

Validating techniques for iron concentration measurement for laboratory applicati...

[Environment](#), [Pollution](#)



Abstract

This experiment sort to find an accurate and most viable method that would be used in laboratories to the measurement of iron concentration in aqueous samples. The methodologies that were identified for further analysis included; colorimetric assay (molecular spectroscopy), flame atomic absorption spectroscopy, redox titration (volumetric) and cation exchange chromatography (EMA technique). These methods were analyzed of their advantages and later subjected to a validation test. In the process, a wide range of CRMs were used alongside six other samples that contained unknown iron concentration.

The experiment was thus, geared towards providing answers to statements like; the necessity of quantification of blood iron concentration, determining the suitability of the named methodologies in the measurement of iron concentration in various aqueous samples and giving detailed illustrations on how the identified techniques are deployed in the process of determining the iron concentration in aqueous extracts.

The results that were obtained after performing the various experiments were then used to critically analyze the methodologies. The results were also used to validate the techniques.

Introduction

It is highly important to accurately determine the iron content of aqueous solutions. This is mainly important because it helps to protect the wider environment, helps promote chemical processes like photosynthesis, and it

is also important for hydrogeology studies. Sensitive techniques like molecular spectroscopy have been widely applied in the process of quantifying the iron concentration of various sample extracts. Unfortunately, it has proved over time that the precise amount of iron in a given sample is quite difficult to analyze because of the susceptibility of the sample to contamination.

This experimentation basically consisted of a set of four different techniques that were being analyzed. These methodologies included colorimetric assay, flame atomic absorption spectroscopy, redox titration and equivalent mineral acid technique. The methods were performed sequentially and the results obtained used to critically analyze the methods before choosing the best among them.

In the method of flame atomic absorption spectroscopy, energy is availed by a system of lamps that consist of a hollow cathode that is specific to a given metallic material. Therefore, the quantity of energy that is usually absorbed by the valence electrons that change state is assumed to be directly proportional to the quantity or concentration of ions present in the gaseous sample. In addition to a cathode lamp, the instrument used in flame AAS has the following parts; a monochromator and a burner-sampler system (C. Broekaert, 1998).

The EMA method is used to express the number of hydroxide (H⁺) ions that are given off from a surface of an ion resin. This method employs the use of calcium carbonate as the main reference material/compound.

The colorimetric method is a more accurate method for determining the iron concentration of a wide sample of extracts. In this technique, an octahedral complex is formed between iron (II) ions and bi-dentate ligand. The complex formed has a deep red color. Therefore, the complex formed provides a more sensitive way for testing for the presence of iron (II) ions.

This report also goes a further to give an illustration of the analysis of iron concentration in extract samples by use of a redox titration method. This is an easy to use method as long as the relationship of the reacting species is known quantitatively. It is an oxidation-reduction technique.

Validation of Analytical Methods

Furthermore, this report constitutes validation procedure of analytical methods. By definition, method validation is the demonstration of the fitness of a given method to its intended purpose. The validation procedure entails a plan which covers the method scope, performance characteristics of the method being validated and the limits acceptable (ICH, 1995). Some of the parameters that are keenly considered and monitored include detection limits, precision, accuracy, linearity, selectivity and ruggedness. The process of method validation is essential as it ensures that only proven methods meet all requirements for analytical applications. The method to be validated must first be fully demonstrated in laboratory experimentations by use of standards identical to the unknown specimen samples that are routinely analyzed. Some of the steps that are often followed in validation of methods include:

Development of a validation protocol

Definition of the purpose, scope and application of the method

Definition of the acceptance criteria and performance parameters

Definition of the validation experiments to be performed

Verification of all relevant equipment performance characteristics

Qualification of materials, that is, the reagents and standards

Performance of pre-validation of experiments

Adjustment of method parameters if necessary

Validation experiments then performed fully at an internal or external level

Development of standard operating procedures to be used to execute the method routinely

Definition of a criteria to be used for revalidation

Frequency and type definition of suitability tests for routine checks

Documentation of the validation experiments and the accompanying results

Generally, this experiment had the following three main areas of concern (EURACHEM, 1993);

- i. To analyze the iron concentration in sample extracts by using four different methods
- ii. To evaluate and compare these methods by use of the results obtained in the analysis process
- iii. To discuss the validation of methods process by using the results obtained in the experiments

