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A New Spectrophotometric Method for the Determination of Ferrous Iron in the Presence of Ferric Iron

Leandro Herrera, Pauline Ruiz, Juan C. Aguillon & Alexis Fehrmann

Departamento de Ingenieria Quimica, Facultad de Ciencias Fisicas y Matematicas, Universidad de Chile, Beaucheff 861, Santiago, Chile

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ABSTRACT

A new spectrophotometric method for the determination of ferrous iron concentration in samples containing ferric iron is presented. This new method is a modification of the Muir o-*phenanthroline ferrous iron determination method.*

The new method consists of the quantitative spectrophotometric determination of Fe(II) by o-*phenanthroline, but in which the ferric iron is complexed with sodium fluoride to eliminate interferences.*

A critical experimental comparison between the Muir spectrophotometric method, the Kolthoff titrametric method and the new proposed method is presented. Whenever Fe(III) accounts for more than 50% of the total iron in a sample, the method of Muir overestimates Fe(II).

The proposed method has a range of 1–10 μg ferrous iron. It is as insensitive to other metal ions as the original method of Muir and is insensitive to Fe(III) up to a point where Fe(III) might be as high as 95% total iron.

The proposed method is suitable for Fe(II) determination in bacterial leaching systems, where Fe(II) might be as low as 1% of total iron.

Key words: iron, ferrous, ferric, method, spectrophotometric, bacterial leaching.

1 INTRODUCTION

Spectrophotometric methods tend to be preferred as they offer a number of advantages such as simplicity, speed and sensitivity over other methods (e.g., titrametric analyses).

For biological experimental systems the importance of good, reliable quantitative methods cannot be overstressed. For example, an overestimated result might be falsely interpreted as evidence of substrate and/or product inhibition in the reacting system.

We have observed that, for a given bacterial leaching system, the use of different analytical methods gave different results, particularly for the quantitative determination of ferrous iron. The difficulty of obtaining reliable growth parameters for *Thiobacillus ferrooxidans* might be related to problems with the analytical methods currently used in this field.

The spectrophotometric method of Muir[1] for the quantitative determination of Fe(II) in solution was proposed for metallurgical samples containing many ions. However, if Fe(III) was present in the sample, Muir[1] mentioned the possibility of interference catalysed by sunlight.

A more precise but cumbersome method is the titrametric determination of Fe(II), originally proposed by Knop[2] and later modified by Kolthoff,[3] using potassium dichromate to oxidize the ferrous iron and barium diphenylamine-4-sulfonate as internal indicator.

A most important feature of the titration method is the presence of 85% orthophosphoric acid to complex Fe(III) so that it does not interfere with the analysis.

Fe(II) is a common energy source of *T. ferrooxidans*. The oxidative action of the bacteria produces situations where the remaining Fe(II) in solution might be as low as 1% of total Fe. This ratio of Fe(III) to Fe(II) makes the use of the Muir[1] method impractical.

A new method is reported here which uses the same colorimetric reagent as Muir[1] but in which the Fe(III) in the sample is complexed, in a manner similar to Kolthoff's[3] complexing reaction.

2 EXPERIMENTAL METHODS

2.1 Reagents and samples

All reagents used in this research were analytical grade obtained from Merck or Sigma.

In the preparation of synthetic samples, Fe(II) [ammonium iron(II) sulfate hexahydrate] was held constant (at around 50 ppm) and Fe(III) (ferric sulfate heptahydrate) was added at levels from 0 to 10 000 ppm. Fe(II) was hence between 100% and 0·5% of the total iron.

As well as working with synthetic samples, 'real' samples were also assayed. A 'real' sample was defined as a liquid sample from different industrial and experimental ore bacterial leaching systems that might contain *T. ferrooxidans*.

Spectrophotometric readings were taken against a blank containing all reagents except the sample, which was replaced by distilled water. Absorbance was read at 510 nm.

2.2 Kolthoff[3] titration method

A computer-based autotitrator was developed for the quantitative determination of Fe(II). The analytical system used a constant flow rate peristaltic pump (to feed the titrant) and a redox potential (Eh) electrode.

The reaction took place in a 100-ml beaker containing 1 ml sample, 1 ml 85% orthophosphoric acid, 40 ml 1 N sulfuric acid, a magnetic agitator and the Eh electrode. The titrant added by the control system was 0·2 mN pH 1 potassium dichromate (which is a primary standard) as directed by Kolthoff.[3] Titrant addition was stopped by the automatic system when the maximum inflection point was attained (see e.g. Ref. 4).

