DETERMINATION OF TRACE AMOUNT OF COPPER (Cu) USING
UV-VIS SPECTROPHOTOMETRIC METHOD.

A DISSERTRATION SUBMITED IN PARTIAL FULFILMENT OF THE
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4TH 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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DECLARATION

This thesis work has been done by candidate himself and does not contain any material extracted from elsewhere or from a work published by any body else. The work for this thesis has not been presented elsewhere by the author for any degree or diploma.

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Certification of Thesis

A thesis on
"DETERMINATION OF TRACE AMOUNT OF COPPER (Cu) USING
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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 certify that the student has demonstrated a satisfactory knowledge on the field covered by this thesis in an oral examination held on 4th September, 2010.

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DEDICATED
TO
MY BELOVED MOTHER

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....
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Abstract

Trace amount of Copper has determined by spectrophotometric technique using 1-(2-pyridylazo)-2-naphthal, as a new spectrophotometric reagent. 1-(2-pyridylazo)-2-naphthal reacts in highly acidic solution at pH 2.45 to 2.55 with Copper to give a pink chelate which has an absorption maximum at 550 nm. The reaction is instantaneous and absorbance remains stable for over 48 hrs. The average molar absorption co-efficient was found to be 2.05×10^4 L mol^{-1} cm^{-1}. Linear calibration graphs were obtained for 0.1-2.5 µg mL^{-1} of Cu. The stoichiometric composition of the chelate is 1: 2 [Cu: 1-(2-pyridylazo)-2-naphthal]. Large excess of over 50 cations, anions, and some common complexing agents (e.g, oxalate, phosphate, tartarate, thio-urea) do not interfere in the determination. The method was successfully used in the determination of Copper in Several Standard Reference Materials (alloys, steels and water) as well as in some environmental and industrial waste water. The method has high precision and accuracy.

Contents

PART -I

GENERAL INTRODUCTION

(A) SPECTROPHOTOMETRY
(B) TOXICOLOGY
(C) ENVIRONMENTAL POLLUTION
(D) AIM OF THE PROJECT

REFERENCS

SUMMARY

This thesis comprises of two parts, Part I and part II. To maintain the continuity and conformity with the title of the thesis, part I deals with the general introductions covering on-

    (a) Spectrophotometry.

    (b) Toxicology.

    (c) Environmental pollution and

    (d) Aim of the Project.

(a)   Spectrophotometry : It includes a general introduction of molecular absorption spectrophotometry, basic essentials of molecular spectrophotometry as trace and ultra- trace analytical technique and its advantage over other techniques, their merits and demerits.

(b)   Toxicology: It deals with the different terms frequently met with while studying toxicology and irritology, classification of toxicology, essential elements with potential for toxicity in detail discussion of essentiality and irriticity of copper (Cu) which embodied in the thesis. Some hygienic standards of exposure, treatment and therapy for copper intoxication.

(c)   Environmental Pollution: It deals with different terms frequently met with while studying pollution and its control; e. g. classification of environmental pollution, detail discussion about inorganic and organic pollutants, pollution growing hazards and some preventive measures for pollution control in Bangladesh. Summary of elemental content in the human body and the standard values for water quality standard for Bangladesh have been discussed.

(d)   Aim of the Project : The concluding section of part I is the "Aim of the Project" where essential characteristics of absorbing reagents, merits and demerits of both direct and indirect spectrophotometry have been discussed.

Part II describes extensively with experimental work. It is divided into two sub-chapters:

(A)   Reviews of existing spectrophotometric methods for determination of the metal with some important physical, properties, occurrences, uses, etc.

(B)   Sub-chapter (b) is entirely devoted to experimental works and finally details ultra-trace determination procedures are outlined. This part has its own Bibliography tagged at the end.

Sub-Chapter (A) opens up with the review of available Spectrophotometric methods for determination of copper.

Sub-Chapter (B) is devoted to experimental works. A very simple, ultra, sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amount of toxic element copper using 1-(2- pyridylazo)-2-naphathal (PAN) as a new spectrophotometric reagent has been developed. PAN in highly acidic solution (P^H = 1.5-2.5) with copper give a deep pink colour chelate which has an absorption maximum at 550 nm, reaction is instantaneous and absorbance remains stable for over 24hrs. The average molar absorption co-efficient was found to be 2.0636×10^4 L mol^{-1} cm^{-1}. Linear calibration graphs were obtained for 0.1ppm – 5.0ppm. The stoichiometric composition of the chelate is 1:2 (Cu:PAN). Large excess of over 50 cations, anions and same common complexing agents (e.g. oxalate, citrate, tartarate, phosphate, thio-urea) do not interfere in the determination. The method was successfully used in the determination of copper in several standard reference materials such as alloys, steels and water as well as in some environmental water and industrial waste water. The method has high precision and accuracy.

(A) SPECTROPHOTOMETRY

"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) [1]. Spectrophotometry, particularly in the visible region of the electromagnetic spectrum, is one of the most widely used methods of analysis. It is very widely used in clinical chemistry and environmental laboratories because many substances can be selectively converted to a colored derivative. [2] Spectrophotometry is extremely sensitive so much so that sometimes pictogram (10^{-12}g) per gram level can be determined. The instrumentation is readily available and generally fairly easy to operate.

Spectrophotometry as a Trace Analytical Technique:

Trace and Ultratrace : 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 [3] (i.e. 100 µg/gm). Thought the lower limit was not fixed at that time (neither as yet), perhaps it was originally indented somewhere in the order of microgram/gm levels then the lowest possible levels that could be instrumentally detected/ estimated.

The triumph of electronics, the development of sophisticated instrumentation and methodology in recent times have led analytical chemists 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-certainly a very wide range which needs at least two subdivisions. Therefore, some authors are in favour of calling those utramicrogram quantities as 'ultratrace' to differentiates it from 'trace'. While others are in opinion to preserve the classical 'trace' now be reserved for ultramicrogram quantities. Because there is and will be every demand and necessity 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 'ultrace' 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, picotrace, microtrace to pinpoint the smallness of the material used.

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

(1) Molecular absorption spectrophotomery
(2) Molecular fluorscence spectrophotometry

Molecular absorption spectrophotometry is more sensitive technique in inorganic trace analysis [4-15]. Molecular fluorescence spectrophotometry, on the other hand, experimentally similar and akin to molecular absorption spectrophotometry, fortunately is free from all limitation of absorptiometry [16-19].

Theory of UV-VIS Spectroscopy:
Radiation energy can be emitted by substances under high excitation conditions, such as high temperature or by an electric discharge. It can be absorbed, transmitted, reflected and refracted by various substances in different states (solid, liquid, solution and gas) if the incident radiation energy is of appropriate wavelength. These phenomena serve as the basis of the branch of measurement known as photometry. In spectrophotometry the measurements are concerned

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with radiation energy of a single wavelength (monochromatic radiation) or, for practical reasons, a narrow band of wavelengths. A few subdivisions of photometry used in analytical chemistry are as follows.

(i) **Emission spectrography:** In this type of photometry energy emitted by excited atoms, ions or molecules is recorded photographically and measured for wavelength and intensity.

(ii) **Absorption spectrophotometry:** In this method radiant energy of a definite wavelength (or narrow band of wavelength) is absorbed by a homogenious medium. Often, the spectral region concerned is designed, such as ultra-violet, visible or infrared. Actually, the measurement is usually made of the energy transmitted by the sample and various schemes can be used to translate this absorption measurement.

(iii) **Colorimetry :** The method, in its usual sense, applies to the visual observation of the fraction of "White light" from an incandescent source, Which has passed through a liquid or solution medium. By interposing a light filter, such as a plate of colored glass, between the source and the sample, the radiant energy incident upon the sample consists of a more of less wide band or wavelengths and this sharpens the sensitivity of the measurement of observation.

Although all branches of photometry have analytical applications, the present discussion will be limited mainly to the absorption and transmission of radiation energy in the visible region of the spectrum by substances in solution.

Interaction of Electromagnetic Radiation with Matter :

In spectrometric methods, the sample solution absorbs electromagnetic radiation from an appropriate source and the amount absorbed is related to the concentration of the analyte in the solution. A solution of copper is blue because it absorbs the complementary color (yellow) from white light and transmits the

remaining blue light. The more concentrated the copper solution, the more yellow light is absorbed and deeper the resulting blue color of the solution. In spectrometric method, the amount of this yellow light absorbed would be measured and related to the concentration. We can obtain a better understanding of absorption spectrometry from a consideration of the electromagnetic spectrum and how molecules absorb radiation.

The Electromagnetic Spectrum:

Electromagnetic radiation, for our purposes, can be considered a form of radiant energy that is propagated as a transverse wave. It vibrates perpendicular to the direction of propagation and produces a wave motion. The wave is described either in terms of its wavelength, the distance of one complete cycle or in terms of the frequency, the number of cycles passing a fixed point per unit time. The reciprocal of the wavelength is called the wave number, which is the number of waves in unit length or distance per cycle.

The relationship between the wavelength and frequency is

$$\lambda = {}^C\!/\upsilon \ \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \ (1)$$

Where λ is the wave length in centimeters (cm.) υ is the frequency in reciprocal seconds (s $^{-1}$) or hertz (Hz) and c is the velocity of light (3×10^{10} cm/s). The wave number is represented by υ in cm^{-1}.

$$\ddot{\upsilon} = \frac{1}{\lambda} = \frac{\nu}{c} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

The wave length unit preferred for the ultra-violet and visible regions of the spectrum is nanometer, while the micrometer (μm) is preferred for the infrared region. In the last case, wave numbers are often used in place of wavelength and the unit is cm^{-1}.
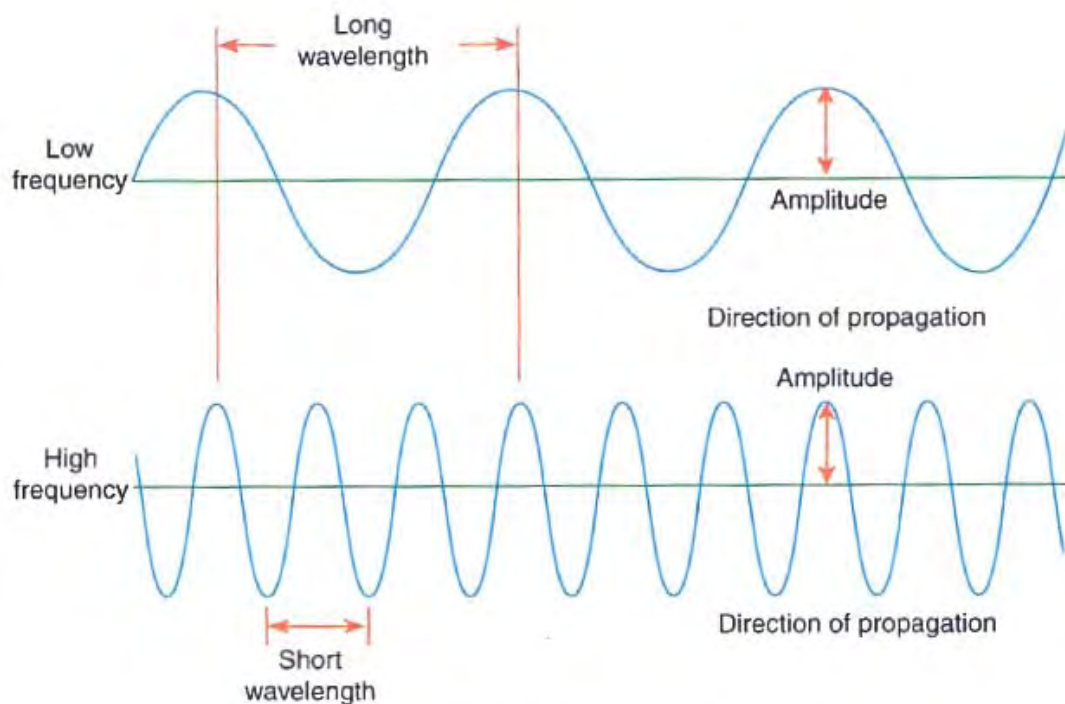
Fig.-1 : Wave motion of electromagnetic radiation.

Electromagnetic radiation possesses certain amount of energy. The energy of a unit of radiation, called photon, is related to the frequency or wavelength by:-

$$E = h\upsilon = {}^{hc}/\lambda \quad \text{.. (3)}$$

Where E is the energy of the photon in erg and h is plank's constant, 6.62×10^{-27} erg s. It is apparent, the shorter the wavelength or the greater the frequency, the greater is the energy.

As indicated above, the electromagnetic spectrum is arbitrarily broken down into different regions according to wavelength. The various regions of the spectrum are shown in figure-2.

Fig.-2 : Parts of the electromagnetic spectrum which employed for spectrophotometry.

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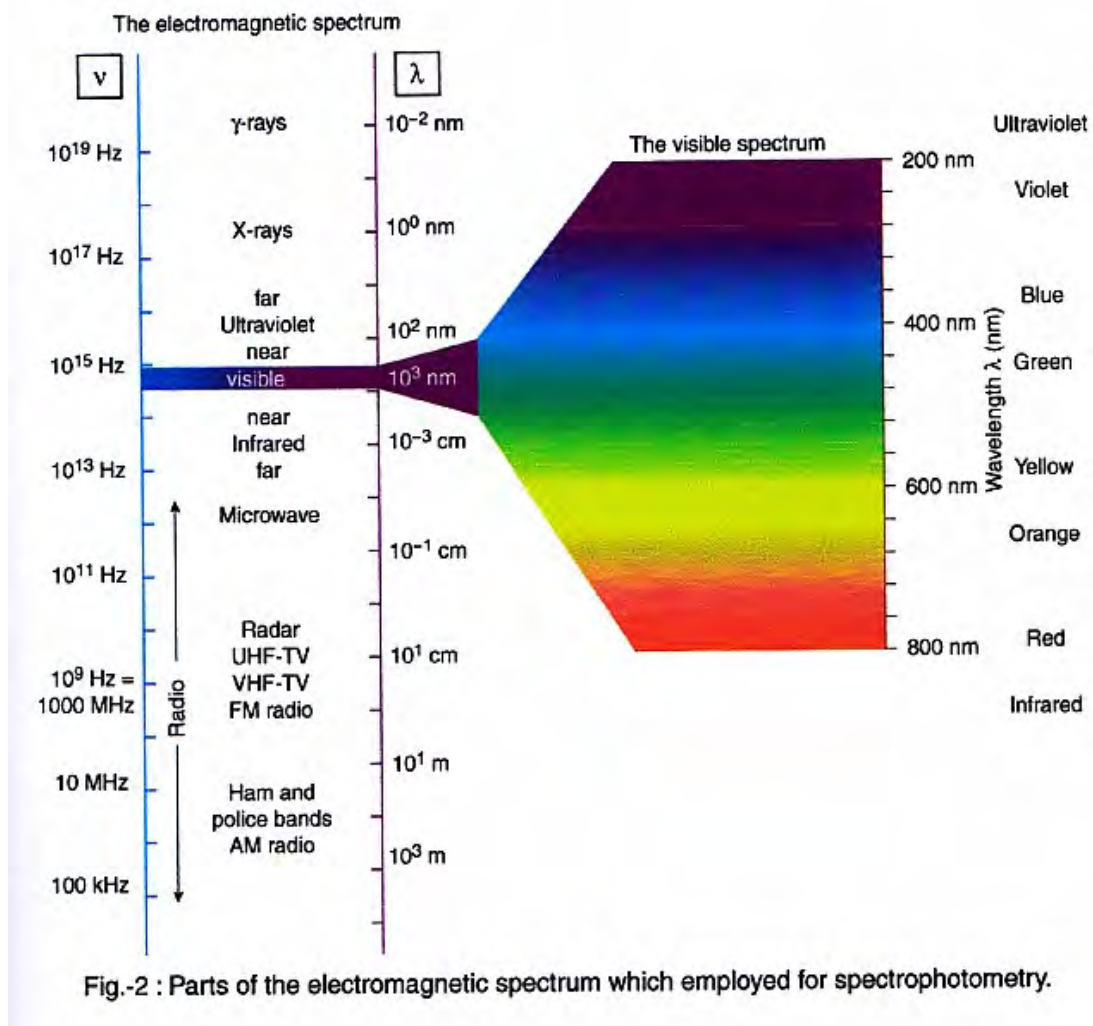
The ultra-violet region extends from about 10 to 380 nm, but the most analytically useful region is from 200 to 380 nm, called the near ultra-violet region. Below 200 nm, the air absorbs appreciable and so, the instruments are operated under a vacuum; hence, this wavelength region is called the vacuum ultra-violet region. The visible region is actually a very small part of the electromagnetic spectrum and it is the region of wavelengths that can be seen by the human eye, that is, the light appears as a color. The visible region extends from near ultra-violet region (380nm) to about 780 nm. The infrared region extends from about 0.78 µm (780 nm) to 300 µm, but the range from 2.5 to 15 µm is the most frequently used for analysis. The 0.8 to 2.5 µm range is known as the near-infrared region, the 2.5 to 15 µm range as the NaCl- infrared region and longer wavelengths as far infrared region.

The Absorption of Radiation:

The qualitative picture of the absorption of radiation can be obtained by considering the absorption of light in the visible region. We 'see' objects because they transmit or reflect only a portion of the light in this region. When polychromatic light (white light), which contains the whole spectrum of wavelengths in the visible region, is passed through an object the object will absorb certain of the wavelengths leaving the unabsorbed wavelength to be transmitted. These residual transmitted wavelengths will be seen as a color. This color is complementary to the absorbed colors. In a similar manner, opaque objects will absorb certain wavelength, leaving a residual color to be reflected and 'seen'.

Quantitative Application of UV-Visible Absorption :-

(a) BEER LAW:

The amount of monochromatic radiation absorbed by a sample is described by the Beer-Bouguer-Lambert law, commonly called Beer's law. Consider the absorption of monochromatic intensity of the incident radiation P_o which passes through a solution of an absorbing apecies 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,

$$p = p_o 10^{-kb}$$

$$\frac{p}{P_o} = 10^{-kb} = T \quad \text{...} \quad (4)$$

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

$$\log T = \log \frac{p}{p_o} = -kb \text{..}(5)$$

In 1852, Beer and Bernard, each stated that a similar law holds for the dependence of T on the concentration, C

$$T = \frac{p}{p_o} = 10^{-k'c} \text{...}(6)$$

or $$\quad \log T = \log \frac{p}{p_o} = -k'c \text{...}(7)$$

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

$$T = \frac{p}{p_o} = 10^{-abc} \text{...}(8)$$

Where 'a' is a combined constant of K and K'.

The logarithmic form of (8) is

$$\log T = \log \frac{p}{p_o} = -abc \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(9)$$

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

$$A = -\log T = \log \frac{1}{T} = \log \frac{P_o}{P} = abc \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots(10)$$

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 (10) 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/litre; b in cm, the constant 'a' is replaced by ϵ and the Beer's law is written as

$$A = \epsilon bc \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (11)$$

This new quantity, ϵ is known as Molar Absorptivity. Since A is unitiless, ϵ has the unit of $L\ mol^{-1}\ cm^{-1}$. 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.

APPLICABILITY OF BEER'S LAW:

Beer's law is the basis of all quantitative applications of uv-visible 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 is chemically unreactive with the colored components do not usually affect the light absorption; large amounts of electrolytes may results in a shift of the

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maximum absorption, and may also change the value of the absorptive. Discrepancies are usually found when the colored solute ionizes, dissociates or associates in solution, since the nature of the species in solution will vary with 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.

The slope of the spectrum increases as the concentration is increased, with the result that the fractions of each wavelength absorbed may change, particularly if the instrument setting should drift over the period of the measurement. A negative deviation in the absorbance-versus-concentration plot will, in this case, be observed. The greater the slope of the spectrum, the greater is the deviation.

Other instrumental factors that may contribute to deviation from Beer's law include stray radiation entering the monocromator and being detected, internal reflections of the radiation within the monochromator and mismatched cells used for different analyte solutions in double-beam instruments. It has been calculated that the minimum error should occur at T = 0.136 of A= 0.87. All Instruments have a working range of about 0.1 to 1.5A and some sophisticated instruments have a range of 0.001 to 2.5A.

Any chemical reaction that can alter the concentration of an absorbing species can result in a deviation from Beer's law. It the concentration is decreased because of the chemical reaction and the product does not absorb radiation at the wavelength at which the measurement is made, a negative deviation occurs. If a product of the chemical reaction absorb more strongly than the assayed substance, a positive deviation occurs. Among the types of chemical reactions

which can lead to a deviation from Beer's law are association-dissociation reactions, acid-base reactions, polymerization reactions, complexation reactions and reactions with the solvent.

Selection Rules:

Requirements for the absorption of light by matter are summarized in the selection rules. Transitions which are possible according to these rules are referred to as 'allowed' transition and those not possible as 'forbidden' transitions. The following selection rules are pertinent to electronic absorption spectroscopy.

(i) Transitions between states of different multiplicity are multiplicity forbidden, that is, electronic transitions in which the spin of an electron changes are forbidden.

(ii) In a molecule which has a center of symmetry, transitions between two grade or two ungraded state (that is, g< -> g or u <-> u) are Laporte forbidden. The allowed transitions are g < - > u and u < - > g. As a result of this rule, d < - > d transitions in octahedral complexes are Laporte forbidden.

(iv)   Simultaneous excitation of more than one electron is forbidden.

However, the term "forbidden" refers to rules set up a simple model and while the model is a good one "forbidden" transitions may occur by mechanisms not included in the simple model. Since intensity of absorption or emission accompanying a transition is related to probability of the transition, the more probable transition give rise to more intense absorption, forbidden transition (low probability) give absorptions of very low intensity.

Absorption Instrument:

The quality and cost of uv-visible 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.

Spectroscopic instruments contain five components, such as;

(i)   a stable source of radiant energy;

(ii)   a wavelength selector that permits isolation of a restricted wavelength regions;

(iii)   a transparent container for holding the sample;

(iv)   a radiant detector or transducer that converts radiant energy to a useable signal (usually electrical); and
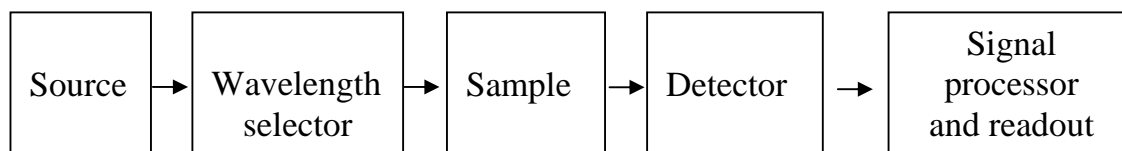
(v)   a signal processor and readout.

| Source | → | Wavelength selector | → | Sample | → | Detector | → | Signal processor and readout |

Fig.- 3: Shows a block diagram of uv-vis absorption instrument.

A brief description of the components follows:

(i)   Source: In instruments for the visible range (about 400 to 750 nm) the source is usually a tungsten-filament lamp. For ultra-violet region the hydrogen discharge tube is commonly used. Close voltage control is required for a stable source.

(ii)   Wavelength selector: In the simple instruments, filters may be used to eliminate or diminish the radiant energy of certain wavelength and pass other selected regions.

A suitable filter for a given substance should transmit well in the spectral region where the desired constituent absorbs strongly. In spectrophotometers, the radiant energy from the source is dispersed into a continuous spectrum by passage through a prism or by diffraction from a grating consisting of fine parallel rulings on a solid surface. Prisms or

diffraction gratings; with their accessory lenses; mirrors and slits; are called monochromators. From the continuum of wavelengths emitted by the source, a single wavelength or usually a very narrow band of wavelengths can be selected for use in the measurements. Different spectral regions require optional components of different materials. Glass is not transparent far outside of the visible region. For use in the ultra-violet region, silicon prisms, lenses and absorption cells are used.

