

Modern Sensors

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In last chapters we have discussed on micro probes fitted with membrane and electrodes for measurement purposes. Modern day technological advances ensure analysis of almost every type of Biosecretion like enzymes, blood glucose, other biocatalysts, crude cell extract, viable plant cells etc. with the help of properly designed biprobe or nanoprobe. Measurement of nonviable microbial cells or microorganisms or viable microbial cells is also possible with these types of biosensors. The different types of biological materials are possible starting from enzyme, antibody organell, hormones, nucleic acid or a whole cell which may be immobilized or mobilized. Immobilised enzymes are prepared by soluble enzyme protein into a solid form of catalyst by different methods. The immobilization technique is mainly developed due to reuse, or continuous use of the enzyme. The biosensor, with its tip coated with immobilized enzyme and fitted with a ion selective semi permeable membrane, is with intimate contact of the bio-material. The biological signal produced is converted into electrical signal. The process electrode used, or detection by oxygen electrode or sensing by photocell or a thermistor. The bio-substances to be analysed pass through the membrane and interact with immobilized bio-material at the tip of the probe and transformed into heat or gas (oxygen) or layer of electrons or ions like Ammonium ions. The product passes through another membrane above the immobilised bio-tip to the transducer *i.e.* gas (oxygen) detector or ion detector or thermistor for heat detection or capacitive detection for electrons or ions, and milivolt order electrical signal is generated. Also, there is colour change at the bio tip which can be analysed by colorimetric detection. This signal is amplified and processed and the read out is recorded.

Referring to Nanoscience, which is the study of the fundamental principle of molecule or atoms whose diameter is in between 1–100 nanometers. The structure is referred to a nanostructure. Nano technology is used to analyse the nanostructure in which biostructure and micro organism is included. For example photorefractive polymers have highly unusual structure, with mobile electronic charges like metal. The mobile charges can be excited with sunlight or electric field so that the new position of these changed particles forms a code which is read by shining it with different colour of light. This code is a characteristic of the analytical properties of the polymer. This type of nanoscale analysers or sensors are called nano-sensors. Nanosensors have wide range of applications ranging from water, temperature, light, sound, electricity, molecules, biological secretions, *i.e.* enzyme, catalyst etc. and micro organisms, toxins and DNA. These sensors use the principle of molecules recognition. Dr. Hupp made some elegant molecules that are identified as molecule metal required and designed to recognise particular target molecules called as analytes. Another major direction of work is nanoscale biostructure. These structure are designed in the nanoscale, can affect a biological process or interact with biological entity. As the biological entities are generally nanostructures, biomedical applications and investigation constitute a major part is the Nanoscience. One of the development in Nanscience is Graetzel cell, which is a dye molecule used to capture energy from sun light. The excited molecule jumps to higher energy state. In the high energy state the molecule separates charge by passing of electron from the dye molecule to a nanoparticle of white crystal of titanium dioxide. The positively charged dye molecule and the negatively charged titanium dioxide (nanoparticle) are allowed to recombine chemically with the solar energy converted to a current passing in the external circuit. So this is an example of nanoscale solar energy capture and transformation to electricity and torque. This electricity is used to produce light in the light emitting diode. This is an instance of sensing of electromagnetic radiation.

Now-a-days, there is a break through in transducer technology with the invention of porous silicon technology. In porous silicon the active surface to volume ratio is very high. Measurement, starting from pressure, temperature, strain, distance, acceleration and electrical voltage, current and true power, can be

measured with porous silicon sensors. The design and fabrication of porous silicon wafer has been machined mechanically, hence, they are grouped as Micromachined devices or MEMS. The main advantage of MEMS is the on-chip fabrication of detector, controller or display or it is called System-ON-Chip (SOC). The other advantage is the customized IC designed (ASIC) which are fabricated for particular industry specific use.

Biocatalyst : Biocatalysts are special type of bio product which accelerates reactions happening in Animal body. The most prominent biocatalysts are enzymes. There are other types of biocatalyst like cell extract. Plant and animal cell, viable microbial cells or microorganism and nonviable microbe cell. Commonly used animal enzymes are lipases, rennets, triprin etc. and plant enzymes are papain, analyses, soyabean lipoxy guase. Enzymes are widely used in leather plant for softing of leather and detergent manufacturing. Enzymes are found in three methods (1) Due to natural secretion from cell *i.e.* extracellular secretion, (2) intracellular enzymes which remain in the cell, and (3) microbial enzyme which is prepared by the way of fermentation in presence of microorganism like bacteria virus or toxin. The production of primary and secondary metabolides is caused in presence of various enzymes.

Intracellular enzymes, due to its presence in the cell, need the cell wall to be destroyed during its preparation. The cell wall is destroyed in presence of high electric field or in some biochemical processes like homogenizer or bread mill. Whereas the extracellular enzymes is prepared with the help of microorganism like cellulose, polymethylgalacturonase etc. After the extraction of proper enzymes, its purification has been done and other elements of cell and different acids forming the cell has been eliminated.

The enzyme prepared due to fermentation process in presence of microorganism are more economical for large scale enzyme production and its amount depends on the size of farmenter. The process of preparation of microbial enzymes is of less cost, easily extraction is possible and easy to purify. For growth of intracellular and extracellular enzyme specific environmental condition *i.e.* temperature pressure and humidity and pH should be reached and control of the ambient condition is necessary. But microbial enzyme produced by the fermentation with microorganism is prepared in varying environmental conditions and a wide variety of enzymes can be made by microbial fermentation.

The microbial enzymes can use Bacteria as microorganism (*Bacillus crews*) to reach to a enzyme named penicillinease. Similarly the residual enzyme urate oxides is the product when *asperyilus flavus fungi* (microorganism) is used. These microbial enzymes are used to get fructose from glucose, widely used in food industry and detergent industry.

Properties of enzyme

The main properties of enzyme are given below :

1. For two closely related, species the enzyme protein macromolecule, they differ inspite of some similar molecular structure.
2. The extracellular unlike intracellular enzymes are influenced by environmental conditions *i.e.* temperature, pressure, pH, humidity etc. in which they are prepared and their stability, activity differs with the ambient condition of microbial growth.
3. A particular microorganism may not decompose a organic matter whereas it is possible that a community of different microorganism can cause secretion of a particular enzyme. It is also some specific enzyme, that can cause the secretion of a particular enzyme in higher amount and utilize the substrate is a more economical way.
4. The same enzyme secretion from different sources show different responses in presence of the same activator or inhibitor.

Enzymes Production : The different methods used in the preparation of enzyme are (1) Isolate microorganism or development of strain (2) Culture medium formation and inoculum (3) Sterilization and inoculation of medium (4) Purification of enzyme (5) Immobilization.

Isolation of Microorganism : Production of enzyme pass through this process of isolation of microorganism because the fermentation process occurs in a short time for production of high amount of enzyme rather than other notabilities and to develop a low cost culture medium. The suitable microorganism for an enzyme, is used to form a cultured medium at particular temperature and pH of the medium and in this

optimised culture mediums improvement of strains is developed. The development of strains in microorganism is catered by the use of mutagenic chemical in the cultured medium with exposure to ultraviolet light.

The other constituents of culture medium are sources of carbon, nitrogen, amino acids, growth promoters trace element and a little salt. Proper pH value and temperature is maintained although the fermentation process in the cultured medium.

The culture medium is sterilized continuously before fermentation. After proper sterilization sufficient amount of inoculum is done so that fermentation starts. The inoculum, for enzyme production, floats on the upper surface of cultured medium and this is called surface culture technique. Also, there is submerged culture medium, so that it is less prone to infection and better yield of enzyme. The growth of enzyme is optimised with proper temperature, pH and oxygen flow into the fermentation process.

Enzymes are purified by preparation of concentrated solution by vacuum evaporation technique with low temperature. It is also done by ultra filtration to remove stresses of microorganism. The addition of preservative like calcium salt, protein starch, sugar as stabilizer is an important process.

Drying and packaging of enzyme is also a process in enzyme production.

Immobilisation : Immobilisation is a process in which enzyme has been separated from the bulk phase by some means. Polymer has been created from the enzyme particularly for reuse of the enzyme product. The bulk phase contains substrate, effector or inhibitor molecules in a dispersed way and by close monitoring, the enzyme as a polymer has been separated.

The pure enzyme may not be economical until they are prepared for reuse. The immobilised enzyme is reused, not only that, they are useful for continuous use and needs less labour incentive.

The different ways for immobilisation are (1) Absorption (2) Covalent bonding (3) Entrapment (4) Encapsulation (5) Cross linking.

The process of immobilisation is the absorption of loosely bound enzyme molecules on a polymer carrier surface. Absorption is a static process enzyme is immobilised on polymer carrier by stirring a container containing enzyme and polymer. Besides absorption, there may be other processes like covalent bonding or cross-linking of enzyme with polymer molecule (Fig. 32.1).

