

## Estimation of Fe(III) using $^{59}\text{Fe}$ and acetylacetonone by substoichiometric isotope - dilution analysis

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A highly precise and rapid method for the quantitative estimation of iron traces by substoichiometric isotope dilution analysis has been described. The method involves the extraction of spiked ( $^{59}\text{Fe}$ ) iron(III) with substoichiometric amounts of acetylacetonone into chloroform at pH 2.0. The proposed method is free from interferences of various closely related foreign ions, thereby establishing the selectivity of the proposed technique. The present method has also been applied to different certified alloys without any pre-separation and the trace quantities of iron are successfully evaluated.

**Keywords:** Isotope dilution analysis, Radiotracer  $^{59}\text{Fe}$ , Substoichiometric extraction

Iron is one of the elements of major importance in both environmental and biological chemistry owing to its unique chemical properties such as solubility and degree of complex formation<sup>1,2</sup>. It is present in the environmental samples mainly as oxides such as magnetite and haematite comprising about 5% of the Earth's crust. These iron oxides have a wide range of applications particularly in the field of telecommunication and television industry<sup>3</sup>. There is abundant iron in the human body most of which is bound to hemoglobin, myoglobin and the cytochromes which helps in the absorption and distribution of oxygen to the tissues. Several health hazards are associated with iron deficiency and iron overload in the human body system. Deficiency of iron in the body system leads to anaemia<sup>4</sup> while iron overload severely damages heart and liver cells<sup>5,6</sup>, thereby leading to metabolic acidosis, coagulopathy, coma and even death. Disturbed iron metabolism may lead to various neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease<sup>7</sup>. The wide array of applications in different fields and the various toxic effects in biological samples require the development of a rapid and highly precise technique for the estimation of iron.

Various techniques such as spectrophotometric<sup>8-10</sup>, solvent extraction<sup>11,12</sup>, solid-phase extraction (SPE)<sup>13-15</sup>, atomic absorption spectroscopy<sup>16,17</sup> and flow-injection methods<sup>18</sup> have been reported on quantitative

estimation of iron. In addition, radiochemical methods such as charged particle activation analysis<sup>19</sup> substoichiometric isotope dilution analysis<sup>20-23</sup> and isotope dilution coupled with mass-spectrometry (SIDMS)<sup>24,25</sup> have also been widely exploited for the determination of iron species.

The present work describes the determination of iron(III) by extraction with substoichiometric amount of acetylacetonone into chloroform using the principle of isotope dilution analysis. The method has exploited in the successful estimation of iron from various certified ores and alloys.

### Experimental Procedure

#### Apparatus and reagents

The  $\beta$ -activity of  $^{59}\text{Fe}$  was measured with well-type NaI(Tl) detector of Nucleonix, India make. The adjustments of pH were carried out with a digital systronics pH meter (Model 335).

The radioisotope  $^{59}\text{Fe}$  in the form of its chloride salt was supplied by BRIT, India. An iron(III) standard solution was prepared by dissolving ~1g ferric chloride (A.R.) in deionised water. An appropriate aliquot of the stock solution was diluted to 100 mL with prior addition of a few drops of radioactive  $^{59}\text{Fe}$ . One millilitre of this working stock corresponded to 32.1  $\mu\text{g}/\text{mL}$  of Fe(III). All other reagents and solvents used were of AR grade.

#### General extraction procedure

A 5 mL aqueous solution of spiked ( $^{59}\text{Fe}$ ) ferric chloride, containing 32.1  $\mu\text{g}/\text{mL}$  Fe(III) and maintained

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at  $pH$  2.0, was extracted with 2 mL 57.40  $\mu\text{g/mL}$  of acetylacetonone in 5 mL chloroform. After equilibration the two phases were disengaged and 1 mL of each of the two phases was measured for their  $\gamma$ -activities using a NaI(Tl) detector connected to a single-channel analyzer. Radioisotope  $^{59}\text{Fe}$  was assayed by its characteristic photopeak at 1.0993 and 1.2993 MeV.

