Sudan University of Science and Technology

College of graduate studies

Fabrication of ZnO Nanorods Supported on Silver Electrode and its Utilization in Potentiometric Measurements

تصنيع قطب اكسيد الزنك النانوي المدعم بقطب الفضة واستخدامه في القياسات الجهدية

A Dissertation Submitted in Partial Fulfillment of the Degree of Master of Science in Chemistry

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الأية

بسم الله الرحمن الرحيم

قال الله تعالى :

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

سورة النور الأية (34)

صدق الله العظيم

I

Dedication

dedicate to:

My parents ,

my husband,

my brother,

and my sister.

Acknowledgment

All praise is due to Allah the most Gracious the most merciful for giving me health and patience to accomplish this work.

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Abstract

On this study we design the simple device that contents two electrodes (working electrode) and (reference electrode).

The reference electrode is a silver-sliver chloride, and the working electrode is zinc oxide nanoparticles which were prepared by ahydrothermal method at low temperature (60^0C), also zinc oxide nanoparticles were grown at silver wire.

The working electrode and reference electrode were connected by coaxial cable in pH-meter. Then this simple device was used to measure the pH and mV of milk sample.

المستخلص

في هذه الدراسة تم تصميم جهاز بسيط مكون من قطبين الاول عبارة عن قطب العمل وهو فضة\كلوريد فضة اما القطب الاخر عبارة عن القطب المرجع وهو مكون من اكسيد الزنك النانوي المحضر بالطريقة الهيدروثيرمية في درجة حرارة منخفضة (60)م^{0}وتم تغطية سلك الفضة باكسيد الزنك النانوي ثم تم توصيل القطب المرجع مع قطب العمل بجهاز الاس الهيدروجينى وتم استخدام هذا الجهاز لقياستركيز ايونات الهيدروجين والفولتمترية لعينات اللبن .

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1.**GENERAL INTRODUCTION**

1.1 The nanotechnology science

Today, nanotechnology (NT) is operating in various fields of science via its operation for materials and devices using different techniques at nanometer scale. Nanoparticles are a part of nanomaterial's that are defined as a single particles 1–100 nm in diameter. From last few years, nanoparticles have been a common material for the development of new cutting-edge applications in communications, energy storage, sensing, data storage, optics, transmission, environmental protection, cosmetics, biology, and medicine due to their important optical, electrical, and magnetic properties. In particular, the unique properties and utility of nanoparticles also arise from a variety of attributes, including the similar size of nanoparticles and biomolecules such as proteins and polynucleic acids. Additionally, nanoparticles can be fashioned with a wide range of metal and semiconductor core materials that impart useful properties such as fluorescence and magnetic behavior. Moreover, unlike their bulk counterparts, nanoparticles have reduced size associated with high surface/volume ratios that increase as the nanoparticle size decreases. As the particle size decreases to some extent, a large number of constituting atoms can be found around the surface of the particles, which makes the particles highly reactive with prominent physical properties. Nanoparticles of particular materials show unique material properties, hence, manipulation and control of the material properties via mechanistic means is needed. In addition, synthesis of nanoparticles having uniform shape and size via easy synthetic routes is the main issue in nanoparticle growth. For the past decade, scientists have been involved in the development of new synthetic routes enabling the precise control of the morphology and size of the nanoparticles. In addition, nanoparticle synthesis can be possible via liquid (chemical method), solid, and gaseous media, but due to several advantages over the other methods, chemical methods are the most popular methods due to their low cost, reliability, and environmentally friendly synthetic routes, and this method provides rigorous control of the size and shape of the nanoparticles.

In general, nanoparticles with high surface-to-volume ratio are needed, but the agglomeration of small particles precipitated in the solution is the main concern in the absence of any stabilizer. In this regard, preparations of stable colloids are important for nanoparticle growth. In addition, nanoparticles are generally stabilized by steric repulsion between particles due to the presence of

surfactant, polymer molecules, or any organic molecules bound to the surfaceof nanoparticles. Sometimes van der Waals repulsion (electrostatic repulsion) also plays important role in nanoparticles stabilization. With all the issues related to nanoparticle synthesis, there are various types of nanoparticles reported in the literature, e.g., metal nanoparticles, metal oxide nanoparticles, and polymer nanoparticles. Among all these, metal oxide nanoparticles stand out as one of the most versatile materials, due to their diverse properties and functionalities. Most preferentially, among different metal oxide nanoparticles, zinc oxide (ZnO) nanoparticles have their own importance due to their vast area of applications, e.g., gas sensor, chemical sensor, bio-sensor, cosmetics, storage, optical and electrical devices, window materials for displays, solar cells, and drug-delivery. ZnO is an attractive material for short-wavelength optoelectronic applications owing to its wide band gap 3.37 eV, large bond strength, and large exciton binding energy (60 meV) at room temperature. As a wide band gap material, ZnO is used in solid state blue to ultraviolet (UV) optoelectronics, including laser developments. In addition, due to its non-Centro symmetric crystallographic phase, ZnO shows the piezoelectric property, which is highly useful for the fabrication of devices, such as electromagnetic coupled sensors and actuators.

1.2 The structure of ZnO

Crystalline ZnO has awurtzite structure has a hexagonal unit cell with two lattice parameters, a and c, andbelongs to the space group of C_{6v}^4 or $P6_3mc$. (Figure 1.1) clearly shows that the structure iscomposed of two interpenetrating hexagonal closed packed (hcp) sublattices, in whicheach consist of one type of atom (Zn or O) displaced with respect to each other alongthe threefold c-axis. It Crystalline ZnO has a wurtzite (B4) crystal structure at ambient conditions. The ZnO can be simply explained schematically as a number of alternatingplanes stacked layer-by-layer along the c-axis direction and composed of tetrahedral coordinated Zn^{2+} and O^{2-}. The tetrahedral coordination of ZnO gives rise to the noncentrosymmetric structure. In wurtzite hexagonal ZnO, each anion is surrounded by fourcations at the corners of the tetrahedron, which shows the tetrahedral coordination andhence exhibits the sp^3 covalent-bonding.

Due to its vast areas of application, various synthetic methods have been employed to grow a variety of ZnO nanostructures, including nanoparticles, nanowires, nanorods, nanotubes, nanobelts, and other complex morphologies.

1.3 nanoparticle of Zn

Figure 1 the hexagonal wurtzite structure model of ZnO. The tetrahedral coordination of Zn-O is shown. O atoms are shown as larger white spheres while the Zn atoms are smallerbrown spheres [1]
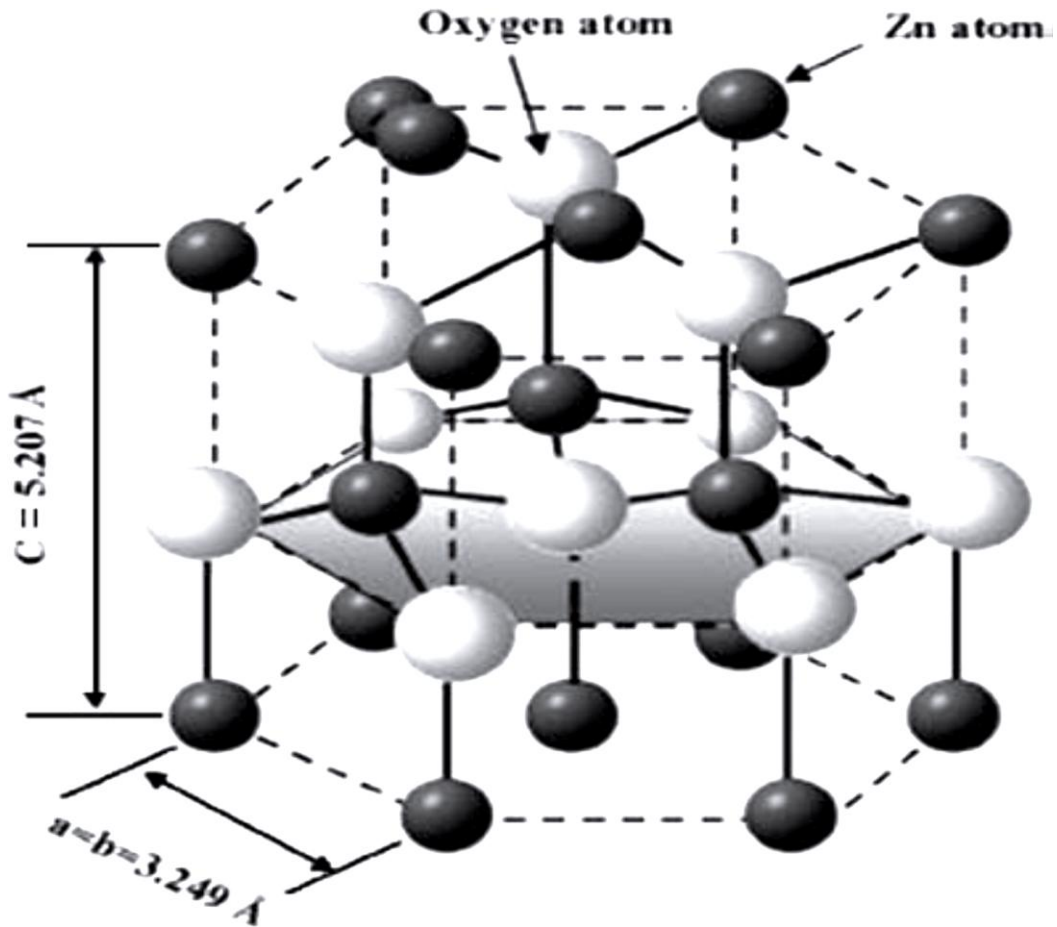
Fig (1):the hexagonal wurtzite structure model of ZnO

1.4 Material properties of ZnO

During the last decade, new nanomaterial's, nanostructures based device structures have Attracted a great attention because of their fascinating properties and potential as building Blocks for electronics, optoelectronics, and sensor applications. These properties make the ZnO a promising material for the fabrication of the Nano devices such as light emitting diodes, electrochemical sensors, ultra-violet (UV) detectors, Nano

generators.Currently, zinc oxide is the most studied material among metal oxides due to its broad Application list related to its semiconducting, optical and piezoelectric properties and Etc.,

Respectively. For instance, ZnO-based devices can be used in optoelectronics,

Sensors/transducers and lasers etc. Here some of the properties of ZnO are highlighted:

* Basic properties of ZnO.

* Physical and chemical properties of ZnO.

* Electrochemical sensing aspect of ZnO.

1.4.1Basicproperties of ZnO

ZnOnormally forms in the hexagonal crystal structure, the lattice parameter a= 3.25 Å and c = 5.12 Å. The large difference in the values of electronegativity (Oxygen = 3.44 and Zinc = 1.65) responsible for the strong ionic bonding between them. In the structures, the zinc (Zn) atoms are tetrahedralcoordinated to four oxygen (O) atoms stacked alternately along the c-axis. Generally, ZnO unit cell is neutralin which an oxygen anion is encircled by four zinc cations at the corner of a tetrahedron, and vice versa. The distribution of the cations and anions could take specific configuration as determined by crystallography technique, so that some surfaces can be terminated entirely withcations or anions, resulting in positively or negatively charged surfaces, called polar Surfaces. These polar surfaces of the ZnO have transferable and unchangeable ionic charges and their interaction at the surface depends on their distribution. Thus, in results the structures have been shaped with a minimal electrostatic energy which is responsible for the fabrication of polar surface dominated nanostructures. This phenomenal effect results for the fabrication of different ZnO one-dimensional (1D) nanostructure such as nanowires, Nano rods, nanotubes, Nano spring, Nano cages and Nano belts .

1.4.2Chemicaland physical properties of ZnO

The most common polar surface is the basal plane. The presence of polarized charged ions, different surfaces like positively charged Zn-(0001) and negatively charged O-(0001) polar surfaces are produced, resulting in a normal dipole moment and spontaneous polarization along the c-axis as well as a divergence in surface energy. To maintain a stable structure, the polar surfaces generally have facets or exhibit massive surface reconstructions, but ZnO ± (0001) are exception, which are atomically flat, stable and

without reconstruction . Understanding the superior stability of the ZnO ± (0001) polar surfaces is a forefront research in today's surface physics. The several properties of ZnO nanostructured materials depend on its polarity, growth, etching, defect generation and plasticity, spontaneous polarization and piezoelectricity. ZnO is a versatile wideband semiconductor as compared to its contenders like GaN in properties and applications. In fact, ZnO have several advantages as compared to the existing devices fabricated from other wideband semiconductors in which the most important property of ZnO is its high exciting binding energy of ZnO i.e. 60 meV at room temperature compared to its counterpart GaN (25 meV). This high exciting binging energy is responsible to enhance the efficiency of light emission.

1.4.3 ZnO nanostructures based electrochemical sensors

The rapid advancements in development of a miniaturized nanodevices based on semiconductornanomaterial's have attracted a significant interest among the researchers due to the special physical properties of these materials at low dimensions. Determination of biological or biochemical/chemical processes is of utmost importance for medical, environmental and biotechnological applications.