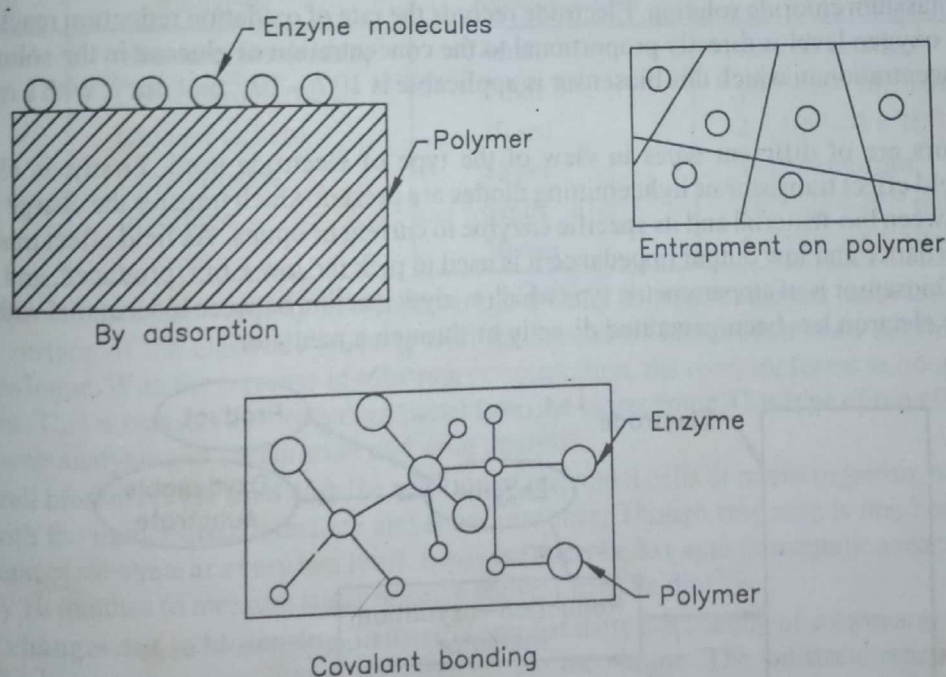


Fig. 32.1.

Biosensor : The biological product when comes to intimate contact to a biological sensor, with immobilised layer, the sensor transduces biological signal to electrical sensor. These immobilised layer sensor which analyse biological product like enzyme, hormone, antibody, nucleic acid, protein, organelle is called the biosensor. There may be any type of electrode like ion-sensitive electrode, oxygen electrode, thermistor or photocell. The

immobilized biological material in contact with the electrode polymer tip is fitted with a semi permeable membrane through which the biological material to be analysed, can pass and interact with immobilised bio-material on the sensor tip. This interaction may produce gas, heat, electrons, oxygen, hydrogens ions and other ions. The product passes through other membrane with specific permeability of the product to the sensor which either analyse the ionic activity or by thermistor to measure temperature or oxygen ion concentration to produce an electrical signal which is recorded.

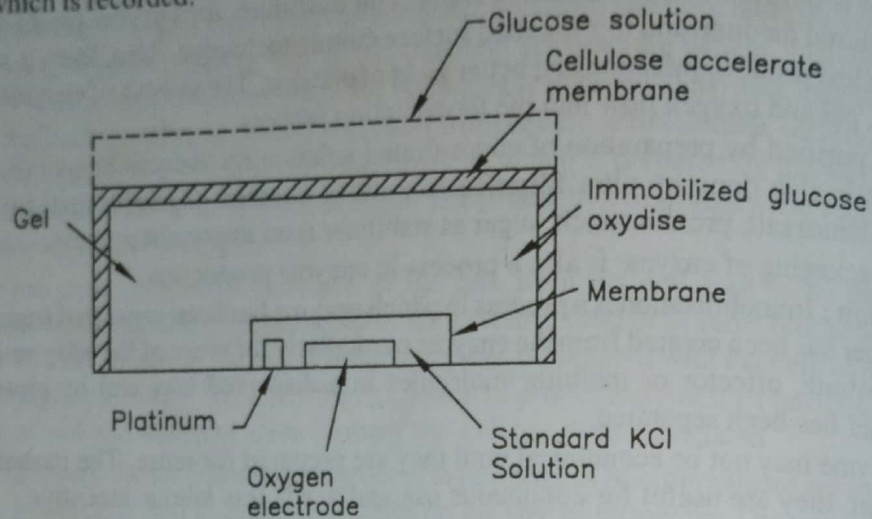


Fig. 32.2.

Glucose Electrode : The glucose electrode shown in Figure 32.2, is separated from immobilized glucose oxydase solution with a semipermeable membrane of cellulose acetate with specific permeability for glucose ion and oxygen. The glucose and oxygen penetrate through the membrane and the reaction with immobilized glucose oxydase produces water, gluconic acid, hydrogen peroxide, water, oxygen and glucose oxide. In this way oxygen concentration in the gel is low. This oxygen concentration has been sensed by the oxygen electrode in potassium chloride solution. Electrode records the rate of oxidation reduction reaction and the rate of decrease in oxygen level is directly proportional to the concentration of glucose in the solution. The range of glucose concentration in which this biosensor is applicable is $10^{-1} - 10^{-5} \text{ mol dm}^{-3}$, with a response time of 1.5 minutes.

Biosensors are of different types in view of the type of electrode used. Firstly, is electro-chemical biosensors, Field effect transistor or light emitting diodes are present which transfer the charge layer produced by reaction between bio-material and its specific enzyme to current or optics. As field effect transistor provides high input impedance and low output impedance it is used to pick the low e.m.f. produced, and amplify it. The second form of biosensor is of amperometric type which analyse reaction between bio material with corresponding enzyme so that electron has been generated directly or through a mediator.

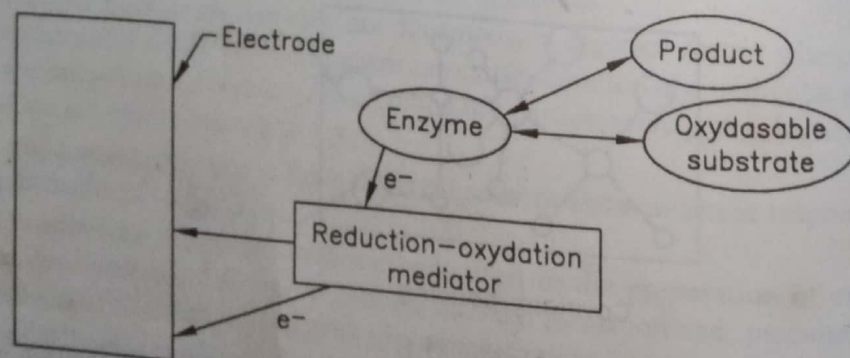


Fig. 32.3.

The enzyme plays the part of catalyst in reduction oxidation reaction in presence of bio-product and substrate and coupled to the electrode. The electron transfer is from oxydasable substrate to electrode through enzyme. In this (enzymatic) type of biosensors, the oxygen requirement is replaced by oxydase.

In enzymes electrode, the substrate which may be protein, amino acid, glucose, alcohol or lactic acid, reacts with an enzyme separated by thin permeable membrane. The reaction produce O_2 , hydrogen ion, Ammonium ion, carbon dioxide or small molecules due to enzyme reaction. The potentiometric response from the electrode depends on the concentration of the substrate. The biochemical reaction between immobilized enzyme and bio-product sometimes generate heat which may be sensed by a thermistor and changed to electrical signal. As thermistor has a measurable sensitivity in the range of temperature $0.1-0.001^\circ C$ this type of biosensors are widely used.

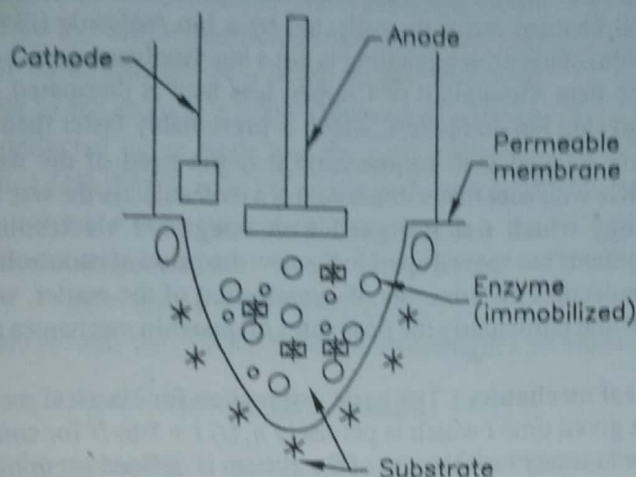


Fig. 32.4.

Electrochemical Electrode : The table show the substrate with its specific enzyme and response time for electro-chemical electrodes.

Enzyme based biosensors

Substrate	Enzyme	Response time	Range
Amines	Monoamines oxidase	4 min	$50-200 \mu \text{mol m}^{-3}$
Cholesterol	Cholesterol oxidase	2 min	$10^{-2} - 2 \times 10^{-5} \text{mol dm}^{-3}$
Glucose	Glucose oxidase	20 sec	$2 \times 10^{-3} - 3 \times 10^{-6} \text{mol dm}^{-3}$
Penicillin	Penicillinases	25 sec	$1-10 \text{m mol dm}^{-3}$
Sucrose	Invertage	6 min	$10^{-2} - 2 \times 10^{-3} \text{mol dm}^{-3}$
Uric acid	Uricase	30 min	$5 \times 10^{-3} - 5 \times 10^{-3} \text{mol dm}^{-3}$

Other type of biosensor, recently developed is bio-affinity sensor. A charged radio labelled receptor is bound on the surface of the electrode reacting with the substrate. The immobilized substrate is called the determinate analogue. With the increase in substrate concentration, the receptor forms as bound complex with the determinant. This is measured by reducing signal from the bioreceptor. This type of bio affinity sensors are used for hormone analysis and saccharide and drug analysis.

Whole cell biosensors are used with the addition of microbial cells or micro organism or their organelles which react with the immobilised substrate and show response. Though response is one hour, it can analyse the concentration of substrate at a very low level. Likewise microbe like acto fermentation reacts with assimilase sugar in nearly 10 minutes to measure sugar linearly above 1m mole dm^{-3} .

Colour changes due to biosensing. In these biosensor there is a coating of enzymes or antibodies on the membrane. The biosensor is also coated with dye on the membrane. The substrate reacts with enzyme or antibody and due to change in pH level of the yield there is a colour change is the dye-membrane coating. These changes in the colour of dye is sensed by light emitting diode.