The computer (an IBM PC with one analog input and one digital output) switched the pump on to feed the titrant. The computer followed the Eh potential and stored the time at which the maximum inflection point was reached.

The Fe(II) concentration in the sample was calculated from the volume of titrant added to reach the maximum inflection point. The computational details (hardware and software) of the system can be obtained from the authors.

2.3 Muir spectrophotometric method

The method of Muir[1] is based upon the color developed by Fe(II) reacting with 1,10-phenanthroline. This reagent will be referred to as '*o*-phen'.

The total analysis volume was modified to 2·5 ml from Muir's 25 ml. Therefore, in a 10-ml test tube, 0·1 ml sample was mixed with 0·4 ml *o*-phen solution and made up to 2·5 ml with distilled water. Total iron was measured, as proposed by Muir,[1] by reducing the Fe(III) in the sample with a 10% aqueous solution of hydroxylamine hydrochloride and measuring the resulting Fe(II) by the *o*-phen method.

2.4 The proposed method

2.4.1 Complexing reagent

Sodium fluoride (2·1 g) was dissolved in 98 ml distilled water and 2·0 ml sulfuric acid to make a solution which was approximately 0·5 M and pH 1. The solution was prepared daily in polyethylene bottles.

2.4.2 Colorimetric reagent

This reagent is the same as in the original *o*-phen method. An *o*-phen solution was prepared by dissolving 10·0 g 1,10-phenanthroline monohydrate in 300 ml distilled water containing 10 drops concentrated hydrochloric acid, and the volume was made up to 500 ml with distilled water. An acetate buffer was prepared by dissolving 125 g ammonium acetate in 75 ml distilled water to which 300 ml glacial acetic acid was added; the volume was made up to 500 ml with distilled water.

2.4.3 o-Phen reagent

One volume of the *o*-phen solution was mixed with one volume of the acetate buffer.

2.4.4 Procedure

The sample (0·1 ml), in the range 10–100 ppm Fe(II) was placed in a 10-ml tube. Complexing fluoride reagent (1·0 ml) was added, followed by agitation of the tube.

Next, 0·4 ml of the *o*-phen reagent was added and the tube was again agitated. The mixture was diluted to 2·5 ml with distilled water and the tube was agitated. After 5 min (minimum) at ambient temperature, the absorbance was read at 510 nm.

A standard containing a known concentration of ferrous iron was analysed with each set of tests. The addition of hydroxylamine ensured that all the iron present in the standard was in the form of Fe(II). It was verified that the reducing agent did not develop any color when used with this new method.

3 RESULTS AND DISCUSSION

All reported results are averages of four measurements and significant deviations were not observed. An analysis of the error in the titration method follows.

The automatic titrator was tested with synthetic samples so that it could be used later as a standard method for comparison with the spectrophotometric methods. The significance of complexing the Fe(III) was observed. The usefulness of the complexing reaction can be appreciated in Table 1 where a comparison is given of the results obtained with the automatic titrator in the presence and absence of the complexing reagent.

If Fe(III) was not complexed and Fe(II) was less than 1·0% of the total iron, the error was greater than a 50% under estimation of Fe(II). Note that the error was inversely dependent on the percent content of Fe(II) in the sample.

Performance was improved by the addition of the complexing agent (85% orthophosphoric acid). A small overestimation error was still obtained but the error was much smaller than before. At worst, when Fe(II) was 0·5%, the error was around 6%.

The error of the automatic titrator at the low end of the results in Table 1 was a function of the response time of the computerized system and of the titrant

TABLE 1
Ferrous Iron Determination for Synthetic Samples[a]

Fe(II) % of total iron	*Fe(II) measured in solution (ppm)*			
	Without complexing		*With complexing*	
	Fe(II)	*% Error*	*Fe(II)*	*% Error*
100·0	49·8	−0·4	50·0	0·0
50·0	49·9	−0·2	50·2	0·1
33·3	49·4	−1·2	50·5	1·0
9·1	44·6	−10·8	50·6	1·2
4·8	31·0	−38·0	51·3	2·6
1·0	22·6	−54·8	51·8	3·6
0·5	21·5	−57·0	52·9	5·8

[a] Titration with and without Fe(III) complexing. Percent error was calculated with respect to the real Fe(II) concentration (50 ppm). The complexing reagent was 85% orthophosphoric acid. Fe(II) concentrations in ppm.

concentration. For applications in bacterial leaching, an error in the determination of Fe(II) of the order of 4% is already within the statistical limits. This fixes the limiting quantitative determination at the low concentration end to a value of Fe(II) of 1% of total Fe. This concentration ratio was below the values we normally find in bacterial leaching systems.