(**iii**) **Sample container:** The cell must be made of material that possesses radiation in the spectral region of interest. This, quartz or fused silica is also transparent in the visible region and to about 3000 nm in the infrared. Silicate glasses can be employed in the region between 350 to 2000 nm.

The quality of spectroscopic data is critically dependent on the way the cell is handled. Fingerprints, grease or other deposits on the walls markedly alter the transmission characteristics of a cell. Thus, through cleaning before and after use is imperative; the surface of the windows must not be touched during handling.

(iv) **Detectors:** The photodetector gives response which varies with the radiation energy reaching it. The less expensive photometers generally use photocells of the barrier-layertype. The more elaborate instruments use photoemmissive type receivers, photomultipliers, thermopiles and bolometers. In each case, the radiant energy is converted to another form of energy (current, potential, heat etc.) which is ultimately measured.

(v) **Signal processors and Readouts:** The signal processor is ordinarily an electronic device that amplifies the electrical signal from the detectors and filters the unwanted ones. The signal processors may permit such mathematical operations on the signal as integration or conversion to a logarithm.
Several types of readout devices are found in modern instruments. Some of these include digital meters, the scales of potentiometers, recorders, computer, etc.

(B) TOXICOLOGY

Before entering into a brief discussion on Toxicology in the author's opinion it will be wish to give some definitions of some useful terms commonly met with the discussion.

DEFINITIONS :

Toxic, Toxicity and Toxicology: The word 'toxic' is derived from the Greek word toxon ('bow') and toxicon or pharmikon ('arrow poison'). Every chemical agent is inherently harmful, depending on form, dose administered, mode of entry and character of the organism. Toxicity is the inherent capacity of any chemical to affect adversely the activity of living organism. Toxicity is a relative term used to compare one chemical or metallic compound with another w.r.t. toxicity. Adverse effects due to toxicity include behavioral changes that affect collective populations. Early mortality, retardation of growth, impaired reproduction, neonatal mortality, neoplasms and varied diseases symptoms are common criteria for metal toxicity in mammals. Thus, 'toxicology' is defined as basic science of poisons (where poison is defined as any agent which is capable of producing injury of death when ingested or absorbed). According to Paracelsus over 400 years ago "all substance are poisons"; there is none, which is not a poison. The does differentiates a poison and a remedy.

Poisons may be administered orally, by injection, by inhalation, by application to a wound of by introduction into the rectum, vagina, and urethra.

CLASSIFICATION OF POISONS :

It is necessary to evolve a basis of classification of poisons, Poisons may be of synthetic, mineral, animal or vegetable origin, but such a classification, based on origin of poisons, is not helpful for their proper study. The best way to

classify them would be on the basis of the chief symptoms they produce on the human body and in this way poisons can be studied in a scientific manner.

I. Corrosives :

(i)   <u>Strong acids</u>, Mineral, e.g. Sulfuric acid, Nitric acid, Hydrochloric acid;

                Organic, e.g. Oxalic acid, Carbolic acid.

(ii)   <u>Strong alkalis,</u> e.g. Caustic Soda or Sodium hydroxide, Caustic Potash or

                Potassium hydroxide, Ammonium Carbonate, Sodium

                Carbonate etc.

II   Irritants :

(i)   <u>Inorganic,</u> Non-metallic e.g. Phosphorus, Chlorine, Bromine, etc.

                Metallic e.g. Copper, Arsenic, Antimony, Lead, Zinc, Mercury,

                Aluminum, Vanadium etc.

(ii)   <u>Organic,</u>   Vegetable e.g. Castor oil seeds, Crouton seeds, Aloes, Abrus

                Precatorius, etc.

                Animal, e.g. Canthatides, Snake and insect bites etc.

                Mechanical, e.f. Powdered glass, Diamond dust, Hair etc.

III.   Neurotics :

(1)   <u>Cerebral</u> (Affecting the barin),

       (i) Somniferous, e.g. opium and its alkaloids,

       (ii) Inebriant, e.g. Alcohol, Ether, Chloroform,

       (iii) Deliriant, e.g. Dhatura, Belladonna, Hyoscyamus, Cannabis indica.

(2)   <u>Spinal</u> (Affecting the spine), e.g. Nux Vomica, Gelsemium.

(3)   <u>Cardiac</u> (Affecting the heart), e.g. Aconite, Digitalis, Oleander etc.

(4)   <u>Asphyxiants</u> (Affecting the lungs), e.g. Carbon Monoxide, Carbon dioxide etc.

(5)   <u>Peripheral</u> (Affecting the peripheral nerves), e.g. Conium Curara etc.

IV   Miscellaneous Group : Food poisoning, botulism etc.

ACTION OF POISONS:

It is now important to understand the mode of action of poisons. Poisons may have local action, remote action or both.

(a)   Local Action: This is due to direct action of the poison on the part or direct interaction with the tissues leading to:

(i)   Corrosion as by strong mineral acids. Although corrosive substances cause lesions, the immediate cause of death may be due to indirectly related phenomenon, e.g. shock which of course should be considered as remote action.

(ii)   Irritation and inflammation, as by cantharides and tartaremetic or by arsenic which causes gastritis mainly due to local action.

(iii) Nervous Effects, i.e. effects on motor and sensory nerves, e.g. tingling following by numbness produced by aconite, local anesthesia due to cocaine, carbolic acid.

(b)   Remote Action: The remote action of a poison should be described as
       (i) Non-specific and (ii) Specific.

(i)   Non-specific remote action has already been referred to, e.g. shock produced reflexly by intense burning pain caused by corrosives.

(ii)   Specific remote action is due to absorption of the poison into the system through the blood and subsequent specific action on certain organs, e.g. strychnine or nux vomica acting on the spinal cord produces tetanic convulsions, cantharides acting on the kidneys produces nephritis, phosphorus acting as a hepatotoxic poison.

(c)   Both Local and Remote Action: Certain poisons have both local action and remote action e.g. Oxalic acid acts locally as a corrosive and remotely by precipitating ionised calcium after absorption into the system, Carbolic acid acts locally as a corrosive and remotely as a narcotic poison.

(i) **Environmental Toxicology:** Environmental is the study of the unwanted effects of chemical environmental agents on living things.

(ii) **Industrial Toxicology or Occupational Toxicology:** Industrial toxicology deals with industrial chemicals in occupational health hazards and industrial hygiene. Occupational exposure to metals is restricted to 'safe' levels defined as the Threshold Limit Value (TLV) for an eight hours day, five-day work week. These levels are intended to provide a margin of safety between maximum exposure and minimum levels that will produce illness. For all these pollutants or toxicants, it is necessary to establish allowable concentration limits and reliable methods for analysis. These levels can be expressed either in terms of 'Maximum Allowable Concentrations' MAC or MAK (used be VDI committee of German Research Association) or 'Threshold Limit Value' TLV (used by American Conference of Governmental industrial Hygienist), the values being published on the basis of known data on the toxic effect of a contaminant. Also in terms of permissible biologic levels for the chemical or their metabolites (biologic TLV). MAC or TLV of a substance (or pollutant) is the value at which a worker can be exposed for 8 hours a day without showing any adverse effect in health and is expressed in units mg/m^3 or $\mu g/m^3$ and mg/litre (for air and water respectively). The values are applicable only for pure substances and not for mixture of toxicants. Permissible levels very widely and the differences reflect, in a sense, the toxicology potency of the metal. As a general rule, the materials that are most abundant in the environment have lesser potential for toxicity as evidenced by the prevailing standard for permissible occupation exposure.

(iii) Synergism and Antagonism: Interactions between metal compounds potentiate or decrease the toxicity of metals. Thus in the presence of mixtures of toxicants, the toxic effects are greatly influenced, enhanced or

attenuated. When the toxic effects by the combination of contaminants is greater than the effect of individual contaminant it is called synergism or potentiation. Synergism possesses a special problem for aquatic and terrestrial species because it is possible that a certain combination by relatively harmless substances may result in an unpredictable high level of toxicity that would seriously threaten the existence of one or more species.

When toxic effect of a substance is reduced on the addition of a substance, the phenomenon is referred to' antagonism' antagonistic substance may or may not be toxic when present by itself . The toxicity of cyanide of mercury in presence of nickel and selenium affect antagonistically. There occurs the reduction in toxicity of vanadium by chromium, cadmium by zinc, selenium by thallium and mercury, arsenic, silver, cadmium and copper by selenium (Frost*, 1972). Since each pair involves one essential metal a stoicheiometric antagonism may be envisioned.

(iv) **Carcinogens and Carcinogenicity:** Carcinogens of oncogens are biological, chemical and physical agents capable of producing uncontrolled cell proliferation in organs and tissues. The capacity to induce cancer by carcinogens is the carcinogenicity. Carcinogenicity varies with different routes of administration, exposure time, dose and physical state of the material as well as with host specific factors. Tumours are abnormal masses of tissue that grow and persist independent of surrounding structures. They have not physiologic function. Tumours that spread to other tissues (metastasize) or are transplantable to other tissues, are called malignant tumours or cancers. Tumours that are usually encapsulated and do not metastasize are benign. Since benign tumours may develop into malignant tumours, the U.S. Environmental protection Agency (Gibney, ** 1976) classified that oncogens are those substances capable of inducing either type of tumour. Carcinogens cause permanent damage and a biologic modification of the sell, making them more susceptible to further carcinogenic effect. Metals such as beryllium,

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* D.V. Frost, CRC Critical Review in Toxicology, **1**(4) 467 (1972).

** L. Gibney, Chemical and Engineering News, **53** 15(1976).

Chromium, cobalt, nickel, cadmium and zinc are proven carcinogens. Special organic complexes of titanium iron, nickel, rhodium and palladium, are established carcinogens, e.g. titanocene and iron dextran. Scandium, arsenic, manganese, selenium yttrium, aluminum, zirconium, silver and lead are reported to possess carcinogenicity, while copper, zinc and tin may possess recondite carcinogenicity. Some metals are primary carcinogens, others are co-carcinogens. Metal carcinogens may enhance or potentiate the carcinogenicity of organic compounds by inhibiting the detoxifying mechanism, e.g. copper potentiates the tumourigenesis of N-hydroxy-2-acetyl-amino-fluorene (Stanton*, 1967).

(ii)   **Genotoxicity or mutagenicity:** Chemical carcionogens are a type of toxic agent that exhibits a specific, defining adverse effect-the production of cancer in animals or humans. Chemical carcinogenesis is classified into two general categories: DNA- reactive (genotoxic) and epigenetic. The DNA reactive (genotoxic) category comprises carcinogens that chemically interact with and alter DNA. These carcinogens are of course mutagenic. Some metals have displyed genotoxic effects suggestive of DNA interaction, these have been placed in this occurring and industrially produced chemicals, including metallic compound may constitute genetic hazard [20]. Among the inorganic chemicals, chromium, titanium, nickel, selenium, cobalt, manganese, lead, beryllium and certain of their derivatives have been found carcinogenic under specific experimental conditions [21].

(vi) **Essential elements or micronutrients and metabolism:** Essential elements or micronutrients serve their biological function satisfactorily and can be regarded like vitamins, as normal dietary constituents without which healthy life and growth are not possible e.g. iron (hemoglobin), iodine (thyroid function) selenium (glutathione-peroxidase). Chromium (glucose tolerance factor), manganese (pyruvate carboxylase). It is only in the last two decades cadmium, chromium, selenium, manganese, silicon and tin each has been proposed an essential nutrient for humans,

* M.F. Stanton, Cancern Res., 28 1000 (1967).

though classically have long histories as toxic elements. Even the well-known toxic elements arsenic, lead, copper, zinc and cadmium are required in trace quantities for the growth of animals. It will be safe to state that nontoxic means a low toxicity. Many metals listed as environmental hazards are essential dietary trace elements required for normal growth and development of animals and human beings. These elements are Ag, Al, As, Ba, Be, Bi, Cd, Co, Cu, Ce, Cr, Fe, In, Mn, Mo, Pb, Se, Sn, Te, Tl, Ti, U, V, W, and Zn.

Nutrients are elements essential to the growth and reproduction of plants and animals and aquatic species depend on the surrounding water to provide their nutrients. Although a wide variety of minerals and trace elements can be classified as nutrients, those required in most abundance by aquatic species are carbon, nitrogen phosphorus. Carbon is readily available from many sources. Carbon dioxide from the atmosphere, alkalinity and decay products of organic matter all supply carbon to the aquatic system. In most cases, nitrogen and phosphorus are the nutrients that are the limiting factors in aquatic plant growth.

Schwartz* used the term 'concentration window' to draw the arbitrary lines of demarcation: (a) essential at trace level for substance of life processes, (b) 'deficient' at lower level than (a) causing metabolic disorder (c) 'toxic' at higher level than (a) causing adverse effects. Many carcinogenic metals are essential nutrients. They induce acute toxicity at high concentration. Metabolism is defined as the chemical changes in molecule within living organisms. In other words, the sum of anabolism and catabolism processes can be shortened as metabolism.

* k. Schwartz, 'Clinical Chemistry and Toxicology of Metals, Elsevier, 1977, P.3.

(a) **Classification of Toxicology:**

Toxicology embraces different scientific disciplines such as chemistry, biology, physiology, nutrition, pharmacology, pathology, immunology and medicine. Toxicology comprises many areas of service and research. Environmental toxicology is that branch of toxicology that deals with the incidental exposure of man and other animals to harmful contaminants of the environment. Forensic toxicology deals with the medical and legal aspects of the adverse effects of chemicals on humans. Clinical toxicology deals with the study of the diagnosis and treatment of diseases resulting from adverse effect of chemicals. Experimental toxicology studies the effect of toxic levels of chemicals and drugs. Industrial toxicology deals with materials involved in occupational health hazards and industrial hygiene. Economic toxicology is the study of such agents as insecticides, herbicides defoliants and their effects on pests, domestic animals and humans. Behavioral toxicology is a new concept (N.K. Mello, Fed. Proc., 34 (1975) 1832; J.M. Spyker, ibid. 34 (1975) 1945].

(b) **Heavy Metal Toxicity:**

The heavy metals are among the most dangerous and yet least understood of contaminants. Because they exist in nature as part of earth crust, so they occur in all soils, rivers and oceans. Perhaps mercury shares the unique undesirable characteristics of being not only deadly but entirely without any useful compensating satisfactory function in biological systems when they turn up as contaminants. Some heavy metals on the other hand, serve their biological faction satisfactorily only when they are present within fairly specified limits and in particular form, so that either the deficiency or the over abundance of an essential heavy metal can lead to disorder or to toxic effects.

Further similar metals tend to interfere with each other biologically; some lose their biological activity in the presence of abnormal level of other

elements. With the exception of need of iron (hemoglobin) selenium (glutathione-peroxidase), chromium (glucose tolerance factor), manganese (pyruvate carboxylase) and iodine (thyroid function), a growing understanding of the biological effects and role of heavy metals has led to the growth of a parallel concern for their effects, even in small amounts, on the patterns of health. The number of cancer causing agents is large but in terms of controllable environmental contaminants the heavy metals comprise a significant proportion of them. The capacity of heavy metals to form stable and irreversible co-ordinated complexes with O, S, and N donor atoms of the ligands of biologically active macromolecules present in the living systems may be responsible for their toxicity. Increased electropositivity and inherent toxicity are associated with increasing atomic number or weight. The heavier metals in each group have the capacity to form irreversible and stable complexes with biological macromolecules, hence these heavy metals are toxic. The inherent toxicity of heavy metals is enhanced if the solubility of salts of these metals is increased. The toxicity of some heavy metals can be associated with their state of oxidation.

The higher oxides of manganese, chromium, vanadium, molybdenum, lead and barium and polyvalent oxyacid salts such as MnO_4^{2-}, MnO_4^{-}, CrO_4^{2-} and VO_4^{2-} are more toxic than the corresponding lower oxides or lower oxysalts. The higher oxides may undergo spontaneous reduction to lower forms, disrupting the delicate mechanism of cellular electron transport systems. There are also a lot of examples where lower valence states of certain metals (As, Sb, etc.) more toxic than these of higher valence ones.

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 biological activity, trace levels required for optimum growth and development, homeostatic levels (storage levels) and toxic levels. For these elements, environmental accumulations are generally less important routes excess exposure than accidents or occupation.

These essential metals are being investigated for their toxicity in excessive amount. The complete does-response curve for essential metals shows a general complex activity spectrum comparable to the of Fig. 4.
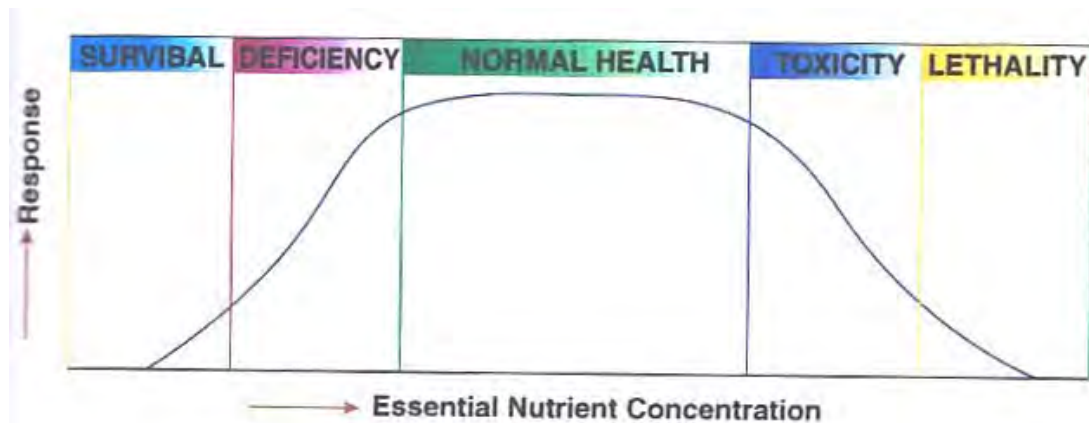

Fig.-4 : Activity spectrum of an essential metal.

[Source: "Metal Toxicity in Mammals, 1"; T. D. Luckey and B. Venugopal (Eds.) Plenum press, New York, 1977, p. 7]

Discussion made so far is of general in nature, the following discussion will be restricted solely to the metal (Copper) as embodied in thesis.

Copper:

Name, symbol, number	copper, Cu, 29
Element category	transition metal
Standard atomic weight	$63.546(3)\,\text{g·mol}^{-1}$
Electron configuration	[Ar] $3d^{10}\,4s^1$
Oxidation states	+1, **+2**, +3, +4 (mildly basic oxide)
Electronegativity	1.90 (Pauling scale)

Copper is a chemical element with the symbol **Cu** (Latin: *cuprum*) and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Pure copper is rather soft and malleable, and a freshly exposed surface has a pinkish or peachy color. It is used as a thermal conductor, an electrical conductor, a building material, and a constituent of various metal alloys.

Copper occupies the same family of the periodic table as silver and gold, since they each have one s-orbital electron on top of a filled electron shell which forms metallic bonds. This similarity in electron structure makes them similar in many characteristics. All have very high thermal and electrical conductivity, and all are malleable metals. Among pure metals at room temperature, copper has the second highest electrical and thermal conductivity, after silver.[25]

Copper metal and alloys have been used for thousands of years. In the Roman era, copper was principally mined on Cyprus, hence the origin of the name of the metal as Cyprium, "metal of Cyprus", later shortened to Cuprum. There may be insufficient reserves to sustain current high rates of copper consumption.[22]

Copper compounds are known in several oxidation states, usually +2, where they often impart blue or green colors to natural minerals such as turquoise and have been used historically widely as pigments.

Copper(II) ions (Cu^{2+}) are soluble in water, where they function at low concentration as bacteriostatic substances, fungicides, and wood preservatives. For this reason, copper metal can be used as an anti-germ surface that can add to the anti-bacterial and antimicrobial features of buildings such as hospitals [23]. In sufficient amounts, copper salts can be poisonous to higher organisms as well. However, despite univer sal toxicity at high concentrations, the Cu^{2+} ion at lower concentrations is an essential trace nutrient to all higher plant and animal life. In animals, including humans, it is found widely in tissues, with concentration in liver, muscle, and bone. It functions as a co-factor in various enzymes and in copper-based pigmets.

History of Copper

Copper Age:

Copper, as native copper, is one of the few metals to occur naturally as an un-compounded mineral. Copper was known to some of the oldest civilizations on record, and has a history of use that is at least 10,000 years old. Some estimates of copper's discovery place this event around 9000 BC in the Middle East [24]. A copper pendant was found in what is now northern Iraq that dates to 8700 BC [25]. It is probable that gold and meteoritic iron were the only metals used by humans before copper [26]. By 5000 BC, there are signs of copper smelting: the refining of copper from simple copper compounds such as malachite or azurite. Among archaeological sites in Anatolia, Çatal Höyük (~6000 BC) features native copper artifacts and smelted lead beads, but no smelted copper. Can Hasan (~5000 BC) had access to smelted copper but the oldest smelted copper artifact found (a copper chisel from the chalcolithic site of Prokuplje in Serbia) has pre-dated Can Hasan by 500 years. The smelting facilities in the Balkans appear to be more advanced than the Anatolian forges found at a later date, so it is quite probable that copper smelting originated in the Balkans. Investment casting was realized in 4500–4000 BC in Southeast Asia [24].

Copper smelting appears to have been developed independently in several parts of the world. In addition to its development in the Balkans by 5500 BC, it was developed in China before 2800 BC, in the Andes around 2000 BC, in Central America around 600 AD, and in West Africa around 900 AD [27]. Copper is found extensively in the Indus

Valley Civilization by the 3rd millennium BC. In Europe, Ötzi the Iceman, a well-preserved male dated to 3300–3200 BC, was found with an axe with a copper head 99.7% pure. High levels of arsenic in his hair suggest he was involved in copper smelting. Over the course of centuries, experience with copper has assisted the development of other metals; for example, knowledge of copper smelting led to the discovery of iron smelting.

In the Americas production in the Old Copper Complex, located in present day Michigan and Wisconsin, was dated back to between 6000 to 3000 BC [28] [29].

Some reports claim that ancient American civilizations, such as the Mound Builders knew of a method of tempering copper which has not yet been rediscovered. According to historian Gerard Fowke, there is no evidence of any such "lost art", and the best technique demonstrated for strengthening copper in this era was hammering [30].

CHARACTERISTICS

Color: Copper has a reddish, orangish, or brownish color because a thin layer of tarnish (including oxides) gradually forms on its surface when gases (especially oxygen) in the air react with it. But pure copper, when fresh, is actually a pinkish or peachy metal. Copper, osmium (blueish) and gold (yellow) are the only three elemental metals with a natural color other than gray or silver [31]. The usual gray color of metals depends on their "electron sea" that is capable of absorbing and re-emitting photons over a wide range of frequencies. Copper has its characteristic color because of its unique band structure. By Madelung's rule the 4s subshell should be filled before electrons are placed in the 3d subshell but copper is an exception to the rule with only one electron in the 4s subshell instead of two. The energy of a photon of blue or violet light is sufficient for a d band electron to absorb it and transition to the half-full s band. Thus the light reflected by copper is missing some blue/violet components and appears red. This phenomenon is shared with gold which has a corresponding 5s/4d structure [32]. In its liquefied state, a pure copper surface without ambient light appears somewhat greenish, a characteristic shared with gold. When

liquid copper is in bright ambient light, it retains some of its pinkish luster. When copper is burnt in oxygen it gives off a black oxide.

