Spectrophotometric determination of fe biology essay

Science, Biology

Abstract

In this paper , the complex reaction of water - Soluble meso -tetra (4-Sulfophenyl) porphyrin (TPPS4) with Fe(III) ion has been studied in the presence of small amount of hydroxyl amine . The porphyrin was found to be a highly sensitive and highly selective reagent for direct spectrophotometric determination of iron at µg level . The soret band of the complex lay at 394 nm (€: 3. 88 x 105L. M-1cm-1) , which is well separated from that of the reagent 432 nm ,(€= 2. 2 x 105 L. M-1. Cm-1) below PH 2The complex formation is slow at room temperature and PH 5. 0 , however , it can be accelerated by heating on boiling water bath and completed within 10 min at 100 Co . five determinations of standard solutions containing 0. 7 mg /10mL of iron gave a relative standard deviation of ±2. 61%. Beer's law is obeyed for iron (III) in the range of 0. 01-2. 0 µg/10ml . The molar ratio of complex is 1: 1. This method has been applied to determine iron (III) in water samples with satisfactory results. The complex is highly stabie . The interference of many ions and salts have been examined and found to be minimal . Large amounts of rare earth do not interfere with the determination . The complex reaction of Fe (III) TPPS with reducing agent of Na2S2O4 was studied as well, it was found the reaction is time dependent , and the reduction can be completed within 6 min . The soret band of Fe TPPS at PH= 12. 5 lay at 408 nm but in the presence of sodium dithionite moves to long wave length of 438 nm that assign of Fe(II) TPPS. The reduction was take place at room temperature, however it can be used this reaction to determine the S2O4-2 ion in aqueous solutions or solids . Beer's law is obeyed for S2O4-2 in the range of 0. 01-1. 85 mg/10ml and the molar absorptivity of Fe (II) TPPS is 3.

48 x 105 L. M-1, Cm-1 , and , for S2O4-2 is 1. 47x 103 L. M-1, Cm-1. Five determinations of standard solutions containing 3. 52 mg /10ml of Na2S2O4gave a relative standard deviation of ±0. 315 . The interference of lactose, starch and sodium chloride was found effective. The recovery percentage was in the range of 87. 5 -98. 29 % and the RSD% in the range of 0. 57-1. 28 (n= 3) . Also in this paper the determination of Fe (II) in Fe (III) TPPS was studied by reduction the Fe(III) TPPS complex at PH 6. 5 by using Na2S2O4 in the presence of bipyridyl reagent, it has been found the reagent destroy the porphyrin ring and Fe(II) ion come out of ring and react directly with bipyridyl reagent due to steric effect, the amount of Fe (II) equal to the third amount of bipyridyl moles react with Fe(II) ion to from a new red complex of Fe(bipy)3 with soret band lay at 522nm and (€= 30 L. M-1. Cm-1)Beer's low obeyed for Fe(II)in the range of 0. 01-15. 6 mg/10ml. Moler ratio was found 3: 1 bipyridyl to Fe(II)ion The sensitivity For the 0. 001 absorbance sendell's index was 0. 00014, 0. 087 and 1. 8 For Fe(III), S2O4-2 and Fe(II) respectively . This method can be used to determine the Fe(II) ion in hemoglobin.

[key words] Tetra (4-sulfophenyl) porphyrin ; Iron spectrophotometry

1. Introduction

The porphyrins are class of naturally occurring macro cyclic compounds , which play a very important role in the metabolism of living organisms . These have been extensively studied due to their biological importance as well as analytical application[1-3]. Because the porphyrins are the highly sensitive chromo genetic reagents for spectrophotometric determination of several metal ions based on the use of the so-called soret band at 380-500nm[4-6] . Many papers have been published on the mechanism of metal ions in corporation into the porphyrinnucleus[6-8]. Synthetic metaloprophyrin are suitable sensitivers for photodynamic therapy of cancer [9]. TPPS4 was applied previously as a complexing reagent of many metal ions such as Hg(II), Pb(II), Cd(II), and Zn(II) ions [5, 7, 10, 11] and many workers have been used water soluble porphyrin to determined trace amount of many transition metals such as Cu, Ni, Pb, Zn, Fe, Cd, Mn and other elements [2, 12-17] based on the color complexation . In this work , we report the spectrophotometric study on the reaction between 5, 10, 15, 20-tetra (4-sulfophenyl) porphine (TPPS4) and Fe(III) with the aim of determining the iron (III), iron(II) ions and the reducing agent Na2S2O4 by using the calibration curve method . Bipyridyl ligand was used for determining the Fe(II) ion in Fe(II) TPPS in aqueous solution at PH below 7. 0 by destroying the iron(II) TPPS and forming a new Fe(II) (bipy)3 red complex . Reductive titration of Fe(III) TPPS with a solution of sodium dithionite resulted in the formation of a Fe(II) TPPS complex at high PH was reported too.

