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Estimation of Iron Metal Contents in Natural Samples by Spectrophotometric Methods

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Abstract: ***Determination of Iron in Natural samples was carried out using the Spectrophotometric methods. For each Natural Samples (determined in duplicate) ash solution was prepared and its aliquot for analysis by Spectrophotometric methods has been done. Mean value for duplicate analysis of each sample determined by the method was tabulated according to Natural sample. The describe method is very simple, rapid, sensitive and selective for estimation of Iron. Result of the study suggests theta method can be used satisfactory for the analysis of iron.***

Keywords: Spectrophotometric methods, Natural sample and Analysis.

1. Introduction

Iron deficiency anemia has long been recognized and is still an important nutritional deficiency problem in the country, afflicting particularly the vulnerable groups. Thus, there has always been an interest in identifying local product rich in iron. Early methods for the determination of iron in natural sample had relied on the gravimetric procedure. Determination of iron using Spectrophotometer has been introduced in recent years. This report presents results of a study of the determination of iron in a wide variety of natural sample using the Spectrophotometric methods. Iron is a vital element in life, Iron is an mineral in human health, playing a role in immune function, cardiovascular health. Iron is available for supplement in two forms; heme and nonheme. Heme iron is found in meat, fish and poultry. Heme iron is absorbed very efficiently our body. Nonheme iron can be found in vegetables (spinach, corn) and beans (soyabeans, kidney beans) but its bioavailability is poor. Absorption of heme iron is very efficient and not significantly affected by the composition of our diet. Only 1% to 7% of the nonheme iron in vegetables staples such a rice, corn, black beans, soyabeans and wheat is adsorbed when consumed as a single food. Meat protein and vitamin C will improve the absorption of non heme iron.

Calcium, polyphenols and tannins found in tea and phytates , which are a component of plants food such as rice and grains , can decrease the absorption of nonheme iron . some protein found in soyabeans also inhibit nonheme iron absorption. Iron from animal products is better absorbed than iron from plant products. If we do not eat any animal products, we will need to eat twice as much non-heme iron.

The amount of absorption of non-heme iron can be increased or decreased by other foods in the diet. Here are some tips to help our body absorb the iron from food:

- Combine heme iron rich foods with non-heme iron rich foods when possible.
- Include a source of vitamin C with our meals. Vitamin C helps iron absorption. Some sources of vitamin C include:

Broccoli, grapefruit, potatoes, green/red peppers, strawberries, peas, cabbage, tomatoes, oranges, orange juice, tomato juice

Causes of low Iron

Low iron can be caused by Low intake of iron rich foods, Donating blood or bone marrow, Surgery, Pregnancy, Large blood loss through menstruation and Other health conditions

Iron plays an important role in biology. Forming complex with molecular oxygen in hemoglobin and myoglobin ; these two compounds are comman oxygen transport proteins in vertebrates. Iron is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals. Iron is a critical component of:

- Hemoglobin (oxygen-transport metalloprotein in red blood cells)
- Myoglobin (oxygen-binding protein in muscle tissue)
- Ferritin (a protein acting as an iron buffer to stabilize the body's iron usage)
- A number of important internal enzymes.

Hemoglobin accounts for nearly two-thirds of the iron found in our body, Carrying essential oxygen to tissues and organs. about one-sixth of our body's Iron is stored in ferritin for use when dietary intake is not sufficient. The remainder of the iron in our body is found in protein tissues that help our body function. Adult men and post-menopausal women lose very little iron except through bleeding. Women with heavy menstrual cycles can lose a significant amount of iron.

Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans. The colour of blood is due to the hemoglobin, an iron-containing protein. As illustrated by hemoglobin, iron often is bound to cofactors, e.g. in hemes.

A Slight deficiency in iron causes anemia, and a chronic deficiency can lead to organ failure. Conversely, too much iron leads to production of harmful free radicals and interferes with metabolism, causing damage to organs like the heart and liver. The body is able to uptake the iron , so overdose is rare and usually only occure when people take supplements.

Iron is an essential trace element, meaning a healthy diet must include this nutrient. Dietary sources of iron are abundant, although vegetarian sources of this iron are more difficult to absorb. Iron occurs naturally in plant and animal life. An extremely important mineral for general well-being and energy, iron is the essential element within the hemoglobin molecule, which carries the oxygen in every red blood cell. It also functions in myoglobin, a molecule that supplies oxygen to muscles.

