ISOLATION, CHARACTERIZATION AND NANOENCAPSULATION OF BIOACTIVE COMPOUND OF *ANNONA MURICATA* LEAF EXTRACT

BY

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FACTULTY OF PHYSICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

APRIL, 2023.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE (M.sc. HONS) DEGREE IN INDUSTRIAL CHEMISTRY.

APRIL, 2023.

CERTIFICATION

This is to certify that this work described in this project was conducted by **Ndubuisi Philip EGBOLUCHE** under the supervision of **Dr (Mrs) S.I Omonmhenle** during the 2020/2021 session, in the department of chemistry, university of Benin, Benin City, Edo State, Nigeria.

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ABSTRACT

Sodium alginate is a commonly used encapsulation matrix for variety of materials such as plant cells, food products, oil and flavor. This compound is biodegradable, biocompatible and non-toxic. Also, it is cheap, available, has chelating ability and forms stable reversible gels. However, plant extracts have challenges of poor utilization, poor stability, chemical degradation, low bioavailbility and high molecular weight. Therefore, this study was aimed at isolation, characterization and use of sodium alginate nanoparticles to encapsulate acetogenin compound isolated from *Annona muricata* leaf. Sodium alginate nanoparticles loaded with acetogenin compound were prepared and characterized using Transmission Electron Microscope (TEM) and Fourier Transform Infrared Spectroscope (FTIR). TEM revealed a spherical and irregular nature of sodium alginate nanoparticles loaded with acetogenin compound with particle size of 280nm. The encapsulation efficiency was 89% and this result shows that encapsulation in nanosized matrics with different concentrations of sodium alginate nanoparticles produced higher encapsulation efficiencies which therefore, enhances solubility, biovailability of acetogenin compound.

CHAPTER ONE

INTRODUCTION AND LITERATURE

1.1 INTRODUCTION

Many medicinal plants have been explored globally by researchers due to their huge benefits to mankind and its consistent use in food and pharmaceutical industries. The medicinal values of these plants lies in their phytochemical constituents that produce definite physiological action in human body (Alara *et al*., 2019). Recently, developing countries have found interest in the use of medicinal plants because, it has been reported safe and without adverse side effects (Usunobun *et al*., 2015). More than 80% of global population relies on herbal medicine for their primary health care according to WHO. *Annona muricata* is one of these medicinal plants that have been globally explored by researchers due to their anti-inflammatory and anti-cancer properties. This plant is known as soursop because of sour and sweet taste of its fruit.

In Indonesia, the plant is called Sirsak or Nangka belanda, while in Portuguese and latin America, it's called Graviola and Guanabana respectively. It belong to the family of Annonaceae and the specie is *Annona muricata* (*A.muricata*) (Siti *et al*., 2018).

A.muricata is found in tropical and subtropical regions of south and Central America, Southeast Asia and West Africa. In Nigeria, it's found in the southern part of Nigeria.

The plant produces edible fruit all year round and is widely used in traditional medicine in many countries to cure various ailments and diseases. *A. muricata* contain acetogenins, alkaloids and phenolics as bioactive components and to improve their stability, nanoencapsulation is required. This process help to protect the bioactive components against environmental degradation and also, help to retain the nutritional and their functional qualities in a nano scale. Biopolymers such as fats, starchs, dextrins, protein, lipids and alginates can be used as encapsulation materials (Shaba *et al*., 2021).

1.1.1 Background of the Study

Nano-encapsulation is a process that forms a thin layer of protection against environmental degradation and retains the nutritional and functional qualities of bioactive compounds in a nano-scale by employing fats, starches, dextrins, proteins, lipids and alginates materials as encapsulation materials (Shaba *et al*., 2021). The use of these biopolymers as an encapsulating materials has increased significantly over the years because of their biocompatibility, non-toxic, and biodegradability. Arwa (2018) reported high encapsulation efficiency when he used sodium alginate nanoparticles to encapsulate doxorubicin. Acetogenin is one of the isolated bioactive components of *A.muricata* that has shown anti-cancer properties, although the cytotoxic effects could also destroy the healthy cells. Therefore, there is a need to develop a techniques that will enhance the stability and provides required concentration of acetogenin at the tumor site without adverse effects on the healthy cells.

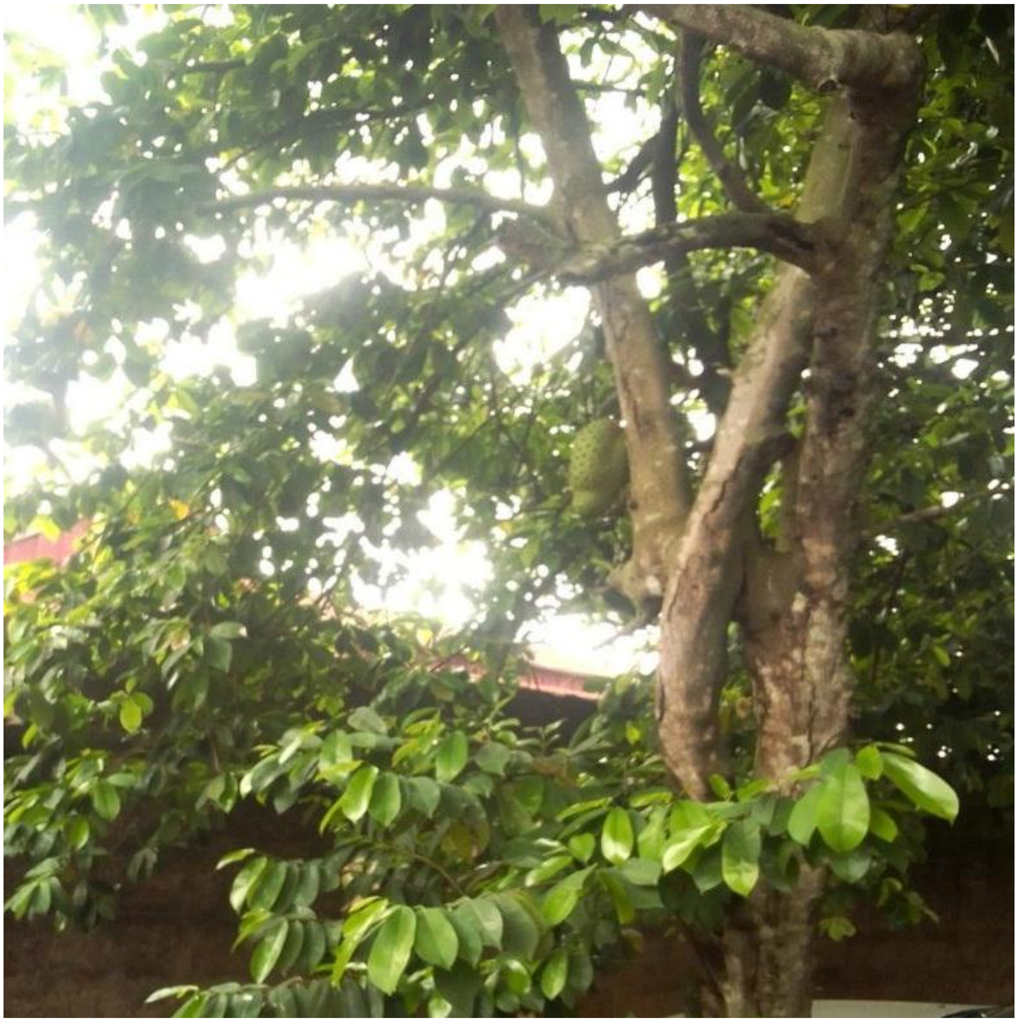

Plate 1: *Annona muricata* (Soursop) plant

1.1.2 Statement of Problem

Acetogenins has a wide range of activities against many cancer diseases. It inhibits tumor cells and promotes cells destruction. It induces adenosine triphosphate (ATP) production, therefore decreases intracellular ATP levels and induces tumor cell destruction. However, these cytotoxic effects are not limited to cancer cells, it can harm healthy cells as well (Escobar-Khondiker *et al*., 2007). Therefore, these effects can be controlled through enhanced stability and specific concentration of acetogenin at the tumor site. Biopolymers such as fats, starch, dextrin, protein, lipids and alginates can be applied to enhance the stability and concentration of acetogenin within the therapeutic range (Shaba *et al.*, 2021).

1.1.3 Relevance of the Study

Acetogenin has anti-inflammatory and anti-cancer properties. They block tumor cells from receiving enough energy , therefore promote cellular destruction. It can also be used as pesticides and for treatments of parasitic, viral and microbial diseases. Therefore, there is need to retain the nutritional and functional qualities of this bioactive compound, and this study is designed to provide a technique that will preserve the qualities of this bioactive compound.

1.1.4 Scope of the Study

The study focused on the use of sodium alginate nanoparticles for encapsulation of acetogenin, one of the bioactive components of *A. muricata* leaf extract. This is because sodium alginate is biodegradable, non-toxic, biocompatible, chelating ability and mucoadhesive (Tonnesen and Karisen, 2002). It is a salt of alginic acid. It’s natural source is from marine brown algae. It contains apha-L-guluronic and beta-D-mannuronic acids. Guluronic acids exchanged sodium ion (Na^+) and react with calcium ion (Ca^{2+}). Reactions of alginate chains help them to join with many other chains and this results to gel formation. Furthermore, it has been used for encapsulation of cells such as; venoms, protein, DNA and vaccines. Sodium alginate is widely used due to its ionotropic gelatin properties and cross-linked structures (Murano, 1998). Therefore, this study covers the phytochemicals screening, isolation of bioactive component of *A.muricata* and structural elucidation of the isolated compound, preparation and characterization of sodium alginate nanoparticles containing acetogenin compound and nanoencapsulation of acetogenin compound with sodium alginate.

1.1.5 Aim and Objectives

The aim of this study was to isolate, characterize and nanoencapsulate acetogenin component of *A. muricata* leaf extract.

The objectives are to:

1. identify and extract *A. muricata* leaves
2. subject the leaf extract to phytochemical analyses
3. isolate bioactive components of *A. muricata* leaf extract
4. characterize the acetogenin component of *A. muricata* leaf extract
5. prepare sodium alginate nanoparticles containing acetogenin compound and nanoencapsulate it.
6. characterize sodium alginate nanoparticle containing acetogenin compound

1.2 LITERATURE REVIEW

1.2.1 Bioactive compounds

A bioactive compound is a compound that has effect on a living organism, tissue or cell. Therefore, the ability of a compound to incite a specific biological reaction is called bioactivity. While dietary nutrients are essential as the body can function without them . bioactive compounds lack sufficient evidence of effect or safety, and consequently unregulated.

Bioactive compounds are also known as secondary metabolites are widely present in plant matrix and over the past few decades, several in vitro and in vivo reports including epidemiological and cohort studies provides that consumption of plant-based foods provides protection against several diseases. These bioactive extracts are cable of treating chronic diseases such as cancer, cardiovascular and diabetes mellitus. Nutraceuticals and pharmaceutical sectors use these extract to develop functional food and plant-based medicines, which have a potential to cure and deliver health benefits (Talmaciu *et al.*, 2016). According to WHO, about 80% of global population depends on natural medicines.

The initial steps to utilize these bioactive compounds from plant matrix are:

- Extraction
- Pharmacological testing
- Isolation
- Characterization
- Clinical evaluation

The quality and yield of the bioactive compounds depends on two factors:

- The method chosen for its extraction
- The extractions parameters such as; nature of plant matrix, extracting solvent, time and temperature.

The most conventional methods employed for bioactive extraction is soxhlet extraction, maceration and hydro-distillation (Talmaciu *et al.*, 2016).

Although, these techniques are commercially employed, but excessive use of solvents and longer processing time are the shortcomings of these methods. Presently, demand for sustainable, chemical-free, advanced extraction processes with enhanced overall yield of bioactive compounds are known as green techniques which include ultra-sound assisted, enzyme-assisted, microwave-assisted, pulse electric-assisted, high pressurized liquid extraction are gaining attention.

Treating the plant matrix with these green technologies help in breaking the cell structure which allows the bioactive compound to leach or rinse out from the cell wall through solvent, as a result enhances extraction yield. Further purification of the extracted bioactive poses another technological challenges as each of these compounds has a unique molecular structure depending on their type, source and biological activity. The extracted compound can be further purified to isolate the respective bioactive compounds efficiently at an optimized temp and pressure. In addition, it is essential to protect the extracted bioactive compound post extraction and purification, as these compounds are highly sensitive to environmental exposure such as moisture and high temperature.

Therefore, protection techniques such as nanoencapsulation are used to ensure that biological activities of these compounds are preserved until they reach and perform their function at the targeted location in the human body.(shaba *et al.,* 2021). Encapsulation plays a vital role in protecting the bioactive compounds from getting degraded. They are two types of encapsulation.

- Micro-encapsulation
- Nanoencapsulation

Nanoencapsulation is preferred over micro-encapsulation because of its nano-scale size, as the smaller the size of the capsules, the higher their bioavailability and their release can be modified and controlled in a better way.

1.2.2 Nanoencapsulation

Nanoencapsulation is a process that forms a thin layer of protection against environmental degradation and therefore, retains the nutritional and functional qualities of bioactive compounds in a nano-scale (Shaba *et al.*, 2021). European Food Safety Authority (2018) defines nanoparticles as particles with at least one size measurement between 1 and 100nm. However, pharmaceutical science considers particles at the range of 1- 1000nm as nanoparticles because of their unique physicochemical properties (Li *et al.*, 2015).