A sample containing 0·5% Fe(II) was tested in all experiments in order to emphasize the limit of quantitative determination of all the methods.

3.1 Analysis of the error in the *o*-phen method

It was found that the spectrophotometric determination of traces of Fe(II) by the *o*-phen method in presence of high concentrations of Fe(III) was liable to significant errors.

Muir[1] postulated that the interference of Fe(III) in the *o*-phen analysis was due to the reduction of Fe(III) to Fe(II) by the *o*-phen. This reduction was assumed to be catalysed by sunlight. In this work it was not possible to verify catalysis by sunlight.

A more plausible explanation could be the direct formation of color by reaction of Fe(III) with *o*-phen. The molar extinction coefficient of the colored complex formed by Fe(II) with *o*-phen has a value of 11 000.[5] The coefficient of the Fe(III)–*o*-phen complex was experimentally found to be only 182 (5% error). Therefore, the contribution of Fe(III) to the absorbance would be significant only if the ratio of Fe(II) to Fe(III) is of the order of 0·1. This ratio is very common in cultures of *T. ferrooxidans* using Fe(II) or minerals as sole energy source, where *o*-phen results of 10% Fe(II) are typical (see e.g. Ref. 6).

The kinetics of color development by Fe(III) were found to be slower than those of Fe(II) and were characterized by a nonlinear response.

In addition to the time dependency of color development it was found that, if the samples contained a significant amount of Fe(III), the dilution of a sample had a contradictory effect.

To analyse the effect of dilution and time, a synthetic sample containing only Fe(III) was diluted and assayed. The resulting absorbance (510 nm) increased with time, as shown in Fig. 1. Figure 2 gives similar results for different dilutions of a sample taken from a continuous reactor where *T. ferrooxidans* was being grown on Fe(II) at pH 1·6. The sample was known to contain very little Fe(II).[7]

Note that in both cases (Figs 1 and 2), the resulting absorbance at any time did not reflect the dilution of the sample. A two-fold dilution should have given about half the absorbance. Instead, the diluted sample interpreted as Fe(II) through the calibration curve, gave a value more than half that of the undiluted original sample.

This effect was found to be due to the nonlinear nature of the response of *o*-phen to Fe(III). To fix orders of magnitude, the response at 1 min was fitted to a line for absorbance between 0·3 and 0·6 (range within which it was linear) and compared with the response to Fe(II) by their respective parameters:

$$A[\mathrm{Fe(II)}] = a + b \times \mathrm{Fe(II)} \quad \text{ferrous iron calibration}$$
$$a = 5{\cdot}44 \times 10^{-3} \qquad b = 2{\cdot}01 \times 10^{-1}$$

$$A[\mathrm{Fe(III)}] = c + d \times \mathrm{Fe(III)} \quad \text{ferric iron calibration}$$
$$c = 1{\cdot}12 \times 10^{-3} \qquad d = 9{\cdot}66 \times 10^{-4}$$

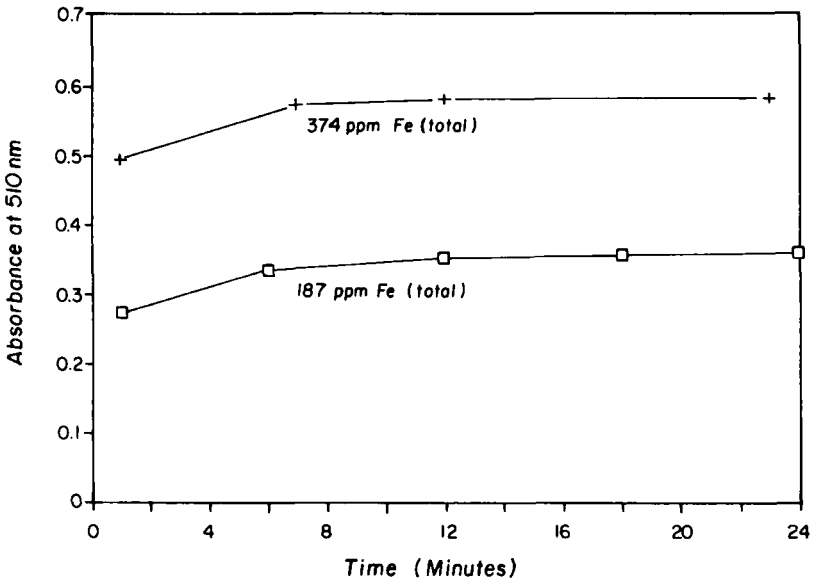

Fig. 1. Evolution of absorbance with time for a synthetic sample containing 370 ppm Fe(III) and diluted 1:1 to give 185 ppm Fe(III); *o*-phen method.