Method

Four analytical methods were chosen to be used in this experiment. The four were; colorimetric assay, flame atomic absorption spectroscopy, reduction-oxidation titration and equivalent acidity mineral method. The procedures and explanation of these methods has been included in the appendix part of this report. The standard solutions were prepared by weighing 9.8g of A. R. salt which was then placed in a 250ml flask. 150ml of 2M sulphuric acid was then added to the volumetric flask. The flask's contents were then thoroughly mixed and then deionised water was added. 25ml of the solution was pipette and then titrated using potassium permanganate solution. The end point was a deep ink color. The standardized solution of permanganate was then used to determine and analyze for the concentration of iron in the extract samples. Several samples containing an unknown concentration of iron were analyzed by four different methods using the standardized solution. The method of flame atomic absorption spectroscopy involves energy being availed by a system of lamps that consist of a hollow cathode that is specific to a given metallic material (M. Sperling, 1999). Therefore, the quantity of energy that is usually absorbed by the valence electrons that change state is assumed to be directly proportional to the quantity or concentration of ions present in the gaseous sample. In addition to a cathode lamp, the instrument used in flame AAS has the following parts; a monochromator and a burner-sampler system.

Results

The results of all the four methodologies that were used in these experiments were a little bite different. The deviation of the results from experiment methodology to the other was mainly noted in the areas of accuracy, consistency of results and precision. The results that were obtained from the method of flame AAS were more consistent than those obtained from other methods. Also, the accuracy of the values generated was within the expected limits as they compared well with those found in literature sources. When validation of analytical methods' parameters were checked against the results during the validation of the methods, the results were more in touch with the atomic absorption spectroscopy method than was the case with the results of the other methods. Therefore, the best method to adopt for laboratory application in testing for iron concentration in extract samples would be flame AAS as it produced results of higher accuracy and precision. The other methods would require to be subjected to additional modifications so that the results produced would meet the necessary requirements of analytical chemistry systems.

All samples that were used in these experiments were thoroughly diluted and mixed to the required standard. They were also kept free of any environmental contamination so as to avoid interference with the results. The samples were carefully handled and all the variations in the results are not as a result of contamination or over/under dilution. The standard solutions were tested first before the real specimen samples were tested and critically analyzed. The results that were obtained were then used to validate

the methods that were used in the experiment. Parameters that were checked of the results included calibration of curves and linearity. The results from flame AAS method showed linearity property as the resulting graph demonstrated that the concentration of iron ions in the sample were linear to the test results. The intercept of the graph was close to the zero origin. Also, the range of the results obtained was analyzed and used in the validation process. The lower and upper limits of the results were obtained with absolute accuracy, linearity and precision by use of the flame AAS method. For the other methods, accuracy and linearity were lacking. The accuracy of the results obtained by the method of flame AAS were agreeable to those in other literature sources thus, this method is the most accurate of all the four methods tested. The other methods did not only lack in accuracy, but also they were not easily reproducible. This might have been due to factors like;

Performance characteristics of the equipment used being variable

The materials might have been of differing quality

Variations in equipment conditions after first run

The validation results were documented and the best method which was easily reproducible and produced accurate results of high precision was then used to do the experiments once again. Standard solutions of known iron concentration were used to generate data that was then used to plot calibration curves. The curves that were plotted were then compared to check for accuracy and precision of each method under study. The standard solutions were at all times kept free of any environmental contamination and

physical interferences. This was highly crucial so that graph of high accuracy might be produced and later used to validate the methods that were being tested. Therefore, every method was critically analyzed based on the results obtained and compared to the results of the other methods before settling on one which was most appropriate to be utilized at the laboratory level.

The results displayed above showed great consistence with those found in literature and other reference books. From the experimental results, the method of flame atomic absorption spectroscopy gave consistent results and thus, it is the best method to be employed at a laboratory level to analyze for the concentration of iron ions in various samples. The graph of comparison of this method to others indicates that flame AAS is by far the most appropriate method. By using the validation parameters such as detection limits, precision, accuracy, linearity, selectivity and ruggedness the method of AAS-flame still proves to be more reliable than the rest. These parameters especially accuracy and precision are more outlined in the method of flame atomic absorbance spectrophotometry.

