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**Determination of Zinc in Biologocal samples by**

**Atomic Absorption Spectrometry**

A project submitted to the scientific committee in the chemistry department in partial fulfillment of the requirement for the degree of bachelor science chemistry

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وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا

# صدق الله العظيم

# سورة الٳسراء

الآية (85-84)

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Abstract

Discovery of zinc in numerous proteins demonstrated the key role of zinc for life. Indeed, it’s an old wisdom that metal ions are indispensable for life, one of these metals is zinc, that's the transition element, occurring at trace level. It’s the first element in group 12 of the periodic table. The total amount of zinc in a human (70kg)is about 2 to 3g. Low levels of zinc or lack of zinc in the body result in zinc deficiency,it can be treated by taking supplements of zinc andİncreasing its levels through diet,zinc deficiency remains a serious health problem worldwide. Zinc toxicity due to acute or chronic ingestion of high quantities of zinc supplements can also occur and lead to impaired immune response. Toxicity occurs almost exclusively from zinc supplements rather than food. .The techniques now used to determine zinc in serum or plasma include colorimetry, neutron activation analysis, polarography, x-ray fluorescence, emission spectrography, fluorometry, and atomic absorption spectrosopy (AAS).

AAS techniques are preferred in the clinical laboratory because of their specificity, sensitivity, precision, simplicity, and relatively low cost per analysis.

**Chapter one**

**1. İntroduction**

Zinc Represented in the periodic table as( Zn) , zinc is **a transition metal, grouped with cadmium and mercury**. With the middling atomic number 30 and stable isotopes with mass 66, 67,68,70. Is often define as a trace mineral required by humans(Sigel *et al.,*2013 ). It is a natural element found in all plants and animals, the body only needs a small amount,yet it  required for the metabolic activity of 300 of the body's enzymes, It plays a crucial part in the health of our skin, teeth, bones, hair, nails, muscles, nerves and brain function and in the creation of DNA, building proteins, in cell growth,healing damaged tissue, and supporting a healthy immune system (Popkin,2001). Adults have an absolute need for 2-3mg zinc per day To compensate for the relatively small loss of zinc in urine, stool and sweat,(Maret and sand stead,2006).as well as a large family of zn proteins involved in a gene transcription, Since it’s necessary for life but toxic in excess. zinc is involved in a much wider variety of processes and molecular mechanisms than vitamins and other Cofactors With more specific chemical functions (Sigel et al.,2013).Zinc deficiency was a major etiological factor in the syndrome of adolescent nutritional dwarfism that had been identified in mid-eastern countries. Zinc deficiency is an important public health problem even small deficiency is a disaster, the world health organization (Who) has identified zinc deficiency as the fifth most important risk factor for morbidity and mortality in developing countries. Nutritionists have been concerned that zinc deficiency affects large numbers of women and children in India and worldwideZinc deficiencies can lead to a variety of health effects, such as diarrhea**,** coldsymptoms**,** rash, vision problems, or weight loss. Pregnant women, the lack of zinc may lead to fetal brain cells decreasing, and affect their mental development. Children’s lack of zinc will hinder their normal growth and development. Zinc is available in supplement form as pills and lozenges but zinc supplementation may not readily remedy zinc deficiency if other factors limit the capability of a cell to control zinc. The role of zinc in human disease require a general understanding of wide spectrum of functions of zinc, how zinc is controlled, how is interact with the metabolism of the other metal ions in particular copper and ion. (Bhowmik *et al.,*2010)

**1.1 Chemistry of Zinc**

Zinc is a metallic element with atomic number 30 and stable isotopes of mass 66, 67,68 and 70 averaging 65.38 a.m.u (Barak and Helmke, 1993). Zinc is aslightly brittle metal and has a shiny-grayish appearance ...,



 figure(1):zinc metal

 it is a naturally occurring element found in the earth's s surface rocks. Because of its reactivity, zinc metal is not found as the free element in nature. It is stable in dry air, but upon exposure to moist air, it becomes covered with a film of zinc oxide or basic carbonate (e.g., 2ZnCO3·3Zn(OH)2) isolating the underlying metal and retarding further corrosion. Bonding in zinc compounds tends to be covalent, as in the sulfide and oxide. In solution, four to six ligands can be coordinated with the zinc ion. Zinc has a strong tendency to react with acidic, alkaline,and inorganic compounds. Since zinc is amphoteric (i.e., capable of reacting chemically either as an acid or a base), it also forms zincates (e.g., [Zn(OH)3H2O]- and [Zn(OH)4]2-)[9] . There are approximately 55 mineralized forms of zinc. The most important zinc minerals in the world are sphalerite (ZnS), smithsonite (ZnCO3), and hemimorphite (Zn4Si2(OH2)H2O). it is the first element in group 12 of the periodic table. It is brittle and crystalline at ordinary temperatures, but it becomes ductile and malleable when heated between 110°C and 150°C . It is a fairly reactive metal that will combine with oxygen and other non-metals, and will react with dilute acids to release hydrogen. has two common oxidation states, Zn(0) and Zn(+2). The terrestrial chemistry of zinc is that of Zn(+2) rather than Zn(0) ( Krzaklewski and Pietrzykowski, 2002). The Zn(II) ion has electron configuration of [1s2,2s2,2p6,3d10 ] and therefore lacks unfilled d subshells in the well known oxidation state. Zn(II) is colorless unlike the true transition metals wich often form brightly colored compounds and complexes. Those compounds and solutions of Zn(II) that are not colorless, or white in powder form take thier color from the other constituents present .(Barak and Helmke, 1993)