The electrochemical sensing based on various nanomaterials with a wide variety of low dimensional nanostructures has attracted considerable attentions due to their special physical properties. Among these materials, ZnO has attracted great interests in the applications of sensors/transducers because it has a wide variety of nanostructures such as Nanowires/nanorods, nanotubes, nanoporous/nanoflakes and etc., and their remarkable Properties such as large surface-to-volume ratio, biosafety, bio-compatibility, nontoxicity,

High-electron transfer rates, enhanced analytical performance, increased sensitivity, easy Fabrication, and low cost. In addition, a high isoelectric point of ZnO (IEP 9.5) provides Convenient micro-environment to form a good matrix with low isoelectric point acidic Proteins or DNA for immobilization by electrostatic interactions with high binding stability Moreover, ZnO possess high ionic bonding (60%), and its dissolution is slow at Biological pH values. Reported that ZnOnanorods are bioCompatible and bio-safe when they are used in biological environment at normal Concentration range. In addition, ZnOis relatively stable around biological pH-values which make ZnO compatible with

biological fluids and species which also makes it attractive in vivo environment. Currently, we have successfully demonstrated that ZnOnanorods/nanowires can be used to measure the intracellular glucose and K+ concentrations using micro injection technique in human adipocytes and frog oocytes.

The main effort has been focused to fabricate the ZnOnanorods/nanowires selectively on the borosilicate glass capillary tips (0.7 µm outer diameters), suitable and capable to gently penetrating the cell membrane and immobilized with glucose oxidase (GOD) and coating of ionosphere (Valinomycin) for the selective determination of glucose and K+ ions concentrations respectively. Thus, the ZnO nanostructures are suitable for extra and intracellular sensing applications [2].

1.5techniques of preparation nanostructure:

A-top-down technique.

B-bottom-up technique.

The "bottom-up" approach first forms the nanostructured building blocks and then assembles them into the final material. An example of this approach is the formation of powder components through aerosol techniques and then the compaction of the components into the final material. These techniques have been used extensively in the formation of structural composite materials. One "top-down" approach begins with a suitable starting material and then "sculpts" the functionality from the material. This technique is similar to the approach used by the semiconductor industry in forming devices out of an electronic substrate (silicon), utilizing pattern formation (such as electron beam lithography) and pattern transfer processes (such as reactive ion etching) that have the requisite spatial resolution to achieve creation of structures at the nanoscale .This particular area of nanostructure formation has tremendous scope,warranting its own separate study, is a driving issue for the electronics industry, and will not be a principal theme of this study. Another top-down approach is "ball-milling," the formation of nanostructure building blocks through controlled, mechanical attrition of the bulk starting material. Those

nano building blocks are then subsequently assembled into a new bulk material.
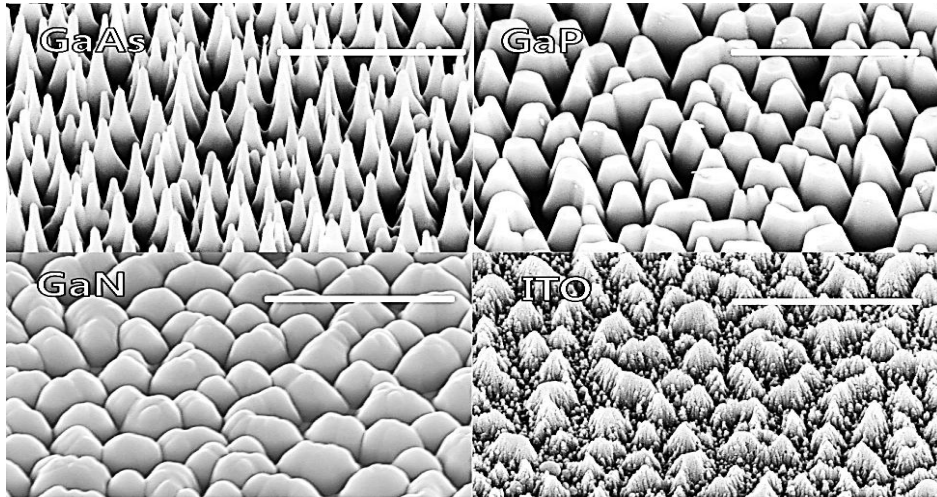
Fig(2) : bottom up approach of nanostructure.

Why is Bottom-Up Processing needed?
- Allows smaller geometries than photolithography.
- Certain structures such as Carbon Nanotubes and Si nanowires are grown through a bottom-up process.
- New technologies such as organic semiconductors employ bottom-up processes to pattern them.
- Can make formation of films and structures much easier.
- Is more economical than top-down in that it does not waste material to etching**Applications of bottom-up processing :**
- Self-organizing deposition of silicon nanodots.
- Formation of Nanowires.
- Nanotube transistor.
- Self-assembled monolayers.
- Carbon nanotube interconnects.

Milling Process (top-down approach) :

The mechanochemical process is a cheap and simple method of obtaining nanoparticles on a large scale. It involves high-energy dry milling, which initiates a reaction through ball–powder impacts in a ball mill, at low temperature. A "thinner" is added to the system in the form of a solid (usually NaCl), which acts as a reaction medium and separates the nanoparticles being formed. A fundamental difficulty in this method is the uniform grinding of the powder and reduction of grains to the required size, which decreases with increasing time and energy of milling. Unfortunately, a longer milling time leads to a greater quantity of impurities. The advantages of this method are the low production costs, small particle sizes and limited tendency for particles to agglomerate, as well as the high homogeneity of the crystalline structure and morphology.The starting materials used in the mechanochemical method are mainly anhydrous $ZnCl_2$ and Na_2CO_3.NaCl is added to the system; this serves as a reaction medium and separates the nanoparticles. The zinc oxide precursor formed, $ZnCO_3$, is calcined at a temperature of 400–800 °C. The process as a whole involves the following reactions (1) and (2):

$$ZnCl_2 + Na_2CO_3 \rightarrow ZnCO_3 + 2NaCL$$

$$ZnCO_3 \xrightarrow{temperature} ZnO + CO_2$$
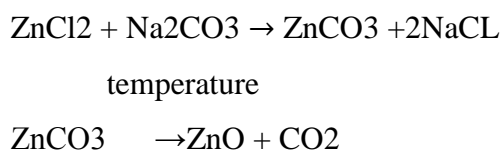
The mechanochemical method was proposed A_0et al.by they synthesized ZnO with an average crystallite size of 21 nm. The milling process was carried out for 6 h, producing $ZnCO_3$ as the zinc oxide precursor. Calcination of the precursor at 600 °C produced ZnO with a hexagonal structure. Tests showed that the size of the ZnO crystallites depends on the milling time and calcination temperature. Increasing the milling time (2–6 h) led to a reduction in the crystallite sizes (21.5–25 nm), which may indicate the existence of a "critical moment". Meanwhile an increase in the calcination temperature from 400 to 800 °C caused an increase in crystallite size (18–35 nm).

The same system of reagents was used by Tsuzuki and McCormick They found that a milling time of 4 h was enough for a reaction to take place between the substrates, producing the precursor $ZnCO_3$, which when calcite at 400 °C produced Nano crystallites of ZnO with an average size of 26 nm. Tsuzuki *et al.* showed that milling of the substrates without a thinner

leads to the formation of aggregates measuring 100–1000 nm. This confirmed the important role played by zinc chloride in preventing agglomeration of the nanoparticles.

A milling process of ZnCl2 and Na2CO3 was also carried out by Moballegh et al and by Aghababazadeh*et al* .Moballegh*et al.*, investigated the effect of calcination temperature on particle size. An increase in the temperature of the process (300–450 °C) caused an increase in the size of the ZnO particles (27–56 nm). Aghababazadeh*et al.* obtained ZnO with an average particle size of approximately 51 nm and a surface area of 23 m2/g, carrying out the process at a temperature of 400 °C.

Stanković*et al.* extended their previous study to investigate mechanical-thermal synthesis (MTS)—mechanical activation followed by thermal activation of ZnO from ZnCl2 and oxalic acid (C2H2O4·2H2O) as reactants with the intention of obtaining pure ZnOnanopowder. The study also aimed to examine the effects of oxalic acid as an organic PCA, and different milling times, on the crystal structure, average particle size and morphology of ZnOnanopowders. The mixture of initial reactants was milled from 30 min up to 4 h, and subsequently annealed at 450 °C for 1 h. Qualitative analysis of the prepared powders was performed using X-ray diffraction (XRD) and Raman spectroscopy. The XRD analysis showed perfect long-range order and the pure wurtzite structure of the synthesized ZnO powders, irrespective of the milling duration. By contrast, Raman spectroscopy indicates a different middle-range order of ZnO powders. From the SEM images, it is observed that the morphology of the particles strongly depends on the milling time of the reactant mixture, regardless of the further thermal treatment. A longer time of milling led to a smaller particle size. [3]

1.6 growth of nanostructure of ZnO

There are different approaches to grow composite ''hybrid'' nano-materials. Usually the growth using the wet ''aqueous'' chemical route is a very easy and popular approach. Before this growth usually a seed layer can be used to control the growth of the first nanomaterial. This nanomaterial will work as a support to grow the other secondary nanomaterial. Hence the wet-chemically seed-mediated growth provides an effective method for the synthesis of the hybrid nanoparticles with well controlled structures, where the secondary species attach and sequentially grow on the preformed seeds. To ensure the deposited species well-dispersed on the supports, heterogeneous nucleation and growth through atomic addition must be achieved and

homogeneous nucleation should be avoided. Significant progress has been achieved in the synthesis of the core shell and dumbbell nanoparticles combining two different components. When the individual components involved have similar crystal structures and lattice parameters, each component fuses together giving the dumbbell shape . In a dumbbell structure consisted of one particle bounded another, charged transfer across nanoscale junction could significantly change local electronic configuration that give the remarkable properties. While large lattice space difference of the individual components results in core–shell-shaped structure obtained by growing a uniform layer of a shell material around colloidal particles. Hybrid materials are becoming very popular due to the multi-functions possible to gain by employing them ino devices. An example is in nano-medicine. A major goal in nanomedicine is the coherent implementation of multifunctional platforms within a single targeted nano-delivery system that would simultaneously perform diagnosis, targeted delivery and efficient therapy. The hybrid nanomaterials are becoming a hot research area as they enable the tracking of cells to simultaneous medical therapy and diagnosis. The Zinc oxide is a (II-VI) material and possesses a variety of excellent properties. These properties are of interest for technological as well as medical applications (devices). Beside of interest for photonic applications, it is a bio-safe and biocompatible and has the strongest electromechanical coupling. The relatively large electro-mechanical coupling makes ZnO an excellent piezoelectric material. Moreover, ZnO possesses self-organized growth property. His makes it possible to grow good crystal ZnO material on any surface being amorphous or crystalline. This property combined with the excellent other properties makes ZnO interesting for many applications, among it, light emitting didoes, laser diodes, energy harvesting components. Since the waste ambient mechanical energy is the most abundant source of energy, ZnO is of potential for developing such energy providing materials.

On the other hand, low temperature chemical growth can be used to grow ZnO nanostructures of good quality. The temperature can be as low as room temperature (but longer duration of growth is needed). This enables the use of soft and foldable material, like e.g. plastic as a substrate, and hence develops plastic electronics. This will lead to reduce the cost of electronic components considerably. The chemical growth can be combined with first apply a seed layer to lead to nucleation cites and hence achieve a uniform growth [4].

