“DETERMINATION OF TRACE Zn (II) ION BY FORMING METAL-LIGAND CHELATE COMPLEX IN AQUEOUS MEDIUM USING UV-VIS SPECTROPHOTOMETRIC METHOD.”

SUBMITTED BY

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SEPTEMBER 2010.

CERTIFICATE

This is to certify that the research work embodied in this thesis has been carried out under my supervision. The work presented herein is original. This thesis has not been submitted elsewhere for the award of any other degree or diploma in any University or institution.

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A THESIS ON

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Submitted by
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has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Master of Philosophy (M.Phil) in Chemistry and the student has demonstrated a satisfactory knowledge on the field covered by this thesis in an oral examination held on September, 2010.

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TO

MY CREATOR

“ ALLAH ALMIGHTY ”

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ABSTRUCT

Trace amount of Zinc has been determined by spectrophotometric method using 1,2-dihydroxy anthraquinone-3-sulphonic acid sodium salt (Alizarin Red S) as a spectrophotometric reagent. The ligand Alizarin Red S reacts in slightly acidic solution (pH 4.00±0.02) with Zinc to give an orange-red chelate that has an absorption maximum at 502.2 nm. The reaction is instantaneous and absorbance remained constant for over 48 hrs. The average molar absorption co-efficient was found to be $2.1\text{x}10^2$ $\text{Lmole}^{-1}\text{cm}^{-1}$. Linear calibration graphs were obtained for 0.30-100 μg mL^{-1} of Zn. The stoichiometric composition of the chelate has been found 1:2 (Zn:ARS). Large excess of cations, anions and some complexing agents have been studied. The method was successfully used in the determination of Zinc in several standard reference material, alloys & environmental water and made a comparison with those obtained by Atomic Absorption Spectrophotometer.

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CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND.

The choice of any analytical methods depends on the sensitivity, selectivity, accuracy, availability of reagents, cost effectiveness of instruments and the time required for analysis as well as safety and easiness of operation.[1] Generally, expensive and time consuming Mass Absorption Spectro photometric (MAS), Atomic Absorption Spectrophotometric (AAS) methods are using for the determination of trace (ppm/ppb) amount of many metal ions.[2,3] Recently scientists are interested to use cheaper method for the determination of trace (ppm/ppb) amount of metal ions.[4] In the literature survey it has been found that UV-Vis spectrophotometric method was used in few cases for the determination of trace level (ppm/ppb) of metal ions.[5,6] In some cases, it is the only suitable technique. It encompasses practically all the fields of chemical science and is so broad that it can be rated as a versatile technique. The key to the wide success of spectrophotometry in varied fields of chemical analysis lies in its manifold advantages; compared to any modern trace and ultra trace analytical technique.

Zinc in trace amounts is industrially important. Zinc is becoming an ever more widely used metal in industry. Zinc metal is used in protective coatings for iron, copper and steel. Zinc oxide is used in storage batteries, Zn-Cu alloys, semiconductors, photography, lithography, painting and dyeing etc. Zinc is an essential in human and animal nutrition. Zinc also is a very toxic metal to all systems studies in man and animal, and has been responsible for a number of deaths. The most serious situation being the disease called " Zincurea" disease. According to Schwartz term "Concentration Window" metals are **(a)** Essential at definite trace level, **(b)** Deficient than that level cause metabolic disorder, **(c)** Higher than that level termed "Toxic level", and causes adverse effects.[7]

As Zn is both toxic and essential element, so it is necessary to monitor and control the trace balanced level of Zn by cheaper, easier and rapid analytical method like UV -Vis spectroscopy rather than AAS or MAS.

Substances those do not absorb electromagnetic radiation in the visible range are colorless. Hence, these substances are not determinable, by uv-visible spectroscopic methods. Present analytical methods (AAS, MAS) used to measure the trace level of Zn is too expensive and time consuming. As a result, it can't monitor the trace level of Zn at various monitoring points. So it is necessary to develop an analytical procedure/technique to measure the trace level (ppm/ppb) of Zn with the help of UV -Vis spectrophotometer.

1.2 PRESENT PROBLEMS:

The analysis of colorless substances by visible spectroscopy is not a new, reacting the colorless analyte with a suitable reagent; a color product is produced and can be measured optically. As long as the product concentration is directly proportional to that of the analyte the measurement of the color intensity ultimately leads to the determination of the analyte. A method of calibration enables the estimation of the analyte.

For the determination of Zinc only a few example of such method is cited in the literature, but these methods are limited by the complexities of the procedure such as low sensitivity, less selectivity due to many interferences, temperature and pH dependent and unfavorable detection limit.

1.3 AIM OF THE PROJECT:

The results of this Dissertation are:

(a) To establish an easy, low cost and rapid analytical procedure/technique to measure the trace (ppm/ppb) amount of Zn by UV-Vis spectrophotometer.

(b) To establish the optimize reaction condition for the formation of zinc-ligand complex, which will give an identical absorbance in UV-Vis range and follows the Beer-Lambert laws.

Finally, the aim of this study was to develop a simple spectrophotometric method for the determination of Zinc with 1,2-dihydroxyanthra quinone-3-Sulphonic acid, sodium salt (alizarin red s). The method was optimized individually and results of the measurements were checked by comparison with AAS method. Alizarin red S (ARS) has been reported as spectrophotometric reagent for arsenic (v) & cadmium[8] but has not previously been used for the determination of Zinc by rapid spectrophotometric method. The present thesis deals with successful attempt toward the establishment of new type of spectrophotometric reagent. In the very sensitive procedure alizarin red S complexes with Zinc, is proposed here.

CHAPTER TWO:

ANALYTICAL CHEMISTRY AND SPECTROSCOPY

2.1.1 INTRODUCTION OF ANALYTICAL CHEMISTRY:

Analytical chemistry is concerned with the chemical characterization of matter. Chemicals make up everything we use or consume and knowledge of the chemical composition of many substances is important in our daily life. To tell in alternative way, every thing is made of chemicals, analytical chemistry determine what and how much. Analytical chemistry plays an important role in nearly all aspects of chemistry, for example, agricultural, clinical, environmental, forensic, manufacturing, metallurgical, and pharmaceutical chemistry. The nitrogen content of a fertilizer determines its value. Foods must be analyzed for contamination (e.g., pesticide residues), vitamin, or mineral content like Zinc. The air in cities must be analyzed for carbon monoxide. Blood glucose must be monitored in diabetics (and, in fact, most diseases are diagnosed by chemical analysis).[9]

The discipline of analytical chemistry consists of qualitative analysis and quantitative analysis. Qualitative tests may be performed by selective chemical reactions or with the use of instrumentation. The formation of a white precipitation occurred when adding a solution of silver nitrate to a dissolved sample indicate the presence of chloride ion. Certain chemical reactions will produce colors to indicate the presence of classes of compounds for example, ketones. On the other hand, Infrared spectra give the “finger prints” of organic compounds or their functional groups. [10]

For quantitative analysis the analysts have to perform a qualitative test prior to performing the more difficult quantitative analysis. There are three phases in the analysis: a) Fast-screening phase (rapid class identification), b) The identification phase (specific identification) & c) The possible quantification phase. To a proper quantification a quantitative analysis involves several steps and procedures. The analytical process may be defined as the following sequence of events: 1) Defining the problem, 2) Obtaining a representative sample, 3) Preparing the sample for analysis, 4) Performing necessary chemical separations, 5) Performing the measurement, and 6) Calculating the results and presenting data.[11]

There are a number of measurements or methods employed may be classified as figure-1, [12]

The method employed for the actual quantitative measurement of the analyte will depend on a number of factors like the amount of analyte present, the accuracy and the precision required the degrees of selectivity, the sensitivity, the cost, and the rapidity. [13] The table-1 gives comparison of different analytical methods.

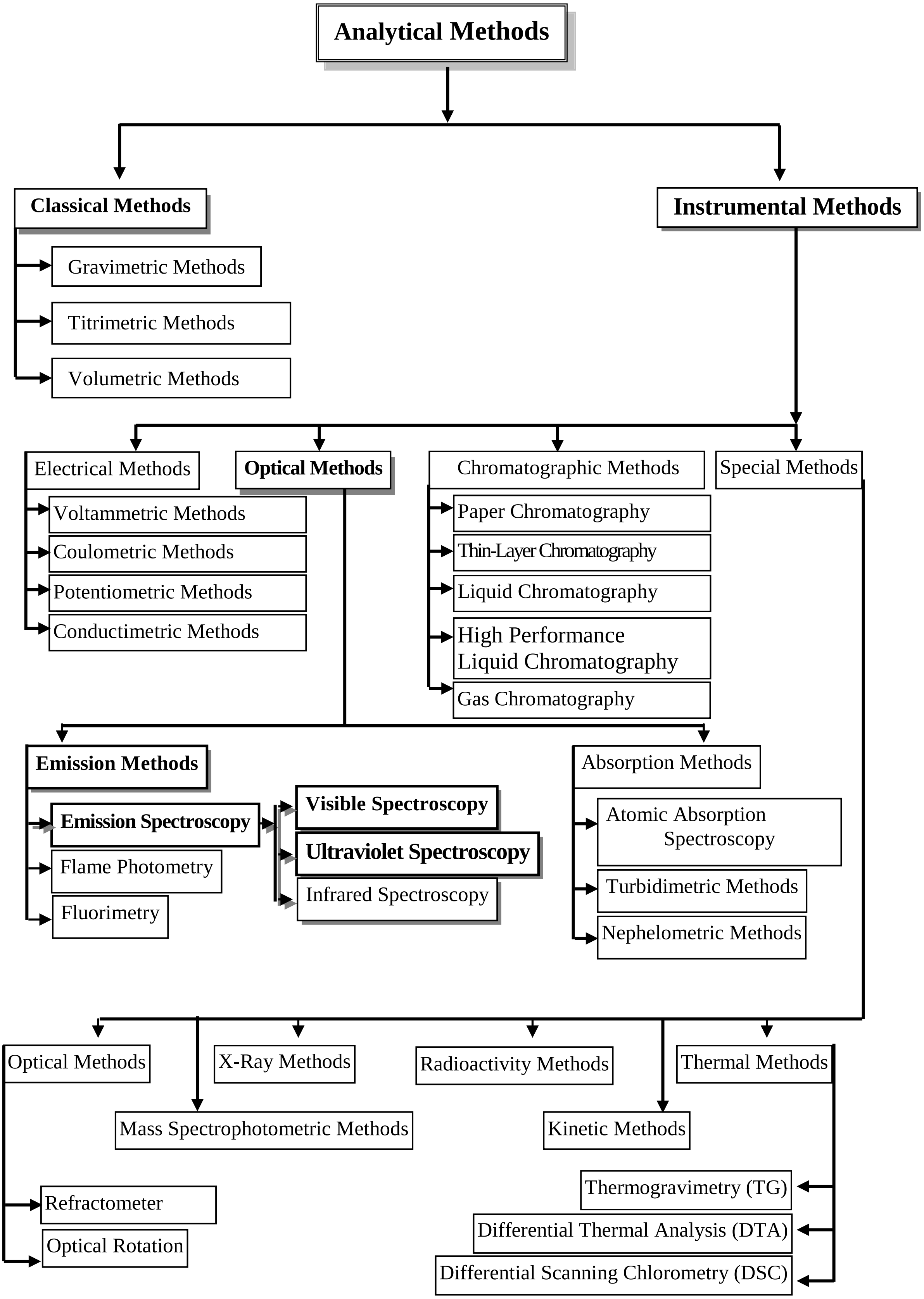

Fig- 1:
Classification of the methods of analytical chemistry. [12]

Generally, expensive and time consuming Mass Absorption Spectrophotometric methods (MAS), Atomic Absorption Spectrophotometric methods (AAS) are using for the determination of trace (ppm/ppb) amount of many metal ions [14, 15]. Recently scientists are interested to use the cheaper methods for the determination of trace (ppm/ppb) amount of metal ions, like uv-vis spectroscopy method. [16]

In the literature survey it has been found that UV-Vis Spectrophotometric method was used in few cases for the determination of trace level (ppm/ppb) of metal ions.[17,18] Zn has not yet been determined by UV-Vis Spectrophotometric method where as, it has determined by the Flame Atomic Absorption Spectrophotometer (FAAS).[19]

2.1.2 INTRODUCTION OF SPECTROPHOTOMETRIC METHOD.

"MOLECULAR ABSORPTION SPECTROPHOTOMETRY IS THE BACKBONE OF MODERN ANALYTICAL TECHNIQUES FOR TRACE ANALYSIS". This prediction was made by an eminent scientist T.S.West in the field of analytical chemistry about spectrophotometry at its infancy (1967). [20] Spectrophotometry is very widely used in clinical chemistry and environmental laboratories because many substances can be selectively converted to a colored derivative. Spectrophotometry is extremely sensitive so that sometimes picogram (10^{-12}g) per gram level can be determined. The instrumentation is readily available and generally fairly easy to operate.[21]

Trace and Ultra trace:

On the outset of this discussion few points are raised to expose the ambiguity associated with the term 'trace'. An analytical chemist is frequently encountered with this term regardless the technique employed spectrophotometry, spectrofluorimetry, conventional atomic absorption, atomic emission, polarography, activation analysis etc. For the academic and scientific interests some clear out distinction between the two extremes is felt urgently necessary. The term 'Trace' originally was used to mean very low concentration and defined as that amount upper limit of which was 100 ppm be weight (i.e. 100 μg/gm). Though the lower limit was not fixed at that time (neither as yet), perhaps it was originally intended somewhere in the order of micro gram/gm levels then the lowest possible levels that could be instrumentally detected/estimated. The improvement of electronics, the development of sophisticated instrumentation and methodology in recent time led the analytical chemistry to measure unbelievably small concentration of elements with incredible success of amazing accuracy. Even femtogram (10^{-15}g) quantities of substances are being measured these days. Thus the term trace amount is pushed back from the range of (10^{-3} to 10^{-6}g) to a range of (10^{-7}g to 10^{-15}g). Therefore, some authors are in favor of calling those ultra microgram quantities as 'ultra trace' to differentiate it from 'trace'. While others are in opinion to preserve the classical 'trace' now is reserved for ultra microgram quantities. Because, there is and will be very demand and necessary of conventional technique, e.g. spectrophotometry, etc. in some sphere of scientific field dealing with micro-quantities, e.g. Geo-science, metallurgy, for example, the term 'trace' will be continued in its classical sense. To fit the smallness of ultra micro concentration 'ultra trace' seems to be more appropriate. It will not only do justice to conventional analytical chemists but also help to remove the ambiguity prevailing now. Some authors also use nano-trace, pico-trace and micro-trace to pinpoint the smallness of the material used. [22]

Spectrophotometry as a Trace Analytical Technique:

The inorganic analytical chemists dealing with chemical spectrophotometry for trace measurement from solutions belong to twin schools of spectrophotometric analysis:

(1) Molecular absorption Spectrophotometry, &

(2) Molecular fluorescence spectrophotometry.