Iron can be found in high amounts in liver and red meats. Vegetable sources include leafy greens, nuts, and seeds. Iron is especially abundant in pumpkin and sunflower seeds, raisins and prunes, and wheat germ and brain. In plants iron is necessary for photosynthesis and is present as an enzymes cofactor in plant. Iron deficiency can result in interveinal chlorosis and necrosis. Iron is not the structural part of chlorophyll but very much essential for its synthesis. Copper deficiency can be responsible for promoting an iron deficiency.

The RDA for Iron

The Recommended Dietary Allowance (RDA) is the daily dietary intake level that is sufficient to meet the nutrient requirements of most all healthy individuals in each life-stage and gender group.

Females
9 to 13 years: 8 mg
14-18 years: 15 mg
19-50 years: 18 mg
51+ years: 8 mg

Males
9 to 13 years: 8 mg
14-18 years: 11 mg
19-50 years: 8 mg
51+ years: 8 mg

Iron is an essential micronutrient because it plays a vital role in oxygen transport, oxidative metabolism, cellular proliferation and many other physiological processes. It is a redox metal and participates in most of the reversible one-electron oxidation-reduction reactions by switching between the two oxidation states, ferrous and ferric. This redox activity of iron can produce free radicals responsible for cell signaling processes and iron mediated toxicity. Iron is also an essential mineral for all known pathogens, because of which many have developed complex mechanisms for iron acquisition and proliferation in an iron-restricted environment.

The human body has therefore developed intricate but exquisitely controlled mechanisms to absorb, transport and store iron, thus ensuring a ready supply for cellular growth and function, but limiting its participation in reactions that produce free radicals and its availability to invading pathogens. However, anaemia is widespread in India in spite of diversity in food habits, particularly in the consumption of cereals and such tight metabolic regulation. The causality between poor dietary iron density, bioavailability and high prevalence of anaemia in our population has not been well established, as anaemia has a multi-factorial aetiology.

The World Health Organization estimated that about 40% of the world's population (more than 2 billion individuals) suffers from anaemia (WHO, 2000). The groups with the highest prevalence are: pregnant women and the elderly, about 50%; infants and children of 1-2 years, 48%; school children, 40%; non-pregnant women, 35%; adolescents, 30-55%; and preschool children, 25% (Allen & Gillespie, 2001). WHO suggested the classification of anaemia of public health significance according to the prevalence rate, namely <15% as "low", 15-40% as "medium" and >40% as "high". Human body regulates quantity of iron through a protein based reservoir 'Ferritin' (figure I), it releases stored iron in case of iron deficiency in blood while in case of overloading Ferritin stores the excess iron.

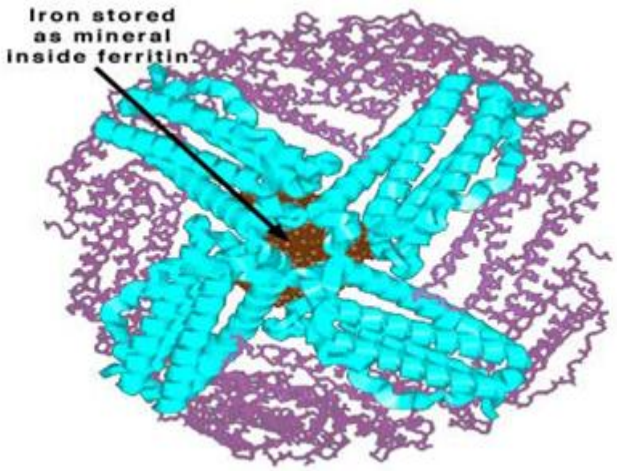

Figure 1: Three-Dimensional X-Ray Crystallographic Representtation of Ferritin.