Use of Biosensors : Biosensors have following applications in medicine, industry, pollution control, detergent industry etc. Glucose analysers are used to find the concentration of blood glucose level and the amount of insulin required. Detection of Mitomycin and aflatoxin, which causes cancer in infants may be

detected by biosensors. The quality control of drinking water has been performed by monitoring with biosensors *i.e.* measuring BOD (oxygen demand).

A microbial cell biosensor is used to detect the mutagenicity and carcinogenicity of some compounds. Biosensors are used in fermentation control to derive different industrial product.

The electrical circuitry has been replaced by biochips in modern days. The biosignal generated are in relation with electrical signal and these signals are tried to be processed by biochip. The main advantage of biochip is that the electrical element mean the reflected by a bio molecule (like protein) whose size is in Nanometer order. The problem of electron tunnelling is not a big disadvantage in biochip. As the bio molecule has less electrical resistance than Aluminium or Copper, less heat is dissipated in the biochip. Now-a-days efforts have been tried to develop bio-computers, which is presumably faster than its electrical counterpart.

Nanosensors : Before starting with nanosensors it is the need of the day to get accustomed with nanoscience. The basic particle with nanometer dimension is a molecule. As the size is reduced from micrometer to nanometer the technology which was designed with integrated electronics or integrated optics or microelectronic machines systems has moved apart in the new direction of nanotechnology. As nanotechnology concerns with atoms and molecules the concepts of quantization of the matter, wave particle duality matter waves Schrodinger equation and particularly the postulates of quantum mechanics and uncertainty principle are of prime interest.

Problem with classical mechanics : The basic assumption for classical mechanics is : (a) There exist dynamical variable q_i and a given time t which is precisely $q_i(t)$ $i = 1$ to N for some integer N . Each of these variables take definite value at time t *i.e.* the state of the system is defined accurately at time t .

The development of $q_i(t)$ ($i = 1$ to N) *i.e.* evolution of the system, with time is deterministic and at a particular time t_1 the set of values $q_i(t)$, $i = 1$ to N gives the state of the system.

The differential equation with $q_i(t)$, $i = 1 \dots N$, was solved by system of particles by taking dynamical variable of position of the particle $q_i(t)$ and their velocities \dot{q}_i ($i = 1$ to N) though only obeyed by classical particles (upto μm in size). The force of law *i.e.* gravitational force between two massive bodies and coulombs inverse square law, Maxwell's equation and Lorentz force are well understood for a distance greater than a few micrometer (10^{-6}m).

But at the end of nineteenth century certain phenomena involving the distance in range 10^{-8}m to 10^{-10}m could not be explained properly. Hence there is a contradiction with classical mechanics at the monolevel or at the molecular level.

One of the departure from classical mechanics is the structure of atoms. When the structure of atoms with orbital motion of electrons around nucleus, the classical picture, is analysed, it is found that a atomic structure is unstable and electrons are predicted to have collapse within the nucleus in much below 1 second. Other discrepancies in classical logic is found in case of black body radiator or harmonic oscillator.

Hence for nanoparticles another law is required to be formulated. The quantum mechanics has two stages of development. One concerns with Planck's introduction, to quantum of action which was not totally satisfactory and the second was the formulation of Schrodinger and Heisenberg, which gives birth to quantum mechanics which the nanoparticles follow. This new mechanics answer satisfactorily, the queries about structure of atoms and molecules, optical spectra from excited atoms, black body radiation and Compton effect.

The structure of atoms and their optical spectra has been solved for Hydrogen atom and Helium atom by Bohr's postulates which state that :

1. There exists a discrete set of stationary states (stable states) of an atom ; from each of these states no electromagnetic radiation is emitted due to accelerating motion of the electrons round the nucleus.
2. Transitions occur from one of these states to another with the emission of radiation. If the initial and final states have energies E_n and E_m , the frequency of the emitted radiation is $\frac{E_n - E_m}{h}$ where h is the Planck's constant.
3. The angular momentum of an electron in motion round the nucleus of hydrogen atom takes the value $0, t, 2t, 3t \dots$ only where $t = \frac{h}{2\pi}$ and the quantisation (discrete values) of angular momentum is the basic of quantum mechanics.

Though, from this Bohr's postulates the structure of hydrogen atom is somehow perceived, yet these postulates are unable to predict the relative intensities of the spectral lines of hydrogen atom. The postulates also fail to predict the actual line frequencies and for more complicated atoms or molecules.

The new turn in the Nanoscience is the wave particle duality. The wave property of matter is defined by the parameter wavelength (λ) whereas the particle property is defined by momentum p . The wavelength of an electron can be measured by the diffraction pattern when the electron passes through a crystal. If the diffraction of a beam of electrons with wavelength λ at right angle to the atom in a plane (separation between atoms is d), the beam is deflected by an angle and the path difference between two adjacent paths (AM' , BM'') is BC is an integral number of wavelength. So if, the electron has a wave nature with wavelength λ , the maxima of Bragg's diffraction will be found as

$$BC = d \sin \theta = n\lambda \quad n = 1, 2, 3, \dots$$

Hence this experiment reveals the relation between wavelength λ momentum p of a nanoparticle by

$$\lambda = \frac{h}{p} \quad h = \text{Planck's constant}$$

and this relation expresses the wave particle duality of matter and the equation is called de Broglie relation.

The immediate consequence of this wave particle dual nature of nanoparticle is the dynamics of the particle given by Schrodinger equation which is stated as

$$i\hbar \frac{\partial \Psi}{\partial t} = \left[-\hbar^2 \frac{\nabla^2}{2m} + V(r) \right] \Psi$$

with the momentum operator as

$$\vec{p} \rightarrow \left(\frac{\hbar}{i} \right) \vec{\nabla}$$

and the Energy operator is

$$\vec{E} \rightarrow i\hbar \frac{\partial}{\partial t}$$

where Ψ is the wave function in view of probability of getting a particle or a wave in a region $\Delta x \cdot \Delta y \cdot \Delta z$ at time Δt . This is connected to the basic Heisenberg uncertainty principle which states

$$\Delta x \cdot \Delta p \geq \frac{\hbar}{2}$$

This uncertainty principle is the limitation in accuracy of simultaneous measurement of position (x) and momentum (p) of a nanoparticle.

Nanoscience also includes the study of molecules. There is different chemical bonding between atoms which is basically caused by interactions between electrons and atoms or ions involved. The different molecular forces between electrons, atoms and ions include Coulomb's force, ionic force and also Van-Der-Waals force which is existent in the nanolevel.

Since electrons are responsible for making and breaking of bonds the chemical properties of atoms and molecules are due to electron transport. These bonds act as mechanical device like hinges, bearings, structural members from nanoscale machines. In greater size (μm) the bonds are related to reaction between two molecules. Hence, bonds are tools in nanoscale. The change in phase, from vapour to liquid, water to ice, the way of packing of molecules is a matter of study in nanoscale. Like, CO_2 molecule is a gas but when many individual molecule cluster they form dry ice which is solid. The study of much larger chain of molecules like polymer, is

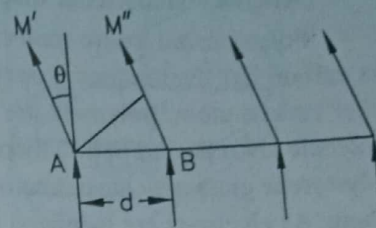


Fig. 32.5.

the key to nanoscience. Most metals shine due to the fact that the electromagnetic radiation is scattered by free electrons on the metal. Some metals, though made up of same atoms, is not metallic but is a insulator as for example, coal, diamond, phosphorous, graphite etc. They are not shiny as they have no free surface electrons.

Generally nanoscience discuss with four basic materials, metals, insulators, polymer and ceramic.

Polymers are macro molecules and generally a long carbon chain. As carbon has the unique property that a carbon atom may bond with other carbon atom, polymers are carbon compounds. The same carbon molecule are repeated in polymers forming a chain of different length. A polystyrene glass may have innumerable polymers molecules of different length. As electrons are localized in polymers chain, polymers generally act like insulator and this is why PVC (polyvinyl chloride) plastic is used for jacketing live electrical wires. In polymers carbon chains are cross linked Fig. 32.6 and they behave like non-metals and they are hard due to their rigid structure. Hence polymeric science is based on nanoscience.

A molecular model of a segment of the polythelene chain. This segment contains 28 carbon atoms (dark), but in commercial polythene there are more than a thousand carbon atoms per strand.

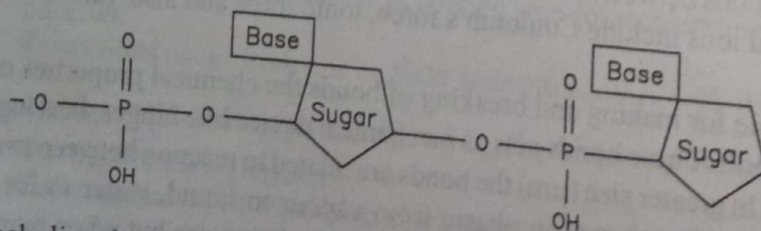
Besides and enzymes are polymers which is responsible for blood oxygen carrier *i.e.* haemoglobin. Another type of plant protein, nitrogense, is responsible for plant growth. Many types of protein is used for structure and growth of plant, animals and the most important one is Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) whose structure is responsible for animal's genetic codification. Cell fraction study reveals that the DNA is a part of cell nuclei whereas RNA is present in both nucleus and cytoplasm.

The fundamental part of DNA and RNA are nuclotide whose formation is given below.

The nucleotides are joined by diester link and they are of molecular weight 50000 units. From X-ray crystallgraphy, it is found that in the model of DNA, nitrogenous bases are at right angles to the axis of the chain and the planes of the sugar molecules and the nitrogenous bases are at right angle. Pauling and Corey postulated a helical structure of DNA from the results.