Occurrence

Copper can be found as native copper in mineral form (for example, in Michigan's Keweenaw Peninsula). It is a polycrystal, with the largest single crystals measuring $4.4 \times 3.2 \times 3.2$ cm [34]. Minerals such as the sulfides: chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), covellite (CuS), chalcocite (Cu_2S) are sources of copper, as are the carbonates: azurite ($Cu_3(CO_3)_2(OH)_2$) and malachite ($Cu_2CO_3(OH)_2$) and the oxide: cuprite (Cu_2O) [33].

Mechanical properties

Copper is easily worked, being both ductile and malleable. The ease with which it can be drawn into wire makes it useful for electrical work in addition to its excellent electrical properties. Copper can be machined, although it is usually necessary to use an alloy for intricate parts, such as threaded components, to get really good machinability characteristics. Good thermal conduction makes it useful for heatsinks and in heat exchangers. Copper has good corrosion resistance, but not as good as gold. It has excellent brazing and soldering properties and can also be welded, although best results are obtained with gas metal arc welding [35].

Copper is normally supplied, as with nearly all metals for industrial and commercial use, in a fine grained polycrystalline form. Polycrystalline metals have greater strength than monocrystalline forms, and the difference is greater for smaller grain (crystal) sizes. The reason is due to the inability of stress dislocations in the crystal structure to cross the grain boundaries [36].

Electrical properties

Copper electrical busbars distributing power to a large building at 59.6×10^6 S/m copper has the second highest electrical conductivity of any element, just after silver. This high value is due to virtually all the valence electrons (one per atom) taking part

in conduction. The resulting free electrons in the copper amount to a huge charge density of 13.6×10^9 C/m^3. This high charge density is responsible for the rather slow drift velocity of currents in copper cable (drift velocity may be calculated as the ratio of current density to charge density). For instance, at a current density of 5×10^6 A/m^2 (typically, the maximum current density present in household wiring and grid distribution) the drift velocity is just a little over ⅓ mm/s [37].

Corrosion

Copper should not be in direct mechanical contact with metals of different electropotential (for example, a copper pipe joined to an iron pipe), especially in the presence of moisture, as the completion of an electrical circuit (for instance through the common ground) will cause the juncture to act as an electrochemical cell (like a single cell of a battery). The weak electrical currents themselves are harmless but the electrochemical reaction will cause the conversion of the iron to other compounds, eventually destroying the functionality of the union. This problem is usually solved in plumbing by separating copper pipe from iron pipe with some non-conducting segment (usually plastic or rubber).

Solutions

Copper does not react with water, but it slowly reacts with atmospheric oxygen forming a layer of brown-black copper oxide. In contrast to the oxidation of iron by wet air, this oxide layer stops the further, bulk corrosion. A green layer of copper carbonate, called verdigris, can often be seen on old copper constructions, such as the Liberty. Copper reacts with hydrogen sulfide- and sulfide-containing solutions, forming various copper sulfides on its surface. In sulfide-containing solutions, copper is less noble than hydrogen and will corrode. This is observed in everyday life when copper metal surfaces tarnish after exposure to air containing sulfur compounds.

Copper is slowly dissolved in oxygen-containing ammonia solutions because ammonia forms water-soluble complexes with copper. Copper reacts with a combination of oxygen and hydrochloric acid to form a series of copper chlorides. Copper(II) chloride (green/blue) when boiled with copper metal undergoes a

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symproportionation reaction to form white copper(I) chloride.Copper reacts with an acidified mixture of hydrogen peroxide to form the corresponding copper salt:

$$Cu + 2\ HCl + H_2O_2 \rightarrow CuCl_2 + 2\ H_2O$$
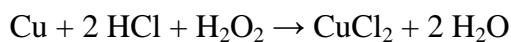
Antibacterial properties

Copper is antibacterial/germicidal, via the oligodynamic effect. For example, brass doorknobs disinfect themselves of many bacteria within a period of eight hour [38]. Antimicrobial properties of copper are effective against MRSA [39]. Escherichia coli [40]. and other pathogens [41] [42] [43]. At colder temperatures, longer times are required to kill bacteria.

Copper has the intrinsic ability to kill a variety of potentially harmful pathogens. On February 29, 2008, the United States EPA registered 275 alloys, containing greater than 65% nominal copper content, as antimicrobial materials [44]. Registered alloys include pure copper, an assortment of brasses and bronzes, and additional alloys. EPA-sanctioned tests using Good Laboratory Practices were conducted in order to obtain several antimicrobial claims valid against: methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes*, *Escherichia coli* O157: H7 and *Pseudomonas aeruginosa*. The EPA registration allows the manufacturers of these copper alloys to legally make public health claims as to the health effects of these materials. Several of the aforementioned bacteria are responsible for a large portion of the nearly two million hospital-acquired infections contracted each year in the United States [45]. Frequently touched surfaces in hospitals and public facilities harbor bacteria and increase the risk for contracting infections. Covering touch surfaces with copper alloys can help reduce microbial contamination associated with hospital-acquired infections on these surfaces.

Isotopes

Copper has 29 distinct isotopes ranging in atomic mass from 52 to 80. Two of these, [63]Cu and [65]Cu, are stable and occur naturally, with [63]Cu comprising approximately 69% of naturally occurring copper [46].

The other 27 isotopes are radioactive and do not occur naturally. The most stable of these is ^{67}Cu with a half-life of 61.83 hours. The least stable is ^{54}Cu with a half-life of approximately 75 ns. Unstable copper isotopes with atomic masses below 63 tend to undergo β^+ decay, while isotopes with atomic masses above 65 tend to undergo β^- decay. ^{64}Cu decays by both β^+ and β^- [46].

^{68}Cu, ^{69}Cu, ^{71}Cu, ^{72}Cu, and ^{76}Cu each have one metastable isomer. ^{70}Cu has two isomers, making a total of 7 distinct isomers. The most stable of these is ^{68m}Cu with a half-life of 3.75 minutes. The least stable is ^{69m}Cu with a half-life of 360 ns [46].

Biological role

Rich sources of copper include oysters, beef or lamb liver, Brazil nuts, blackstrap molasses, cocoa, and black pepper. Good sources include lobster, nuts and sunflower seeds, green olives, avocados and wheat bran.

Copper is essential in all plants and animals. The human body normally contains copper at a level of about 1.4 to 2.1 mg for each kg of body weight [47]. Copper is distributed widely in the body and occurs in liver, muscle and bone. Copper is transported in the bloodstream on a plasma protein called ceruloplasmin. When copper is first absorbed in the gut it is transported to the liver bound to albumin. Copper metabolism and excretion is controlled delivery of copper to the liver by ceruloplasmin, where it is excreted in bile.

Copper is found in a variety of enzymes, including the copper centers of cytochrome c oxidase and the enzyme superoxide dismutase (containing copper and zinc). In addition to its enzymatic roles, copper is used for biological electron transport. The blue copper proteins that participate in electron transport include azurin and plastocyanin. The name "blue copper" comes from their intense blue color arising from a ligand-to-metal charge transfer (LMCT) absorption band around 600 nm.

Most molluscs and some arthropods such as the horseshoe crab use the copper-containing pigment hemocyanin rather than iron-containing hemoglobin for oxygen transport, so their blood is blue when oxygenated rather than red [48].

It is believed that zinc and copper compete for absorption in the digestive tract so that a diet that is excessive in one of these minerals may result in a deficiency in the other. The RDA for copper in normal healthy adults is 0.9 mg/day. On the other hand, professional research on the subject recommends 3.0 mg/day [49]. Because of its role in facilitating iron uptake, copper deficiency can often produce anemia-like symptoms. Conversely, an accumulation of copper in body tissues are believed to cause the symptoms of Wilson's disease in humans.

Chronic copper depletion leads to abnormalities in metabolism of fats, high triglycerides, non-alcoholic steatohepatitis (NASH), fatty liver disease and poor melanin and dopamine synthesis causing depression and sunburn. Food rich in copper should be eaten away from any milk or egg proteins as they block absorption.

Copper is an essential trace mineral that is vitally important for both physical and mental health. However, its importance for health is still largely unappreciated. The following article is an introduction to the large subject of copper imbalance.

COPPER'S ROLE IN THE BODY

Copper has a number of important functions in the human body. The problem usually occurs when there is too much of it in the soft tissues of the body. Here are some of the important roles of copper.

1. Bones and connective tissue. Copper is required to fix calcium in the bones and to build and repair all connective tissue. This includes the tendons, ligaments, skin, hair, nails, arteries, veins and a few other tissues.

Imbalances can contribute to osteoporosis and bone spurs. Others are most conditions of the skin, hair and nails. Others include most cardiovascular problems and many skeletal and structural imbalances as well.

2. Energy production in the cells. Copper is needed in the final steps of the Krebs energy cycle called the electron transport system. This is where most of our

cellular energy is produced. Any problem here causes fatigue, depression and other imbalances related to low energy.

3. Immune Response. Copper must remain in balance with zinc. When imbalances occur, one is more prone to all infections, in particular fungal and yeast infections that are so common today. For example, most people have some intestinal yeast if they eat sugars and most people have chronic sinus infections if they have common symptoms such as post-nasal drip and others.

4. The glandular system, particularly the thyroid and adrenal glands. The thyroid gland is extremely sensitive to copper. In part this is due to its nature and how easily it is influenced by the sympathetic nervous system. Common conditions seen with copper imbalance include hypothyroidism and even hyperthyroidism of a particular type that is very common that I all secondary hyperthyroidism. GraveÕs disease usually due to stress, copper imbalance and often mercury as well. Anyone with a diagnosis of GraveÕs disease or hyperthyroidism should have a hair analysis performed at a lab that does not wash the hair and properly interpreted.

Most often, the problem goes away with a properly designed nutritional balancing program. Reducing all stress and balancing the body chemistry are both required to resolve the condition naturally in my experience. Drugs may be needed temporarily to control the symptoms. Surgery or radioactive iodine treatment and too drastic and not needed, in my experience so far.

5. Reproductive system. Copper is closely related to estrogen metabolism, and is required for women's fertility and to maintain pregnancy. Imbalance can cause every conceivable female organ-related difficulty such as premenstrual syndrome, ovarian cysts, infertility, miscarriages, sexual dysfunctions and more. It affects men less than women in this area, but it may affect menÕs potency and sexual drive as well as that of women.

6. Nervous system. Copper stimulates production of the neurotransmitters epinephrine, norepinephrine and dopamine. It is also required for monoamine oxidase,

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an enzyme related to serotonin production. As a result, copper is involved deeply with all aspects of the central nervous system. Copper imbalances are highly associated with most psychological, emotional and often neurological conditions. These include memory loss, especially in young people, depression, anxiety, bipolar disorder, schizophrenia and others discussed below.

Copper and vitamin C. Copper and vitamin C are direct antagonists. This means that they oppose each other in the body. This is one reason many people feel better taking a lot of vitamin C. Copper tends to oxidize and destroy vitamin C in the body. Meanwhile, vitamin C chelates or removes copper from the body. This requires a dose of vitamin C of at least about 500 mg daily, far higher than the minimum daily requirement of about 60 mg. Many readers know that vitamin C is critical for connective tissues. One of the prominent symptoms of scurvy, or vitamin C deficiency, is bleeding, such as bleeding gums. This is due to connective tissue weakness.

Thus, a copper excess can easily lead to a deficiency of vitamin C in the body and with it many symptoms of vitamin C deficiency. Oddly, however, a copper deficiency also causes connective tissue problems, especially in the heart and cardiovascular system where it is associated with a tendency for aneurisms and atherosclerosis.

Symptoms. Symptoms associated with connective tissue and joints include arthritis, osteoporosis, stretch marks and joint problems of other kinds. Others include scoliosis, kyphosis (bad posture) and many of the conditions of the skin, hair and fingernails and toenails. Others are some diseases the muscles, ligaments and tendons.

Among the most common, for example, are hair loss, especially in women, tendonitis, back problems due to muscle weakness and others.

Copper and cancer

Copper imbalance impairs the immune system. Research is underway investigating the role of excess copper in tumor angiogenesis. Elevated copper on a hair mineral analysis, when the level is above about 12 mg% and persists at this level, is often related to a tendency for infections and even cancer.

Cancer is associated with all three copper imbalances Ð deficiency, excess and biounavailable copper, which is a combination of the other two. This is one reason for the cancer epidemic we experience today. The important topic of cancer and natural approaches to it, is discussed in other articles on this website. Here are just a few ways cancer is linked to copper imbalance:

1. The levels of estrogen and copper have direct relationships. This means that as copper rises, often estrogen rises, too. This is one reason many women and even men are so-called Òestrogen dominantÓ today. Really, they have too much copper and cannot detoxify estrogen well enough. This imbalance is tied to cancer because estrogen is a potent carcinogen. It is the reason we never recommend supplementing even natural estrogen unless it is done with extreme caution. It is rarely needed if the body chemistry can be balanced using nutritional balancing science.

2. Copper causes liver toxicity when it is in excess or when it ibiounavailable. The liver is important to protect to avoid and to control cancer in every case, according to Dr. Max Gerson, MD, a pioneer in non-toxic cancer therapies.

3. Copper alters thyroid gland activity in most cases. This can also contribute to cancer and many other illnesses such as GraveÕs disease, for example.

4. Copper imbalance is associated with fungal and other infections. These can often be at the root of a cancer situation. For example, it is known that root canal-filled teeth can give off bacterial toxins that help predispose the body to cancers of certain kinds.

5. Copper blocks anerobic metabolism when it is in balance. This can help prevent cancer when copper is in balance, but not when it is too high or too low in the body.

6. Copper in excess often interferes with zinc metabolism. Zinc is required for the immune response and for over 100 enzymes in the body from helping digestion to protecting the skin from invasion from infections and even some skin cancers.

Copper and children

Copper has an incredible impact on children, particularly young ones. Common conditions such as ear infections, skin rashes and dandruff usually involve an imbalance between copper and zinc in children. Others in which we commonly find copper imbalance, along with other metal imbalances are learning and developmental disorders, colic, ADD and ADHD, sleep problems and childhood cancers.

This has to do with the extreme importance of copper in childhood development, especially of the developing nervous and immune systems. Children are born with high copper levels. Young children are very sensitive and intuitive. They often lose some of their sensitivity as their copper levels diminish around age four. Today, however, persistently elevated copper levels in children are commonly seen. At times, the copper is hidden.

Why children have copper imbalances. Copper imbalance problems for a child often begin when still in the womb. High-copper mothers pass on excessive copper (and often low zinc) to the fetus through the placenta. This is called congenital, rather than genetic high copper. It can be prevented by correcting one's copper metabolism before becoming pregnant. It can also be corrected after a baby is born, though this takes much more effort in most cases.

Once a baby is born, copper imbalance can develop as well. Inadequate zinc or high copper in the breast milk, in fact, is one reason children stop breastfeeding. ChildrenÕs diets are usually not great and often atrocious. Stress in the home or at school is another critical factor in sensitive children that can literally push them over the edge. Stress of any kind can lower zinc and raise the copper level.

Vaccination and the use of prescription drugs can aggravate a child's copper imbalance, usually by depleting the zinc level. Copper imbalance in children is associated with delayed development, attention deficit disorder, anti-social and hyperactive behavior, autism, learning difficulties and infections such as ear infections.

Beware of fast oxidizing young children. Do not restrict their copper. Most of them absolutely require extra copper. This is because they are fast oxidizers. This body type must have extra copper or they will exhibit violence, sleep problems or anti-social

behavior such as ADD or ADHD. So beware, since avoiding copper will make these children decidedly worse.

COPPER AND THE CARDIOVASCULAR SYSTEM

Low or biounavailable copper is associated with atherosclerosis and a tendency for aneurisms as well. The arteriosclerosis or atherosclerosis is secondary, usually, to weakened arterial walls. The body tries to reinforce inflamed or weakened arteries by coating them on the inside with calcium or fatty plaques. High or biounavailable copper is associated with mitral valve prolapse and other cardiovascular problems as well. It is not directly associated with high blood pressure, but may be secondarily due to the reasons for arteriosclerosis explained above.

VEGETARIAN DIETS AND THE COPPER BALANCE

Excess copper interferes with zinc, a mineral needed to make digestive enzymes. Too much copper also impairs thyroid activity and the functioning of the liver. If severe enough, a person will become an obligatory vegetarian. This means they are no longer able to digest meat very well. Conversely, if one becomes a vegetarian for other reasons, most likely one's copper level will increase. Vegetarian proteins are higher in copper, and lower in zinc.

At times, the vegetarian orientation is health-producing. In many people, however, restricted diets do not work well. Fatigue, spaciness and other symptoms begin to appear. Many people, including the author, felt they were becoming more Òspiritual Ó on a vegetarian diet, when in fact it was just copper poisoning! The taste for meat often returns when copper is brought into better balance.

Some people with high copper dislike all protein. They crave high-carbohydrate diets. Protein feels heavy or causes other symptoms. Eating protein stimulates glandular activity. This releases stored copper, which causes the symptoms. However, these individuals usually need to eat protein. The symptoms will eventually disappear.

REDUCING EXCESS COPPER

I have dealt with severe copper imbalance in myself and experienced many of the symptoms of it. He has not had difficulty with it for many years and helps thousands of others, literally, with the same issues. Seven methods can be used at the same time to reduce copper in the tissues and are described below.

This section is divided into methods to reduce toxic levels of copper and methods for improving the activity of the adrenal glands.

Reducing Copper In The Tissues.

At least several of the following methods should be used at the same time for best results. This is often overlooked, leading to temporary or incomplete restoration of health.

1) Balance body chemistry, enhance energy production and improve adrenal gland activity. To support the adrenal glands, avoid sweets, eat protein with each meal. Supplements that assist the adrenals include vitamins A,C and E, manganese, zinc, adrenal glandular and B-complex vitamins. Animal protein is very helpful due to its higher content of zinc, B-vitamins and sulfur amino acids including cysteine and taurine. Adrenal glandular substance is also frequently helpful.

2) Reduce fear and stress. Methods range from a change in location or work to meditation, therapy, more rest and other changes.1) Inhibit the sympathetic nervous system. This is easier said than done. Copper toxic individuals often complain of their mind racing. Turning off the sympathetic or fight-or-flight nervous system can be a challenge. Methods that are helpful include electric light sauna therapy, meditation, relaxation techniques, deep breathing, supplemental calcium, magnesium, ox bile, pancreatin, kidney glandular and coffee enemas.

3) Reduce exposure to sources of copper like copper intra-uterine devices, swimming in pools and high-copper vegetarian diets.

4) Enhance the eliminative organs, such as the liver, skin and colon. Digestive enzymes, especially pancreatin, are very important. Also excellent is sauna therapy, especially with an near infrared electric light sauna. Other methods of enhancing the eliminative organs are coffee enemas, colonic irrigation and skin brushing.

5) Antagonists such as zinc, manganese and iron compete with copper for absorption and utilization. Other antagonists include vitamins B6, folic acid and selenium. Research indicates copper may be excreted by binding with glutathione and metallothionine which require these nutrients.

6) Chelators are not needed or helpful in most cases. These are substances that bind to copper and help remove it from the body. Click here to read more about why we avoid Chelation Therapy. Synthetic chelators may be used, but can have harmful side effects. The most common one is penicillamine, sold under the names Cupramine or Depen. This toxic method has been around for years. It is not recommended ever, due to side effects that can include kidney damage, blurred vision, B6 deficiency, ringing in the ears, ulcers, jaundice and other liver damage, abdominal pain, bloody urine and more.

Note that just taking copper antagonists and chelators may not work very well. This is because these, of themselves, do not assist to rebalance body chemistry. In fact, they can make the overall balance of the electrolytes worse. This is why a complete program of balancing body chemistry with nutritional balancing science is far preferable. I will assist any practitioner who wishes to learn about this method of copper removal.

For example, zinc is often used to correct a high copper. However, it lowers the hair tissue sodium level, which is often dangerous if persisted in. Molybdenum, another excellent copper antagonist and chelator, raises sodium. Vitamin C, when used in high doses, tends to cause other imbalances because it can remove other metals besides copper.

Each vitamin and mineral affects overall body chemistry. For best results, I strongly recommend an integrated nutrition, lifestyle and detoxification program based on a properly performed and interpreted hair mineral analysis. It is worth the extra time, cost and energy to get better results. It can also avoid the purchase of unnecessary and costly supplements and other problems that come from their use. Also, be careful with chelating methods, including natural products such as Metal Free, NDF and others. We do not like these products, as they often remove some essential minerals as well as removing toxic metals.

Adrenal Gland Restoration.

Restoring the adrenal glands is often absolutely necessary to prevent copper from accumulating over and over again in the body. This is because the adrenal glands signal the liver to produce ceruloplasmin, the principal copper binding agent in the body, along with metallothionein. To restore the adrenal glands, the following methods may be extremely helpful and necessary in many instances.

1. Rest is number one. Get at least 11 hours of sleep daily. This may be broken into nighttime plus a nap or two. A year of more of extra rest is often needed.

2. Diet is also critical. The diet should be high in fresh vegetables, in particular, as well as healthful proteins and some whole grain rice and corn, if these can be tolerated well. For the recommended diet, see the document Slow Oxidizer Diet. This diet is appropriate for most of those with copper imbalance, though not all. A small number are fast oxidizers. They must have much more fat and oil in their diets, and less protein at times. Other special cases also exist due to more complex nutritional imbalances, food sensitivities and other rarer conditions.

Equally important, the diet must be as low as possible in sweets and sugars as possible. These foods, along with all stimulants, stress the adrenal glands and tend to make copper imbalance worse. Stimulants include sugars, caffeine and food additives such as MSG, aspartame and other excitotoxins in the diet. Many other food chemicals and additives, however, even including too much salt, also stress the body and are not helpful for copper imbalance. Strict vegetarian diets usually aggravate copper imbalance badly. Wheat and refined flour products, in general, are also not helpful at all. These are some of the most important dietary considerations, especially for slow oxidizers.

2. Carefully chosen nutritional supplements are also extremely important to reduce copper imbalance. These are best determined by the use of a hair mineral analysis, in our view.

Many doctors give symptomatic remedies for copper These are nutrients that are known to lower or to balance copper in the body. They include vitamin C, garlic, zinc, molybdenum, vitamin B6 and others. I do not endorse or recommend this method at all. It is far too likely to fail, though it will often give some quick relief. Please beware of using remedies to lower copper without getting a hair mineral analysis from a doctor or nutritional consultant who understands how to interpret the test.

Programs we design always help to support the adrenal and thyroid glands and include a digestive aid along with targeted nutritional support depending on the mineral ratios in the body. I also do not like the use of most herbs for the adrenal glands. We do not use ginseng, licorice and other herbs that may be stimulating, as these eventually cause more severe problems, though, once again, they offer quick results in many cases.

We also do not need to use any hormones such as DHEA, pregnenolone, testosterone, progesterone or cortisol. These also give quick relief in most cases, but upset the delicate hormone balance and eventually usually worsen copper imbalance.

4. Lifestyle modifications. Most people with copper imbalance are very sensitive. Many also need to slow down, relax more, let go of anger and learn to meditate, perhaps. Some also need to make big life changes in their relationships, location, work and other important aspects of their lives so that they Òlive their truthÓ to a greater degree. Living a lie can be an important problem, in fact.

5. Detoxification. Copper imbalance responds beautifully to the use of coffee enemas and colonic irrigation if enemas are not possible. In addition, the use of a near or far infrared sauna is absolutely essential for some people, especially those with emotional problems connected to their copper imbalance.

Other methods of detoxification are less effective, in our experience. These include methods such as cleansing diets, which can make the copper problem worse.