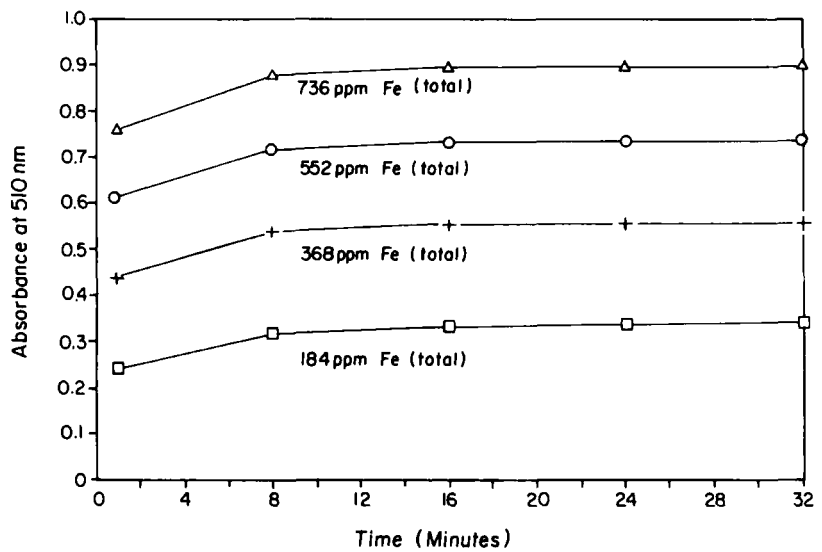

Fig. 2. Evolution of *o*-phen absorbance with time for a sample from a culture of *Thiobacillus ferrooxidans*.

where $A(x)$ stands for the absorbance of x; a and c are intercepts and b and d are slopes. As the slopes ratio (b:d) is of the order of 2:100, a sample containing 2% Fe(II) in Fe(III) would be seen as having 4% Fe(II).

This relative response was valid only within the given linear range and, as the nature of the response is nonlinear, it cannot be extrapolated. Due to this nonlinear

TABLE 2
Affinity Constants Involved in the Fluoride *o*-Phen Method

Reaction	*log(k)*	*Reference*
Fe(III)/*o*-phen	15·9	Lee[5]
Fe(II)/*o*-phen	22·7	Lee[5]
Fe(III)/F	16·2	Seel[8]
Fe(II)/F	< 1·5	Dodgen[9]

TABLE 3
Ferrous Iron Determination for Synthetic Samples[a]

Fe(II) (%)	*Titration*		o-*Phen*		*Fluoride* o-*phen*	
	Fe(II)	% *error*	*Fe(II)*	% *Error*	*Fe(II)*	% *Error*
100·0	49·9	0·0	49·9	0·0	49·9	0·0
95·2	50·0	0·2	50·0	0·2	49·9	0·0
90·9	49·9	0·0	50·2	0·6	49·8	−0·2
80·0	50·1	0·4	50·3	0·8	49·9	0·0
69·4	50·0	0·2	50·7	1·6	49·7	−0·4
60·6	49·9	0·0	51·2	2·6	50·0	0·2
50·0	50·0	0·2	51·6	3·4	50·1	0·4
40·0	50·1	0·4	52·2	4·6	49·9	0·0
30·3	50·2	0·6	52·6	5·4	50·0	0·2
20·0	50·2	0·6	54·1	8·4	50·3	0·8
10·0	50·5	1·2	56·9	14·0	50·5	1·2
5·0	50·6	1·4	64·1	28·5	51·1	2·4
1·0	51·3	2·8	96·3	93·0	53·5	7·2
0·5	51·9	4·0	124·1	148·7	55·9	12·0

[a] Titrametric, *o*-phen, and fluoride *o*-phen methods. Percent error was calculated with respect to the real Fe(II) concentration (49·9 ppm). Titration results correspond to the Fe(III) complexing system. The fluoride *o*-phen results were obtained by addition of fluoride to the reaction system. Fe(II) concentrations in ppm.

nature of the assay, no method has yet been found to correlate reliably the true Fe(II) concentration of a sample to the results obtained with the *o*-phen method.