Nanoencapsulation provides a protective shield around bioactive compounds. It is a system where a suitable nano-carrier, resistant to enzymatic degradation especially in gastrointestinal tract including chitosan, zein and alginate, are widely used to encapsulate bioactive compounds employing several delivery methods including association colloids, nano-particles, nano-emulsions, nanofibres/nano-tubes, nano-laminates.

The selection encapsulation method is based on two main factors:

Nature of the core material

Nature of wall material including wall material size, thickness, solubility, permeability and its rate of delivery. Basically, these techniques are classified into three main genres including chemical (emulsion and interfacial polymerization), physical-chemical methods (spray-drying/spray cooling/spray-congealing/prilling, freeze-drying, electrodynamic methods and extraction). However, combination of these techniques are practiced as in the case of emulsification, first using homogenization, the emulsions are prepared and later converted to dry powder state using spray drying or freeze drying. Reports indicates that about 80-90% of flavor encapsulation is done using spray drying, while 5-10% is done using spray chilling,2-3% by melt extraction and 2% by melt injection (Talmaciu *et al*., 2016). Pateiro *et al* (2021) reported encapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products.

Natural compounds such as plant extracts poses some challenges of poor utilization due to low bioavailability, chemical degradation (during storage), poor stability and high molecular weight.

Therefore, encapsulating materials can be used to preserve these bioactive materials presents in plants, such as vitamins, minerals, phenolics, flavonoids, acetogenins etc. Some of these compounds are hydrophobic, therefore, application of nanoencapsulation can enhance their solubility.

1.2.3 Sodium Alginate

Sodium alginate is extracted from brown seeweeds (Phaeophyceae). The alginic acid, the free acid form of alginate, is extracted from the seaweed in alkaline conditions, then precipitated and ion exchanged. The most commonly used alginate in food is sodium alginate.

Alginate is a cold –soluble polymer. It does not require any heating to hydrate. It has very high affinity with calcium to which it bonds to form thermo-irreversible gels. During the hydration step, it is therefore critical to avoid all contact with free calcium. To meet the target viscosity for a defined application, a choice is made from many different viscosity grades of alginate. In a non-ionic environment such as water, alginate is a pure thickener. In order to increase the viscosity at low alginate concentrations in non-dairy system a small amount of a slightly soluble calcium salt (such as calcium sulphate, calcium citrate) may be added after hydration of the alginate. Calcium ions will react immediately with the alginates to create a three-dimensional network. Depending on the level of calcium and nature of the alginate (high gel strength or low gel strength) this will either only increase the viscosity or create a sufficient network to suspend particles in a water system (acid or neutral). It is important to note that this system will be highly sensitive to any change in pH and in calcium content and alginate precipitates below its pk_a value (pH:3.5) (Onsoyen, 1999).

In neutral milk, alginate provides a nice, smooth, rich and fat-like mouth-feel provided. It is either in an integrated form with emulsifiers, calcium sequestrants (tetra sodium pyrophosphate is added) or it is added alone above75^{0}C (under agitation and preferably mixed with other dry ingredients). If lumps are formed at this stage with the free calcium, they will be irreversible.

Alginate building blocks are beta-(1-4)-linked d-mannuronic acid units and alpha-(1-4) linked l-guluronic acid units, commonly known as M and G blocks respectively. The polysaccharide can exit in homopolymeric M-M and G-G forms, or alternating sequence of M-G blocks (Hay *et al.*, 2010; Reddy and Yang, 2015). Due to the rigid, extended structure of alginate, electrospinning of alginate tend to be challenging.to overcome this issue, Nie *et al*. (2008) utilized glycerol and water as co-solvent where glycerol improved flexibility and the entanglement of alginate chains in solution, by distrupting the inter and intramolecular hydrogen bonds among the alginate chains, allowing the formation of alginate fibers ranging from 120-300nm. Bonino *et al*. (2011) electrospun alginate fibers of low (37kDa) and high (196 kDa) molecular weights, using PEO as a spinning aid and Triton X-100 (p-Tertiary-octylphenoxy polyethyl) as a surfactant. Alginate concentrations were 13.5 and 4 wt% for 37 and 196 kDa polymers with alginate:PEO:surfactant component ratio at 8.0:1.6:2.0 and 2.8:1.2:2.0 respectively. To render the electrospun fibers insoluble, the researchers crosslinked the resulting nonwoven first in ethanol for 1min followed by a 10s treatment in 2% $CaCL_2$ in 1:5 ethanol:water. Since, PEO is soluble in ethanol, this process selectively removes PEO from the fiber while retaining the integrity of the alginate fiber matrices (Bonino *et al*., 2011). Alborzi *et al*. (2010) reported that a blend of alginate with pectin, at 70:30 (w/w) ratio, could not be electrospun unless PEO (900KDa;20-50% (w/w) of total alginate/pectin content) was added to the polymer solution. Depending on polymer concentration (3-5%, w/w), fibers (smooth fibers or beade fibers) of 39-147nm were formed. The fiber-promoting properties of PEO were attributed to its electrical conductivity and surface tension lowering effects on the spin dope solutions. Using a similar technique as Bonin *et al.* (2011), Alborzi *et al.* (2014) subjected folic acid-loaded electrospun alginate/pectin/PEO fiber to 5min 95% ethanol treatment, followed by 10min in a solution of 1% $CaCl_2$ in ethanol to crosslink the alginate. The resulting carrier exhibited pH-dependent folic acid release behaviours. Approximately 21% and 97% (w/w) of folic acid were released in 2h from the electrospun fibers when exposed to water at pH 3 and 7.8 respectively (Alborzi *et al.*, 2014). The lower release rate under the acidic condition, can be attributed to the protonation of alginate and pectin, resulting in a collapsed network trapping folic acid. On the other hand, the alkaline condition induced a negative charge on the polymers, causing chain-chain repulsion and matrix swelling, favouring the release of the micronutrient.

Electrosprayed alginate particles have been exploited for the encapsulation of viable bacteria. Laelorspoen *et al.* (2014) prepared alginate-zein core-shell microcapsules by an electrospraying process to encapsulate viable lactobacillus acidophilus. The core was made of electrosprayed alginate/glycerol solution (1.4% w/w) alginate; 8% (w/w) glycerol) collected in an electrically grounded acidic zein solution (7% w/w) zein, 0.10-0.15% (w/w) citric acid, 75% (v/v) aqueous ethanol, and 1.5% (w/w) $CaCl_2$. The viability of the encapsulated cells was reduced by one log cfu/ml, while the non-encapsulated bacteria exhibited a 5log cfu/ml reduction after a 2hr incubation in stimulated gastric fluid at pH 1.2 containing pepsin. In another study, an alginate solution (2.5%, w/v) containing 8% (w/v) glycerol and viable L. acidophilus, was electrosprayed directly into the gelling bath, containing egg albumen (EA; 8%, w/v), stearic acid (SA)/Tween 40 (1:1.25 ratio), and $CaCl_2$ (1.5% w/v). The bath was acidified to pH 3.65 using citric acid (Pitigraisorn *et al.*, 2017). The resulting capsules were coated with cassava starch granules in fluidized bed dryer using cassava pearls drying aid. This method resulted in encapsulation efficiencies of higher than 90%.

1.2.3.1 Nanoencapsulation with sodium alginate.

Sodium alginate is a salt of alginic acid. It contains apha-L-guluronic and beta-D-mannuronic acids. Guluronic acids exchanged sodium ion (Na^+) and react with Calcium ion (Ca^+). This reaction help them to join with many other chains and this results to gel formation. It has been used for encapsulation of cells such as venoms, protein, DNA and vaccines. It is widely used due to its ionotropic gelatin properties and cross-linked structures (Murano, 1998).

Saeed *et al*. (2013) studied the effect of encapsulating ICD-85 (Venom derived piptides) with sodium alginate. ICD-85 was encapsulated with sodium alginate nanoparticles.

The authors observed that their encapsulation increased the overall stability of ICD-85. The loading capacity and encapsulation efficiency were 89.5%(w/w) and 89%(w/w).

The results showed that the encapsulation of ICD-85 with sodium alginate nanoparticles can significantly increase the stability and cytotoxicity of ICD-85 on HEp-2-cells line compared to uncoated ICD-85.

Mehrasa *et al.* (2015) studied the use of sodium alginate nanoparticles as a potential protein delivering system. Sodium alginate was used to coat bovin serum albomin (BSA). The author observed that the encapsulation efficiencies were depended on the concentration of the encapsulating material.

Arwa *et al*. (2018) studied the characterization of doxorubicin nanoparticles prepared by ionic gelation. The author observed an interesting advantages over uncoated doxorubicin nanoparticles. The result shows that the doxorubicin loaded nanoparticle prepared using sodium alginate had a higher encapsulation efficiency when compare to uncoated doxorubicin in the previous studies.

1.2.4 Nanoparticles (NPs)

Nanoparticles are wide class of materials that include particulate substances, which have one dimension less than 100nm at least (Laurent *et al*., 2010). But pharmaceutical science consider nanoparticle as particle at the range of 1-1000nm (Li *et al*., 2015). The importance of these materials were realized when researchers found that size can influence the physicochemical properties of a substance such as the optical properties.

Nanoparticles are not simple molecules therefore, composed of three (3) layers.

- The surface layer
- The shell layer
- The core

The surface layer contains varieties of small molecules, metal ions, surfactants and polymers. The shell layer is chemically different from the core in all aspects. The core, is the central portion of nanoparticles and usually refers as the nanoparticle itself (Shin *et al*., 2016). Therefore, these materials become a huge interest of researchers in multidisciplinary fields due to these exceptional characteristics.

1.2.4 Classification of Nanoparticles

Nanoparticles are broadly divided into various categories depending on their morphology, size and chemical properties. Some of the well known classes are:

1.2.4.1 Carbon-based Nanoparticles

Fullerenes and carbon nanotubes (CNTs) represent two major classes of carbon-based nanoparticles. Fullerenes contain nanomaterials that are made of globular hollow cage such as allotropic forms of carbon. They have created commercial interest due to their electrical conductivity, high strength, structure, electron affinity, and versatility (Astefanei *et al*., 2015). These materials possess arranged pentagonal and hexagonal carbon units, while each carbon is sp^2 hybridized.

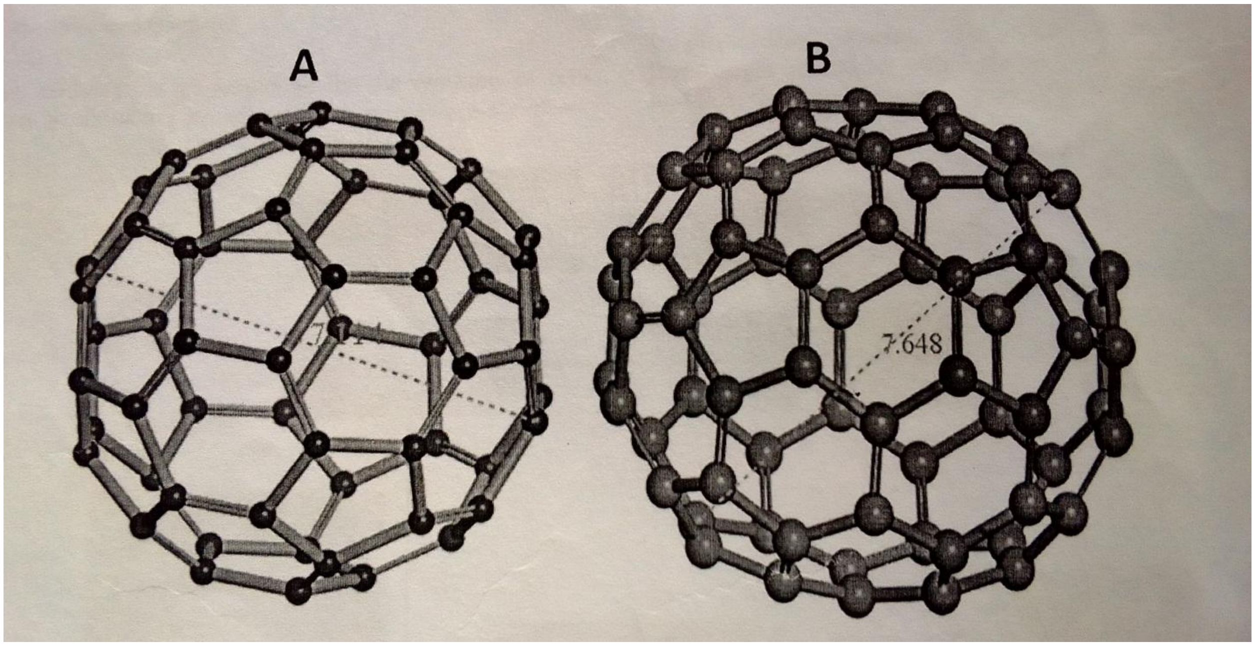

Figure 1: different form of fullerenes/buck balls (A) C_{60} and C_{70}

Carbon nanotubes are elongated, tubular structure, 1-2nm in diameter. These can be predicted as metallic or semiconducting reliant on their diameter telicity (Aqel *et al*., 2012). These are structurally resembling a graphite sheet rolling upon itself.