Molecular absorption spectrophotometry is more sensitive technique in inorganic trace analysis. [23-35] Molecular fluorescence spectrophotometry, on the other hand, experimentally similar and akin to molecular absorption spectrophotometry, fortunately is free from all limitations of absorptiometry. [36-39]

2.1.3 QUANTITATIVE APPLICATION OF UV-VISIBLE ABSORPTION:

BEER LAW: The amount of monochromatic radiation absorbed by sample is described by the Beer -Bouguer-Lambert Law, commonly called Beer’s Law. Consider the absorption of monochromatic radiation of radiant power P_0 which passes through a solution of an absorbing species at concentration ***c*** and path length ***b***, and the emergent (transmitted) radiation has radiant power ***P***. This radiant power is the quantity measured by spectrometric detectors. Bouguer in 1729 and Lambert in 1960 recognized that when electromagnetic radiation is absorbed, the power of the transmitted energy decreases in exponential manner as,

$$P = P_0 10^{-kb}$$

$$\frac{P}{P_0} = 10^{-kb} = T \quad \text{...} \quad (1)$$

Where K is a constant and T is called the transmittance, the fraction of radiant energy transmitted. Logarithmic form of the equation is

$$\log T = \log \frac{P}{P_0} = -kb \quad \text{...} \quad (2)$$

In 1852, Beer and Bernard, each stated that a similar law holds for the dependence of T on the concentration, ***‘c’***

$$T = \frac{P}{P_0} = 10^{-k'c} \tag{3}$$

or,

$$\log T = \log \frac{P}{P_0} = -k'c \tag{4}$$

Where k' is a new constant. Combining these two laws is obtained which is known as Beer’s law. It described the dependence of T on the path length and the concentration of the absorbing species as,

$$T = \frac{P}{P_0} = 10^{-abc} \tag{5}$$

Where ***‘a’*** is a combined constant of k and k'. The logarithmic form of (5) is

$$\log T = \log \frac{P}{P_0} = -abc \tag{6}$$

It is more convenient to omit the negative sign on the right hand side of the equation and to define a new term, absorbance:

$$A = -\log T = \log \frac{1}{T} = \log \frac{P_0}{P} = abc \tag{7}$$

Where, A is the absorbance. This is the common form of Beer’s law. It is the absorbance that is directly proportional to the concentration. The path length ‘b’ in Equation (7) is expressed in centimeters; the concentration ‘c’ is in gram per liter. The constant ‘a’ is then called the absorptivity or extinction coefficient. When ‘c’ is expressed in moles/liter; ‘b’ in cm, the constant ‘a’ is replaced by ε and the Beer’s law is written as

$$A = \varepsilon bc \quad \cdots\cdots\cdots \quad (8)$$

This new quantity **ε** is known as Molar Absorptivity. Since A is unit less, **ε** has the unit of liter mol^{-1} cm^{-1}. Molar absorptivity and absorptivity are dependent on the nature of the absorbing material and the wavelength of measurement. Beer's law holds strictly for monochromatic radiation, since the absorptivity varies with wavelength. **[40]**

2.1.4 APPLICABILITY OF BEER'S LAW:

Beer's law is the basis of all quantitative applications of spectroscopy. Generally a method of calibration of standardization is used for determining the concentration of the analyte.

Beer's law will generally hold over a wide range of concentration if the structure of the colored ion or of the colored non electrolyte in the dissolved state does not change with concentration. Small amounts of electrolytes, which chemically un-reactive with the colored components, do not usually affect the light absorption; large amounts of electrolytes may results in a shift of the maximum absorption, and may also change the value of the absorptivity. Discrepancies are usually found when the colored solute ionizes, dissociates or associates in solution, since the nature of the species in solution will very with the concentration. The law does not hold when the colored solute forms complexes, the composition of which depends upon the concentration. Beer's law holds strictly for the monochromatic radiation. But no instrument can attain such resolution of wavelength. In practice a narrow band is used . With the broadening of band width, the system tends to show increasing deviation from Beer's law. The behavior of a substance can, however, always be tested by plotting absorbance-versus-concentration; a straight line passing through the origin indicates conformity to the law.

2.1.5 ABSORPTION INSTRUMENT:

The quality and cost of uv-vis absorption instruments may differ tremendously from one another. But their basic components are remarkably similar. The required properties of these components are the same regardless of the spectral regions for which they are designed. [41]

A Spectroscopic instrument contains five basic components are shown if figure-2.

1) A stable source of radiant energy;
2) A wavelength selector that permits isolation of a restricted wavelength regions;
3) A transparent container for holding the sample ;
4) A radiant detector or transducer that converts radiant energy to a useable signal (usually electrical); and
5) A signal processor and readout.

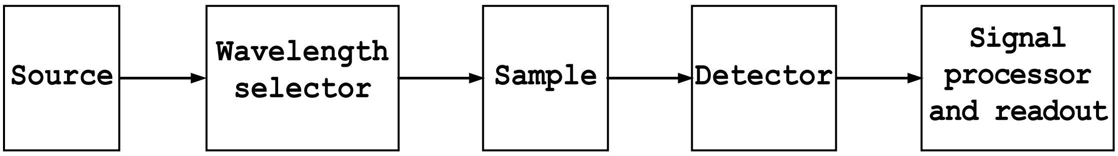

Fig.– 2: The block diagram of uv-vis absorption instrument..

2.2.1 THE USE OF ZINC.

The name "**Zinc**" Originated from the German word "**ZINK**". Alchemists burned zinc in air to form what they called "***lana philosophica***", "**philosopher's wool** " or "white snow".[42] Zinc is a blue-gray, metallic element, at room temperature it is brittle . It is the 24th most abundant element.[43]

Modern life is inconceivable without zinc. Zinc is an essential mineral of "exceptional biologic and public health importance".[44] Zinc deficiency affects about two billion people in the developing world and is associated with many diseases. In children it causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhea, contributing to the death of about 800,000 children worldwide per year.[44] Zinc has many other uses like:

1) The most important use is for alloying with copper in making brass or composition.[45]

2) When zinc is exposed to air or water, a durable and impermeable coating of zinc carbonate is formed on the surface of the metal. This makes it useful for galvanizing iron to protect against rusting. Zinc sheets may be used for sheathing or roofing.[46]

3) In automotive equipment and household appliances, fittings, tools, toys.[47]

4) A zinc disc attached to a ship's iron rudder will slowly corrode while the rudder stays unattached.[48]

5) Zinc is the most electro-positive of the common metals. This makes it particularly useful as a

protection against galvanic corrosion in steam boilers, and on the hulls of steel ships. Copper, brass and bronze may also be protected by its use. However, when the zinc becomes much corroded and its metallic surface is no longer exposed, the zinc compound resulting from the corrosion acts electro-negative to the protected metal and this metal is itself destroyed. The protection of iron by galvanizing is due not only to a coating of zinc but also to the fact that when a part of the zinc coating is broken and the iron is exposed, dampness sets up an electric current which consumes the zinc instead of the iron. When exposed to air and water, iron and steel rust. A coating of zinc provides threefold protection. First, zinc provides a tough and adherent coating which seals the underlying metal from contact with its corrosive environment. Second, zinc corrodes more slowly than iron. Third, if the coating is damaged and the steel exposed (accidentally or through cutting, drilling, etc.), adjoining zinc protects the exposed steel (this remarkable property is called "sacrificial protection"). Life of the coating is further extended because zinc corrosion products tend to seal off damaged areas.

"No substitute for galvanizing exists for protecting large tonnage iron and steel products from corrosion." *

* (U.S. Congress, Office of Technology Assessment)

6) Powdered zinc uses in alkaline batteries and sheets of zinc metal form the cases for and act as anodes in zinc-

carbon batteries. Zinc uses as the anode or fuel of the zinc-air battery/fuel cell. [49-53]

7) Oxide and sulphide of zinc are used to make a superior grade of white paint.[54]
8) The semiconductor properties of zinc oxide make it useful in varistors and photocopying products.[55]
9) Zinc sulfide (ZnS) is used in luminescent pigments such as on the hands of clocks, X-ray and television screens, and luminous paints.[56]
10) In building and construction.[57]
11) Zinc oxide is widely used as a white pigment in paints, and as a catalyst in the manufacture of rubber.[58]
12) In pharmaceuticals, medical equipment and cosmetics. [59-60]
13) Zinc chloride is often added to lumber as a fire retardant [61] and can be used as a wood preservative.[62]
14) In fertilizers and animal feed.[63]
15) Micronutrient for humans, animals and plants.[64]

Many zinc compounds have commercial applications. Some examples include zinc acetate [Zn($C_2H_3O_2$)], used as a wood preservative, a dye for textiles, an additive for animal feeds, and glazing for ceramics; zinc chloride ($ZnCl_2$), used as a solder, for fireproofing materials, as a food preservative, as an additive in antiseptics and deodorants, as a dental cement, in petroleum refining, and in embalming and taxidermy products; zinc hydrosulfite (ZnS_2O_4), as a bleaching agent for textiles, straw, vegetable oils and other products and as a brightening agent for paper and beet and cane sugar juice; zinc oxide (ZnO), used in rubber production, as a white pigment in paint, to prevent the growth of mold on paints, and in the manufacture of glass, ceramics, tiles, and plastics, and zinc sulfate ($ZnSO_4$), used in the manufacture of rayon, as a supplement in animal feeds, and in the dyeing of textiles.

Zinc is also an essential element that is indispensable for human health and for all living organisms. Zinc is included in most single tablet over-the-counter daily vitamin and mineral supplements.[65] It is believed to possess antioxidant properties, which protect against premature aging of the skin and muscles of the body, although studies differ as to its effectiveness.[66] Zinc also helps speed up the healing process after an injury.[66] Zinc gluconate is one compound used for the delivery of zinc as a dietary supplement. [67]

The Age-Related Eye Disease Study determined that zinc can be part of an effective treatment for age-related macular degeneration.[68]

Zinc lactate is used in toothpaste to prevent halitosis.[69] Zinc pyrithione is widely applied in shampoos because of its anti-dandruff function.[70] Zinc ions are effective antimicrobial agents even at low concentrations.[71]

The life of zinc-containing products is variable and can range from 10-15 years for cars or household appliances, to over 100 years for zinc sheet used for roofing. Street lighting columns made of zinc-coated steel can remain in service for 40 years or much longer, and transmission towers for over 70 years. For example, zinc coated steel poles placed in the Australian outback a hundred years ago are still in excellent condition. [72]

The relative reactivity of zinc and its ability to attract oxidation to itself also makes it a good sacrificial anode in cathodic protection. Cathodically protecting (CP) buried pipelines requires a solid piece of zinc to be connected by a conductor to a steel pipe.[73] Zinc acts as the anode (negative terminus) by slowly corroding away as it passes electric current to the steel pipeline.[73] Zinc is also used to cathodically protect metals that are exposed to sea water from corrosion.[74] A zinc disc attached to a ship's iron rudder will slowly corrode while the rudder stays unattacked.[75]

Zinc has been proposed as a salting material for nuclear weapons[76].Zinc dithiocarbamate complexes are used as agricultural fungicides; these include Zineb, Metiram, Propineb and Ziram. [77] Zinc naphthenate is used as wood preservative.[78] Brass (a zinc-copper alloy) is bacteriostatic and thus helps to diminish the spread of bacteria.[79]

The second largest use of zinc is as an alloy and the remaining zinc consumption is for making paint, chemicals, agricultural applications, in the rubber industry, in TV screens, fluorescent lights and for dry cell batteries. [80]

Zinc (Zn) is an essential element for all life, from man to the smallest micro-organism. Zinc deficiency affects about two billion people in the developing world and is associated with many diseases.[81]

A comprehensive review (Walsh et al. 1995) of current knowledge about zinc and human health concluded that there is a potential for zinc deficiency on a worldwide scale. [81]

Adequate daily intake of zinc is vital for the proper functioning of the immune system, digestion, reproduction, taste and smell and many other natural processes. Zinc deficiency is now recognized as one of the most important risks to human health and is one of the leading causes of illness and disease in low-income countries.[82]

In children it's defecence causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhea, contributing to the death of about 800,000 children worldwide per year. Enzymes with a zinc atom in the reactive center are widespread in biochemistry, such as alcohol dehydrogenase in humans. Consumption of excess zinc can cause ataxia, lethargy and copper deficiency. [82]

There is no life without zinc. Zinc is found in all parts of our body; it is in our organs, tissues, bones, fluids and cells. As zinc is used to generate body cells; is especially important during pregnancy ; for the growing fetus whose cells are rapidly dividing. Zinc is vital in activating growth (height, weight and bone development) in infants, children and teenagers. Among all the vitamins and minerals, zinc shows the strongest effect on our all-important immune system.[83]

Zincurea (elevated serum zinc) accompanies albuminuria and post alcoholic cirrhosis. Hypozincemia (lowered Zinc Level) accompanies hepatitis, cardiac infarction, pregnancy, oral contraception, and stagnant skeletal growth.[84]

Zinc contained mainly in bones, teeth, hair, skin, liver, muscle, leukocytes, and testes. Zinc is a component of several hundred, leukocytes, and testes. Zinc is a component of several hundred enzymes, including many nicotinamide adenine dinucleotide (NADH) dehydrogenases, RNA and DNA polymerases, and DNA transcription factors as well as alkaline phosphatase, superoxide dismutase, and carbonic anhydrase.[85]

Zinc plays a vital role in fertility. In males, zinc protects the prostate gland from infection (prostates) and ultimately from enlargement (prostatic hypertrophy). Zinc helps maintain sperm count, mobility and normal levels of serum testosterone. In females, zinc can help treat menstrual problems and alleviate symptoms associated with premenstrual syndrome (PMS).[86]

Among all the vitamins and minerals, zinc shows the strongest effect on our all-important immune system. Zinc plays a unique role in the T-cells. Low zinc levels lead to reduced and weakened T-cells that are not able to recognize and fight off certain infections. An increase of the zinc level has proven effective in fighting pneumonia and diarrhea and other infections. Zinc can also reduce the duration and severity of a common cold.[87]

Zinc activates areas of the brain that receive and process information from taste and smell sensors. Levels of zinc in plasma and zinc's effect on other nutrients, like copper and manganese, influence appetite and taste preference. Zinc is also used in the treatment of anorexia.[87]

Zinc accelerates the renewal of the skin cells. Zinc creams are used for babies to soothe diaper rash and to heal cuts and wounds. Zinc has also proven effective in treating acne, a problem that affects especially adolescents. Zinc is important for healthy hair. Insufficient zinc levels may result in loss of hair, hair that looks thin and dull and that goes grey early. There are also a number of shampoos which contain zinc to help prevent dandruff.[87]

High concentrations of zinc are found in the retina. With age the retinal zinc decline that seems to play a role in the development of age-related macular degeneration (AMD), which leads to partial or complete loss of vision. Zinc may also protect from night blindness and prevent the development of cataracts.[87]

Zinc is an essential trace element, necessary for plants,[88] animals,[89] and microorganisms.[90] Human contains about 2g of Zn. This is the second largest amount of a transition metal after Fe, 4g. [91] There are more than 200 enzymes containing Zn, for the stabilization of DNA and the expression of genes, and for the transfer of nervous signals.[92] Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye.[93] Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth.[94] Some of the best known roles are as follows:

Carbonic anhydrase, which is present in red blood cells, is involved in respiration. Carboxypeptidase, which is present in the pancreatic juice, is involved in the digestion of proteins by animals. Alkaline phosphatase (energy release). Dehydrogenases and aldolases (sugar metabolism). Alcohol dehydrogenase (metabolism of alcohol). Zinc is a lymphocyte mitogen (causes expansion of immune cells). Zinc Regulate Immune functions. Zinc Deficiency cause abnormal development of immune organs. Zinc Deficiency susceptibility to infections increases.[95]

In the years since the original discovery of how zinc influences the immune system, many groups have studied zinc[96] and other links between nutrition and immunity. Indeed there is growing evidence that nutrients have actually evolved as co-factors in development and maintenance of immune response.[97] Zinc has a unique role in thymus dependent "T" cell mediated immune response.[98]

Zn is one of the elemental contents in human body.