Iron is stored in the Fe (III) oxidation state inside ferritin. It is stored in the ferritin core as Fe(III) in a crystalline solid having chemical formula $[FeO(OH)]_8[FeO(H_2PO_4)]$. This mineral can be represented by ferrihydrite, FeO(OH) and mineral is attached to the inner wall of the sphere. Iron can only release the ferritin by first changing from the Fe(III) to the Fe(II) oxidation state. In the Fe(II) state, iron breaks away from the lattice as the Fe^{2+} ion. The positive charge of the Fe^{2+} ion attracts the electronegative oxygen atoms of water, and so water "cage" forms around the ion. (In the water cage, six water molecules surround the ion at close range). Thus, iron becomes soluble as a hydrated Fe^{2+} ion, $Fe(H2O)_6^{2+}$, and can be released from the ferritin protein via the channels in the spherical shell (shown above). Once the iron is soluble [as $Fe(H2O)_6^{2+}$] it will be released from ferritin shell through the three-fold channels of the ferritin due to polarity.

Iron is an essential metal used as a single ingredient or as a component in various pharmaceutical products, for the formation of hemoglobin and for the oxidative process of living tissues, to treat anemia (Martaindale, 1999).A large number of methods have been reported for the determination of iron in different matrices, with and without complex formation.

The ferric-ferrous ratio of natural silicate liquids equilibrated in air was studied by Kilinc et al[1].Determination of Ferrous Iron in the Presence of Ferric Iron With Bathophenanthroline was done by Lee et

al[2].Determination of arsenic, boron, carbon, phosphorus, selenium, and silicon in natural waters by direct current plasma atomic emission spectrometry was observed by Urasa[3].Colorimetric flow-injection analysis of dissolved iron in high DOC waters was studied by Pullin et al[4].The new method utilizes two selective ligands to stabilize Fe(III) and Fe(II), thereby preventing changes in Fe reduction−oxidation distribution. Complexed Fe(II) is cleanly removed using a silica-based, reversed-phase adsorbent, yielding excellent isolation of the Fe(III) complex. Iron(III) concentration is measured colorimetrically or by graphite furnace atomic absorption spectrometry (GFAAS) [5].

Solid Phase Colorimetry of Trace Metal Ions Based on a Tristimulus Chromaticity Diagram and Simultaneous Determination of Iron(II) andIron(III) were observed by Yokota et al[6].A procedure for the removal of free iron from soils and clays by a single extraction at 50 °C. with sodium hydrosulphite in a citrate buffer at pH 4.75 is developed by D. E. Coffin[7].The effect of the iron ore tailings on the coastal environment of Tolo Harbour, Hong Kong was studied by Wong et al [8].Development of a dispersive liquid–liquid microextraction method for iron speciation and determination in different water samples was done byTabrizi[9].Visual estimation of iron in saprolite was done by Hurst[10].

Solvent extraction and fluorometric determination of fluoride ion at ppb level in the presence of large excess of aluminum(III) and iron(III) by using an expanded porphyrin, sapphyrin were determined by Nishimoto et al [11].

Determination of iron(II) in natural waters by capillary zone electrophoresis using on-capillary complexation with 2,4,6-tri(2′-pyridyl)-1,3,5-triazine was observed by Dahlen et al[12].

Al(III) and Fe(III) binding by humic substances in freshwaters, and implications for trace metal speciation was studied by Tipping et al[13].

2. Material and Method

The instrument used for the study was Spectophotometer 118 (Systronics).

All Solutions were prepared in deionized water because we were working with dilute solutions of Iron in this experiment and because tap water contains significant concentrations of iron, we have to be very careful to avoid contamination of our solutions with tap water. In other words, all glassware should be rinsed thoroughly with deionized water before use.

Diluted sulfuric acid 0.7 M : This solution was prepared by adding 40 ml of concentrated sulfuric acid to approximately 1 L of deionized water in a beaker, mixing thoroughly and allowing to cool to room temperature.

Hydroxylamine Hydrochloride 10 g/100 ml : This solution was prepared by dissolving approximately 2.5 g of hydroxylamine hydrochloride ($H_2NOH{\cdot}HCl$) in approximately 25 ml of water in a small beaker.

1,10-phenanthroline, 0.1 g/ 100 ml : This solution was prepared by dissolving approximately 0.2 g of orthophenanthroline monohydrate in 200 ml of water. If necessary, the mixture is warmed gently and stirred to ensure complete dissolution. The solution stored in the dark until it is used. If the solution darkens at any step in the process, discard it and prepare another solution.