The rolled sheets can be single, double or many walls and therefore named as single-walled, double-walled or multi-walled carbon nanotube respectively. They are widely synthesized by deposition of carbon precursors especially the atomic carbons, vaporized from graphite by laser or by electric are on metal particles. Recently, they have been synthesized through chemical vapour deposition technique (Elliott *et al.*, 2013). Because of their unique physical, chemical and mechanical characteristics, these materials are not only used in pristine form but also in nanocomposites for many commercial applications such as fillers, efficient gas adsorbents for environmental remediation and as support medium for different inorganic and organic catalysis (Saeed, 2016; Ngoy *et al.*, 2014; Mabena *et al.*, 2011).

1.2.4.2 Metal Nanoparticles

Metal nanoparticles are purely made of the metals precursors. Nanoparticles of alkali and noble metals such as Cu, Ag and Au, have a broad absorption band in the visible zone of the electromagnetic solar spectrum. The facet, size and shape controlled synthesis of metal nanoparticles is important in present day cutting-edge materials (Dreaden *et al.*, 2012). Metal nanoparticles find applications in many research areas due to their advanced optical properties. Gold nanoparticles coating is widely used for the sampling of Scanning electron microscope (SEM), to enhance the electronic stream, which helps in obtaining high quality SEM images.

1.2.4.3 Ceramics Nanoparticles

Ceramics nanoparticles are inorganic non-metallic solids, synthesized through heat and successive cooling. They can be found in amorphous, polycrystalline, dense, porous or hollow forms (Sgmund *et al.*, 2006). Therefore, these nanoparticles are getting great attention of researchers due to their applications in catalysis, photocatalysis, photodegradation of dyes, and imaging applications (Thomas *et al.*, 2015).

1.2.4.4 Semiconductor Nanoparticles

Semiconductor materials possess properties between metals and nonmetals and therefore found various applications due to this property (Ali *et al.*, 2017). Semiconductors nanoparticles possess wide bandgaps and therefore showed significant alteration in their properties with bandgap tuning. Therefore, they are very important materials in photocatalysis, photo optics and electronic devices (Sun, 2000).

Inorganic nanoparticles gives a strong support for nanostructure than organic based structures because, they are made from heavy metals and semi-conductors such as carbon, silica and gold. However, these nanoparticles have some limitations such as non-degradability and high toxicity (Kim *et al.,* 2019).

1.2.4.5 Lipid-based Nanoparticles

These nanoparticles contain lipid moieties and is used in many biomedical applications. It has been widely researched due to their biocompatibility, gradual release from matrix and low toxicity. The use of lipid nanoparticles has few limitations such as low loading efficiency and prone to drug expulsion during storage (Poovi *et al.*, 2018). Like polymeric nanoparticles, lipid nanoparticles possess a solid core made of lipid and a matrix contains soluble lipophilic molecules. Surfactants or emulsifiers stabilized the external core of these nanoparticles (Rawat *et al.*, 2011). Lipid nanotechnology is a special field that focus on designing and synthesis of lipid nanoparticles for various applications such as drug carriers and delivery and release in cancer therapy (Puri *et al*., 2009; Gujrati *et al*., 2014).

1.2.4.6 Polymeric Nanoparticles

These are organic based nanoparticles. They are mostly nanospheres or nanocapsular shaped. Nanosphere are matrix particles whose overall mass is generally solid and the other molecules are adsorbed at the outer boundary of the spherical surface. In the latter case, the solid mass is encapsulated within the particle completely (Rao and Geckeler, 2011).

The polymer based nanoparticles can be natural or synthetic. Some of the natural polymers used for nanoparticles are chitosan, cellulose, gelatin and alginates, while synthetic polymers are polylactic and polylactideglycolactide. These nanoparticles are biodegradable, biocompatible and non-toxic (Tonnesen and Karisen, 2002).

1.2.5 Characterization of Nanoparticles

Different characterization techniques have been used for the analysis of various physicochemical properties of nanoparticles. These techniques are:

- X-ray diffraction (XRD)
- X-ray photoelection spectroscopy (XPS)
- Fourier transform infrared (FTIR)
- Scanning electron microscope (SEM)
- Transmission electron microscope (TEM)
- Brunauer- Emmett-Teller (BET)
- Particle size analysis.

1.2.5.1 Morphological Characterization

The morphological features of nanoparticles always attain great interest since morphology always influences most of the properties of the nanoparticles. There are different characterization techniques for morphological studies, but microscopic techniques such as polarized optical microscopy (POM), SEM and TEM are the most important of these.

SEM techniques is based on electron scanning principle, and it provides all available information about the nanoparticles at nanoscale level. The morphological features of Zinc oxide (ZnO) modified metal organic frameworks (MOFs) were studied through SEM techniques, which indicates the ZnO nanoparticles dispersion and morphologies of MOFs at different reaction conditions.

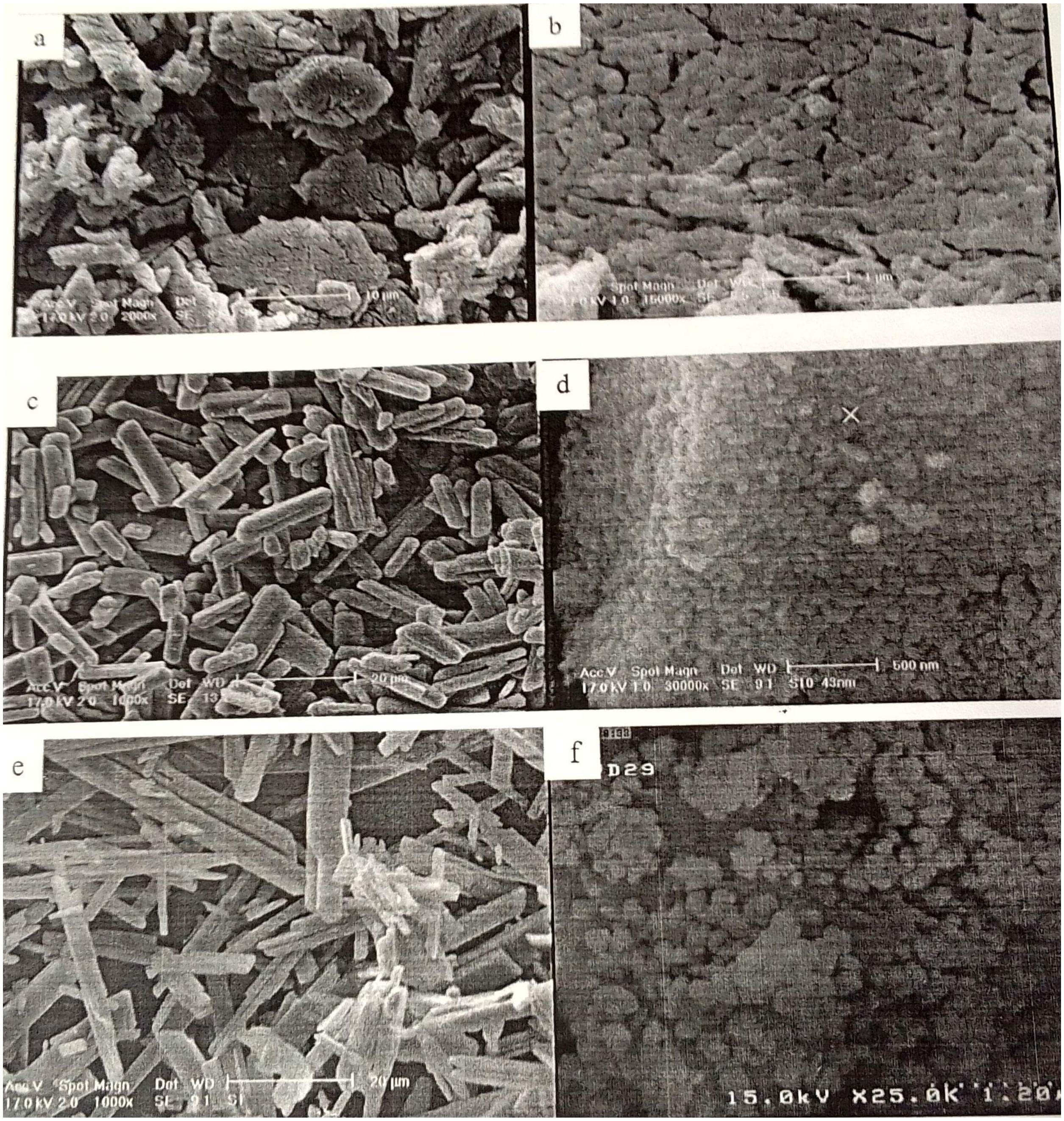

Figure 2: SEM images of ZnO modified MOFs at different temperatures (Mirzadeh and Akhbari, 2016)

Similarly, TEM is based on electron transmittance principle, therefore, it can provide information of the bulk material from very low to higher magnification. The different morphologies of Gold nanoparticles are studied through this technique.

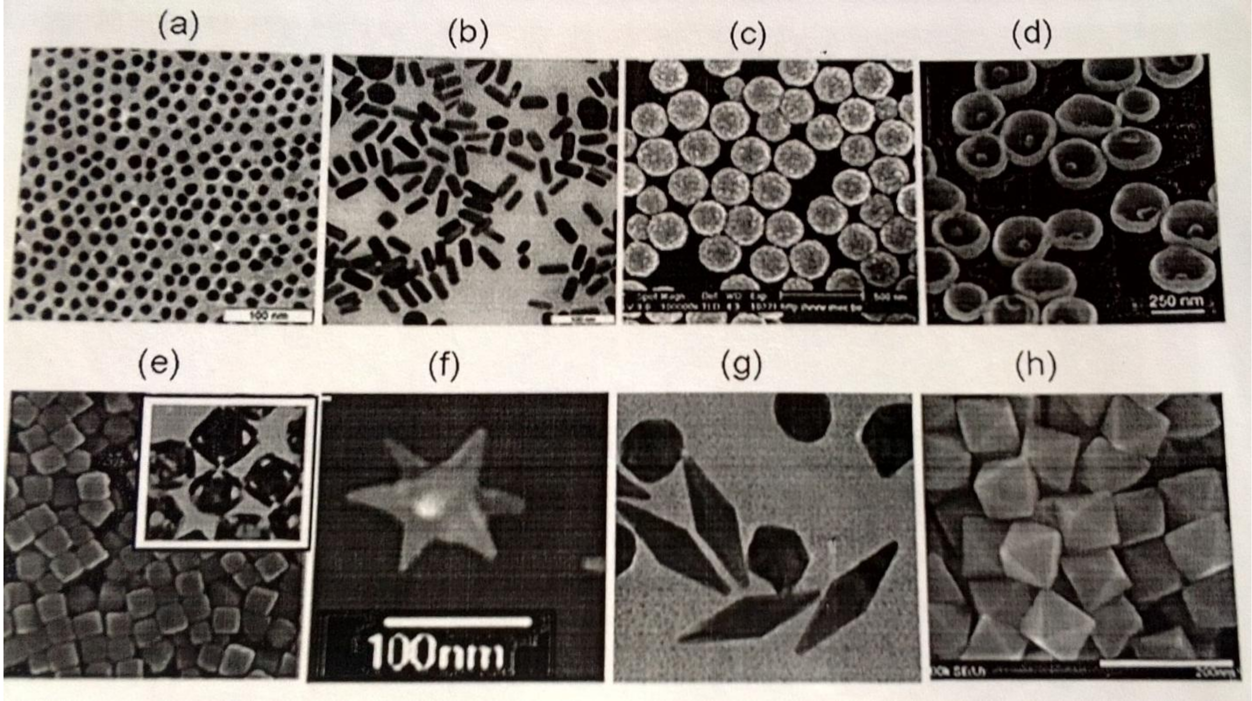

Figure 3: TEM images of different form of gold nanoparticles, synthesized by different techniques (Khlebtsov and Dykman, 2011).

TEM also provides essential information about two or more layer materials, such as the quadrupolar hallow shell structure of Co_3O_4 nanoparticles observed through TEM. These nanoparticles founded to be exceptionally active as anode in Li-ion batteries.

1.2.5.2 Structural characterizations

The structural characteristics are of the primary importance to study the composition and nature of bonding materials. It provides diverse information about the bulk properties of the subject material. XRD, energy dispersion X-ray (EDX), XPS, FTIR, Raman, BET and Zieta size analyzer are the common techniques used to study structural properties of nanoparticles.

XRD is one of the most important characterization techniques to reveal the structural properties of nanoparticles. It gives enough information about the crystallinity and the phase of nanoparticles.

The EDX techniques are used to determine the elemental composition of ultra-sonochemically synthesized pseudo-flower shaped $BiVO_4$ nanoparticles (Khan *et al*., 2017). Similarly, by utilizing similar technique the elemental confirmation and carried out, which showed C, In and O as contributing elements.

XPS is considered to be the most sensitive technique and it is widely used to determine the exact elemental ratio and bonding nature of the elements in nanoparticles materials. It is surface sensitive technique and can be used in depth profiling studies to know the overall composition and the compositional variation with depth. XPS is based on the basic spectroscopic principles and typical XPS spectrum is composed of the number of electrons on Y-axis plot versus the binding energy (eV) of the electrons on X-axis. Each element has their own finger binding energy value and thus gives specific set of XPS peaks.