1.6.1 Growth of ZnO at low temperature:
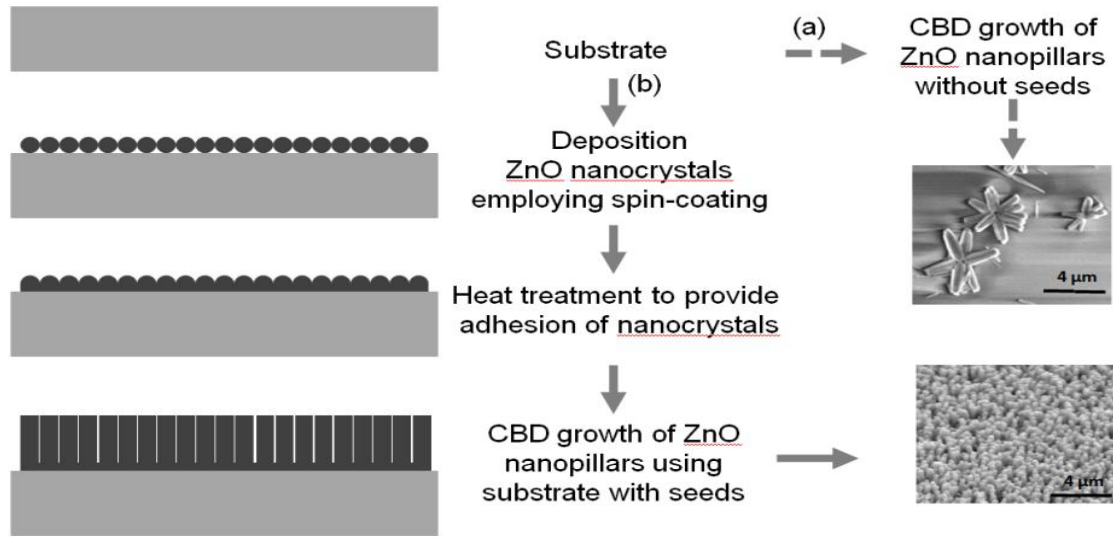
Fig (3) : growth of ZnO at low temperature

1.6.2 growth of ZnO at (50-70) C^0:-
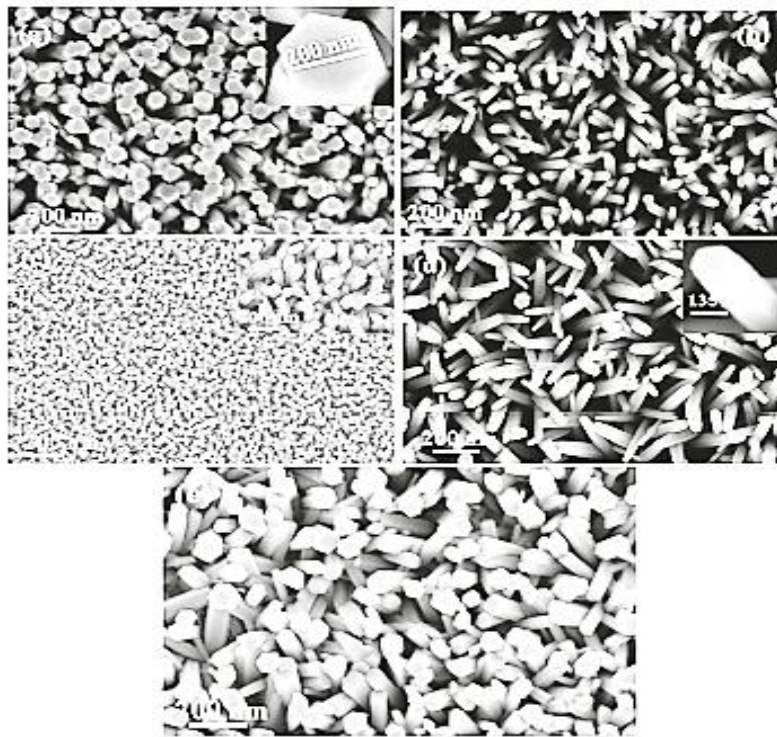
Fig(4) : growth of ZnO at 50-70 c^0
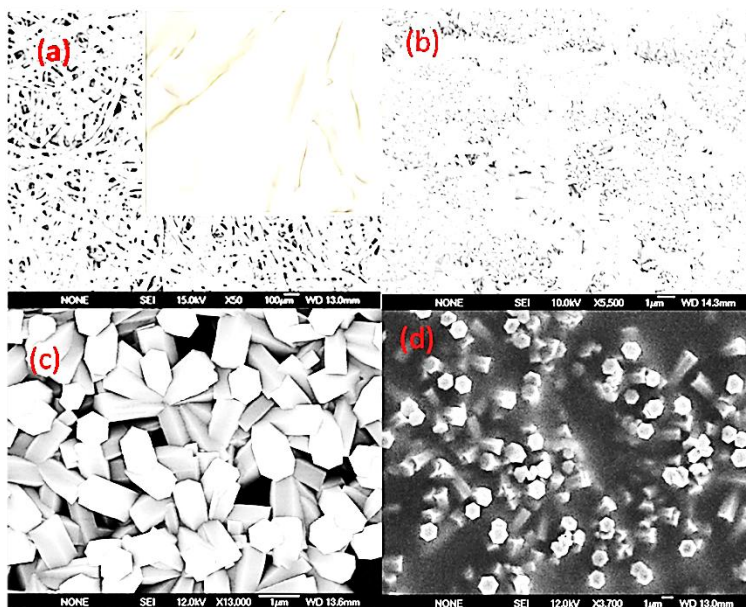
Fig (5): ZnOnanorodes grown at 50^{0}C

1.7Characterization of zinc oxide nanoparticles:-

1.7.1X-ray diffraction techniques:-

The distance between the atoms in a solids has been estimated around one angstrom (Å) and

The energy corresponds to this distance can be estimated by the formula given below;

E= hc / λ

forλ= 1 Å E ≈ 12 × 103 eV

Generally, we applied the X-ray diffraction (XRD) characterization technique for

Semiconductor materials in order to determine the material quality, composition, lattice

Parameters, orientation, defects, stress and strain. X-rays have approximately same energy

(KEV) as the interatomic distance energies of solids. In 1913 W.H and W.L. Bragg demonstrated

That the X-rays have the characteristics patterns after reflection from crystalline materials and

These patterns were different from those that were obtained from liquids. After this successful

Demonstration, X-ray diffraction patterns became an important tool for the material science

Research. Showing a schematic diagram of Bragg reflection from crystalline planes

 Having inter-plane distance d. The incident and reflected X-rays from the two planes are also

Shown. Bragg concluded that the path difference between the two X-rays diffracted from two

12

Consecutive lattice planes is 2dsinθ and it leads to Bragg's law, which states that the condition
For diffraction of X-rays for a crystalline material is:

nλ = 2d sinθ

Here θ is the angle of incidence and λ is the wavelength of the X-rays, n is an integer and it is
The order of reflection, and d is the distance between the lattice planes[2].

1.7.2 X-ray diffraction analysis

Figure 6shows XRD diffraction pattern of ZnO nanoparticles. The peaks are indexed as 31.82º
(100), 34.54º (002), 36.42º (101), 47.46º (102), 56.74º (110), 62.92º (103), 66.06º (200), 68.42º
(112), 69.06º (201) and 78.82º (202) respectively. All diffraction peaks of sample correspond to
the characteristic hexagonal wurtzite structure of zinc oxide nanoparticles (a = 0.315 nm and c =
0.529 nm). Similar, X-ray diffraction pattern were reported by C. Chen et. al. and Y. Pong et. al.
Average particle size of ZnO nanoparticles is found to be 10.0 nm using Scherrer equation
.Diffraction pattern corresponding to impurities are found to be absent. This proves that pure
ZnO nanoparticle were as synthesized [5].

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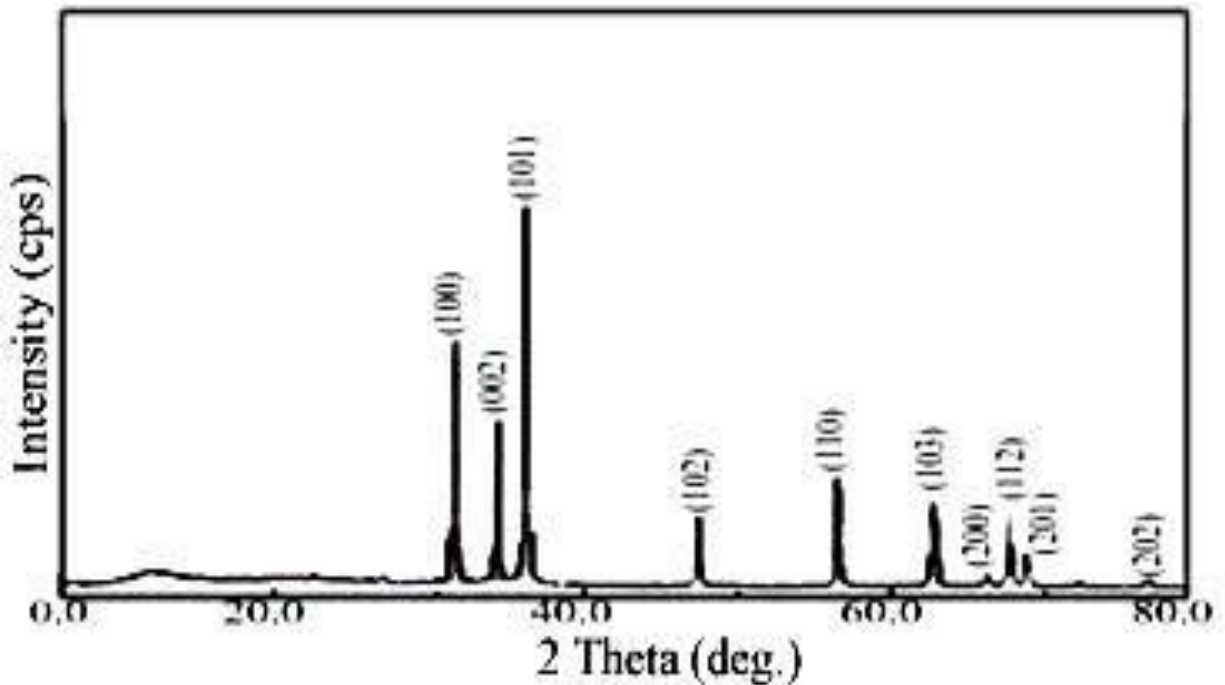
Fig 6:XRD patterns of ZnO nanoparticle

1.8 Electrochemical measurements using ZnO nanostructure based sensors:-

Electrochemical measurements were carried out using a two-electrode configuration Consisting of ZnO nanowires/Nano rods as the working electrode and an Ag/AgCl as a Reference electrode. The electrochemical responses were measured using pH meter at room temperature (25 °C). The electrochemical response was observed until the equilibrium potential has reached and stabilized, then the electrochemical potential was measured.[2]
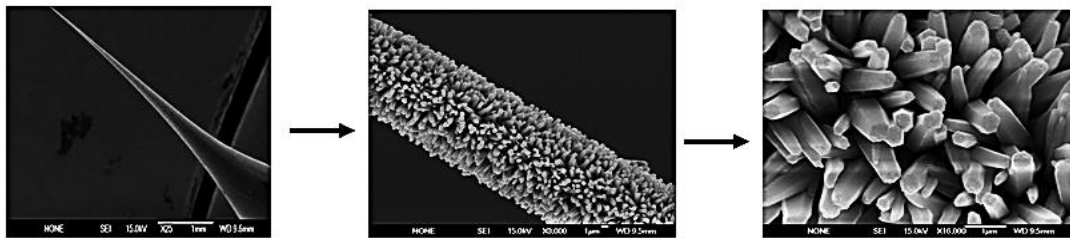
Different sub-micron electrodes coated with ZnO nanowires acting nano-sensing elements. These nano-sensing elements are highly sensitive and can be made selective by functionalization.
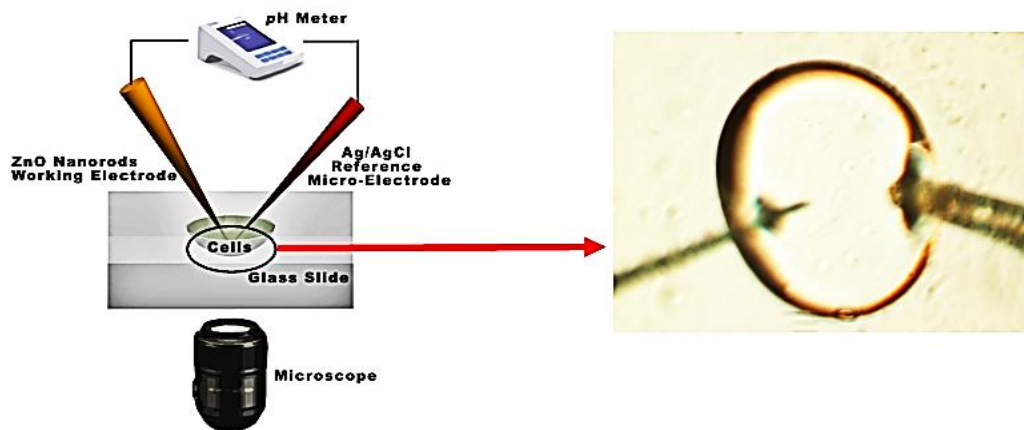
(Left) Inter cell measurement set up and (Right) Digital microscope photograph of inter cell selective calcium ion measurement for human fat cell (adipocytes) .

Fig 7: the schematic diagram of measuring set up for inter cell selective calcium ion.

1.2Milk

1.2.1 Milk components

Analysis of food and drink is a major activity for the industry and a very important part of product development and quality assurance. Food analysis is required for a wide variety of reasons - from testing suitability for purpose, through checking shelf-life or authenticity, to assuring legal compliance. Food testing is integral to the efficient production of safe, quality products. Apart from the well-known food analysis areas such as chemical and microbiological analyses, Food testing also includes the analysis of the physical properties of foods, (e.g. glass, insects, stones and metal).Milk has long been a popular beverage, not only for its flavor, but because of its unique nutritional package. Chemically, milk is a complex fluid in which more than 100 separate chemical compounds have been found. Its major components are water (more than87%), fat, lactose, casein, whey proteins, and minerals (calcium, magnesium, phosphorus, potassium, selenium, and zinc),see**Fig(8)**
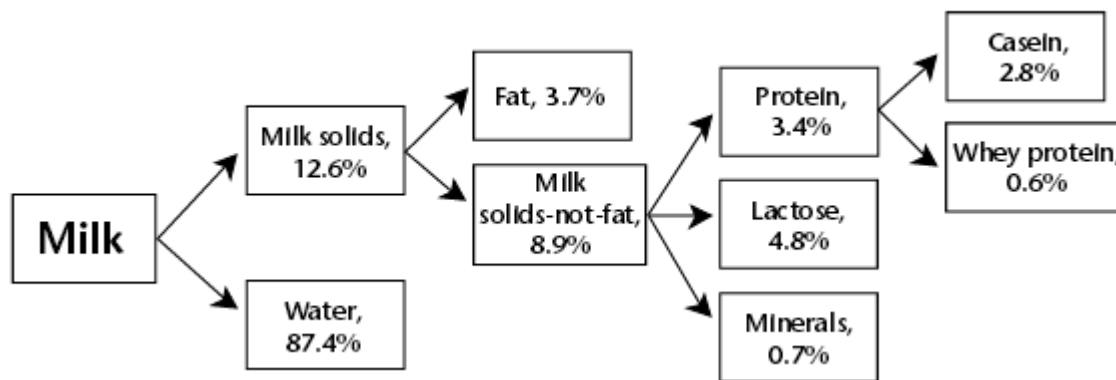
Fig(8): Gross composition of milk, showing major constituents.