Element	Serum (ppm)	Urine	Tissues (ppm)
Zn	1·2	0·3-0·6 mg/day	12-100

Source:
G.D. Christian, *Analytical Chemistry*, 4th Edn. John Wiley and Sons, New Yourk, 1986, P, 531.

2.2.2 ESSENTIAL ELEMENTS WITH POTENTIAL FOR TOXICITY:

Eight metals generally accepted as essential are cobalt, copper, cadmium, chromium, nickel, lead, zinc and molybdenum. Each of these eight essential elements has three levels of biologic activity, trace levels required for optimum growth and development, homeostatic levels (storage levels) and toxic levels. [99]

These essential metals are being investigated for their toxicity in excessive amount. The complete dose-response curve for essential metals shows a general complex activity spectrum comparable to that of Fig.3.

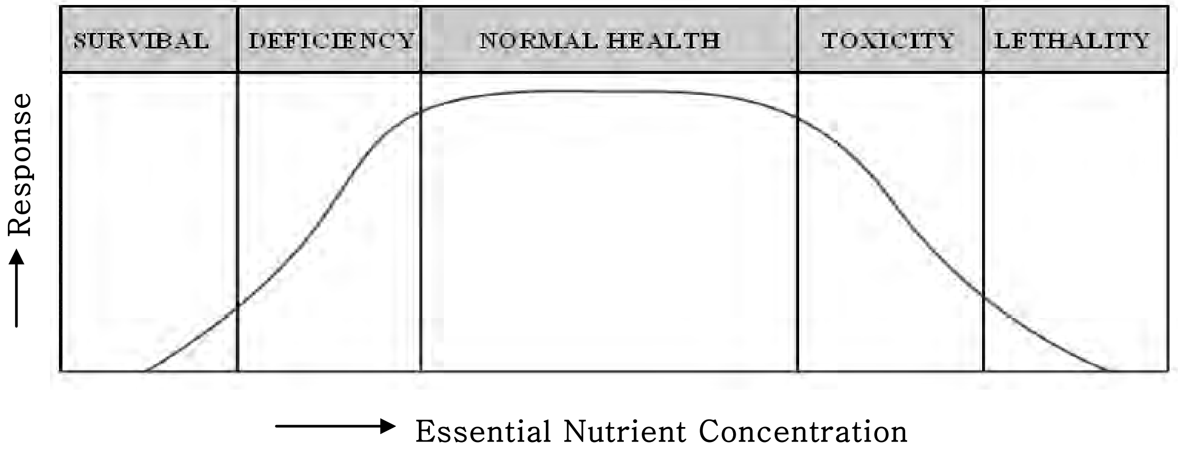

Fig:-3 Activity spectrum of an essential metal.

[Source: ``*Metal Toxicity in Mammals,* **1**''; T. D. Luckey and B. Venugopal (Eds.) Plenum press, New York, 1977, p. 7]

2.2.3 TOXICITY OF ZINC:

Zinc is an essential mineral, but too much is not beneficial. Excessive absorption of zinc suppresses copper and iron absorption. [100] The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. [101] The free ion activity model is well-established in the literature, and shows that just micromolar amounts of the free ion kills some organisms. A recent example showed 6 micromole killed 93% of all *Daphnia* in water.[102] Symptoms of zinc toxicity include nausea/vomiting, fever, cough, diarrhea, fatigue, neuropathy and dehydration.[99]

The U.S. Food and Drug Administration (FDA) has stated that zinc damages nerve receptors in the nose, which can cause anosmia.[103]

Some other examples are:

1.) Early toxic effects of heavy metals (HMs) Zn, Co, and Cd in concentration from 0.01 to 100 mM on photosynthetic activity of the green alga Chlorella pyrenoidosa Chick S-39 were studied. The early effect of HMs was manifested as a rapid (0.5 -2 h) reduction of photoinduced oxygen release by the algal cells. The suppressed relative yield of variable chlorophyll fluorescence ratio (Fv/Fm) by the metals as well as its dynamics in C. pyrenoidosa demonstrated rapid inactivation of photosystem II (PS II). Analysis of the induction curve of delayed chlorophyll fluorescence in Chlorella cells suggested that the early toxic effects of the tested concentrations of Zn, Co, and Cd included both reduced electron transport in PS II and decreased photosynthetic membrane energization. Hence, the early toxic effect of Zn, Co, and Cd was primarily related to the decreased efficiency of the light reactions of photosynthesis which further reduced the alga productivity later.[104]

2). Zinc toxicity from a normal diet is highly unlikely. Isolated outbreaks of acute zinc toxicity have occurred as a result of the consumption of food or beverages contaminated with zinc released from galvanized containers. Signs of acute zinc toxicity are abdominal pain, diarrhea, nausea, and vomiting. Single doses of 225mg to 450mg of zinc usually induce vomiting . Milder gastrointestinal distress has been reported at doses of 50mg to 150mg/day of supplemental zinc. Metal fume fever has been reported after the inhalation of zinc oxide fumes. Profuse sweating, weakness, and rapid breathing may develop within 8 hours of zinc oxide inhalation and persist 12-24 hours after exposure is terminated.[105]

3.) Zinc deficiency decreased cellular immune response. Zinc supplementation reverses this response . High concentration of zinc intake is reported to alter immune response. The higher concentration of zinc affects expression of IL-2, IL-2Rα, and TNF-α, and NF-κB activation in HUT-78 (Th_0) cells. The results show that HUT-78 cells incubated in 15, 50, and 100 μM zinc medium had significantly higher intracellular zinc contents and faster growth after 4 days of incubation, compared to the cells incubated in 1 μM zinc medium. After PMA/PHA stimulation, 1 μM zinc showed significant decreases in NF-κB activation, and in the levels of IL-2, IL-2Rα and TNF-α production and mRNAs compared to 15 μM zinc. The cells incubated in higher concentrations of zinc (50 and 100 µM zinc) showed mild to moderate decreases in the levels of IL-2, IL-2Rα, and TNF-α production and mRNAs, and in NF-κB activation compared to those incubated in 15 µM zinc medium. These data indicate that not only low level of zinc, but also high levels of zinc decrease Th1 function.[106]

4.) Toxicity of zinc is extremely rare. Taking of elemental zinc ranging from 100 mg to 150mg/day interferes with copper metabolism and causes low blood copper levels, RBC microcytosis, neutropenia, and impaired immunity. Ingesting large amounts (200 to 800 mg/day), usually by consuming acidic food or drink from a galvanized (zinc-coated) container, can cause vomiting and diarrhea. Metal fume fever, also called brass-founders' ague or zinc shakes, is caused by inhaling industrial zinc oxide fumes; it results in neurologic damage. Symptoms resolve after 12 to 24 h in a zinc-free environment.[107]

5). 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 [108]. One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate (570 mg elemental zinc) [109]. Reductions in a copper-containing enzyme, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks. Due to the finding of zinc induced copper deficiency, some researchers are examining the benefits of zinc on Wilson's disease [108].

6) Drug interactions:

Captopril, chlorthalidone, deferoximine, loop diuretics, thiazides increase urinary zinc excretion. Amiloride, ACE inhibitors, spironolactone decrease urinary zince excretion. Chlorthalidone increases serum zinc levels. Penicillamine decreases serum zinc levels. Use with tetracyclines and fluoroquinolones decreases both antibiotic and zinc absorption. Caffeine, bran, calcium, iron, protein and phosphorus decrease zinc absorption.[109]

Zinc supplements have the potential to interact with several types of medications. A few examples are provided below.

Both quinolone antibiotics (such as Cipro®) and tetracycline antibiotics (such as Achromycin® and Sumycin®) interact with zinc in the gastrointestinal tract, inhibiting the absorption of both zinc and the antibiotic.[109,110] Zinc can reduce the absorption and action of penicillamine, a drug used to treat rheumatoid arthritis. [111] Thiazide diuretics such as chlorthalidone (Hygroton®) and hydrochlorothiazide (Esidrix® and HydroDIURIL®) increase urinary zinc excretion by as much as 60%.[112]

"Zinc is an essential element in the environment. The possibility exists for both a deficiency and excess of this metal. For this reason it is important that regulatory criteria for zinc, while protecting against toxicity, are not set so low as to drive zinc levels into the deficiency area."

CHAPTER THREE: LITERATURE REVIEW

The Properties of Zinc:

Discovery : 16th century.
Position in Modern Periodic Table : 12
Position in Old Periodic Table : II B
Atomic Number : 30
Electronic Configuration : [Ar] $3d^{10}$ $4s^{2}$
Relative Atomic Mass : 65.39
Oxidation states chiefly : + 2
Melting Point : 692.7^{0} K, 419.53 °C
Boiling Point : 1180^{0} K, 907 °C
Density : 7.14 g·cm^{-3}

3.1.1. AVAILABLE METHODS FOR QUANTITATIVE DETERMINATION OF ZINC:

There are a several methods for the quantitative determination of Zinc. Some of the established methods of quantitative determination of Zinc are as follows:

A. The complexometric determination of zinc in the presence of large quantities of magnesium.

Zinc is converted into the complex cyanide and the resultant solution is passed through a column of cation-exchange resin (Amberlite IR -120) when the bulk of the magnesium is separated off and the zinc is collected unchanged as the complex cyanide. Zinc ions are liberated from the complex by means of formaldehyde and are subsequently titrated at pH 6.8 with disodium dihydrogen ethylenediamine tetra-acetate solution (Complcxone III).[113]

B. Determination of zinc and copper with Morpholine.

In morpholine has been found an organic base which is useful as an analytical reagent, and may serve for the purpose of quantitative determination of zinc and copper. In both cases, the analytical procedure is of a very simple nature, and yields good results. Morpholine may be useful for the separation of amphoteric ions of electropositive character from those of electronegative character.[114]

C. High sensitive determination of zinc with novel water-soluble small molecular fluorescent sensor.

A high sensitive method of quantitative analysis for the determination of zinc in the nutrition supplements has been developed by using a novel water-soluble fluorescent sensor HQ3: (8-pyridylmethyloxy-2-methyl-quinoline). Under the optimized condition of 67 mM phosphate buffer, pH 7.4, and 5% (v/v) DMSO, the zinc concentration showed good linear relationship with fluorescence intensity in the range of 7.5×10^{-8} to 2.5×10^{-5} M with the detection limit of 1.5×10^{-8} M. HQ3 exhibited high selectivity to zinc comparing with other metal ions except for cadmium. The developed analytical method was successfully used for determining the content of zinc in a real sample of zinc gluconate solution of Sanchine.[115]

D. Determination of Zinc as 8-hydroxyquinaldinate:

Zinc may be precipitated by 8-hydroxyquinaldine (2-methyloxine) in acetic acid-acetate solution: it can thus be separated from aluminium and magnesium. It can be weighted as $Zn(C_{10}H_8ON)_2$ after drying at 130-140^{0}C.[116]

E. An improved dithizone method for the determination of small quantities of zinc in blood and tissue samples.

The study of zinc metabolism in experimental animals and humans is dependent upon a reliable analytical method. Numerous methods, gravimetric, turbidimetric, and colorimetric (*1), have been tried and found wanting. Fischer (*2) first introduced diphenylthiocarbazone (dithizone) for the analysis of metals with relation to industrial processes. This organic dye combines with a number of the heavy metals (Sandell (*3)). The combination is selective for any one metal, depending upon the pH of the solution containing the metals, and the presence of salts that form complexes with other metals in solution. It was not until 1937 that J.Fischer and Leopoldi (*4) adapted the dithizone method to the analysis of Inorganic Zinc.

Holland and Ritchie (*5) and Cowling and BIiller (*6) used this dye for zinc analyses in plants, and Hove et al. (*7) in the measurement of carbonic anhydrase. The procedure employed by these authors. However, involved a preliminary extraction of other metals, principally copper, and a final extraction of zinc. Gettler (*8) simplified the method by the use of a buffered solution of complex-forming salts, obviating the preliminary separation. We have further refined the method to obtain greater accuracy in the analysis of the zinc content of samples of whole blood, plasma, erythrocytes, and leucocytes and samples of tissue. Samples of the size that can be practicably obtained may contain as little as 1 to 5 y of zinc.

At pH 5.5 and in the presence of a titrate solution and complex-forming buffer, dithizone combines with zinc in stoichiometric proportions to form zinc dithizonate, but does not combine with other metals which may be present. The completion of this reaction is accompanied by a change of color of the dithizone from green to bright red. In routine extraction of samples this color change occurs slowly and appears to pass through an intermediary purple stage. In our experience, this color change takes place more rapidly when the dye is dissolved in carbon tetrachloride than in chloroform.[117]

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1. Todd, W. R., and Elvehjem, C. A., J. Biol. Chem., 98.609 (1932).
2. Fischer, H., Wiss. Verdfentl. Siemens-Kongern, 4,158 (1925).
3. Sandell, E. B., Calorimetric determination of traces of metals, New York (1944).
4. Fischer, H., and Leopoldi, G., 2. anal. Chem., 107,241 (1936).
5. Holland, E. B., and Ritchie, W. S., J. Assn. Agr. Chem., 22,333 (1939).

F. A complexometric method based on selective masking and de-masking has been developed for the rapid determination of aluminium, lead and zinc from the same solution in glass and glass frit samples.

The determination is carried out using potassium cyanide to mask zinc, and excess disodium salt of EDTA to mask lead and aluminium. The excess EDTA was titrated with standard Mn(II)SO$_4$ solution using Erichrome Black-T as the indicator. Subsequently selective de-masking agents– triethanolamine, 2,3-dimercaptopropanol and a formaldehyde / acetone mixture – were used to determine quantities of aluminium, lead and zinc in a stepwise and selective manner.[118]

G. An indirect complexometric method is described for the determination of zinc (II) using 2,2'-bipyridyl as masking agent.