1.2.5.3 Vibrational Characterization of Nanoparticles

Vibrational characterization of nanoparticles is studied through FTIR and Raman spectroscopies. These techniques are the most developed and feasible when compared to other elemental analytical methods. The most important range for nanoparticles is the fingerprint region, which provides signature information about the material. Dablemont *et al.* (2008) used FTIR and XPS technique to characterize platinum nanoparticles (1.7nm mean size) and it's interaction with

Alumina substrate. FTIR confirms the functionalization as it showed the signature vibrational peaks of carboxylated C-O at 2033cm^{-1} and a broader O-H peak at 3280cm^{-1}. The degree of functionalization was revealed from red shift values of FTIR bands.

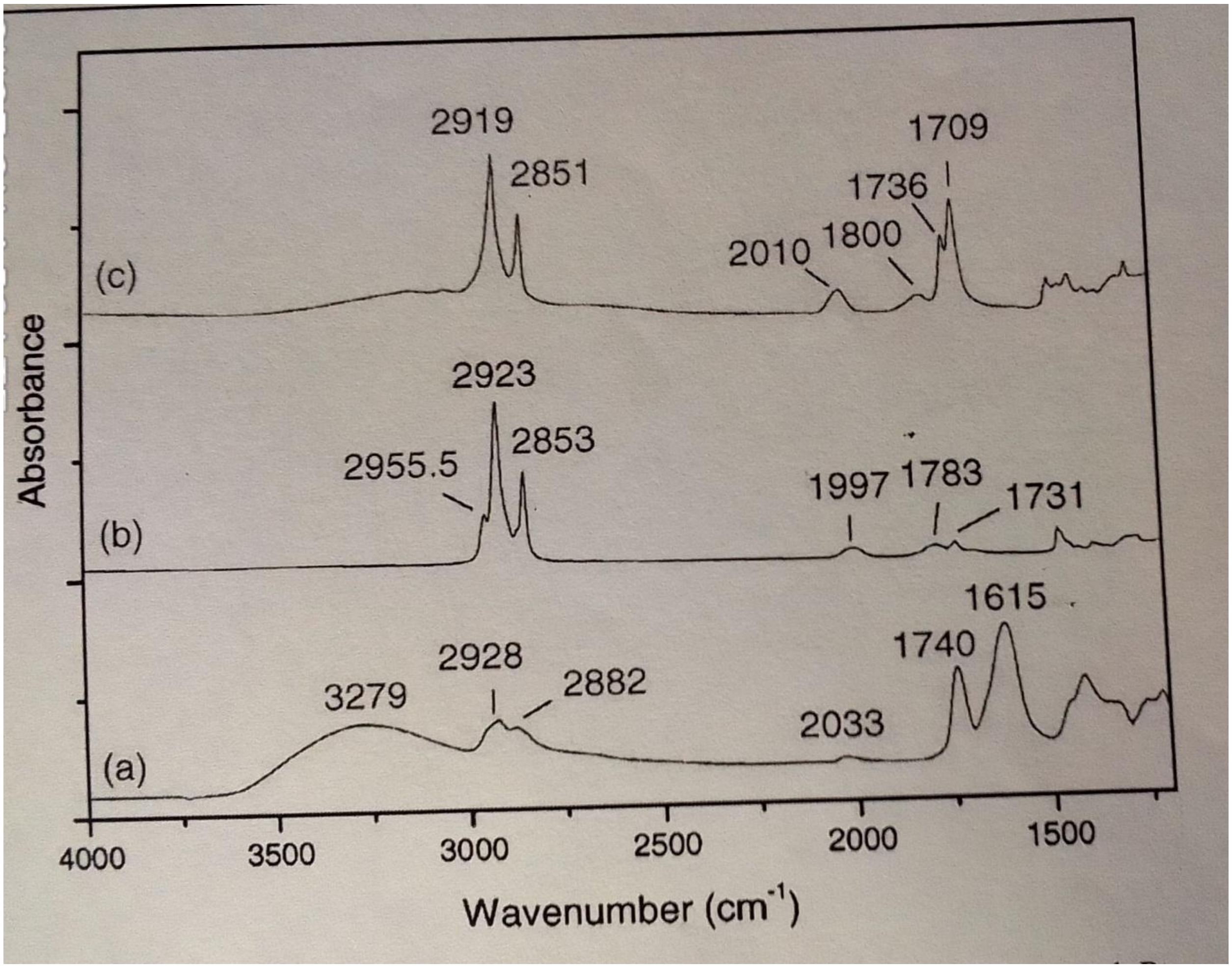

Figure 4: FTIR spectra of platinum (1.7nm) (a) extracted from polyol, (b) dodecanethiol coated pt, and (c) MUDA coated pt (Dablemont *et al*., 2008).

Recently, surface enhanced Raman spectroscopy (SERS) is evolving as vibrational conformational tool due to its signal enhanced capability through SPR phenomenon. Ma *et al*., (2011), used SERS technique to study the vibrational properties with phonons modes in nanostructured and quantum dots nanoparticles of TiO_2, ZnO and PbS. they concluded that the enhanced spectra can be attributed to the plasmonic resonance in semiconductor systems.

1.2.5.4 Particle Size and Surface Area Characterization

Different techniques can be used to estimate the size of nanoparticles. These include SEM, TEM, XRD, AFM and dynamic light scattering (DLS). SEM, TEM and AFM can give better idea about the particle size but, the zeta potential size analyzer/DLS can be used to find the nanoparticles size extremely low level. Sikora *et al*., (2016), used DLS technique to investigate the size variation of silica nanoparticles with absorption of proteins from serum. The results showed that size increased with acquisition of protein layer. However, in case of agglomeration and hydrophilicity, DLS might prove incapable of accurate measurement, so in that case high resolution technique of differential centrifugal sedimentation (DCS). Besides DSC, nanoparticles tracking analysis (NTA) is relatively newer and special technique, which can be helpful in case of biological systems such as protein and DNA. In NTA method, we can visualize and analyze the nanoparticles in liquid media that relates to Brownian motion rate to particle . This techniques allow us to find the size distribution profile of nanoparticle with diameter ranging from 10-100nm in liquid medium (Filipe *et al*., 2010). This technique produced some good results as compared to DLS and found to be very precise for sizing mono dispersed as well as polydisperse samples with substantially better peak resolution. Gross *et al*., (2016), detected the particle size and concentration of different sized nanoparticles in suspension of polymer and protein samples and provided an overview on the effect of experimental and data evaluation parameters. Large surface area of nanomaterials offers great room for various applications and BET is the best technique to determine the surface area of nanoparticles materials.

This technique is based on adsorption and desorption principle and Brunauer-Emmett-Teller (BET) theorem.

1.2.5.5 Optical Characterization

Optical characterization is used to reveal the mechanism of their photochemical processes. These characterization are based on beer-lambert's and basic light principle. These techniques give information about the absorption, reflectance, luminescence and phosphorescence properties of nanoparticle.

Nanoparticles especially metallic and semiconductor nanoparticles possess different colours and therefore best harmonized for photo-related applications. So, it is always interesting to know the value of absorption and reflectance of these materials to understand the basic mechanism for each application. Ultraviolet-visible (UV-Visible), photoluminescence (PL) and the null ellipsometer are known optical instruments, which can be used to study the optical properties of nanoparticles materials.

The UV-Visible-diffuse reflectance spectrometer (DRS) is a fully equipped device which can be used to measure the optical absorption, transmittance and reflectance. the former two are supplementary to each other while the later (DRS) is a special technique use for mostly solid samples. The method is exceptionally acceptable for the determination of band gaps of nanoparticles and other nanomaterials. Band gap of materials is very important to conclude about the photoactivity and conductance of the material. The carbon nanodot-carbon nitride (C_3N_4) was found to be a metal free water splitting photocatalyst. The photo ability of this material is directly correlated to the band gap value of 2.74-2.77eV, which was calculated using UV-Visible spectroscopy (Li *et al.*, 2015). Similarly, this technique also uses to see the absorption shift in case of doping, composite formation or heterostructure nanoparticle materials. Peng *et al.* (2016) synthesized MMT, LaFeO3 and $LaFeO_3$/MMT nanocomposites and studied variation in their electromagnetic radiations absorption through UV-Visible DRS to reconnoiter their optical characteristics. The strong red shift observed in the case of nanocomposite as compared to pristine MMT and $LaFeO_3$ nanoparticles. $LaFeO_3$ and $LaFeO_3$/MMT displayed rather broad absorption band from 400-620nm, showing decrease in their band gap. This property makes these catalysts considerable for solar light driven photocatalysis (Peng *et al*., 2016).

In addition to UV, PL is also considered as a valuable technique to study the optical properties of the photoactive nanoparticles and other nanomaterials. This technique offers additional information about the absorption or emission capacity of the materials and their effect on the overall excitation time of photoexcitation. Thus, it provides significant information about the charge recombination and half-life of the excited materials in their conductance band which are useful for all photo related and imaging applications. The photoluminescence (PL) spectrum can be recorded as emission or absorbance depending on the nature of study.

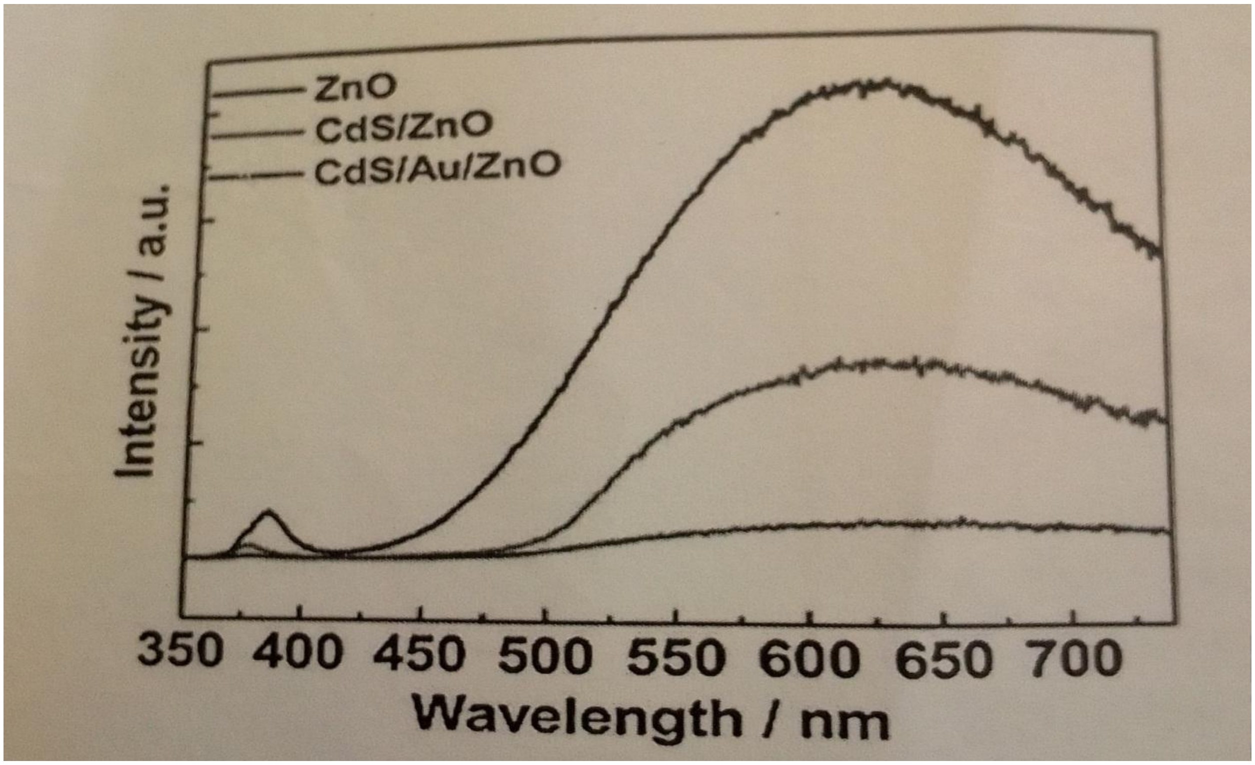

Figure 5: A typical PL spectrum of pristine and modified ZnO nanoparticles.

It is evident from this figure that pristine ZnO nanoparticles show high PL intensitu/Zy as compared to CdS modified ZnO nanoparticles. The gold embedded CdS/Au/ZnO composite shows the lowest intensity. This quenching from pure ZnO to CdS/Au/ZnO can be attributed to the decrease in the rate of charge recombination and larger lifetime of photoexcitation in the latter case (Yu *et al.*, 2013). In addition, this technique is successfully used to determine the thickness of layer, doping quantity of material and defects/oxygen vacancies determination of nanoparticles (Lin *et al.*, 2015; Gupta *et al.*, 2013; Pal *et al.*, 2012; Torchynska *et al.*, 2016).

Similarly, Wan *et al.* determined the values of refractive index and extinction coefficient for hollow gold nanoparticles (HG-NPs) through spectroscopic ellipsometry. They prepared a series of HG-NPs, with different morphologies and plasmonic properties and the optical constant was calculated. The values were compared with the optical constant values of solid gold nanoparticles, which gave good indication to use these materials in chemical sensing applications due to their sensitive nature as revealed from ellipsometric values (Wan *et al*., 2009).