The structure of DNA has been shown in figure 32.8.

The nucleotides were joined by diester links between the phosphoric acid and the sugar molecule, viz:



and such dinucleotides were first synthesized by Todd in 1955.

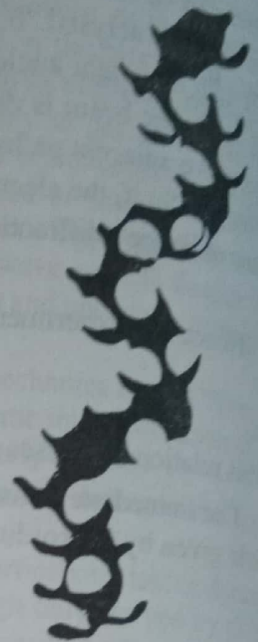


Fig. 32.6.

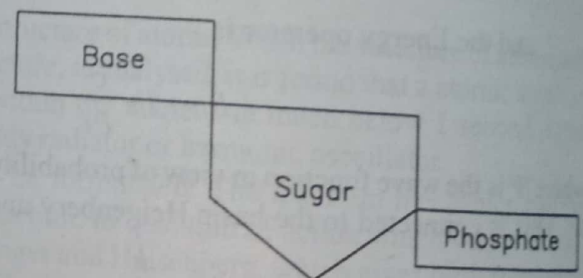


Fig. 32.7.

During this period it became clear from chemical analyses that both DNA and RNA were macromolecules with molecular weights of the order of 50,000 units.

X-ray crystallography showed that the nitrogenous bases were at right angles to the axis of the chain and that the planes of the sugar molecules and the bases were mutually at right angles. Pauling and Corey (1953) then postulated a helical structure for the molecule which was, however, of incorrect form.

Crick and Watson's model of DNA consists of two coaxial polynucleotide right-handed helices, each of which were of equal pitch and subtended an angle of 120 degrees at the axis (Fig. 32.8).

If we temporarily forget about the helical twisting of the molecule, we may use the analogy of a chain ladder. The rungs of the ladder are formed by the hydrogen bonding of two nitrogenous compounds.

The DNA structure contains negative charge due to presence of phosphorus and oxygen. DNA structure is double helix with a pile of chips : Each base pair can have one of the four combinations namely *AT*, *TA*, (*G* or *GC*). For each position of the staircase the two planar molecules are any of the four molecules adenines Thymine, guanine and cytosine i.e. *A*, *T*, *G*, *C*. *A* and *T* bond between themselves and *G* and *C* can be bonded. So there are four combinations possible. The genetic code in the structure of DNA is read by proteins and RNA in the cell. The nanoscience and associated technology is a way to read the DNA code.

Another nanomaterial is ceramics. Ceramics, most of the time is oxide, but, it is not always oxide. In ceramic, a long chain has been produced with an extended structure of oxygen. Like clay is aluminium oxide, sand is silicon dioxide and fire brick is magnesium silicon oxide.

Unlike metals, ceramics are non-conductor of electricity and generally not glazy. They are hard and sometimes brittle.

Molecular Recognition : The bonding affinity between one large molecule with other molecule due to coulombic attraction is called to be molecular recognition. Like allergens is a large molecule which is recognised by large molecules in the body and the body molecule bind to the allergens and affected by foreign molecules. Our sensory experiences are also molecular recognition. The nasal bulb can, recognise large foreign molecules find with it and creates schration in our body. Due to molecular recognition insects attract at another and higher biological organisms is structured with proteins.

Measurement of Nanostructure

Probe Type Sensing : There are a few ways of measuring nanostructure. The probe type instruments are popular. Probe type instrument with microscopic observation type fitted with modern processor can measure

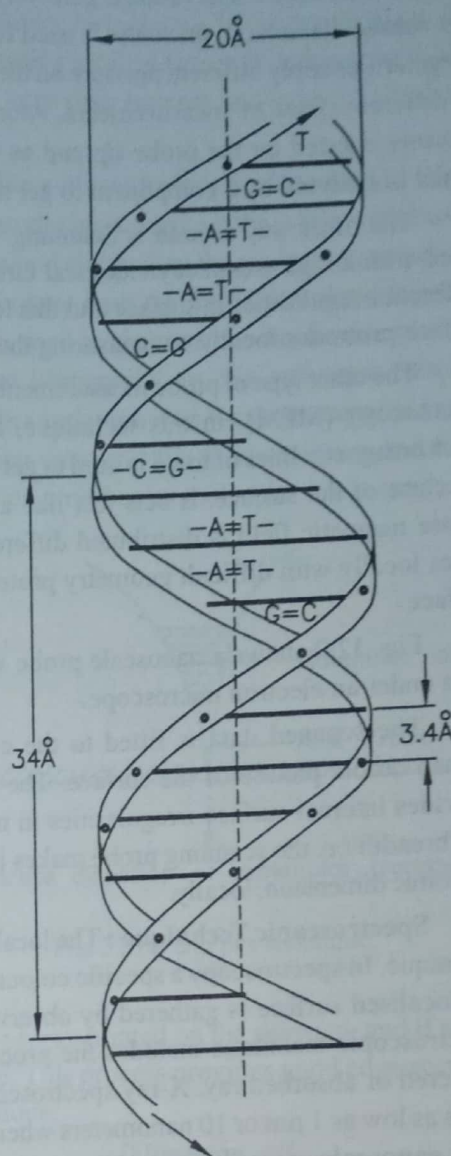


Fig. 32.8. Model of DNA The Biological Role of the Nucleic Acids, Arnold.

upto one nanometer. The probe is generally made of tungsten wire whose tip is of nanoscale diameter and often one atomic diameter in thickness is used for scanning the surface with nanoscale irregularities. The nanoscale irregularities apply different pressure on the tip of the probe as the probe slides on the surface, which corresponds to different types of measurements. Atomic Force Microscopic (AFM) technique ensures the sensing of pressure exerted on the probe tip and as the probe scans the surface a composite signal is generated. This signal is analysed by a component to get the picture of irregularities on the surface in atomic scale.

The other way around is Scanning Tunnelling Microscopy (STM). In this technique, the probe tip is fitted with a high frequency electrical circuit which generates different current at the touch of probe tip on different irregularities in surface and this technique test the local geometry of the surface *i.e.* how much of the surface protrudes locally on measuring the local electrical conducting characteristics.

The other type of probe measurement is Magnetic Force Microscopy (MFM), in this technique, a magnetic tip or electromagnetic lines of force is used to get the local magnetic structure of the surface. It acts just like a proximity sensor whose magnetic field is distributed differently as the probe scans locally with difficult geometry protruding parts of the surface.

Fig. 32.9 shows a nanoscale probe with dimension as seen under an electron microscope.

The scanned data is fitted to the computer to get a human usable picture of the surface. The computer picture provides internal surface irregularities in micrometer length and breadth *i.e.* the scanning probe makes it possible to view in atomic dimension, locally.

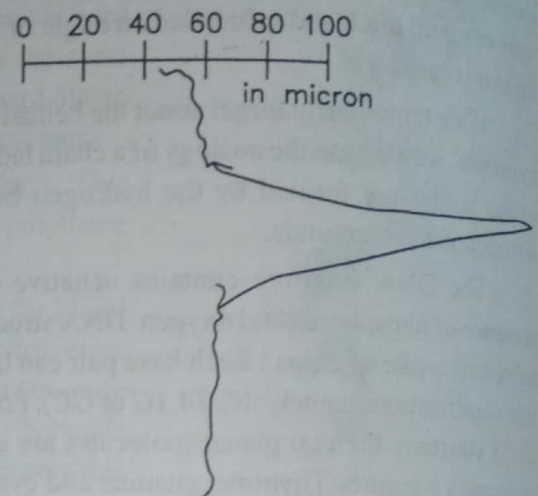


Fig. 32.9.

Spectroscopic Technique : The localised scanning of nanoscale structure can be made by spectroscopic technique. In spectroscopy a specific colour light is focused on the nanoscale surface and the information from the localised surface is gathered by observing the scattered or absorbed or refracted rays from the surface. Spectroscopic technique includes the processing of intensity or phase sensitive data from the incident and scattered or absorbed ray. X-ray spectroscopy is advantageous to use for nanostructure as wavelength of X-ray is as low as $1\text{ }\mu\text{m}$ or 10 nanometers whereas visible light spectroscopy provides a wavelength much greater than nanoscale irregularities. Now-a-days Magnetic Resonance Imaging (MRI) is used as nanoscale spectroscopic technique.

Electrochemical Probe : Electrochemical analysis surface with nanoscale irregularities is based on the principle of generation of charged layer at a specific permeable membrane due to chemical reaction of the probe tip with the surface. The membrane provided, is permeable to the surface atoms. The electrochemical analysis of the surface atoms in an array provides information about the nature of surface.

Electron Microscopy : Electron microscopy is one type of nano-surface scanning technique where the object is exposed to electron beam rather than visible light and the transmitted electron beam intensity is analysed to get idea about nanostructure. The popular Transmission Electron Microscopy (TEM) is what, the instrument is called.

The advantage of using TEM for nanomeasurement is that, it can measure local nanoscale physical structure rather than forces like electric or magnetic field. The TEM image is processed and viewed at length of few μm .

Generation of Nanostructure : Probe type nanostructure generation instruments are available now-a-days. Though expensive, and, to develop a nanostructure is time consuming, the probe type instrument is a

way to create a nanostructure. The probe which is used to analyse nanostructure, is the same probe with its tip a few nanometer. The assembling of the structure on an atom-by-atom or molecule by molecule basis. The probe tip interacts with the surface by giving specific pressure or creating localized electric or magnetic field or by electrochemical process. The nanoscale probe generation is a very slow process and is unable to satisfy mass demand.