3.2 Analysis of the fluoride modified *o*-phen method

The importance of the orthophosphoric acid as a complexing agent for Fe(III) in the titration method suggested that a similar reagent might be found to modify the *o*-phen method. This complexing agent had to be colorless, stable and soluble under the conditions of the assay.

Fluoride ions react with Fe(III) to form ferric fluoride and the complex complies with the requirements mentioned above. Considering the affinities involved in the reaction (Table 2) fluoride was selected as the complexing agent to modify the *o*-phen method. Results for synthetic samples are shown in Table 3 and Fig. 3, where the new method is compared with the *o*-phen and titration methods.

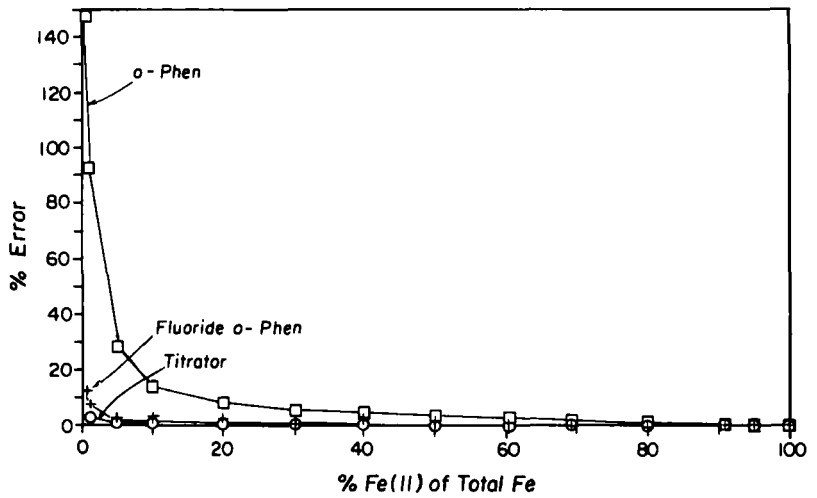

Fig. 3. Percent error in the determination of Fe(II) for synthetic samples containing 49·9 ppm of Fe(II) and a varying amount of Fe(III). Tested methods: titration, *o*-phen and fluoride *o*-phen.

TABLE 4
Ferrous Iron Determination for Real Samples[a]

Sample number	*Fe(II)* (%)	o-*Phen Fe(II)*	*Fluoride Fe(II)*	*Relative* % *error*
1	0·5	8·0	3·6	122·2
2	9·0	140·2	122·7	14·2
3	16·0	2100·0	1917·0	9·4
4	18·0	1861·0	1725·0	7·9
5	20·0	2543·0	2341·0	8·6
6	24·0	1882·0	1755·0	7·2
7	24·0	2003·0	1869·0	7·2
8	26·0	1492·0	1433·0	4·1
9	26·8	2233·0	2084·0	7·1
10	29·0	2840·0	2688·0	5·6

[a] *o*-Phen and fluoride *o*-phen methods. Percent error was calculated with respect to fluoride *o*-phen results. The percent content of Fe(II) was calculated by measurement of total Fe. Fe(II) concentrations in ppm.

Clearly, the fluoride *o*-phen method was superior to the original *o*-phen method; but titration was still better with very low levels of Fe(II), i.e. when Fe(II) was below 5% of total iron.

It should be noted that the *o*-phen method can be a gross overestimator whenever Fe(III) is present in large amounts. Also, as with the noncomplexed titration method, the error was a function of the relative amount of Fe(III) present in the sample. On the other hand, Table 4 summarizes the results obtained with both spectrophotometric methods using real samples. The error was evaluated by taking the fluoride *o*-phen results as reference. To compare the errors associated with real