Sodium acetate, 1.22 M : This solution was prepared by dissolving approximately 10 g of sodium acetate in 100 ml of water in a small beaker. Obtain about 100 ml of this solution in a small beaker.

Preparation of Standard ferrous ammonium sulfate solutions:

Stock 1: Weigh approximately 0.210 grams of reagent grade Ferrous Ammonium Sulfate Hexahydrate ($FeSO_4(NH_4)_2SO_4{\cdot}6H_2O$, MM = 392.14) to the nearest fourth of a milligram, transfer the salt carefully to a small beaker and dissolve the salt in 12.5 ml (graduated cylinder) of the diluted sulfuric acid solution. When the salt was completely dissolved, transfer the solution quantitatively to a 500-ml volumetric flask using at least five rinses with small volumes of deionized water to ensure that all the solution is transferred to the volumetric flask. Dilute the solution in the volumetric flask to the calibrated mark and mix thoroughly.

Stock 2:Pipette out 25.00 ml of the Stock 1 solution into a clean 250-ml volumetric flask, add 5 ml of the diluted sulfuric acid solution to the flask, dilute to volume and mix thoroughly. Natural samples of various groups were purchased from local markets and retail stores for analysis. In Natural sample the edible portions was blended and aliquots taken for analysis.

Preparation of Natural Sample
An amount of 5-15 g of the homogenized sample was dried in an air oven at 105°C for 3 hours. The dried sample then charred until it ceased to smoke. The charred sample was kept in a muffle furnace until a whitish or grayish ash was obtained. The ash was treated with dilute sulfuric acid, transferred to a volumetric flask and make up to 50 ml. For each food to be studied, two ash solutions were prepared, i.e. duplicate analysis was carried out. Each ash solution was used for the determination of iron by the Colorimetric method. Details of the Colorimetric methods used are described in Tee et al. (1987).
Reaction step (Colour development)

Standard and unknown solutions prepared were used to develop the Iron/Phenanthroline complex ion in a blank, calibration standards and an aliquot of our unknown solution. prepare one blank and one set of standard solutions and prepare unknown sample. In Spectophotometer 118 (Systronics) was used. Wave length was set at 508 nm. A standard curve was prepared using Ferrous Ammonium Sulphate and used for calculation of iron in the test (unknown) solution.

3. Result and Discussion

The Spectrophotometric method were found to give satisfactory result. In the Spectrophotometric method several steps for the observations recordings. The red colour complex formed is stable for a number of hours. The procedure is relatively economicaly cheaper, thats requires only a low cost instrument for the measurements.

For the Spectrophotometric Method : In Spectrophotometer model no. CL-320 (Chemi line) was used.wave length was set at 505 nm. A standard curve was prepared using Ferrous Ammonium Sulphate and used for calculation of iron in the test (unknown) solution.

The amount of Iron in different Natural Samples were found up to 10^{-4} mg/ml. A total seven natural samples were studied. Mean values for duplicate analysis of samples determined by Spectrophotometric method were tabulated. The Common name and the scientific name of the samples are also tabulated appropriate.

The Spectrophotometric method the calculated amount of iron is lowest in Sample 2 i.e. In case of Beta Vulgaris linn while the highest concentration of Iron has been found in Sample 4 i.e. Trigonella Foenum (Methi). The Spectrophotometric method gives reproducible results. There was generally good agreement in the result obtained by this methods.

Table 1: Comman and botanical names of Natural Samples

S.no.	Sample No.	Comman name	Botanical name
1.	Sample 1	Fig	Ficus carica
2.	Sample 2	Beet root	Beta vulgaris linn
3.	Sample 3	Spinach	Spinacia oleracea
4.	Sample 4	Fenugreek leaves(Methi)	Trigonella foenum
5.	Sample 5	Dry fenugreek leaves(Kasoori Methi)	Trigonella foenum graecum
6.	Sample 6	Mint	Mentha arvensis/ piperita
7.	Sample 7	Curry leaves	Murraya koenigii