Toxicity:

Copper toxicity refers to the consequences of an excess of copper in the body. Copper toxicity can occur from eating acid food that has been cooked in un-coated copper cookware, or from exposure to excess copper in drinking water or other environmental sources.

Copper in the blood exist in two forms: bound to ceruloplasmin (85–95%) and the rest "free" loosely bound to albumin and small molecules. Free copper causes toxicity as it generates reactive oxygen species such as superoxide, hydrogen peroxide, the hydroxyl radical. These damage proteins, lipids and DNA.[52].

Toxicity can occur from eating acidic food that has been cooked with copper cookware. Cirrhosis of the liver in children (Indian Childhood Cirrhosis) has been linked to boiling milk in copper cookware. The Merck Manual states that recent studies suggest that a genetic defect is associated with this cirrhosis [50]. Since copper is actively excreted by the normal body, chronic copper toxicosis in humans without a genetic defect in copper handling has not been demonstrated [47]. However, large amounts (gram quantities) of copper salts taken in suicide attempts have produced acute copper toxicity in normal humans. Equivalent amounts of copper salts (30 mg/kg) are toxic in animals [51].

Symptoms and presentation

Symptoms of copper poisoning are very similar to those produced by arsenic. Fatal cases are generally terminated by convulsions, palsy, and insensibility. The DRI Tolerable Upper Intake Level for adults of dietary copper from all sources is 10 mg/day. In toxicity, copper can inhibit the enzyme dihydrophil hydratase, an enzyme involved in haemopoiesis and homeostasis.

Treatment

In the cases of suspected copper poisoning, ovalbumin is to be administered in either of its forms which can be most readily obtained, as milk or whites of eggs. Vinegar should not be given. The inflammatory symptoms are to be treated on general principles, and so are the nervous.

Cookware

When acidic foods are cooked in unlined copper cookware, or in lined cookware where the lining has worn through, toxic amounts of copper can leech into the foods being cooked.[53]. This effect is exacerbated if the copper has corroded, creating reactive salts [54]. Many countries and states prohibit or restrict the sale of unlined copper cookware.

Drinking water

With an LD50 of 30 mg/kg in rats, "gram quantities" of copper sulfate are potentially lethal in humans.[55] The suggested safe level of copper in drinking water for humans varies depending on the source, but tends to be pegged at 2.0 mg/l[56].

Pathophysiology

A significant portion of the toxicity of copper comes from its ability to accept and donate single electrons as it changes oxidation state. This catalyzes the production of very reactive radical ions such as hydroxyl radical in a manner similar to Fenton chemistry.[57] This catalytic activity of copper is used by the enzymes that it is associated with and is thus only toxic when unsequestered and unmediated. This increase in unmediated reactive radicals is generally termed oxidative stress and is an active area of research in a variety of diseases where copper may play an important but more subtle role than in acute toxicity.

It has been suggested that some of the effects of aging may be associated with excess copper [58]. In addition, studies have found that people with mental illnesses such as schizophrenia had heightened levels of copper in their systems. However it is unknown at this stage whether the copper contributes to the mental illness, whether the body attempts to store more copper in response to the illness, or whether the high levels of copper are the result of the mental illness.

Indian Childhood Cirrhosis

One manifestation of copper toxicity, cirrhosis of the liver in children (Indian Childhood Cirrhosis), has been linked to boiling milk in copper cookware. The Merck Manual states that recent studies suggest that a genetic defect is associated with this particular cirrhosis [59].

Wilson's Disease

An inherited condition called Wilson's disease causes the body to retain copper, since it is not excreted by the liver into the bile. This disease, if untreated, can lead to brain and liver damage.

Alzheimer's disease

Elevated free copper levels exist in Alzheimer's disease [52]. Copper and Zinc are known to bind to amyloid beta proteins in Alzheimer's disease [60]. This bound form is thought to mediate the production of reactive oxygen species in the brain [61].

In marine life

Too much copper in water has also been found to damage marine life [62]. The observed effect of these higher concentrations on fish and other creatures is damage to gills, liver, kidneys and the nervous system. It also interferes with the sense of smell in fish, thus preventing them from choosing good mates or finding their way to mating areas [63].

Generally, sheep require about 5 ppm (parts per million or mg/kg) of Cu in their total diet. Toxicity can occur at levels above 25 ppm. However, dietary molybdenum (Mo) levels also affect copper requirements, as Mo forms an insoluble complex with Cu to prevent copper absorption. If molybdenum levels are low (less than 1 ppm), sheep are more susceptible to Cu toxicity. If Mo intakes exceed 10 ppm, Cu deficiency may occur on diets that would normally be adequate. Sulfur (S) further complicates the Cu:Mo relationship by binding with the Mo.

Copper toxicity in sheep usually results from the accumulation of excess Cu in the liver over a period of a few weeks to more than a year with no clinical signs, followed by a sudden release of liver Cu stores to cause toxicity (rapid breakdown of red blood cells).

In these situations, chronic Cu poisoning may result from excessive Cu intakes or from low intakes of Mo, S, zinc, calcium or following liver damage. Stresses, such as

weather, environment, poor nutrition, transportation and handling can also cause the liver cells to die and release the stored copper into the bloodstream.

Affected sheep are lethargic and anemic. They may grind their teeth incessantly and experience extreme thirst. Membranes are very pale and may appear yellow, as jaundice sets in. Urine is a bloody color. Death usually occurs 1 to 2 days after the onset of clinical symptoms. At post-mortem, tissues are pale to dark yellow and the kidneys are a very dark color.

In contrast, cattle require about 10 ppm of Cu in their diet and can tolerate Cu levels ten times higher than sheep.

<u>Copper Toxicity Treatment</u>

The main thing to do is to increase Zinc and Magnesium levels to calm the mind and nervous system. However, just taking Zinc alone can cause serious side effects as Zinc will cause a Copper detox as the body dumps this excess copper and Mercury and other heavy metals from the tissues. This can cause rashes and flu-like symptoms. Zinc will also lower Sodium levels which may make you feel extremely tired.

I recommend a hair Analysis test to evaluate your soft tissue levels of copper, zinc, sodium, potassium, calcium, magnesium and other trace minerals and toxic metals to help design a custom tailored Nutritional Balancing program. Hair Analysis is a very accurate and non-invasive means of analyzing the Mineral Levels in the Soft tissues. These readings can help us determine how much Heavy Metal and Copper toxicity there actually is and what type of Supplement and Nutritional therapy will be able to correct your Biochemical imbalance.

By balancing your Hair mineral levels and ratios, the adrenal glands are strengthened, Zinc levels will rise, and the body is able to produce ceruloplasmin and metallothioneine, which will help eliminate the copper toxicity.

Meditating, practicing Qigong, and enjoying a relaxed and stress free lifestyle is also extremely important in recovering from adrenal fatigue and copper toxicity. Try to remember to breathe deeply and slowly and not react to every little stressor that you may encounter. Peace of mind is essential for copper toxicity recovery.

Copper detoxification symptoms

One of the difficulties in reducing excess copper are symptoms that arise during the process of elimination. As the body begins to mobilize excess copper from tissue storage sites, it enters the bloodstream on its way to the liver and kidneys for elimination. While in the bloodstream, the copper can cause headaches, skin rashes, racing thoughts, strange odors, digestive upset, mood swings and energy fluctuations. In men, testicular pain is not uncommon. Women's periods may be affected.

Certain methods of lowering copper cause these symptoms more than others. Zinc, vitamin C and manganese tend to cause more symptoms, perhaps because zinc and manganese replace copper in the liver. Molybdenum and sulfur compounds such as Russian black radish tend not to produce copper elimination effects.

If one knows what is occurring, it is possible to take measures to minimize these temporary elimination symptoms. Enemas, sweating, and drinking more water can help promote copper elimination. Reducing the nutrition program for a few days may also help slow the reactions and reduce symptoms if they are severe. Supplements of molybdenum, bile acids, laxative herbs and vitamin B6 may also mitigate elimination symptoms.

Resources Eck, P. and Wilson, L., *Toxic Metals in Human Health and Disease*, Eck Institute of Applied Nutrition and Bioenergetics, Ltd., Phoenix, AZ, 1989.
2. Gittleman, A.L., *Why Am I Always So Tired?*, Harper San Francisco, 1999.
3. Nolan, K., "Copper Toxicity Syndrome", J. Orthomolecular Psychiatry, 12:4, p.270-282.
4. Pfeiffer, C., *Mental and Elemental Nutrients*, Keats Publishing, New Canaan, CT., 1975.
5. Wilson, L., *Nutritional Balancing and Hair Mineral Analysis*, L.D. Wilson Consultants, 2005, 2010.
6. Hundreds of technical articles on the sources, symptoms and correction of copper imbalance are available on the worldwide web. They are too numerous to list here. The books and articles mentioned above contain more complete references.

Miscellaneous hazards

The metal, when powdered, is a fire hazard. At concentrations higher than 1 mg/L, copper can stain clothes and items washed in water.

(C) ENVIRONMENTAL POLLUTION

Before entering into a brief discussion of Environmental pollution it will be wise to give some definition of some useful terms commonly met with the discussion.

(a) **Definitions:**

(i) **Environmental Pollution:** "Environmental pollution is partly rapacity and partly a conflict of interest between the individual multimillions of individuals and the commonwealth, but largely, in our generation it is the exaggerated effects of specialization with no sense of ecology i. e. the balance of nature" .... G. G. McClellan.

Environmental pollution is any degree of contamination of air, water, soil or food which is likely to produce a significant adverse health effect to a significant number of persons in a foreseeable of time.

(ii) **Pollutant:** A substance present in nature, is greater than natural abundance due to human activity, which ultimately has a detrimental effect on the environment and therefrom on living organisms and mankind.

Example: Lead, chromium, mercury, sulphur dioxide, carbon dioxide etc.

(iii) **Contaminant:** A material which does not occur in nature, but is introduced by human activity into the environment, affecting its composition. A contaminant is classified as a pollutant when it exerts a detrimental effect.

Example: Chlorine gas, Bromine gas, etc.

(iv) **Receptor:** The medium which is affected by a pollutant. Man is the receptor of photochemical smog causing irritation of the eyes and respiratory tract. Trout fingerlings are receptors of dieldrin in water which causes their death.

(v)   **Speciation:** The different chemical forms or species of inorganic, organic or organo-metallic compounds present in the environment. It is important to identify the chemical species of a pollutant since some species are more toxic than other. Thus, chromium (VI) is much more toxic than chromium (III).

(vi)   **Dissolved Oxygen (DO):** Oxygen is a vitally important species in water. It is consumed by oxidation of organic matter/ reducing agents etc. It is an important water quality parameter. The optimum value is for good water quality is 4-6 mg L^{-1} of DO, which ensures healthy aquatic life in a water body. Lower DO values indicate water pollution.

(vii)   **Threshold Limit Value (TLV) :** This indicates the permissible level of toxic pollutant in atmosphere to which a healthy industrial worker is exposed during an eight-hour day without any adverse effect. TLV values for Cd. Cr, Be, Al and Zn are 0.05, 0.05, 0.002, 10.0 and 1.00 mg m^{-3}, respectively.

(viii)   **Tolerance Limit:** Tolerance is defined as ability to endure the continued and increasing administration of a toxicant. Tolerance is also the capacity of an organism to exhibit less response to a test does of a chemical than it did previously to the same dose.

(b)   **Sources and Distribution of the Contaminants:**
The main sources of contaminants in the atmosphere can broadly be divided into natural and anthropogenic groups. The former includes windborne dust, forest fires, volcanic eruptions, sea mist plant decomposition products etc. The anthropogenic sources are mines, factories where the metals contaminants are processed and factories where different metal compounds are manufactured. Modern industrialization has introduced harmful metals into the environment by redistributing them immobilized ones and minerals. Thus main anthropogenic sources of contamination of the environment arise from manufacture from their ores from which industrial waste airborne emission and industrial products take the leading role in contaminating the biosphere. This can be illustrated with the following typical diagram.

Fig.-5 : Scheme illustrating the migration of metal in the biosphere.
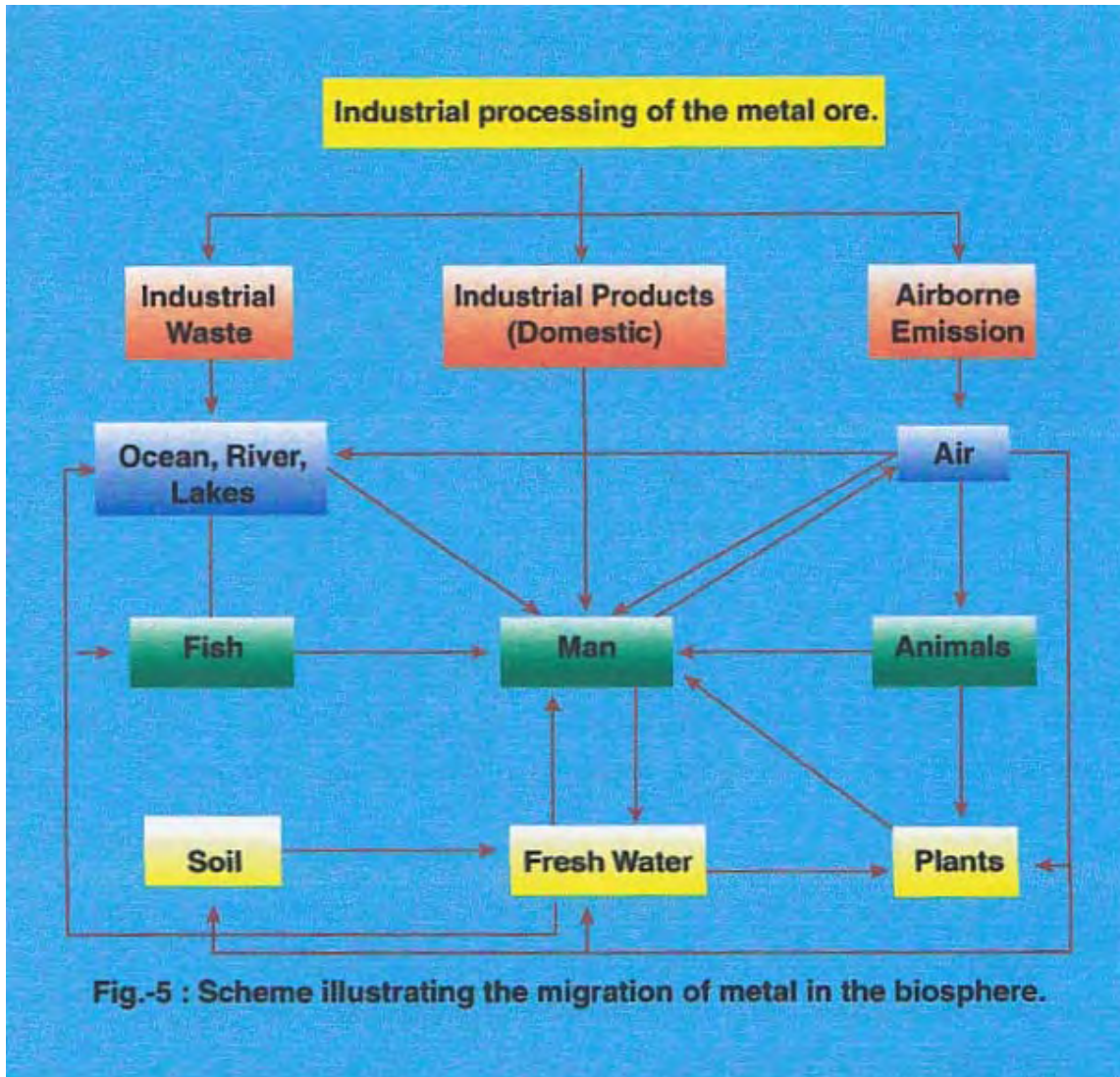
(c)   Public Health and Pollution:

Mankind has been confronted with pollution problems since early times. Until the end of the 19th century, it was universally accepted that health was equivalent to the absence of disease. It was only at the beginning of the present century that, thanks to achievements in the social field, a more positive concept of health began to crystallize. It came to be recognized as a state of physical, mental and social well-being, a definition which is in fact embodied in the constitution of WHO adopted in 1948.

The general public has become increasingly concerned about the pollution of the environment and in number of countries the problems caused by pollution have been met by a whole range of statutory and regulatory provisions aimed at protecting of public health. The term pollution is generally defined as the presence in the ambient environment of chemical, physical or biological factors capable of inducing disturbances in the normal physiology and functioning of human organs. If we bear in mind that the respiratory system and digestive tract are merely extensions (without the body) of the external environment, the tendency to associate the pollution of foodstuffs and drugs with that of air, water and soil will be readily understood.

The fields in which pollution can occur are manifold, since no activity can be performed without entailing pollution. It is precisely this linkage between human activities and the resultant pollution that constitutes the nub of the problem. It is at juncture that we begin to reflect on protection of basic human rights.

Worker's demands during the last century and especially in the last 50 years, have led to the recognition of a large number of occupational diseases associated with the presence of harmful agents in the working environment. Measures to protect workers against occupational hazards have shown a remarkable expansion in recent years.

The effects of pollution on individual depends on various factors. These include the toxicity or harmfulness of the pollutants involved, their concentration and the conditions under which they exert their effect. Having in mind that the human being has a remarkable capacity for adaptation and recovery following exposure to external agents, one can judge how complexing the problems are-

The rising incidence of bronchial diseases among the workers and the populations of certain large cities is certainly connected with the increase in the emission of sulfur dioxide from factories plants and heating installations.

The development of lung tumors in cities could be the consequence of air pollution caused by motor vehicles or from certain domestic sources.

Pollution of the sea was responsible for the notorious outbreak of mercury poisoning among the population living around Minimata Bay in Japan. Prior to "the epidemic intoxication" in 1971 /2 which was one of the most catastrophic with 6530 cases of poisoning with methyl mercury in farmers and their families in Iraq, about 1000 cases had been ascribed in the world as methyl and ethyl mercury poisoning.

Cadmium and poisoning with cadmium between workers and itai-itai disease caused by cadmium in general population are also known in Literature. A study of the USA public Health service on the chromate producing industry indicates that the incidence of a whole service of ill effects ranging from dental caries to bronchiogenic cancer, was abnormally high.

(d)   **The Growing Hazards:**
The increasing demand of chemicals in day-to-day life and the residue of these compounds are likely to change the composition of air, water, soil, food chain and living tissues. The persistence of DDT, bis-chloroisopropyl ether, polyvinyl chloride, trace elements etc. is an alarming concern and hence needs a through investigation of their impact on the total environment.

In this context the disastrous Bhopal gas tragedy needs a worthy mention. The leakage of methylisocynate gas claimed the lives of several thousand people. People are still suffering from its after affects. The dangerous gas did not even spare the plant kingdom. Ammonia gas escaped from a tanker which burst at Ghorasal Fertilizer Factory near Dhaka in August, 1991 and killed eleven persons working in a nearby room. The release of waste water from Ghorasal and Polash Fertilizer claimed the lives of fishes and aquatic animals and polluted the water of river Shitalakhaya. The release of carbon monoxide gas from automobile exhaust, dust

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particles from Cement Factory at Chittagong and chlorine gas from different industries in Bangladesh, show the negligence of the governing bodies. Evidence of contamination of products used in food packing and pipes for drinking water [64] by unpolymerised vinyl chloride monomer have also been reported.

An examination of the location of industries show that paper mills are situated on the banks of rivers karnaphuli, Surma, Padma and Bhairab. These mills are established long ago and do not have elaborate effluent processing systems. They discharge alkali hypochlorite, chlorinated/ sulphonated lignin compounds etc. in the rivers. Urea and TSP fertilizer factory are situated on the banks of the river karnaphuli, kushiyara, Maghna and Shitalakaya. These industries too were established at a time when awareness for preserving the environment was not much. As a result these industries too have only limited effluent processing arrangement. Steel and oil refinery industries have scarcely and effluent treatment systems. Effluents from tannery industries, mostly located near cities of Chittagong and Dhaka, find its way to the adjoining water systems. Tannery industries use chromium salts which are toxic even in low doses. No measure worth the name have so far been taken to regulate discharge of this toxic materials in the rivers. Sugar mills, dotted around North West part of the country. Do not have any effluent processing system.

Hence there is a great need of regulation of environmental protection act of banning the use of hazardous compounds recklessly in Bangladesh.

Elemental contents in the human body and environmental water quality standard for Bangladesh are summarized in the Table 1 and 2, respectively

TABLE-1 ELEMENTAL CONTENT IN THE HUMAN BODY[a].

Element	Serum, ppm	Urine	Tissues ppm[b]
Li	0.01		
Na	3200	1000-5000 mg/day	0.07 g/gN
K	120-214		
Rb			20-200 (dry)[c]
Be			0.00012 (liver)
Mg	18-29 (ave-22)	60-120 mg/day	300-500
Ca	90-100	96-800 mg/day	60-90
Sr		0.4 mg/day	0.1-0.5 (dry)
Ba			0.02-0.10 (dry) 1.0 in lung
Ti			0.3-0.6 (dry) 10 in lung
V	0.005 ± 0.008		<0.02-0.03 (dry) [b] 0.6 in lung
Cr	0.03		0.01-0.13[e] 036 in spleel
Mo	0.10-0.16[e]	0.01-0.03 mg/day	0.1-0.2 (dry)
Mn	0.01-0.02	0.04-0.07 mg/day	0.2-1.7
Fe	0.80-1.6 (ave.1-25) men 0.65-1.3 (ave.0.90) women	0.1-0.3 mg/day	
Co	0.00007-0.017 (whole blood)	0.001-0.007 ppm	0.1-0.2 (dry)
Ni	0.025 (range 0.001-0.08)	0.025mg/day (range 0.007-0.04)	
Cu	**1.05-1.10**		**5-20(dry), 10-15 in liver**
Ag			<0.01-0.2 (dry)
Au			<0.1-1 (dry) (occurrence< 20%)
Zn	1.2	0.3-0.6 mg/day	12-100
Cd	0.0033-800 ± 0.0024	0.002-0.02 ppm	0.2-0.8,2.0-6.0 in kidney
Hg		<0.03 ppm	
B			0.5-1 (dry)

Element	Serum, ppm	Urine	Tissues ppm[b]
Al	0.13-0.17	0.05 mg/day	0.2-0.6, 20-60 in lung
Tl[1]	3-17	>0.35 ppm	
Si			20-40 (dry), 400 in lung
Sn			5-30 (ash)
Pb	0.3-0.4[g]	0.01-0.07 mg/day	1-10 (dry)
As	0.04-0.2	$\leq$0.1 ppm	0.2-0.3
Bi	0.02		<1 (ash)
Se	0.14		0.2-0.6
P	0.3-0.65	0.4-1.3 g/day	0.2-0.45
N(asNO_3^-)	0.5-1.0		0.2-0.6

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

[b]Fresh weight, [c]Rats, [d]Organs, [e]Sheep and Cattle, [f]Concn, During thallium poisoning, [g]Whole blood.

TABLE-2: STANDARD VALUES FOR WATER.