Table(1) : properties for zinc

| **Element properties** |
| --- |
| **Atomic number** | 30 | **Boiling point** | 907 |
| **Atomic weight** | 65.38 | **Density** | 70133 gm/cm3 |
| **Melting point** | 420 | **Oxidation state** | +2 |

**1.2 - Zinc in biochemistry**

Almost all our knowledge about the molecular role of zinc is based on the interaction of Zinc with proteins. Discovery of Zinc in numerous proteins demonstrated the key role of zinc for life. They occurred in the order of (i) zinc as a catalytic ion in enzymes. (ii) Zinc in the structure of protein, and (iii) zinc in the regulation of protein. (Maret, 2013)

Zinc is required for the activity of 300 enzymes, covering all six classes of enzymes.

**1.2.1- Catalytic role**

zinc is essential and directly involved in catalysis and co\_catalysis by the enzymes, wich control many cell process including DNA synthesis, normal growth, brain development, behavioral response , reproduction, membrane stability, bone formation and wound healing.

1.2.2- **Structural role**

Zinc due to its physio\_chemical properties, plays structural and functional role in several proteins involved in DNA replication and reverse transcription and it’s critical for the functions of a number of metalloproteins ( Chasapis *et al.*,2012). Zinc binding sites in proteins are often distorted tetrahedral or trigonal bipyramidal geometry, made up of the sulfur of cysteine, the nitrogen of histidine or the oxygen of aspartate and glutamate, or a combination. Zinc in proteins also can be important for maintaining protein structure and stability. In all catalytic sites, the zinc ion functions as a Lewis acid.( McCall *et al.*,2000)

**1.2.3- Regulatory**

 Zinc finger proteins have been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission( Bhowmik *et al.*,2010).

For the most part, Zinc enzymes seem to be absent in the major biochemical pathways of intermediary metabolism. This absence does not mean, however, that Zinc has no roles in metabolism. It appears that rather than being a permanent constituent of metalloenzymes in these pathways, zinc has a role in controlling some of them. (Maret , 2013)

**1.3- Zinc deficiency**

Low levels of zinc or lack of zinc in the body result in zinc deficiency, zinc deficiency can develop if the amount of zinc in the diet is insufficient relative to the requirements(Deshpande *et al*.,2013) , presence of Zn inhibitors, excessive Zn loss during diarrhea(Hussain *et al*., 2022) or if the patients have such conditions as malabsorption, liver cirrhosis, diabetes mellitus, renal diseases, or intestinal fistula . Zinc deficiency can be induced by continued use of low mineral purified foods, foods containing additives with chelating activity, gastrointestinal surgery and other digestive diseases all decrease zinc absorption and increase zinc loss from the body( Deshpande *et al*., 2013) . And can assess zinc deficiency as a stress factor that makes it susceptible to the influence of the social context on intellectual development. Also it must be taken into account wich zinc deficiency occurs global, and in numerous cases, it’s combined with different nutrient And micronutrient deficiencies (M.J Salgueiro *et al.,*2004). Zinc deficiency is more likely to develop during childhood, when the daily requirement of zinc is higher and among adult women on weight reducing diets and in elderly people whose dietary consumption of nutrients is poor ,because the amount of zinc ingested per day. Also 30\_50% of alcoholics have low levels of zinc because alcohol decreases zinc absorption and increase urinary excretion of zinc (Deshpande *et al*.,2013).

Zinc deficiency remains a serious health problem worldwide affecting developed as well as developing countries (M.J Salgueiro *et al.,*2004) . It contributes to a number of diseases and lead toward adverse functional disorders, such as blindness, cognitive losses, decreased IQ level, stunting, premature mortalities during pregnancy, and increased exposure to infectious disease during pregnancy (Hussain *et al*., 2022). There is evidence proving that zinc deprivation during periods of rapid growth negatively affects sexual developments. It has been described on various occasions that boys are more sensitive than girls to zinc deficiency, probably because of their greater requirements for this mineral[pdf5 viber].fecal zinc excretion is also increase during diarrhea, which may contribute to zinc deficiency increase with high rates of enteric infections( Brown *et al., 2001)*.