## Results and Discussion

### Effect of solvent

The reagent (acetylacetonone) used in the present study is found to be soluble in a variety of organic solvents. Thus, the extraction process was carried out in several different solvents and the extraction efficiency of iron(III)-acetylacetonone complex in different organic solvents is found to be in the following order :

Chloroform > *n*-butanol > amyl alcohol > cyclohexanol > nitrobenzene

### Optimisation of $pH$

In extraction with chelating agent like acetylacetonone, the  $pH$  of the reaction media plays an important role in determining the extent of reaction, hence the extraction was carried out under varying  $pH$  condition. For this purpose, the extent of extraction of iron(III) with acetylacetonone into chloroform under varying  $pH$  (1-3) of the aqueous phase was studied. After phase separation, 1 mL organic phase of each set of extraction was measured for their activities. The extracted  $\gamma$ -activity in the organic phase is plotted as a function of  $pH$  of the aqueous solution (Fig.1). The optimum  $pH$  for the extraction corresponds to a value of 2.0 and hence further extraction processes were carried out at  $pH$  2.0. At higher  $pH$ , hydrolysed species of Fe(III) hinders the extraction. On the other

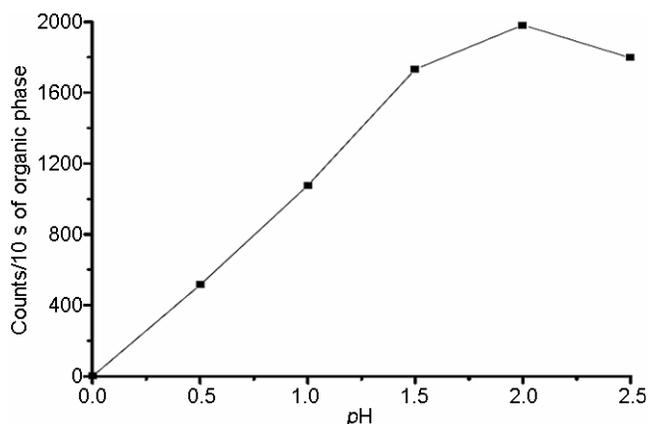


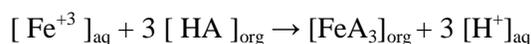
Fig. 1—Variation in extraction of iron(III) with  $pH$  of aqueous solution.

hand, prospect of extraction is not very high in strong acid medium as the ligand itself is a weak monobasic acid and dissociates weakly under such condition.

### Reproducibility of substoichiometric extraction

In order to establish the stoichiometry of the extracted complex in the organic phase, 5 mL of the aqueous phase maintained at  $pH$  2.0 and containing 32.1  $\mu\text{g}$  of Fe(III) was extracted with varying amount (0 – 5 mL) of 57.40  $\mu\text{g/mL}$  acetylacetonone in chloroform as solvent. The volumes of the two phases were kept equal for all set of extractions. After equilibration, the two phases were separated and 1 mL organic phase for each set of extractions was monitored for the  $\gamma$ -activity. The measured activity of iron(III) extracted into the organic phase is plotted against the amount of reagent added (Fig.2).

Analysis of the reproducibility curve (Fig.2) indicates that maximum extraction corresponds to 3 mL 57.40  $\mu\text{g/mL}$  of the ligand. With further increase in concentration of acetylacetonone beyond 172.2  $\mu\text{g}$  there is no further increase in the  $\gamma$ -activity of the organic phase since the plot is almost parallel to the X-axis in this region, thereby indicating that the extraction of 32.1 $\mu\text{g}$  iron(III) has reached a saturation value corresponding to 172.2  $\mu\text{g}$  of ligand. This particular point is called the stoichiometric point which corresponds to metal:ligand ratio of 1:3 in the extracted complex and the extraction equilibrium can be represented as:



HA = undissociated ligand

Hence, it is obvious that below the stoichiometric point the extraction is always quantitative.