The process for the validation of the analytical methods used in chemistry is very critical in the design and development of crucial methods that meet all the requirements for various analytical process applications. In this experimental report, the method of flame atomic absorption spectroscopy was found to be more appropriate and as thus validated accordingly for laboratory applications. Other methods that were used could not be disqualified as inappropriate but they too can be further modified to curb their inaccuracy and lack of precision.

B. Welz, M. Sperling (1999), Atomic Absorption Spectrometry, Wiley-VCH, Weinheim, Germany

J. A. C. Broekaert (1998), Analytical Atomic Spectrometry with Flames and Plasmas, 3rd Edition, Wiley-VCH, Weinheim, Germany

EURACHEM Guide 25, 1993, Accreditation for chemical laboratories:

Guidance on the interpretation of the EN 45000 series of standards and ISO/IEC

ICH-Q2A, Geneva 1995, Registration of Pharmaceuticals for Human Use, Validation of analytical procedures

Appendices

Here are the experimental procedure and the results that were obtained in the experiments.

1. Determination of the Iron Concentration by Ion Exchange Chromatography

The following procedure is a rapid one for the determination of the total cations present in water. When water containing dissolved ionised solids is passed through a cation exchanger in the hydrogen form all cations are removed and replaced by hydrogen ions. Any alkalinity present in the water is destroyed and the neutral salts present in solution are converted into the corresponding mineral acids. The effluent is titrated with 0.02 mol dm⁻³ sodium hydroxide using screened methyl orange as indicator (magenta-red to green).

Prepare a 25-30cm column of resin in a chromatography column. Pass 250cm³ of hydrochloric acid (2 mol dm⁻³) through the tube over about 30

minutes. Rinse the column with deionised water until the effluent is just alkaline to screened methyl orange. The resin is now ready for use. Pass 50.0 cm³ of the sample of water under test through the column at a rate of 3-4 cm³ per minute discarding the effluent. Now pass 2 x 50.0 cm³ through the column at approximately the same rate and collect the effluent separately, and titrate each with standard 0.02 mol dm⁻³ sodium hydroxide using screened methyl orange as indicator.

Calculation of EMA and conversion to ppm of Fe present for different forms of Iron.

Normal EMA

where V - volume of NaOH of molarity M for a volume V₁. Units - ppm of CaCO₃

$$1 \text{ ppm} = 1 \text{ mg/dm}^3$$

E. M. A - Equivalent Mineral Acidity is a measure of the number of H⁺ ions released from the surface of the ion exchange resin after exchange with other ions. The reference measurement is that of a CaCO₃ solution - i. e., the number of H⁺ released when a 1M solution of CaCO₃ is passed through the resin. The figures in the formula above allow you to calculate the number of moles of NaOH required to neutralise the amount of H⁺ released after exchange, (V x M). This in effect is the number of H⁺ released.

This is then correlated to mg/dm³ (ppm) of CaCO₃ ($50 \times 1000/V_1$). Mw of CaCO₃ is 100g per mole but each Ca²⁺ releases 2 x H⁺ on exchange so $100/2 = 50$

So to revise this formula to relate the number of H⁺ released by exchange with Fe in your samples you need to know whether you have Fe²⁺ or Fe³⁺ present.

Revised Calculation for Iron Determination

ppm of if you suspect the sample contains Fe²⁺

ppm of if you suspect the sample contains Fe³⁺

The results should not be quoted as EMA = ppm CaCO₃ but simply as ppm of Fe²⁺ or Fe³⁺ depending on what you have calculated.

Results:

Titrate/50 ml NaOH

Initial reading cm³

Final reading cm³

Volum/cm³

0. 1ml

0

261. 0

261. 0

0. 02ml

0

140. 6

140. 6

Mole/colour

Plus Indicator

Colour

0. 1 m

Light orange

Blue/green

0. 02 m

Light orange

purple

1st 0. 02m NaOH= 261 cm³

2nd 0. 1m NaOH= 140. 6

First ppm of = 1983. 6

First conc = 1983. 6

Second PPM of Fe= ppm of = 5342. 8

2. Determination of Iron by a Colorimetric Method

Iron can be conveniently determined with some accuracy by a colorimetric method. Iron(II) forms an octahedral complex with the bidentate ligand 1, 10-phenanthroline that has an intense red colour.