**1.3.1 Symptoms of zinc deficiency**

Some of the symptoms of zinc deficiency include:

 • Hair loss

 • Skin lesions

• Loss of body tissues

• Malfunction of different Organs

• Acne

 • Congenital abnormalities, leading to zinc deficiency

 • Anorexia (Betsy *et al.,* 2013)



**1.3. 2. Correlation of zinc deficiency to hemoglobin and its treatment**

Nutritional zinc deficiency, can be treated by taking supplements of zinc and İncreasing its levels through diet . Foods rich in zinc are pumpkin seeds, eggs , meat, milk , peanuts , etc . If someone is diagnosed with zinc deficiency, they should consult a doctor to get the correct dose of supplements because excessive intake can cause headache and also abdominal pain. Relationship of maternal zinc to hemoglobin and pregnancy, has been described in the past decades. The state of hemoglobin and zinc during pregnancy is crucial for women’s health and fetal growth. Micronutrient deficiency remains a major problem for women in the age of Reproducibility in many developing countries. One of the most common nutritional deficiencies is zinc deficiency.It is said that about 82 % of pregnant women in the world suffer from this kind of deficiency. The World Health Organization (WHO) reported that the prevalence of anemia among pregnant women in developed countries and developing ones, is respectively 14 % and 51 %. Low levels of hemoglobin in pregnancy are linked to increased morbidity, infections, preterm births and high perinatal mortality in children. (Ladipo , 2000)

**1.4- Source of zinc**

Adults have an absolute need for 2-3mg zinc per day To compensate for the relatively small loss of zinc in urine, stool and sweat, about 0.1% of which are replenished daily.

|  |  |
| --- | --- |
|  |  |

On this basis and based on estimates of bioavailability of zinc, dietary recommendations are made for apparently healthy individuals (Popkin,2001)**.** Main food groups contributing to zinc intakes were meat and meat products, grains and grain-based products and milk and dairy products(EFSA *et al.,* 2010).Also Zinc is found in liver, fish, chicken and cereals, eaten alongside vegetables to  enhance zinc absorption. Fats and various non nutritive foods, fruits, Poultry, pork, dairy products and whole grains, cereals, lamb,  beef, leafy grains, root vegetables, shellfish , Oysters, lobster and organ meats. Major plant sources of zinc include cooked dried beans, sea vegetables, fortified cereals, soyfoods, nuts, peas, and seeds. It is well known that zinc is present in many foods, but in most developing countries children have a low intake of foods rich in readily absorbable zinc, such as liver, red meat, poultry, fish, oysters, and crabs. Good sources of its mushrooms, day lily flowers, edible fungus,  cabbage, black sesame, black rice, dates, hazelnuts, ebony and other vegetables, food crops  and fruit. Traditional staple foods, such as cereals, legumes, and tubers, contain zinc, but the presence of phytate, fiber, and lignin reduces its bioavailability. These substances form insoluble  complexes with zinc, preventing its absorption. Cow’s milk, because of its high concentrations of calcium and casein, and soymilk, because of its phytate content, may further reduce the absorption of zinc from the diet. In contrast, zinc in breast milk is well absorbed. Vegetables and fruits contribute very little to dietary zinc intake, but fruits eaten with cereals may increase the bioavailability of zinc. The concentration of zinc in plants varies based on levels of the element in soil. When there is adequate zinc in the soil, the food plants that contain the most zinc are wheat and various seeds. Zinc is also found in beans, nuts,almonds, whole grains,  pumpkin seeds, sunflower seeds and blackcurrant. Millions of people throughout the world may have inadequate levels of zinc in the diet due to limited access to zinc-rich foods (animal products, oysters and shellfish) and the  abundance of zinc inhibitors, such as phytates common in plant-based diets.Calcium can retard zinc absorption, so calcium and zinc supplements should be taken at different times of  the day. Coffee is known to contain tannin which  can potentially inhibit zinc absorption. Because zinc is not well conserved in the body and because zinc deficiency is directly related to dietary zinc intake, an indirect approach to quantify the prevalence of zinc deficiency would be to examine the adequacy of zinc in the diet in various regions.Dietary surveys are conducted in many countries, but few such surveys exist in developing countries.Dietary supplements contain several forms of zinc, including zinc gluconate, zinc sulfate, and zinc acetate. The percentage of elemental zinc varies by form.(Deshpande *et al*., 2013)

**1.5- Health benefits of zinc**

**1.5.1- zinc and testosterone**

Zinc is necessary to maintain normal serum testosterone . Insufficient zinc levels prevent testosterone production. Zinc also inhibits the aromatase enzyme that converts testosterone into excess estrogen.

**1.5.2 zinc boosts brain activity**

Zinc also boosts brain activity, because it is found in the vesicles of the mossy fiber system of the brain hippocampus. These fibers play a role in enhancing memory and thinking skills. ( Bhowmik *et al.*,2010)

**1.5.3- zinc heals, and protect skin**

Antioxidants play a critical role in keeping skin healthy. The antioxidant benefits of vitamin C and E are well known, but the importance of the trace mineral, zinc, has been overlooked, Zinc protects against UV radiation. Topical zinc, in the form of divalent zinc ions, has been reported to provide antioxidant photoprotection for skin .Two antioxidant mechanisms have been proposed for zinc: zinc ions may replace redox active molecules, such as iron and copper, at critical sites in cell membranes and proteins; alternatively, zinc ions may induce the synthesis of metallothionein, sulfhydryl-rich proteins that protect against free radicals (Rostan *et al*., 2002)

Zinc is essential for the healthy skin, zinc sulfate in a water based solution has been used for treating acne, sores and burns. Remember zinc sulfate is a salt and can be quite caustic to raw tissue in high or moderate concentration.