1.2.5.6 Physicochemical Properties of Nanoparticles

Physicochemical properties such as large surface area, mechanically strong, optically active and chemically reactive make nanoparticles unique and suitable applicants for various applications.

1.2.5.7 Electronic and Optical Properties

The optical and electronic properties of nanoparticles are inter-dependent to greater extent. For instance, noble metals nanoparticles have size dependent optical properties and exhibit a strong UV-Visible extinction band that is not present in the spectrum of the bulk metal. This excitation band result when the incident photon frequency is constant with collective excitation of the conduction electrons and is known as the localized surface plasma resonance (LSPR). LSPR excitation results in the wavelength selection absorption with extremely large molar excitation coefficient resonance Ray light scattering with efficiency equivalent to that of ten fluorophores and enhanced local electromagnetic fields near the surface of nanoparticles that enhanced spectroscopies. It is well established that the peak wavelength of the LSPR spectrum is dependent upon the size, shape and interparticle spacing of the nanoparticles as well as its own dielectric properties and those of its local environment including the substrate, solvents and adsorbates (Eustis and El-Sayed, 2006). Gold colloidal nanoparticles are accountable for the rusty colours seen in blemished glass door/windows, while Ag nanoparticles are typically yellow (d electrons in Ag and gold) are freely transportable through the nanomaterial. The mean free path for Ag and gold is ~50nm, which is more than the nanoparticles size of these materials. Thus, no scattering is expected from the bulk, upon light interaction, instead they set into a standing resonance conditions, which is responsible for LSPR in these nanoparticles.

1.2.5.8 Magnetic Properties

The uneven electronic distribution in nanoparticles leads to magnetic property. These properties are also dependent on the synthetic protocol and various synthetic methods such as solvothermal, co-precipitation, micro-emulsion, thermal decomposition, and flame spray synthesis can be used for their preparation (Qi *et al*., 2016; Wu *et al*., 2008). The literature revealed that nanoparticles perform best when the size is less than critical value i.e 10-20nm. At such low scale, the magnetic properties of nanoparticles dominated effectively, which make these particles priceless can be used in different applications (Reiss and Hutten, 2005).

1.2.5.9 Mechanical Properties

The distinct mechanical properties of nanoparticles allow researchers to look for novel applications in many important fields such as tribology, surface engineering, nanofabrication and nano-manufacturing. Different mechanical parameters such as elastic modulus, hardness, stress and strain, adhesion and friction can be surveyed to know the exact mechanical nature of nanoparticles. Besides these parameters surface coating, coagulation, and lubrication also aid to mechanical properties of nanoparticles (Guo *et al*., 2014). Nanoparticles show dissimilar mechanical properties as compared to microparticles and their bulk materials. Moreover, in a lubricated or greased contact, the contrast in the stiffness between nanoparticles and the contacting external surface controls whether the nanoparticles are indented into the plan surface or deformed when the pressure at contact is significantly large.

Decent controls over mechanical features of nanoparticles and their interactions with any kind of surface are vital for enlightening the surface quality and elevating material removal. Fruitful outcomes in these fields generally need a deep insight into the basics of the mechanical properties of nanoparticles, such as elastic modulus and hardness, movement law, friction and interfacial adhesion and their size dependent characteristics (Guo *et al*., 2014).

1.2.5.10 Thermal Properties

Metal nanoparticles have thermal conductivities higher than those of fluids in solid form. Example, the thermal conductivity of copper at room temperature is about 700 times greater than that of water and about 300 times greater than that of engine oil. Even oxides such as alumina (Al_2O_3) have thermal conductivity higher than that of water. Therefore, the fluids containing suspended solid particles are expected to display significantly enhanced thermal conductivities relative to those of conventional heat transfer fluids. Nanofluids are produced by dispersing the nanometric scales solid particles into liquid such as water, ethylene glycol or oils. Nanofluids are expected to exhibit superior properties relative to those of conventional heat transfer fluids and fluids containing microscopic sized particles. It is desirable to use the particles with large total surface area., because the heat transfer takes place at such surface. The large total surface area also increases the stability suspension (Lee *et al*., 1999). Recently, it has been demonstrated that the nanofluids consisting of CuO or AL_2O_3 nanoparticles in water or ethylene exhibit advance thermal conductivity (Cao, 2002).

1.2.6 Applications of Nanoparticles

Nanoparticles can be used in variety of applications such as:

- Manufacturing and materials

Nanocrystalline materials provide very interesting substances for material science since their properties deviate from respective bulk material in a size dependent manner. Manufactured nanoparticles display physicochemical characteristics that induce unique electrical, mechanical, optical and imaging properties that are extremely looked for in certain applications within the medical, commercial, and ecological sectors (Dong *et al.*, 2014). Nanoparticles focus on the characterization , designing and engineering of biological as well as non-biological structures less than 100nm which show unique and novel functional properties. Among the nanotechnology consumer products, health fitness products form the largest category as well as home and garden category. Nanotechnology has been touted as the next revolution in many industries including food processing and packing.

Metals nanoparticles such as noble metals, including Au and Ag have many colours in the visible region based on plasmon resonance, which is due to collective oscillations of the electrons at the surface of nanoparticles. The resonance wavelength strong depends on size and shape of nanoparticles, the interparticle distance, and the dielectric property of the surrounding medium. The unique plasmon absorbance features of these noble metals nanoparticles have been exploited for a wide variety of applications including chemical sensors and biosensors (Unser *et al.*, 2015).

1.2.6.1 Environment

The increasing area of engineered nanoparticles in industrial and household applications leads to the release of such materials into the environment. Assessing the risk of these nanoparticles in the environment requires an understanding of their mobility, reactivity, eco toxicity and persistency (Ripp and Henry, 2011). The engineering material applications can increase the concentration of nanoparticles in groundwater and soil which presents the most significant exposure avenues for assessing environmental risk (Golobic *et al*., 2012). Due to high surface to mass ratio, natural nanoparticles play an important role in the solid/water portioning of contaminants to be absorbed by the surface of the nanoparticles, co-precipitated during the formation of natural nanoparticles or trapped by aggregation of nanoparticle of nanoparticles which had contaminants absorbed to their surface. The interaction of contaminants with nanoparticles is dependent on the nanoparticles characteristics such as, size, composition, morphology, porosity, aggregation/disaggregation and aggregate structure. The luminophores are not safe in the environment and are protected from the environmental oxygen when they are doped inside the silica network (Swadeshmukul *et al*., 2001).

Environmental applications of nanotechnology fall into three categories:

- Environmentally sustainable products example, green chemistry or pollution prevention.
- Remediation of materials contaminated with hazardous substances.
- Sensors for environmental stages.

The removal of heavy metals such as mercury, lead, thallium, Cadmium and arsenic from natural water has attracted considerable attention because of their adverse effects on environment and human health. Superparamagnetic iron oxide nanoparticles are an effective sorbent material for this toxic soft material. So, there is no measurements of engineered nanoparticles in the environment due to the absence of analytical methods to quantify trace concentration of nanoparticles

(Nuller and Nowack, 2008). Photodegradation by nanoparticles is also very common practice and many nanomaterials are utilized for this purpose. Rogozea *et al* (2017), used NiO/ZnO nanoparticles modified silica in the tandem fashion for photodegradation purpose. The high surface area of nanoparticles are due to very small size less than 10nm, facilitated the efficient photodegradation reaction. They also reported the synthesis of variety of nanoparticles and their optical, florescence and degradation applications.

1.2.6.2 Electronics

There has been growing interest in the development of printed electronics in last few years because printed electronics offer attraction to traditional silicon techniques and the potential for low cost, large area electronics for flexible displays, sensors. Printed electronics with various functional inks containing nanoparticles such as metallic nanoparticles, organic electronic molecules, CNTs and ceramics nanoparticles have been expected to flow rapidly as a mass production process for new types of electronic equipment (Kosmala *et al*., 2016).

Unique structure, optical and electrical properties of one dimensional semiconductor and metals make them the key structural block for a new generation of electronics, sensors and photonic materials (Millstone *et al*., 2010). The good example of synergism between scientific discovery and technological development is the electronic industry, where discoveries of new semiconducting materials resulted in the revolution from vacuumed tubes to diode and transistors, and eventually to miniature chips (Cushing *et al*., 2004).

The important characteristics of nanoparticles are facile manipulation and reversible assembly which allow for the possibility of incorporation of nanoparticles in electric, electronic and optical devices such as “bottom up” or selfassembly" approaches are the bench mark of nanotechnology (O'Brien *et al.*, 2001).

1.2.6.3 Energy Harvesting

Nanoparticles are widely used to generate energy from photoelectrical (PEC) and electrochemical water splitting due to their large surface area, optical behavior and catalytic nature (Avasare *et al.*, 2015). Besides water splitting, electrochemical CO_2 reduction to fuels precursors, solar cells and piezoelectric generators also offered advance options to generate energy. Nanoparticles is also use in energy storage application to reserve the energy into different forms at nanoscale. (Lie *et al.*, 2015). Recently, nanogenerators are created , which can convert the mechanical energy into electrical using piezoelectric, which is an unconventional approach to generate energy (Wang *et al.*, 2015).

1.2.6.4 Mechanical Industries

Nanoparticles can offer many applications in mechanical industries especially in coating, lubricants and adhesive applications. Besides, this property can be useful to achieve mechanically stronger nanodevices for various purposes. Tribological properties can be controlled at nanoscale by embedding nanoparticles in the metal and polymer matrix to increase their mechanical strengths. This is because, the rolling mode of nanoparticles in the lubricated contact area could provide very low friction and wear. In addition, nanoparticles offer good sliding and delamination properties, which could also effect in low friction and wear, and hence increase lubrication effect (Guo *et al.*, 2014). Coating can lead to various mechanically strong characteristics, which improves toughness and wear resistance. Alumina, Titania and Carbon based nanoparticles successfully demonstrated to get the desirable mechanical properties in coatings (Kot *et al.*, 2016).

1.2.6.5 Drugs and Medications

Nano-sized inorganic particles of either or complex nature, display unique physical and chemical properties and represent an important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications (Loureiro *et al.*, 2016). Nanoparticles have drawn increasing interest from every branch of medicine for their ability to deliver drugs in the optimum dosage range often resulting in increased therapeutic efficiency of the drugs, weakened side effects and improved patient compliance (Alexis *et al.*, 2008). Iron oxide particles such as magnetite (FeO_4) or its oxidized form magnemite (Fe_2O_3) are the most commonly employed for biomedical applications. The selection of nanoparticles for achieving efficient contrast for biological and cell imaging applications as well as for photo thermal therapeutic applications is based on the optical properties of nanoparticles. The development of hydrophilic nanoparticles as drug carrier has represented over the last few years an important challenge. Among the different approaches, polyethylene oxide (PEO) and polylactic acid (PLA) nanoparticles have been revealed as very promising system for the intravenous administration of drugs (Calvo *et al.*, 1997). Superparamagnetic iron oxide nanoparticles with appropriate surface chemistry can be used for numerous in vivo applications such as MRI contrast enhancement, tissue repair and immunoassay, detoxification of biological fluids hyperthermia, drug delivery and cell separation. All these biomedical applications require that nanoparticles have high magnetization value, a size smaller than 100nm and a narrow particle size distribution (Laurent *et al.*, 2010). The detection of analytes in tissue sections can be accomplished through antigen-antibody interactions using antibodies labeled with fluorescent dyes, enzymes, radioactive compounds or colloidal Au (Khlebtsov and Dykman, 2010).

Over the past few decades, they have been increasing interest in developing biodegradable nanoparticles as effective drug delivery devices (Zhang and Saltman, 2013). Various polymers have been used in drug delivery research as they can effectively deliver the drugs to the target site therefore, increases the therapeutic benefit, while minimizing side effects. The controlled release of pharmacologically active drugs to the precise action site at the therapeutically optimum degree and dose regimen has been a major goal in designing such devices.

Liposomes has been used as a potential carrier instead of conventional dosage forms because of their unique advantages which include ability to protect drugs from degradation, target to the site and reduce the noxiousness and other side effects. However, developmental work on liposome drugs has been restricted due to inherent health issues such as squat encapsulation efficiency, rapid water leakage in the commodity of blood components and very poor storage, and stability. On the other hand, polymeric nanoparticles promise some critical advantages over these materials.

Most of the semiconductor and metallic nanoparticles have immense potential for cancer diagnosis and therapy on account of their surface plasmon resonance (SPR) enhanced light scattering and absorption. Au nanoparticles efficiently convert the strong absorbed light into localized heat which can be exploited for selective laser photo thermal therapy of cancer (Prashant *et al*., 2007). Silver nanoparticles are being used increasingly in wound dressings, catheters and various household's products due to their antimicrobial activity. Antimicrobial agents are extremely vital in textile, medicine, water disinfection and food packaging. Therefore, the antimicrobial characteristics of inorganic nanoparticles add more potency to this important aspect, as compared to organic compounds, which are relatively toxic to the biological systems (Hajipour *et al*., 2012). These nanoparticles are functionalized with various groups to overcome the microbial species selectively. TiO_2, ZnO, $BiVO_4$, Cu and Ni-based nanoparticles have been utilized for this purpose due to their suitable antibacterial efficacies (Yin *et al*., 2016).

