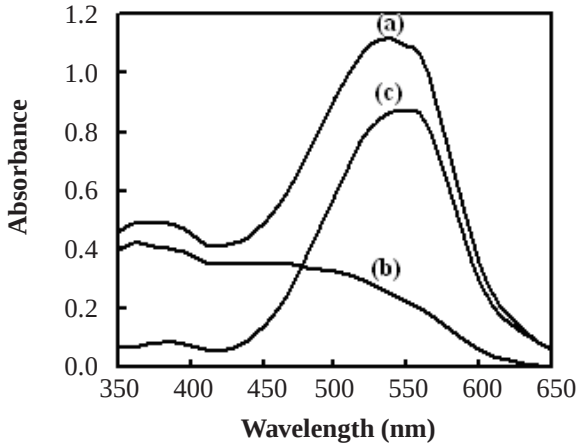


Fig. 3. Absorption spectra: (a) DBC-CPA + KIO_4 (against water)-non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA (A_0 - A); [Fe(III)] = 2.7×10^{-8} mol/L; [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating temperature T = 100 ºC; Heating time t = 2 min

Effect of the amount of sulfuric acid: The effect of different acidity on the reaction was reviewed. Under the condition that other experimental conditions were kept optimum, 0.1, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5, 1.8 and 2.0 mL of 0.10 mol/L sulfuric acid solution was added respectively and contrasted with a blank reagent. The results

showed (Fig. 4) that under the measurement condition ΔA increased as the amount of sulfuric acid increased over 0.1-1.0 mL. At 1.0 mL, ΔA was maximum and the sensitivity of the reaction was maximum. When the amount of sulfuric acid was more than 1.0 mL, ΔA decreased. Therefore, 1.0 mL of 0.10 mol/L sulfuric acid solution was selected. At this time in the reactive system the concentration of H_2SO_4 was 1.0×10^{-2} mol/L.

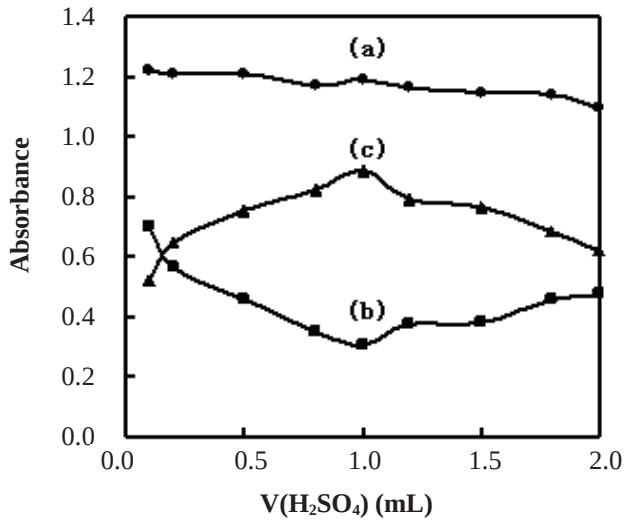


Fig. 4. Effect of acidity: (a) DBC-CPA + KIO_4(against water)-non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA; [Fe(III)] = 2.7×10^{-8} mol/L; [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; Heating temperature T = 100 ºC; Heating time t = 2 min; λ = 548 nm

Effect of the amount of DBC-CPA: When other experimental conditions were kept optimum, 0, 0.2, 0.5, 0.8, 1.0, 1.2 and 1.5 mL of 5.9×10^{-4} mol/L DBC-CPA solution was respectively added and contrasted with a blank reagent. The results showed (Fig. 5) that under the measurement condition ΔA distinctly increased as the amount of DBC-CPA increased over the range 0.1-0.8 mL. Over 0.8-1.2 mL, the change of ΔA was smooth and smaller. After 1.2 mL, as the amount of DBC-CPA increased, ΔA further increased. Considering that the reaction has a proper sensitivity and a suitable linear range, in 10 mL solution system 1.0 mL of 5.9×10^{-4} mol/L DBC-CPA solution was selected to be most appropriate.

Effect of the amount of KIO_4: When other experimental conditions were kept optimum, 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5 and 2.0 mL of 0.010 mol/L KIO_4 solution was respectively added and contrasted with a blank reagent. The results showed (Fig. 6) that under the test condition ΔA increased as the amount of KIO_4 gradually increased over the range 0-0.5 mL. Over the range 0.5-1.5 mL, ΔA was maximum and nearly constant. After 1.5 mL, ΔA decreased. Thus, 1.0 mL of 0.010 mol/L KIO_4 solution was selected to be most appropriate.