2. Experimental section

2. 1 APParatus

Spectrophotometer model 6800 Jenway with 1Cm silica cells was used throughout this research work.

2. 2 Materials and Reagents

All reagents used were of analytical grade and were obtained from fluka. Tetra (p-phenyl) porphine (TPP) was prepared by refluxing of 5ml pyrrole with 7. 24 ml benzaldehyde 1: 1 mole ratio in propanic acid media for two hours [1] after cooling to the room temperature the solution was filterated and the product washed with a minimum quantity of ethanol, a blue crystals of TPP were obtainted . Meso-Tetra (p-sulfophenyl) porphine was prepared by mixing 2gm of TPP with 50ml of concentrated sulfuric acid, the mixture was heated on a steam bath for 4-5 hr due to the method was reported by reference[12]

2. 3 Standard solutions

Stock solution (10-3m) TPPS4 was prepared by dissolution of 0. 308 gm in 250ml of buffer solution (PH= 5. 0). 1gml-1 stoek solution of Fe (III) was prepaed by dissolution (7. 0)gm of Ferrous ammonium sulfate in 250 mlStock solution (0. 012M) bipyridyl was prepared by dissolution of (18. 9)gm in(10)ml of buffer solution PH 0. 5 . Stock solution of 0. 051M Na2S2O4. H2O was prepared by dissolution of 90 mg in 10ml buffer solution of NaoH at PH 12. 5 . Buffer components were of analytical grade and used without further purification. The acetate buffer was used for PH <5, phosphate buffer for PH 6-8, borate buffer for PH 8-10 , NaOH solution was used for PH> 10.

2. 4. Composition of complexes.

2. 4. 1. Composition of FeTPPS complex

Determination of composition of the forming complex was conducted using mole ratio method. Metal (Fe(III)) was added to 5 x 10-6M porphyrin solution in the concentration range 10-6-1. 5 x 10-5 M . After 10 min of reaction in the hot water bath , all the reactants were at PH 5. 0 buffer solution, the absorbance at wave length of 394 nm was measured.

2. 4. 2 Composition of Fe(II)bipy complex

iron(II) concentration in the range10-6-1 x10-5 M was added to 6 x 10-6M of bipyridyl at PH 6. 5 and after a few minutes the absorbance was measured at wavelength of 522nm

2. 4. 3 Determination of iron (III) ion in tap water .

Tap water samples were just filtered through 0. 45um pore size Millipore membrane filter. 1ml of sample was taken in to a calibration flask and 2. 5ml of TPPS (10-3M) , 5ml of buffer solution (PH5. 0) were added successively. After dilution to the mark (25ml), the absorbance was measured at 394nm against reagent blank.

2. 5 Construction of calibration curve for ions .

2. 5. 1. Calibration curve for Fe(III)

In to a series of ten of 10 ml volumetric flasks containing a liguots of standard Fe(III) ions solutions in the rang of (0. 01-2. 0) µg /10ml. To each of these were added 1mlTPPS4 solution(10-3M)After dilution to the mark, the solutions were heated for 10 min in boiling water bath adjusted at 100 Co, then cooled and diluted to the mark with distilled water and mixed well . The absorbance of complex was measured at 394nm against reagent blank at pH 5. 0, T= 25Co .

2. 5. 2 Calibration Curve of S2O4-2

Into a series of ten of 10ml volumetric flasks containing aliguots of standard Na2S2O4 solution (0. 01-1. 85)mg/10ml . To each of these FeTPPS were added at final concentration of 5. 11x10-6 M at pH= 12. 5, T= 25C0 , after dilution to the mark (10ml) by using distilled water and mixed well for ten min the absorbance was measured at 438nm and 408nm against water blank.