Therefore, these nanoparticles were considered for nanoencapsulation of acetogenin, one of the bioactive components of *A.muricata* leaf extract.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

- Sodium alginate and calcium chloride were purchased from a sales agent of sigma Aldrich, Lagos, Nigeria.
- Organic solvents and reagents were analytical grade.
- Precoated thin layer chromatography plate.
- Silica gel (both column and TLC grade)

Equipment

- Chromatographic column
- Ultraviolet lamp
- UV-visible spectrophotometer
- Rotary evaporator
- Centrifuge
- Whatman filter paper, no 42(125mm)

2.2 Methods

2.2.1 Sample collection, identification and preparation of *annona muricata* leaves for extraction.

A fresh leaves of *A. muricata* were obtained from a soursop plant garden in Akokwa, ideato North local government Area, 1mo state, Nigeria and were identified in Botany department of university of Benin, Benin City, Edo State. The *A. muricata* leaves were air-dried at room temperature (26°C), ground to uniform powder, sieve through standard 105mm mesh size and were extracted with ethanol.

2.2.2 Extraction of *A. muricata* leaves.

100g of dried powdered plant leaves were soaked in 1000ml of absolute ethanol (at room temperature) for 48hours. Thereafter, the extract were filtered through a whatmann filter paper No. 42(125mm) and was concentrated using a rotary evaporator with a water bath set at 50^{o}C . The concentrated extracts were therefore used for phytochemical analysis, isolation and encapsulation of bioactive compounds (Usunobun, *et al*., 2015).

2.2.3 Phytochemical analyses

Phytochemical analyses was carried out using standard procedures (Bargah, 2015; Ayoola *et al*., 2008).

Flavonoids test

0.1g of the extract was weighed and heated with 10ml of ethyl acetate in a water bath for few minutes. It was filtered and 2ml of the filtrate was shaken with few drops of dilute ammonia. A yellow colour appears and become colourless when few drops of dilute sulphuric acid was added to it. This indicates the presence of flavonoids.

Tannins test

0.1g of the extract was weighed into a test tube, 10ml of water was measured and added in the test tube. The solution was boiled and filtered. 2ml of 0.1% ferric chloride was added and the solution appeared brownish green colour. This indicates the presence of tannins.

Saponins test

0.1g of the extract was added to 2ml of distilled water in a test tube and the solution was shaken vigorously. A persistent frothing was observed. Few drops of olive oil was added and was shaken vigorously, the solution appeared like an emulsion.

Terpenoids (salkowski test)

0.1g of the extract was weighed and dissolved in 2ml of chloroform, 2ml of acetic anhydride and 3ml of suphuric acid was added. A reddish brown colouration was formed. This indicates the presence of terpenoid.

Anthraquinone test

0.1g of the extract was boiled with 10ml of concentrated H_2So_4 and was filtered. The filtrate was shaken with 5ml of chloroform. A layer was formed, transferred to a test tube and 2ml of dilute ammonia was added. The appearance of a pink red colour in the ammonical lower phase shows the presence of free anthraquinones.

Alkaloids test

The extract was disolved in dilute Hcl and was filtered. The filtrate was treated with Dragendroff's reagent and a red precipitate was formed. This indicates the presence of alkaloid. Dragendroff reagent is a solution of potassium bismuth iodide.

Steroids test

0.1g of the extract was weighed and dissolved in 5ml of chloroform. 5ml of concentrated H_2SO_4 was added by the sides of the test tube. No reddish brown colour at the interface. This shows absence of steroids.

Cardiac glycosides test

0.1g of the extract was weighed and dissolved in 5ml of water, and glacial acetic acid solution (containing 1 drop of ferric chloride solution) was added. A brown ring at the interface was observed when 1ml of concentrated H_2SO_4 was added. This indicates the presence of cardiac glycosides.

2.2.4 Isolation of acetogenin component of. *A muricata* leaf extract using Column Chromatography

The column was prepared by dissolving a certain mass of silica gel in an eluting solvent and poured in the column. The crude ethanolic extract of *A.muricata* was dissolved and introduced slowly from the top of the column. Then, isolation using column chromatography technique with silica gel as stationary phase was established. The isolation procedure was carried out in three different steps, and each step was carried out with solvent of different polarities as the eluent. Eluted fractions were identified using the kedde reagent which formed a dark green complex with the unsaturated y-lactone group present in all annonaceous acetogenin compounds (Mulia *et al*., 2013). Further analysis were conducted on these fractions to establish the level of their purities.

2.2.5 Separation of eluted fractions using thin layer chromatography (TLC).

TLC plate (coated with silica gel) was used for the analysis. Each of the eluted fractions were applied on activated TLC plate with the help of capillary tube at 1cm apart from lower edge of TLC plate marked with a lead pencil and was kept in a developing chamber containing suitable solvent for specific time, until the developing solvent reaches top of the upper edge of TLC plate.

The plate was taken out from developing chamber, dried and solvent front was marked with lead pencil. The spots on TLC plate was detected using iodine solution. The detected spots were marked and the retention factor value (R_f) of each spot was calculated by the formular.

R_f = distance travelled by the sample (cm)/distance travelled by solvent (cm) (Bajpai *et al*., 2016).

2.2.6 Characterization of the isolated compound

The isolated compound was characterized using FT-IR, ^{1}HNMR and ^{13}CNMR (Ghosh *et al*., 2017).

2.2.7 Preparation of sodium alginate nanoparticles containing acetogenin compound.

Sodium alginate nanoparticles were prepared using ionic gelation method (Arwa *et al*., 2018) Calcium chloride was used as cross-linker. Different ratios of sodium alginate and calcium chloride were used to prepare different formulations of acetogenin loaded nanoparticles that is designated as; ACE-1, ACE-2, ACE-3, ACE-4 and ACE-5. Solutions of sodium alginate at different concentrations (0.2-1mg/ml) were prepared by dissolving a known quantity of the salt in distilled water at 37°C and stirred for 25min at 700rpm. Thereafter, acetogenin was added and the solution was stirred for 20mins for complete homogenization. Calcium chloride ($Cacl_2$) solution was prepared by dissolving 1110mg in 100ml of deionized water to produce 100mM. The calcium chloride solution was added drop-wise into the solution of sodium alginate loaded with acetogenin and stirred for 1hr at 1500rpm. The nanoparticles formed were purified using ultracentrifugation.

Table 1: Composition of acetogenin nanoparticles

Formulation	Acetogenin(mg/ml)	Na-alginate(mg/ml)	$CaCl_2$(mM)
ACE-1	0.1	0.2	100
ACE-2	0.1	0.4	100
ACE-3	0.1	0.6	100
ACE-4	0.1	0.8	100
ACE-5	0.1	1.0	100

2.2.8 Determination of particle size

The particle size of sodium alginate and acetogenin loaded sodium alginate nanoparticles were determined using particle size analyzer. Meanwhile, the particle suspension was diluted using deionized water and was equilibrated at 25^{o}C before the analysis was performed using Malvern zetazizer (Malvern Instruments, UK).

2.2.9 Determination of shape and surface morphology

The physical properties of acetogenin loaded sodium alginate nanoparticles were studied using transmission electron microscope (TEM).

The samples were placed onto copper grid and the grid was stained with 2% phosphotungstic acid solution and allowed to stand for 1min before excess stain was removed. Thereafter, the samples were air-dried and nanoparticles shape and surface morphology were examined using Philip 400 transmission electron microscope (Netherland).

2.2.10 Fourier transform infrared spectroscopy (FTIR)

FT-IR was used to establish cross-linking between sodium alginate and calcium chloride. Acetogenin loaded nanoparticles were centrifuged at 15,000 rpm for 30mins at 15^0C and freeze-dried. Thereafter, the dried nanoparticles were mixed with potassium bromide and used for measurement. The FT-IR spectral data were obtained using FT-IR – 400 Jasco Colchester, UK).

2.2.11 Determination of the encapsulation efficiency

The separation of nanoparticles was carried out by ulta-centrifugation of the formulation at 15000 rpm for 1h at 15^0C. Thereafter, the separated nanoparticles were sonicated for 20min to obtain a homogenized solution. The amount of encapsulated acetogenin was determined using uv-visible spectrophotometric method at 480nm (Mitra *et al*., 2001) Encapsulation efficiency (EE) of acetogenin was calculated.

$$\%\ \mathrm{EE} = \frac{(A\text{-}AS)}{A} \ \mathrm{X}\ \frac{100}{1}$$

$$\%\ \mathrm{LC} = \frac{(A\text{-}AS)}{W} \ \mathrm{X}\ \frac{100}{1}$$

A = Total amount of acetogenin in added solution.
AS = Total amount of acetogenin in supernatant after centrifugation (Dounighi *et al*., 2012)
W = Weight of the nanoparticles measured after freeze drying.

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

Table 2: Results of the phytochemical analysis of ethanolic leaf extract of *Annona muricata*.

Phytochemicals	*A. muricata*
Flavonoids	+
Tannis	+
Saponins	+
Terpenoids	+
Anthraquinone	-
Alkaloids	+
Steroids	-
Glycosides	+

+ = Present

- = Absent

The ^{1}HNMR spectrum revealed the existence of 3 proton triplet at 1.0

1 proton singlet was observed at 3.3

4 proton quartet was also observed at 3.5

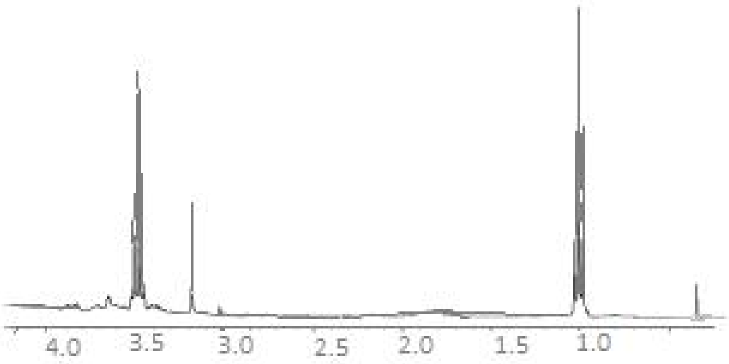

Figure 6: ^{1}HNMR of the isolated compound

The Shift from 19-30ppm and 30 -38ppm correspond to CH_3CO and RCH group respectively.

Shift from 60-65ppm and 67-82 correspond to RCH_2OH and akyne group respectively.

Shift from 92ppm correspond RC=CH group and shift at 104 correspond to alkene and aromatic group.

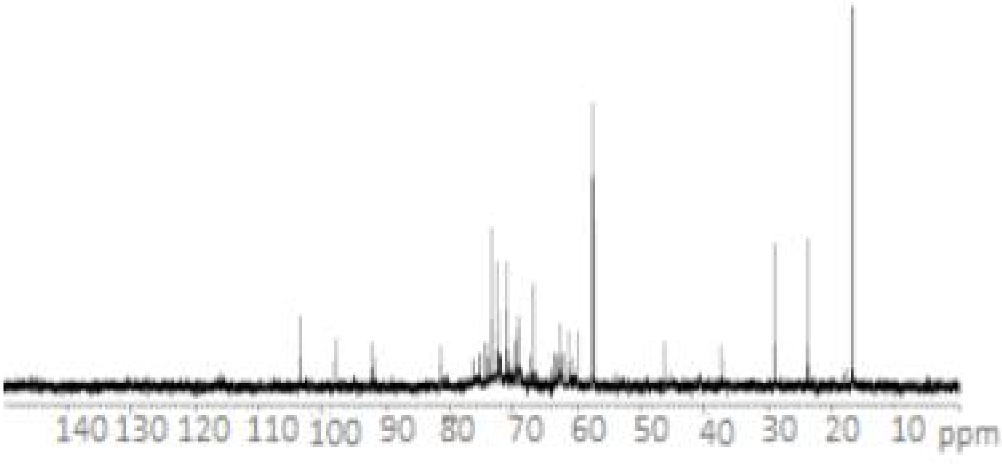

Figure 7: ^{13}CNMR of the isolated compound

Table 3: Gradient elution pattern of fractions

Fraction	Hexane%	Ethylacetate%	Ethanol%	Spot
1	100	-	-	1
2	50	50	-	2
3	-	100	-	2
4	-	50	50	2
5	-	-	100	1

Plate 2: Eluted fractions from Column Chromatography

- Broad peak at 3230cm^{-1} correspond OH group.
- Peak at 2930cm^{-1} correspond CH_2 alkenes group.
- Peak at 1390cm^{-1} correspond to CH_3 alkane group
- Peak at 1260cm^{-1} correspond to CO ester

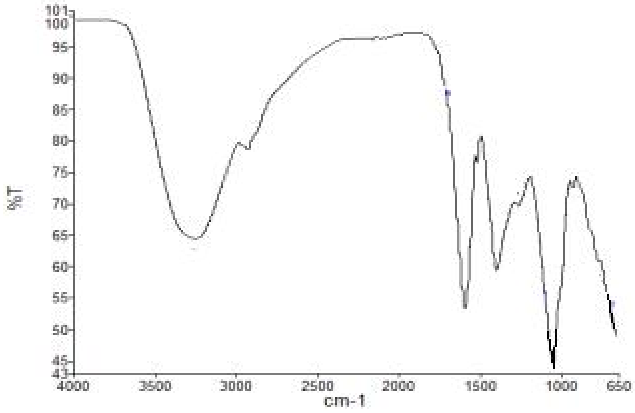

Figure 8: FTIR spectrum of the isolated compound

3.1 Particle size

Sodium alginate nanoparticles were in the range of 230nm.