Parameters	Unit	Standard value		Recreational	Fishing	Industrial	Irrigation	Livestock	Coastal
		Drinking Water		Water	Water	Water	Water	Water	Water
Determinants		EQS[a]	WHO[b]	Water	Water	Water	Water	Water	Water
pH		6-8	6-8.5	NYS	NYS	NYS	NYS	NYS	NYS
Aldehyde	mg/L	NYS	NYS	<20	NYS	NYS	NYS	NYS	NYS
Aluminum	mg/L	NYS	NYS	NYS	20-100	NYS	1	NYS	NYS
Ammonia (NH_3)	mg/L	0.5	0.5		0.2-5	NYS	3	NYS	NYS
Elemental Nitrogen (as N)	mg/L	NYS		NYS	1.2	NYS	15	NYS	60
Arsenic	mg/L	0.05	0.05	0.2	NYS	NYS	1	1	1
Barium	mg/L	0.5		NYS	NYS	NYS	NYS	NYS	NYS
Benzene	mg/L	0.01	10	NYS	NYS	NYS	NYS	NYS	NYS
Bicarbonate	mg/L	NYS	339	NYS	NYS	NYS	NYS	500	NYS
BOD	mg/L	0.2	6.0	3	6	10	10	NYS	NYS
Boron	mg/L	1.0	1.0	NYS	NYS	NYS	not<1	NYS	NYS
Cadmium	mg/L	0.005	0.01	NYS	NYS	NYS	0.01	0.5	0.3
Carbon dioxide	mg/L	NYS	NYS	NYS	6	NYS	NYS	NYS	NYS
Chloride	mg/L	150-600	500	600	600	NYS	600	2000	NYS
Calcium	mg/L	75	750	NYS	NYS	NYS	NYS	700	NYS
Carbon Tetrachloride	mg/L	0.01	5	NYS	NYS	NYS	NYS	NYS	NYS

		Standard value							
Parameters	**Unit**	**Drinking Water**		**Recreational**	**Fishing**	**Industrial**	**Irrigation**	**Livestock**	**Coastal**
1,1-Dicloro-ethylene	mg/L	0.001		NYS	NYS	NYS	NYS	NYS	NYS
1,2-Dichloro-methylene	mg/L	0.03	NYS	NYS	NYS	NYS	NYS	NYS	NYS
Tetrachloroethylene	mg/L	0.03		NYS	NYS	NYS	NYS	NYS	NYS
Trichloroethylene	mg/L	0.09		NYS	NYS	NYS	NYS	NYS	NYS
Pentachloropenol	mg/L	0.03	0.001	NYS	NYS	NYS	NYS	NYS	NYS
2,4,6-Tri-chlorrophenol	mg/L	0.03		NYS	NYS	NYS	NYS	NYS	NYS
Chlorine (residual)	mg/L	0.2	1.0	0.3	<0.01	NYS	NYS	NYS	2
Chloroform	mg/L	0.03	10	NYS	NYS	NYS	NYS	NYS	NYS
Chromium (Cr^{6+})	mg/L	0.05	0.05	0.05	NYS	0.5	NYS	NYS	NYS
Chromium (Total)	mg/L	0.05	0.05	NYS	0.05	NYS	NYS	NYS	NYS
COD	mg/L	4	4	4	NYS	3-10	NYS	NYS	8
Coliform (Faocal)	mg/L	n/	o		NYS	NYS	10	NYS	NYS
Coliforms (Total)	n/100	n/	2^t	200	5000	NYS	1000	100	1000
Color	Hazen	15		Clear	Normal	Normal	Normal	Normal	Normal
Copper	**mg/L**	**2**	**2.0**	**NYS**	**<0.4**	**NYS**	**0.2**	**NYS**	**0.3**
Cyanide (as CN)	mg/L	0.1	0.05	0.1	NYS	NYS	NYS	NYS	0.2
Detergente	mg/L	0.2		NYS	NYS	NYS	NYS	NYS	NYS

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Standard value									
Parameters	**Unit**	**Drinking Water**		**Recreational**	**Fishing**	**Industrial**	**Irrigation**	**Livestock**	**Coastal**
DO	mg/L	6	4-6	4-5	4-6	5	5	4-6	6
Fluoride	mg/L	1	1.5	1.5	NYS	NYS	NYS	4	NYS
Formaldehyde	mg/L	NYS		NYS	NYS	NYS	NYS	NYS	NYS
Hardness (CaCO$_3$)	mg/L	200-500	500	NYS	80-120	250[b]	NYS	NYS	NYS
Hydrogen Sulfide	mg/L		10			1-5[c]			NYS
lron	mg/L	0.321[c]	0.3	NYS	NYS	0.5[c]	NYS	NYS	NYS
kjeldahl nitrogen (Total)	mg/L	1	1	1	1	NYS	NYS	NYS	NYS
Lead	mg/L	0.05	0.05	NYS	0.05	2.01	0.1	0.05	0.2
Magnesium	mg/L	30-50	30	NYS	NYS	NYS	NYS	NYS	NYS
Manganese	mg/L	0.1	NYS	NYS	1.1-1[f]	2	NYS	NYS	NYS
Mercury	mg/L	0.001	0.001	NYS	0.001	NYS	NYS	NYS	NYS
Nickel	mg/L	0.1	0.2	NYS	NYS	NYS	0.5	NYS	0.2
Nitrate (as N)	mg/L	10	13	NYS	NYS	NYS	NYS	250	NYS
Nitrate (as NO$_2$)	mg/L	0.1	0.1	NYS	0.03	NYS	NYS	None	NYS
Odour		Odour		unobjectionable	Normal	Normal	Normal	Normal	Normal
Oil and Grease	mg/L	0.01	0.01	0.1	0.1	NYS	NYS	NYS	NYS
Phosphate	mg/L	0.01	0.8	0.05	0.8	0.8	0.2	0.1	NYS

2 per 100 mL in two consecutive samples or in more than 100 of the samples examined for drinking water.

(a) For boiled feed water depending on boiler pressure.
(b) For boiler feed water 2-40 mg/L depending on boiler pressure, tanning 50-130 mg/L.
(c) For cooling water- 5mg/L. For air conditioning water – 1mg/L.
(d) Textile dyeing 0.25 mg/L, tanning 0.2 mg/L.
(e) 2 in some areas the maximum tolerable limit may be up to 5 mg/L in absence of better source for drinking water.
(f) For air conditioning water – 0.5 mg/L, for textile dyeing 0.2 mg/L.

* **NYS- Not yet sustainable.**

Source: "Environmental Quality Standard for Bangladesh, Department of Environment, People's Republic of Bangladesh, 1991.

WHO-World Health Organization (International).

D) AIM OF THE PROJECT

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. Among various modern trace analysis techniques employed in solution, molecular absorption spectrophotometry has been rated to be one of the most useful powerful and successful tools recognized today. In some cases, it is the only suitable technique. Spectrophotometry is very sensitive so that sometimes picogram (10^{-12}g) per gram levels can be determined. 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 spetrophotometry in varied fields of chemical analysis lies in its manifold advantages. Compared to any modern trace and ultra trace analytical technique.

Copper in trace amounts is industrially important. Copper is becoming an ever more widely used metal in industry. Copper metal is used in protective coatings for iron, zinc and steel. Copper electroplated parts are used in radio and television sets. Telephone wires are made of Cu-Cd alloys and Ni-Cd rechargeable batteries are extensively used in electronic equipment.

Copper is not essential in human and animal nutrition, but its toxicity is tremendously dangerous. Copper 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 carcinogen disease. The major effects of copper poisoning are experienced in the lungs, kidneys and bone. Acute effects of inhalation are bronchitis and pneumonitis and toxemia in the liver.

The acute effects of oral intakes of copper are excess salivation, nausea, vomiting, abdominal pains, diarrhea, vertigo and for large doses, loss of

consciousness. Chronic inhalation of copper compounds as fumes or dust produce pulmonary emphysema. The industrial waste from an upstream copper mine was responsible for the contamination of food and drinking water. The carcinogenic effect of copper has been established in animals. Testicular destruction has been induced in animals by copper poisoning occur in animals manure, sea-food especially oysters and commercial phosphate fertilizers. Therefore, its accurate determination of trace level using simple and rapid method is of paramount importance.

Substances which do not absorb electromagnetic radiation in the visible range are colorless. These substances, hence not determinable, as such by visible spectroscopic methods. The project aims at the development of such a method for the quantitative determination of trace and ultra trace amount of copper in above spectral range.

The analysis of colorless substances by visible spectroscopy is not new, reacting the colorless analyte with a suitable regent, a color product is produced and 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 copper only a few examples of such a method is cited in the literature, but these methods are limited by the complexity of the procedure, low sensitivity, less selectivity due to many interferences, temperature and pH dependent and unfavorable detection limit.

The Ultimate Aims of Present Dissertation are primarily:

(a)   To introduce a good spectrophotometric reagent through novel reaction techniques.

(b)   To develop the non-extractive, direct spectophotometric method in very simple, rapid and highly selective and sensitive ways, particularly for some inorganic poisons such as copper for which either spectrophotometric methods are non-existence or scarce in literatures.

Finally, the aim of this study was to develop a simple spectrophotometric method for the determination of copper with 1-(2-pyridylazo)-2-naphthal (PAN). The method was optimized individually and result of the measurements was checked by comparison with congenital analysis. PAN has been reported as spectrophotometric reagent for Ni, Co, Zn, Mn, Ca, but has not previously been used for determination of copper in spectrophotometic method. The present thesis deals with successful attempt toward the establishment of new type of spectrophotometric reagent.

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PART-II

EXPERIMENTAL SECTION

(C)   REVIEW ON SPECTROPHOTOMETRIC METHODS
       OF COPPER

(D)   SPECTROPHOTOMETRIC DETERMINATION OF
       TRACE AMOUNT COPPER IN AQUEOUS MEDIA

(I)   INTRODUCTION

(II)   EXPERIMENTAL

(III) RESULTS & DISCUSSION

(IV) APPLICATIONS

(V)   CONCLUSION
       REFERENCES

(A) REVIEW ON SPECTROPHOTOMETRIC METHODS OF COPPER

Copper is a chemical element with the symbol **Cu** (Latin: *cuprum*) and atomic number 29. It is a ductile metal with very high thermal and electrical conductivity. Pure copper is rather soft and malleable, and a freshly exposed surface has a pinkish or peachy color. It is used as a thermal conductor, an electrical conductor, a building material, and a constituent of various metal alloys.

Different spectrophotometric and other methods for determination of copper

Determination of Copper by diethyldithio-carbamate:

Discussion. Small quantities of copper may be determined by the diethyldithio-carbamate method or by the neo-cuproin method an extraction being necessary in both cases. In another, somewhat simpler procedure, the copper is complexed with biscyclohexanone oxalyldihydrazone and the resulting blue colour is measured by a suitable spectrophotometer within the range 570-600 nm (orange filter). The solution measured should contain net more than 100 µg of copper.

Reagents. Bicyclohexanone oxalyldihydraxone solution (copper reagent). Dissolve 0.l g of the solid reagent in 10 mL ethanol (or industrial methylated spirit) and 10 mL hot water, and dilute to 200 mL. Filter, if necessary.Synthetic standard solution (for analysis of steel). Dissolve an appropriate weight of pure iron (Johnson Matthey) in a mixture of equal volumes of concentrated hydrochloric acid and concentrated nitric acid: with this solution as base add a suitable amount of copper nitrate solution containing 0.01 g copper per L.

Procedure (copper in steel). Weigh out accurately a 0.1g sample of the steel* into a 150 mL conical beaker. add 5 mL concentrated hydrochloric acid and 5 mL. concentrated nitric acid. and warm gently. In the presence of interfering amounts of chrominum, add 5 mL perchloric acid, sp. gr. 1.70 an evaporate until strong fuming occurs. When the sample has dissolved or after the fuming with perchloric acid. add 50 mL cold distilled water, followed by 10 mL and solution (1:1 HCl/ HNO_3). Carefully add 10 mL concentrated ammonia solution, sp. gr. 0.88, cool to room temperature, and dilute to 100 mL in a graduated flask. Return the solution to the

original beaker and transfer a 10 mL aliquot to a 100 mL graduated flask. Add 20 mL of the copper reagent, dilute to 100 mL with distilled water, and transfer to a 100 mL dry beaker. Allow to stand for 10-15 minutes, and then measure the absorbance with a spectrophotometer.

Construct a calibration curve using the synthetic standard solution: add the standard copper solution immediately before the reagent [1].

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* The following British Chemical Standard or Euronorm Certified Reference Materials may be used for practice in this determination: BCS No. 402: ECRM 084-1.

DETERMINATION OF LEAD AND COPPER IN STEEL

In the application of the polarographic method of analysis to steel a serious difficulty arises owing to the reduction of iron (III) ions at or near zero potential in many base electrolytes. One method of surmounting the difficulty is reduce iron (III) to iron (II) with hydrazinium chloride in a hydrochloric acid medium. The current near zero potential is eliminated, but that due to the reduction of iron (II) ions at about -1.4 volts us S.C.E. still occurs. Other metals (including copper and lead) which are reduced at potentials less negative than this can then be determined without interference from the iron. Alternatively, the Fe^{3+} to Fe^{2+} reduction step may be shifted to more negative potentials by complex ion formation.

The following procedure may be used for the simultaneous determination of copper and lead in plain carbon steels. Dissolve 5.0g of the steel, accurately weighed, in a mixture of 25 mL of water and 25 mL of concentrated hydrochloric acid: heat gently to minimize the loss of acid. Add a few drops of saturated potassium chlorate solution to dissolve carbides, etc. and boil the mixture until the solution is clear. Cool and dilute to 50 mL with water in a graduated flask. Pipette 2.00 mL of this solution into a polarogaphic cell and add: 1.0mL of 20 per cent hydrazinium chloride solution to reduce any iron (III) to the iron (II) state. 1.0 mL of 0.2 per cent methyl cellulose to act as a maximum suppressor, and 5.5 mL of 2.0 M sodium formate solution to adjust the pH of the solution to that at which reduction of Fe (III) and Cu (II) ions takes place. Place the cell in a nearly boiling water bath for 10 minutes in order to complete

the reduction. Cool. Analyze the solution polarographically: use a saturated calomel reference electrode. The first step in the polarogram is due to the reduction of copper (I) ions to the metal and has a half –wave potential of -0.25 volt us S.C.E. The second step, which is due to lead , has a half-wave potential of -0.45 us S.C.E. Carry out a calibration by adding known amounts of copper and lead to a solution of steel of low copper and lead content, and determine the increase in wave heights due to the additions [2].

Calculate the percentage of copper and of lead in the sample of steel.

DETERMINATIONS OF COPPER

Copper (II) compounds. Many other metallic ions which are capable of undergoing oxidation by potassium iodate can also be determined. Thus, for example. copper (II) compounds can be analysed by precipitation of copper (I) thiocyanate which is titrated with potassium iodate:

$$7IO_3^- + 4CuSCN + 18H^+ + 7CI^- = 7ICI + 4Cu^{2+} + 4HSO_4^- + 4HCN + 5H_2O$$

As a typical example, 0.8 g of copper (II) sulphate ($CuSO_4$, $5H_2O$) is dissolved in water, 5mL of 0.5M sulphuric acid added, and the solution made up to 250 mL in a graduated flask; 25.0mL of the resulting solution are pipetted into a 250mL conical flask, 10-15mL of freshly prepared sulphurous acid solution added, and then after heating to boiling, 10 per cent ammonium thiocyanate solution is added slowly from a burette with constant stirring until there is no further change in colour, and then 4mL of reagent is added in excess. After allowing the precipitate to settle for 10-15 minutes, it is filtered through a fine filter paper and then washed with cold 1 per cent ammonium sulphate solution until free from thiocyanate. It is then transferred quantitatively into the vessel in which the titration is to be performed, and after adding 30 mL of concentrated hydrochloric acid, followed by 20 mL of water, the titration is carried out in the usual manner either with an organic solvent present, or with an internal indicator being added as the end point is approached [3].

DETERMINATION OF COPPER AS THE DIETHYLDITHIIOCARBAMATE COMPLEX

Discussion : Sodium diethyldithiocarbamate (B) reacts with a weakly acidic or ammoniacal solution of copper (II) in low concentration to produce a brown colloidal suspension of the copper (II) diethyldithiocarbamate. The suspension may be extracted with an organic solvent (chloroform, carbon tetrachloride or butyl acetate) and the colored extract analyse spectrophotometrically at 560 nm (butyl acetate) or 436 nm (chloroform or carbon tetrachloride).

Many of the heavy metals give slightly soluble products (some white. some coloured) with the reagent most of which are soluble in the organic solvents mentioned above. The selectivity of the reagent may be improved by the use of masking agents, particularly EDTA.

The reagent decomposes rapidly in solutions of low pH.

Procedure. Dissolve 0.0393 g of pure copper (II) sulphate pentahydrate in I L of water in a graduated flask. Pipette 10.0 mL of this solution (containing about 100 μg Cu) into a beaker, and 5.0 mL of 25 per cent aqueous citric acid solution, render slightly alkaline with dilute ammonia solution and boil off the excess of ammonia: alternatively, adjust to pH 8.5 using a pH meter. Add 15.0 mL of 4 per cent EDTA solution and cool to room temperature. Transfer to a separatory funnel, add 10 mL of 0.2 percent aqueous sodium diethyldithio-carbamate solution, and shake for 45 seconds. A yellow-brown colour develops in the solution. Pipette 20 mL of butyl acetate (ethanoate) into the funnel and shake for 30 seconds. The organic layer acquires a yellow colour. Cool, shake for 15 seconds and allow the phases to separate. Remove the lower aqueous layer; and 20 mL of 5 percent sulphuric acid (v/v), shake for 15 seconds, cool, and separate the organic phase. Determine the absorbance at 560 nm in 1.0 cm absorption cells against a blank. All copper is removed in one extraction [4].

Repeat the experiment in the presence of 1 mg of iron (III); no interference can be detected.

DETERMINATION OF COPPER AS THE 'NEO-CUPROIN' COMPLEX

Discussion. 'Neo-cuproin' (2,9-dimethyl-1, 10-phenanthroline) can, under certain condition, behave as an almost specific reagent for copper (I). The complex is soluble in chloroform and absorbs at 457 nm. It may be applied to the determination of copper in cast iron, alloy steels, lead-tin solder, and various metals.

Procedure. To 10.0 mL of the solution containing up to 200 µg of copper in a separatory funnel. add 5.0 mL of 10 percent hydroxyl ammonium chloride solution to reduce Cu (II) to Cu (I), and 10 mL of a 30 per cent sodium citrate solution to complex any other metals which may be present. Add ammonia solution until the pH is about 4 (Congo red paper), followed by 10 mL of a 0.1 percent solution of 'neo-cuproin' in absolute ethanol. Shake for about 30 seconds with 10 mL of chloroform and allow the layers to separate. Repeat the extraction with a further 5 mL of chloroform. Measure the absorbance at 457 nm against a blank on the reagents which have been treated similarly to the sample [4].

Determination of copper as copper (I) thiocyanate:

Discussion. This is an method. Since most thiocyanates of other metals are soluble. Separation may thus be effected from bismuth. cadmium, arsenic, antimony, tin, iron, nickel, cobalt. manganese, and zinc. The addition of 2-3g of tartaric acid is desirable for the prevention of hydrolysis when bismuth, antimony, or tin is present. Excessive amounts of ammonium salts or of the thiocyanate precipitant should be absent, as should also oxidizing agents: the solution should only be slightly acidic, since the solubility of the precipitate increases with decreasing pH Lead, mercury. The precious metals, selenium, and tellurium interfere and contaminate the precipitate.
The essential experimental conditions are:

1. Slight acidity of the solution with respect to hydrochloric acid or sulphuric acid, since the solubility of the precipitate increases appreciably with decreasing pH;

2. The presence of a reducing anent, such as sulphurous acid or ammonium hydrogensulphite, to reduce copper (II) to copper (I);

3. a slight excess of ammonium thiocyanate, since a large excess increases the solubility of the copper (I) thiocyanate due to the formation of a complex thiocyanate ion;

4. The absence of oxidizing agents.

The reaction may be represented as :

$$2Cu^{2+} + HSO_3^- + H_2O = 2Cu^+ + HSO_4^- + 2H^+$$

$$Cu^+ + SCN^- = CuSCN$$
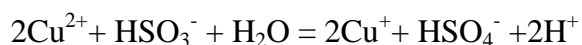
The precipitate is curdy (compare silver chloride) and is readily coagulated on boiling. It is washed with dilute ammonium thiocyanate solution; a little sulphurous acid or ammonium hydrogensulphite is added to the wash solution to prevent any oxidation of the copper (I) salt.

Procedure. Weigh out accurately about 0.4g of the copper salt (Note 1) into a 250 mL baker, and dissolve it in 50 mL water. Add a few drops of dilute hydrochloric acid, and then a slight excess (about 20-30 mL are required) of freshly prepared saturated sulphurous acid solution. Alternatively, add 25mL ammonium hydrogensulphite solution; the latter is prepared by diluting to ten times its volume the commercial concentrated solution, which has a specific gravity of 1.33 and contains about 54 per cent sulphur dioxide, Dilute the cold liquid 150-200 mL, heat nearly to boiling, and add freshly prepared 10 per cent ammonium thiocyanate solution, slowly and with constant stirring from a burette until present in slight excess. The precipitate of copper (I) thiocyanate should be white; the mother liquor should be colorless and smell of sulphur dioxide. Allow to stand for two hours, but preferably overnight. Filter through a weighed filtering crucible (sintered glass, or porcelain), and wash the precipitate 10 to 15 times with a cold solution prepared by adding to every 100 mL of water 1 mL of a 10 per cent solution of ammonium thiocyanate and 5-6 drops of saturated sulphurous acid solution, and finally several times with 20 per cent ethanol to remove ammonium thiocyanate (Note 2). Dry the precipitate to constant weight at 110-120°C (Note 3). Weigh as CuSCN[5].

Note. (1) Copper sulphate pentahydrate is suitable for practice in this determination 0.4g of this contains about 0.1 g of Cu.

(2) Alternatively, but less desirably, the precipitate may be washed with cold water until the filtrate gives only a slight reddish coloration with iron (III) chloride, and finally with 20 percent ethanol.

(3) The precipitate, collected in a sintered-glass (porosity No. 4) or porcelain filtering crucible may be weighed more rapidly as follows. Wash the copper (I) thiocyanate five or six times with ethanol. followed by a similar treatment with small volumes of anhydrous diethyl ether, then suck the precipitate dry at the pump for 10 minutes, wipe the outside of the crucible with a clean linen cloth and leave it in a vacuum desiccators for 10 minutes weight as CuSCN.

DETERMINATION OF COPPER (CONSTANT CURRENT PROCEDURE)

Discussion. Copper may be deposited from either sulphuric or nitric acid solution, but usually, a mixture of the two acids is employed. If such a solution is electrolyzed with an e.m.f. of 2-3 volts the following reactions occur:

Cathode: $Cu^{2+} + 2e = Cu$

$2H^+ + 2e = H_2$

Anode: $4 OH^- = O_2 + 2H_2O + 4e$
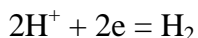
The acid concentration of the solution must not be too great, otherwise the deposition of the copper may be incomplete or the deposit will not adhere satisfactorily to the cathode. The beneficial effect of nitrate ion is due to its depolarizing action at the cathode:

$NO_3^- + 10 H^+ + 8e^- = NH^{4+} + 3H_2O$

The reduction potential of the nitrate ion is lower than the discharge potential of hydrogen, and therefore hydrogen is not liberated. The nitric acid must be free from nitrous acid, as the nitrite ion hinders complete deposition and introduces other complication. The nitrous acid may be removed (a) by boiling the nitric acid before adding it, (b) by the addition of urea to the solution:

$2H+ + 2NO_2^- + 2H^+ + 2NO_2^- + CO(NH_2)_2 = 2N_2 + CO_2 + 3H_2O$

or most efficiently (c) by the addition of a little sulphamic acid:

$H^- + NO_2^- + {}^-O.SO_2. NH_2 = N_2 + HSO_4^- + H_2O$

The action is rapid, and the acidity of the electrolyte is unaffected. The error due to nitrous acid increased by the presence of a large amount of iron; iron is reduced by the current to the iron (II) state, whereupon the nitric acid is reduced. This error may be minimized by the proper regulation of the pH and by the addition of ammonium

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nitrate instead of nitric acid or, best by the removal of the iron prior to the electrolysis, or by complication with phosphate or fluoride.