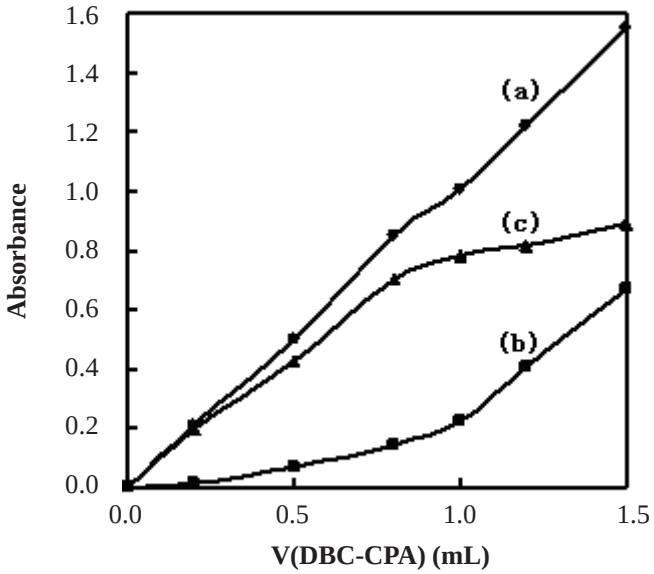


Fig. 5. Effect of the amount of DBC-CPA: (a) DBC-CPA + KIO4 (against water)-non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA (A_0 - A); [Fe(III)] = 2.7×10^{-8} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating time T = 100 ºC ; Heating time t = 2 min; λ = 548 nm

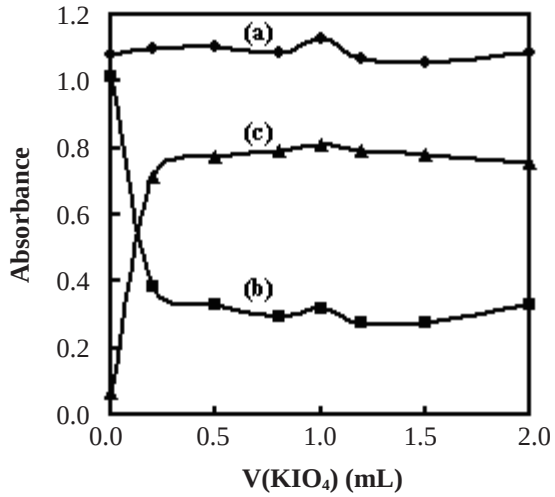


Fig. 6. Effect of the amount of KIO_4: (a) DBC-CPA + KIO_4 (against water)-Non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA (A_0 – A); [Fe(III)] = 2.7×10^{-8} mol/L; [DBC-CPA] = 5.9×10^{-5} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating temperature T = 100 ºC; Heating time t = 2 min; λ = 548 nm

Effect of heating temperature: When other experimental conditions were kept unchanged, temperature effect were studied throughout. At the water baths of 40, 50, 60, 70, 80, 90 and 100 ºC, the experiments were made according to the standard procedure, respectively and contrasted with a blank reagent. The results showed (Fig. 7) that under the test condition below 50 ºC, ΔA is nearly zero. The rate of catalytic reaction and non-catalytic reaction is approximately equal. As the temperature increased, ΔA gradually increased. When the temperature of water bath was 100 ºC, ΔA was maximum. Thus, the water bath of 100 ºC was selected for heat and the reaction was terminated by running water. The determined data over 50-100 ºC was regressed and disposed to obtain a linear regression equation: $\log \frac{Ao}{A}$ = -1547.81/T (K) + 4.8165, with a correlation coefficient r = 0.9995. According to the slope of the equation the activation energy of the reaction was obtained to be Ea = 64.575 kJ/mol.

Effect of heating time: When other experimental conditions were kept unchanged, the experiments of the effect of heating time were put up. A heating time was 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300 s, respectively, the result was contrasted with that of a blank reagent. The results are presented in Fig. 8. The results showed that ΔA in the range 20-120 s and time presented a linear relationship. At 120 s, ΔA was a maximum. After 120 s, it gradually decreased. Therefore, the heat time selected was 120 s. A graph was made with ΔA to t its linear regression equation was obtained as follows: ΔA = 0.4875t - 0.0220, with a correlation coefficient r = 0.9927. The rate constant of the reaction calculated was $k = 8.125 \times 10^{-3}$ s^{-1} and a half-life period was 1.422 min.