Internally, zinc stimulates cell division, healing, proper connective tissue formation, and also increases the transport of Vitamin A to the skin from the liver. ( Bhowmik *et al.*,2010)

**1.5.4 zinc stimulates taste, smell**

Taste is one of the main senses in humans, it’s the sensory system devoted principally to check the quality of the food being ingested.Zinc Stimulates Taste, Smell İn activates areas of the brain that receive and process information from taste and smell sensor (Bhowmik *et al.*,2010). Physiological and environmental factors hold responsible for taste distortions in humans including aging and disease conditions. Truthfully, age-related decline in taste acuity may be both a cause and an effect of zinc depletion. Zinc is an important substance for healthy taste buds because taste buds are known to contain different zinc containing enzymes so if there’s a zinc deficiency it can cause taste disorder(Barak and Helmke, 1993).

Taste disorder in the basis of the state impairment classified into three types :

* The first involves external damage of the gustatory papillae and taste buds and induced by dry mouth.
* The second type involves internal damage of the gustatory papillae and taste buds, and one of the factors that caused by is zinc deficiency
* The third type involves disturbance of the taste sensation neural pathway as a result of peripheral or central nerve damage.(Soni *et al.,* 2017)

**1.5.5- Zinc Improves Appetite**

Levels of zinc in plasma were found to influence appetite and taste preference . (Bhowmik *et al.*,2010)

**1.5.6 - Zinc in pregnancy and lactation**

Zinc is essential for normal embryogenesis And fetal growth. Moderate zinc deficiency During pregnancy has adverse effects on pregnancy outcome in laboratory animals, including altered gestational length, delivery Complications, and intrauterine growth retardation . Some evidence suggests that zinc Deficiency during human pregnancy causes Similar complications although findings Are inconsistent . Zinc is also important to the growth of the infant postnatally. Mild zinc deficiency during infancy and early childhood Has resulted in stunted growth, decreased Midarm muscle circumference, and altered Taste acuity .The average zinc content in breast milk in the first 3 months of lactation is 1-2 mg/d.( Fung *et al.,*1997). It is critical that a pregnant woman satisfy her body’s need for zinc. The official RDI for pregnant women is 19 milligrams per day (Bhowmik *et al.*,2010). Zinc absorption increase during lactation in human .( Fung *et al.,*1997)

**1.5.7- zinc and immune system**

 The innate immunity as the first line of defense represents a natural protection Against infections. Zinc is known to play a Vital role in normal immune system. The Functions of the innate immunity are Disturbed by altered zinc levels because it is Essential for high proliferating cells in the human Body. It is well known that zinc affects Multiple aspects of the immune system from The barrier of the skin to gene regulation to Lymphocytes. Zinc is crucial for normal Development and function of cell mediating, specific immunity such as B & t cells and Nonspecific immunity (active) such as Neutrophils and natural killer cells.Vivo and vitro effect of zinc on immune cells Mainly depends on the zinc concentration. By zinc deficiency all functions of Monocytes and activation function of T lymphocytes are impaired and B lymphocytes development and antibody production are reduced, particularly immunoglobulin G is compromised , whereas in natural killer cell’s cytotoxicity is decrease, and in neutrophils granulocytes, phagocytosis is reduced.B and T cells of the specific immune system have a great variety of specific receptors (antibodies and T-cell receptors) and can produce memory cells that respond quickly and powerfully to antigens to which they Have been primed. B cells were shown to be less dependent on zinc for proliferation than T cells; Compared to T lymphocytes, B Lymphocytes and their precursors (especially Pre-B and immature B cells) are started to More reduced in absolute number during Zinc deficiency than T lymphocytes. According to all aspect the effect of zinc on these key immunological mediators is rooted in the myriad roles .(Soni *et al*., 2017)

**1.5.8 Zinc Improves Mood**

Zinc abnormalities also often exist in mood disorder patients. Zinc sulfate, taken as a supplement, appears effective in reducing fatigue, mood swings and changes in appetite. (Bhowmik *et al.*,2010)

**1.6- Cellular zinc distribution**

In cells, zinc is distributed in the cytoplasm (50%), nucleus (30 – 40%), and membrane (10%) .The total cellular zinc concentration is thought to range between tens and hundreds of micromolar . Zinc is, however, bound with a myriad of proteins and sequestered into organelles and vesicles, and thus the cytosolic labile.The labile zinc concentrations in intracellular organelles have been measured as 0.14 pM in the mitochondria , 0.2 pM in the mitochondrial matrix , 0.9 pM in the ER, and 0.2 pM in the Golgi , while much higher concentrations in the mitochondria and the ER are also described . This huge discrepancy in zinc concentrations in the ER and Golgi has not been explained in convincing detail, although the differences may be due to changes of ligand binding or improper folding caused by variables such as pH or the oxidizing environment in their lumen, because the values were measured by different FRET systems. The precise determination of labile zinc concentrations in the cytosol and lumen in subcellular compartments is of current interest and further importance.The cellular and subcellular zinc homeostasis is achieved through sophisticated regulation of uptake, distribution, storage, and efflux, in which ZnT and ZIP transporters play fundamental roles.(Kambe et al., 2015)