Zinc (II) in a given sample solution is initially complexed with an excess of EDTA and surplus EDTA is titrated with lead nitrate solution at pH 5.0 -6.0 (hexamine), using xylenol orange as indicator. An excess of 2,2'-bipyridyl is then added, the mixture shaken well and the EDTA released from the Zn -EDTA complex is titrated with standard lead nitrate solution. Results are obtained for 3 -39 mg of Zn with relative errors < 0.5% and standard deviations ± 0.06 mg. The interference of various ions are studied. The method is applied for the determination of zinc in its alloys and ores.[119]

H. Copper, zinc and manganese determination in saline samples employing FAAS after separation and preconcentration on Amberlite XAD-7 and Dowex 1x-8 loaded with Alizarin Red S.

Two procedures have been proposed and compared for separation and preconcentration of trace amounts of manganese, copper, and zinc in saline samples. The procedures are based in the use of Amberlite XAD-7 and an anion-exchanger Dowex 1 X 8–50 loaded with Alizarin Red S (ARS). In order to obtain quantitative recoveries of metal ions, various experimental parameters such as pH, shaking time, sample volume, amounts of solid phase , effects of concomitants, capacity and cations desorptions from solid phases were optimized. For Amberlite XAD -7 impregnated with ARS (XAD 7 -ARS), Mn, Cu, and Zn were quantitatively retained, in the pH range 8.5 -10.0, by using 0.50 g of solid phase, stirring time of 5 min and a total mass up to 200 µg of each cation. The sorbed elements were subsequently eluted with 5 mL of 3 mol/L HNO_3, with recovering over 95 %, and a fifty-fold preconcentration factor for Zn and a ten-fold preconcentration factor for Mn and Cu were obtained. For Dowex 1X8-50 impregnated with ARS (Dowex-ARS), Cu and Mn were quantitatively retained in the pH range 8.1 to 9.0, and Zn pH 8.1, by using 0.75 g of solid phase, 90 min of stirring time and a total mass up to 50 µg of each cation. The sorbed elements were subsequently eluted with 20 mL of 2 $molL^{-1}$ HNO_3, and a five-fold preconcentration factor to Cu and Zn was obtained. The precision of the procedure was determined by running 10 replicate samples, each one containing 250 µg L^{-1} of each element and the relative standard deviations were 0.2% (Cu), 0.4 % (Mn), and 0.4% (Zn), to XAD 7-ARS and 0.3% (Cu), 0.5 % (Mn), and 0.3% (Zn), to Dowex-ARS. The procedures were used for determining of Mn, Cu, and Zn in physiological solutions and seawater samples, from Salvador-Bahia. The analyte addition technique was used and the recoveries obtained (95%) revealed that the proposed procedure shows good accuracy and precision.[120]

I. Quantitative separation of zinc traces from cadmium matrices by solid-phase extraction with polyurethane foam.

A system for separation of zinc traces from large amounts of cadmium is proposed in this paper. It is based on the solid-phase extraction of the zinc in the form of thiocyanate complexes by the polyurethane foam. The following parameters were studied: effect of pH and of the thiocyanate concentration on the zinc extraction, shaking time required for quantitative extraction, amount of PU foam necessary for complete extraction, conditions for the separation of zinc from cadmium, influence of other cations and anions on the zinc sorption by PU foam, and required conditions for back extraction of zinc from the PU foam. The results show that zinc traces can be separated from large amounts of cadmium at pH 3.0±0.50, with the range of thiocyanate concentration from 0.15 to 0.20 $molL^{-1}$, and the shaking time of 5 min. The back extraction of zinc can be done by shaking it with water for 10 min. Calcium, barium, strontium, magnesium, aluminum, nickel and Iron(II) are efficiently separated. Iron(III), copper(II) and cobalt(II) are extracted simultaneously with zinc, but the iron reduction with ascorbic acid and the use of citrate to mask copper(II) and cobalt(II) increase the selectivity of the zinc extraction. The anions nitrate, chloride, sulfate, acetate, thiosulphate, tartarate, oxalate, fluoride, citrate, and carbonate do not affect the zinc extraction. Phosphate and EDTA must be absent. The method proposed was applied to determine zinc in cadmium salts using 4-(2-pyridylazo)-resorcinol (PAR) as a spectrophotometric reagent. The result achieved did not show significant difference in the accuracy and precision (95 % confidence level) with those obtained by ICP–AES analysis.[121]

J. Application of a new spectrophotometric method for Zinc determination in beverages and condiments.

A new spectrophotometric method has been applied to the determination of zinc in bottled mineral water (with and without gas), refreshments, vinegar, and table salt. The method introduces the 1-[di-(2-pyridyl)-methylidene]-5-(salicylidene)-thiocarbohydrazone (DPST) as chromogenic reagent.[122]

K. A Rapid Spectrophotometric Method for Trace Determination of Zinc.

A simple, sensitive, and highly selective method is proposed for the determination of zinc (II) using a bis-azo dye, 2, 6-bis(1-hydroxy-2-naphthylazo)pyridine as spectrophotometric reagent. At pH = 7.8, in 50 % (*V*/*V*) ethanol–water medium, the complex is found to obey Beer's law up to 1.3 mg/L with an optimum concentration range between 0.19 and 1.0 mg/L. Sandell's sensitivity of the color reaction was calculated to be 0.0011 µg cm^{-2} with molar absorptivity of 6.0×10^4 L mol^{-1} cm^{-1} at 560 nm. The optimum conditions for the determination of Zn(II) with the reagent were ascertained. The complexation at different pH was studied in water–ethanol medium. The composition of the complex is 1:2. The action of some interfering ions was verified, and the developed method applied successfully for the estimation of zinc levels in food and milk samples, and the results were then compared with those obtained by using AAS.[123]

3.2.1. THE CHARACTERS OF ALIZARIN RED S (THE LIGAND USED HERE FOR QUANTITATIVE DETERMINATION OF ZINC BY UV-VIS SPECTROPHOTOMETRIC METHOD).

Alizarin is an organic compound that is historically important as a prominent dye. It is an anthraquinone originally derived from the root of the madder plant. Alizarin is also the name for a variety of related dyes, such as "Alizarine Cyanine Green G", "Alizarine Brilliant Blue R." and "Alizarine Red S." The word ***alizarin*** derives from the Arabic ***al-usara***, juice.[124]

Alizarin (l,2-dihydroxy-9,10-anthraquinone) was the first of the hydroxyanthraquinones to be proposed as an analytical reagent for qualitative and quantitative determination of metals. Its water-soluble derivative alizarin red S (l,2 -dihydroxy-9,10-anthraquinone-3-sulfonic acid) became one of the most widely used and popular analytical reagents.[125]

The Chemical properties of Alizarin red S:

Alizarin red S is the derivative of **Anthraquinone** (**9,10-dioxoanthracene**) which is an aromatic organic compound. and is a derivative of anthracene.

Molecular Formula = $C_{14}H_7NaO_7S$
Molecular Weight = 342.26
pH Range = 3.5 - 6.5 ; 9.4 - 12.0
Color changes at pH = Yellow (3.5) to Orange (6.5); Red (9.4) to Violet (12.0).
pK_a = 4.5, 11.
Physical Form = Orange Yellow Powder
Melting Point = > 250^0C
Uv-Vis (λ_{max}) = 423nm (Acidic), 546nm (Basic).
Isosbestic point = 447 nm

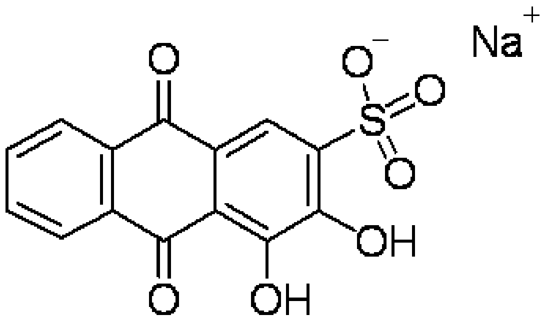

Fig-4: Structure of ALIZARINE RED S.

1,2-dihydroxy anthraquinone-3-sulfonic acid, sodium salt.

Spectrum:-1

The absorption spectrum of 0.012 % Alizarin Red S at pH 4.0 .

a = pH 3.50   b = pH 3.75   c = pH 4.00   d = pH 4.25   e = pH 4.50
f = pH 4.75   g = pH 5.00   h = pH 5.25   i = pH 5.50

Spectra:-2
The absorption spectra of 0.012% Alizarin Red S at pH 3.50, 3.75, 4.00, 4.25, 4.50, 4.75, 5.00, 5.25 & 5.50.

3.2.2 EVIDENCE FOR SPECTROPHOTOMETRIC REAGENT OF ALIZARIN RED S:

Alizarin red S (ARS) previously has been used as a spectrophotometric reagent for several metals. Some special works are:

1. **“Spectrophotometric determination of aluminium with alizarin red S sensitized with polyvinylpyrrolidone.”**

For the determination of aluminium by alizarin red s, A molar absorptivity of 1.70 × 10^4 L mol^{-1} cm^{-1} has been obtained at 510 nm for acetate-buffered solutions at pH 4.75;[126]

2. **Spectrophotometric determination of molybdenum with Alizarin Red S in the presence of poly sulfonylpiperidinylmethylene hydroxide.**

The ARS modified by PSPMH reacts with molybdenum(VI) in the solutions of pH 3.4 −4.0 to produce a red complex. The composition of the complex is 1:4:1 mol ratio of Mo(VI): ARS:PSPMH. The complex obeys Beer's law from 0.05 to 5.50 µg mL^{-1} with an optimum range. The molar absorptivity is 2.1×10^4 l mol^{-1} cm^{-1} at 500 nm. The interference effects of the foreign cations have been examined and it has been determined that only Cu(II), Al(III) and Fe(III) have to be masked by EDTA. The method has been applied to the determination of geological samples without solvent extraction or separation steps.[127]

3. "Spectrophotometric determination of Toxic elements in aqueous media".

Trace amount of toxic element (Cadmium) was determined by spectrophotometric method by using 1,2-dihydroxy anthraquinone-3-sulfonic acid, sodium salt (Alizarin Red S) as a new spectrophotometric reagent. Alizarin Red S reacts in slightly acidic solution (0.005 – 0.05 M H_2SO_4) with cadmium to give a deep greenish yellow chelate which has an absorption maximum at 442 nm. The reaction is instantaneous and absorbance remains stable for over 24hrs. The average molar absorption coefficient and sandell's sensitivity were found to be 2.24 x 10^3 $Lmol^{-1}cm^{-1}$ and 20ng cm^{-2} of Cd sensitivity. Linear calibration graphs were obtained for 0.1 -40µg mL^{-1} of Cd. The stoichiometric composition of the chelate is 1:2 (Cd: Alizarin Red S) . Large excess of over 50 cations, anions and some common complexing agents (e.g EDTA, Oxalate, citrate, phosphate, thio-urea, SCN^-) do not interfere in the determination. The method was successfully used in the determination of cadmium in several standard reference materials (alloys, steels and water) as well as in some environmental waters (in land and surface), biological samples (human blood and urine) , soil samples and complex synthetic mixtures. The method has high precision and accuracy. (S= ± 0.01 for 0.5 µg mL^{-1}).**[128]**

CHAPTER FOUR: EXPERIMENTAL

4.1.1. EXPERIMENTAL PREPARATION:

Apparatus:

A shimadzu (Kyoto, Japan) (Model–1601PC) double beam UV/VIS recording spectrophotometer and Denver (UB-10) UltraBasic pH-meter were used for the measurements of absorbance and pH, respectively. A Shimadzu (Model-AA6401F) atomic absorption spectrophotometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used for comparison of the results.

Reagents and Solutions:

All the chemicals used were of analytical-reagent grade or the highest purity available. Doubly distilled de-ionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Glass vessels were cleaned by soaking in acidified solutions of $K_2Cr_2O_7$ followed by washing with nitric acid (1 + 1) and rinsed several times with high-purity de-ionized water. Stock solutions and environmental water samples (1000 mL each) were kept in polypropylene bottles containing 1 mL of concentrated HNO_3. More rigorous contamination control was used when the Zinc levels in the specimens were low.

Alizarin Red S Solution, 0.5% or 14.605 x 10^{-3} M:

Reagent solution was prepared by dissolving the requisite amount of alizarin red S. (1, 2-dihydroxyanthraqui-none-3-sulphonic acid, sodium salt) (BDH chemicals) in a known volume of de-ionized water. More dilute solutions of the reagent were prepared as required.

Zinc Standard Solutions: A 100-mL amount of stock solution with 100 ppm or 100 mg/L or 0.001529 M of divalent Zinc was prepared by dissolving 0.0440 gm of AR grade crystallized Zinc sulfate ($ZnSO_4.7H_2O$) (Merck) in doubly distilled de-ionized water. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required.

Other Solutions: Solutions of a large number of inorganic ions and complexing agents were prepared from their analar grade or equivalent grade water soluble salts (or the oxides and carbonates in hydrochloric acid).

4.1.2 PROCEDURE:

Alizarin red S solution was taken in a 10-mL calibrated flask with the Zn solution, the molar ratio of Zn:ARS was maintained in between 1:2 → 1:9 fold. The solution's pH was maintained at pH 4.00. The pH was controlled by $HClO_4$ as acid and NaOH as base as required. The solution was prepared with the double distilled de-ionized water. The absorbance was measured at 502.2nm against a corresponding Zinc blank solution. The Zinc content in an unknown sample was determined using a concurrently prepared calibration graph.

4.2 THE SELECTION OF PARAMETERS.

4.2.1 •$_{max}$ DETERMINATION:

On the time of survey, we have observed that Zn-ARS complex formed a distinguishable color than ARS at the same pH. This color variation was followed in the pH range 3.50 to 4.50. A comparison spectra has been shown in spectra-3

Four samples of pH at 3.50 to at 4.25 have been studied to determine the λ_{max} for the Zn-ARS complex. Absorbances were measured from 350nm to 650nm and presented in spectra-4. Except the pH all parameters were kept same as mentioned bellow. The ARS = 0.15%, Zn = 100ppm & pH = 3.50, 3.75, 4.00 and 4.25. The results have been presented in table-2 and Fig-5.

Table-2:
•$_{max}$ determination of the Zn-ARS complex, studied in the range of 350nm to 650nm.

pH	•$_{max}$ (nm)	Absorbance of Zinc - ARS Complex w.r.t ARS.
3.50	492.60	0.03667
3.75	496.80	0.16511
4.00	**502.20**	**0.31303**
4.25	502.80	0.14504

Spectra-3 Comparison of absorption spectra of ARS, Zn-ARS complex & Zn-ARS complex w.r.t. ARS.

Spectra:-4

Absorption spectra of Zinc-ARS complex w.r.t. ARS at pH 3.50, 3.75, 4.00 and 4.25 in the range of 350nm to 650nm.

