May, 1986] © 1986 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 59, 1571—1573 (1986) 1571

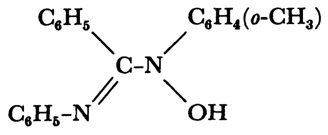
Determination of Iron in Drug Biochemical and Biological Samples as a Thiocyanato Mixed Ligand Complex with Hydroxyamidine

Raghunandan SINGH,* Lalta Prasad CHANDRAKAR, and Rajendra Kumar MISHRA†
Department of Chemistry, D. B. Science College, Gondia (M.S.), India
†Department of Chemistry, Ravishankar University, Raipur (M.P.), India

(Received April 25, 1985)

N-Hydroxy-*N*-*o*-tolyl-*N'*-phenylbenzamidine (HTPB) is presented as a new sensitive and highly selective reagent for determination of iron in drug, biochemical and biological samples in presence of thiocyanate. The red organge Fe(III)–HTPB–SCN mixed ligand complex is quantitatively extracted into benzene. The extraction of iron(III) as thiocyanato mixed ligand complex is quantitative in the acidity range between 0.2—0.7 M (1M=1 mol dm^{-3}) hydrochloric acid. The effective molar absorptivity of the complex is 12000 l mol^{-1} cm^{-1} with Sandell's sensitivity of 0.0046 μg of iron per cm^2. On the basis of this sensitive color reaction, a simple, rapid, selective, and highly reproducible method for the extractive spectrophotometric determination of microgram amounts of iron in various drug, biochemical and biological samples has been developed. The method is free from most of the common metal ions and commonly used sequestering agents.

Among the several methods reported for the spectrophotometric determination of iron in various complex materials,[1–13] the thiocyanate and 1,10-phenanthroline methods are used over a long period. The iron(III)–thiocyanate method is simple, rapid, and sensitive, but it suffers from interferences due to a large number of experimental factors. These include time of standing, thiocyanate concentration, deviation from Beer's law and reaction of the thiocyanate with other elements to give colored products. The iron(II)–1,10-phenanthroline method is widely applicable to routine determination of iron but it suffers from serious interference from common ions such as Ni(II), Cu(II), Bi(III), and Ag(I). Hence it is thought worthwhile to describe a better method for determination of iron in complex materials. In this communication, the reaction of *N*-hydroxy-*N*-*o*-tolyl-*N'*-phenylbenzamidine(HTPB) with iron(III) in presence of thiocyanate was investigated looking towards the possible use of this reagent for spectrophotometric determination of iron in biochemical and biological samples and separation from other metal ions associated in complex materials. The proposed method offers several advantages over other established methods.[1–13] It is sensitive and highly selective and can be used over wide acidity range. The reagent is easy to prepare and if kept in amber glass bottles, solution can be stored for at least a month without any deterioration. It is applicable for the determination of iron in all complex materials because most of the common ions do not interfere.



HTPB

Experimental

Standard Iron(III) Solution. The standard solution of iron(III) was prepared by dissolving 1.0 g pure iron wire (E. Merck) in 50 ml of 1:3 nitric acid. The oxides of nitrogen were removed by boiling and diluted to one liter with distilled water. The metal content of the solution was evaluated gravimetrically with 8-quinolinol.[14]

Apparatus. A Carl-Zeiss specord recording ultraviolet spectrophotometer and an ECIL UV-VIS spectrophotometer model GS-865 equipped with 1-cm matched silica and quartz cells were employed for all photometric measurements.

