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COLORIMETRIC DETERMINATION OF IRON IN VITAMIN SUPPLEMENTS

BONG LEE KIM

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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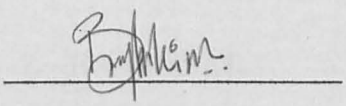
DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries each of which have been fully acknowledged.



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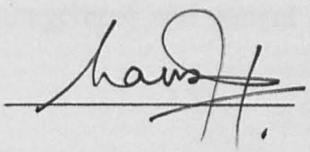

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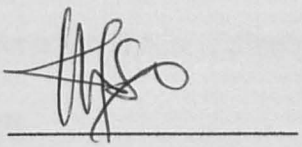

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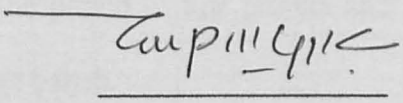

4. **DEAN**

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ABSTRACT

A study was done to determine the concentration of iron (Fe) from five different brands of vitamin supplement, namely Seven Sea (S1), Revicon (S2), Obimin (S3), Pharmaton (S4) and Vita-Kaps-R (S5). All samples were obtained from the local pharmacy. The sample chosen was first powdered and diluted with HCl. Then, the solution was filtered and being prepared for Fe analysis by adding *o*-phenanthroline as a color forming reagent and NH_2OHCl as a reducing agent. Subsequent analysis was carried out by using UV-Visible Spectrophotometer based on absorbance at 508 nm. The relationship between absorbance and concentration was in accordance to the Beer-Lambert law for the Fe concentration range 0-1 mg/L while higher concentrations resulted a negative deviation from the Beer's law. The concentration of each sample was calculated according to the linear equation of the calibration curve made earlier. The Fe content per tablet for each vitamin sample was 11.32 ± 1.47, 6.41 ± 0.33, 59.1 ± 22.20, 7.69 ± 0.64 and 6.75 ± 1.12 mg/tablet respectively. The proposed method was more applicable for the determination of Fe in vitamin supplement with low Fe contents.

PENENTUAN Fe DALAM TABLETS VITAMIN SECARA METRIWARNA.

ABSTRAK

Kajian telah dijalankan untuk menentukan kepekatan ferum(Fe) dalam lima jenis jenama sampel vitamin iaitu Seven Sea (S1), Revicon (S2), Obimin (S3), Pharmaton (S4) and Vita-Kaps-R (S5). Semua sampel diperolehi dari farmasi tempatan. Sampel yang telah dipilih dijadikan serbuk dan dilarut dalam HCl. Kemudian, larutan itu dituras dan sedia untuk tujuan analisis Fe dengan penambahan o- phenanthroline sebagai larutan pewarna dan NH_2OHCl sebagai agen penurunan. Analisis selanjutnya dijalankan dengan menggunakan UV-Visible Spectrophotometer berdasarkan serapan pada 508nm. Hubungan antara nilai serapan dengan kepekatan adalah mematuhi hukum Beer-Lambert pada julat kepekatan 0-1 mg/L. Sebaliknya sisihan negatif daripada hukum Beer berlaku pada kepekatan yang lebih tinggi daripada 1 mg/L. Kepekatan bagi setiap sample dikira berdasarkan persamaan linear lengkuk kalibrasi yang telah dibuat terlebih dahulu. Kandungan Fe per tablet dalam sampel vitamin adalah masing-masing 11.32 $\pm$ 1.47, 6.41 $\pm$ 0.33, 59.1 $\pm$ 22.20, 7.69 $\pm$ 0.64 dan 6.75 $\pm$ 1.12*mg/tablet. Kaedah ini lebih sesuai digunakan untuk penentuan Fe) dalam sampel vitamin yang mempunyai kandungan Fe yang rendah.*