** A = Absorbance at PH = 3.50; λ_{max} = 492.60nm; absorbance = 0.03667
B = Absorbance at pH = 3.75; λ_{max} = 496.80nm; absorbance = 0.16511
C = Absorbance at pH = 4.00; λ_{max} = 502.20nm; absorbance = 0.31303
D = Absorbance at pH = 4.25; λ_{max} = 502.80nm; absorbance = 0.14504

It is observed in the table-2 that the maximum absorbance (0.31303) has been occurred at λ_{max}=502.2 nm. It has also been observed that with the increase of pH the λ_{max} increases, after the λ_{max} 502.2 nm the absorbance decreases. That is at λ_{max} 502.2 nm the absorbance is highest.

4.2.2 pH ADJUSTMENT:

Some samples of Zinc-ARS complex of 100 ppm Zn and 0.15% Alizarin Red S have been prepared with the continuous change of pH at 3.85, 3.90, 3.95, 4.00, 4.05 and 4.10 by the standard procedure . The absorptions measured were presented in spectra-5. The absorbances were measured at λ_{max} 502.20nm and the results have been presented in table-3 and fig-6.

Table-3:
Adjustment of pH for Zn-ARS complexes, studied at pH 3.85, 3.90, 3.95, 4.00, 4.05 & 4.10.

pH	Absorbance of Zinc - ARS Complex w.r.t ARS at 502.2 nm
3.85	0.23104
3.90	0.26510
3.95	0.30480
4.00	**0.31303**
4.05	0.30250
4.10	0.28691

The results of the table-3 indicate that the absorbance increased continuously from pH 3.85 to 4.00 at λ_{max}=502.2nm and decreased from pH 4.00 to 4.10. The maximum absorption has been found at pH 4.00 (λ_{max} 502.2nm, abs 0.31303).

Spectra:-5

Absorption spectra of Zinc-ARS w.r.t. ARS at pH 3.85, 3.90, 3.95, 4.00, 4.05 & 4.10.

4.2.3 EFFECT OF TIME ON THE ABSORBANCE OF Zn-ARS COMPLEX AT pH 4.00:

A standard sample of Zn-ARS complex at pH 4.00 was prepared to observe the effect of time. Absorbances were measured at λ_{max} 502.2nm with the time interval and the data obtained have been presented in table-4 and fig-7.

From the results it has been observed that for 48 hours the absorbance (0.31303) remained constant at λ_{max} 502.2nm.

Table-4:
Effect of time on the absorbance of Zn-ARS complex w.r.t ARS at pH 4.00.

Time (Min)	2	5	8	13	22	30	90	250	300	1440	2880
Abs 502.2nm	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303	0.31303

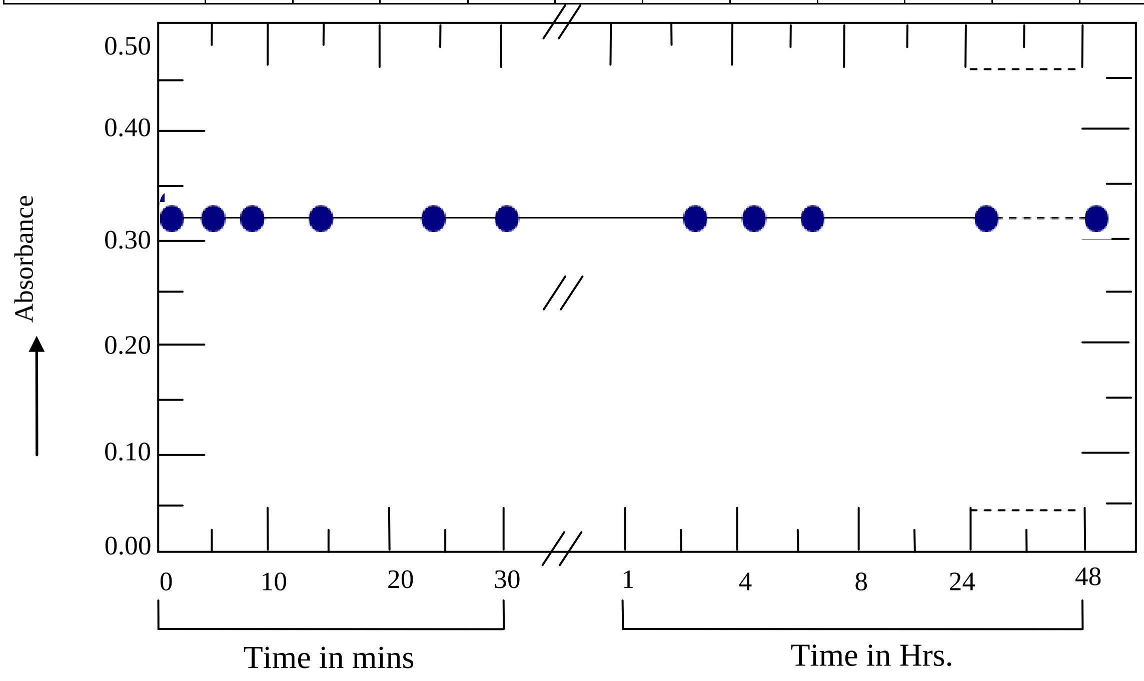

Fig.–7:

Effect of time on the absorbance of Zn-ARS complex w.r.t ARS at pH 4.00.

4.2.4 EFFECT OF TEMPERATURE ON THE ABSORBANCE OF Zn-ARS COMPLEX:

The effect of temperature on Zn-ARS complex and ARS have been studied over 25 ^{0}C to 60 ^{0}c at pH 4.00 and the absorbances were measured at λ_{max}=502.2nm (Spectra-6). The corresponding data have been presented in table-5.

Table-5:

Effect of Temperature on the Absorbance of Zn-ARS Complex.

Temperature (^{0}C)	60^{0}C	55^{0}C	50^{0}C	45^{0}C	40^{0}C	35^{0}C	30^{0}C	25^{0}C
Abs (502.2nm) Zn-ARS	0.4855	0.4497	0.4218	0.3943	0.3783	0.3638	0.3439	0.3375
Abs (502.2nm) ARS	0.0973	0.0970	0.0968	0.0967	0.0965	0.0964	0.0963	0.0963

From the result of table-5 it has been observed that the absorbances of ARS negligibly changed within the temperature range; were as, for the Zn-ARS complex the rate of increase of absorbances have been increased with the increase of temperature.

4.2.5 EFFECT OF ACIDS AND BASE ON THE ABSORBANCE OF Zn-ARS COMPLEX:

The effects of various acids and base have been studied on the standard solutions of Zn-ARS complex. The absorbances were measured at λ_{max} 502.2nm. For all subsequent measurement pH was 4.00, ARS was 0.15% and Zn was 100ppm. The results have been presented in table-6 and fig-8.

Table-6:

Effect of various acids and base on the absorbance of Zn-ARS complex.

Sl No	Acids and Base	Absorbance of Zn-ARS with respect to ARS at 502.2nm
1	**$HClO_4$**	**0.313**
2	HCl	0.207
3	NH_3	0.192
4	HNO_3	0.171
5	H_2SO_4	0.137
6	Oxalic Acid	0.089
7	Citric Acid	0.064
8	H_3PO_4	0.020
9	CH_3COOH	0.001

The results of table-6 indicate that the absorbance of Zn-ARS complex was low in the case of organic acids & NH_3 and high in the case of inorganic acids. More over the absorbance was highest in the case of $HClO_4$ acid.

4.2.6 VERIFICATION OF THE BEER LAMBERT LAWS FOR ALIZARIN RED S AT 447 nm, pH 4.00:

The well known equation of spectrophotometric analysis is Beer’s law. The Beer’s laws have been studied at 447.0 nm over 0.35% to 0.006372 % of ARS. The corresponding data have been presented in table-7 and fig-9.

Table-7: Beer Lambert Laws for ARS at 447.0 nm, pH 4.00.

Sl No	% of ARS	Abs of ARS at 447 nm
1	0.350000	2.87207
2	0.306250	2.58630
3	**0.267969**	**2.29309**
4	0.234473	2.03162
5	0.205164	1.78650
6	0.179518	1.56860
7	0.157078	1.37317
8	0.137444	1.20581
9	0.120263	1.05774
10	0.105230	0.92419
11	0.092076	0.80652
12	0.080567	0.70300
13	0.070496	0.61414
14	0.061684	0.53613
15	**0.053974**	**0.46631**
16	0.047227	0.40625
17	0.041323	0.35266
18	0.036158	0.30713
19	0.031638	0.26770
20	0.027684	0.23242
21	0.024223	0.20251
22	0.021195	0.17554
23	0.018546	0.15259
24	0.016228	0.13281
25	0.014199	0.11450
26	0.012424	0.09937
27	0.010871	0.08594
28	0.009512	0.07446
29	0.008323	0.06470
30	0.007283	0.05664
31	0.006372	0.05005

From the results it has been found that the absorbances were liner with the concentration of ARS and followed the Beer’s law. The molar absorption co-efficient was found 2.92×10^{2} $Lmol^{-1}cm^{-1}$ at 447.0 nm.[Appendix -1]

Fig-09:
Verification of the Beer Lambert Laws for ARS at 447.0 nm & pH 4.00.

4.2.7 VERIFICATION OF THE BEER LAMBERT LAWS FOR ZINC-ARS COMPLEX AT 502.2 nm, pH 4.00.

To verify the Beer’s laws for the Zn-ARS complex, the complex has been studied over 100 ppm to 6 ppm of Zn at pH 4.00. The absorptions were measured according to the standard procedure (Spectra-7). All the absorbances were measured at λ_{max} 502.2nm by using ARS as a reference sample and the results have been presented in table-8 and fig-10.

Table-8: Absorbance of Zinc-ARS complex w.r.t. ARS with the change of concentration of Zn.

Sl No	Concentration of Zinc (ppm)	Concentration of Zn (Molarity)	Absorbance of Zn-ARS w.r.t ARS at 502.2 nm
1	100.000	0.001529286	0.3131
2	87.500	0.001338125	0.2780
3	**76.563**	**0.001170867**	**0.2434**
4	66.992	0.001024499	0.2116
5	58.618	0.000896437	0.1869
6	51.291	0.000784386	0.1629
7	44.880	0.000686343	0.1412
8	39.270	0.000600551	0.1252
9	34.361	0.000525478	0.1073
10	30.066	0.000459795	0.0976
11	26.308	0.000402325	0.0859
12	23.019	0.000352026	0.0754
13	20.142	0.000308029	0.0653
14	17.624	0.000269521	0.0533
15	15.421	0.000235831	0.0467
16	**13.493**	**0.000206347**	**0.0405**
17	11.807	0.000180563	0.0339
18	10.331	0.000157991	0.0298
19	9.040	0.000138247	0.0267
20	7.910	0.000120967	0.0239
21	6.921	0.000105842	0.0215
22	6.056	0.000092614	0.0194

The results indicate that the absorbance of Zn-ARS complex increases linearly with the increase of the concentration of Zn. That is, the complex formation followed the Beer's law. The molar absorption co-efficient of Zinc has been calculated 2.10×10^2 $Lmol^{-1}cm^{-1}$. [Appendix -1]

Spectra:-7

Observation of Beer Lambert laws for Zinc-ARS complex at 502.2 nm.

4.2.8 EFFECT OF LIGAND CONCENTRATION ON THE ABSORBANCE OF Zn-ARS COMPLEX:

To observe the effect of ligand (ARS) concentration on the Zn-ARS complex formation; different solutions were prepared containing different molar ratios of Zn & ARS.

The absorption spectra were measured according to the standard procedure (spectra-8). The absorbances have been measured at λ_{max} 502.2nm and presented in the table-9 and fig-11.

The results indicate that the absorbance increases with the increase of the ratio of ARS and remain constant after the molar ratio of Zn:ARS is 1:2.

Table-9:

Absorbances of Zn-ARS complex w.r.t. ARS with the change of molar ratio (Zn:ARS).

Sl No	Molar ratio (Zn:ARS)	Absorbance of Zn-ARS with respect to ARS at 502.2nm
1	**1 : 9.00**	**0.1706**
2	**1 : 6.00**	**0.1700**
3	**1 : 4.00**	**0.1710**
4	**1 : 3.00**	**0.1706**
5	**1 : 2.50**	**0.1706**
6	**1 : 2.00**	**0.1703**
7	1 : 1.90	0.1695
8	1 : 1.50	0.1653
9	1 : 1.20	0.1553
10	1 : 1.00	0.1489

Spectra:-8

Effect of ligand’s (ARS) molar concentration on the absorbance of Zn-ARS complex.

4.2.9 DETECTION LIMIT (ppm) OF ZINC:

To observe the detection limit of Zinc, Zn-ARS solutions were prepared containing 100 ppm to 0.15 ppm of Zn. The absorption spectra were measured according to the standard procedure (Spectra-9).

The absorbances were measured at λ_{max} 502.2nm and the data have been presented in table-10 & fig-12, 13.

Table-10:

Detection limit of Zn, studied on 100 ppm to 0.15 ppm of Zn.

Sl No	Concentration of Zinc in ppm	Absorbance of Zn-ARS with respect to ARS at 502.2 nm
1	100.000	0.3131
2	87.500	0.2780
3	76.563	0.2434
4	66.992	0.2116
5	58.618	0.1869
6	51.291	0.1629
7	44.880	0.1412
8	39.270	0.1252
9	34.361	0.1073
10	30.066	0.0976
11	26.308	0.0859
12	23.019	0.0754
13	20.142	0.0653
14	17.624	0.0533
15	15.421	0.0467
16	13.493	0.0405
17	11.807	0.0339
18	10.331	0.0298

Sl No	Concentration of Zinc in ppm	Abs of Zn-ARS with respect to ARS at 502.2 nm
19	9.040	0.0267
20	7.910	0.0239
21	6.921	0.0215
22	6.056	0.0194
23	4.5420	0.0144
24	3.4065	0.0106
25	2.5549	0.0080
26	1.9162	0.0060
27	1.4371	0.0045
28	1.0778	0.0034
29	0.8084	0.0025
30	0.6063	0.0019
31	0.4547	0.0014
32	**0.3410**	**0.0012**
33	0.2558	0.0010
34	0.1918	0.0009
35	0.1500	0.0009

From the result, it has been observed that the absorbances were increases linearly with the increase of the concentration of Zinc for 0.3- 100 ppm of Zinc at 502.2nm. Below 0.3 ppm of zinc the absorbances were not liner, which indicate the lower limit of detection of Zn is 0.3 ppm.

Fig-12:
Absorbances of Zn-ARS complexes at 502.2nm Vs Concentration of Zn (100-6) ppm.

Fig-13:
Absorbances of Zn-ARS complexes at 502.2nm Vs Concentration of Zn (6-0.150) ppm.

Spectra:-9

Absorption spectra for the detection limit of Zn (100-0.150 ppm).

The following parameters in table -11 have been selected for the determination of Zinc.

Table-11:

Selected analytical parameters to determine the concentration of Zinc.