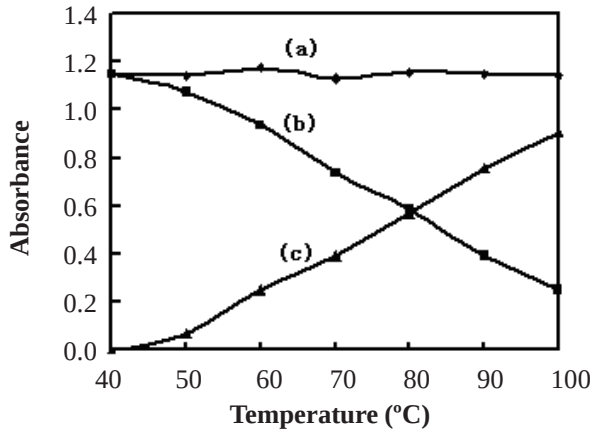


Fig. 7. Effect of heating temperature: (a) DBC-CPA + KIO_4 (against water)-non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA (A_0 - A). [Fe(III)] = 2.7×10^{-8} mol/L; [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating time t = 2 min; λ = 548 nm

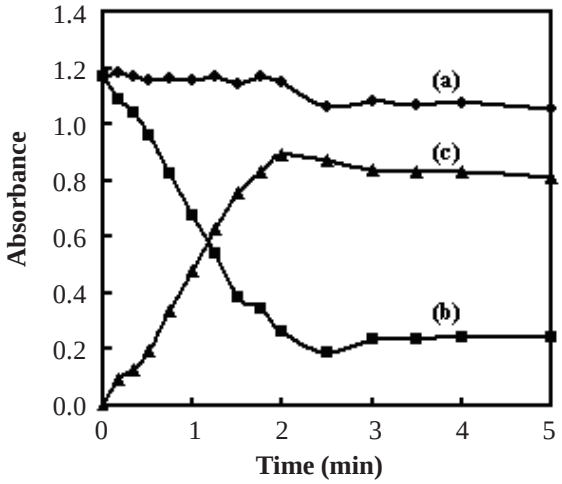


Fig. 8. Effect of heating time: (a) DBC-CPA + KIO_4 (against water)-non-catalytic reaction A_0; (b) DBC-CPA + KIO_4 + Fe(III) (against water) – Catalytic reaction A; (c) Net catalytic reaction ΔA (A_0 - A); [Fe(III)] = 2.7×10^{-8} mol/L; [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L; Heating temperature T = 100 ºC; λ = 548 nm

Stability of system: Under the optimum conditions the determination experiments of system stability were made. After running water cooled non-catalytic and catalytic reactions, absorbance kept unchanged. Within 3.5 h the absorbance varied less than ± 5 % and the system kept stable.

Calibration curve: Under the optimum experimental conditions the experiments of a linear range was made. The standard solution containing 0, 0.1, 0.2, 0.5, 0.8, 1.0, 1.2 and 1.5 µg of Fe(III) was respectively added to a series of 10 mL test-tube and determined the absorbance according to the standard procedure and the results were contrasted with that of a blank reagent as shown in Fig. 9. The results showed that under the optimum conditions in a 10 mL solution of Fe(III) quality over the range 0.10-1.5 µg and ΔA presented a good linear relationship. Its regression equation was: $\Delta A = 5.810C$ (C: µg/mL) + 0.1592, with a correlation coefficient r = 0.9936. For 11 replicate determinations of 1.00 µg/mL Fe(III), the relative standard deviation of the present method calculated was 1.10 %, indicating high precision and reproducibility of the present method. Eleven replicate determinations of a blank reagent were made and the detection limit of the method calculated by 3S/K (S is standard deviation of 11 replicate determinations of a blank reagent and K is the slope of the calibration curve, respectively) method was 5.06 ng/mL.