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LIST OF SYMBOLS

Fe	Iron
g	Gram
mg	Milligram
ml	Milliliter
mg/L	Milligram per liter
cm	Centimeter
nm	Nanometer
MVM	Multiple vitamin/mineral
%	Percentage
<	Less than
λ_{max}	Maximum wavelength
Abs	Absorbance
UV	Ultra violet
SD	Standard deviation

CHAPTER 1

INTRODUCTION

1.1 IRON AND HUMAN HEALTH

Iron, Fe, is one of the most abundant metals on earth (Fergusson, 1982). It is an essential nutrient in the human diet. The average adult human body contains 4-6 g of iron (Bothwell *et al.*, 1979; Haas & Brownlie, 2001). In the body, the majority of iron present is found in the blood in a protein called hemoglobin, which carries oxygen from the lungs to the various tissues in the body where it is used to produce energy (Bloomfield & Stephene, 1996). One of the byproducts of this metabolism, carbon dioxide, is then transported back to the lungs by hemoglobin. Both the oxygen and carbon dioxide molecules bind to the iron ion present in hemoglobin during transport. Besides, iron also plays an equally essential role in respiratory enzymes such as cytochromes, which allows us to use oxygen (Oliveira & Masini, 2001).

Some dietary iron is required in order to replace quantities lost by various natural processes, especially bleeding. Principal dietary sources of iron include legumes, eggs, whole grain wheat, oatmeal, and red meat (Bloomfield & Stephene,

1996). However many people, even in our well-fed society, suffer from iron deficiency. This is common during infancy, pregnancy and adolescence (Araujo *et al.*, 1997). Symptoms include tiredness, apathy, inability to concentrate and a tendency to feel cold. In order to avoid iron deficiencies, an adequate supply of iron is needed. Some people take dietary supplements, which contain iron, such as multi-vitamins or single vitamin. Various brands are available with variable content of iron. Meanwhile excessive iron can cause problems such as cirrhosis of the liver, a greater risk of infections, and possibly increased incidence of heart disease (Corbett, 1995).

1.2 ANALYSIS OF IRON

Analysis of iron in pharmaceuticals and environmental samples can be carried out by atomic absorption spectrometry (Aleixo & Nobrega, 2003; Bag *et al.*, 2001), chromatographic (Harrington *et al.*, 2001), potentiometric (Mahmoud, 2001), fluorometric (Palanche *et al.*, 1999; Zhu *et al.*, 2002), sequential injection spectrophotometric (Tesfaldet *et al.*, 2004) and colorimetric (Atkins, 1975) methods. Comparatively, colorimetric method is very simple, rapid and less expensive. This method can be categorized into several techniques, depending on the type of color-forming reagent used such as *o*-phenanthroline, bathophenanthroline, ferrozine and bipyridine.

1.3 OBJECTIVES OF STUDY

The objectives of this study are:

a. To investigate a colorimetric method for Fe analysis.

b. To determine the amount of Fe in selected vitamin supplements according to colorimetric method.

c. To compare the Fe content with the respective reported values.

1.4 SCOPE OF STUDY

In this study, colorimetric analysis of Fe based on the *o*-phenanthroline method was investigated. Subsequently, the Fe content of selected brands of Fe supplements was determined using UV-Visible spectrophotometer.

CHAPTER 2

LITERATURE REVIEW

2.1 IRON AND THE HUMAN BODY

2.1.1 Introduction

Iron is a chemical element with the symbol Fe and atomic number 26. Iron is a group VIII and period IV metal (Fergusson, 1982). Iron is normally obtained through the food in the diet in form of ferrous citrate, ferrous gluconate, ferrous succinate and ferrous sulfate. In humans, iron is essential to all body cells for the regulation of cell growth and differentiation (Bothwell *et al.*, 1979). Almost two-thirds of iron in the body is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues. Smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to muscle, and in enzymes that assist biochemical reactions.