Consumers currently determine milk spoilage by checking the "sell by" and "best if used by" dates on milk cartons provided by suppliers. These dates are simple estimates of milk shelf life and are often inaccurate due to the variable processing, shipping, and storage conditions of the milk .In reality,milk may spoil before the printed expiration, and spoiled milk can lead to food poisoning if consumed [6]

1.2.2 Physical and chemical properties of milk :-

Density:

Density is defined as an object's mass divided by its volume. It depends on the temperature of the object, composition of the material, and whether or not the object contains air. The density of milk products can be used to convert volume into mass and mass into volume, to estimate the amount of solids present in milk, and to calculate other physical properties. The density of cow's milk usually varies between 1.028 and 1.038 g/cm3.

Appearance:

The opacity of milk is due to its content of suspended particles of fat, proteins, and minerals. The color varies from white to yellow depending on the carotene content of the fat. Skim milk is more transparent and has a slightly bluish color.

Freezing Point:

The freezing point of milk is lower than the freezing point of water because of the dissolved components in milk. Measuring the freezing point is used as a legal standard to determine if milk has been diluted with water. The freezing point of milk is -0.552oC or 31oF [6].

1.2.3 Changes in milk during storage

* Oxidation of fat

* Oxidation of protein

* Hydrolysis

Oxidation of fat

Oxidation occurs at the double bonds of the unsaturated fatty acids. The presence of iron and copper salts accelerates the onset of auto-oxidation and the development of a metallic flavor. Also, the presence of dissolved oxygen and exposure to light promotes oxidation. Oxidation of milk fat may limit the shelf life of whole milk powders, but is not usually a serious problem. Oxidation is catalyzed by light, so if milk is exposed to light for too long a period of time, off-

flavors will occur. Fat oxidation can be counteracted by microorganisms in milk such as lactic acid bacteria because they consume oxygen thereby reducing oxidation. Also, pasteurization helps to reduce oxidation because reducing compounds such as sulfhydryl groups are formed when milk is heated. The metallic oxidation off-flavor is more common in the winter than in the summer because of differences in the cow's diet. Summer feeds are higher in Vitamins A and C. These vitamins increase the amount of reducing substances in milk.

Oxidation of protein:

When exposed to light, the amino acid methionine is degraded to methional by riboflavin and vitamin C. Methional causes the "sxxunlight flavor" in milk. However, methionine is present in milk as part of the milk proteins. So fragmentation of the proteins must occur to release the free amino acid methionine. Factors that relate to sunlight flavor development are:Intensity of the light, especially light from fluorescent tubes, Duration of exposure, Homogenized milk is more sensitive that non-homogenized milk, Nature of the package - opaque packages such as plastic and paper give good protection

Hydrolysis:

Hydrolysis is the liberation of free fatty acids from the glycerol backbone. This reaction requires the presence of a lipase, which is an enzyme that catalyzes the hydrolysis of ester bonds of lipids. A large amount lipoprotein lipase is present naturally in milk, but fortunately fat globules with an intact milk fat globule membrane are not susceptible to hydrolysis by the enzyme. Also, lipoprotein lipase is inactivated at temperatures required for pasteurization. Spoilage bacteria provide a heat stable lipase, but the spoilage bacteria must exceed normal levels, and routine bacterial control should prevent such an occurrence [8].

1.2.3Milk spoilage:

Milk spoilage is an indefinite term and difficult to measure with accuracy. This uncertainty can cause suffering for both milk manufacturers and consumers. Consumers who have been misled by ambiguous expiration dates on milk cartons waste resources by disposing of unspoiled milk or experience discomfort from drinking spoiled milk. Consumers are often unwilling to purchase products close to their inaccurate expiration

dates. This consumer behavior has a negative financial impact on milk producers. Inaccurate milk spoilage detection methods also force milk producers to use overly conservative expiration dates in an effort to avoid the legal and economic consequences of consumers experiencing illness from drinking spoiled milk. Over the last decade, new methods have been researched with the purpose of developing more accurate and efficient means of detecting milk spoilage. These methods include indicators based on pH bacteria counts and gas-sensor arrays. This article explores various methods of spoilage detection designed to prevent such consequences. The respective level of effectiveness of each method is discussed, as well as several further approaches to contain freshness regardless of detection.

Consumers currently determine milk spoilage by checking the "sell by" and "best if used by" dates on milk cartons provided by suppliers. These dates are simple estimates of milk shelf life and are often inaccurate due to the variable processing, shipping, and storage conditions of the milk. Retailers and consumers discard billions of pounds of unspoiled milk each year while relying on inaccurate printed expiration dates. Conversely, milk may spoil before the printed expiration, and spoiled milk can lead to food poisoning if consumed. Another issue demonstrating the urgency of developing more accurate milk packaging is that unless there are local restrictions for dairy products, the manufacturer determines the date; if an established regulation does not exist, manufacturers can legally sell the expired product past the posted date. The cities or processors that do have regulations have different sets of rules. For example, until 2010, milk could only be legally sold in New York City up to 96 hours after 6:00 AM on the day after pasteurization. Dairylea Cooperative, Inc., the biggest processor in the region, allows milk to be sold 10 to 12 days after pasteurization. Researchers have recently begun investigating applications of current technologies for the detection of milk spoilage. While the meat, fish, and fruit industries have continually advanced new methods of packaging, packaging innovation in the milk packaging industry has remained stagnant,. Although a handful of packaging companies have proposed ideas for updated milk packaging designs, none of the proposed alternatives have succeeded in the market. Food industries and consumers have shown a trend of increasing interest in environmentally friendly, health-conscious intelligent packaging, and these market trends

indicate the need to invest heavily in these novel developments. Innovative milk packaging will further revolutionize the packaging industry and transform the way consumers think about packaging.

The aims of this article are to review the current state of the intelligent food packaging industry and present an update on the considerations that intelligent food packaging.

Milk producers are required to exclude bacteria from milk, but some may still gain entry. In the US, the bacterial count in Grade A raw milk may not exceed 300,000/ml. When the cow has mastitis, microorganisms associated with infections are found, including Staphylococcus aureus, Streptococcus uberis, and Streptococcus agalactiae. Milk from cows with mastitis cannot be used for human consumption. Pasteurization destroys most of the microorganisms in milk and all of the pathogens. In the US, the upper limit of bacteria in pasteurized milk is 20,000/ml. •Milk has a pH of 6.6, which is ideal for the growth of many organisms. Milk is sterile at secretion in the udder, but it becomes contaminated by bacteria before it leaves the udder. At this point, the bacteria are few and harmless, unless the cow has mastitis. Further infection of the milk by microorganisms happens during milking, storage, handling, and other activities [9].

1.2.3.1 Spoilage microorganisms:

Spoilage microorganisms cause degradation of proteins, fats, and carbohydrates and the milk's texture, color, taste, and smell is deteriorated. Psychrotrophs (bacteria that grow at refrigeration temperatures) are usually involved in spoilage reactions. Most are destroyed by pasteurization, but can still produce heat stable lipolytic and proteolytic enzymes that can cause spoilage. •However, some strains and species of Clostridium, Bacillus, Arthrobacter, Cornebacterium, Microbacterium, Micrococcus, Streptococcus, and Lactobacillus can survive pasteurization and grow at refrigeration temperatures and cause spoilage. •Coliforms: Coliforms are closely associated with the presence of pathogens, but they are not always pathogenic themselves, thus they are called indicator organisms. They are killed by proper pasteurization treatment. Their presence indicates improper or inadequate pasteurization or it indicates contamination after pasteurization. Escherichia coli is an example of a coliform. They are facultative anaerobes and grow best at 37oC or 98.6oF. Coliforms are able to degrade milk proteins and ferment lactose, causing milk to be spoiled quickly.

1.2.3.2 Lactic acid bacteria:

Lactic acid bacteria can produce chemical changes that are desirable in the production of fermented dairy products because they are able to ferment lactose to lactic acid. They are naturally present in the milk and are used as starter cultures in the production of cultured dairy products, such as yogurt and cottage cheese. Lactic acid bacteria include lactococci, lactobacilli, and Leuconostoc.

1.2.3.3 Spoilage of milk and milk products

Raw milk contains many types of microorganisms coming from different sources. The average composition of cow's milk is 3.2% protein, 4.8% carbohydrates, 3.9% lipids, and 0.9% minerals. Besides casein and lactalbumin, it has free amino acids that provide a good N-source. As the main carbohydrate is lactose, those microorganisms with lactose-hydrolyzing enzymes (lactase or β-galactosidase) have an advantage over those unable to metabolize lactose. Milk fat can be hydrolyzed by microbial lipases, with the release of small molecular volatile fatty acids (butyric, capric, and caproic acids).

Microbial spoilage of raw milk can potentially occur from the metabolism of lactose, proteinaceous compound, fatty acids (unsaturated), and the hydrolysis of triglycerides. If the milk is refrigerated immediately following milking and stored for days, the spoilage will be predominantly caused by the Gram-negative psychrotrophic rods, such as Pseudomonas, Alcaligenes, Flavobacterium spp., and some coliforms. Pseudomonas and related species, being lactose-negative, will metabolize proteinaceous compounds to change the normal flavor of milk to bitter, fruity, or unclean. The growth of lactose-positive coliforms will produce lactic, acetic, and formic acids, CO_2, and H_2 leading to curdling and souring of milk. Some Alcaligenesspp and coliforms can also cause ropiness (sliminess) due to production of viscous polysaccharides. However, if the raw milk is not refrigerated soon, growth of mesophiles predominates e.g, Lactococcus, Lactobacillus, Enterococcus, Bacillus, and coliforms, along with Pseudomonas, Proteus, and others causing changes like souring and curdling of milk. Yeast and mold growth, under normal conditions, is generally not expected [10].

1.2.4Current Methods of Milk Spoilage Detection

pH

The pH of milk is higher, or more alkaline, outside of the cow than inside the cow due to loss of carbon dioxide to the air. The pH of milk is never determined immediately after milking because the processing milk goes through removes dissolved gasses. The pH is determined after processing the milk to assure that lactic acid is being produced at the desired rate by added microorganisms during the preparation of cheeses and fermented milk. The casein in milk forms into a curd or a gel at a pH of 4.6 [8].