The solution should be free from the following which either interfere or lead to an unsatisfactory deposit: silver, mercury, bismuth, selenium, tellurium, arsenic, antimony, tin, molybdenum, gold and the platinum metals, thiocyanate, chloride, oxidising agents such as oxides of nitrogen, or excessive amounts of iron (III), nitrate or nitric acid. Chloride ion is avoided because Cu (I) is stabilised as a chloro-complex and remains in solution to be re-oxidized at the anode unless hydrazinium chloride is added as depolarizer.

The electrolytic deposit should be salmon-pink in colour, silky in texture, and adherent. If it is dark, the presence of foreign elements and or oxidation is indicated. Spongy or coarsely crystalline deposits are likely to yield high results; they arise from the use of too high current densities or improper acidity and absence of nitrate ion.

Procedure. The solution (100 mL) may contain 0.2-0.3g of Cu (see Note). Add cautiously 2mL of concentrated sulphuric acid, 1mL of concentrated nitric acid free from nitrous acid by boiling or by the addition of a little urea, or, better, 0.5 g of sulphamic acid), and transfer to, unless already present in the electrolysis vessel. Clean, dry and weight the platinum gauze cathode as described in section 12.7 then assemble the apparatus (complete with a magnetic stirrer) on the electro-deposition apparatus; the electrodes should be clamped so that they are about 80-90 per cent covered by the solution. Cover the beaker with the split clock glass, and following the instructions pertaining to the electrolysis and, switch on the stirrer, and then (at a minimum setting) the electrolysis current: finally adjust the latter so that a potential difference of 3-4 volts is applied to the cell and the current is 2-4A. Continue the electrolysis until the blue colour of the solution has entirely disappeared (usually somewhat less than I hour), reduce the current to 0.5 – 1A, and test for completeness of deposition by rinsing the split clock glass, raising the level of the liquid by about 0.5 cm by the addition of distilled water, and continuing the electrolysis for 15-20 minutes. If no copper plates out on the fresh surface of the cathode electrolysis may regarded as complete.

Lower the beaker very slowly, or raise the electrodes, and at the same time direct a continuous stream of distilled water from a wash bottle against the upper edge of the cathode. This washing must be done immediately the cathode is removed out of the solution, and the circuit must not be broken during the process. When the cathode has been thoroughly washed, break the circuit, dip the cathode into a beaker of distilled water, and then rinse it with analytical grade acetone. Dry at $100\text{-}110^0C$ for 3-4 minutes, and weigh after cooling in air for about 5 minutes.

From the increase in weight of the cathode, calculate the copper content of the solution. After the cathode has been weighed, It should be cleaned with nitric acid and re-weighed; the loss in weight will serve as a check [6].

It is needless to emphasize further that the direct spectrophotometric method in non-extractive way is more useful if it offers higher sensitivity and selectivity. Search should be directed to a new method in order to develop simpler and simple spectrophotometric method for non-extractive estimation of copper in very sensitive and selective way suitable for environmental chemical analysis. The present method [35] described here records for the first time the direct spectrophotometric determination of copper in aqueous media without recourse to any "clean up" step. The method [35] is far more selective (virtually specific) sensitive, non-extractive, simple and rapid than all the existing spectrophotometric method [7-34] and has been successfully tested upon some industrial waste water, environmental water. The method is very reliable and concentration in the µg range in aqueous medium at room temperature (25^0C) can be measured in a very simple and rapid way.

B. SPECTROPHOTOMETRIC DETERMINATION OF TRACE AMOUNT COPPER IN AQUEOUS MEDIA.

INTRODUCTION:

Different types of legend were used with about 30 toxic metal ions to obtain color chelate through the novel reaction techniques. Finally Trace amount of toxic element copper was determined by spectrophotometric method using 1-(2-pyridylazo)-2-naphthal (PAN) as a new spectrophotometric reagent.

Copper in trace amounts is important industrially [36], as a toxicant [37] and biological non-essential [37], as an environmental pollutant [38] and as an occupational hazard [39] It is a toxic metal, has been responsible for a number of diseases [40]. The symptoms of copper poisoning are instantaneous hypertension, shortening of life-span; Kidney damage, bronchitis, retardation of growth, cirrhosis, Wilson's, Alzheimer's diseases, gross abnormalities of the vital organs and the risk of skin cancer [41]. It also caused generalized cancers in laboratory animals and has been linked epidemiologically with certain human cancers [41]. The most serious situations being the disease called cirrhosis, Wilson's, Alzheimer's diseases and skin cancer which causes gradual weakening of the bone structure, diminution of stature and ultimately the total collapse of the entire skeletal system [42]. Its extreme toxicity towards marine and fresh water organisms is also well known [42]. Copper is a potential health hazard due to its presence in drinking water, food cooked in copper utensil [42]. The permissible limit of copper in drinking water is 2.0 mgL^{-1} according to EPA [43]. Increasing copper pollution of the environment resulting from the growth of copper based industries and the use of fossil fuels makes the development of method for the trace and ultra-trace analysis of this toxic metal essential.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. 1-(2-pyridylazo)-2-naphthal (PAN) has been reported as a spectrophotometric reagent for Co, Ni, Zn, Mn, Ca [44] but has not previously been used for spectrophotometric determination of Copper in aqueous media. This paper reports its use in a very sensitive, highly specific spectro-photometric method for the trace determination of copper. The method possesses distinct advantages over existing methods [7-34] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision and ease of operation. The method is based on the reaction of non-absorbent 1-(2-pyridylazo)-2-naphthal (PAN) in highly acidic solution (1M HCl) with copper to produce a highly absorbent deep pink chelate product, followed by direct measurement of the absorbance in aqueous solution. With suitable masking, the reaction can be made highly selective.

EXPERIMENTAL

Apparatus :

A shimadzu (Kyoto, Japan) (Model-1601PC) double beam UV/VIS recording spectrophotometer and Jenway (England, U.K.) (Model-3010) pH-meter were used for the measurement of absorbance and pH, respectively. A Shimadzu (Model-AA 6200) atomic absorption spectrophotometer equipped with a micro computer-controlled nitrous oxide-acetylene flame was used for comparison of the results.

Reagents and Solutions:

All the chemicals used were of analytical-reagent grade of 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 $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with nitric acid (1+1) and rinsed several times with high-purity de-ionized water. Stock solutions and environmental water samples (1000mL each) were kept in polypropylene bottles containing 1 mL of concentrated HNO_3. More rigorous contamination control was used when the copper levels in the specimens were low.

1-(2-pyridylazo)-2-naphthal (PAN) Solution, 4.01x 10^{-4} M :

Prepared by dissolving the requisite amount of 1-(2-pyridylazo)-2-naphthal (PAN) (BDH chemicals) in a known volume of highly acidified (HCl) de-ionized water. More dilute solutions of the reagent were prepared as required. PAN is insoluble in water but soluble in acidic water and organic solvent.

Copper Standard Solutions:

A 100-mL of stock solution of divalent copper was prepared by dissolving 0.03929mg of AR crystallize copper sulfate (Cu SO_4. $5H_2O$) (Merck) in doubly distilled de-ionized water. Aliquots of this solution were standardized by EDTA titration using Sulfon black-T as indictor. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water and when required.

EDTA Solution :

100-mL stock solution of EDTA (0.01% W/v) was prepared by dissolving 10 mg of A.C.S.-grade ($\geq$ 99%) of disodium dihydrogen ethylenediamine tetraacetate dihydrate in (100-mL) de-ionized water.

Potassium permanganate Solution:

A 1% potassium permanganate Solution (Merck) was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid. Sodium azide solution(2.5% W/v) (Fluka purity >99%) was also used.

Tartarate Solution :

A 100-mL stock solution of tartarate (0.01% W/v) was prepared by dissolving 10 mg of A.C.S grade (99%) potassium sodium tartarate tetrahydrate in (100-mL) de-ionized water.

Aqueous Ammonia Solution :

A 100-mL solution of aqueous ammonia was prepared by diluting 10-mL concentrated NH_4OH (28 – 30%, A.C.S grade) to 100-mL with deionized water. The solution was stored in polypropylene bottle.

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); those of niobium, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukharjee [45] In the case of insoluble substances, special dissolution methods were adopted [46].

Procedure :

To 0.1 – 1.0 mL of a neutral aqueous (pH= 4) solution containing 1 – 10 µg of copper in a 10-mL calibrated flask was mixed with 1 : 5 -1 : 10 fold molar excess of 1-(2-pyridylazo)-2-naphthal (PAN) reagent solution preferably 0.01% or 4.01 x 10^{-4}M followed by the addition of 0.5 M hydrochloric acid (HCl) and NaOH or NH_4OH to control pH of solution at around 2.5. The mixture was diluted to the mark with de-ionized water. The absorbance was measured at 550 nm against a corresponding reagent blank. The copper content in an unknown sample was determined using concurrently prepared calibration graph.

Factor Affecting the Absorbance:

Absorption spectra :

The absorption spectra of the copper-1-(2-pyridylazo)-2-naphthal (PAN) solution system in 1(M) HCl medium was recorded using the UV-Vis spectrophotometer. The absorption spectra of the copper-PAN is a symmetric curve with the maximum absorbance co-efficient is shown in Fig-1. In all instances measurements were made at 550 nm against a reagent blank. The reaction mechanism of the present method is as reported earlier [47].

Identification:

Name: 1-(2-Pyridylazo)-2-naphthol, Synonyms: PAN, Molecular Structure
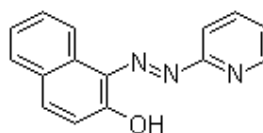
Fig: Structure of 1-(2-pyridylazo)-2-naphthal (PAN).

Molecular Formula: $C_{15}H_{11}N_3O$, Molecular Weight: 249.27

Properties Melting point: 138-141 ℃ Water solubility: Insoluble but soluble in organic solvent. We have used acidic water to dissolve PAN.
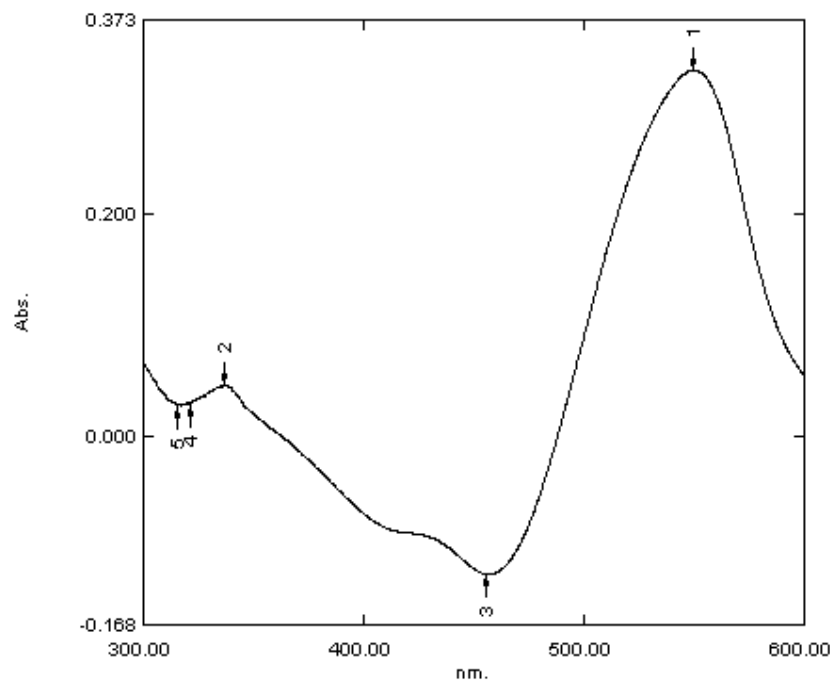
Fig.-1 : Absorption spectra of copper-1-(2-pyridylazo)-2-naphthal (0.005%) against the reagent blank (at pH=2.5, λ_{Max}= 550 nm) in aqueous solution.

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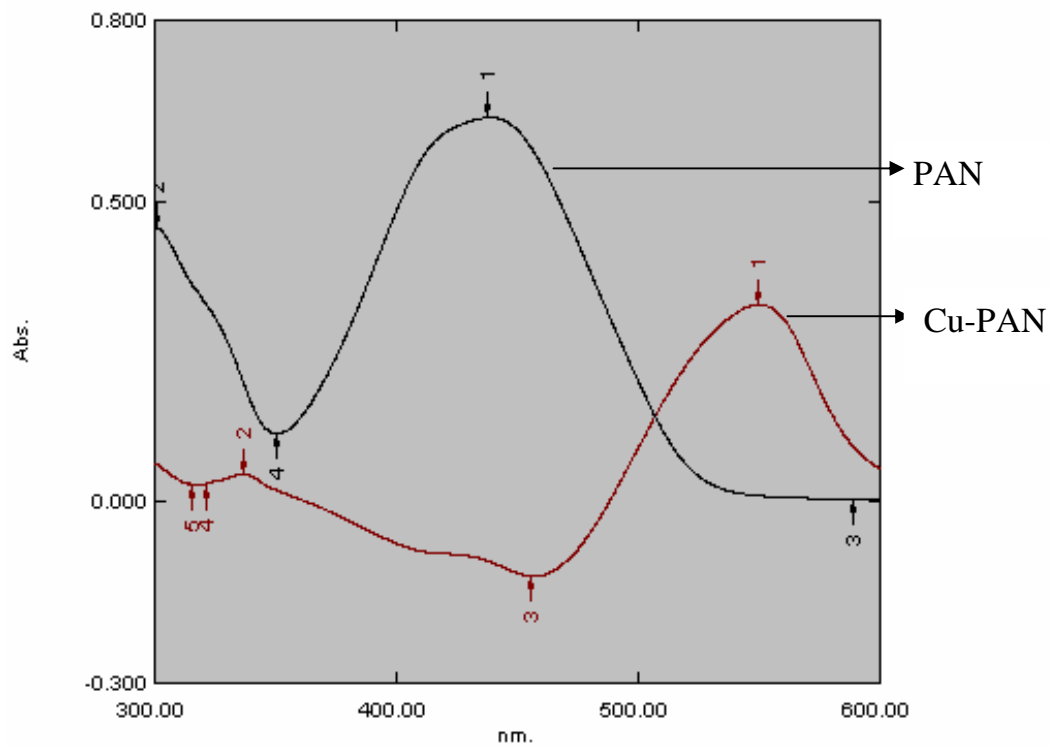
Fig. : Absorption spectra of 1-(2-pyridylazo)-2-naphthal (PAN) and copper-PAN in aqueous solution.
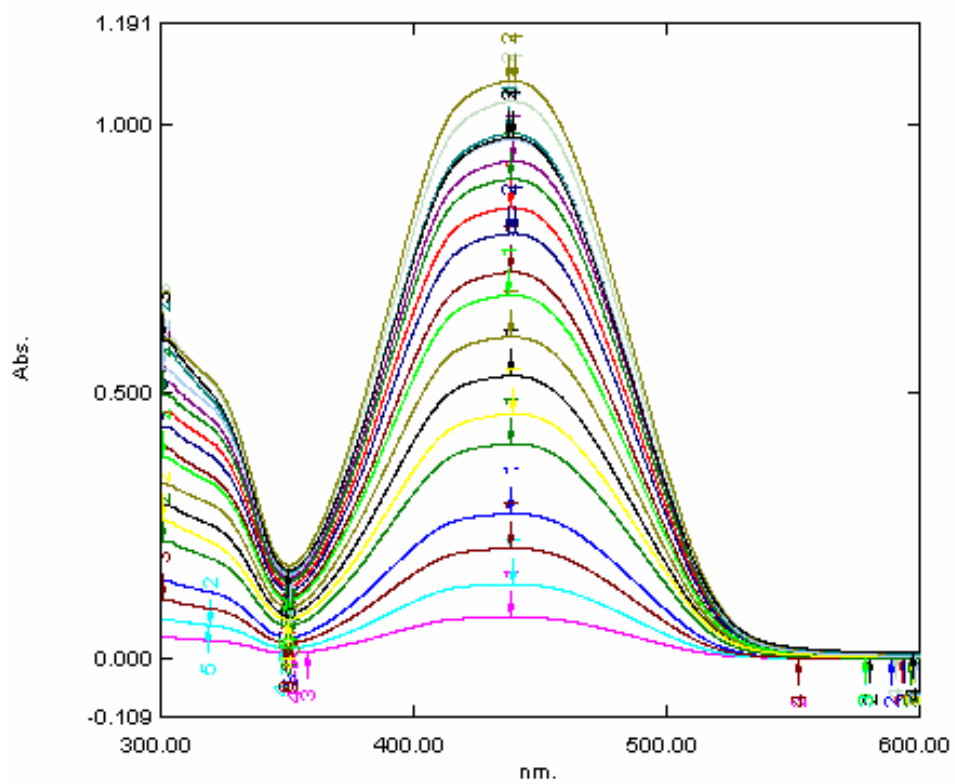
Fig. : Absorption spectra at different concentration of 1-(2-pyridylazo)-2-naphthal (4.01 x 10^{-6}M to 8.03 x 10^{-5}M) at pH=2.5, λ_{Max}= 440 nm in aqueous solution.
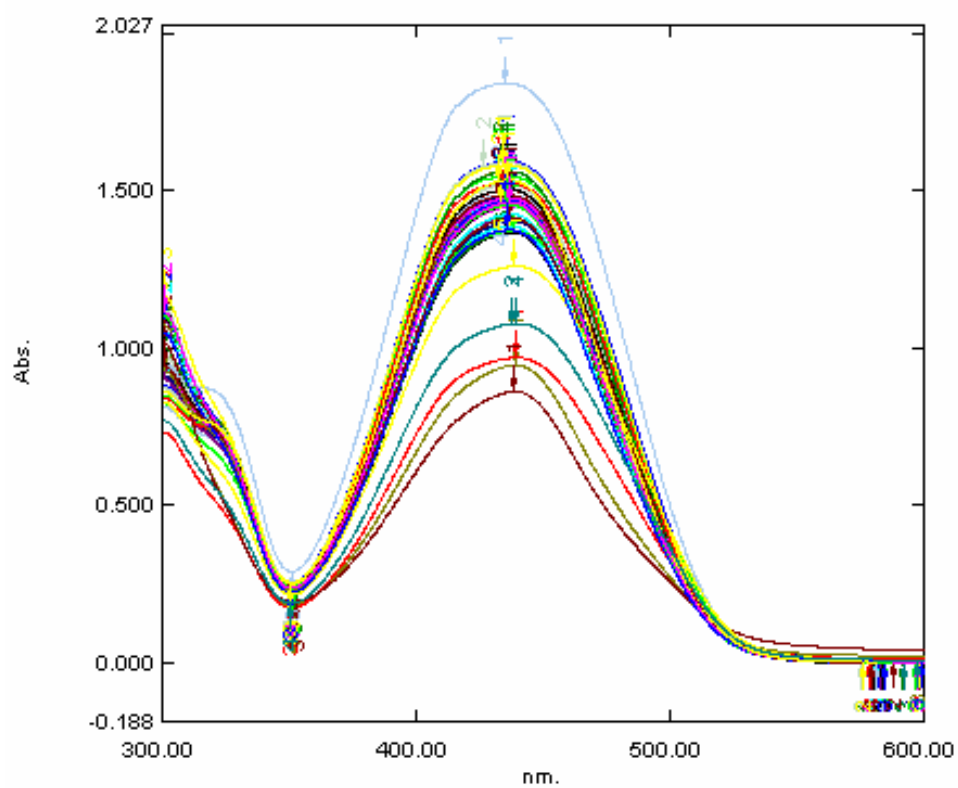
Fig.-: Absorption spectra at different pH (1.0 -3.0) of 1-(2-pyridylazo)-2-naphthal (8.03 x 10^{-5}M) at λ_{Max}= 440 nm in aqueous solution.

Effect of Acidity:

To see the effect of different pH we have taken 0.5 ppm copper solution. Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied hydrochloric acid was found to be the best acid for the system. The absorbance was maximum when the pH of the solution is 2.5 at room temperature $(25 \pm 5)^0$C. We have controlled the pH of the solution using hydrochloric acid and ammonium hydroxide, NaOH or KOH as a base and double distilled water. Outside this range of acidity, the absorbance decreased (Fig.-2). At λ_{Max}= **550 nm** the absorbance values of Copper-PAN complex are as follows. At higher concentration of PAN solution starts to form ppt. at pH 2.0. So we have used the lower concentrated solution (0.01% or 4.0 x 10^{-4}M) of PAN.
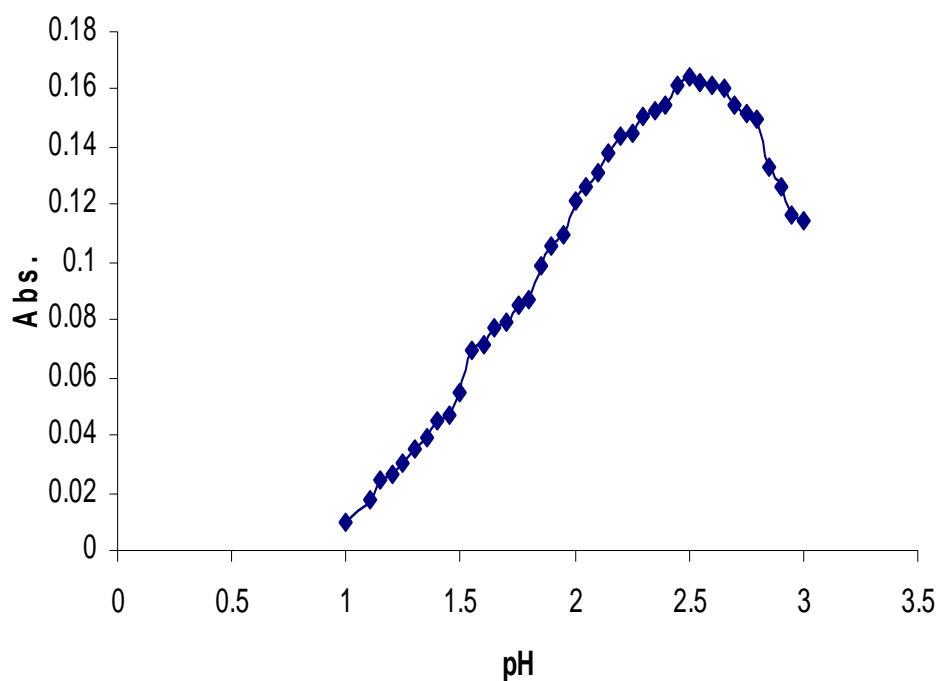
Fig.-2: Effect of pH on the absorbance of Cu-1-(2-pyridylazo)-2-naphthal (1:10) complex.