2.1.2 Chemistry and Metabolism of Iron

Iron is transported in the bloodstream bound to a protein called transferrin (Bothwell *et al.*, 1979). Ferritin, another iron containing substance, is also found in the bloodstream as well as in tissues such as the bone, liver, spleen and muscle. Ferritin levels parallel

the storage of iron in the body. It is used as an indirect measurement of tissue supplies of iron. The degree of saturation of transferrin is also used as a measurement of body stores of iron (Kiechl *et al.*, 1997).

There are two forms of dietary iron, heme and nonheme (Hurrell, 1997). Iron in meat is in the heme form while iron in plants or animal products such as milk, eggs and cheese is referred to as non-heme iron. In the heme form, once it is cleaved from the food, iron is directly absorbed intact into the blood. In the non-heme form, iron must be cleaved from its food source, and then reduced from the ferric to the ferrous form before it can be absorbed (Monson, 1988). This chemical change takes place with the help of HCl from the stomach or from vitamin C found in foods. Absorption takes place mostly in the upper part of the small intestine. The reduced iron or ferrous form must then be chelated to an amino acid so that it can be readily absorbed across the intestinal mucosa. In human body, the absorption of heme iron is about ten times that of non-heme iron depending on whether body stores are replete (Hurrell, 1997).

Iron is transported within the mucosal cell by transferrin. Transferrin is usually saturated to about one third of its total iron binding capacity. If no iron is needed, then transferrin remains saturated and less is absorbed from the intestinal mucosal cells. The transferrin that remains in the cells eventually gets sloughed away with the mucosal cells at the end of their 2-3 day life cycle. If iron is needed, the transferrin is less saturated when it reaches the intestinal mucosal cells and more iron passes from the mucosal cell to the transferring (Bothwell *et al.*, 1979).

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Excretion of iron takes place through bleeding. It may also be excreted via the feces, sweat and normal exfoliation of hair, skin and nails in very small amounts, usually between 0.9 to 1.2 mg per day (Monson, 1988). In menstruating women, however, iron losses are considerably greater and vary widely from individual to individual.

2.1.3 Iron Deficiency

Iron deficiency, which can cause anemia, is the most common nutritional deficiency in the world (Haas & Brownlie, 2001). It occurs when there is not enough iron in the red blood cells. It may result from inadequate iron intake in some infants, adolescent girls, and pregnant women. Blood loss may produce an iron deficiency in any person (Tapiero *et al.*, 2001). There is a variety of possible symptoms of iron deficiency including lack of energy or tiredness, extreme fatigue, pale skin, light headedness, brittle nails and headaches. Other symptoms of iron deficiency may include hair loss, decreased endurance, and impaired mental ability (Haas & Brownlie, 2001).

2.1.4 Iron Toxicity

Excess iron in human body is toxic, causing vomiting, diarrhea, and damage to the intestine. There is considerable potential for iron toxicity because very little iron is excreted from the body. Thus, iron can accumulate in body tissues and organs when normal storage sites are full. Hemochromatosis is iron overload disease which is characterized by deposits of iron-containing pigments in many tissues. This results in

tissue damage. In children, death has occurred from ingesting 200 mg of iron (Corbett, 1995). It is important to keep iron supplements tightly capped and away from children's reach.

2.2 VITAMIN

Vitamins are, by definition, compounds necessary in trace amounts for the normal functioning of the human body (Williams, 1995). A balanced diet usually supplies sufficient vitamin intake. However, in individually identified situations, a designated supplement amount may be needed. Vitamins are needed in order to grow, to make bones and connective tissue, to fight infections and cancer, to heal wounds, to stop from bleeding to death, and to keep our teeth from falling out (Burton & Foster, 1988). Vitamins are usually classified on the basis of their solubility as either fat soluble or water soluble. The fat-soluble vitamins are A, D, E and K. Their metabolic tasks are mainly structural in nature. The water-soluble vitamins are vitamin C and the eight B vitamins. Their major metabolic tasks related to their roles as coenzyme factors, except for vitamin C, which helps protein build strong tissue (Williams, 1995).