1.2.4.1 Utilizing pH Indicators as a Measure of Spoilage

Bacteria growth varies from one species of bacteria to another. While one bacteria species may prosper under certain conditions, another species may weaken. These conditions are interdependent and include nutrient avail- ability, moisture, oxygen levels and the level of other gases, the presence of inhibitors, temperature, and pH . The pH of unspoiled milk is approximately 6.7, a level at which many forms of bacteria thrive . At lower pH levels of 4.0 - 5.0, lactic acid bacteria can grow and pro- duce lactic acid. While these organisms inhibit the growth of many pathogenic bacteria and are also inten- tionally employed to ferment milk to make other dairy products such as yogurt and cheese, they can also induce undesirable spoilage in certain products. Coliforms, a common form of bacteria, have been an indicator of the presence of pathogens in assessing the contamination of water as well as dairy products. Coliforms can cause rapid spoilage in milk because they ferment lactose with the production of acid and gas, and they can also degrade milk proteins. Escherichia coli is a well-known example of a coliform. Studies have shown that other properties of milk also promote bacteria growth, such as the high availability of moisture and dissolved oxygen which supports both aerobic and facultative anaerobic microorganisms. Temperature is frequently controlled to limit bacteria growth. Extreme heat is lethal to many organisms, such as coliforms, which explains the process of milk pasteurization (63°C for 30 minutes). Two types of bacteria exist in pasteurized milk: thermoduric bacteria, which are capable of surviving the extreme heat during pasteurization, and bacteria that originate from unsanitary conditions post- pasteurization. Psychrotrophs comprise the largest percentage of bacteria in milk and cause spoilage in refrigerator temperatures at or below 7°C . Acidity

increases as milk spoils; thus, acidity can be quantified to measure milk quality. Acidity in dairy prod- ucts can be expressed in two ways: 1) titratable acidity, which shows total acidity but not acid strength; and 2) hydrogen ion concentration or pH, which indicates acid strength. The natural acidity of milk is 0.16% - 0.18%, and samples with higher figures indicate developed acidity. At normal levels of pH, the main protein in milk, casein, remains evenly dispersed. At lower levels of pH

below 4.6, the protein can no longer remain in solution, so it coagulates due to acid generated from fermentation. Two studies confirm the link between pH change in milk and spoilage: Fromm and Boor (2004) researched the attributes of pasteurized fluid milk (2% High Temperature/Short Time, HTST milk) during its shelf life. Milk samples were randomly collected from three fluid milk processing plants in the state of New York. A group of 13 panelists evaluated 2% HTST processed fluid milk products based on a quantitative descriptive analysis. They tasted and scored the perceived intensity of the aroma, taste, and aftertaste of milk samples varying in degree of freshness using a numeric scale ranging from 0 to 15 and the descriptive terms listed. The free fatty acid (FFA) content of the samples significantly increased throughout shelf life. Though not significantly different between day one and day seven, the FFA content drastically increased between days four- teen to seventeen due to milk fat lipolysis. The higher the FFA, the more likely sensory panelists were able to detect lipolyzed or rancid off-flavors in 2% fat milk. Casein levels in all milk samples also decreased approximately 2% at a relatively rapid rate following:

This study concludes that each processing plant has different microflora species and needs to have plant- specific strategies to identify and reduce sources of con- tamination. However, these species, while different, all cause milk to decrease in pH. Increases in FFA and drops in casein levels correlate with a decrease in pH. This suggests that pH can serve as a measurement not only of milk spoilage but also of milk edibility, since panelists determined FFA and casein levels affect rancidity and off flavors in fluid milk. This specific study, however, does not establish a lactic acid level and corresponding pH at which milk remains drinkable. Though pH meters are available, modern versions are inconvenient and cumbersome for individual consumers. Ostlie, Helland, and Narvhus (2003) conducted a study to analyze the amount of metabolic products produced by five

specific probiotic strains in ultra-high temperature (UHT) treated milk [18]. The study used a pH meter with a combined glass electrode and temperature probe to measure pH during fermentation. Volatile compounds were analyzed with headspace gas chromatography and organic acids were analyzed with high pressure liquid chromatography. Quantitative analysis of carbon dioxide production was determined with an infrared CO_2 gas analyzer. Below, Table 2 shows the results of survival and storage stability: Preliminary studies showed that the growth varied considerably with the concentration of the added supplements. After 6 - 16 hours of incubation, all strains attained viable cell numbers above 8.7 - 9.18 log CFU/mL. Depending on the probiotic strain used, the pH of the ultra-high temperature milk decreased from 6.7 initially to 3.9 - 4.4 after 24 hours of incubation. A disadvantage of the study was that in fortified milk, the various probiotic strains possessed different metabolic profiles, which affected the sensory quality of products containing these different organisms. The in- crease in strain growth (cell numbers), led to an increase

in the amount of lactic acid produced. This increase in lactic acid in turn led to a drop in pH. Whereas the earlier methodology correlated levels of pH with amounts of protein, this study correlated pH directly with the number of bacteria cells. These studies conclude pH is quantifiable and can measure spoilage in milk. However, there must be further research to address the shift in metabolism of probiotic bacteria in response to environmental changes, as well as the effect of different milk treatment regimens on the metabolism of probiotic bacteria in milk. In addition, the pH range where milk meets the definition of "spoiled" does not coincide with the colloquial definition of spoilage. As in the earlier study, most consumers consider milk to be inedible at those pH levels. For the future development of pH as an indicator of milk quality, a more accurate pH range must be established to define the point at which milk is no longer drinkable. This goal can be accomplished by combining Fromm and Boor's methodology involving a group of panelists to determine the level of consumable spoilage with Ostlie,Helland, and Narvhus' study of pH values and acidic byproducts. There are currently a few devices that can determine the acidity levels of milk. These are typically used as a means of quality control by manufacturers, rather than by end consumers. Many pH electrodes/meters and titra- tors/meters quickly and accurately measure pH of dairy

products. A prototype, the Milkmaid smart jug, is a new product that detects when milk starts to spoil with a pH sensor in the base. The jug informs users of spoilage via a change in the color of the jug's light-emitting diode lights. This product is not yet sold and the price has not yet been determined. A disadvantage of this design is that consumers must pour milk into a separate container, because the pH sensor is not incorporated into the plastic container milk is sold in. Thus, while the Milkmaid jug shows that detecting spoilage with pH is applicable to commercial products, it remains to be seen if this particular design becomes commercially successful [9].

1.3 literature review :-

*An electrochemical biosensor based on ZnOnanorods for potentiometric cholesterol determination is proposed. Hexagon-shaped ZnOnanorods were directly grown on a silver wire having a diameter of 250 μm using low temperature aqueous chemical approach that produced ZnOnanorods with a diameter of 125– 250 nm and a length of ~1 μm. Cholesterol oxidase (ChOx) was immobilized by a physical adsorption method onto ZnOnanorods. The electrochemical response of the ChOx/ZnO/Ag biosensor against a standard reference electrode (Ag/AgCl) was investigated as a logarithmic function of the cholesterol concentration ($1\times10-6$ M to $1\times10-2$ M) showing good linearity with a sensitivity of 35.2 mV per decade and the stab output signal was attained at around 10 s[11]

*Using zinc oxide (ZnO) nanostructures, nanorods (NRs) and nanoparticles (NPs) grown on different substrates (submicrometer glass pipettes, thin silver wire and on plastic substrate) different bio-sensors were demonstrated. The demonstrated sensors are based on potentiometric approach and are sensitive to the ionic metals and biological analyte in question. For each case a selective membrane or enzyme was used. The measurements were performed for intracellular environment as well as in some cases (cholesterol and uric acid). The selectivity in each case is tuned according to the element to be sensed. Moreover we also developed photodynamic therapy approach based on the use of ZnO NRs and NPs. Necrosis/apoptosis was possible to achieve for different types of cancerous cell. The results indicate that the ZnO with its UV and white band emissions is beneficial to photodynamic therapy technology.

*ZnO possesses high Isoelectric Point (IEP) of 9.5 which makes it suitable for absorption of proteins with low IEPs where the protein immobilization is primarily driven by electrostatic interaction [12].

*ZnOnanorods were grown on a silver-coated tip of a borosilicate glass capillary (0.7_min tip diameter) and used as selective potentiometric sensor of intracellular free Mg^{2+}. To functionalize the ZnOnanorods for selectivity of Mg^{2+}, a polymeric membrane with Mg^{2+}-selective ionophores were coated on the surface of the ZnOnanorods. These functionalized ZnOnanorods exhibited A Mg^{2+}-dependent electrochemical potential difference versus an Ag/AgCl reference microelectrode within the concentration range from 500 nM to 100mM. Two types of cells, human adipocytes and frog oocytes, were used for the intracellular Mg^{2+} measurements. The intracellular concentration of free Mg^{2+} in human adipocytes and frog oocytes were 0.4–0.5 and

0.8–0.9mM, respectively. Such type of nanoelectrode device paves the way to enable analytical measurements in single living cells and to sense other bio-chemical species at the intracellular level [13].

*One-dimensional nanostructures exhibit interesting electronic and optical properties due to their low dimensionality leading to quantum confinement effects. ZnO has received lot of attention as a nanostructured material because of unique properties rendering it suitable for various applications. Amongst the different methods of synthesis of ZnO nanostructures, the hydrothermal method is attractive for its simplicity and environment friendly conditions. This review summarizes the conditions leading to the growth of different ZnO nanostructures using hydrothermal technique. Doping of ZnO nanostructures through hydrothermal method are also highlighted.

*Due to its wide band gab, ZnO is transparent in the visible part of the electromagnetic spectrum. Highly n-doped ZnO: Al   can therefore be used as a transparent conducting oxide (TCO). The constituents Zn and Al are much cheaper and less poisonous compared to the generally used indium tin oxide (ITO). One application which has begun to be commercially available is the use of ZnO as the front contact for solar cells, which avoids the shadow effect necessarily associated with metal-finger contacts. Other appearing applications use ZnO as the front contact of liquid crystal displays or ZnO: Al in the production of energy-saving or heat-protecting windows. A coating with TCO results in a glass which lets the visible part of the spectrum in but either reflects the IR back into the room (energy saving) or does not let the IR radiation into the room (heat protection), depending on which side of the window has the TCO coating [14].

*investigate the antibacterial activity of ZnO nanoparticles with various particle sizes. ZnO was prepared by the base hydrolysis of zinc acetate in a 2-propanol medium and also by a precipitation method using Zn (NO3)2 and NaOH. The products were characterized by x-ray diffraction (XRD) analysis, transmission electron microscopy (TEM) and photoluminescence (PL) spectroscopy. Bacteriological tests such as minimum inhibitory concentration (MIC) and disk diffusion were performed in Luria-Bertani and nutrient agar media on solid agar plates and in liquid broth systems using different concentrations of ZnO by a standard microbial method for the first time. Our bacteriological study showed the enhanced biocidal activity of ZnO nanoparticles compared with bulk ZnO in repeated experiments. This demonstrated that thebactericidal efficacy of ZnO nanoparticles increases with decreasing particle size. It is

proposed that both the abrasiveness and the surface oxygen species of ZnO nanoparticles promote the biocidal properties of ZnO nanoparticles.

*We demonstrate intrinsic white light emission from hybrid light emitting diodes fabricated using an inorganic–organic hybrid junction grown at 50 °C on a paper substrate. Cyclotene was first spin coated on the entire substrate to act as a surface barrier layer for water and other nutrient solutions. The active area of the fabricated light emitting diode (LED) consists of zinc oxide nanorods (ZnO NRs) and a poly (9, 9-dioctylfluorene) (PFO) conducting polymer layer. The fabricated LED shows clear rectifying behavior and a broad band electroluminescence (EL) peak covering the whole visible spectrum range from 420 nm to 780 nm. The color rendering index (CRI) was calculated to be 94 and the correlated color temperature (CCT) of the LED was 3660 K.The low process temperature and procedure in this work enables the use of paper substrate for the fabrication of low cost ZnO–polymer white LEDs for applications requiring flexible/disposable electronic devices [15].

*Nanotechnology is an emerging inter-disciplinary paradigm which encompasses diverse fields of science and engineering converge at the nanoscale. Nanotechnology is not just to

Grow/fabricate nanostructures by just mixing nanoscale materials together but it requires the

Ability to understand and to precisely manipulate and control of the developed nanomaterials

In a useful way. Nanotechnology is aiding to substantially improve, even revolutionize, many

Technology and industry sectors like information technology, energy, environmental science,

Medicine/medical instrumentation, homeland security, food safety, and transportation, among

Many others. Such applications of nanotechnology are delivering in both expected and

Unexpected ways on nanotechnology's promise to benefit the society.

The semiconductor ZnO with wide band gap (~ 3.37 eV) is a distinguish and unique material

And its nanostructures have attracted great attention among the researchers due to its peculiar

Properties such as large exciton binding energy (60 meV) at room temperature, the high

Electron mobility, high thermal conductivity, good transparency and easiness of fabricating it

In the different type of nanostructures. Basedj on all these fascinating properties, ZnO have

Been chosen as a suitable material for the fabrication of photonic, transducers/sensors,

Piezoelectric, transparent and spin electronics devices etc. The objective of the current study

is to highlight the recent developments in materials and techniques for electrochemical

Sensing and hetrostructure light emitting diodes (LEDs) luminescence properties based on the

Different ZnO nanostructures. The sensor devices fabricated and characterized in the work

Were applied to determine and monitor the real changes of the chemical or biochemical

Species. We have successfully demonstrated the application of our fabricated devices as

Primary transducers/sensors for the determination of extracellular glucose and the glucose

Inside the human fat cells and frog cells using the potentiometric technique. Moreover, the

Fabricated ZnO based Nano sensors have also been applied for the selective determination of

Uric acid, urea and metal ions successfully. This thesis relates specifically to zinc oxide

Nanostructure based electrochemical sensors and photonic device (LED) applications.

The first part of the thesis includes paper I to V. In this part, we have demonstrated the

Electrochemical sensing characterization and wireless remote monitoring system for glucose

Based on the well aligned vertically fabricated ZnO nanowires based sensors [2].

 *Milk spoilage is an indefinite term and difficult to measure with accuracy. This uncertainty can cause suffering for both milk manufacturers and consumers. Consumers who have been misled by ambiguous expiration dates on milk cartons waste resources by disposing of unspoiled milk or experience discomfort from drinking spoiled milk. Consumers are often unwilling to purchase products close to their inaccurate expiration dates. This consumer behavior has a negative financial impact on milk producers. Inaccurate milk spoilage detection methods also force milk producers to use overly conservative expiration dates in an effort to avoid the legal and economic consequences of consumers experiencing illness from drinking spoiled milk. Over the last decade, new methods have been researched with the purpose of developing more accurate and efficient means of detecting milk spoilage. These methods include indicators based on pH bacteria counts and gas-sensor arrays. This article explores various methods of spoilage detection designed to prevent such consequences. The respective level of effectiveness of each method is discussed, as well as several further approaches to contain freshness regardless of detection [9].