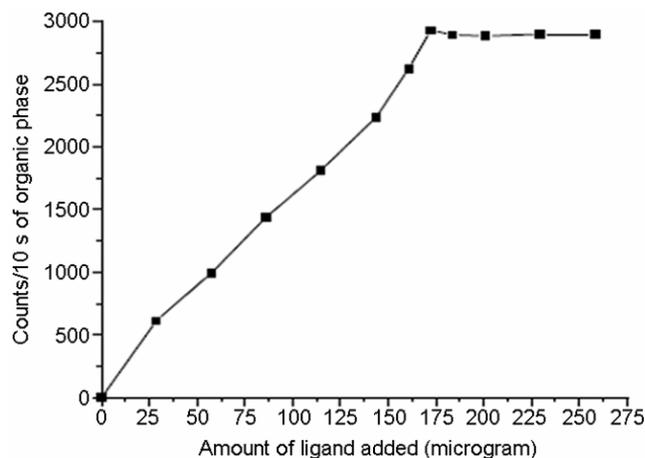


Fig. 2—Reproducibility of substoichiometric extraction of iron (32.1 g) with acetylacetonone into chloroform

### Chemical interferences

The interference effects from various closely related foreign ions was critically examined by extracting various sets of spiked Fe(III) solution with acetylacetone into chloroform media in presence of 10-fold amounts of diverse ions under identical experimental conditions. After disengagement of the two phases the activity of 1 mL organic extract of each set was measured ( $a$ ). Another set of extraction was carried out in the absence of any interfering ions and after phase separation, the activity of 1 mL organic extract was measured ( $a_0$ ). The hindrance of these diverse ions on the extraction efficiency is summarized in Table 1 in terms of ratio  $a/a_0$ . The closer the value of the ratio to unity, the more efficient is the extraction and lesser interference is expected. It can be seen from Table 1 that the extraction system is free from hindrances of different allied ions.

### Calibration

The quantitative estimation of iron(III) from various complex matrices requires the need of a calibration curve. In order to obtain the calibration plot, a series of extraction was performed. In each set of extraction, 5 mL aqueous phase consisting of spiked ferric chloride solution corresponding to 32.1 g iron(III) and varying (known) amount of inactive iron(III) was extracted with 2 mL 57.40  $\mu\text{g/mL}$  acetylacetone diluted to 5 mL with chloroform i.e. under substoichiometric condition. After attainment of equilibrium the two phases were disengaged and the  $\gamma$ -activity of 1 mL organic extract was recorded as A. The process of extraction was repeated under the same set of conditions but in absence of inactive iron(III). The extracted activity in this case is  $A_0$ .

Table 1—Effect of foreign ions on extraction of iron(III)

Diverse ions	Amount taken, g	$a/a_0$
Cr(III)	341	0.9232
Mn(II)	350	0.9533
Co(II)	356	0.9739
Ni(II)	329	0.9678
Cu(II)	325	0.9814
Zn(II)	350	0.9679
Pb(II)	317	0.9884
Hg(II)	317	0.9765
$\text{PO}_4^{3-}$	320	0.9123
F <sup>-</sup>	374	0.9561

Amount of iron(III) taken = 32.1 g.

$a$  = Activity extracted in presence of foreign ion.

$a_0$  = Activity extracted in absence of foreign ion.

The ratio of  $A_0/A$  is plotted against the amount of inactive iron(III) added (Fig. 3).

The figure shows a linear variation in  $A_0/A$  against the amount of inactive iron(III) added. With gradual addition of incremental amounts of inactive iron a competition arises between active and inactive iron(III) to be effectively extracted by acetylacetone. As a result, the activity of iron(III) extracted in the organic phase (A) falls off proportionately with amount of inactive iron added. The linear variation permits determination of microgram amounts of iron with a fair degree of precision.

