



Fig. 1: 3, 5-di-tert-butyl-4-hydroxy styrene used in this study.

BA=0.19827R2L+0.32270 R2B1+0.84462 R2B5+0.8442 (3), where, n=15, r=0.475, r<sup>2</sup>=0.226, f=0.729, t=0.854 and STD=0.281. The above Eqn. 3 is statistically not significant having poor coefficient of determination, but it indicates that the over all value of B5 (maximum width of substituent) is more contributing than L (length of substituent) along the axis of the parent skeleton Table 4. From all the above observations, it can be concluded that, the antiinflammatory activity of the analogues is highly correlated with the thermodynamic (MR) and Sterimol (B5, L) parameters. Hence, Eqns. 2 and 3 indicates a lipophilic group, which is less bulkier at R1 and more bulkier at R2, is required for good biological activity. The above QSAR studies may throw some light on the substitutional requirement for further development of these compounds for more potent activity.

## ACKNOWLEDGEMENTS

Authors would like to express their thanks to the Director, SGSITS, Indore, for providing the facilities for this work. Geeta Kant is also thankful to the Ministry of Human Resource Development, New Delhi, for providing a research fellowship for this project.

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## A Modified Sensitive Micro Spectrophotometric Determination of Iron(III) by Thiocyanate Method

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Accepted 8 February 2005

Revised 6 September 2004

Received 1 August 2003

A systematic study of the colour reaction formed between iron(III) and thiocyanate reagent has been carried out by using micro-scale spectrophotometric method. The optimized data resulted conditions in obtaining unusual highest sensitivity with molar absorption of  $2.9565 \times 10^4 \text{ l/mol.cm}$  at wavelength of maximum absorption 480 nm in 0.2-1.4 N nitric acid medium containing 60%

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acetone and 25,000 fold molar excess of the thiocyanate reagent. Beer's law is valid for 0.1-4.0 ppm of iron(III). Sandell's sensitivity is 0.002  $\mu\text{g}/\text{cm}^2$ . Effects of reagent concentration, order of addition, stability, acidity, types of solvents and diverse ions are reported. It has been found to give accurate results of iron estimations in pharmaceutical preparations and mustard seeds. The proposed optimized method has highest sensitivity, unusual stability and simplicity in operation, without involving cumbersome extractive procedure. Further, the procedure has additional advantages of economy, safety and less time consuming with minimum waste disposal.

Thiocyanate reagent for the determination of iron(III) is one of the most convenient methods, which was first proposed in 1826 by Berzelius<sup>1</sup>. Since then many scientists studied the above reaction to improve the sensitivity and stability of the colour formation by varying acids<sup>2-3</sup>, solvents<sup>2,4-7</sup>, pH of the reaction medium<sup>4-8</sup> and combining other reagents<sup>9</sup>. Hayashi *et al.* developed a sensitive method for the determination of iron(III) using thiocyanate, containing Triton X-100 and methyloctylammonium chloride in the presence of amines. But this method involves the use of other costly reagents. In addition, information regarding reagent concentration, order of addition and interference studies is lacking. A few methods of extractive spectrophotometric determination of iron as iron-thiocyanate with other organic reagents were proposed<sup>10-13</sup>. But these methods suffer disadvantages of less sensitivity, use of costly reagents and the cumbersome extractive procedures. A systematic study of the reaction for a particular set of conditions with respect to the type of solvent, reagent concentration, Beer's law limit, order of addition, rate of colour formation, stability and interferences is lacking. This resulted in problems in using the method for practical applications. Further, small-scale chemistry is increasingly popular because of its significant reduction in the cost of the chemicals, less problem of waste disposal, greater safety, rapidity and less space requirements. However, to secure the same order of accuracy in the final results of the small-scale experiment special care must be taken. It is always not satisfactory to mere scale down the existing macro scale experiments. So, a systematic study in micro scale of the reaction of iron(III) with thiocyanate under novel conditions has been carried out. The proposed method offers the advantages of simplicity, selectivity, highest sensitivity, wider range of determination and very good stability without the need for cumbersome extraction using additional costly reagents.