**1.7 Plasma zinc concentration**

 In apparently healthy subjects, plasma and serum zinc concentration is affected by intake, both inadequate and excessive. Concluded that plasma zinc concentration responds to an increase in intake over short periods, but that the homeostatic mechanisms that act to maintain plasma zinc concentration within the physiologic range may prevent high plasma concentrations from being sustained over a prolonged period.Plasma zinc concentrations are reduced with severe inherited and acquired zinc deficiency states. However, sensitivity as a biomarker is poor and, with more moderate zinc deficiency states, lacks specificity . Plasma zinc concentration has been recommended as a biomarker of zinc status and the population‟s risk of zinc deficiency by the World Health Organization (WHO), the United Nations Children’s Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition Consultative Group(IZiNCG). (EFSA,2010)

**1.8- Supplements**

Zinc is available in supplement form as pills and lozenges. Excess zinc can interfere with the absorption of iron and copper. High doses can also cause nausea and even vomiting. Therefore it is important not to take supplemental zinc unless it is known that the diet is low in foods containing zinc or a zinc deficiency is confirmed. A registered dietitian can help to evaluate one’s diet and determine if zinc intake is low. . (Bhowmik *et al.,*2010)

**1.8.1- Health Risks from Excessive Zinc**

Zinc toxicity due to acute or chronic ingestion of high quantities of zinc supplements can also occur and lead to impaired immune response, hypocupremia, microcytosis, and neutropenia. Toxicity occurs almost exclusively from zinc supplements rather than food. There have been no reports of eating too much zinc from the diet alone [22link] .Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. Intakes of 150–450 mg of zinc per day have been associated with such chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins.( Deshpande *et al.,* 2013)

**1.8.2- Recommendations on Zinc Supplements**

 Remember that the RDI (Recommended Daily Intake) and RDA (Recommended Daily Allowance) numbers are a statistical estimate of the amounts that prevent individuals from demonstrating deficiency signs and symptoms. The optimum nutrition level is generally higher than the RDI numbers, sometimes much higher. Unfortunately, we do not know what those numbers are. Furthermore, individual needs vary with age, sex, health status and individual genetic makeup. The simplest estimate of optimum zinc intake is assumed to be between the RDI numbers and the maximum tolerance numbers as from the table 2 . It is also likely that foods are deficient in zinc in general, therefore, zinc supplementation is appropriate. In general, the sum of the zinc from foods and supplements should not exceed the maximum tolerance estimates, at least for long periods of time. Because zinc competes with iron and copper, those taking high doses of zinc supplements may develop deficiencies of these minerals. One way to avoid this is to take a good broad spectrum multi-mineral supplement. It is best to take these at different times to avoid conflict over the absorption channels. Zinc is water soluble and is not stored effectively in body tissues. Therefore, for maximum absorption and utilization.( Bhowmik *et al.,2010)*

Table2:

|  |
| --- |
| Zinc Requirements Daily Reference Intakes |
| İnfants:0 - 6 months 2 Zinc(mg/day)7 - 12 months 3 Zinc(mg/day) | Lactation:< 18 years 14 Zinc(mg/day)19 - 30 years 12 Zinc(mg/day)31 - 50 years 12 Zinc(mg/day) |
| Females:9 - 13 years 8 Zinc(mg/day)14 - 18 years 9 Zinc(mg/day)19 - 30 years 8 Zinc(mg/day)31 - 50 years 8 Zinc(mg/day)51 - 70 years 8 Zinc(mg/day)> 70 years 8 Zinc(mg/day) | Males:9 - 13 years 8 Zinc(mg/day)14 - 18 years 11 Zinc(mg/day)19 - 30 years 11 Zinc(mg/day)31 - 50 years 11 Zinc(mg/day)51 - 70 years 11 Zinc(mg/day)> 70 years 11 Zinc(mg/day) |
| Pregnancy:< 18 years 13 Zinc(mg/day)19 - 30 years 11 Zinc(mg/day)31 - 50 years 11 Zinc(mg/day) | Children :1 - 3 years 3 Zinc(mg/day)4 - 8 years 5 Zinc(mg/day) |

**1.9 - Atomic Absorption Spectroscopy**

 Atomic absorption spectroscopy was first used as an analytical technique, and the underlying principles were established in the second half of the 19th century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany. The modern form of AAS was largely developed during the 1950s by a team of Australian chemists. They were led by Sir Alan Walsh at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Chemical Physics, in Melbourne, Australia.

 Atomic absorption spectrometry has many uses in different areas of chemistry such as clinical analysis of metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue, semen, in some pharmaceutical manufacturing processes, minute quantities of a catalyst that remain in the final drug product, and analyzing water for its metal content.