2. 5. 3 Calibration Curve for Fe (II) ion.

Deferent concentration of bipyridyl in the rang (0. 01-0. 84)mmoles were placed in series oftenof10ml volumetric flasks, containing 7. 84x10-6 M of FeTPPS in the present excess of Na2S2O4 , then after ten min the absorbance was taken at wave length of 522nm at pH= 6. 5, T= 25Co against water blank .

3. Results and discussion .

3. 1 Spectrophotometric study of porphyrin

Solution of 3. 1x10-5 M TPP in chloroform solvent is purple and exhibit six absorption maxima one is soret band at 395nm (€= 1. 06x105L. M-1. cm-1), 356nm as sholder(€ = 6. 25x104LM-1. cm-1) , 515nm(€= 3. 96x104LM-1. cm-1), 545nm(€= 1. 74x104LM-1. cm-1) , 590nm(€= 1. 25x104LM-1. cm-1) and645nm(€= 1. 03x104LM-1. cm1) . TPPS solution at pH 1. 7 exhibit four bands one in soret at 432nm (€= 2. 2x105LM-1cm-1), 486nm(€= 8. 2x104LM-1cm-1) , 644nm (€= 6. 6x104 LM-1cm-1)and702nm(€= 3. 5x104 LM-1cm-1), but at high pH= 12. 5 the spectra shows soret band at406nm(€= 5x104LM-1cm-1) , 518nm(€= 6. 5x103LM-1cm-1), 558nm (€= 3. 8x103LM-1cm-1)and650nm(€= 1. 45x103 LM-1cm-1). TPPS4 has an empirieal formula of C44H26N4O12S4Na4 with 12H2O : Analysis . Calc% N= 4. 48, S= 10. 3 . Found % N= 4. 50, S= 10. 4

F. g. 1 Structure of the porphyrin

R= SO3H 5, 10, 15, 20- tetrakis (4-sulfonatophenyl) porphyrin, TSPPFeTPPS spectra shows band at low pH 2. 6 as monomer of 394nm (€= 5x105 LM-1cm-1) and 530nm (€= 1. 1x105 LM-1cm-1) , but at high pH about 12. 5 shows at 408nm (€= 3×105 LM-1cm-1) as a soret band of dimer form. Addition of Fe (III) ion to 10-5 M of TPPS at pH 5. 0 then heated in boiling water bath for 10min the color change from green to brown with moving the soret band from 432nm to the 394nm. The mole ratio is 1: 1 of Fe(II) to ligand as shown in figure . 2.

Fig. 2: The mole ratio of TPPS4 / Fe(lll).

3. 2 Calibration Curve for Fe(III) , S2O4-2 and Fe(II) ions and the interferences .

Table. 1: shows the analytical and regression parameters , Figure 3, 4and 5 : shows the calibration curve for Fe(III), Fe(II) and S2O4-2 with TPPS4ligand. Under the condition employed , the calibration graphs were liner up to 2. 0 µg , 1. 85 mg and 15. 68mg per 10 ml for Fe(III) , S2O4-2 and Fe(II) respectively. The sensitivity for the 0. 001absobance (Sandell's index) was 0.

00014, 0. 087 and 1. 8µg Cm-2 for Fe(III), S2O4-2 and Fe (II) respectively , thus the sensitivity is superior to that obtained previously for porphyrin]8, 20, 21]. Application of TPPS4 allowed the determination of small amount of Fe(III) , S2O4-2 and Fe(II) in aqueous solution or solid materials . The precision of five replicate measurements at 0. 7 mg , 3. 52 mg and 5. 7 mg per 10 ml for Fe(III) , S2O4-2 and Fe(II) concentration level was in the range 0. 315-2. 16% RSD. The effect of foreign ions was investigated for the determination of Fe(III) and S2O4-2 using TPPS4 as color reagent see table . 2The method shows a good tolerance to the great excess of alkali and alkaline earth metals. No significant interferences from common inorganic anions, such as chlorids, nitrates and sulfates , were also found for Fe(III) , but not for determination of S2O4-2 ions, it was found the recovery percentage can be affected in the present of interferences such as lactose , starch , and sodium chloride as shown in Figure 6 . The effect of the presence of some heavy metals was examined such as lead and tin and was found caused increasing in the amount of Fe(III) recovery percentage in the range of 2. 2 - 2. 96%. even they were at similar or smaller level, owing to formation of their complexes with TPPS4 by metal substitution.