Nanoscale Lithography : Lithography is the process to make a object from a single stone : In silicon technology lithographic image is produced on a silicon wafer by producing a mask on the wafer with a oxide coating on it and by exposing the wafer to UV ray or by chemical method eliminating the mask to get actual chip structure. The UV lithography and more modern lithographic technique limit the dimension of mask greater than $0.4\ \mu\text{m}$ or 4 nanometer. Hence X-ray lithographic technique is used to generate nanoscale structure. The new lithographic technique that is accepted today is the imprint lithography. In this technique nanoink/molecular ink is used to develop a nano structure on a material rubber and press it on the target material so that nanoscale structure has been imprinted. This process of generating nanostructure is inexpensive and faster than other processes but the technology to create a nanostructure on a rubber by molecular ink is new.

Dip Pen technique : Atomic Force Microscopy (AFM) probe is used to read nanostructure with a dip pen. The molecular ink is used in the pen and as AFM scans and put force on the surface a nanoetching is developed on the surface with molecular ink. The figure 32.10 shows how Dip Pen nanolithography technique is used.

Electron beam may be used to eliminate the mark on the substrate in a desired way and this type of nanolithography is called electron beam lithography. Electron beam lithography is an accepted technique and it is better than X-ray lithography in the sense the 1\AA X-ray is more particle in nature than wave nature and can damage the substrate molecule in a undesired way.

Lift off Nanolithography : In this process molecular ink is spray printed on the substrate and if an ink molecule is lifted off the surface, it produces a dot on the substrate. This process provides good edge accuracy at the edge of the drops. The figure 32.11 shows the lift off technique.

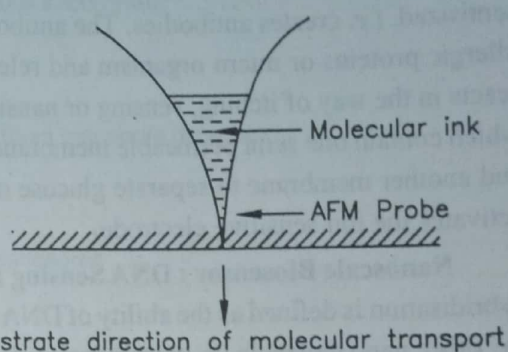


Fig. 32.10. Dip pen technique.

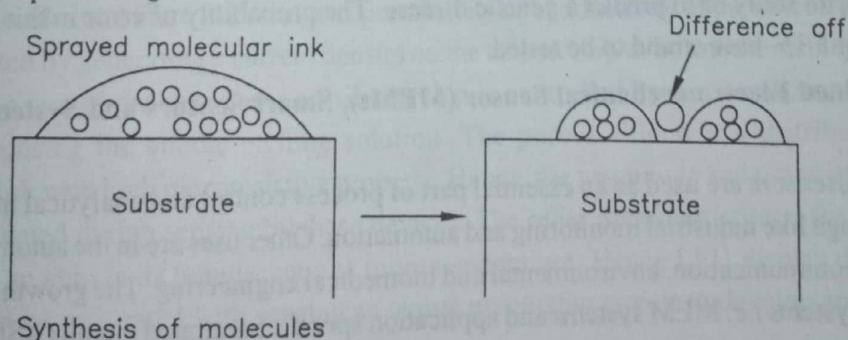


Fig. 32.11. Lift off Lithography

Synthesis of molecules : The production of molecules to a target structure is called molecular synthesis. This includes synthesis of protein, catalyst, enzymes and last but not the least the DNA synthesis. Synthesis molecules are used for drug, detergent industries. This nanosynthesis can be realised by a process called self-assembly. As the molecule will seek the lowest energy level an atom or a molecule can be placed at a desired position by relating particular energy level diagram.

The other method for synthesis of particular molecular pattern is polymerization. A polymer like PVC contains million of molecular in chain. Modern day technology permits controlled polymerization *i.e.* a monomer molecule is added to the polymeric growth at a time.

In DNA synthesis, when a synthetic DNA sample is added on to a bacterial DNA the DNA of microorganism *i.e.* bacteria, many copies of a particular protein or enzymes is produced. This bio-synthesis of nanoscale DNA is an important process in Drug industry.

Nanoscale Sensors : Nanosensor are generally of two types (1) natural nanosensors (2) synthetic nanosensors. The first instance of a natural nanosensor is the nasal bulb which works in the process of molecular recognition. The rule behind sex attraction in the insect world is nanosensual and also a type of molecular recognition. The complementary shapes in the dog's nose sensor or the receptors of the insects recognise the shape of the signal molecule and sensors are actuated by particular distribution of electronic charge due to the generation of new polymeric molecule which is molecular recognition.

Synthetic Sensors : Synthesis sensing of biological entities. One type of synthetic sensor is biosensor. When a animal body has been exposed to allergen or a microorganism like bacteria or fungi, the body is sensitized, *i.e.* creates antibodies. The antibodies in view of the principle of molecular recognition, sense the allergic proteins or micro organism and release histamine. This histamine is a substance due to which body reacts in the way of itching, sensing or nansia. The blood glucose level has been identified with an electrode which contain one semi permeable membrane to separate glucose oxidase (an enzyme) from glucose solution and another membrane to separate glucose oxidase from electrode. The reaction of glucose with its enzyme activates the ion sensitive electrode.

Nanoscale Biosensor : DNA Sensing : DNA sensing is another aspect of nanosensor technology. DNA hybridisation is defined as the ability of DNA to bind to a complementary strand (structure) and not binding to any other nano molecule *i.e.* protein molecule. Like, a DNA sequence GCCGTTC could be sensed with a DNA sequence CGGCAAG obtained by translation. Different DNA sequences can be sensed using the complementary strand, therefore, the amino acid sequence of corresponding protein or enzyme is determined. The number of different combinations possible for 4 bases in a single strand combinations possible for 4 bases is a single strand is 4^6 . Hence if a particular biological molecule has a known DNA sequence, it is possible to get a short list of 10 to 15 sequence, which can sense it uniquely, without error. This type of biosensor with DNA sensing is used for finger print study or to predict a genetic disease. The probability of error in this type of biosensing is one in million for a 15-base strand to be tested.

Micromachined Electromechanical Sensor (MEMs), Smart Sensors and Systems and System on Chip (SOC).

Silicon microsensors are used as an essential part of process control and analytical instrumentation in a wide application range like industrial monitoring and automation. Other uses are in the automobile engineering, transportation, telecommunication, environmental and biomedical engineering. The growth in micromachined electromechanical systems *i.e.* MEM systems and application specific integrated circuit (ASIC) design, sensing and signal processing has become more and more precise and reliable. The integration of sensing part and signal processing part on the same integrated chip, the idea of SMART sensor has been developed. The same technology has been used for system on chip (SOC) in which the sensor, the logic to control the system, the micro actuator and also the dedicated display unit with a processor has been developed on the same chip. The display unit is preferably built with porous silicon or some other optoelectronic material. Silicon transducer are generally of two types. The first one is MEMs (Micromachined electromechanical Systems) and the other porous silicon based mechanical structure.

Micromachined technology ensures the fabrication of mechanical devices on a silicon wafer of very small dimension ($.1 - 10 \mu\text{m}$) and these devices may be cantilevers, nozzles, cavities, diaphragms or membranes, small bridges, mechanical resonators etc. The silicon wafer is chosen for the development of mechanical devices as yield strength of silicon is double the yield strength of steel, whereas, Young's modulus is chosen to that of steel and density of silicon is one third of steel. As the yield strength is high and density of silicon is lower than steel it is easy to be machined in the micron order. Other advantage of silicon is that its plasticity is small and hence it yields less permanent deformation in stress and show very low hysteresis. Hence, the MEMS and porous silicon devices are ideal sensing device with high repeatability.

The technology used for micromachining of silicon wafer is controlled etching in a preferential direction with anisotropic etchants like potassium hydroxide, TMAH, EDP. To develop microstructures like cantilever cavity, nozzle on the silicon chip, hydrazine and complexing agents like isopropyl alcohol are used in a controlled fashion. The crystal structure of the etchant and controlled temperature used during etching process are the parameters on which etching rate is dependent. The other way of etching is electrochemical etching to develop the MEMS and this type of etching provides better control.

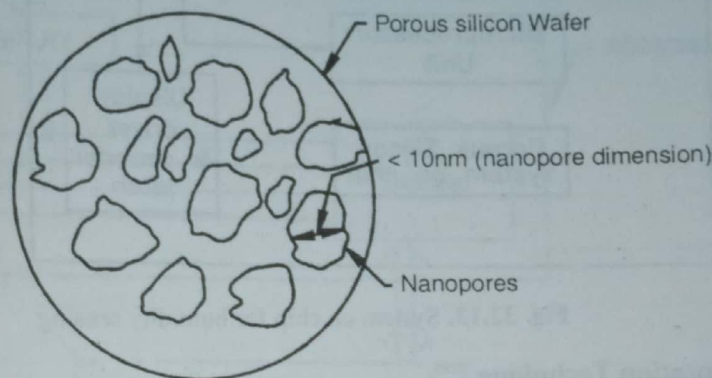


Fig. 32.12. Porous silicon humidity or gas sensor.