Parameter	Studied range	Selected Value
Wavelength/ λ_{max}(nm)	350 – 650	502.2
Acid Selection	HCl, $HClO_4$, H_2SO_4, Oxalic acid, HNO_3, H_3PO_4, Acetic Acid	$HClO_4$
pH	3.5 – 4.25	4.00 ± 0.02
Time/h	0 – 48	Instantaneous
Temperature/°C	25°C – 60°C	25°C ± 2°C (Room Temp)
Reagent fold molar (Zn : ARS)	1:1 – 1:9	1:2 – 1:9
Linear range in ppm	0·15 – 100	0·30 – 100

4.2.10 PRECISION AND ACCURACY:

The precision of the present method was evaluated by determining different concentrations of Zinc (each analyzed at least five times).

The samples were prepared and measured the concentration of Zn according to the standard procedure. The results have been presented in table-12 to calculate the standard deviation.

Form the result, the relative standard deviation (n=11) has been found 1.098 %, which was in between 0 -2% indicates that the method is precise & reproducible.

The results of alloy analysis by the spectrophotometric method were excellent agreement with those obtained by AAS (Table-15). Hence, the precision and accuracy of the method were found to be excellent.

Table–12:

Standard deviation and relative standard deviation of Zn(II)-ARS system.

Sample No.	Zn(II) taken mg L^{-1}	Zn(II) Found X_1 mg L^{-1}	Mean $\bar{X}$ mg L^{-1}	$X_1 - \bar{X}$	$(x_1 - \bar{X})^2$	Standard deviation (± s)	Relative standard deviation (s_r)%
1	100·0	99.0	100·32	1.32	1.74	± 1.102	1·098
2	100·0	99.5		0.82	0.67		
3	100·0	101.0		0.68	0.46		
4	100·0	101.5		1.18	1.40		
5	100·0	99.0		1.32	1.74		
6	100·0	101.5		1.18	1.40		
7	100·0	101.0		0.68	0.46		
8	100·0	99.5		0.82	0.67		
9.	100·0	99.0		1.32	1.74		
10	100·0	101.5		1.18	1.40		
11	100.0	101.0		0.68	0.46		
N = 11		ΣX_1 = 1103.5		$\Sigma X_1 - \bar{X}$ = 11·1818	$\Sigma (x_1 - \bar{X})^2$ = 12.1364		

$$\text{Mean, } \bar{X} = \frac{\Sigma X_1}{N} = \frac{1103\cdot5}{11} = 100\cdot32$$

$$\text{Standard deviation, } S = \sqrt{\frac{\Sigma\,(X_1 - X)^2}{N-1}} = \sqrt{\frac{12.136}{11-1}} = \sqrt{1.2136} = \pm\ 1.102$$

$$\text{Relative standard deviation } (S_r)\ \% = \frac{S}{\bar{X}} \times 100.$$

$$= \frac{1.102 \times 100}{100\cdot32} \qquad = 1.16\ \%$$

$$= 1.098\ \%$$

4.2.11. EFFECT OF FOREIGN CATIONS:

The effect of over 30 cations and complexing agents on the determination of Zinc was studied. The interference on an absorbance value varying not more than ±5 % are expected.[129] The results are summarized, in Table-13.

From the results, it has been observed that a large number of ions have no significant effect on the determination of Zinc. The most serious interference was found from Ag(I), Ba (II), Pb(II),Cu(II) and Fe(III) ions. Interference from these ions were probably due to complex formation.

A greater tolerance limits for these ions can be achieved by using several masking methods.

Table-13:

Tolerance limits of foreign ions[1].

[SPECIES (X)]/ Zn (W/W).

Species X	Tolerance ratio X/Zn	Species X	Tolerance ratio X/Zn
Ammonium (I)	100	Chromium(III)	More than 200
Arsenic (III)	100	Chromium (VI)	More than 200
Silver (I)	More than 200	Copper (II)	100
Manganese(II)	200	Vanadium (V)	200
Chloride	More than 200	Arsenic (V)	50
Fluoride	More than 200	Lead (II)	50
Barium	200	Nickel (II)	200
Nitrate	More than 200	Iodide	200
Nitrite	100	Phosphate	100
Bismuth (III)	100	Oxalate	More than 200
Citrate	More than 200	Thiocyanate	100
Aluminium	More than 200	Sodium	More than 200
Bromide	100	Cadmium	More than 200
Cobalt (II)	100	Mercury (II)	200
Cobalt (III)	100	Calcium	More than 200
Iron (II)	50	Potassium	More than 200
Iron (III)	40	Molybdenum(VI)	100

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[1] Tolerance limit was defined as ratio that causes less than 5 per cent interference.

4.2.12. PROBABLE STRUCTURE OF ZINC-ALIZARIN RED S COMPLEX:

Job's method [130] of continuous variation and the molar-ratio [131] method were applied to ascertain the stoichiometric composition of Zn-ARS complex. A Zinc-Alizarin Red S (1:2) complex was indicated by both methods. The assumed structure is like fig-14.

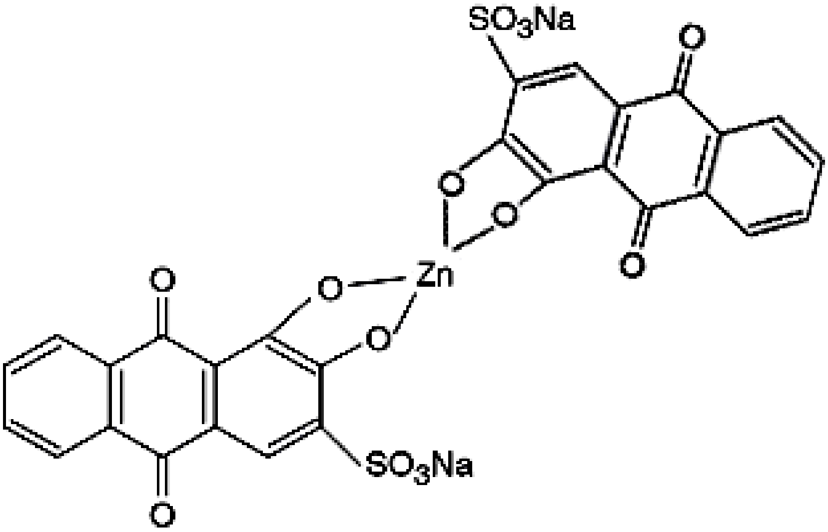

Fig:-14 Probable structure of
Zinc-Alizarin Red S (1:2) complex.

This is an assumed structure of Zn -ARS complex in fig-14. We observed that Zn formed the complex that is 5 member ring a stable complex. More over the study of molar ratio also supported the stoichiometric composition of this structure.

4.3 APPLICATIONS:

The present method was successfully applied to the determination of Zinc (II) in a series of mixtures of various compositions (Table-14). The results of alloy analysis by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table-15). In view of the unknown composition of environmental water samples, the same equivalent portions of each of such sample was analyzed for Zinc content, recoveries in both the 'spiked' (added to the samples before the mineralization or dissolution) and the 'un spiked' samples are in good agreement (Table-16). The precision and accuracy of the method were excellent.

4.3.1. DETERMINATION OF ZINC IN A MIXTURE OF IONS:

Several mixtures of compositions containing Zinc and various ions of known concentrations were determined by the present method using potassium thiocyanate as a masking agent. The results have been presented in Table-14. The results were found to be highly reproducible. Accurate recoveries were achieved in all solutions.

Table-14:

Determination of zinc in some mixtures of ions.

Sample No.	Composition of Mixture/ mg L^{-1}	Zinc / mg L^{-1}	
		Added	Found[a]
A	Zn	0·50 1·00	0·49 0·99
B	As in A + $Mn^{2+}_{(25)}$ + $Na_{(25)}$ + $KSCN_{(10)}$	0·50 1·00	0·49 1·01
C	As in B + $Hg^{2+}_{(50)}$ + $Ni^{2+}_{(50)}$ + $KSCN_{(10)}$	0·50 1·00	0·53 1·02
D	As in C + $Cd_{(25)}$ + $K_{(25)}$	0·50 1·00	0·54 1·04
E	As in D + $Ba_{(25)}$ + $Br^{-}_{(50)}$	0·50 1·00	0·53 1·08

[a] Average of five analyses of each sample.

4.3.2. DETERMINATION OF ZINC IN ALLOY WITH THE COMPARISON OF AAS METHOD.

A 0·0175 g amount of an alloy sample was accurately weighed into a 50-mL flask following the method recommended by parker et .al[132]. To it, 1 mL of concentrated nitric acid was added, carefully covering with a watch-glass until the brisk reaction subsided. The solution was heated gently to the dryness. Then 2 mL of 1:1 (V/V) $HClO_4$ was added and the solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen, and then cooled to room temperature (25±5)^{0}c. After suitable dilution with deionized water, the contents of the Erlenmeyer flask were wormed to dissolve the soluble salts. The solution was then cooled and neutralized with dilute NaOH. The solution was then filtered through sintered glass crucible if necessary. The volume of the solution was adjusted to 50mL by adding D.D. water , and the zinc was determined from this solution by the following methods.

1) Proposed Method: Sufficient amount of ARS, approximately 1:3 molar of ARS was added with the 5mL of quantitatively measured stock solution. The sample solution was prepared by the standard procedure to 10mL. The absorbance was measured at 502.2nm using ARS as a reference sample (Spectrum-10). The absorbance value was found at 0.18031. From this result the Zn content was calculated as 32.027% of Zn in the brass alloy.

2) AAS Method:

A 10 mL of stock solution was packed in a sample container then sent it to examine the percentage of Zn by AAS method. The amount of Alloy on the sample is (0.0175/50x10) g or 0.0035 g or 3.5 mg. The result has been presented in the table-15.

Table-15:

Determination of zinc in a Brass sample.

Sample Source	% of Zn W/W	
	Proposed method	AAS method
Brass	32. 027 %	32. 000 %

Spectrum 10:-

The absorption spectrum of Brass-ARS solution to determine the percentage of Zn.

4.3.3 DETERMINATION OF ZINC IN ENVIRONMENTAL WATERS:

Environmental water sample (1000mL) filtered with Whatman No.40 was evaporated nearly to dryness with a mixture of 5mL of con.$HClO_4$ and 10 mL of con.HNO_3 in a fume cupboard following a method recommended by Greenberg et al[133], and was then cooled to room temperature. The residue was then heated with 10-mL of de-ionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NaOH in the presence of 3-4 mL of dilute KSCN solution. The resulting solution was filtered and quantitatively transferred into a 25mL calibrated flask and made up to the mark with deionized water.

Most spectrophotometric method for the determination of metal ion in natural water require pre-concentration of metal.[134] An aliquot (1–2 mL) of this pre-concentrated water sample was pipetted into a 10-mL calibrated flask and the Zinc content was determined as described under standard procedure.

The analyses of environmental water samples from various sources for Zinc determination are presented in Table-16.

Table-16:

Determination of zinc in some environmental water samples.

Sample	**Zinc / mg L^{-1}**		**Recovery ± S (%)**
	Added	**Found**	
Tap water	0	3·5	
	100	105·0	99 ± 0·1
	500	504·0	100 ± 0·3
Drain water (Olympic Battery).	0	120	
	100	223	101 ± 0.5
	500	630	102 ± 0.3

CONCLUSION

In this thesis a new simple, sensitive, selective and inexpensive method with Zn-Alizarin Red S complex has been introduced for the determination of Zinc by uv-vis spectrophotometer. This method has been developed for the determination of zinc in industrial, environmental, health issue and many others for continuous monitoring to establish trace levels of Zinc in different samples. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES (Inductive Coupled Plasma – Atomic Emission spectroscopy), and ICP-MS (Inductive Coupled Plasma – Mass Spectroscopy), are available for the determination of Zinc at trace levels in numerous factors such as the low cost of the instrument, easy handling, lack of requirement for consumables etc. have caused uv-spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of relative standard deviation of the presented method is very reliable for the determination of Zinc in real samples minimum to $\mu g\ g^{-1}$ levels in aqueous medium at room temperature (25±2)°C.

ACKNOLEDGEMENT

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APPENDIX - I

Calculations.

Calculation of “•” for ARS:-

From Beer Law we know that A = •CL

Where, A = Absorbance

• = Molar Absorption co-efficient

C = concentration in moles/Liter

L = path length in cm;

Our cell’s path length was 1 cm then A = •C or • = A/C

From the data of table-7 on page 58 we calculate that

• = A/C

= • y / • X

= (2.29309-0.46631) / (0.0078294-0.0015770)

= 1.826780/ 0.006252

= 292.171811 L mol $^{-1}$cm^{-1}.

The Molar Absorption coefficient of ARS at 447.0 nm is

• = 292.171811 L mol^{-1}cm^{-1}.

= 2.92 x 10^{2} L mol^{-1}cm^{-1}.

Calculation of "•" for Zn-ARS:-

From Beer Law we know that A = •CL

Where, A = Absorbance

ε = Molar Absorption co-efficient

C = concentration in moles/Liter

L = path length in cm;

Our cell's path length was 1 cm then A = εC or • = A/C

From the data of table-8 on page 61 we calculate that

ε = A/C

= • y / • X

= (0.2434-0.0405) / (0.001170867-0.000206347)

= 0.2029 / 0.000964521

= 210.3635801 L $mol^{-1}cm^{-1}$.

The Molar Absorption co-efficient for Zn at 502.2nm is

ε = 210.3635801 L $mol^{-1}cm^{-1}$.

= 2.10 x 10^{2} L $mol^{-1}cm^{-1}$.

Calculation of Zn on Brass sample by Proposed method:-

From Beer Lambert Laws we know that A = •CL

Where,

A = Absorbance = 0.18031

• = Molar Absorption co-efficient = **210.3635801** L mol^{-1}cm^{-1}

C = Concentration in moles/Liter = ?

L = Path length in cm = 1 cm

Then,

C = A/• moles/Liter

= 0.18031 / 210.3635801 moles/Liter

= 0.000857135 moles/Liter

or, 56.048 ppm

56.048 ppm denote,

1000 mL solution contain **56.048** mg Zinc

10 mL solution contain (**56.048** x 10)/1000 mg Zinc

= 0.56048mg Zinc

50 mL stock contains 0.0175 gm Alloy,

So, 5 mL stock contains 0.00175 gm or 1.75 mg Alloy.

1.75 mg alloy contain 0.56048 mg Zinc

100 mg alloy contain (0.56048 x 100)/1.75 mg Zinc

= 32.027 mg Zinc

i.e,. the percentage of Zinc in the Brass alloy is 32.027 %.

APPENDIX - II

Abstract of the publication based on the present work.

"DETERMINATION OF TRACE Zn (II) ION BY FORMING METAL-LIGAND CHELATE COMPLEX IN AQUEOUS MEDIUM USING UV-VIS SPECTROPHOTOMETRIC METHOD."

Analytical Sciences, 2010 (communicated).