Fig3: The calibration curve of Fe(lll)ion .

Fig4: The calibration curve of S2O4-2ion .

Fig5: The calibration curve of Fe(ll) ion.

Table . 1: Shows the analytical and regression parameters.

Parameters

Fe(lll) ion

S2O4-2 ion

Fe(ll) ion

Beer, s law limits(µg/10ml)

0. 01-2. 0

0. 01-1. 85

mg/10ml

0. 01-15. 6

mg/10ml

Molar absorptivity(L. mol-1. cm-1)

3. 88×105

1. 47x103

30

Sandell, s sensitivity(µg ml-2)

0. 00014

0. 087

1. 8

Average recovery(%)

98. 96

99. 16

99. 90

Correlation coefficient

0. 9965

0. 982

0. 96

Regression equationY*

Slope. a

0. 0068

0. 0098

0. 0001

Intercept. b

-0. 0165

0. 0

0. 094

RSD%

2. 34

0. 315

1. 40

*Y= ax+b, where x is the concentration of ions.

Table. 2: shows the effect of foreign ions on determination of Fe(lll) and S2O4-2 ions.

Compoutd nameA394 ɿ nmCmg Fe(lll)/10mlRecovery %Interference mg/10ml addedRSD%n= 4Recovery Increasing %12. 284. 38100Fe(lll)= 4. 38

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1+Zn2. 2894. 40100. 4Zn= 0. 590. 990. 41+pb2. 334. 48102. 2Pb= 0. 751. 332. 21+Sn2. 354. 51102. 96Sn= 0. 730. 832. 961+Ni2. 314. 44101. 36Ni= 0. 641. 21. 362438 =ɿnmμg/10ml Fe(ll)

=

=

n= 3Recovery decreasing %21. 763. 13100

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2+Glu1. 733. 0798. 291. 580. 57-0. 062+La1. 662. 9594. 311. 770. 06-0. 182+St1. 672. 9794. 880. 15gm/10ml1. 36-0. 162+Nacl1. 542. 7987. 53. 151. 28-0. 401 = Fe TPPS , 2= Fe(ll) TPPS, Glu = glugose , la = lactose , st = starchFig. 6: The visible spectra of Fe(ll) TPPS in Presence of Glugose , lactose, Starch and sodium chloride at PH 12. 5 , 438 ɿnm. 3. 2. 1 Analytical application of method for determination of Ferric ion in tap water. The proposed method was applied to the determination of Fe(III) ion in some natural water samples and nutritional supplement. The results are given in table 3 and comparative data obtained by AAS method are also presented. The . proposed spectroph-otometric method with TPPS4 allows the sensitive determination of traces of Ferric with quite simple instrumentation .

Table 3. Determination of Ferric ion in tap water

Sampleproposed method(n= 5)AAS method(n= 5)Tap water1. 72±0. 1ppm1. 73±0. 04ppmWell water0. 3±0. 01ppm0. 32±0. 01ppmNutritional Supplement2. 6±0. 1mg per tablet2. 2±0. 05mg per tablet**contain 2. 5mg/tablet.

3. 2. 2 The reduction of FeTPPS with Na2S2O4

figure 7. Shows changes in visible spectrum of titration Na2S2O4 with FeTPPS 5. 11x10-6 M at high pH= 12. 5, T= 25Co, it is very clear from spectra that the soret band for FeTPPS move to long weve length from 408nm to 438nm due to the reduction of iron(III) to the iron (II) porphyrin as shown in this equation. Fe p (H2O) —O — Fe p(H2O) Na2S2O42Fe (II) P(oH)2+2e-

Fig. 7: the changes in visible spectrum upon titration Na2S2O4 with Fe TPPS , of 5. 11x10-6 M at PH 12. 5 , T= 25 Co .

Figure 8. shows the relation between reduction percentage and the absorbance at ג max 438nm.

Also it has been found the FeTPPS reduction is time dependent , when 100 µg /10ml of Na2S2O4 was added to the FeTPPS solution at pH= 9. 5 , the reductiom was found completed after 6 min and the progress of absorbance change as shown in figure 9 . and 10

F. g. 9 : The absorbance change upon addition 100µg to the FeTPPS at pH= 9. 5, The ɿmax of final Fe(II)TPPS complex was at 425nm.