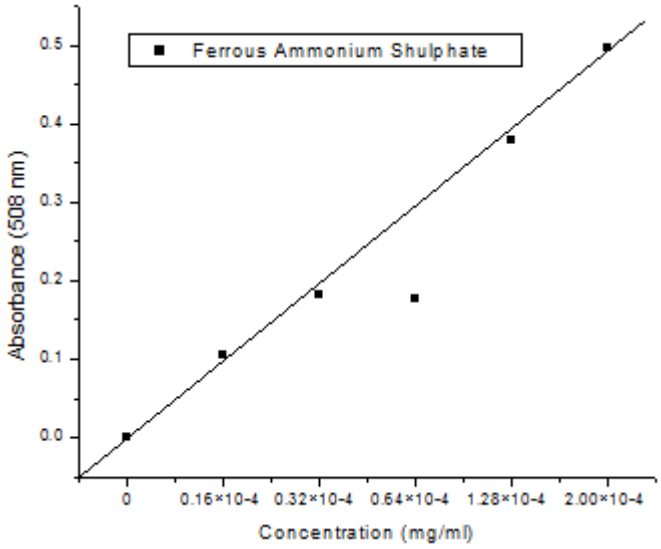

Figure 1: Absorbance-Fe Concentration Calibration curve

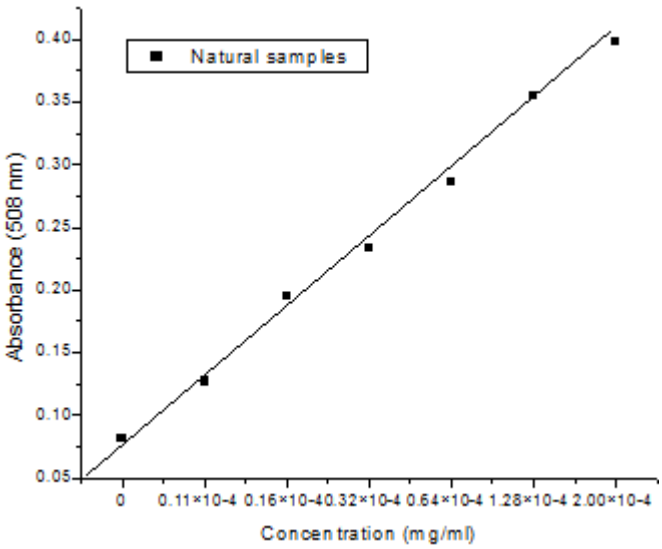

Figure 2: Absorbance-Fe Concentration curve

Table 2: Calibration Curve Data for Natural Sample by Spectrophotometer

Concentration(mg/ml)	Absorbance
Blank	0.000
0.16×10^{-4}	0.106
0.32×10^{-4}	0.182
0.64×10^{-4}	0.177
1.28×10^{-4}	0.378
2.00×10^{-4}	0.496

Table 3: Curve Data For Natural Sample by Spectrophotometer

S.no.	*Sample No.*	*Absorbance*
1.	Sample 1	0.082
2.	Sample 2	0.127
3.	Sample 3	0.195
4.	Sample 4	0.234
5.	Sample 5	0.286
6.	Sample 6	0.355
7.	Sample 7	0.398

4. Conclusion

In the spectrophotometric methods, the red colour complex is formed which is stable for number of hours. The procedure is also relatively much cheaper, requiring only a low cost Instrument. The deficiency of iron cause anemia. Most of the people of our country are poor. So they cannot buy rich food, The person, who suffer from iron deficiency can get rid of their diseases by selecting the iron rich vegetables, fruits, pulses and multivitamins tablets or iron tablets including as source of vitamin C in our meal too. Vitamin C helps Iron absorption. In case of unavailability of Iron rich vegitables, fruits the people can grow such vegetable, fruits and pulses in their gardens or fields.

From this research it is concluded that vegetables are nutritious foods that provide sufficient amount of nutrients needed for normal body function, maintenance and reproduction. It was found that nutrients composition in all the selected vegetables was different. Some vegetables contained high amount of starch while other contained maximum amount of protein. Some vegetables were rich in

some minerals such as Fe, P, Na, K etc. but their concentration in other vegetables were poor. Vitamins concentration was also different in all the vegetables. Moisture content was found maximum in all the selected vegetables. Vegetables are poor sources of fat that make them good food for obese people. They are good source of fiber and can decrease the concentration of high cholesterol level in body. From this trial we found that vegetables intake in different combination is essential for the maintenance of healthy life and normal body functioning. However, further investigations are required to notice the effect of cooking and storage conditions on these valuable nutrients.

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