A Systronics 117 spectrophotometer with 1 cm path length square glass cells were used for absorbance measurements. A Systronics  $\mu$  pH-362, was used for pH measurements. All reagents were of analytical grade and double distilled water was used for preparing all the solutions. Stan-

dard iron(III) solution of ammonium ferric sulphate was prepared with 10ml of concentrated nitric acid in 100ml volumetric flask to obtain  $2 \times 10^{-2}$ M (9.74 mg/ml) solution. Ammonium thiocyanate solution of 7.5 M was prepared by dissolving 57.1 g ammonium thiocyanate in a 100 ml volumetric flask with double distilled water. Suitable dilutions were made to obtain required solutions. Nitric acid of 10 N was prepared by diluting 62.5 ml of concentrated nitric acid to 100ml.

Solutions 0.1, 0.2, 0.25, 0.3 and 0.4 ml of 50 ppm iron(III) were placed in separate 5 ml five volumetric flasks. To each flask, 1.3 ml of 7.5 M ammonium thiocyanate, 3.0 ml of acetone and 0.2 ml of 10 N nitric acid were added. Then the solutions were made up to mark with double distilled water. After 10 min of mixing, absorbance readings were taken against the reagent blank at 480 nm. Calibration graph was prepared by plotting absorbance versus concentration of iron(III) in ppm. About 20 mg of the powdered iron tablet was weighed accurately into a 50 ml beaker and dissolved using 1.0 ml of 2.0 N nitric acid and 2.0-3.0 ml of double distilled water (warm if necessary). The solution was filtered into a 25 ml volumetric flask using Whatmann No. 41 filter paper and washed with double distilled water. The solution was made up to the mark with double distilled water and shaken to obtain uniform concentration. A suitable volume of the solution was transferred into a 5 ml volumetric flask. All the reagents except iron(III) were added as explained in the calibration procedure and then absorbance measurement was made as above. Amount of iron(III) present was calculated using the calibration curve. The results are summarized in Table 1.

The colour reaction between iron(III) and thiocyanate reagents has been investigated by studying the effects of various factors like nature of acid, solvent, spectral characteristics, optimum concentration range, sensitivity of the reaction, effect of reagent concentration, order of addition, rate of colour formation, stability of the colour reaction and interferences. Micro-scale approach has been investigated for the above reaction and suitable procedure is proposed for the analysis. Order of the reaction sensitivity observed

TABLE 1: DETERMINATION OF IRON(III) IN THE PHARMACEUTICAL SAMPLES\*

Sample	Iron reported (mg)	Iron found (mg)	Relative Standard deviation (%)	Relative error (%)
Tofe	100	99.87	0.3706	0.450
Ferose	100	99.87	0.4126	0.936
Ferium	100	99.89	0.7438	0.855

\*The results presented in the table are based on average of six determinations

to be  $\text{HNO}_3 > \text{HCl} > \text{CH}_3\text{COOH} > \text{H}_2\text{SO}_4$ . Optimum nitric acid concentration range was found to be 0.2-1.4 N. Higher concentration of the acid is not recommended because it gave yellow colour with thiocyanate reagent<sup>3</sup>. Intensity of the colour reaction for a given iron concentration has been found to be invariable with ammonium thiocyanate, sodium thiocyanate or potassium thiocyanate. However, ammonium thiocyanate preferred to other salts as it is not hygroscopic and is economical. Acetone (60 %) increased the sensitivity to more than 275%. The red coloured species obtained in the presence of nitric acid and acetone exhibited an absorption maximum at 480 nm. The reagent solution without iron(III) under similar condition showed no absorbance around this

wavelength, thus promoting an excellent analytical condition. A 25,000 fold molar excess of the reagent is necessary for the full development of the red colour. Addition of excess reagent has no effect on absorbance. The effect of order of addition of reagents has no effect on the colour of the reaction. Maximum value of absorbance was observed after 10 min of mixing of the reagents. The maximum colour developed remained stable for more than three days. Beer's law is obeyed in the range 0.1-4.0 ppm of iron(III). The optimum concentration range for the effective spectrophotometric determination of iron as evaluated by Ringbom's method<sup>14,15</sup> has been found to be 0.5-4.0 ppm. Sandell's sensitivity of the reaction is 0.002  $\mu\text{g}/\text{cm}^2$  and molar absorptivity is