Porous silicon Humidity Sensor, Porous Silicon Devices : The porous silicon technology have delivered a convenient way to integrate sensor, filters, waveguides electroluminescent display on a single chip. The high surface to volume ratio of porous silicon ($> 500 \text{ m}^2/\text{cm}^3$) ensure a good sensing medium for pressure, humidity or as Bio-sensing. In a vapour sensor the small pores ($< 10 \text{ nm}$) *i.e.* nonpores of the porous silicon absorb the vapour or gas molecules and there is a change in permittivity or conductivity of the silicon chip. The nanopores are generally created by generating a current density on the silicon chip or by anodic etching of the silicon chip. The shape and position of the nanopores on silicon chip is controlled by the formation of current density or by properly composing the anodic etching solution. The porous silicon is a distributed type resistance capacitance network with high piezoresistive property. Hence, the magnitude and phase of the electrical signal both can be modulated during sensing by these devices. The other important criteria for porous silicon to be used as a system on chip is its luminescence at room temperature. Hence LED display developed on chip of porous silicon can be integrated with sensing and signal processing circuit to develop application specific IC (ASIC).

The MEMS and porous silicon devices are excellent for pressure sensing. The fabrication of the sensor require direct wafer bonding technology and better control of the thickness of the diaphragm for pressure sensing. The MEM pressure sensor shows high sensitivity, good linearity and good stability.

The process other than particularly diaphragm as MEMS, for measurement of ambient pressure, is the use of a layer resulting in the formation of cavity which interact with ambient pressure.

Biosensors : As silicon is bio-compatible, it can be embedded in the living cell, without toxic effect. A typical MEMS has been designed in which two electrodes on a free standing membrane has been developed to analyse the rate and amount and monitoring of fermentation process. Besides MEMS and porous silicon biosensors are used for blood pressure, blood flow, oxygen saturation and measurement of body temperature.

The humidity measurement scheme with fabrication of porous sensor and the system on chip (SOC) arrangement has been shown in Fig. 32.13.

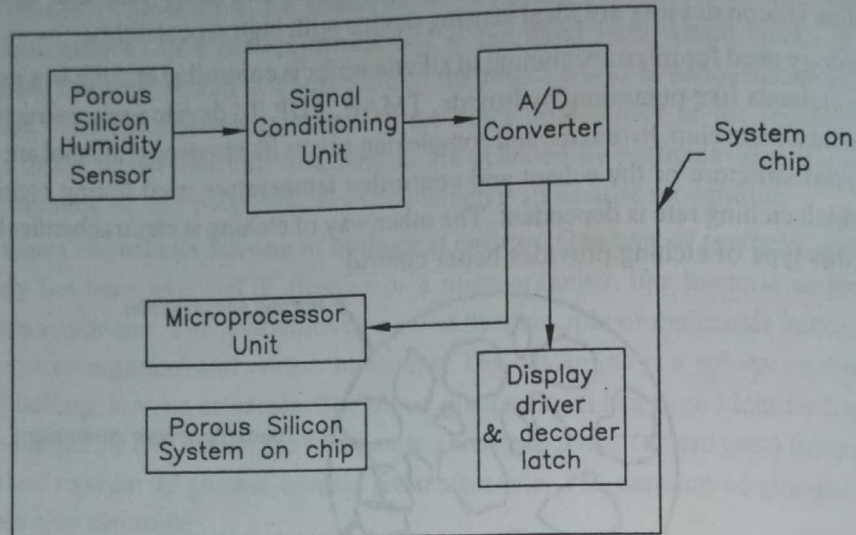


Fig. 32.13. System on chip for humidity sensing.

Advanced Instrumentation Technique

Virtual Instruments : Virtual instrument laboratories are associated with the modelling of instrument for a particular application with specified proper range resolution, accuracy, repeatability : A novel virtual measurement laboratory is based on a software support like internationally accepted software like CORBA or Jini which provides the tools and methodologies to assess the required specifications and deliver the inputs and output pattern of the sensor *i.e.* instruments in a Java accepted objects. The applications software for virtual instrumentation is in a internet acceptable Java environment which can accept object *i.e.* measuring instrument from the internet and place the developed instrument as an object in the internet service. Hence, the environment of the virtual instrumentation laboratory is a distributed system with networking facility. Like, a Jini virtual instrumentation laboratory, the measurement instrument concern the monitoring of the power network characteristic. Attention is focussed on (1) the power network analyzer services and the monitoring services.

The work aims at experimenting in a distributed environment over the internet using industry based software techniques. This virtual instruments are developed in modular approach and the major idea is the modular design and exploitation of heterogeneous computing platforms for supporting a open and dynamically configurable measurement system.

The monitoring system of a smart power electrical network analyzer is shown in the following Figure 32.14.

In the last few years a number of tools have been developed for designing virtual distributed system in a distributed way. CORBA software or Java related software JAVA-RMI, Java spaces, Jini and some mobile agent system are few of them. Like CORBA aims at developing interactions between object based, interoperable

applications in different implementation language. JAVA technologies are able to transform a instrument developed of heterogeneous machine in a network of homogeneous virtual machine.

The Fig. 32.14 shows a distributed measurement example. In a reported work a series of measurement experiments were developed in JINI software. They include the instrument control, monitoring current and voltage at a remote load (Fig. 32.14) frequency response of an amplifier and calibration of a DAQ load.

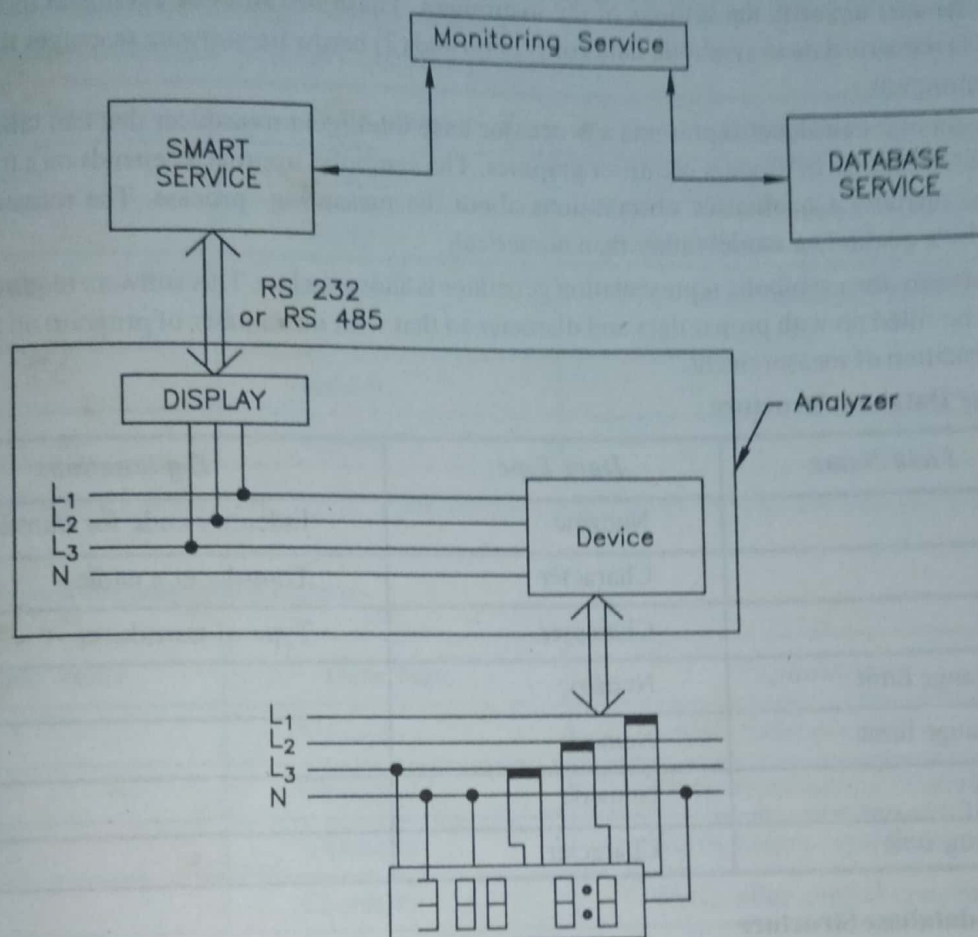


Fig. 32.14. Typical virtual instrumentation power measurement object.

The measurement test in Fig. 32.14 is organised in three services.

- The power electrical network analyzer service
- Database service
- The monitoring service, which implements the measurement algorithm and controls the other two services.

At last all the measurement services in a modelling environment can be remotely controlled through distinct graphical user interface (GUI) which can be viewed in an acceptable way by the enduser.

Intelligent Instrumentation

The manifestation of a measurement system or instrumentation in the real world so that human being can perceive the situation by high level of interaction preferably in English language is called intelligent instrumentation. The perception of the temperature in a room can be in form of a voltage level or symbolic like "too cold", "cold", "hot", "too hot" and warm. The symbolic representation provides a person newly acquainted

with the instrument a idea about the room temperature. Similarly, for a level control system, the set point specification or the measurement output is of no meaning to a person who handles the instrument for the first time. Here another symbolic representation, which is called Man Machine Interface (MMI) which shows how level is getting changed in the reservoir with time helps a new user to adopt to the system quickly. Many software has been developed which includes the engine that converts the measurement results and a simulator that allows the user to verify the settings of the instrument. These software for intelligent measurement does two works (1) numeric data to symbolic data conversion and (2) hardware/software resources that will host and execute the program.

The symbolic transducer represents a processor base intelligent transducer that can talk to the user not in number but in English or process identifier graphics. The symbolic approach depends on a functional model based on the operator's qualitative observations about the measuring process. The measured quantity is represented by a qualitative model rather than numerical.

The software for a symbolic representation generator is shown below. This software requires four different databases to be filled up with proper data and diameter so that with the number of program on these databases yeild representation of measurement.