Trace amount of Zinc has been determined by spectrophotometric method using 1,2-dihydroxy anthraquinone-3-sulphonic acid sodium salt (Alizarin Red S) as a spectrophotometric reagent. The ligand Alizarin Red S reacts in slightly acidic solution (pH 4.00±0.02) with Zinc to give an orange-red chelate that has an absorption maximum at 502.2 nm. The reaction is instantaneous and absorbance remained constant for over 48 hrs. The average molar absorption co-efficient was found to be $2.10 \times 10^2 Lmole^{-1} cm^{-1}$. Linear calibration graphs were obtained for 0.30-100 $\mu g\ mL^{-1}$ of Zn. The stoichiometric composition of the chelate has been found 1:2 (Zn:ARS). Large excess of cations, anions and some complexing agents have been studied. The method was successfully used in the determination of Zinc in several standard reference material, alloys & environmental water and made a comparison with those obtained by Atomic Absorption Spectrophotometer.

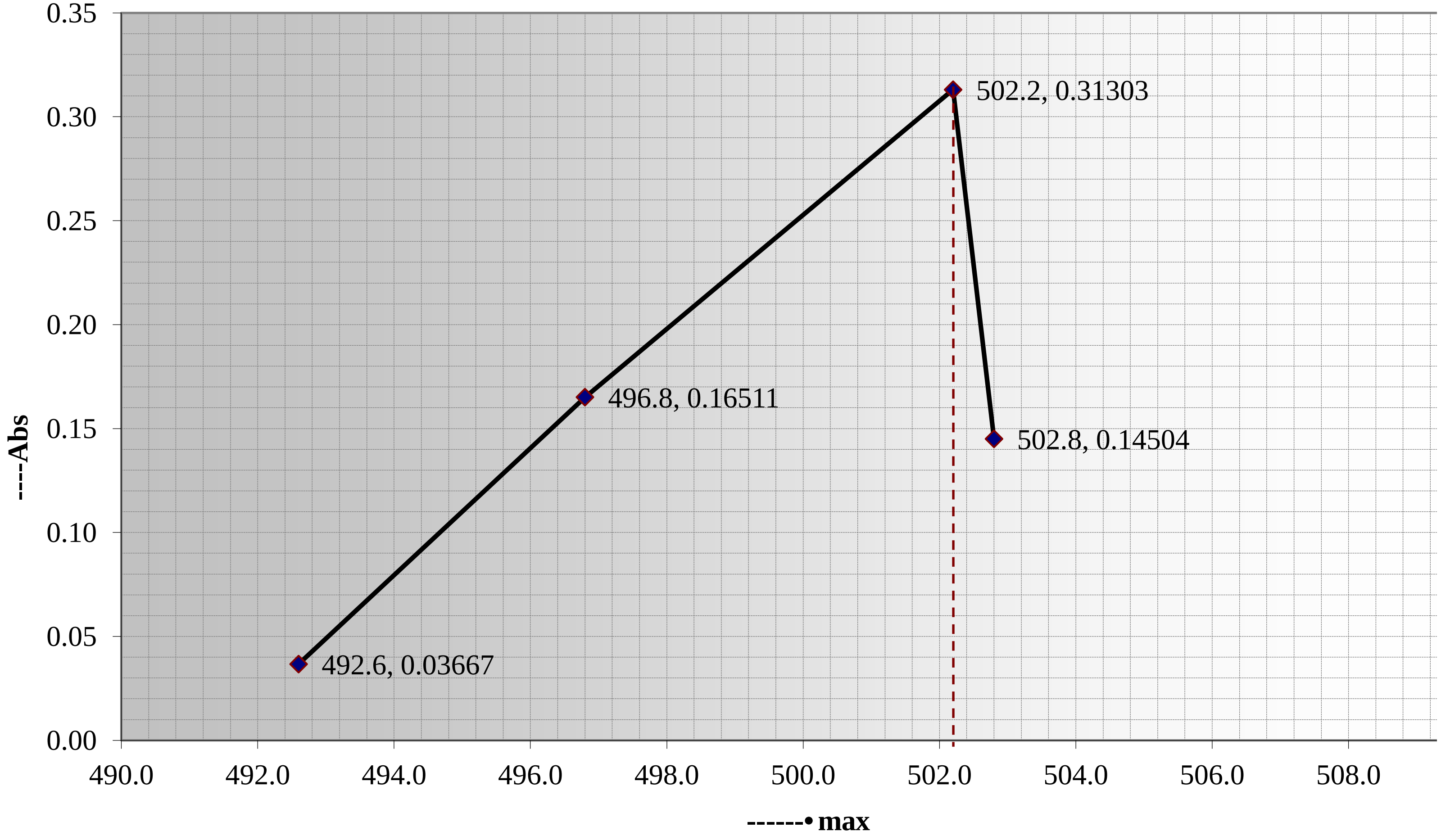


Fig:-5 Absorbance of Zinc-ARS Complex w.r.t. ARS at pH 3.50, 3.75, 4.00 & 4.25. Vs • max in the range of 350nm to 650n

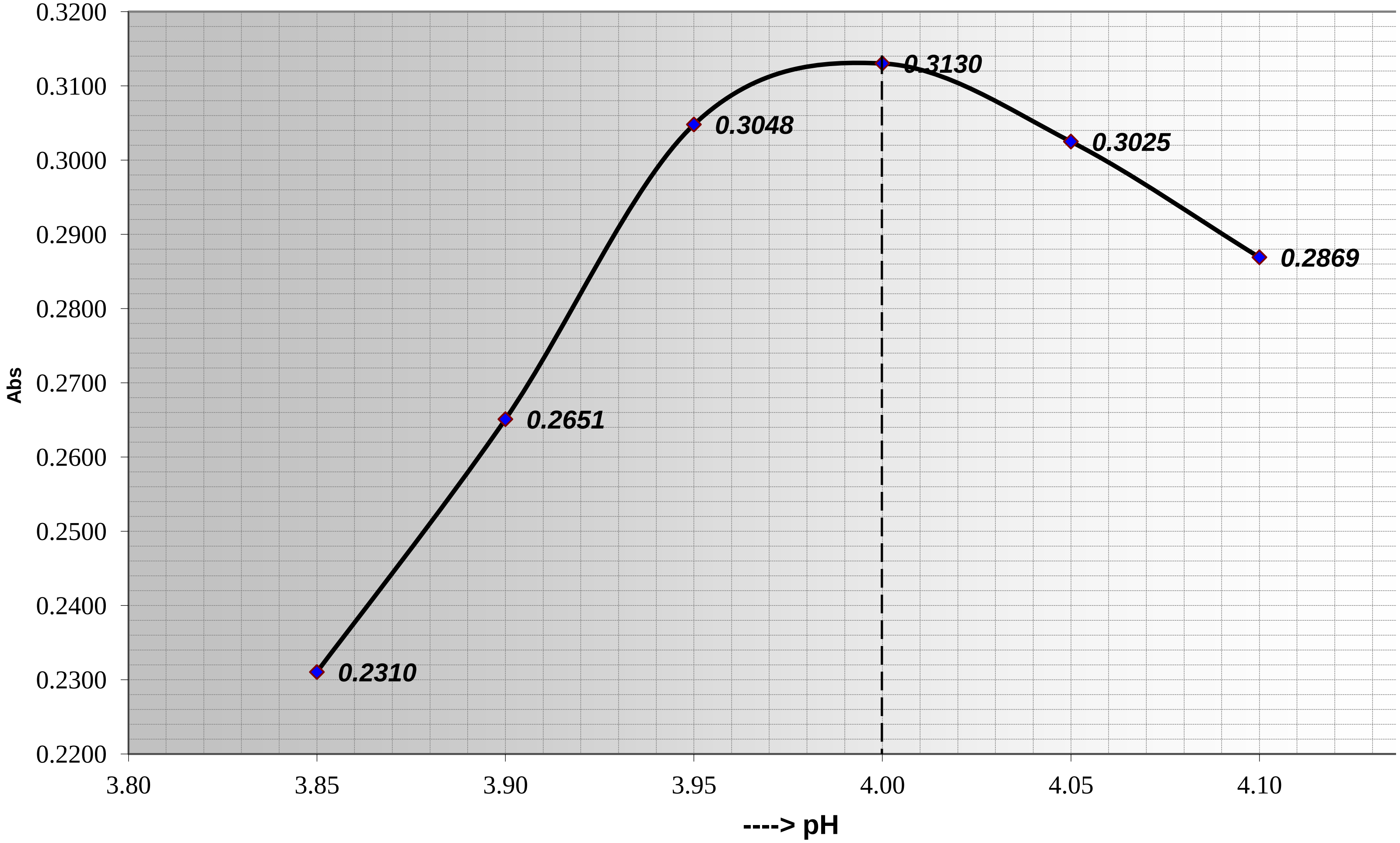


Fig-6 Absorbances of Zn-ARS complexes at 502.2 nm w.r.t. ARS Vs at pH 3.85, 3.90, 3.95, 4.00, 4.05 & 4.10.

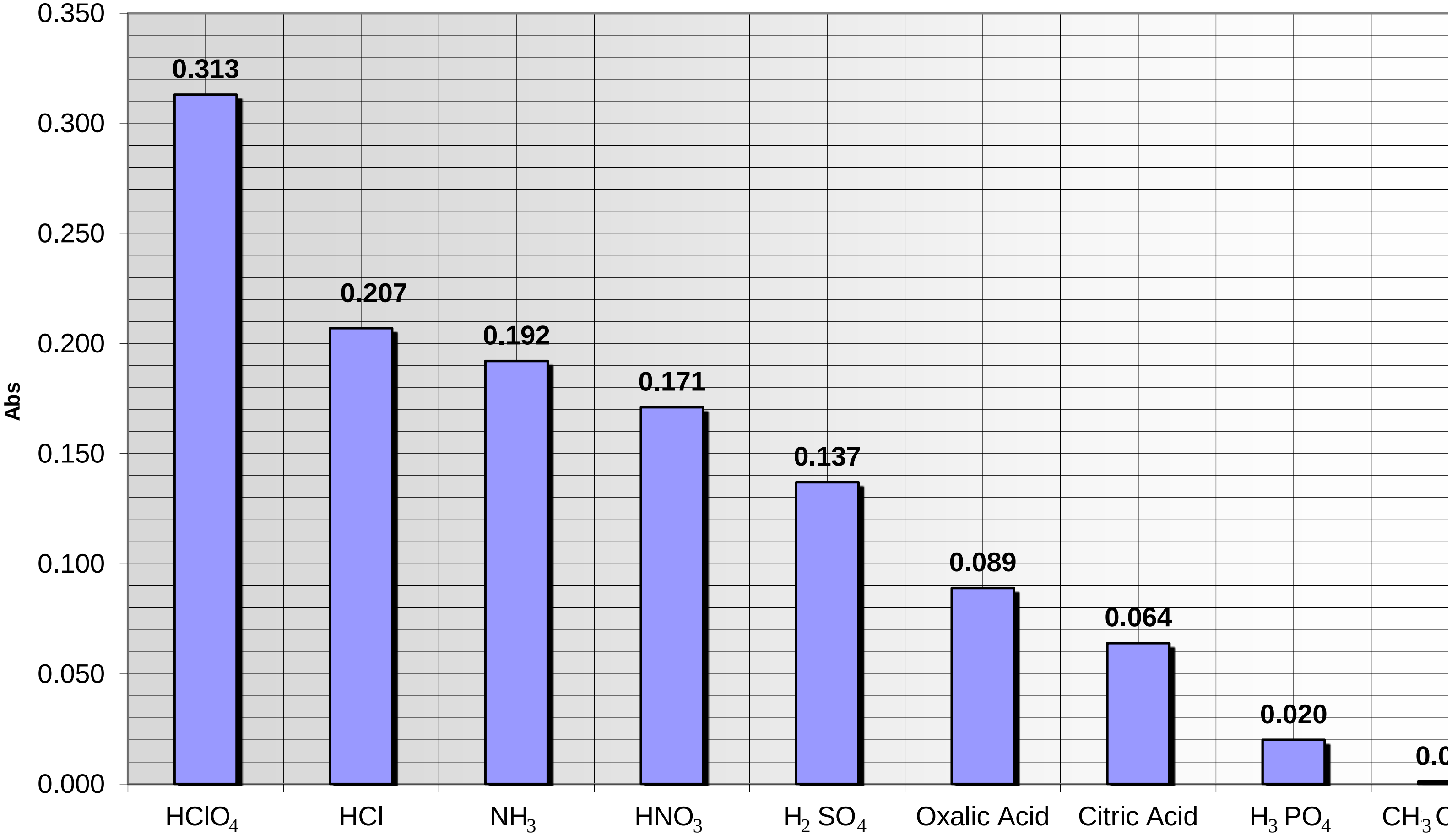
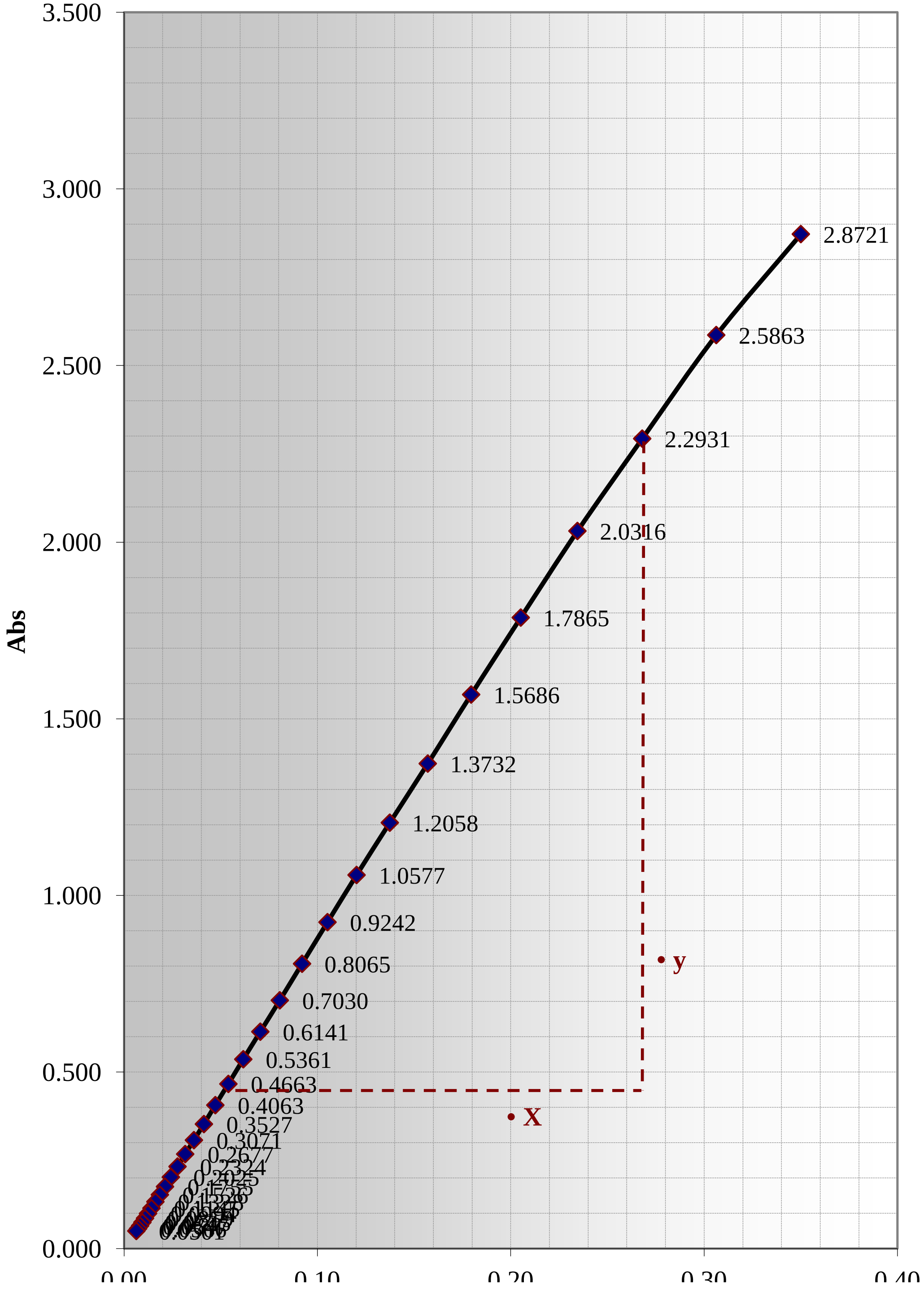


Fig:- 08. Effect of Acids and Base on the absorbance of Zn-ARS complex at 502.2nm. Absorbance Vs Acid or Base used.