Preparation of *N*-Hydroxy-*N*-*o*-tolyl-*N'*-phenylbenzamidine. *N*-*o*-Tolylhydroxylamine 3.2 g (0.027 mol) was taken in 50 ml absolute ether in a 500 ml conical flask. To this, 5.6 g (0.027 mol) of *N*-phenylbenzimidoyl chloride in 100 ml ether was added in small quantities during the course of 15 min with constant stirring. A light brown oil slowly separated out. Stirring and scratching were continued till the oil solidified and separated in the form of white crystals. This was filtered off and washed with 3×20 ml portions of ether and air dried. The resulting hydrochloride was treated with dilute aqueous ammonia to liberate the free base. This was recrystallized from petroleum ether–benzene (1:2) at 60—80°. Yield 45%, mp 131 °C. Analysis showed C, 79.08; H, 5.65; N, 8.98%. $C_{20}H_{18}N_2O$ requires: C, 79.47; H, 5.96; N, 9.27%.

Extraction Solution. A 0.1% W/v benzene solution of reagent was employed for the extraction work. 2% W/v aqueous solution of potassium thiocyanate was used throughout the experiment.

Recommended Procedure. Place and aliquot of iron(III) solution containing 40—80 μg of metal in a 100 ml separatory funnel. To this, add 5 ml of potassium thiocyanate solution. Adjust the volume of the solution to about 25 ml and the acidity to between 0.2 and 0.7 M with hydrochloric acid. Introduce 25 ml benzene solution of the reagent and equilibrate for 2 min. Allow the phases to separate. Dry the colored extract over anhydrous sodium sulfate 2 g in a 50 ml beaker. Measure the absorbance at wavelength of maximum absorption against reagent blank.

Results and Discussion

Absorption Spectra. The absorption spectra of the ternary iron(III) complex and the reagent in benzene are shown in Fig. 1. The Fe(III)–HTPB–SCN complex shows a sharp absorption maximum at 460 nm having the value for molar absorptivity to be 12000 l mol^{-1} cm^{-1}. As a reagent blank prepared under similar conditions does not absorb in the region 450—700 nm, it can be replaced by benzene.

Choice of Solvent. Various water immiscible organic solvents such as chloroform, benzene, carbon tetrachloride, toluene, xylene, etc. were found to extract the mixed ligand complex quantitatively. However, benzene was found to be the better extracting solvent as in this the sensitivity of the complex is enhanced and complete extraction is relatively rapidly accomplished.

Effect of Acidity. The acidity of the solutions was maintained with 2 M hydrochloric acid. If the hydrochloric acid concentration of aqueous phase lies in the range 0.01 to 1.0 M the position of the absorption band of the benzene extract remains intact. However, the optimum acidity range was found to be 0.2 to 0.7 M hydrochloric acid in which iron(III) was quantitatively extracted. The fatty acids and other inorganic acids were unsuitable for extraction work due to low absorbance of complex.

Effect of Reagents. A 30 and 120 fold molar excess of HTPB and thiocyanate respectively was found to be adequate for complete extraction of iron. A further excess of these (HTPB upto 100 fold and thiocyanate upto 5000 fold molar) did not interfere with the determination. The order of addition of reagents was not critical.

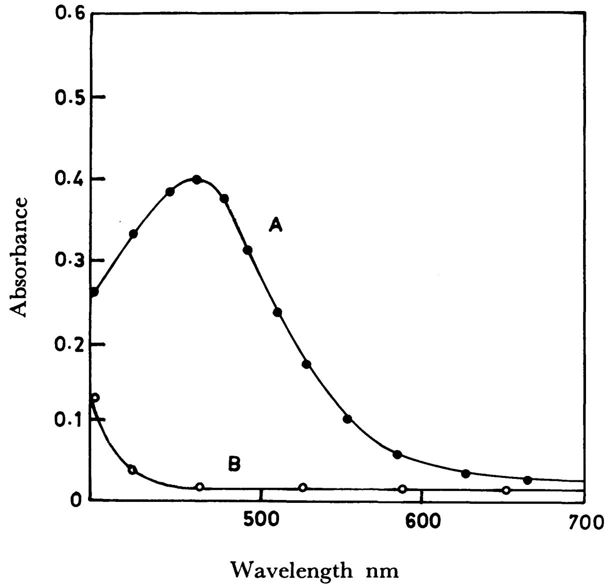


Fig. 1. Absorption spectra.
A: Fe(III)-HTPB-SCN complex (Fe=3.21 × 10^{-5}M), B: *N*-Hydroxy-*N*-*o*-tolyl-*N*′-phenylbenzamidine 0.003 M in benzene.