Various vitamins supplement are available in the market and pharmacy in form of single or multiple vitamins. Multiple vitamin/mineral (MVM) supplements contain a variable number of essential and non-essential nutrients. Their primary purpose is to provide a convenient way to take a variety of supplemental nutrients from a single product, in order to prevent vitamin or mineral deficiencies, as well as to achieve higher intakes of nutrients believed to be of benefit above typical dietary levels. An MVM

supplement should not take the place of a healthful, well-balanced diet, but it will help prevent deficiencies that often arise (Pao & Mickle, 1981).

2.3 IRON SUPPLEMENT

Iron supplements are not recommended for everybody. According to Williams (1995), MVM that contain iron should be taken only by people who are known to be iron deficient or have a history of frequent past iron deficiencies. Chronically high intakes of iron can lead to the accumulation of iron in tissues such as the heart and liver, which can lead to toxic damage and increased risk for disease. Endurance athletes engaged in heavy training almost certainly have an elevated iron requirement due to inadequate intake and excessive losses. For this purpose, iron can be found in virtually every general purpose multi-mineral supplement. The daily value for iron is 18 mg per day (Burton & Foster, 1988). Women typically consume about 10 mg of iron each day and men get closer to 15-20 mg. Monthly iron losses from menstrual blood flow may lead to increased iron requirements in some premenopausal women. Athletes participating in intense training or competition on a regular basis, may be at risk for excessive iron loss and may want to consider a dietary supplement with 10-15 mg per day (Burton & Foster, 1988).

2.4 SPECTROPHOTOMETRIC ANALYSIS

2.4.1 General Principles

The most common use of spectrophotometric method in chemistry is to measure concentration of an analyte in solution. Spectrophotometer is the instrument in which the intensity of the color can be measure. The quantitative basis of spectrophotometric analysis is that the amount of radiation absorbed at an appropriate wavelength is proportional to the concentration of the light-absorbing chemical in the sample. Since absorption occurs in less than one second, it can be measured very rapidly, thus, spectrophotometry is a very fast and convenient method of quantitative analysis (Willard *et al.*, 1988).

There are two major approaches to spectrophotometric analysis. One is to measure the radiant energy absorbed by the ion or molecule itself. For example, highly colored species such as the permanganate ions or organic dyes obviously absorb light and can be measured by spectrophotometric or colorimetric analysis. Colorless species, such as most organic molecules or colorless cations, do not absorb light, but they may absorb ultraviolet or infrared radiation and so can be measured only by spectrophotometric analysis. The other major approach involves species that do not absorb significant amounts of light. A suitable chemical reagent is added to this species to convert them to a new species that absorbs light intensely (Willard *et al.*, 1988). For example, iron (II) ion, is very light green. At low concentration, it does not absorb a significant amount of light and is virtually colorless in dilute acid solution. However, an

organic compound, 1,10-phenanthroline, reacts with Fe(II) to form an intense red color complex ion that is suitable for colorimetric measurement (Robert, 1997; Sandell, 1978).

In colorimetric analysis, the concentration of colored substance in solution can be estimated by comparing the intensity of its color with that of several standard solutions of known concentrations. This intensity can be measured in a form of a parameter known as absorbance. The relationship between absorbance, *A*, and concentration of the species for which the sample being analyzed, *c*, is known as Beer's law (Peller, 1998). The absorbance measurement can be made at a specific wavelength using a spectrophotometer. In this technique, a calibration curve is constructed from a series of standard solutions. The absorbances of several standard solutions are measured, and these values are plotted as a function of the concentration of the solutions. If the adsorbing species behaves according to Beer's law, such a plot should produce a straight line. The absorbance of sample solution concentration can then be measured with the spectrophotometer and this value can be used in conjunction with the calibration curve to determine the concentration of the sample solution (Peller, 1998; Robert, 1997).

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2.4.2 Spectrophotometer

According to Nitisewojo (1995), a spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers

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