Effect of coexisting substance: Under the optimum experimental conditions the experiments of the effects of coexisting substances were carried through. When 0.10 µg/mL Fe(III) was determined and a relative error was controlled within ± 5 %, the allowable amounts of coexisting ions was as follow (in mass multiple, m/m):

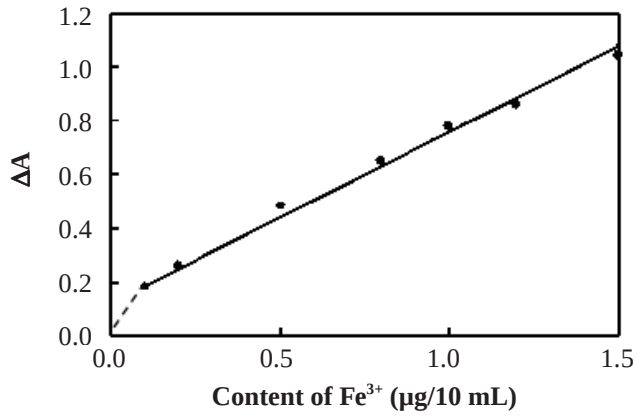


Fig. 9. Calibration curve: [DBC-CPA] = 5.9×10^{-5} mol/L; [KIO_4] = 1.0×10^{-3} mol/L; [H_2SO_4] = 1.0×10^{-2} mol/L. Heating temperature T = 100 ºC; Heating time t = 2 min; λ = 548 nm

MnO_4^- (70); VO_3^- (150); F^- (40); Cl^- (2000); Br^- (1000); I^- (50); SO_4^{2-} (3000); PO_4^{3-} (1200); CH_3COO^- (3000); Ag^+ (40); Mg^{2+} (300); Ca^{2+} (1000); Zn^{2+} (50); Pb^{2+} (120); Mn^{2+} (50); Ni^{2+} (10); Cd^{2+} (15); Cu^{2+} (0.2); Bi^{3+} (60); La^{3+} (15); Eu^{3+} (8); Y^{3+} (8); Cr^{3+} (5); Al^{3+} (1); Ti^{4+} (150); Ce^{4+} (10); Zr^{4+} (8); Th^{4+} (8); H_3BO_3 (200); W^{6+} (50); Mo^{6+} (50); Cr^{6+} (10); $H_2C_2O_4$ (5). From the above results it can be seen that the proposed method has good selectivity.

Application: The recommended procedure was applied for the determination of iron to evaluate the effectiveness of the method. For this purpose, human blood samples were analyzed. The analytical results obtained are listed in Table-1. From the table it can be seen that the analytical results of the samples were excellent agreement with those by atomic absorption spectrometry. The recovery of the addition standard was between 99.98-100.7 % and the relative standard deviation of 11 replicate determinations was between 0.87-1.42 %. It can be seen from the above analysis that the analytical results of the method proposed in the paper were quite satisfactory.

TABLE-1
ANALYTICAL RESULTS OF SAMPLES

Sample	Found (mg/L)	Average (mg/L)	RSD (%)	Added (μg/10 mL)	Recovered (μg/10 mL)	Recovery (%)	Atomic absorption spectrometry (mg/L)
1	528.1, 529.8, 519.5, 509.2, 519.5, 510.9, 528.1, 524.6, 514.3, 522.9, 512.6	520.0	1.42	0.1000	0.1007	100.7	519.8
2	491.1, 495.4, 498.1, 489.3, 486.6, 491.1, 493.3, 495.4, 483.9, 489.3, 498.1	492.0	0.87	0.1000	0.9998	99.98	492.1

Conclusion

The decolouring reaction of dibromo-*p*-chloro-chlorophosphonazo of the oxidation of potassium periodate catalyzed by iron(III) and its optimum experimental conditions were studied. In 1.0×10^{-2} mol/L of sulfuric acid medium, the maximum absorption peak of Fe(III)-(DBC-CPA)-KIO_4 system is at 548 nm. At the wavelength the iron(III) amount over the range 0.010-0.15 μg/mL and the absorbance difference ΔA present a good linear relationship and its regression equation is ΔA = 5.810C (C: μg/mL) + 0.1592, with a correlation coefficient r = 0.9936. The detection limit of the method is 5.06 ng/mL. The present procedure has been successfully applied to the determination of iron in the human blood sample. The recovery of the addition standard was 99.98-100.7 % and the relative standard deviation of 11 replicate determinations was 0.87-1.42 %. The analytical results of the proposed method according to those of atomic absorption spectrometry and are satisfactory.

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