Figure 10: The change in the absorbance of FeTPPS upon addition 100µg of $Na_2S_2O_4$ at pH= 9. 5, ɿmax 425nm with time.

3. 2. 3 Reaction FeTPPS with bipyridyl reagent.

It has been found there is no reaction between bipyridyl and FeTPPS at high pH> 12. 5 in the presence $Na_2S_2O_4$ as a reducing agent due to the steric effect, but when lowering the pH to the 6. 5, the reagent destroy the porphyrin ring , and another red complex form of $Fe(bipy)_3$, because the Fe(II) ion come out of porphyrin ring in the solution , we used this phenomena for determination of Fe(II) in FeTPPS and may be it is useful to used to determine the Fe(II) ion in protein such as hemoglobin in the same way. The visible spectrum of NH_4FeSO_4 with bipyridal was found similar to the FeTPPS with $Na_2S_2O_4$ and bipyridyl with a broad band at 522nm at pH= 6. 5 , T= 25Co. The complexing ratio was found 3: 1 bipy : Fe(II) as shown in Figure 11 : shows the mole ratio of bipyridyl with Fe (II) ion. Lx = bipy moles, Mx = Fe(II) ions moles. figure 11. Figure 12: shows destroying the visible spectra , ɿmax at 425 nm of Fe(II)TPPS with increasing the concentration of bipyridyl in the presence of $Na_2S_2O_4$ at pH= 6. 5 , T= 25Co. The number of moles of Fe(II) calculated from number of moles of bipyridyl added to the solution and that equal third of it . The above results indicates that TPPS4 ligand is very sensitive as a chromogentic reagent, our results are similar to the other workers results using the same porphyrins of TPPS as shown in table 4.

Table 4. Spectrophotometric determination of metal ions with porphyrin reagent.

Metal ion

Reage-nt

Condition

Absorptio-n Spectra

λmax, nm €(x105) LM-1 cm-1

Range of deter- mination

interference

Refs

Zn(ll)

TPPS4

pH 7. 1+ Imidazole+Cd room temp 30 min

425 , 4. 5

10-150 ngl-1

Mn(ll), Co(ll), Fe(ll)

[16]

Pb(ll)

TPPS4

pH4+ ascorbic Acid(100. 7min)

411 , 2. 2

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Cu(ll), Hg(ll)

[21]

Ni(ll)

TPPS4

pH8-9+(Cd(ll) + imidazole(100oC

418 , 2. 3

Up to 150 ngI-1

[22]

U ion

TPPS4

pH 3. 0+NH2OH. HCl

420 , 2. 2

0. 01-120µgl-1

[23]

Cd

T4BPP

pH 10. 0 by HPLC

436

0. 01 – 120 µgl-1

[1]

Hg

T4BPP

10. 0 by HPLC

449

0. 01 -120 µgl-1

[12]

Fe(lll)

TPPS4

PH 5. 0+ NH4OH 100CO 10min

394 3. 88x105

0. 01-200 µgI-1

This work

S2O4-2

TPPS4

PH= 12. 5 room temp.

438 1. 4x103

1. 0 – 185 µgl-1

La, Gu, St

This work

Fe(ll)

TPPS4

PH= 6. 5+Na2S2O4+bipyridyl room temp.

425, 30

1. 0 – 1560 µgl-1

Zn, Cu

This work

Conclusion:

Porphyrins find many application as a ligand for spectrophotometric determination of cations and anions in analytical chemistry. In this study TPPS4 used for determination of Fe (III) , S2O4-2 and Fe(II) ions by three steps,(1) the stable complex between Fe(III) cations and TPPS4 enable us to determination the Fe(III) ion in tap water or solid compounds ,(2) the reaction of FeTPPS at room temperature with Na2S2O4 as a reducing agent used for determine the S2O4-2 ion in aqueous solution , (3) Reaction of FeTPPS with Na2S2O4 in the presence bipyridyl ligand at pH= 6. 5 give a new red complex of Fe(bipy)3 as a result of steric effect and destroying the porphyrin ring , that make Fe(II) ion free in solution to form stable complex , so we used this reaction for determination Fe(II) ion.