Effect of Electrolytes, Volume of Aqueous Phase, Temperature, and Standing Time. The absorption of the colored system and the percentage extraction of iron(III) are not affected by electrolytes such as potassium chloride, ammonium chloride, and sodium chloride (2.0 M). The volume of the aqueous phase can vary between 10 to 60 ml without affecting the mixed complex formation and its subsequent extraction. Variation in temperature from 20 to 40 °C did not influence the absorbance value of the colored system. The extracts were stable for at least 40 h at 27±2 °C. A time of 1 min was sufficient for complete extraction of iron(III).

Beer's Law, Optimum Concentration, Sensitivity, and Precision. The orange-red iron(III) mixed complex adhered to Beer's law over the concentration range 0.2—4.5 ppm of the metal. The optimum concentration range on the basis of Ringbom plot[15)] is 0.8 to 3.8 ppm of iron. The molar absorptivity and

Table 1. The Effect of Foreign Ions in the Determination of Iron(III) (45 μg/25 ml)

Ion	Added form	Tolerated amount[a)]/ppm
Fe(II)	$FeSO_4(NH_4)_2SO_2 \cdot 6H_2O$	800
Cr(III)	$K_2SO_4Cr_2(SO_4)_3 \cdot 24H_2O$	1000
Al(III)	$Al(NO_3)_3$	1600
Bi(III)	$Bi(NO_3)_3$	1200
Cu(II)	$CuSO_4 \cdot 5H_2O$	300
Ni(II)	$Ni(NO_3)_2$	1400
Co(II)	$Co(NO_3)_2$	1400
Mn(II)	$MnCl_2 \cdot 4H_2O$	1000
Zn(II)	$ZnSO_4$	1600
Cd(II)	$CdSO_4$	2000
Pb(II)	$Pb(NO_3)_2$	1500
Ca(II)	$Ca(NO_3)_2$	3000
La(III)	$La(NO_3)_2$	2500
Th(IV)	$Th(NO_3)_4 \cdot H_2O$	1200
Zr(IV)	$Zr(SO_4)_2 \cdot 4H_2O$	80
Ti(IV)	$K_2TiO(C_2O_4) \cdot 2H_2O$	100
Nb(V)	Niobium Citrate	120
Ta(V)	Tantalum Citrate	120
Mo(VI)	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	350
W(VI)	$Na_2WO_4 \cdot 2H_2O$	60
U(VI)	$UO_2(NO_3)_2 \cdot 6H_2O$	500
F^-	NaF	900
I	KI	900
$S_2O_3^{2-}$	$Na_2S_2O_3$	900
AsO_4^{3-}	Na_2HAsO_4	900
Phthalate	$KHC_8H_4O_4$	2500
Citrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	800
Tartrate	$NaKC_4H_4O_6 \cdot 4H_2O$	800
Triethanolamine	$N(C_2H_4OH)_3$	2500
Urea	CH_4N_2O	2500
Thiourea	CH_4N_2S	2500

a) Causing an error of less than 2%.

Sandell's sensitivity of the color reaction are found to be 12000 l mol^{-1} cm^{-1} and 0.0046 µg of iron per cm^2 at 460 nm. The precision of the method was evaluated by measuring the absorbance value of 10 samples, each containing 45 µg iron per 25 ml. A mean absorbance value of 0.388 was observed with a standard deviation of ±0.0025 absorbance unit and relative standard deviation of ±0.69%.

Composition. In iron(III)–HTPB–SCN mixed ligand complex, the ratio of iron to reagent and thiocyanate was determined by curve fitting method[16] (by plotting log absorbance versus log concentration of reagents in terms of molarity). The results obtained indicate the formation of 1:1:2 (metal: HTPB:SCN) mixed ligand complex in benzene.