TABLE 2: EFFECT OF DIVERSE IONS ON THE DETERMINATION OF 1 PPM OF IRON(III)

Cation	Tolerance value in ppm	Anion	Tolerance value in ppm
$\text{Al}^{3+}$	500	$\text{F}^-$	300
$\text{Cd}^{2+}$	400	$\text{Cl}^-$	400
$\text{Ce}^{4+}$	12	$\text{Br}^-$	950
$\text{Co}^{2+}$	0	$\text{I}^-$	0
$\text{Cr}^{6+}$	2	$\text{HCOO}^-$	3000
$\text{Cu}^{2+}$	0	$\text{CH}_3\text{COO}^-$	4000
$\text{Hg}^{2+}$	120	$\text{CO}_3^{2-}$	3000
$\text{Mg}^{2+}$	450	$\text{NO}_2^-$	3
$\text{Mn}^{2+}$	80	$\text{NO}_3^-$	2400
$\text{Mo}^{6+}$	0	$\text{SO}_4^{2-}$	2400
$\text{Ni}^{2+}$	100	$\text{S}_2\text{O}_3^{2-}$	4
$\text{Pb}^{2+}$	10	$\text{PO}_4^{3-}$	>2400
$\text{U}^{6+}$	30	Citrate	4000
$\text{V}^{5+}$	10	Oxalate	35
$\text{W}^{6+}$	55	Tartrate	6000
$\text{Zn}^{2+}$	30		

2.9565x10<sup>4</sup> l/mol cm at 480 nm, highest sensitivity ever observed. In order to assess the possible analytical applications of the above coloured reaction, effects of a wide range of cations and anions which often accompany iron are investigated. An error of 2% in absorbance readings has been considered to be tolerable. The tolerance data are summarized in Table 2. The accuracy of the recommended procedure has been tested by applying the method for the analysis of iron content present in the pharmaceutical preparations and in mustard seeds. The procedural details are given in the experimental section. Data are presented in Table 1. The proposed micro spectrophotometric method with new developed conditions of colour reaction of iron(III) with thiocyanate has unusual highest sensitivity and stability among the methods proposed so far and stability. The result presented in Table 1 has been found to be entirely satisfactory with respect to the accuracy of the method. It is proved very effective for the determination of minute amounts of iron. The optimized procedural details are very simple without involving cumbersome extracting procedure. Further, the method has economy in using small amount of chemicals, improved safety and less time consuming with the minimum quantities of waste disposal.

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## Essential oil Composition of *Coleus amboinicus* Lour

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Accepted 8 February 2005

Revised 8 September 2004

Received 24 April 2004

**The volatile constituents of the whole herb of *Coleus amboinicus* Lour (Labiatae) were analyzed by GC-MS following isolation by hydrodistillation. Ten compounds were identified among which carvacrol (50.7 %)  $\gamma$ -caryophyllene (13.1 %) and patchoulane (8.7%) were dominant.**

*Coleus amboinicus* Lour (Labiatae), commonly called Indian borage or French thyme is a perennial herb found wild and cultivated throughout India. Its leaf juice is a powerful aro-

matic carminative, used in digestive disorders in children<sup>1</sup>. The leaves are considered effective in asthma and chronic cough<sup>2</sup>. Essential oil is reportedly antibacterial<sup>3</sup> and weakly herbicidal<sup>4</sup>. Previous investigations of the herb oil reveal the presence of thymol<sup>5,6</sup>. GC/MS analysis of essential oil of *Coleus amboinicus* of Indian origin has not been attempted earlier.

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