1. Transducer Database Structure

<i>Field Name</i>	<i>Data Type</i>	<i>Explanations</i>
Code	Numeric	Identify code for transducer
Name	Character	Transducer's name
Class	Character	Type of transducer
Lower range limit	Numeric	
Upper range limit	Numeric	
Accuracy	Numeric	
Measuring unit	Character	

2. Application database Structure

<i>Field Name</i>	<i>Data Type</i>	<i>Explanations</i>
Transducer code	Numeric	Links to certain transducer
Application code	Numeric	Internal code for application
Name	Character	Application name
Lower range limit	Numeric	
Upper range limit	Numeric	APPLICATION
Central value	Numeric	RANGE LIMIT
Concepts	Numeric	Concept No.
Form code	Numeric	Code No. corresponding to concept form
Adoptation code	Numeric	The adoption function code

The application database structure contains a field created to link this database to transducer's database. This linking or relations is necessary because the applications are properties of the transducer.

Function Database

Each function has to satisfy several mathematical tricks so that the transducer output has been linked to the linguistic concept via functional output. Say, for examples, the lower limit is equal to lower range limit of transducer database + central value of application database.

In this way the functional database structure has been grown as :

Field Name	Data Type	Explanations
Function code	Character	The name of
Name	Character	The function
Branch J	Numeric	The mathematical expression of 1st function branch
Lower 1	Numeric	
Upper 1	Numeric	

Lower 1 and upper 1 is the first branch of the function which is defined on the interval determined by those two limits.

4. The last date base is the concepts database

Field Name	Data Type	Explanations
Code	Numeric	Central concept code
Text	Character	Words representing central concept
Before Text	Character	Words before central concept
After Text	Character	Words after central concept

After properly filling up these database the processor runs the program with the following algorithm.

The program will read "Temperature Low" for a function on transducer database yields : Lower range 5°C, Upper range 10°C. The program, from the user point of view, emphasize operations like setting, configuring, adding the databases and at last the symbolic representation has been developed which links transducer database to the concept database which involves the linguistic representation. This types of software with object oriented approach are available in the market.

Linearization

The sensor output, not necessarily, is related to the physical parameter (measurand) by a single linear relation. For such type of sensors where the output maintains nonlinear relation with the sensed variable (measurand) there is a need for prediction of the output for a particular input to draw the calibration curve. Again, for a unknown value of measurand (physical parameter) the experiment suggests a output value which is an indication of the measurand. When the input and output maintains a linear relation the prediction is easy, whereas, for nonlinear relation between input and output, there is a need for linearization. In a particular case, when the relation between sensor output and measurand is quadratic the output must be square rooted so that square root quantity makes an linear relation with the input and this linearlization technique ensures a linear

calibration curve. Sometimes, the linearization technique is not as simple as the above mentioned example (Just square rooting the output). The input and output may be related by a more complex mathematical relation which needs more complicated linearization treatment to be processed. There are certain benchmark technique for complex linearization process. The Analog to Digital converters used now-a-days has a reference voltage input (V_{REF}) which coupled with some input resistances and capacitances gives linearization in case of a more

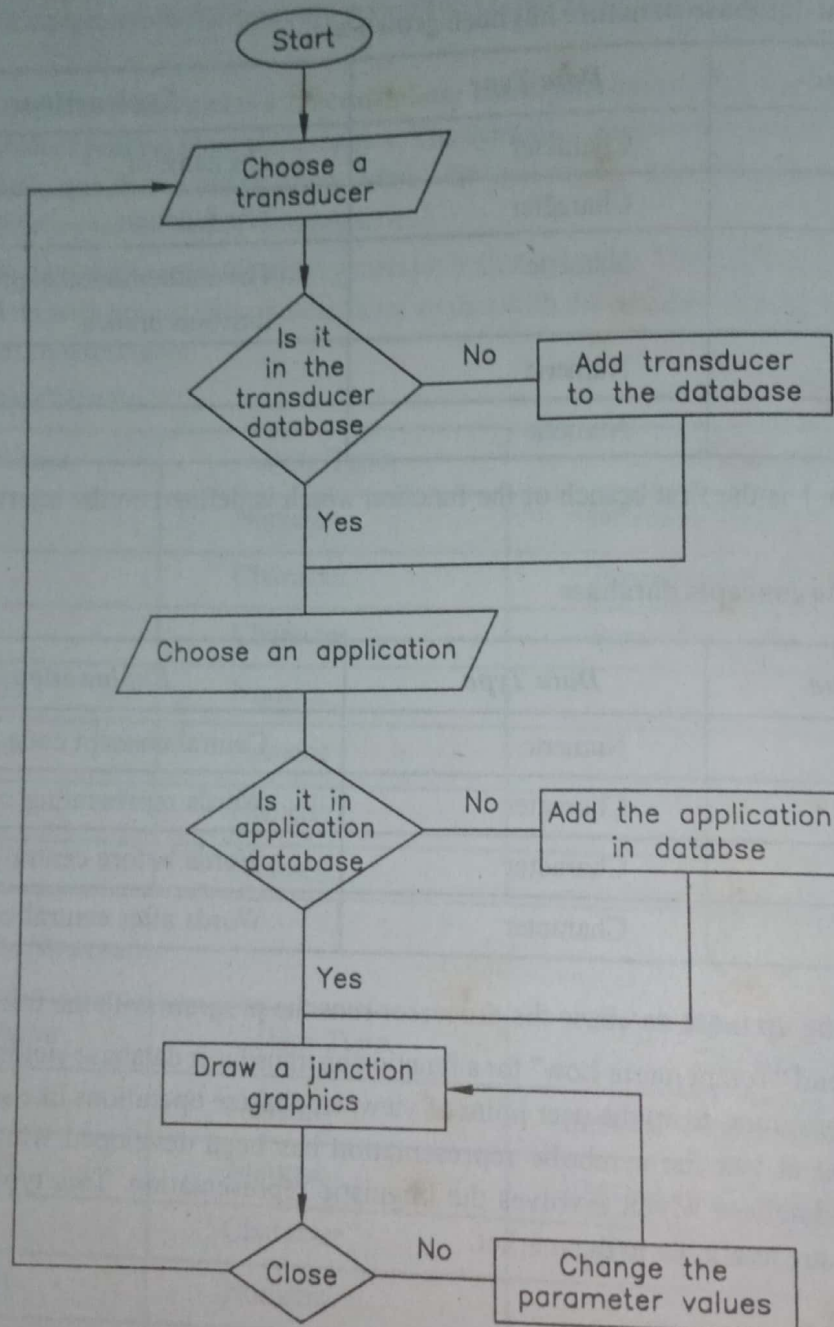


Fig. 32.15. Flowchart for Intelligent Instrumentation.

complex process. The 0804 or 0808 A/D converter ICS have this facility. The physical logic behind this technique is that, the time constant (RC) of the voltage charging has been changed by changing the resistance and capacitance and the charged voltage is compared to the reference voltage V_{REF} by a comparator whose output detects the proper linearization circuit.

The other method used as complex linearization technique is the Artificial Neural Network (ANN) based algorithm which seems to be very efficient. The ANN systems can stimulate any complex relation between input and output with a weight matrix. And the prediction is :

$$[\text{Input Vector}] = [W] \times [\text{Output Vector}]$$

where $[W]$ is the weight matrix. This weight matrix is obtained by supervised learning technique when a output - input pattern is presented as the learning pattern.

Digital Three Phase Real Power and Energy Measurement

There are few disadvantages of real power and energy measurement using power meter or energy meter. Due to reactive components i.e. inductance or capacitance of coils the eddy current is induced in the metal parts of the instrument by varying magnetic field of the current coil. Other faults occur due to fault in potential transformer which cause under registration. This chapter in this book deals with some digital wattmeter or energy meter for power and energy measurement with the help of microprocessor or by optical means.

The schematic diagram of power measurement for three phase four wire system has been shown in the figure 32.16.

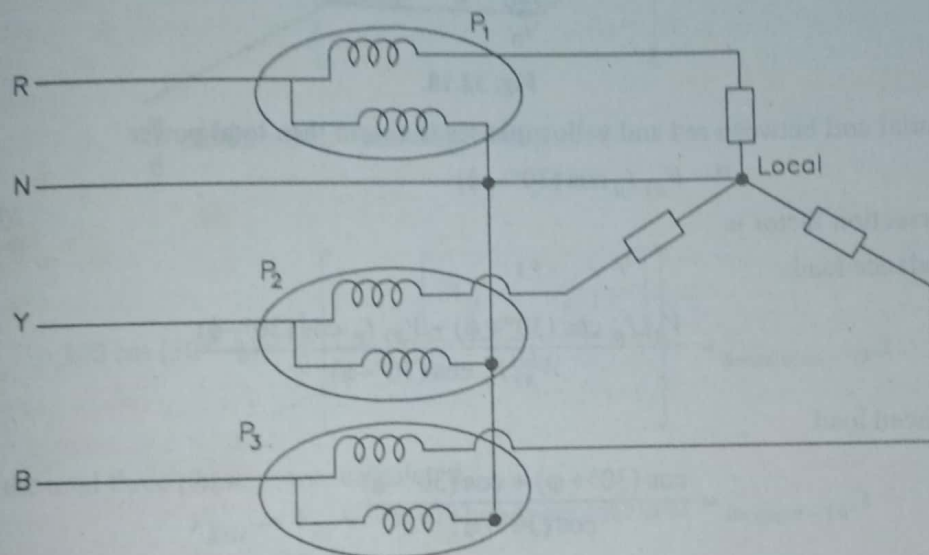


Fig. 32.16.

The same diagram of measurement of power for three phase three-wire system has been given by Fig. 32.17.