### Recovery of added iron

For the recovery of added iron, different amounts of inactive iron were mixed with labeled ( $^{59}\text{Fe}$ ) ferric chloride solution keeping the total iron(III) content of the aqueous phase constant (64.2 g). Then the mixture was subjected to extraction with 4 mL 57.40  $\mu\text{g/mL}$  of ligand into chloroform. The volumes of both phases were kept equal (5 mL). After equilibration, the  $\gamma$ -activity ( $C$ ) of 1 mL organic extract was measured. Another set of extraction was carried out with only 64.2 g labeled iron(III), keeping the amount of ligand constant. The extracted activity of 1 mL organic phase corresponds to  $C_0$ . The amount of inactive iron(III) was computed using the following relationship:

$$W_{\text{inactive}} = [(C_0 - C) \times W_{\text{active}}] / C$$

where  $W_{\text{inactive}}$  = amount of labeled ( $^{59}\text{Fe}$ ) iron, and  $W_{\text{active}}$  = amount of inactive iron(III) added.

The results (Table 2) are in good agreement with actual amount of inactive iron(III) added and the

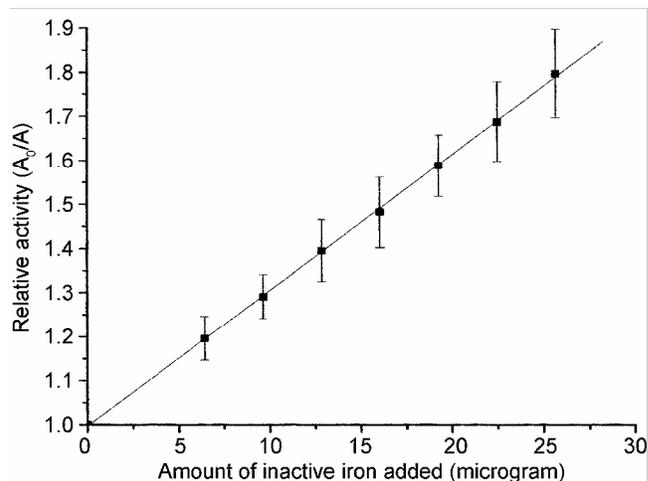


Fig. 3—Calibration curve for estimation of inactive iron(III) added

Table 2—Recovery of inactive iron added

$W_{\text{active}}$ g	$W_{\text{inactive}}$ added, g	Counts / 10 s of organic phase	$W_{\text{inactive}}$ found, g	%Error
64.2	0	3427 ( $C_0$ )	—	—
57.78	6.42	3129	5.503	- 1.43
48.15	16.05	2577	15.88	- 1.06
32.1	32.1	1723	31.75	- 1.09
16.05	48.15	866	47.46	- 1.43

Table 3—Analysis of iron from various certified ores and alloys

Sample	Amount taken, mg	Amount of iron present g	Amount of iron found* g
Mn-bronze**(10e)	100	160	155 ± 2.00
Dolomite**(9h)	100	210	206 ± 1.47
Al-bronze**(32a)	100	267	260 ± 2.00

\*Average of triplicate analysis.

\*\*As per certificate of analyses issued by Bureau of Analyzed Samples Limited.

method can be further extrapolated for analysis of real samples.

### Application

The present method has been applied for the estimation of iron(III) from various certified ores and alloys. The samples were brought into solution by usual methods<sup>26</sup> and subjected to SIDA using the proposed method of analysis and the results obtained are summarized in Table 3. The experimental results are found to be greatly consistent with the certified values. The method can be effectively used for the determination of iron(III) from various other complex matrices.

### Conclusion

The presence of low levels of iron in environmental samples and presence of other associated elements necessitate a pre-separation step prior to estimation of iron. In our proposed work we have studied the interfering effects of various allied cations and few anions which reveals that the extraction process is highly selective.

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