Table-1 (Effect of pH)

pH of Copper-PAN complex solution	Absorbance of Copper-PAN complex at λ_{Max}= 550 nm
1.00	0.010
1.10	0.018
1.15	0.024
1.20	0.026
1.25	0.030
1.30	0.035
1.35	0.039
1.40	0.045
1.45	0.047
1.50	0.055
1.55	0.069
1.60	0.071
1.65	0.077
1.70	0.079
1.75	0.085
1.80	0.087
1.85	0.099
1.90	0.106
1.95	0.110
2.00	0.121
2.05	0.126
2.10	0.131
2.15	0.138
2.20	0.144
2.25	0.145
2.30	0.151
2.35	0.153
2.40	0.155
2.45	0.161
2.50	0.164
2.55	0.162
2.60	0.161
2.65	0.160
2.70	0.155
2.75	0.152
2.80	0.150
2.85	0.133
2.90	0.126
2.95	0.116
3.00	0.114
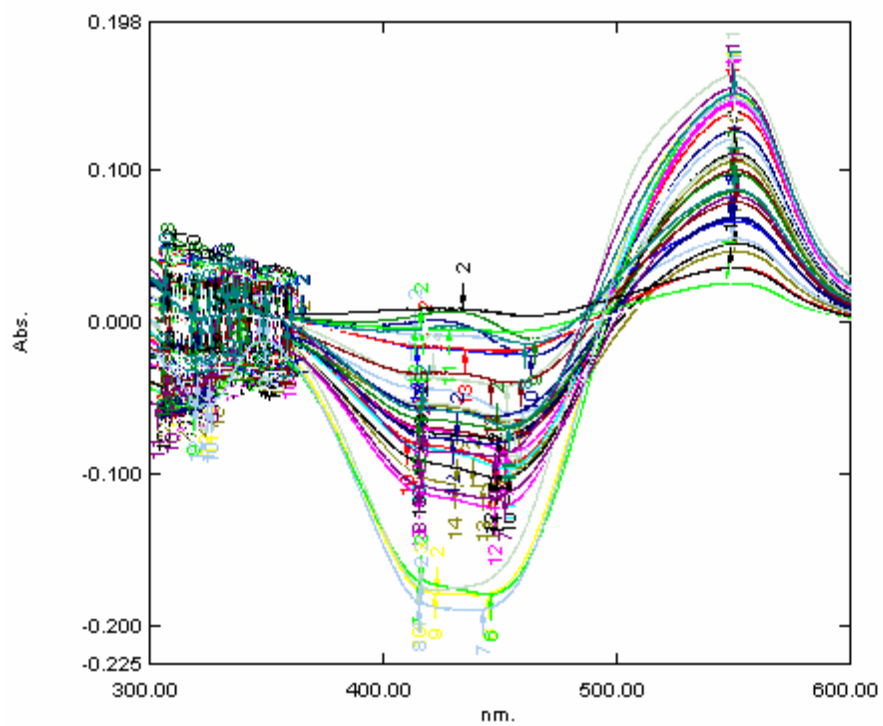
Fig: The overlay of absorption spectra of copper-PAN(1:10) at different pH(1.00-3.00).

Effect of Time:

The reaction is instantaneous. Constant maximum absorbance was obtained just after diluting to volume and remained strictly unaltered for 48 hours (Fig.-3 A,B). At pH over 3.0 copper start to form ppt with the solution of PAN. When the solution from ppt it go out of the measurement of spctrophotomatric analysis. We have fixed the pH at 2.5, in this pH the complex will not form ppt for the long time. We have observed sample of complex for 48 hours and constant maximum absorbance was obtained just after diluting to volume and remained strictly unaltered form beginning to end.

Table-2

Duration of Time (min)	Absorbance of Copper-PAN complex at λ_{Max}= 550 nm
0.0	0.324
05	0.324
10	0.324
15	0.324
20	0.324
25	0.324
30	0.324
60	0.324

Table -3

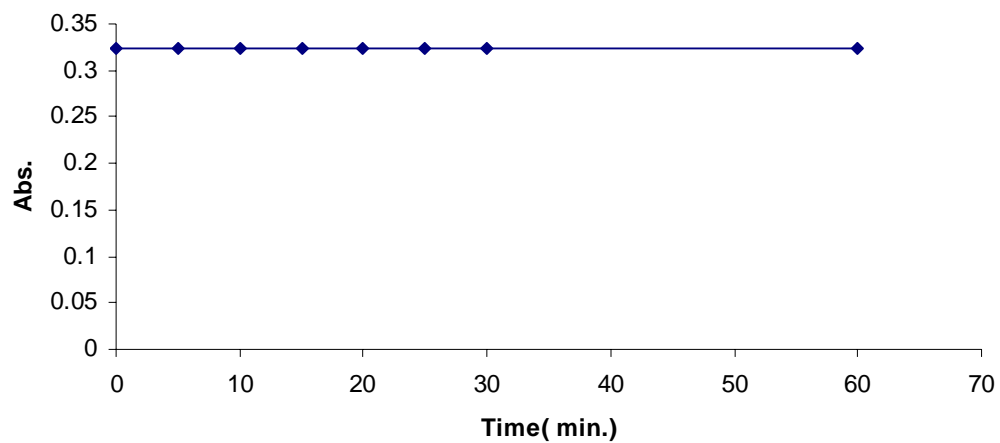| Duration of Time (hrs)	Absorbance of Copper-PAN complex at λ_{Max}= 550 nm
1	0.324
2	0.324
3	0.324
4	0.324
5	0.324
10	0.324
24	0.324
48	0.324

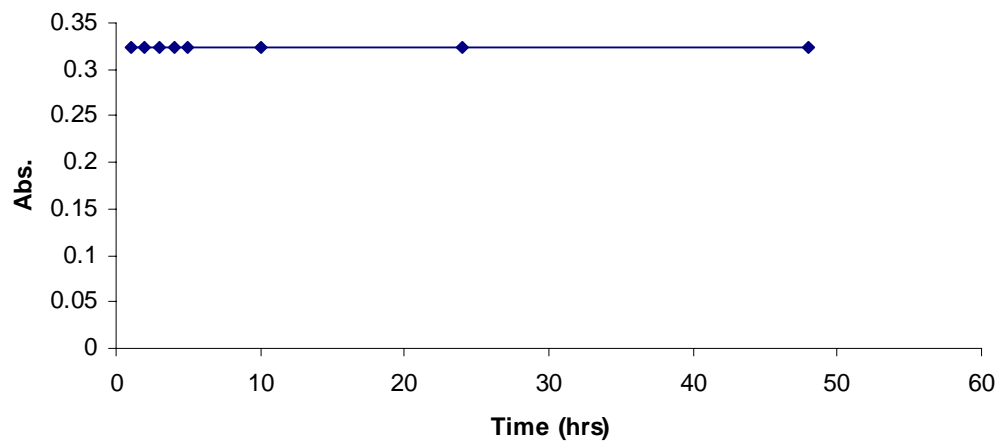**Fig.-3 (A) :** Effect of the time on the absorbance of Copper-PAN(1:10) system.

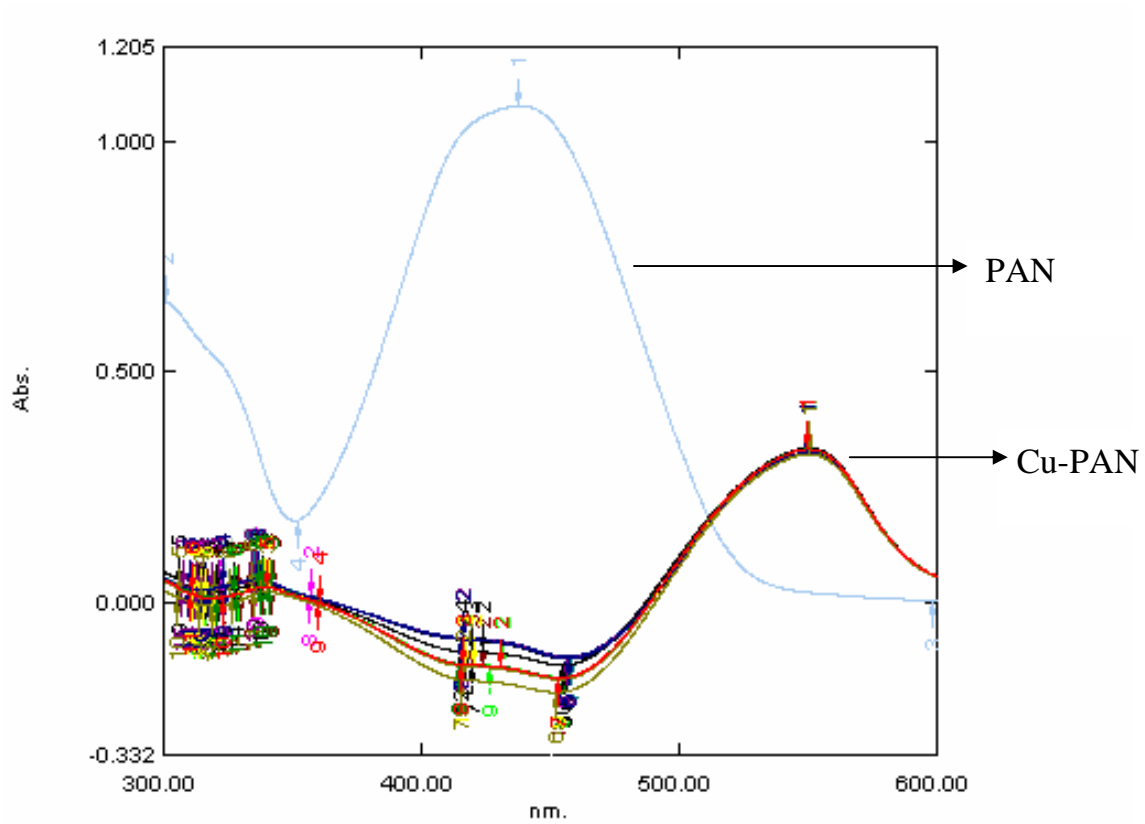**Fig.-3 (B) :** Effect of the time on the absorbance of Copper-PAN(1:10) system.

Fig: The overlay of absorption spectra of copper-PAN(1:10) at different time.

Effect of Temperature:

The copper-1-(2-pyridylazo)-2-naphthal (PAN) system obtained maximum and constant absorbance at room temperature $(25\pm5)^0$C. Outside this range of temperature, the absorbance decrease gradually. The results are as follows:

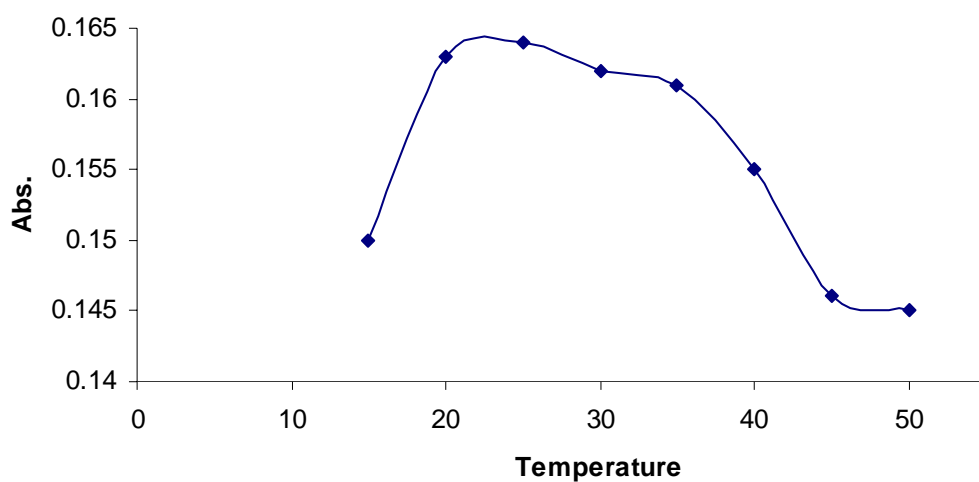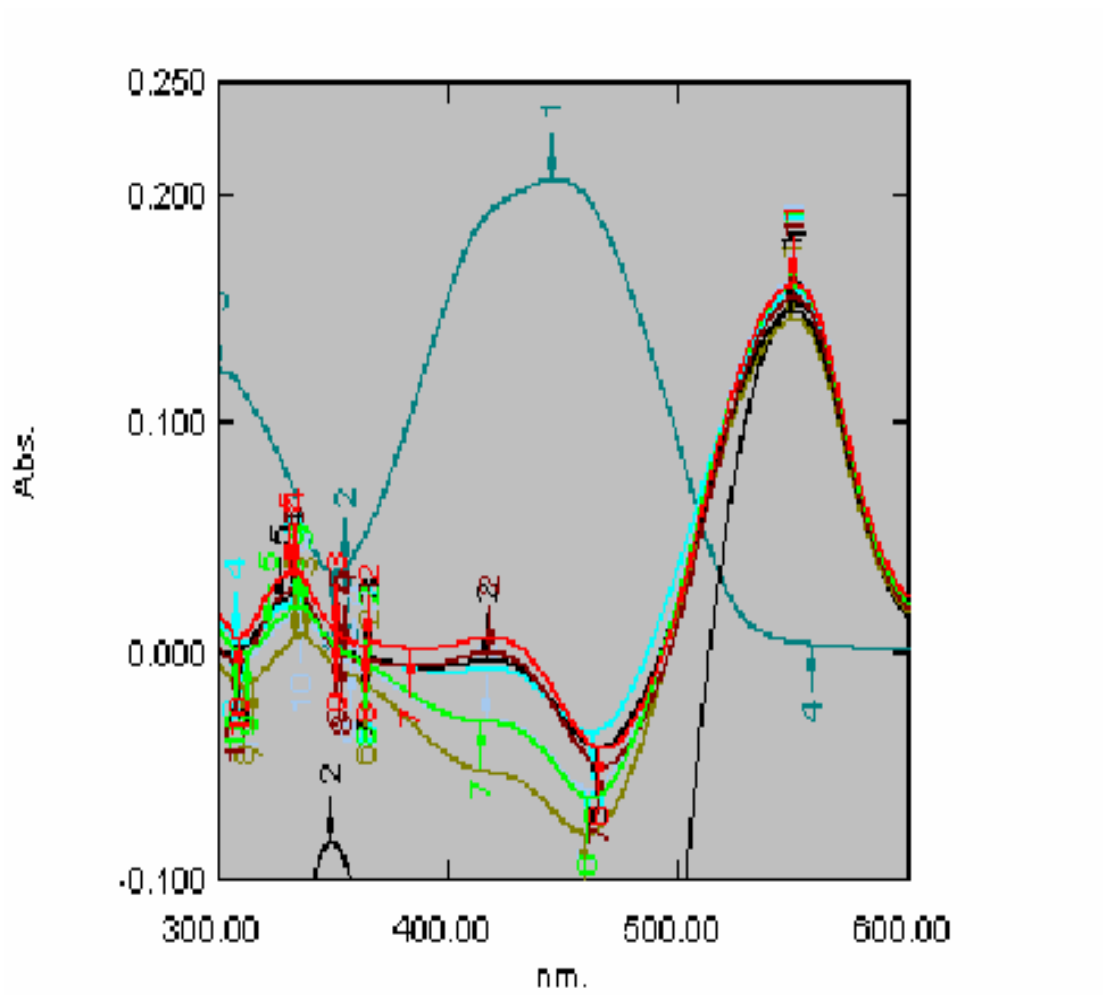| Temperature(^{0}C)	Absorbance of Copper-PAN complex at λ_{Max}= 550 nm
15	0.150
20	0.163
25	0.164
30	0.162
35	0.161
40	0.155
45	0.146
50	0.145

Fig: Effect of Temperature

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Fig: The overlay of absorption spectra of copper-PAN at different Temperature

Effect of Reagent Concentration:

Different molar excesses of 1-(2-pyridylazo)-2-naphthal (PAN) were added to fixed metal ion concentration and absorbance were measured according to the standard procedure. It was observed that at the 1 ppm copper metal the reagent molar ratios of 1:2-1:20 produce a constant absorbance of the Cu-chelate (Fig.-4). For all subsequent measurements different amount (mL) of 4.01×10^{-4}M 1-(2-pyridylazo)-2-naphthal (PAN) reagent was added.

Table-4 (Effect of reagent concentration)

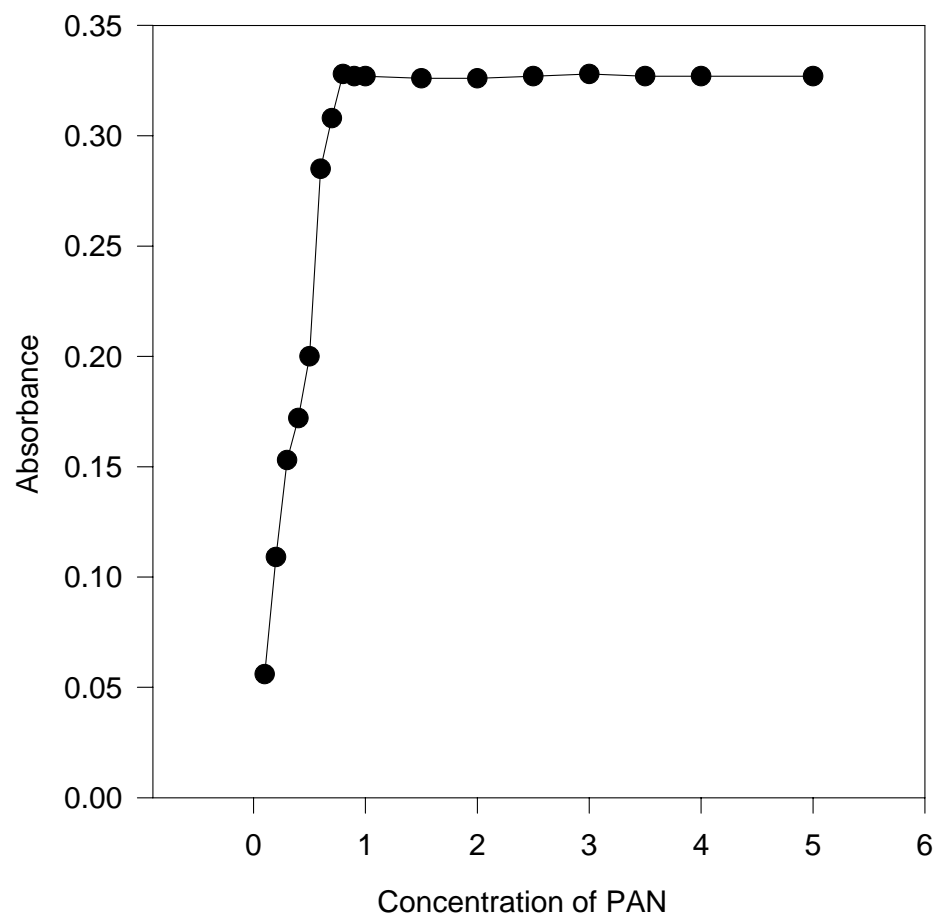| Amount of PAN (ml) of 0.01% solution	Absorbance of Copper-PAN complex at λ_{Max}= 550 nm
0.1	0.056
0.2	0.109
0.3	0.153
0.4	0.172
0.5	0.200
0.6	0.285
0.7	0.308
0.8	0.328
0.9	0.327
1.0	0.327
1.5	0.327
2.0	0.326
2.5	0.327
3.0	0.327
3.5	0.327
5.0	0.327

Fig.-4: Effect of reagent copper-1-(2-pyridylazo)-2-naphthal (PAN) molar concentration ratio on the absorbance of copper-PAN system.
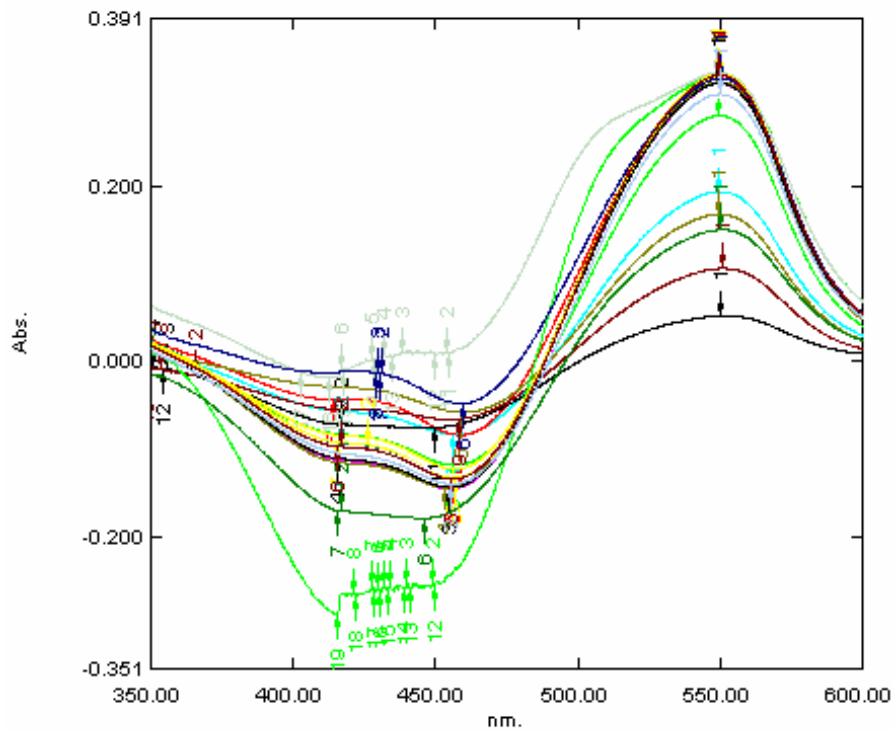
Fig: The overlay of absorption spectra of copper-PAN at different reagent (PAN) concentration (0.0001%-0.005% or 4.01 x 10^{-6}M-2.00 x 10^{-4}M).

Calibration Graph (Beer's law and Sensitivity):

The well-known equation for spectrophotometric analysis in very dilute solution was derived from Beer's law. The effect of metal concentration was studied over 0.1-5.0 µg mL^{-1} distributed in two different sets (0.1 – 2.5 and 1 – 5.0 µg mL^{-1} for convenience of measurement. The absorbance was linear for 0.1 – 2.50µg mL^{-1} of copper at 550 nm. The molar absorption co-efficient [48] was found to be 2.052 x 10^4 L mol^{-1} cm^{-1}. Of the calibration graph which that showing the limit of linearity range is given in Fig.-5 (A).