Influence of Diverse Ions. For studying the influence of diverse ions on the determination, an aliquot of iron(III) solution containing 45 µg of the metal was transferred to a separatory funnel and to this was added a solution containing a known weight of diverse ions. The acidity was adjusted to 0.5 M with hydrochloric acid and the total volume to about 25 ml and iron was extracted with the reagent as described earlier. The effect of various ions in the determination of iron(III) is summarized in Table 1.

Application of the Method. To test the validity of the method, various drugs, biochemicals and biological samples were selected. Iron presented in the form of iron(II) sulfate, iron(II) succinate, and fumarate can be conveniently determined by this method.

A weighed amount of the sample was transferred into a Kjeldahl flask and heated gently with a mixture of concentrated nitric and sulfuric acids (10:1 v/v) until charring commenced. Addition of concentrated nitric acid and boiling were continued until either a colorless or a pale yellow solution was obtained. This was then cooled, a few mililiters of water was added and then solution was evaporated until white fumes were evolved. The procedure was repeated two or three times. A few mililiters of dilute hydrochloric acid were added and heating was continued until fumes of nitric acid had been removed. The solution was then diluted to an appropriate volume. An aliquot of the solution was pipetted and iron was determined by the procedure recommended earlier. The results were compared with those obtained by 1,10-phenanthroline method (Table 2).

Table 2. Determination of Iron in Drug Biochemical and Biological Samples

No. Sample	Calculated iron content[a]/%	
	1,10-Phenanthroline method	Hydroxyamidine method
1 Iberol	0.105	0.103
2 Compoferon	0.110	0.109
3 Folitrin	0.350	0.347
4 Globintone	0.200	0.198
5 Ferronicum	0.325	0.324
6 Blood	0.057	0.057
7 Brassica Oleracia[b] varbotrytis (Leaves)	0.041	0.040

a) Average of six determinations. b) Value obtained from Ref. 17.

References

1) E. B. Sandell, "Colorimetric Determination of Traces of Metals," 3rd ed. Interscience, New York (1959), p. 97.

2) H. Nishida, *Bunseki Kagaku,* **19**, 221 (1970).

3) Y. Horiuchi and H. Nishida, *Bunseki Kagaku,* **19**, 930 (1970).

4) V. Panduranga Rao and P. V. R. B. Sarma, *Mikrochim. Acta,* **4**, 763 (1970).

5) H. Alexaki-Tzivanidou, *Anal. Chim. Acta,* **75**, 231 (1975).

6) M. Katyal, V. Kushwaha, and R. P. Singh, *Analyst,* **98**, 659 (1973).

7) K. Hayashi, Y. Sasaki, and K. Ito, *Bunseki Kagaku,* **21**, 1338 (1972).

8) H. L. Cluley and E. J. Newman, *Analyst,* **88**, 3, (1963).

9) J. M. Zehner and T. R. Sweet, *Anal. Chim. Acta,* **35**, 135 (1966).

10) B. M. Desai and J. V. Parghi, *J. Indian Chem. Soc.,* **54**, 1102 (1977).

11) B. J. Desai and V. M. Shinde, *Analyst,* **104**, 160 (1979).

12) A. J. Pandell, R. A. Montgomery, and R. A. Meissner, *Analyst,* **105**, 181 (1980).

13) D. T. Burns and M. E. M. Abdel Aziz, *Analyst,* **105**, 383 (1980).

14) A. I. Vogel, "A Text Book of Quantitative Inorganic Analysis," Longmans, Green London (1964), p. 524.

15) A. Ringbom, *Z. Anal. Chem.* **115**, 332 (1939).

16) L. G. Sillen, *Acta Chem. Scand,* **10**, 185 (1956).

17) C. Gopalan, B. V. Ramsastri, and S. C. Balasubramanian. "Nutritive Value of Indian Foods," National Institute of Nutrition, Indian Council of Medical Research, Hyderabad (1978), p. 65.