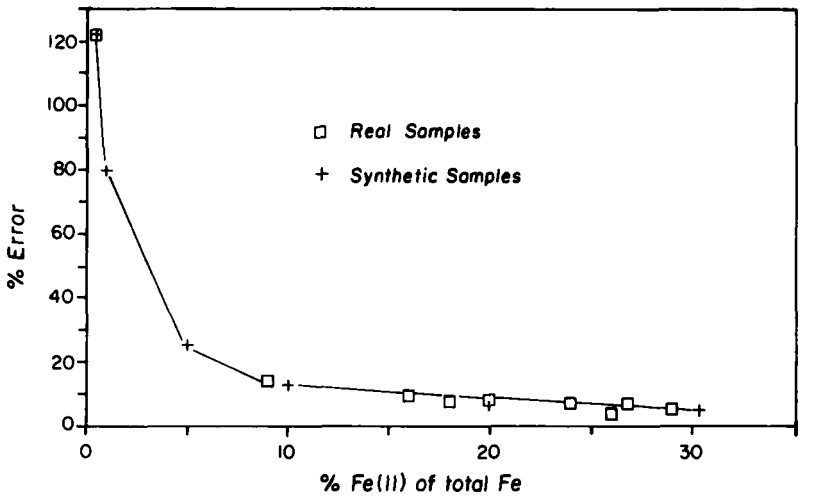

Fig. 4. Percent error in the determination of Fe(II) in real and synthetic samples of *o*-phen with respect to fluoride *o*-phen.

TABLE 5
Ferrous Iron Determination for Real Samples

Sample number	*Total iron*	*Measured Fe(II) concentration in samples*					
		Specific values obtained				*Average*	*% Deviation*
1*	720·0	7·9	7·9	7·9	8·1	8·0	1·10
**		3·6	3·6	3·5	3·5	3·6	1·40
2*	1363·0	140·8	139·6	140·8	139·6	140·2	0·43
**		120·9	124·6	122·1	123·3	122·7	1·10
4*	9581·0	1867·0	1879·0	1842·0	1855·0	1861·0	0·75
**		1712·0	1737·0	1712·0	1737·0	1725·0	0·71
10*	9269·0	2859·0	2834·0	2834·0	2834·0	2840·0	0·38
**		2670·0	2707·0	2682·0	2694·0	2688·0	0·51

*, *o*-phen method; **, fluoride *o*-phen method. Percent deviation was calculated as the percent standard deviation with respect to average obtained. Samples follow the same numbering as those in Table 4. Fe(II) concentration in ppm.

and synthetic samples, the errors in Table 3 were processed in the same way. These errors, from both Tables 3 and 4 are presented together in Fig. 4.

The error for real samples (which contained many different metallic ions) was very much the same as that for synthetic samples; hence it must be due to Fe(III) only. We also concluded that this new method maintains the selectivity towards iron, with respect to other metallic ions, as exhibited by the *o*-phen method.

Regarding statistical errors, Table 5 summarizes the results for the real samples showing that the reproducibility of both methods (viz. *o*-phen and fluoride *o*-phen) was equally high and of the order of 1 %.

The amount of fluoride required depends on the expected Fe(III) concentration in

the sample. As the sample was expected to have Fe(II) between 10 and 100 ppm and the Fe(II) to be down to 1% of total Fe, a fluoride concentration of 3800 ppm was selected so that fluoride would be in excess.

Using synthetic samples, the concentration of fluoride was decreased to verify the insensitivity of the analysis to small variations in its concentration. In fact, the calibration curve slope changed only by 3% when fluoride was halved (to 2000 ppm).

The fluoride method worked well in the range from 1 to 10 μg of Fe(II), which is the same range given by Muir[1] for his *o*-phen method; but the Fe(III) in the sample could be as high as 95% of total iron.

4 CONCLUSIONS

The *o*-phen method of Muir[1] overestimates the level of Fe(II) when Fe(III) is more than 50% of total iron. The size of the error does not depend upon the nature of the sample (either synthetic or from bacterial leaching of natural ores), but is solely dependent upon Fe(III) content.

A second method was examined: the titration method of Kolthoff[3] in which the Fe(III) is complexed, and this performed well. This method was automated and used as a comparison standard in this work. The automated method performs well down to the point where Fe(II) is down to 1% of total iron.

The proposed and tested new spectrophotometric method is based upon the addition of sodium fluoride to complex the ferric iron present in the sample.

The new method performs as well as the titrametric method until a point where ferrous iron is below 5% of total iron, at which point the new method overestimates Fe(II). When Fe(II) is only 0·5% of total iron in the sample, the overestimation is of the order of 12%. As this new method is spectrophotometric it will generally be preferred to titrametric methods.

The absorbance, read at 510 nm after 5 min, did not have any noticeable dependence on time.

No method could be found to correlate reliably the results obtained by the method of Muir to the real concentration of Fe(II) in a sample, if Fe(III) was more than 50% of total iron.

The hypothesis that these analytical effects explain the diversity of opinion about the parameters of growth of *T. ferrooxidans* is currently being investigated.

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