* ZnOnanorods (NRs) with high surface area to volume ratio and biocompatibility is used as an efficient photosensitizer carrier system and at the same time providing intrinsic white light needed to achieve cancer cell necrosis. In this letter, ZnOnanorods used for the treatment of breast cancer cell (T47D) are presented. To adjust themsample for intracellular experiments, we have grown the ZnOnanorods on the tip of borosilicate glass capillaries (0.5 lm diameter) by aqueous chemical growth technique.The grown ZnOnanorods were conjugated using protoporphyrin dimethyl ester (PPDME), which absorbs the light emitted by the ZnOnanorods. Mechanism of cytotoxicity appears to involve the generation of singlet oxygen inside the cell. The novel findings of cell-localized toxicity indicate a potential application of PPDME-conjugated ZnO NRs in the necrosis of breast cancer cell within few minutes[16].

1.4 Objective of the Project :

- To synthesis ZnO nanorodes materials by hydrothermal method.

- To prepare the ZnO nanorodes – silver supported electrode.

- To construct an electrochemical system containing the thus fabricated ZnO nanorodes – silver substrate electrode .

- To utilize the electrode system in potentiometric measurement.

Experimental

2.1 Materials

2.1.1 samples

2.1.1.1   sample collection

Bhry farm sample :

50 ml of fresh caw milk was collected at 8:00 am   directly from the caw . Sample was put into clean and dry bottle and was used immediately after collection .

Halatkoko sample:

50 ml of fresh caw milk was collected at 8:00 am directly from the caw . Sample was taken into clean and dry bottle and used immediately after collection

Khartoum north sample:

50 ml of caw milk was collected at 8:00 am from milk seller and was taken into clean and dry bottle and used immediately after collection .

Khartoum sample:-

50 ml of caw milk was collected at 8:00 am from milk seller and was taken into clean and dry bottle and used immediately after collection.

Omdurman sample:-

50 ml of caw milk was collected at 8:00 am from milk seller and was taken into clean and dry bottle and used immediately after collection.

2.1.2 Chemicals :-

1. Zinc acetate ,Anal R grade .
2. Methanol ,high purity .
3. Potassium hydroxide ,Anal R grade .
4. Hexa methylene tetra mine ,Anal R grade .
5. Silver wire (99.9999% Ag).
6. Hydrochloric acid ,Anal R grade .
7. Deionized water.

2.2 Instrumentations and equipment's

2.2.1 Instruments

- Balance.
- Hot plate with stirrer.
- Oven .
- pH meter .
- XRD

2.2.2   Apparatus :-
- Glass Beakers (250ml, 500ml)
- glass rode
- Thermometer.

2.3 methods :-

2.3.1 Preparation procedure:-

2.3.1.1 Seed solution for ZnO nanostructure growth:-

Zinc acetate dehydrate was prepared in absolute methanol (99%),when 0.01 moles of zinc acetate dehydrate (274mg) was dissolved in 125ml of methanol under stirring (the solution was transparent),it was heated to 60°c under continuous stirring .109 mg of potassium hydroxide was dissolved in 65ml methanol (0.03M concentration), this solution was shacked well until it become transparent and added drop-wise to the heated solution of zinc acetate dehydrate under continuous stirring. The resulting solution was kept under stirring and heating (60°C) for 2 hours before it was become ready for use. Silver wire was cut about 5 cm and cleaned by methanol and deionized water. Silver wires were inserted in the seed solution. Finally silver wires were left to dry, then cleaned with methanol and deionized water.

2.3.1.2 The aqueous chemical growth solution for the ZnO:-

Zinc nitrate (zinc source salt) was added to 100ml of deionized water (25 Mm) and Thoroughly mixed(solution 1). Hexamethylenetetramine (HMTA) in 100ml DI-water (solution 2),Then (solution 2) was added to (solution 1).the final ratio between the Zn concentration and HTMA was 1:1 .The seeded silver wires were loaded in the aqueous solution and then warmed in side an oven adjusted at a temperature of 50-96°C for 4 hours . Samples become ready for characterization and measurement.

2.3.1.3 Preparing of reference electrode:-

The silver wire was cleaned by methanol and deionized water. Then silver wire was

Cut about 5 cm long anddipped in concentrated hydrochloric acid for a few minuts,Then removed and washed with methanol and deionized water .

2.3.1.4 Measuring of pHand voltage :-

pH of samples of caw milk were measured by a pH meter and recorded. Then samplesvoltage wasmeasured by using pH-meter using coaxial cable that connect the working electrode (ZnO/Ag) and the reference electrode (Ag/AgCl).

2.3.2 Characterization procedure:

2.3.2.1 : XRD instrument :-

Samples of zine oxide were finely ground in to very fine homogenous powder were put in holder and pressed to obtain a flat surface,then placed in the diffractometerand exposed to X-rays ,the wide–angle X-ray scattering of samples was measured using an X-rays diffractometer D8 advance powderdiffractometer .

Chapter Three

Results and Discussion

3.1 pH-measuring device:-

In this study a simple pH measuring device has been designed and constructed and utilized for following progress of the fermentation process in cow,s milk samples collected from different milk providers in greater Khartoum .

The main feature of the pH-measuring device is the utilization of a working electrode of silver wire coated with ZnO nanoparticles and silver-silver chloride is a reference electrode.

 A schematic diagram of the layout of the device is shown in fig(9).
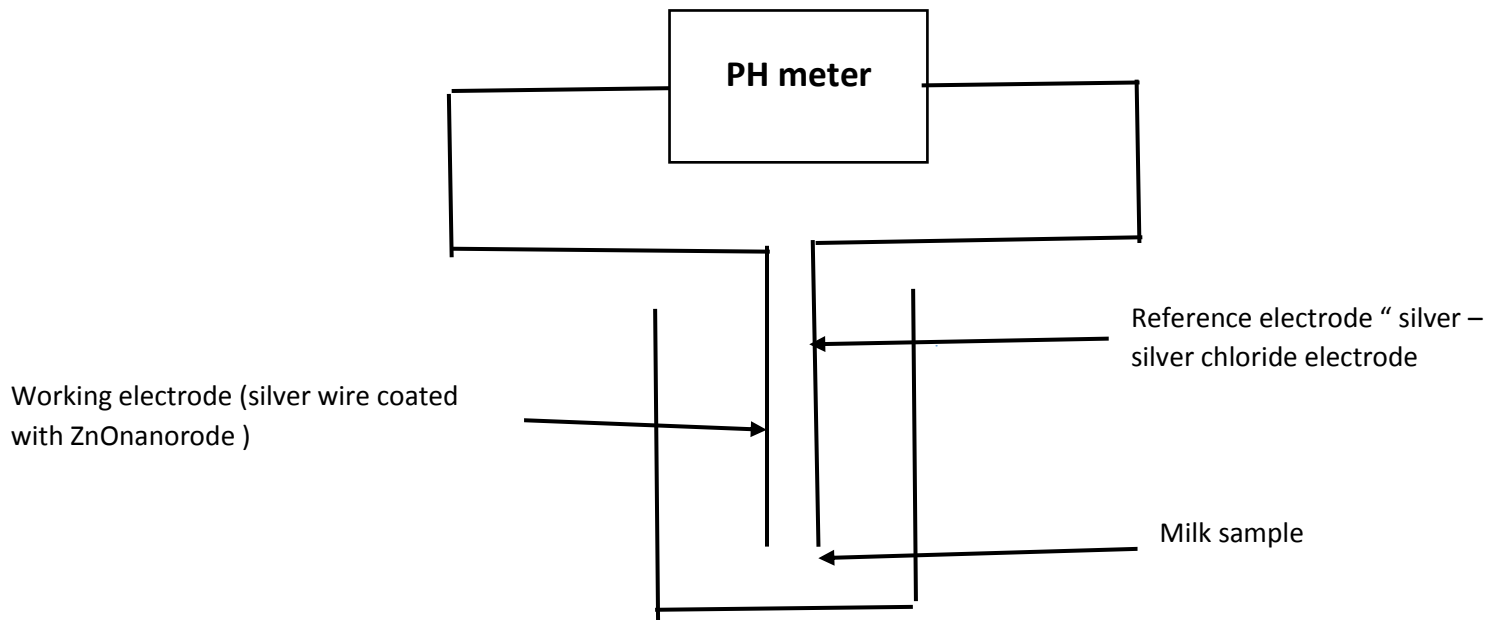
Fig 9: schematic diagram of pH measuring device

The working electrode function with acceptable sensitivity and (stability), giving stable reading of mV .
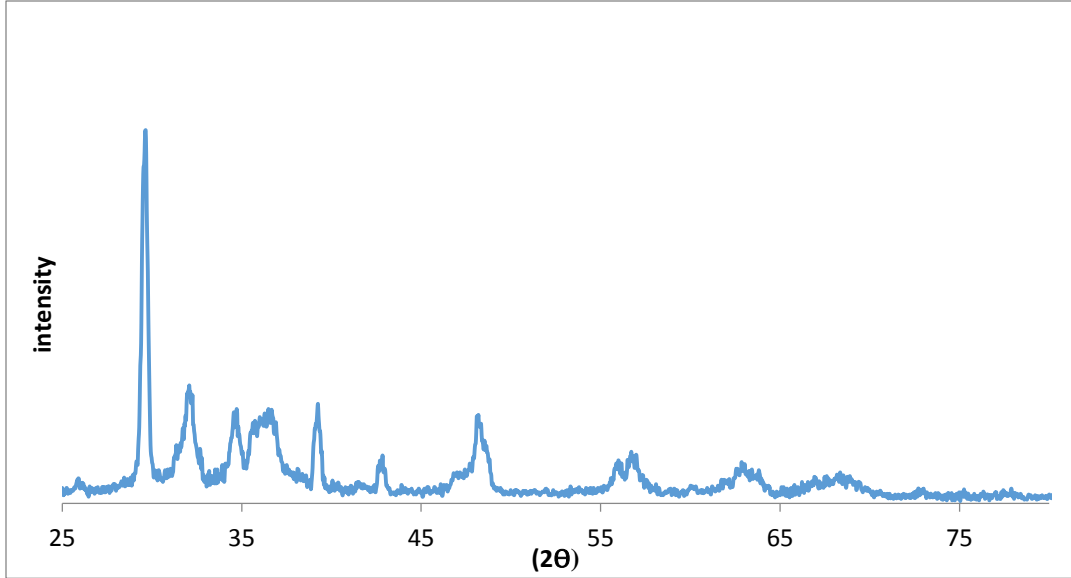
3.2XRD characterization:-

Fig (10) : XRD of zinc oxide nanoparticle

The ZnO nanoparticle coat on the silver wire was characterized by XRD measurements.

Fig(10) show the XRD spectrum of zinc oxide where X-ray intensity was plotted versus the scatting as angle (2Θ).

3.3Computation procedure for characterization of ZnO nanoparticle:-

the lattice parameters were calculated from the XRD spectra using PowderCell computer software. Hence the lattice and shape were ascertained.The crystal structure was hexagonal and lattice parameters were $\alpha=\beta= 90^0$, $\gamma= 120^0$

While a =3.2494 A^0, b = 3.2494 A^0 and c=5.1938 A^0.and table (1): show the ion coordinates.

Table (1): shown the lattice parameters of ZnO sample:

a =3.2494 A^0, b = 3.2494 A^0, c=5.1938 A^0 ($\alpha=\beta= 90^0$, $\gamma= 120^0$)

Ions	X	Y	Z
Zn	0.33333	0.66667	0.00000
O	0.33333	0.66667	0.38210

3.4 pH and mV measurement of the dairy farm cow's milksamples :-

The device was used to measure the pH and mV of caw's milk samples collected from two dairy farms located at Bhary(Khartoum north) and Haltkoko, and three others samples collected for cow's milk distributer in Omdurman, Khartoum north and Khartoum .

The pH and mV of each sample was measured every two hours for a period of time of 12 hours. The deterioration of milk samples due to atmospheric bacterial fermentation was followed by plotting the variation of pH and mV versus time.

Fig (11) and (12) and table (2) show the change of pH and mv with time of sample collected from Bhary dairy farm.