( L'vov, 2005)

Figure 2: Schematic diagram showing the component parts of Atomic absorption spectroscopy.

**1.9.1- Principles**

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer Lambert Law.

 In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer Lambert Law.

**1.9.2- Instrumentation**

**1.Light source:**

The light source is usually a hollow-cathode lamp of the element that is being measured. The disadvantage of these narrow-band light sources is that only one element is measurable at a time.

* Hollow-cathode Lamps

 Hollow-cathode lamps (HCL) are a type of discharge lamp that produces narrow emission from atomic species. The hollow cathode lamp uses a cathode made of the element of interest with a low internal pressure of an inert gas.

* A low electrical current (~ 10 mA) is imposed in such a way that the metal is excited and emits a few spectral lines characteristic of that element (for instance, Cu 324.7 nm and a couple of other lines; Se 196 nm and other lines, etc.).
* The light is emitted directionally through the lamp's window, a window made of a glass transparent in the UV and visible wavelengths. The lamp is filled with an inert gas like argon or neon. When a potential is applied, it causes gas to become excited and it is driven towards the cathode. (García and Báez, 2012).



 Figure 3:Diagram of hollow cathode lamp.

**2. Atomizer**

Flame Atomization: In a flame atomizer, a solution of the sample is nebulized by a flow of gaseous oxidant, mixed with a gaseous fuel, and carried into a flame where atomization occurs. The following processes then occur in the flame.

1. Desolvation (produce a solid molecular aerosol)

2. Dissociation (leads to an atomic gas)

3. Ionization (to give cations and electrons)

4. Excitation (giving atomic, ionic, and molecular emission) (Forbes, 1976)

**3. Types of Flames**

 Table (1-1) lists the common fuels and oxidants found in flame spectroscopy and the approximate range of temperatures achieved with each of these mixtures. Note that, when the oxidant is air, temperatures are in the range of 1700 to 2400°C. At these temperatures, only easily excitable species, such as the alkali and alkaline earth metals, produce usable emission spectra. For heavy-metal species, which are not so easily excited, oxygen or nitrous oxide must be used as the oxidant. These oxidants produce temperatures of 2500 to 3100°C with common fuels (García and Báez, 2012).

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| **Fuel and oxidant** |  **Temperature, C⸰** |
| --- | --- |
| \*Gas/Air | 1700 - 1900 |
| \*Gas/O2 | 2700 - 2800 |
| H2/Air | 2000 - 2100 |
| H2/O2 | 2500 - 2700 |
| C2H2/Air | 2100 - 2400 |
| C2H2/O2 | 3050 - 3150 |
| C2H2/N2O | 2600 - 2800 |

 \*Propane or natural gas

 Table1-1 : lists the common fuels and oxidants.

 Disadvantages of Flame Atomic Absorption Spectroscopy

1. Only solutions can be analyzed

2. Relatively large sample quantities required (1 – 2 mL)

3. Less sensitivity (compared to graphite furnace)

4. Problems with refractory elements

Advantages

1. Inexpensive (equipment, day to day running)

2. High sample throughput

3. Easy to use

4. High precision .( Yeung *et al*.,2017)

4. Flame Structure

 All locations of a flame are not equal in temperature, and are not equal in fuel to oxidant ratio. The three main zones of a flame include the primary combustion zone, secondary combustion zone, and the interzonal region. The interzonal region is prevalent in free atoms and is the hottest area of the flame. It is therefore the region used for spectroscopic analysis. The flame usually rises about 5 cm above the burner tip, with 2.5cm being the max temperature point. The portion of the flame used for AAS is specific as to what element is being analyzed. Due to the formation of oxides, different elements achieve max absorbance at different distances (cm) above the burner. ( L'vov, 2005)

**5. Performance**

 Flame atomic atomization is the most reproducible of all the liquid sample introductions, however it has many disadvantages. Oxides are easily formed which leads to a reduced absorbance of samples, and flame atomization has a lower sensitivity than electrothermal atomization. Samples could be drained as waste and therefore have a low residence time, leading to low efficiency. Another disadvantage of flame atomic atomization is the flame fluctuations which can affect the absorbance of samples

**1.10- Limits of Detection**

 In Flame Atomic absorption Spectroscopy the limit of detection is between 1 ppm for transition metals to 10 ppb for alkali metals. Transition metals need more energy than alkali metals to excite their outer electrons which is why the higher detection limit is needed (Fuwa, K. and Valle, B.L., 1963).