Table-5: Calibration Graph (Beer's Law and Sensitivity)

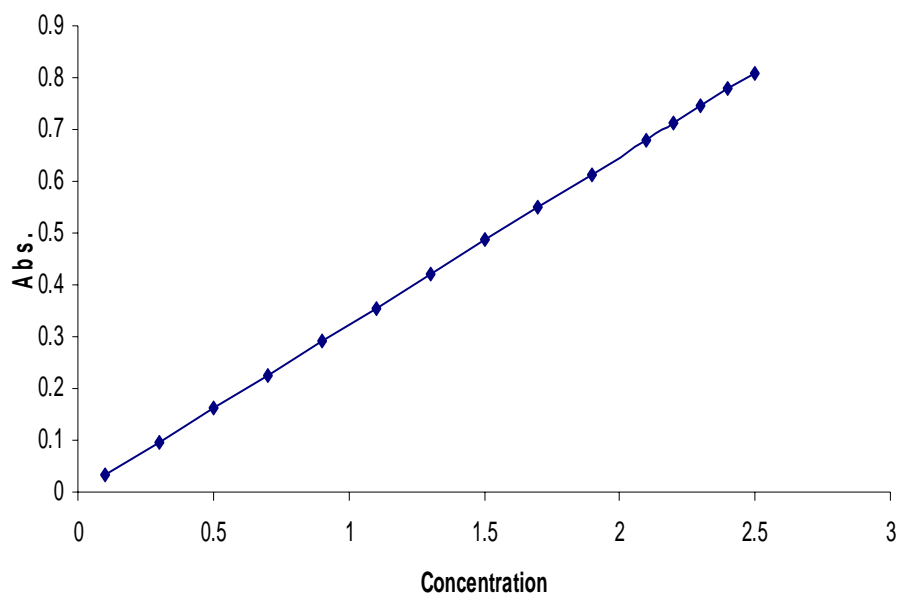| Concentration of copper (ppm)	Absorbance of Cu –PAN(0.005%) complex at pH= 2.5, λ_{max} =550 nm
0.1	0.032
0.2	0.064
0.3	0.097
0.4	0.129
0.5	0.162
0.6	0.194
0.7	0.226
0.8	0.258
0.9	0.291
1.0	0.323
1.1	0.355
1.2	0.388
1.3	0.420
1.4	0.453
1.5	0.486
1.6	0.517
1.7	0.549
1.8	0.581
1.9	0.614
2.0	0.646
2.1	0.680
2.2	0.712
2.3	0.745
2.4	0.778
2.5	0.810
3.0	0.940
3.5	1.089
4.0	1.216
4.5	1.310
5.0	1.400

Fig: 5(A) Calibration graph of absorbance of Cu –PAN (0.005%) complex against the

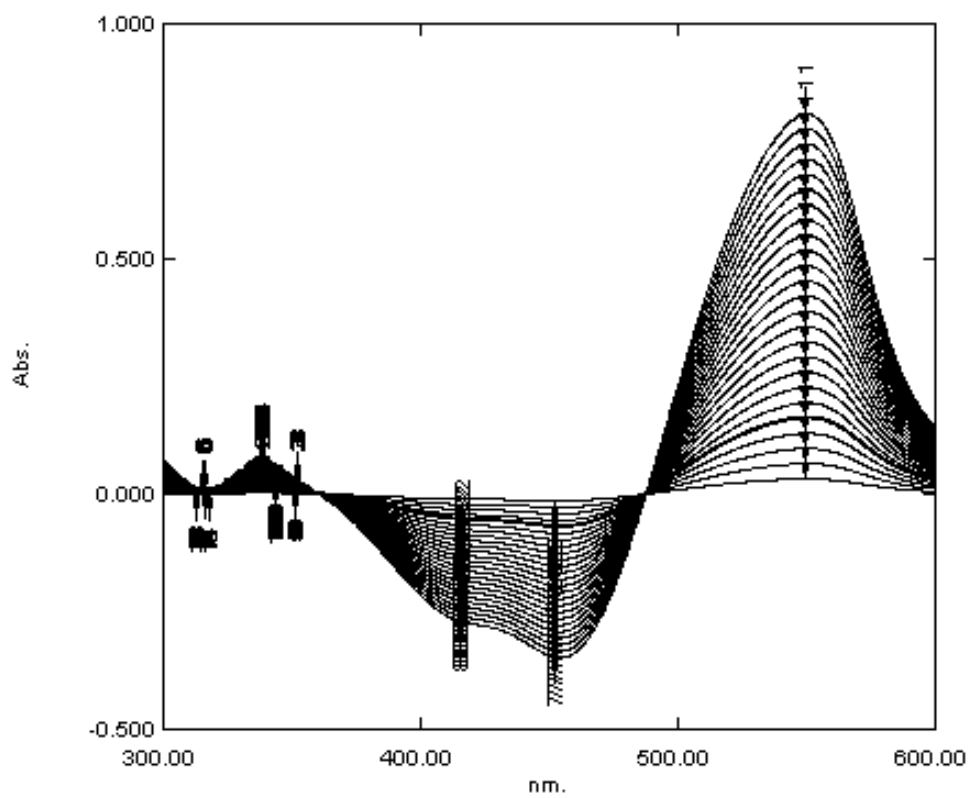different concentration of Cu (0.1 to 2.5 ppm) at pH= 2.5, λ_{max} =550 nm.

Fig: The overlay of absorption spectra of copper-PAN (0.005%) for 0.1 – 2.5 µg mL^{-1} of
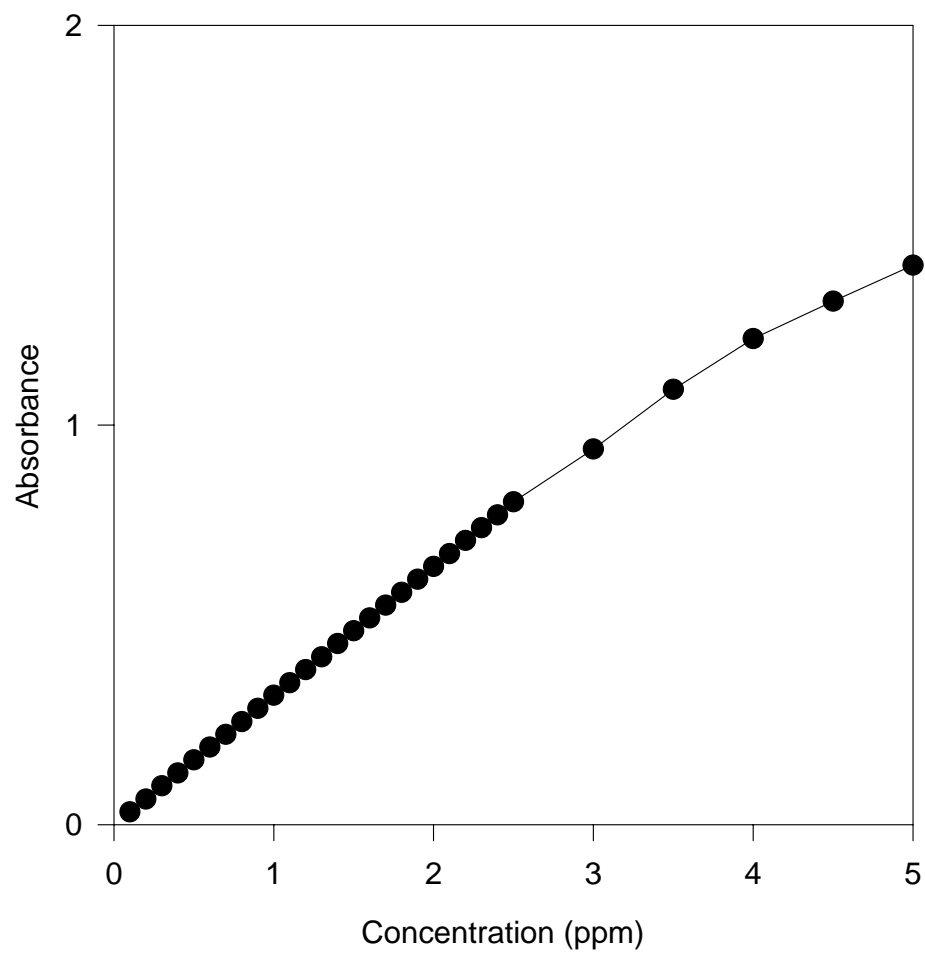Copper at pH= 2.5, λ_{max} =550 nm.

Fig.-5 (B): Calibration graph of absorbance of Cu –PAN (0.005%) complex against the

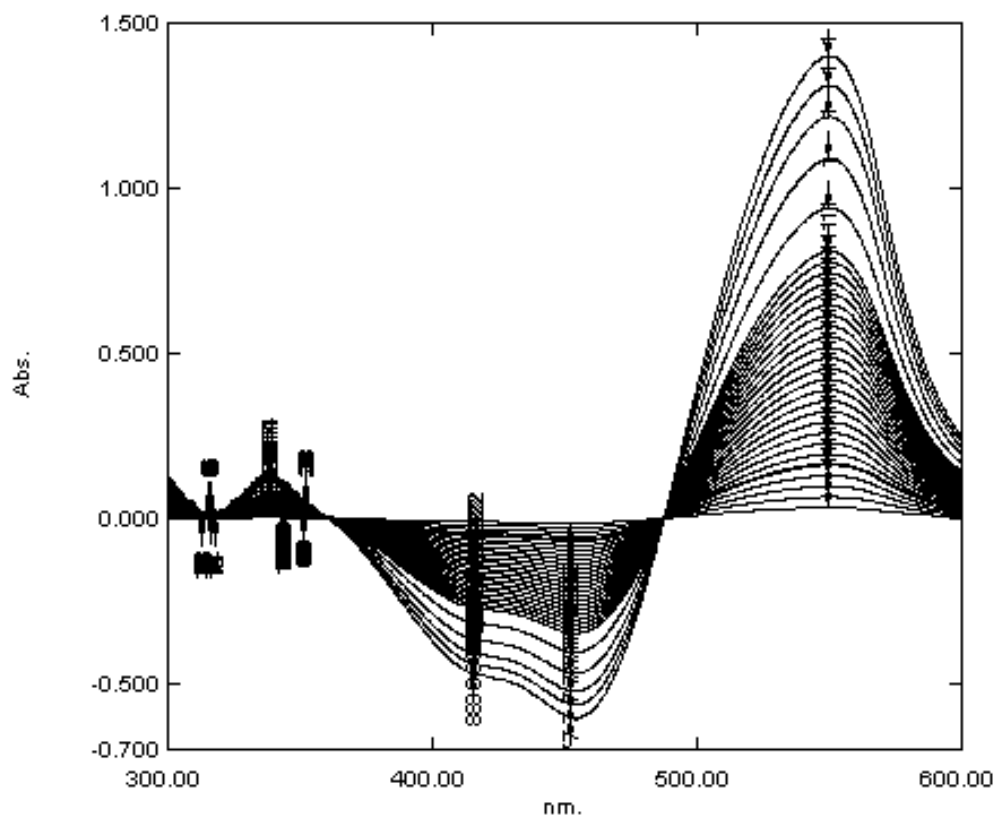different concentration of Cu (0.1 to 5.0 ppm) at pH= 2.5, λmax =550 nm.

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Fig: The overlay of absorption spectra of copper-PAN (0.005%) for 0.1 –5.0 µg mL^{-1} of Copper at pH= 2.5, λmax =550 nm.

The selected analytical parameters obtained with the optimization experiments are summarized in table-6.

Table-6 : SELECTED ANALYTICAL PARAMETERS OBTAINED WITH THE OPTIMIZATION EXPERIMENTS.

Parameter	Studied range	Selected Value
Wavelength/λmax(nm)	200 - 800	550
pH	1.0 – 3.5	2.45-2.55
Time/h	0 - 72	24
Temperature/°C	1 - 50	25 ± 5
Reagent (fold molar excess, M: R)	1 : 2 – 1: 20	1 : 5 – 1: 10
Linear range/µg/mL^{-1}	0.01 - 20	0.1 – 5.0
Detection limit/µg mL^{-1}	1 - 20	0.1–2.50
Reproducibility (% RSD)	0 - 2	0 – 2

Precision and accuracy:

The precision of the present method was evaluated by determining different concentrations of copper (each analyzed at least five times). The relative standard deviation (n = 5) was 2 – 0% for 1 – 2.5µg of copper in 10-mL, indicating that this method is highly precise and reproducible (Table-7). The detection limit is 0.1-2.5 µg. Added copper was accurately recovered from the other metals (Table-9). The reliability of our Cu-Chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of copper to some environmental water and industrial waste water samples was quantitative as shown in (Table-10). The results of water samples analysis by the spectrophotometric method were in excellent agreement with those obtained by ASS. Hence the precision and accuracy of the method were found to be excellent.

TABLE-7: STANDARD DEVIATION AND RELATIVE STANDARD DEVIATION OF Cu (II)- 1-(2-PYRIDYLAZO)-2-NAPHTHAL (PAN) SYSTEM.

Sample No.	Cu (II) taken μgL^{-1}	Cu (II) Found X_1 μgL^{-1}	Mean $\bar{X}$ μgL^{-1}	$X_1 - \bar{X}$	$(X_1 - \bar{X})^2$	Standard deviation $(\pm S)$	Relative standard deviation $(S_r)\%$
1	100.0	98.5		1.64	2.67		
2	100.0	99		1.14	1.29		
3	100.0	101		0.86	0.75		
4	100.0	100.5		0.36	0.13		
5	100.0	99.5		0.64	0.41		
6	100.0	101.5	100.14	1.36	1.85	± 1.16	1.16
7	100.0	101		0.86	0.74		
8	100.0	99.5		0.64	0.41		
9	100.0	98.5		1.64	2.69		
10	100.0	101.5		1.36	1.85		
11	100.0	101		0.86	0.74		
N = 11		$\sum X_1 =$ 1101.5		$\sum X_1 - \bar{X} =$ 11.364	$\sum (X_1 - \bar{x})^2 =$ 13.53		

Mean $\bar{X}$ $\dfrac{\sum X_1}{N} = \dfrac{1101.5}{11} = 100.14$

Standard deviation, $S = \sqrt{\dfrac{\sum (X_1 - X)^2}{N-1}} = \sqrt{\dfrac{13.53}{11-1}} \quad \sqrt{1.353} = \pm 1.16$

Relative Standard deviation $(S_r)\%$ $= \dfrac{S}{X} \times 100$

$= \dfrac{1.16 \times 100}{100.14}$

$= 1.16$

Effect of foreign ions:

The effect of over 50 ions and complexing agents on the determination of only 1µg mL^{-1} of copper was studied. The criterion for interference [49] was an absorbance value varying by more than ± 5% from the expected value for copper alone. The results are summarized, in (Table-8). A large number of ions have no significant effect on the determination of copper. The quantities of these diverse ions mentioned (Table-7) were the actual amounts added and not tolerance limits. The most serious interferences were from Ni (II), Co (II) and Fe (III) ions. Interference from these ions is probably due to complex formation with PAN.

The greater tolerance limits for these ions can be achieved by using several masking agents. In order to eliminate the interference if Ni and Co are present in the sample they are eliminated by extraction from the sample using 15 ml of 10×10^{-3} mole/L DMG solution in 1,2-dichloroethane. If the aqueous phase contains mercury and iron, 1g of KI was added masking agent for Hg and 10g of KF dissolved in 4 mole/L HNO$_3$ solution was used to preclude the interference of iron [50].
 Interference from these metal ions Ni (II), Co (II) and Fe (III) have been effectively removed by a short single step ion- exchange separation process, using an Amberlite XAD-8 resin (100-200 mesh) anion exchanger [51].

TABLE-8: TABLE OF TOLERANCE LIMITS OF FOREIGN IONS, TOLERANCE RATIO.

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

Species X	Tolerance ratio X/Cu	Species X	Tolerance ratio X/Cu
Ammonium (I)	100	Chromium (III)	100
Arsenic (III)	100	Chromium (VI)	100
Ascorbic Acid	100	Cadmium (II)	100
Azide	100	Vanadium (V)	100
Chloride	500	Selenium (IV)	200
Fluoride	1000	Selenium (VI)	200
Barium	200	Nickel (II)	10
Nitrate	500	Iodide	25
Nitrite	100	Cesium	100
Bismuth (III)	100	Cerium	100
Citrate	500	Thiocyanate	10
Tartrate	200	Sodium	100
Bromide	100	Zinc	100
Cobalt (II)	10	Mercury (II)	25
Cobalt (III)	10	Calcium	100
Iron (II)	10	Potassium	100
Silver (I)	25	Molybdenum (VI)	100
EDTA	...	Arsenic (V)	200
Oxalate	500	Lead (II)	200
Phosphate	100	Thallium (I)	50
Aluminum	100	Gallium	50
Manganese (II)	200	Tungsten (IV)	50
Iron (III)	10	Tungsten (VI)	50

Composition of the absorbent Complex :

Job's method [52] of continuous variation and the molar-ratio [53] method were applied to ascertain the stoichiometric composition of the complex. A copper-1-(2-Pyridylazo)-2-naphthal (PAN) (1 : 2) complex was indicated by both methods.
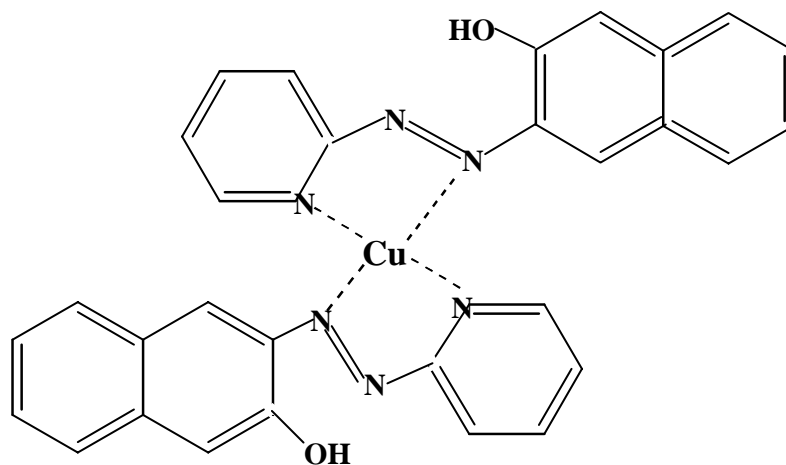
Structure of the Copper-PAN complex.

APPLICATIONS

The present method was successfully applied to the determination of copper (II) in various industrial waste water samples of various compositions (Table-10) and also in number of real samples, e.g several Certified Reference Materials (CRM) (Table-9). The method was also extended to the determination of copper in a number of environmental samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such sample was analyzed for copper content, recoveries in both the 'spiked` (added to the samples before the mineralization or dissolution) and the 'unspiked` samples are in good agreement (Table-10). The results of industrial waste water analysis by spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The precision and accuracy of the method were excellent.

Determination of copper in alloys and Steels:

0.1 g amount of an alloy or steel sample was accurately weighed into a 50-mL flask following a method recommended by Parker [54]. To it, 10-mL of 20% (V/V) sulfuric acid was added, carefully covering with a watch-glass until the brisk reaction subsided. The solution was heated and simmered gently after addition of 5-ml of concentrated HNO_3 until all carbides were decomposed. Then 2-mL of 1: 1 (V/V) H_2SO_4 was added and the solution was evaporated carefully to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature $(25\pm 5)^0C$. After suitable dilution with de-ionized water, the contents of the flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with dilute NH_4OH in the presence of 1 – 2 mL of 0.01% (W/V) tartarate solution. The resulting solution was filtered, if necessary, through a whatman No. 40 filter paper into a 50-mL calibrated flask. The residue was washed with a small volume of hot water and the volume was made up with de-ionized water. A suitable aliquot (0.1 – 1.0 mL) of the above solution was taken into a 10-mL calibrated flask and the copper content was determined as described under procedure using fluoride as a masking agent. The results are shown in (Table-9). Added copper was recovered accurately from the other metals.

TABLE-9: DETERMINATION OF COPPER IN STANDARD BRONZE, BRASS AND STEEL SAMPLE SOLUTIONS.

Sample No.	Certified Reference Material (Composition, %)	Cu spiked		Recovery ± S[b](%)
		Added (µg mL^{-1})	Found[a] (µg mL^{-1})	
1.	BAS032a, Al-Bronze Alloy Cu=85.9, Zn=0.94 Mn=0.27 Fe=2.67, Ni=1.16, Al = 8.8	0.10 0.50	86.06 85.95	100 ± 0.2 99 ± 0.5
2.	BAS-10g HT Brass Cu=60.0, Fe=1.56, Sn= 0.31 Pb=3.34, Zn =32.0, Mn = 1.36	0.10 0.50	60.10 60.49	103 ± 0.6 98 ± 0.5
3.	BAS-646, High speed steel, Te= 0.90, Cr = 4.55 Mo= 4.95, V= 1.99	0.10 0.50	0.102 0.50	100 ± 0.2 100 ± 0.0
4	Brass – 5f Cu= 70.8, Zn = 24.2, Sn= 1.85 Pb = 2.52, Fe=0.31, P= 0.06	0.10 0.50	70.65 71.25	105 ±1.6 108 ± 0.8

a. Value given represents the average of triplicate determination.

b. The measure of precision is the standard deviation (S).

Determination of Copper in Environmental and Industrial Water:

Each filtered (with whatman No.-40) environmental water sample (1000-mL) was evaporated nearly to dryness with a mixture of 5mL of concentrated H_2SO_4 and 10-mL of concentrated HNO_3 in a fume cupboard following a method recommended by Greenberg et al [55], 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 NH_4OH in the presence of 1 – 2 mL of 0.01%(W/V) tartarate solution. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1 – 2 mL) of this pre-concentrated water sample was pipette into a 10-mL Calibrated flask and the copper content was determined as described under procedure using KF as a masking agent. The analyses of environmental water samples from various sources for copper are shown in (Table-10).

Most spectrophotometric method for the determination of copper in natural and sea water require pre-concentration of copper [56]. The concentration of copper in natural and sea water is a few µg mL^{-1}. The suggested safe level of copper in drinking water for humans varies depending on the source, but tends to be pegged at 2.0 mg/L[55].The results of water samples analysis by spectrophotometric method were found to be in excellent agreement with those obtain by AAS. The results are shown in table-11.

TABLE-10: DETERMINATIN OF COPPER IN SOME ENVIRONMENTAL WATER AND INDUSTRIAL WASTE WATER SAMPLES.

Sample		Copper/ µg L^{-1}		Recovery	S_r^b (%)
		Added	**Found^a**	**±S (%)**	
Tap water		0	2.5	±0.2	0.25
		100	102.0	99±0.6	0.58
Waste Water of UFFL		0	1.5		
		100	101.0	100.2±0.4	0.39
Waste Water of PUFFL		0	1.0		
		100	102.0	100.4±0.6	0.59
Waste Water of CUFL		0	1.0		
		100	101.5	100.2±0.8	0.79
Waste Water of JFCL		0	1.5		
		100	102.0	100.0±0.3	0.29
Waste Water of AFCL		0	1.5		
		100	102.0	100.0±0.4	0.39
Waste Water of TSP		0	2.5		
		100	103.5	100.2±0.3	0.29
River water	**Buriganga (upper)**	0	5.0		
		100	107.0	100.9±0.4	0.24
	Buriganga (lower)	0	5.5		
		100	105.5	99.4±0.3	0.37
Sea water	**Bay of Bengal (upper)**	0	12.0		
		100	120.0	99±0.2	0.29
	Bay of Bengal (lower)	0	13.5		
		100	114.8	100.1±0.3	0.21
Drain water	**Berger Paints, Dhaka**	0	15.0		
		100	113.0	99±0.5	0.43
	Asian Paints, Dhaka	0	12.0		
		100	114.0	100.9±0.3	0.26

^{a} Average of five replicate determinations.

^{b} The measure precision is the relative standard deviation(S$_r$).

TABLE-11: DETERMINATIN OF COPPER IN SOME ENVIRONMENTAL WATER AND INDUSTRIAL WASTE WATER SAMPLES.

Serial No	Sample	Copper/μgL^{-1}		Sample source
		AAS	Proposed method	
01	Waste Water	0.9	1.5±0.5	UFFL, Ghorashal, Narshingdi.
02	Waste Water	1.6	1.0±0.6	PUFFL Ghorashal, Narshingdi.
03	Waste Water	1.7	1.0±0.8	CUFL, Chittagong.
04	Waste Water	2.0	1.5±0.4	JFCL, Jamalpur.
05	Waste Water	1.8	1.5±0.3	AFCL, Ashogong.
06	Waste Water	2.9	2.5±0.3	TSP, Chittagong.
07	River water	5.4	5.0±0.4	Buriganga
08	Sea water	12.3	12.0±0.3	Bay of Bengal
09	Drain Water	15.6	15.0±0.6	Berger Paints, Dhaka
10	Drain Water	12.4	12.0±0.3	Asian Paints, Dhaka

*Average of five replicate determinations.

CONCLUSION

In this Thesis a new simple, sensitive, selective and inexpensive technique with Cu-1-(2-pyridylazo)-2-naphthal (PAN) complex was developed for the determination of copper in environmental and industrial waste water samples for continuous monitoring. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES, and ICP-MS, are available for the determination of copper at trace level in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables etc. have caused spectrophotometry to remain a popular techniques particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of relative standard deviation of the present method are very reliable for the determination of copper in real samples down to μgL^{-1} levels in aqueous medium at room temperature $(25\pm 5)^{0}C$.

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