Table (2): variation of pH and the corresponding (voltage) with time of cow milk's sampleBhary farm

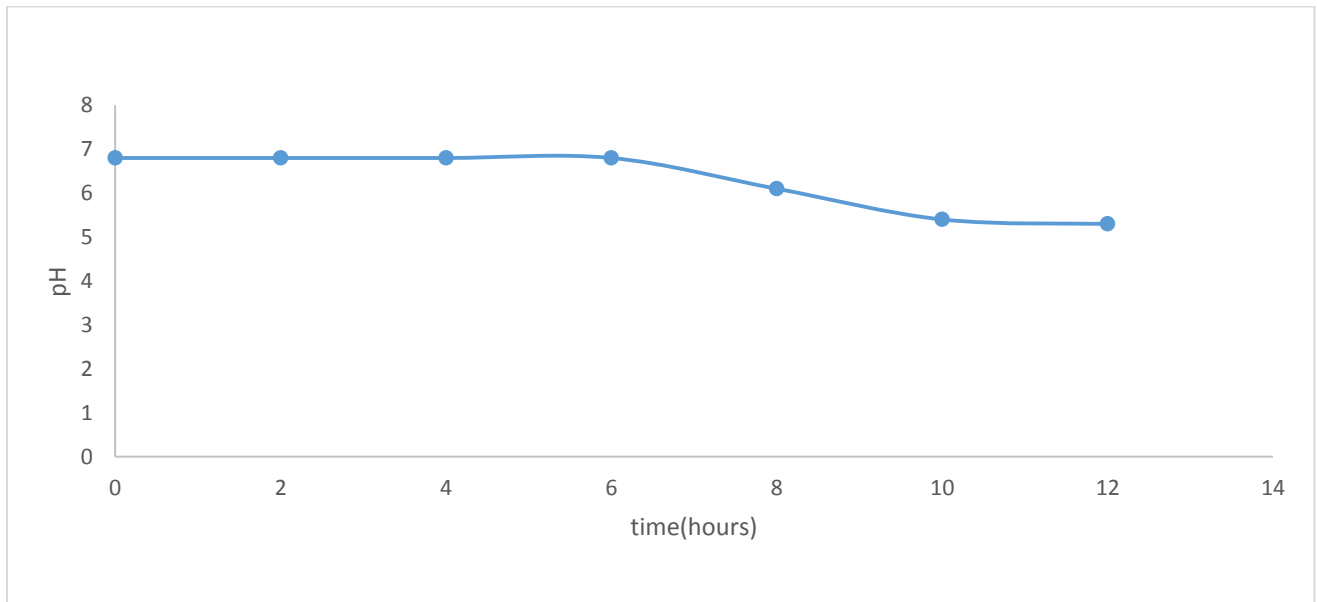| Time	pH	Voltage
0	6.8	86.7
2	6.8	48.6
4	6.8	44.4
6	6.8	42.3
8	6.1	27.7
10	5.4	26.0
12	5.3	13.4

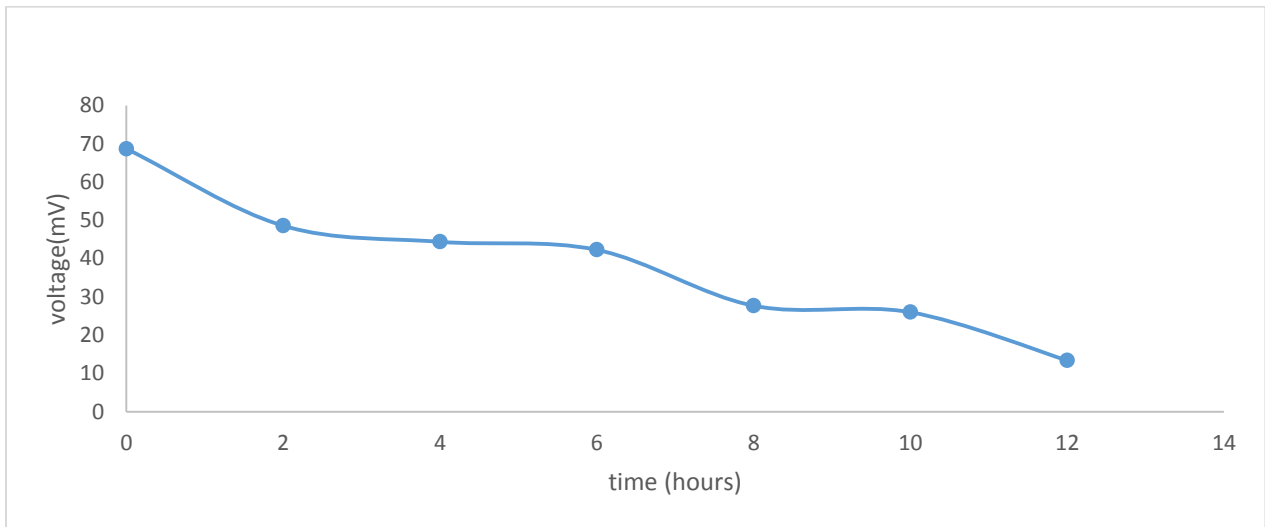**Fig (11): pH – time profile of Bhry's farm caw'smilk sample.**

Fig (12):variation of voltage(mV) with time for Bhary cow's milk sample

From these results it is clear that fermentation process became significant after six hours from the time of cow's milking, where the pH started to decrease as the acidity of the sample started to increase. The increase inacidity became more rapid and the rate of change in pH became greater, till the pH decrease from 6.8 at the onset of the fermentation process to 5.4 after 4 hours form the time of onset of fermentation process.

Fig (13) and (14) and table (3) show the results of pH-time curve as well as mV-time profile for the sample collected from Haltkoko dairy farm.

Table (3): variation of pH and the corresponding (voltage) with time cow's milk Haltkokofarm sample.

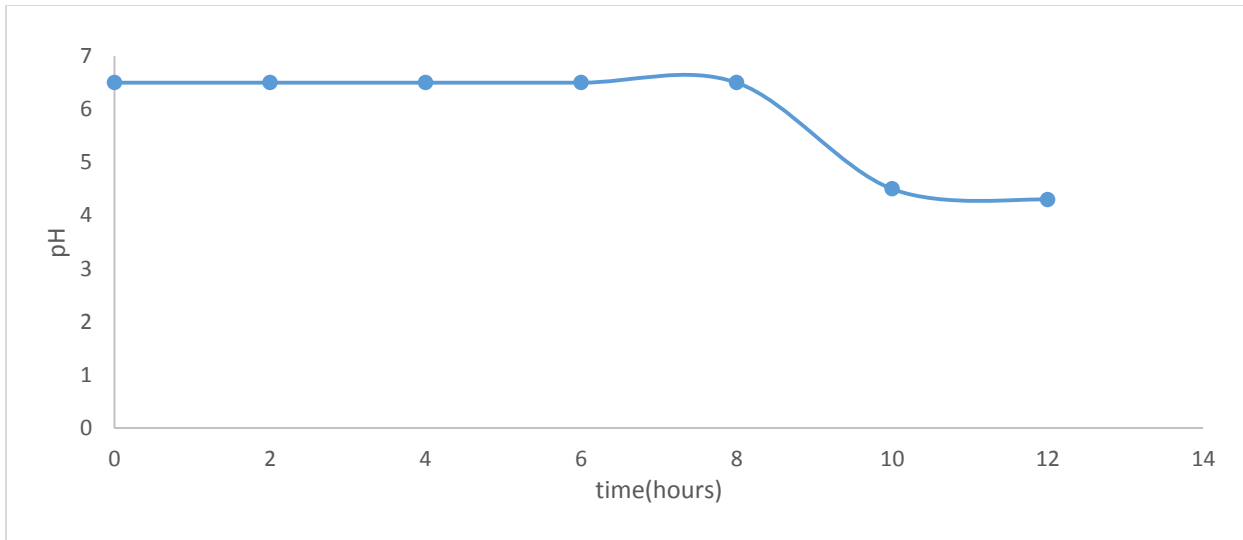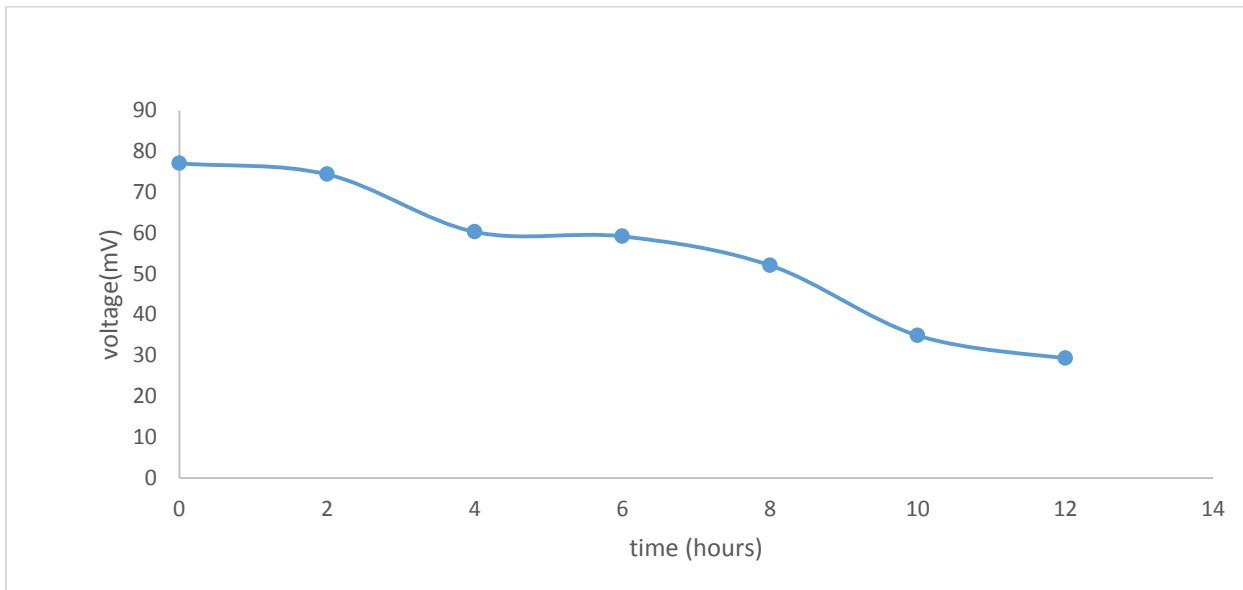| Time	pH	Voltage
0	6.5	77.1
2	6.5	74.4
4	6.5	60.3
6	6.5	59.2
8	6.5	52.1
10	4.5	34.9
12	4.3	29.3

Fig (13):pH – time profile of Haltkoko caw's milk sample

Fig(14):variation of voltage(mV) with time for Haltkoko cow's milk sample

The general feature of the curve is not much different from the one obtained for the previous sample, yet the fermentation onset time for Haltkoko sample was at eight hours from the time of milking and the pH decrease from 6.5 to 4.5 in only two hours' time.

The figures (15) and (16) and table (4) ,(17) and (18) and table (5), (19) and (20) and table (6) shows the variation of pH and mV with time for samples collected from Khartoum north , Khartoum and Omdurman milk distributers respectively.

Table (4): variation of pH and the corresponding (voltage) with time cow's milk Khartoum north sample

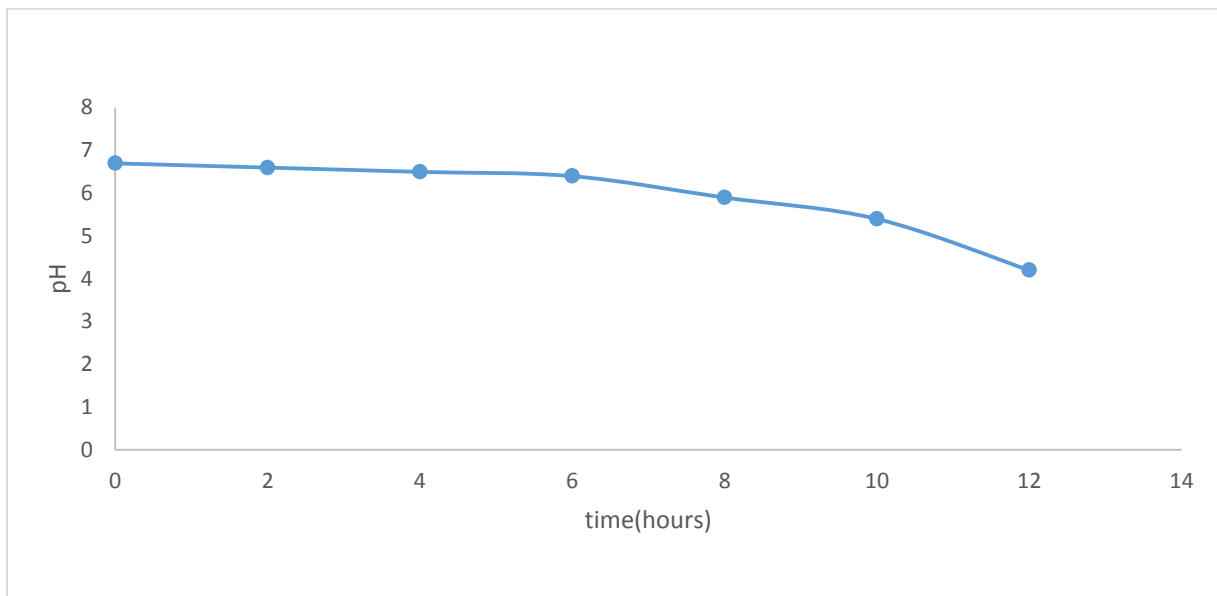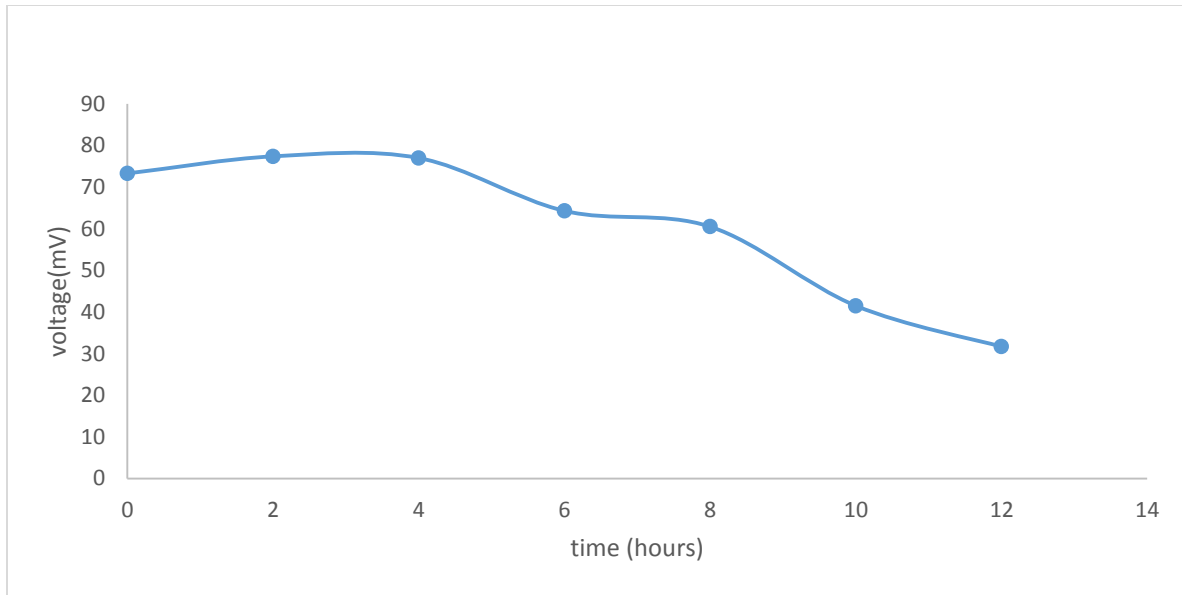| Time	pH	Voltage
0	6.7	73.3
2	6.6	77.4
4	6.5	77.0
6	6.4	64.3
8	5.9	60.5
10	5.4	41.5
12	4.2	31.7