**Chapter Two**

# 2.1 The determination of zinc in blood plasma by atomic absorption spectrometry

 Atomic absorption spectrometry has been used in the analysis of plasma zinc because of its sensitivity and simplicity. Dilution techniques reduce the viscosity of plasma and facilitate direct analysis, but viscosity differences can produce deviations in aspiration rates between sample and standard, and so cause errors. A direct (1 + 4) dilution of plasma with deionized mater is suggested. Working zinc standards are prepared in 5% glycerol to approximate the viscosity characteristics and aspiration rates of the diluted plasma samples. The analytical curve for diluted plasma samples and 5% glycerol working standards proved identical. Plasma zinc concentrations are accurately calculated from a daily working curve. The accuracy of the method exceeds 99% and recovery of added inorganic zinc to a pooled plasma averages 99.8%. The precision is primarily limited by baseline drift. A confidence interval of +- 2 µg /lOO ml was achieved by means of six contiguous 10s-integration readings. The method is free of nebulizer clogging and matrix interferences and is not subject to significant day-today variations. Because the method is accurate, sensitive, reliable and specific. it should be useful in the clinical laboratory\_ Plasma zinc has been assayed by various procedures including calorimetry, neutron activation, fluorimetry and atomic absorption spectrometry (a.a.s.) . A.a.s. has been used for most routine analysis because of its low cost of operation, sensitivity and precision . Even in the presence of a complex ionic matrix, analysis for zinc can be interference-free . Before the advent of the Boiling burner, which is suited to high solids matrices, sample preparation required precipitation of the plasma proteins by trichlor oacetic acid . The method was time-consuming and costly, and trace contaminants could be introduced . Sprague and SIavin suggested a direct method for plasma zinc requiring a (1. + 1) dilution with water. The zinc standards were also prepared in water. Although the method was free of chemical interferences, the burner head had to be modified to prevent clogging

from plasma proteins. Accuracy was poor, perhaps because of flow rate or viscosity differences between sample and standard. also used a (1 + 1) dilution with water; the zinc working standards were prepared in 3% dextran solution to match the viscosity of the diluted plasma and recoveries averaged 99%. further explored the effect of various flow rates and viscosities on plasma zinc analysis. They pointed out that when dilute plasma samples were aspirated through thin capillaries, significant changes occurred in flow rate and absorbance readings, because of apparent viscosity differences between plasma sample and standard. They concluded that a minimum two fold dilution was necessary for precise and accurate meesurements. In order to obviate errors from clogging, others suggested 10- and 20-fold dilutions, but a lowered signal sensitivity necessitated the addition of nitric acid or n-butanol as enhancing agents . Extreme dilution may also contribute to reduced precision from greater pipetting errors, in homogeneity of the dilute sample and a relatively low signal-to-noise ratio . Because the viscosity effect of dilute plasma cannot be totally reduced by extreme dilution, Pekarek suggested a method utilizing (1 + 4) dilution of plasma, which gave higher signal sensitivities without capillary clogging. Although reproducible values were obtained with aqueous standards, the accuracy of the values was not confirmed. It has been suggested that the largest source of error in flame methods arises from differing viscosities and thus flow rates between sample and standard. Glycerol has been previously used as a viscosity adjuster for clinical measurements . Its versatility comes from its capacity to maintain homogeneity with buffer solutions over a wide range of concentrations. Its flow and viscosity characteristics are well defined. An A.A.S. methods handbeok suggests that the viscosity of a (1 + 4) diluted serum sample nearly matches the viscosity of zinc standards prepared in 5% glycerol. In the method described below, the one-step dilution of plasma with a fourfold addition of deionized water is recommended. Working zinc standards are prepared in 5% glycerol solutions to approximate the viscosity and aspiration rates of the diluted plasma samples . Because the technique is accurate, sensitive, reliable and specific, it is suitable for routine clinical laboratories . (Butrimovit and Purdy, 1977).

**2.2 Determination of Zinc in biological fluids by atomic absorption spectrometry in normal and cirrhotic subjects**

A technique using an atomic absorption spectrophotometer (Perkin-Elmer Model 303) for the determination of zinc in plasma, red blood cells, and urine has been deseribed. The values obtained by this technique were comparable to those of the dithizone method. The atomic absorption technique is believed to be much simpler and less time consuming. By this technique, zinc levels in plasma, red blood cells, and urine were measured in normal subjects and in patients with cirrhosis of the liver. The plasma and red blood cell zinc was decreased and urinary zinc excretion was increased in patients with cirrhosis of the liver, confirming reports of earlier investigators who utilized the dithizone method for zinc determination.( Prasad *et al*.,1965)

**2.3 Direct determination of zinc in whole blood, plasma and urine by atomic absorption spectroscopy**

Methods are described for the determination of zinc in plasma diluted twenty-fold with 0.1 N HCl, in whole blood diluted one hundred times and urine diluted tenfold, using a Perkin-Elmer 303 atomic absorption spectrophotometers. An analytical rate of 10 samples per hour can be achieved.

Suppression of up to 15% of the apparent zinc content by inorganic components of the sample was overcome by the addition of the appropriate amounts of those ions to the standard zinc solutions used in the determination. The organic components of the samples had no significant effect on the apparent zinc content. Random contamination presents a problem which was best detected by replicate analysis.