The vector diagram corresponding to Figure 32.18 is given by

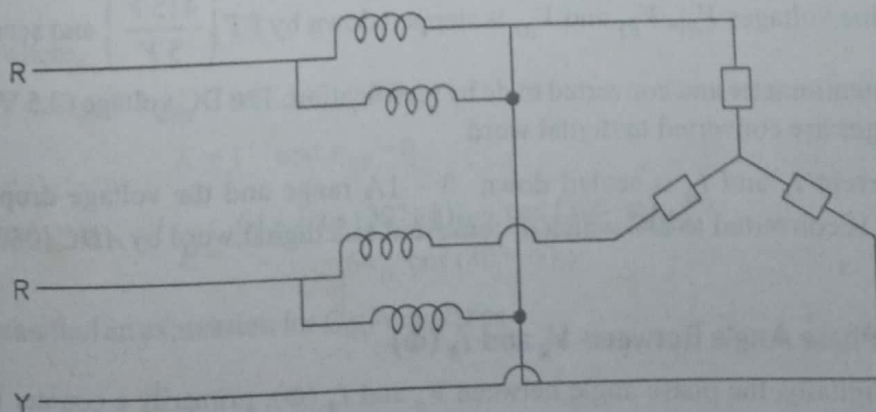


Fig. 32.17.

The total power flowing through the load (Fig. 32.18).

$$P = V_{RY} I_R \cos (30^\circ + \phi) + V_{BY} I_B \cos (30^\circ - \phi) \quad \dots(1)$$

If the load is balanced then

$$V_{RY} = V_{BY} = \sqrt{3} V$$

and

$$I_R = I_B = I$$

or,

$$P = 3 VI \cos \phi \quad \dots(2)$$

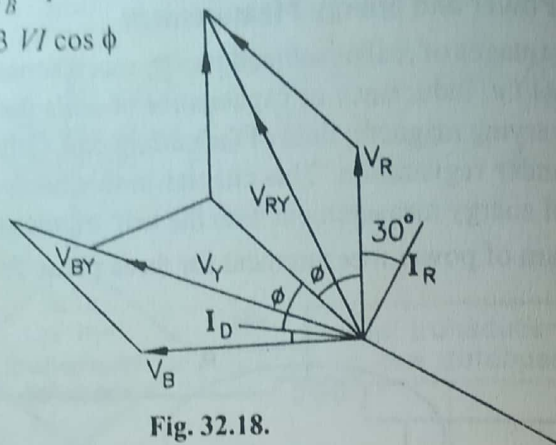


Fig. 32.18.

If the potential coil between red and yellow phases is absent then total power

$$P = V_{RY} I_B \cos (30^\circ - \phi)$$

And the correction factor is

1. For unbalance load

$$C_{RY - \text{unbalanced}} = \frac{V_{RY} I_R \cos (30^\circ + \phi) + V_{BY} I_B \cos (30^\circ - \phi)}{V_{BY} I_B \cos (30^\circ - \phi)}$$

2. For balanced load

$$C_{RY - \text{balanced}} = \frac{\cos (30^\circ + \phi) + \cos (30^\circ - \phi)}{\cos (30^\circ - \phi)}$$

Similarly for fault in PT, between Y and B phases

$$C_{BY - \text{unbalanced}} = \frac{V_{RY} I_R \cos (30^\circ + \phi) + V_{BY} I_B \cos (30^\circ - \phi)}{V_{RY} I_R \cos (30^\circ + \phi)}$$

Digital Measurement Scheme with ADC

Firstly the line voltages V_{RY} , V_{BY} and V_{BY} is stepped down by PT $\left(\frac{415 V}{5 V} \right)$ and secondary of PT further scaled down by potentiometer and converted to dc by rectification. The DC voltage (0.5 V) proportional to V_{RY} and V_{BY} line voltages are converted to digital word.

The line current I_R and I_B is scaled down 0 – 1A range and the voltage drop across a resistance due to this current is converted to DC which is converted to a digital word by ADC (0804) through channels 1 and 2.

Measurement of Phase Angle Between V_R and I_R (ϕ)

To convert digitally, the phase angle between V_R and I_R (ϕ), primarily a counter is set when V_R has a

rising edge and the counter increments with a delay clock pulse and the count stops as soon as I_R has a rising edge. The count value is proportional to ϕ .

Again the rising edge of the V_R voltage is the time when counter is set and the zero of the falling edge of the voltage V_R is the time instant when counter is stopped. The count is proportional to 180° .

So $\phi \propto N$ (from first count value)
 $180^\circ \propto N$ (from second count value)

Some clock pulse is used to identify N and N_1 .

Now,
$$\cos \phi = \frac{3.14 N}{N_1} = \frac{13A_H \times N}{N_1} \text{ (in Hex)}$$

Changing to hexadecimal codes

$$\therefore 100 \cos(30^\circ + \phi) = \frac{1}{2} \left[C8_H - \frac{\left[34_H + \frac{13A_H \times N}{N_1} \right]}{64_H} \right] \quad \left[\text{From } \cos x = 1 - \frac{x}{1} \text{ from (2)} \right]$$

where, $\frac{\pi}{6} \text{ radian} = 34_H$ and $C8_H = 100_{10}$

Similarly,

$$100 \cos(30^\circ - \phi) = \frac{1}{2} \left[C8_H - \frac{\left[34_H - \frac{13A_H \times N}{N_1} \right]}{64_H} \right]$$

Hence the total three phase power calculated.

$V_{RYH} \rightarrow V_{RY}$ Voltage in hex in hexadecimal

$I_{RH} \rightarrow I_R$ current

$$= \left[\frac{64_H mf_{V_{RY}} V_{RYH} mf_{I_R} I_{RH} \cos(30^\circ + \phi)}{64_H} \right] + \frac{64_H mf_{V_{BY}} V_{BYH} mf_{I_B} I_{BH} \cos(30^\circ + \phi)}{64_H} \times K$$

for line fault where,

$$V_{RYH} \neq 0 \text{ and } V_{BYH} \neq 0$$

for

$$K = 1 \text{ and } V_{RY} = 0$$

then

$$P = \frac{64_H [\cos(30^\circ + \phi)_H + \cos(30^\circ - \phi)_H]}{64_H \cos(30^\circ - \phi)_H}$$

Similary we can find an expression for C_{RY} -unbalanced

The fault measurement algorithm is shown in the flow chart.

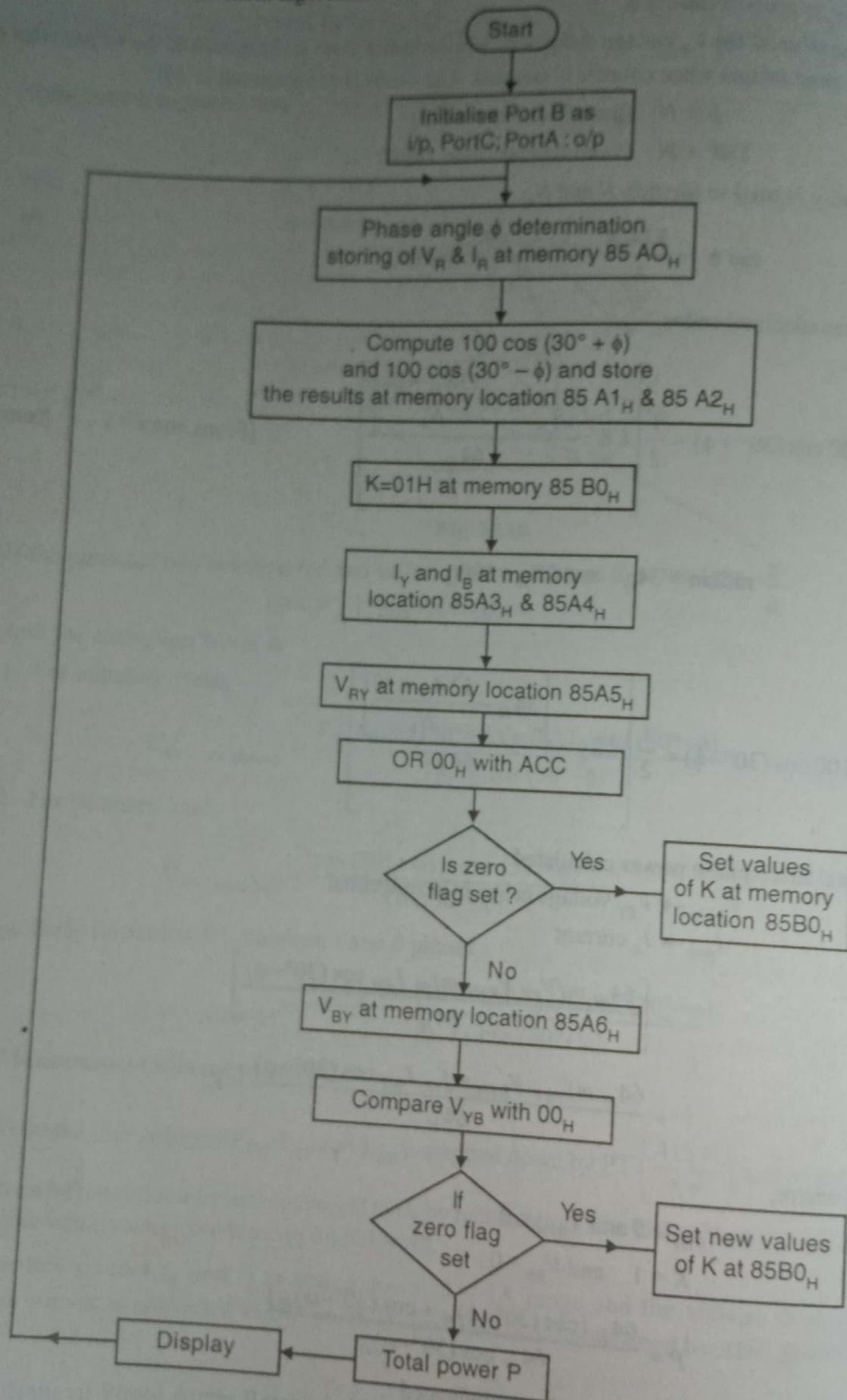


Fig. 32.19.