Fig (15):pH – time profile of Khartoum north caw's milk sample

Fig(16):variation of voltage(mV) with time for Khartoum north cow's milk sample

Table (5):variation of pH and the corresponding (voltage) with time cow's milk Khartoum sample:

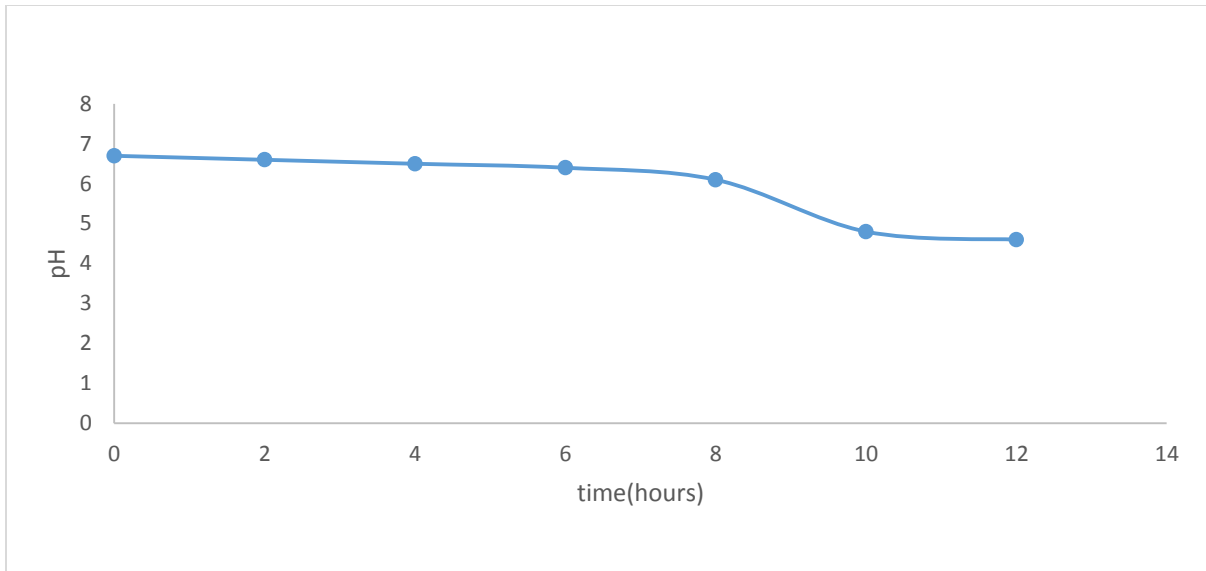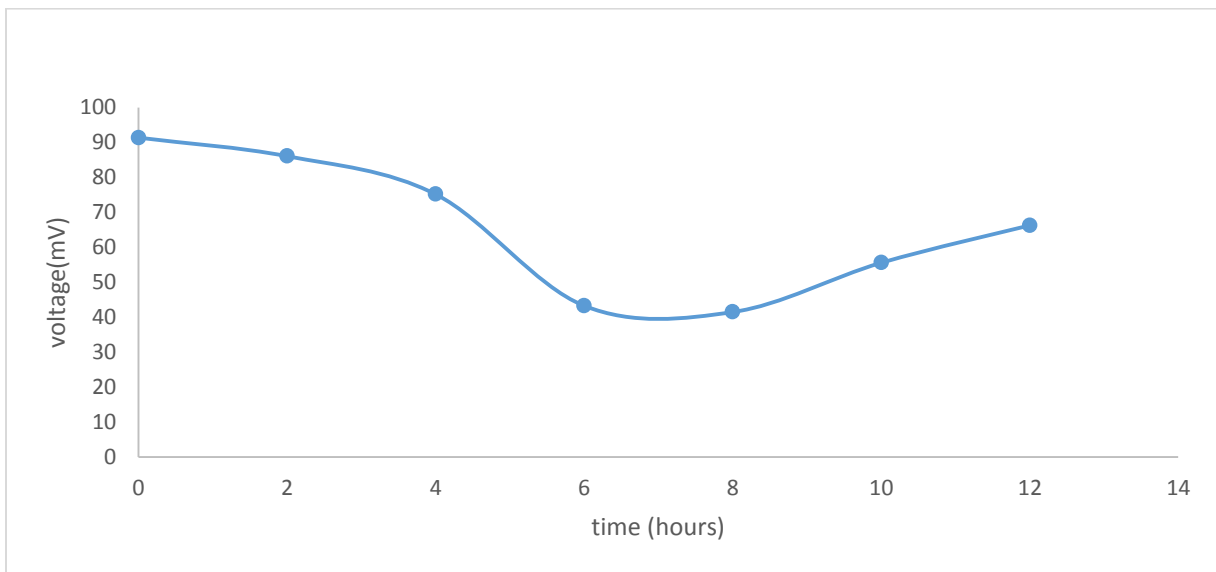| Time	pH	Voltage
0	6.7	91.4
2	6.6	86.1
4	6.5	75.2
6	6.4	43.3
8	6.1	41.5
10	4.8	55.6
12	4.6	66.3

Fig (17):pH – time profile of khartoum caw's milk sample

Fig(18):variation of voltage(mV) with time for khartoum cow's milk sample

Table (6): **variation of pH and the corresponding (voltage) with time cow's milk Omdurman sample**:

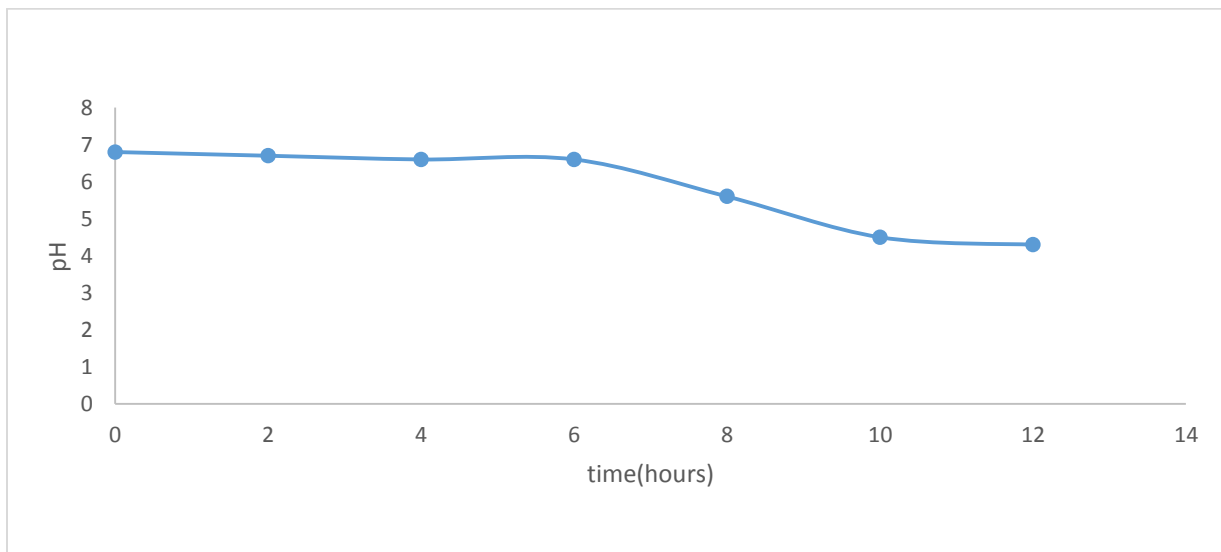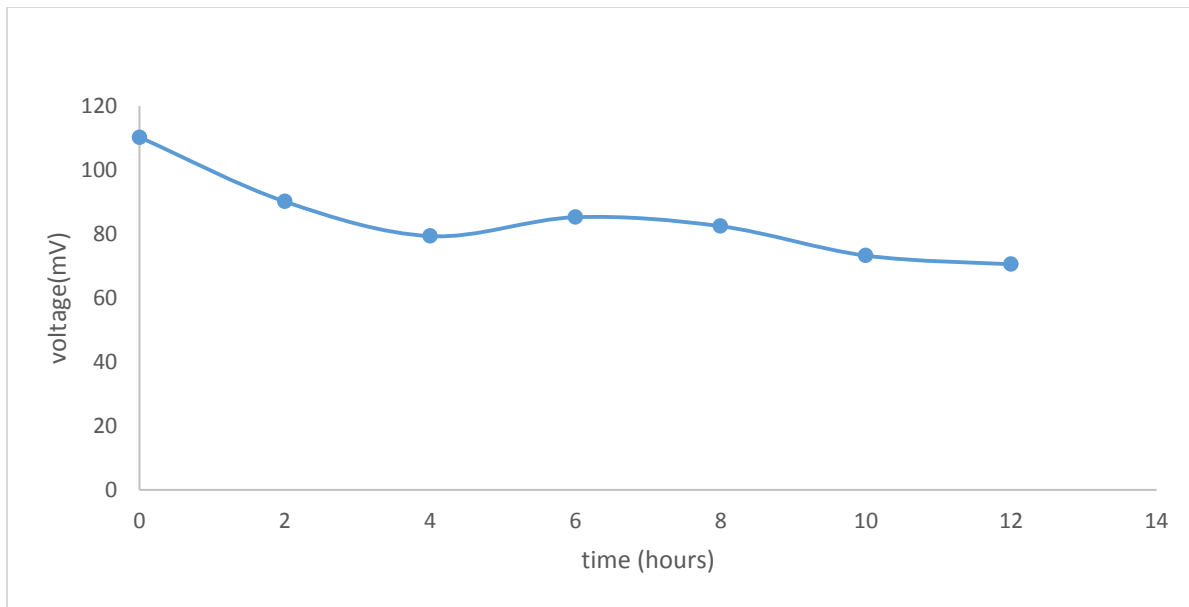| pH	Voltage
6.8	110.2
6.7	90.1
6.6	79.3
6.6	85.2
5.6	82.4
4.5	73.2
4.3	70.5

Fig (19):pH – time profile of Omdurman caw's milk sample

Fig(20):variation of voltage(mV) with time for Omdurman cow's milk sample

Allthe results obtained for the pH-time profiles indicates decrease of pH with progress of time showing commencement and persistence of fermentation progress in all the samples.The samples collected from the Khartoum north milk distributor showed gradual decrease in pH that started immediately after two hours of sample collection. Then the pH continued to decrease till it reached its minimum value after 12 hours from the time of it collection. Thisfeatures is observed also in the other two samples collected form Khartoum and Omdurman as shown in the figures (17),(18),(19) and (20). This indicate that the fermentation process had started some time before the collection of the sample. Which would mean the shelf life of the milk obtained directly from the dairy farms is longer compared to milk obtained from milk distributers.The constructed pH-measuring device was able to measure the pH of all the samples with acceptable stability.

3.5 Conclusion:-

The device constructed for measuring the sample mV using ZnOnanorodes coated on silver wire function in an indicative and acceptable way matching conventional pH-meter, although it was far simpler and easier to fabricate and construct.

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