The formation of this complex ion provides a sensitive test for iron(II). (The reaction may also be used as the indicator in oxidation - reduction titrations)

Under carefully controlled conditions, the measured absorbance of solutions containing the complex may be used to determine the concentration of

iron(II). The Beer Lambert law is obeyed. Note that the corresponding phenanthroline complex of iron(III) is pale blue. It is important to ensure that reducing conditions are maintained in the determination.

Materials

0. 1% solution of phenanthroline in 95% ethanol.

Standard iron (III) solution 0. 1g dm⁻³. Prepared by dissolving 0. 864g iron(III) ammonium sulphate solid in water, adding 10cm³ conc sulphuric acid, then diluting to 1 dm³ . This solution is reduced in the determination to the iron(II) form. 10% solution of hydroxylamine hydrochloride which reduces Fe(III) to Fe(II). 40% solution of hydrated sodium ethanoate which is a buffer solution to control the pH at which reaction occurs.

Method

Prepare a range of standard solutions of iron(II) - phenanthroline complex as per the accompanying table. After mixing the standard iron solution, the buffer solution and the hydroxylamine hydrochloride allow about 15 minutes for the reduction of the iron(III) to iron(II) to occur before addition of the phenanthroline and water. Measure the absorbance of each solution at 510nm using solution 1 as reference. Use solutions 2-6 to plot a graph of absorbance against concentration. Check that the relationship is linear (i. e. Beer - Lambert law is obeyed). Use the graph to determine the concentration of iron in the sample.

3. The Determination of Iron Ions in Solution using Atomic Absorption Spectroscopy

Principle:

At sufficiently high temperatures metal salts can be vaporised, especially the chlorides. The resulting gaseous metal ions consist of a nucleus containing protons and neutrons with surrounding electrons held in orbitals of various energy levels. These energy levels are said to be quantised, that is they have well-defined energies. The absorption of energy of a precise wavelength can cause an electron to be promoted from a filled orbital of energy level E_0 to an empty orbital of energy E_1 such that:

The absorbed quantum $h\nu = E_1 - E_0$

In atomic absorption spectroscopy, the energy is supplied by one of a cluster of lamps each fitted with a hollow cathode specific to the metal being analysed. Usually only the valence electrons are promoted, and the amount of energy absorbed is proportional to the concentration of gaseous ions present.

The instrument consists of four main parts:

- a) The hollow cathode lamp fitted into a magazine of four lamps;
- b) The burner-sampler system. The long burner uses either acetylene/air or acetylene/nitrous oxide mixtures. The flame is therefore much larger and hotter than that used in the flame photometer.
- c) A monochromator to select the required spectral line emitted by the lamp;
- d) A sensitive detector to measure the absorbance in the form of a photomultiplier linked to a sensitive galvanometer.

Method:

You are supplied with a stock solution containing 1000ppm of iron ions made by dissolving the calculated amount of a iron salt in 1000cm³ of de-ionized water.

Dilute 25cm³ of this solution to 250cm³ to give a working standard of 100ppm.

Using this 100ppm standard prepare a series of four or five more dilute standards in the range 0-10ppm.

Tune the Atomic Absorption Spectrometer to the appropriate wavelength, and measure the absorbance of each standard and unknown under identical conditions. A computer will print out the data and a calibration graph, but it is best to pre-plot on graph paper and then read off the concentration of the unknown.

4. Standardisation of 0.02mol dm⁻³ Potassium Permanganate Solution using Ferrous Ammonium Sulphate (Ammonium Iron (II) Sulphate) and subsequent determination of Iron Concentration of an Unknown Sample.



Weigh out accurately about 9.8g of the A. R. salt. Transfer to a 250ml volumetric flask, add 2/3 (approx. 150ml) bench sulphuric acid (2M). Shake until the solid has dissolved and make up to the mark with deionised water. Shake well. Remove 25ml of the solution with a pipette and titrate with the permanganate solution to the first permanent pink colour.

Carry out 2 titrations. These should not differ by more than 0.05ml.

Use the standardised permanganate solution to determine the concentration of iron in each of the unknown samples.