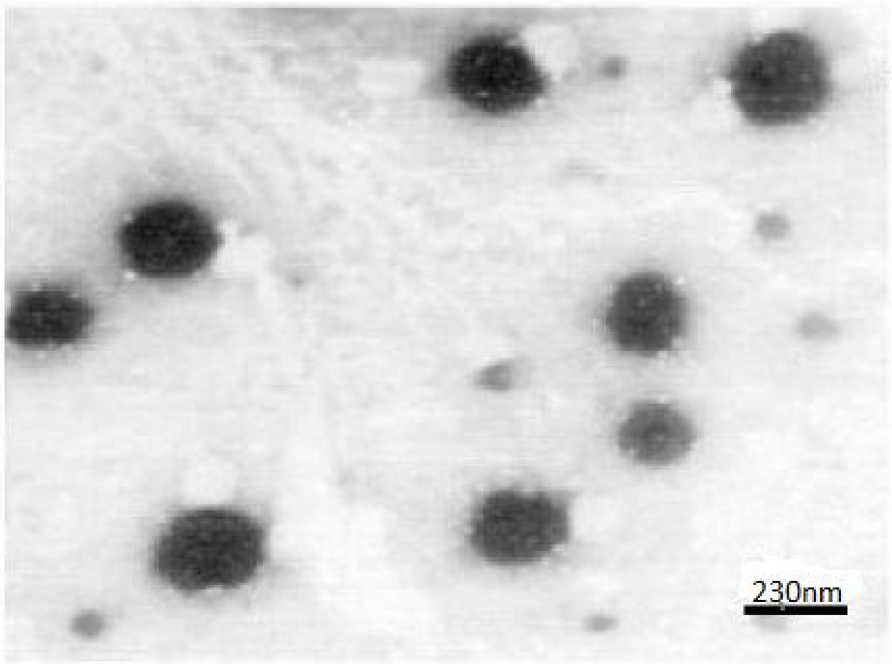

Plate 3: TEM for sodium alginate nanoparticles .

The particle size of sodium alginate nanoparticles loaded with acetogenin compound were in the range of 280nm.

TEM revealed a spherical and irregular shape of sodium alginate nanoparticles loaded with acetogenin compound.

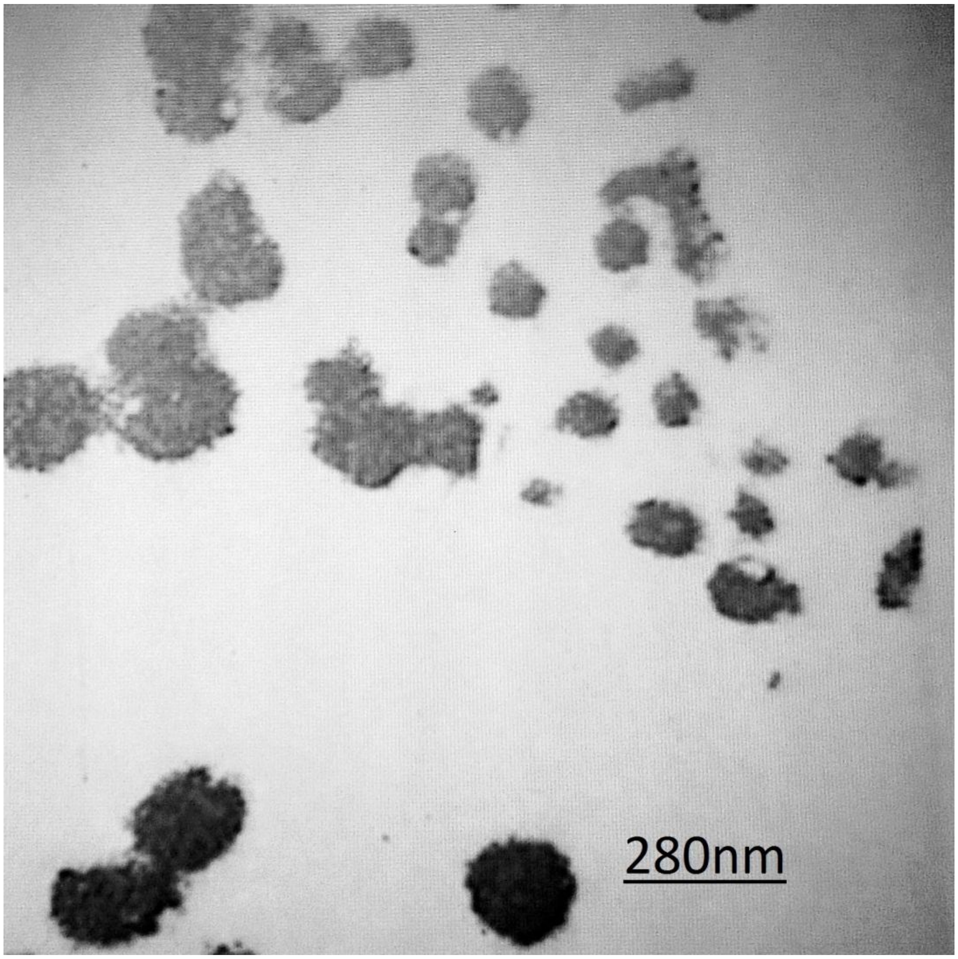

Plate 4: TEM for sodium alginate nanoparticles loaded with acetogenin compound.

Table 4: Particle size and polydispersity of formulation

Formulation	Size(nm)	Polydispersity index
ACE-1	240	0.332
ACE-2	248	0.390
ACE-3	251	0.270
ACE-4	265	0.624
ACE-5	280	0.761

FTIR spectroscopy was used to study the chemical interaction between sodium alginate nanoparticles and acetogenin compound.

- Peak around 3330-3400cm^{-1} correspond to OH group.
- Observed peak at 1600cm^{-1} could be attributed to ionic interaction between the two compound.

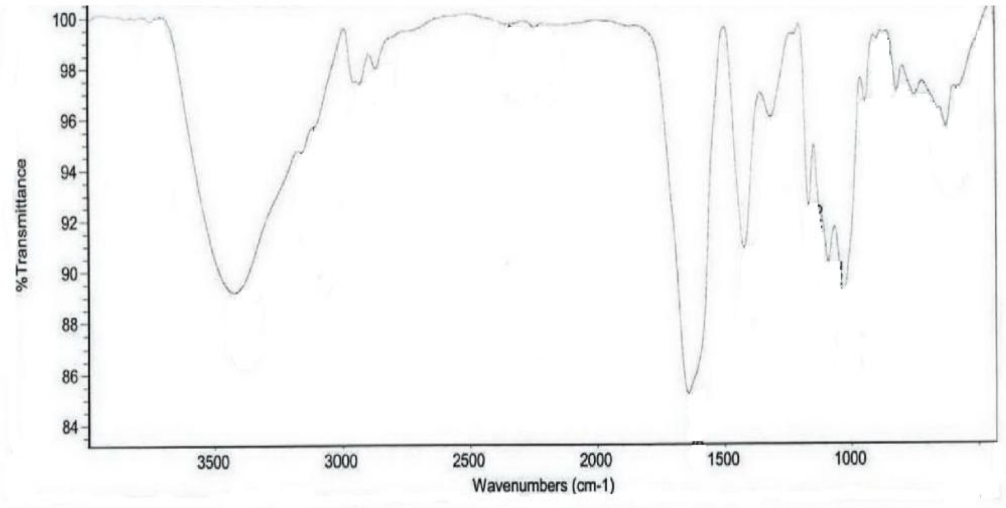

Figure 9: FTIR spectrum of sodium alginate nanoparticles loaded with acetogenin compound.

Table 5: Encapsulation efficiency of acetogenin loaded sodium alginate nanoparticles.

Formulations	Absorbance(530nm)			Mean	EE(%)
ACE-1	0.602	0.600	0.601	0.601	69
ACE-2	0.623	0.621	0.623	0.622	72
ACE-3	0.668	0.700	0.691	0.686	78
ACE-4	0.705	0.7O2	0.698	0.701	84
ACE-5	0.751	0.754	0.750	0.751	89

The percentage encapsulation efficiencies of acetogenin nanoparticles were in the range of 69-89%.

Acetogenin nanoparticles formulations were prepared in triplicate.
The encapsulation efficiency of these formulations (ACE-1-ACE-5) were determined by measuring their absorbance. Three absorbance values of each formulation were taken and the mean values were found (Table 3). The absorbance varied with polymer concentration and encapsulation efficiency increased with increasing concentration of dispersing agent. The formulations showed the highest encapsulation efficiency of 89%.

3.4 Discussion.

Medicinal plants are known to consist many bioactive components with different polarities, which have made their separation and determination a difficult task. Most of them have been purified by combination of several chromatographic techniques and other purification methods.

Acetogenin loaded nanoparticles for nanoencapsulation has been prepared using ionic gelation method. Characterization of nanoparticles formulation revealed high encapsulation efficiency and loading capacity. Polymer based nanoparticles were prepared with biodegradable hydrophilic polymer such as sodium alginate.

3.5 Findings

In this study, acetogenin nanoparticles prepared using ionic gelation had high encapsulation efficiency,

- The encapsulation efficiency increased with polymer concentration.
- The ACE-1-ACE-5 formulations had encapsulation efficiencies range of 69-84% the solution.

3.6 Contribution to knowledge

To the best of my knowledge, higher polymer and acetogenin concentration formulation produced high encapsulation efficiency.

3.7 Conclusion

Identification of this potent compound (acetogenin) in *A.muricata* leaves has been successfully determined and identified using FTIR, ^{1}HNMR and ^{13}CNMR techniques. Meanwhile, acetogenin nanoparticles have been successfully prepared by ionic gelation method. Encapsulation efficiency is high in sodium alginate nanoparticles. Therefore, this technique can be used to increase stability, thereby, retain nutritional and functional qualities of bioactive compounds.

3.8 Recommendation

Further studies on acetogenin loaded sodium alginate nanoparticles as a delivering agent, its release kinetics and absorption methods at the targeted sites are recommended.

REFERENCES

Alara O.R, Abdurahman N.H, Ukaegbu C.I, Kabbashi N.A (2019). "Extraction and characterization of bioactive compounds in vernonia amygdalina leaf ethanolic extract comparing soxhlet and microwave-assisted extraction techniques". *Journal of Talibah University for science*; 4:200-214.

Alexis F, Pridgen E, Molnar L.K, Farokhzad O.C (2008). "Factors affecting the clearance and biodistribution of polymeric nanoparticles" *Molecular Pharmaceutics*; 5:505-515.

Ali A, Zafar H, Zia M, UI Haq I, Phull A.R, Ali J.S, Hussain A (2016). " Synthesis, characterization, applications and challenges of iron oxide nanoparticles" *Nanotechnology, science and Applications*; 9:49-67.

Ali S, Khan I, Khan S.A, Sohail M, Ahmed R, Rehman A, Ur Ansari M.S, Morsy M.A (2017). "Electrocatalytic performance of Ni at Pt core-shell nanoparticles supported on carbon nanotubes" *Journal of Electroanalytical chemistry;* 795:17-25.

Aqel A, El-Nour K.M.M.A, Ammar R.A.A, Al-Warthan A (2012). "Carbon nanotubes, science and Technology part (1) structure, synthesis and characterization" *Arabian Journal of Chemistry*; 5:1-23.

Arwa K, Sajid Bashir, Muhammad Sohail, Muhammad Imran Amirzada (2018). "Characterization of doxorubicin nanoparticles prepared by ionic gelation". *Journal of Pharmaceutical science*; 17(12): 2329-2334.

Astefanei A, Nunez O, Galceran M.T (2011). "Characterization and determination of fullerenes" *A critical review of analytica Chimica Acta*; 882:1-21.

Bajpai V.K, Majumder R, Park J.G (2016). “Isolation and purification of plant secondary metabolites using column chromatographic techniques”. *Journal of Pharmacology*; 11:844-848.

Calvo P, Remuoon-Lopez C, Vila-Jato J.L, Alonso M.J (1997). “Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers” *Journal of Applied Polymer science*; 63:125-132.

Cao Y.C, (2002). “Nanoparticles with Raman spectroscopic finger-prints for DNA and RNA detection” *Science 80*; 297:1536-1540.

Cushing B.L, Kolesnichenko V.L, O’Connor C.J (2004). “Recent advances in the liquid-phase syntheses of inorganic nanoparticles” *Chemical review*; 104:3893-3946.

Dablemont C, Lang P, Mangeney C, Piquemal J.Y, Petkov V, Herbst F, Viau G (2008). “FTIR and XPS study of Pt nanoparticles functionalization and interaction with alumina” *Langmuir*; 24:5832-5841.

Dong H, Wen B, Melnik R (2014). “Relative importance of grain boundaries and size effects in thermal conductivity of nanocrystalline materials” *Scientific report*; 4:7037.

Dounighi M.N, Eskandari R, Avadi M.R, Zolfagharian H, Sadeghi Mir Mohammad A, Rezayat M (2012). “Preparation and in vitro characterization of chitosan nanoparticles containing mesobuthus eupeus scorpion venom as an antigen delivery system”. *Journal of Venomous Animals and Toxins including Tropical Diseases*; 18(1): 44-52.