Studies of the plasma zinc level of 20 normal subjects (10 men, 10 women) showed a significant difference (p < 0.001) between samples taken fasting at 9.00 h and five hours later (14.00 h) after the usual meals. The mean values were: 9.00 h, men: 98 μg/100 ml, women: 96 μg/100 ml; 14.00 h, men: 80 μg/100 ml, women: 83 μg/100 ml. The difference in whole blood values taken fasting at 9.00 h and five hours later was not significant (p > 0.6) and the means of the two samples were: men 584 μg/100 ml (range 414–794 μg/100 ml), women 582 μg/100 ml (range 342–700 μg/100 ml). The 24-h urine excretions were men 585 μg (range 263–817 μg) and women 414 μg (range 276–702 μg). This difference was significant (p < 0.05).( Dawson and Walker, 1969)

# 2.4 Determination of zinc in serum and urine by atomic absorption spectrophotometry; Relationship between serum levels of zinc and proteins in 104 normal subjects

Serum and urine zinc were measured by atomic absorption spectrophotometry. The drift in base-line was continuously registered and corrections for the drift were made in the calculations of zinc measurements to avoid any adjustment in the instrument settings. Food intake was shown to lower the serum zinc concentration by 19% on average (p < 0.001). A diurnal variation of serum zinc was observed with a minimum at 7 p.m. Venous stasis elevated the mean serum zinc concentration by 13% (p < 0.001) and the mean serum protein level by 14% (p < 0.001). Weak correlations between serum zinc and both the serum concentration of proteins and of albumin were found in 104 normal subjects. There was no correlation between serum concentrations of zinc and α2-macroglobulin. The concentration of serum zinc was 9% higher in males than in females (p < 0.005) which was not explained by sex differences in serum protein concentrations. Furthermore, males had a higher excretion rate of zinc in urine than females, when expressed as μmol Zn/24 hour (p < 0.01). This difference disappeared when urine zinc was corrected to urinary excretion rate of creatinine. Our results show that blood samples for serum zinc measurement should be taken in the morning in fasting state. The urinary zinc excretion rate should be related to the creatinine excretion rate to avoid sex differences. ( Kiilerich *et al*.,1980)

**2.5 Direct Measurement of Zinc in Plasma by Atomic Absorption Spectroscopy**

The techniques now used to determine zinc in serum or plasma include colorimetry, neutron activation analysis, polarography, x-ray fluorescence, emission spectrograph, fluorometry, and atomic absorption spectroscopy (AAS) . AAS techniques are preferred in the clinical laboratory because of their specificity, sensitivity, precision, simplicity, and relatively low cost per analysis. Before AAS burner heads capable of aspirating high-solid matrixes became available, preparation of plasma samples had to include precipitation and extraction. These pre-treatment techniques were time consuming, and a source of in accuracies. Therefore, a direct method is preferred if the accuracy of the method can be established. The greatest source of inaccuracy for flame methods, however, appears to be viscosity, with the differences it causes in aspiration rate between sample and working standard. In 1965, Sprague and Slavin suggested a direct AAS method for serum zinc, the serum being diluted with an equal volume of water. The accuracy, as reflected by analytical recovery studies, was poor, and the burner head had to be modified to prevent clogging. In addition, the working standards were prepared in water and were not adjusted for differences in viscosity between sample and standard. In 1969, Hackley et al. described an AAS method for zinc in plasma that was similarly diluted with de-ionized water. They prepared the standards in a dextran solution (30 g/L) to match the viscosities of the diluted plasma and eliminate the major error caused by the use of standards prepared in water. They concluded that “to avoid analytic error, it is necessary for the reference standard and the sample to have a similar viscosity.” In addition, Reinhold et al. illustrated that the rate at which the sample flowed into the instrument was affected by the size of the capillary tubing used for aspiration, and suggested that differences in viscosity between the diluted serum and standards were responsible. They reported that at least a threefold dilution of serum with water was necessary for accuracy. To minimize errors from clogging, ion interferences, and differences in viscosity between sample and standard, others suggested dilutions as great as 20-fold. Such large dilutions weakened the signal, so that enhancing agents such as nitric acid or n-butanol were necessary. In addition to a relatively low signal-to-noise ratio, very dilute samples may also decrease precision because of greater pipetting errors. The optimum dilution is the one that does not clog the capillary and burner head but still yields an adequate signal. It was suggested that serum diluted fivefold (one part plasma + four parts water) could be compensated for by use of zinc standards in glycerol/water (5/95 by vol). Accuracy has not heretofore been established for the use of working standards in glycerol with a direct-dilution method for plasma zinc. Therefore, we tested a one-step dilution of one part by volume of plasma with four parts of de-ionized water . The viscosity of the resulting solution was effectively matched by working standards that were prepared in glycerol/water (5/95 by vol).( Smith *et al*.,1979)

**2.6- Conclusion**

Zinc is a trace mineral,meaning that body only needs a small amounts,yet it is necessary for more than 300 enzymes so it plays a crucial role in human body.Zinc status in human subjects is assessed by measurement of zinc in plasma,urine..etc.there are many analytical technique for determining zinc that the best one is AAS.The evaluation of atomic absorption spectroscopy is based on Beers law, wich says that the absorption of light is directly proportional to the number of atoms absorbing it.

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