3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Abs
0.00
0.10
0.20
0.30
0.40
2.8721
2.5863
2.2931
2.0316
1.7865
1.5686
1.3732
1.2058
1.0577
0.9242
0.8065
0.7030
0.6141
0.5361
0.4663
0.4063
0.3527
0.3071
0.2677
0.2324
0.2025
• y
• x

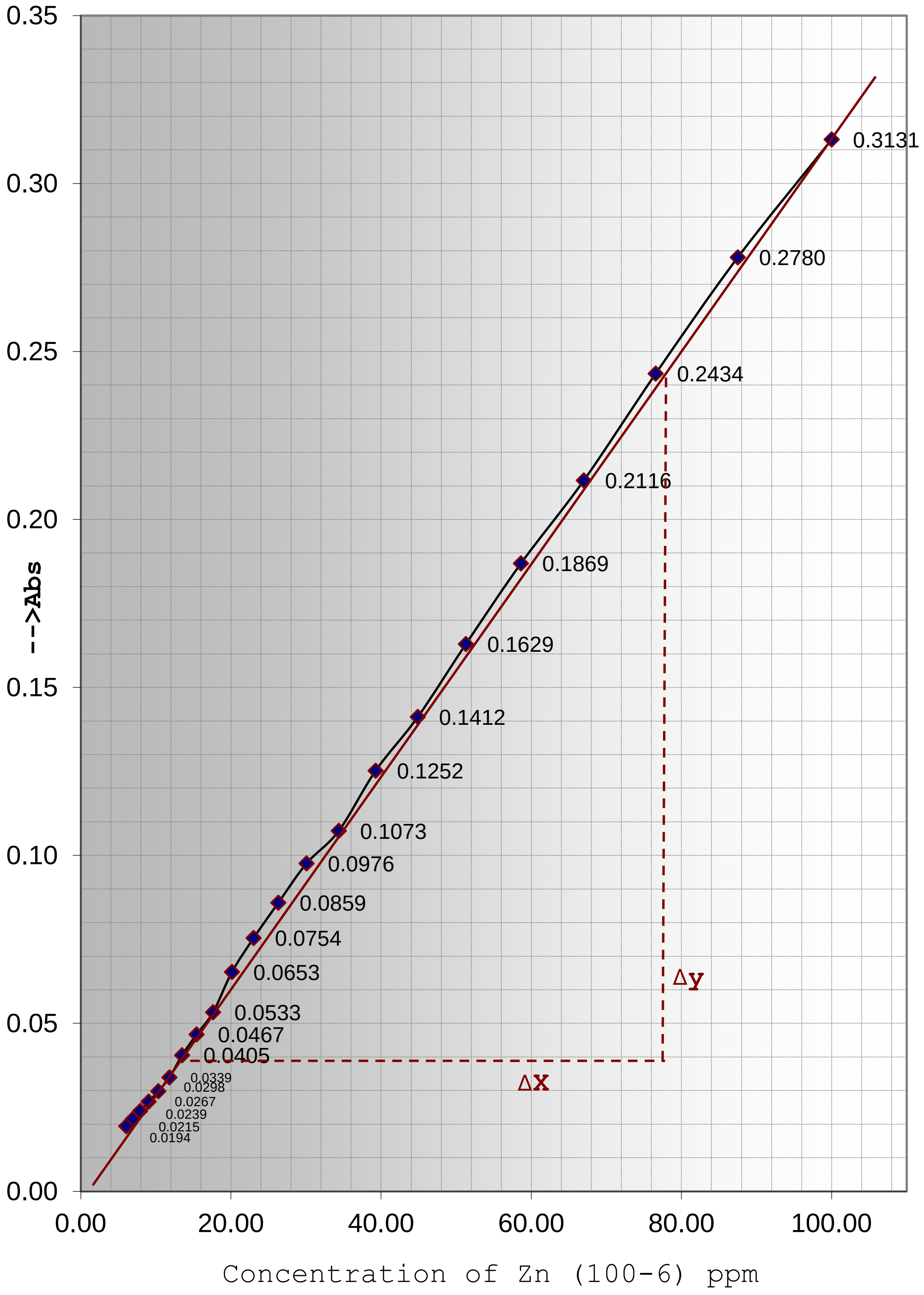


Fig-10:

Verification of Beer Lembart Laws for Zn-ARS Complex at 502.2nm , pH 4.00.
[Absorbance of Zn-ARS at 502.2nm Vs Conc of Zn (100-6 ppm)]

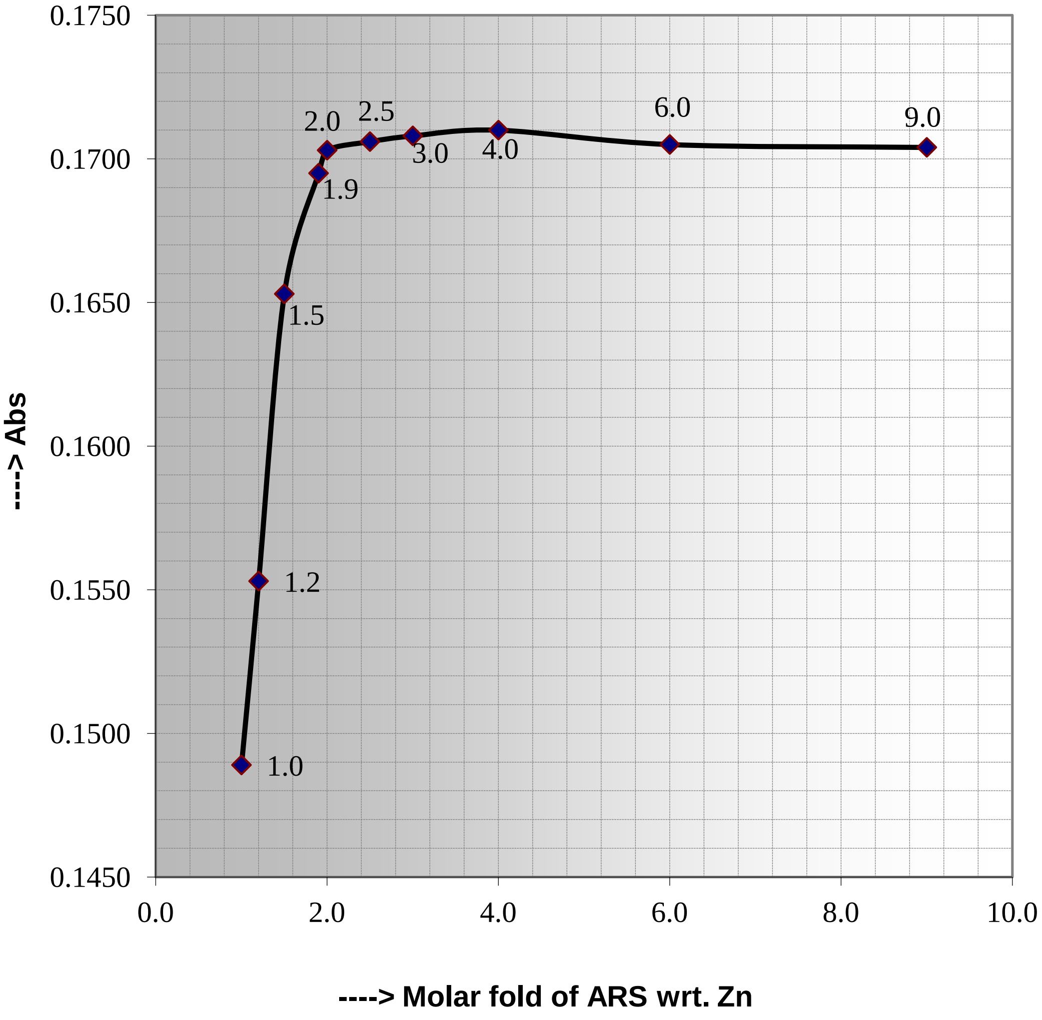
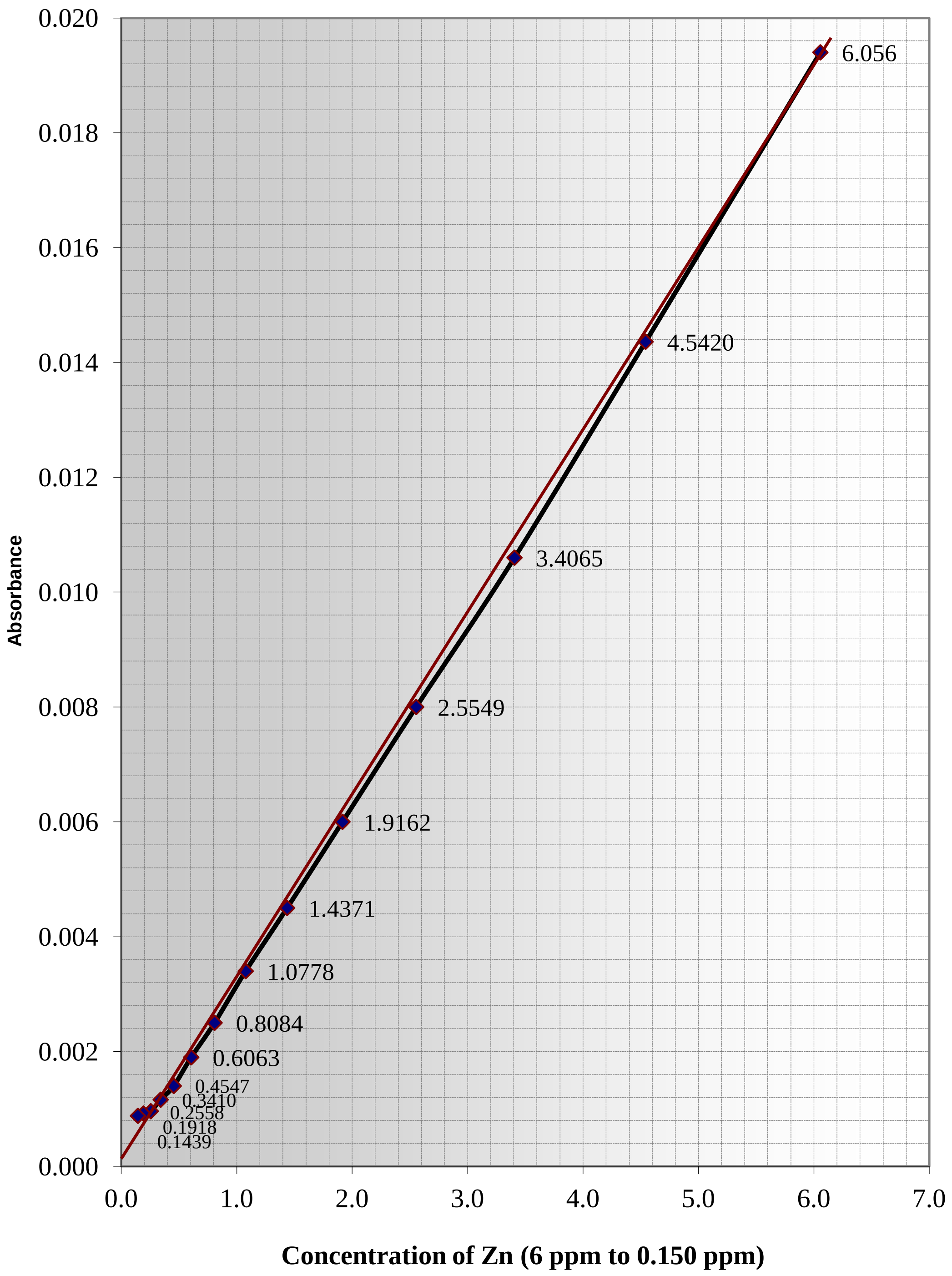


Fig-11:
Absorbances of Zn-ARS complexes with respect to ARS Vs Molar ratio of Zn:ARS.


Absorbance
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
Concentration of Zn (6 ppm to 0.150 ppm)
6.056
4.5420
3.4065
2.5549
1.9162
1.4371
1.0778
0.8084
0.6063
0.4547
0.3410
0.2558
0.1918
0.1439

The table below will give an idea about to choose an analytical method. [3]

Table-1. Comparison of Different Analytical Methods.

Methods	Approx Range, mol / L	Approx Precision, %	Selectivity	Speed	Cost	Principal Uses
Gravimetry	10^{-1} – 10^{-2}	0.1	Poor - Moderate	Slow	Low	Inorg.
Titrimetry	10^{-1} – 10^{-4}	0.1 – 1	Poor - Moderate	Moderate	Low	Inorg,. Org.
Potentiometry	10^{-1} – 10^{-6}	2	Good	Fast	Low	Inorg.
Electrogravimetry (coulometry)	10^{-1} – 10^{-4}	0.01 – 2	Moderate	Slow – Moderate	Moderate	Inorg., Org.
Voltammetry	10^{-3} – 10^{-10}	2 – 5	Good	Moderate	Moderate	Inorg., Org.
Spectrophotometry	**10^{-3} – 10^{-6}**	**2**	**Good - Moderate**	**Fast – Moderate**	**Low – Moderate**	**Inorg., Org.**
Fluorometry	10^{-6} – 10^{-9}	2 – 5	Moderate	Moderate	Moderate	Org.
Atomic spectroscopy	10^{-3} – 10^{-9}	2 – 10	Good	Fast	Moderate – High	Inorg., multielement
Chromatography	10^{-3} – 10^{-9}	2 – 5	Good	Fast – Moderate	Moderate – High	Org., multielement
Kinetic methods	10^{-2} – 10^{-10}	2 – 10	Good - Moderate	Fast – Moderate	Moderate	Inorg., Org., enzymes