Dreaden E.C, Alkilany A.M, Huang X, Murphy C.J, El-Sayed M.A (2012).” The golden age: gold nanoparticles for biomedicine” *Chemical Society Review*; 41:2740-2779.

Elliott J.A, Shibuta Y, Amara H, Bichara C, Neyts E.C (2013). “Atomistic modeling of CVD synthesis of carbon nanotubes and grapheme” *Nanoscale*; 5:6662.

Escobar-Khondiker M, Hollerhage M, Pierre M.M (2007). “Annonacin, a natural mitochondrial complex 1 inhibitor, causes tau pathology in cultured neurons”. *Journal of Neuroscience*; 27(29) 7827-7837.

Eustis S, El-Sayed M.A (2006). “Why gold nanoparticles are more precious than pretty gold: noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes” *Chemical Society Review*; 35:209-217.

Filipe V, Hawe A, Jiskoot W, (2010). “Critical evaluation of nanoparticle tracking analysis (NTA) by nanosight for the measurement of nanoparticles and protein aggregates. *Pharmaceutical Research*; 27:796-810.

Ghosh S, Saha M, Bandyopadhyay P.K, Jana M (2017). “Extraction, isolation and characterization of bioactive compounds from chloroform extract of Carica papaya seed and its in vivo antibacterial potentialility in channa punctatus”. *Microbial pathogenesis*; 111:508-518.

Golobic M, Jemec A, Drobne D, Romih T, Kasemets K, Kahru A(2012). “Upon expose to Cu nanoparticles, accumulation of copper in the isopod *porcellio scaber* is due to the dissolved Cu ions inside the digestive tract” *Environmental Science and Technology*; 46:12112-12119.

Gross J, Sayle S, Karow A.R, Bakowsky U, Garidel P (2016). “Nanoparticle tracking analysis of particle size and concentration detection in suspensions of polymer and protein samples: influence of experimental and data evaluation parameters” *European Journal of Pharmaceutics and Biopharmaceutics* ; 104:30-41.

Gujrati M, Malamas A, Shin T, Jin E, Sun Y, Lu Z.R (2014). “Multifunctional cationic lipid-based nanoparticles facilitate endosomal escape and reduction-triggered cytosolic siRNA release” *Molecular Pharmaceutics*; 11:2734-2744.

Guo D, Xie G, Luo J (2014). “Mechanical properties of nanoparticles: basics and applications” *Journal of Physics D Applied Physics*; 47:13001.

Mulia K, Winarcahyo S.W, Krisanti E, Kurniasuci D (2013). “Practical isolation of bullatacin from Annona muricata leaves extract using an open column chromatography technique”. *Advanced materials research*; 789:545-550.

Gupta K, Singh R.P, Pandey A (2013). “Photocatalytic antibacterial performance of Tio_2 and Ag-doped Tio_2 against S. aureus, P. aeruginosa and E.coli” *Beilstein Journal of Nanotechnology*; 4:345-351.

Hajipour M.J, Fromm K.M, Ashkarran A, Akbar de Aberasturi D, Jimenez de Larramendi I.R, Rojo T, Serpooshan V, Parak W.J, Mahmoudi M (2012). “Antibacteria properties of nanoparticles” *Trends Biotechnology*; 30:499-511.

Khan I, Ali S, Mansha M, Qurashi A (2017). “Sonochemical assisted hydrothermal synthesis of pseudo-flower shaped Bismuth vanadate($BiVO_4$) and their solar driven water splitting application” *Ultrasonics Sonochemistry*; 36:386-392.

Khlebtsov N, Dykman L (2011). “Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies” *Chemical Society Reviews*; 40:1647-1671.

Kim H.M, Jung J.I, Chai C, Imm J.Y (2019). “Characteristics and glucose uptake promoting effects of chrysin loaded phytosomes prepared with different phospholipids matrices”. *MDPI nutrients*; 11 (Google scholar).

Kot M, Major L, Lackner J.M, Chronowska-Przywara K, Janusz M, Rakowski W (2016). “Mechanical and Tribological properties of carbon-based graded coatings. *Journal of Nanomaterials*; 2016:1-14.

Lee S, Choi S.U, Eastman J.A (1999). “Measuring thermal conductivity of fluids containing oxide nanoparticles” *Journal of heat and Mass Transfer*; 121:280-285).

Lei Y.M, Huang W.X, Zhao M, Chai Y.Q, Yuan R, Zhuo Y (2015). “Electrochemiluminescence resonance energy transfer system: mechanism and application in ratiometric aptasensor for lead ion” *Analytical chemistry*; 87:7787-7794.

Li Z, Jiang, H, Xu C, Gu L.A (2015). “Using nanoparticles to enhance absorption and bioavailability of phenolic phytochemicals”. *Food hydrocoll*; 43:153-164.

Lin G, Zhang Q, Lin X, Zhao D, Jia R, Gao N, Zuo Z, Xu X, Liu D (2015). “Enhanced photoluminescence of gallium phosphide by surface plasmon resonances of metallic nanoparticles”

Loureiro A, Azoia N.G, Gomes A.C, Cavaco-Paulo A (2016). “Albumin-based nanodevices as drug carriers” *Current Pharmaceutical Design*; 22:1371-1390.

Ma S, Livingstone R, Zhao B, Lombardi J.R (2011). “Enhanced spectroscopy of nanostructured semiconductor phonon modes” *Journal of Physical Chemistry Letters*; 2:671-674.

Marrache S and Dhar S (2012). “Engineering of blended nanoparticles platform for deliveryof mitochondria-acting therapeutics”. *Proceedings of the National Academy of Sciences of the United States of America*; 109(40): 16288-16293.

MabenaL.F, Sinha Ray S, Mhlanga S.D, Coville N.J (2011). “Nitrogen-doped carbon nanotubes as a metal catalyst support” *Applied Nanoscience*; 1:66-77.

Millstone J.E, Kavulak D.F.J, Woo C.H, Holcombe T.W, Westling E.J, Briseno A.L, Toney M.F, Frechet J.M.J (2010). “Synthesis, properties and electronic applications of sized-controlled poly (3-hexylthiophene) nanoparticles” *Langmuir*; 26:13056-13061.

Mirzadeh E, Akhbari K (2016).”Synthesis of nanomaterials with desirable morphologies from metal-organic frameworks for various applications” *Cryst EngComm*; 18:7410-7424.

Mitra S, Gaur U, Ghosh PC, Maitra AN (2001). “Tumor targeted delivery of encapsulated dextran-doxorubicin conjugate using chitosan nanoparticles as carrier”. *Journal of Controlled Release*; 74(1-3): 317-323.

Murano E (1998) “Use of natural polysaccharides in the microencapsulation techniques”. *Journal of Applied Ichthyology*; 14(3-4): 245-249.

Ngoy J.M, Wagner N, Riboldi L, Bolland O (2014). “A CO_2 capture technology using multi-walled carbon nanotubes with polyaspartamide surfactant” *Energy Procedia*; 63:2230-2248.

O’Brien S, Brus L, Murray C.B (2001). “Synthesis of monodisperse nanoparticles of barium titanate:towards a generalized strategy of oxide nanoparticle synthesis” *Journal of American Chemical Society*; 123:12085-12086.

Pal M, Pal U, Jemenez J.M.G.Y, Perez-Rodriquez F (2012). “Effects of crystallization and dopant concentration on the emission behavior of TiO_2: Eu nanophoshores” *Nanoscale Research Letters*; 7:1.

Pateiro M, Gomez B, Munekata Paulo E.S, Barba F.J, Putnik P, Kovacevic D.B, Lorenzo J.M (2021). “Promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products”. *MDPI*; 26(6):1547.

Peng K, Fu L, Yang H, Ouyang (2016). “Perovskite $LaFeO_3$/ montmorillonite nanocomposites:synthesis, interface characteristics and enhanced photocatalytic activity” *Scientific reports*; 6:19723.

Poovi G, Damodharam N (2018). “Lipid nanoparticles: A challenging approach for oral delivery BSC Class-ll drugs”. *Future Journal of Pharmaceutical Sciences*; 4:191-205.

Rao J.P, Geckeler K.E (2011). “Polymer nanoparticles:preparation techniques and size-control parameters” *Progress in Polymer Science*; 36:887-913.

Rawat M.K, Jain A, Singh S, MehnertW, Thunemann A.F, Souto E.B, Mehta A, Vyas S.P (2011). “Studies on binary lipid matrix based solid lipid nanoparticles of repaglinide:in vitro and in vivo evaluation" *Journal of Pharmaceutical Science*; 100:2366-2378.

Ress G, Hutten A (2005). "Magnetic nanoparticles:applications beyond data storage" *Nature material*; 4:725-726.

Ripp S, Henry T.B (2011). "Biotechnology and Nanotechnology Risk Assessment: Mindingand Managing the potential Threats around Us, ACS Symposium Series" *American Chemical Society, Washington D.C*; 1079.

Rogozea E.A, Peteu A.R, Olteanu N.L, Lazar C.A, Cadar D, Mihaly M (2017). "Tandem adsorption-photodegradatiion activity induced by light on NIO-ZnO p-n couple modified silica nanomaterials" *Material Science in Semiconductor Processsing*; 57:1-11.

Saeed M, Abbas Z.M, Ali S, Nasser M.D, Saman S and Mehrasa R.B (2013). "Preparaction and characterization of nanoparticles containing ICD-85 (Venom derived piptides)". *Journal of innovation & applied studies*; 4:534-542.

Shin W.K, Cho J, Kannan A.G, Lee Y.S, Kim, D.W (2016). "Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO_2 nanoparticle for lithium–ion polymer batteries" *Scientific Reports*; 6:26332

Sigmund W, Yuh J, Park H, Maneeratana V, Pyrgiotakis G, Daga A, Tylor J, Nino J.C (2006). "Processing and structure relationships in electrospinning of ceramic fiber systems" *Journal of the American Ceramic Society*; 89:395-407.

Sikora A, Shard A.G, Minelli C (2016). "Size and potential measurement of silica nanoparticles in serum using tunable resistive pulse sensing" *Langmuir*; 32:2216-2224.

Siti M, Abdul W, Ibrahim J, Md Areeful H, Liaba A (2018). "Exploring the leaves of Annona muricata L. as a source of potential anti-inflammatory andanticancer agents". *Frontiers in pharmacology*; 9:661.

Sun S (2000). "Monodisperse FePt nanoparticles and ferromagnetic FePt nanocrystal superlattices" *Science 80*; 287:1989-1992.

Swadeshmukul S, Peng Z, Kemin W, Rovelyn T, Weihong T (2001). "Conjugation of biomolecules with luminophore-doped silica nanoparticles for photostable biomarkers" *Analytical Chemistry*; 73:4988-4993.

Talmaciu A.I, Ravber M, Volf I, Knez Z, Popa V.I (2016). "Isolation of bioactive compounds from spruce bark waste using sub-superficial fluids". *Journal of supercritical fluids*; 117:143-251.

Thomas S, Harshita, B.S.P, Mishra P, Talegaonkar S (2015). "Ceramic nanoparticles:fabrication methods and applications in drug delivery" *Current Pharmaceutical Design*; 21:6165-6188.

Tonnesen H.H and Karisen J (2002). "Alginate in drug delivery system". *Drug Development and Industrial pharmacy*; 28(6): 621-630.

Torchynska T.V, El Filali B, Ballardo Rodriguez I.C, Shcherbyna L (2016). "Deffect related emission of ZnO and ZnO Cu nanocrystals prepared by electrochemical method" *Physica Status Solidi*; 13:594-597.

Usunobun U, Okolie N.P, Anyanwu O.G, Adegbegi A.J, Egharevba M.E (2015). “Phytochemical screening and proximate composition of Annona muricata leaves”. *European Journal of botany plant sciences & phytology*; 2(1):18-28.

Wan D, Chen H.L, Lin Y.S, Chang S.Y, Shieh J, Chen S.H (2009). “Using spectroscopic ellipsometry to characterize and applythe optical constants of hollow gold nanoparticles” *American Chemical Society Nano*; 3:960-970.

Wang Z, Pan X, He Y, Gu H, Wang Y, Wang Z (2015). “Piezoelectric nanowaves in energy harvesting applications” *Advances in Materials Science and Engineering*; 2015:1-21

Wu W, He Q, Jiang C (2008). “Magnetic iron oxide nanoparticle:synthesis and surface functionalization strategies” *Nanoscale Research Letters*;3:397-415.

Yin Q, Wu W, Qiao R, Ke X, Hu Y, Li Z (2016). “Glucose-assisted transformation of Ni-doped ZnO at void at SiO_2 core-shell nanocomposite photocalytst” *Royal Society of Chemistry Advances*; 6:38653-38661.

Yu Z.B, Xie Y.P, Liu G, Lu G.Q, Ma XL, Cheng H.M (2013) “Self-assembled CdS/Au/ZnO heterostructure induced by surface polar charges for efficient photocataytic hydrogen evolution” *Journal of Material Chemistry A*; 1:2773.

Zhang J, Saltman M (2013). “Engineering biodegradable nanoparticles for drug and gene delivery” *Chemical Engineering Progress*; 109:25-30